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Newsletter of Indian Academy of Medical Genetics

Volume: 7 | Issue: 1 (January - March 2014)

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Editorial

Story of the Diagnosis of Intellectual Disability Continues: New Challenges

I remember our journey through the diagnosis of intellectual disability. We have witnessed a lot of progress over the years in the diagnostic yield for cases with intellectual disability and malformation syndromes. Until a few decades ago, cases with ID were diagnosed largely on the basis of clinical features. Dysmorphology diagnosis was interesting and a relatively easy puzzle to solve and could be done without sophisticated metabolic investigations, to which we did not have access. At that time the sources of information for syndrome diagnosis were atlases for syndromes and Smith's textbook of malformations (Smith's Recognizable Patterns of Human Malformations). Mendelian Inheritance in Man was in book form and difficult to search. The only diagnostic investigations available to us were neuroimaging by CT scan or MRI and traditional karyotyping. It was not unusual that one would suddenly find the diagnosis of an intriguing case seen months ago while browsing through case reports in the American Journal of Medical Genetics or Clinical Dysmorphology. The diagnosis of storage diseases would be based on organomegaly and supportive clinical findings like cherry red spot, etc. The situation changed rapidly with availability of the molecular diagnosis for fragile X syndrome. Discovery of the genetic basis of fragile X syndrome and phenomena such as decreased penetrance were exciting developments in the field. Premutation, Sherman paradox and complexities of genetic counseling for fragile X syndrome were the most discussed highlights of the nineteen- nineties.

Availability of online databases for dysmorphology and metabolic disorders provided easy entry into the maze of diagnostics. With internet-based databases like Pubmed and OMIM, search became easier and one could be reasonably confident that the case one was describing was a newly delineated syndrome, not similar to any of the previously described syndromes. We were waiting for all the causative gene defects to get identified and thought it would solve all confusions surrounding the phenotypically similar syndromes and help clinical geneticists to be confident of the diagnosis. Soon genetic etiologies of many malformation syndromes were identified. Progeria and mandibuloacral disorders were found to be caused by mutations in the LMNA (lamin A) gene. Noonan syndrome, cardio-facio-cutaneous, Costello syndrome, etc. were found to have mutations in different genes in the same molecular pathway. Genetics of syndromes with renal, retinal, neurological disorders with or without polydactyly evolved into a group of ciliopathies. Genetics of craniosynostosis was found to be equally complex and interesting. This gene – syndrome association works both ways. One gene may cause multiple phenotypes and the same phenotype can be caused by different genes. Examples of same phenotypes being caused by mutations in different genes include Cornelia de Lange syndrome and Coffin Siris syndrome. The identification of causative genes has changed the existing classifications of many genetic disorders, with incorporation of the underlying genetic defects into the classification. This is very much reflected in newer versions of classification of genetic bone disorders. Cases with milder phenotypes are also now getting identified as a result of mutation detection.

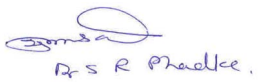
It was expected that the availability of mutation based diagnosis would bypass the need for clinical geneticists. But as has been well said by senior clinical geneticist Dr Judith Hall, phenotype delineation and documentation continues to remain an important exercise for patient care and research. Many genes have been found to cause syndromic as well as non- syndromic mental retardation. Many genes for X linked mental retardation have been identified. Though the genes for many malformation syndromes and syndromes with intellectual disability have been identified, their utility in clinical

practice remains limited due to the large number of genes and the cost involved in testing.

Along with these developments in molecular genetics, there have been significant advances in the field of cytogenetics, with traditional cytogenetics getting transformed into molecular cytogenetics. Many microdeletion syndromes have got delineated. FISH (fluorescence in situ hybridization) has come into clinical practice. However, limitation of tests such as FISH are that they are target based and clinical suspicion is needed before ordering the test as against traditional karyotype which can look at the whole genome, though at a lower resolution. Screening tests and confirmatory tests for metabolic disorders have now become available in India. In spite of this extensive diagnostic armamentarium, 30 to 40% of cases with intellectual disability are left without an etiological diagnosis and genetic counseling is a challenging task in such situations.

On this background, emergence of genomic techniques like cytogenetic microarray to detect microdeletions / duplication syndromes and next generation sequencing is a major milestone. These have revolutionized testing in medical genetics in general and especially in intellectual disability and malformation syndromes. As both techniques cover the whole genome they can be used for etiological diagnosis with or without a clinical diagnosis. The diagnostic yield of microarray for intellectual disability is more than 10 to 15%. Exome sequencing can identify genetic defects of monogenic disorders presenting as sporadic cases without any clinical pointers to the diagnosis. This is fascinating and equally, important in prevention of recurrences of serious and / or handicapping disorders in families. A special diagnostic strategy for autosomal recessive disorders needs to be mentioned especially in the Indian scenario. SNP microarray to identify homozygous areas in patients from consanguineous families can help to locate the candidate genes which later can be sequenced. This has proved a successful strategy in our limited experience with autosomal recessive Osteogenesis imperfecta and intellectual disability. Our experience has shown that in up to 35% of cases, patients with recessive disorders are from consanguineous families. Of course microarray and exome sequencing are equally effective in autosomal dominant and sporadic cases. So the diagnosis appears to be more within reach for the disorders which were considered to be diagnostic challenges. Now the challenge is to decipher the results of genomic techniques and to identify the actual causative mutation from the thousands of non-pathogenic variations spread all over the genome.

Even as clinical geneticists and laboratory scientists are getting more and more experience in these newer diagnostic techniques, diagnosis and research in malformation syndromes / intellectual disability continues to remain a challenge. Best wishes for the New Year and the New Era!



Shubha Phadke
1st January, 2014

Announcement

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An entity which still occurs - Congenital Rubella Syndrome

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Introduction

The rubella virus is a member of the Togaviridae family, genus Rubivirus. It is spread through airborne droplets and infected people are either asymptomatic or present with symptoms mimicking upper respiratory infection. However, primary Rubella infection during pregnancy causes significant concern due to the associated risk of congenital Rubella syndrome (CRS). Primary rubella infection within 11 weeks of pregnancy is associated with 90% fetal transmission and a 90% risk of CRS in the fetus.[1] Common features in CRS include deafness, eye lesions (cataract, retinopathy), congenital heart disease (patent ductus arteriosus, pulmonary stenosis etc.), microcephaly and intrauterine growth restriction (IUGR). We hereby present a case of CRS, with osseous changes and epispadias which though known to be associated with CRS are relatively uncommon.

This case report highlights the morbidity and mortality of a vaccine preventable disorder and reiterates to remind pediatricians and obstetricians to check the immune status of adolescent girls and women of child bearing age.

Case Report

A non-consanguineous couple was referred for counseling at 36 weeks gestation with abnormal antenatal ultrasound findings. There was multisystem involvement with hepatosplenomegaly, cardiomegaly with diffuse cardiac dilatation, lissencephaly, cerebral ventriculomegaly (13mm), porencephalic cysts in the cauda-thalamic region, mild oligohydramnios and growth restriction (gestational age by USG - 33wks and by LMP - 36wks). Previous antenatal scans were normal with no congenital anomaly or growth restriction. The primigravida mother gave a history of high grade fever with rash in the periconceptional (mid cycle) period lasting for a week. There was no history of pregnancy induced hypertension, gestational diabetes or teratogen exposure during pregnancy.

As part of the investigations done early in the first trimester, Rubella IgM (6.12U/ml) and IgG (27.14U/ml) (reactive >1) were positive at 5-6 wks of gestation. However evaluation and follow up for the same was not done. All previous ultrasounds were normal till the current scan at 36 weeks gestation. As the history and investigations suggested congenital rubella syndrome (CRS), the couple were counseled. Other differential diagnosis discussed during counseling included a multiple malformation syndrome and a storage disorder. Fetal cord blood investigations including Rubella IgM and PCR were advised for confirmation. The couple declined testing due to the advanced gestation. Intrauterine death was identified on a follow up scan a week later and the fetus was delivered. Fetal cord blood for Rubella IgM, PCR, chromosomal analysis and enzyme assays for storage disorders was collected. The fetus and placenta were sent for an autopsy.

The fetal anthropometry corresponded to 33-34 weeks gestation, confirming growth restriction. The fetus had coarse facies, bilateral small low set ears, periorbital puffiness, and a horizontal groove present over the chin. (Figure 1) There was no gum

hypertrophy and corneal clouding/cataract could not be appreciated as the fetus was received in formalin. Examination of external genitalia showed a well developed scrotal sac with epispadias (Figure 2). Relevant findings on internal examination were cardiomegaly (46.6gms corresponding to › 42 wks of gestation), splenomegaly (19 gms corresponding to › 42 wks of gestation), and multiple calcified structures resembling lymph nodes in the peritoneum. Examination of the brain showed dilated third and lateral ventricles with no evidence of lissencephaly. The fetogram showed irregular and frayed metaphyses of all bones. There were linear striations of increased radiopacity extending from the epiphysis into the metaphyses of tubular bones. (Figure 3)

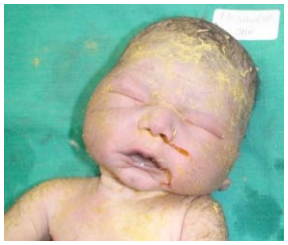

Figure 1: Face of the fetus with Congenital Rubella syndrome

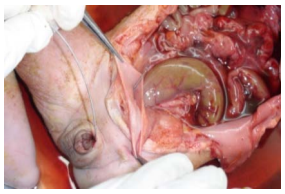

Figure 2: Scrotal sac with epispadias and peritoneal calcification

The histopathological examination confirmed the presence of lymph nodes in the peritoneum. The fetal organs and placenta were otherwise unremarkable.

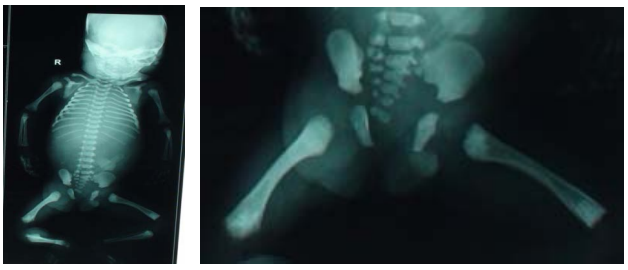

Fig 3: Irregular and frayed metaphyses of all tubular bones

Cord blood IgM was positive (15.36U/ml) while rubella PCR on fetal blood was negative. The cord blood enzyme assays for Gaucher disease, GM1 Gangliosidosis, and Niemann Pick disease were normal. Karyotype was normal (46, XY),

Based on all the above findings a final diagnosis of congenital rubella infection was made.

Discussion

Congenital Rubella syndrome, the most serious complication of rubella infection, occurs when a non immune woman contracts infection during pregnancy. It is characterized by abnormalities of the heart (patent ductus arteriosus, peripheral pulmonary stenosis, ventricular septal defects), intellectual disability, ophthalmological anomalies (cataracts, microphthalmia, chorioretinitis, pigmentary retinopathy), sensorineural hearing loss and microcephaly. Perinatal morbidity includes spontaneous abortion or fetal death. Neonatal manifestations also include hepatosplenomegaly, thrombocytopenia, meningoencephalitis and radiological abnormalities. Dewan and Gupta et al in their study population in India, showed 10-30% of adolescent females and 12-30% of women in the reproductive age-group to be susceptible.[2]

The fetal transmission is maximum when infection occurs in early gestation, being 90% for fetuses exposed before 11 weeks, 67% for fetuses exposed at 11-14 weeks, 25% at 23 - 26 weeks and increases to 53 % in third trimester infection.[1] The most important determinant of fetal outcome is gestational age at the time of infection as the risk of fetal congenital defects varies with the gestational age. It is 90% for infection before 11 weeks of gestation, 33% for infection during weeks 11 - 12, 11% for weeks 13 to 14, and 24% for weeks 15 to 16 with no reported evidence after 16 wks. Rubella infection in our patient occurred in the first trimester and the fetus had clinical features of congenital rubella syndrome with stillbirth which was most likely due to the infection.

The Advisory Committee on Immunization Practices (ACIP) recommend vaccination of all non-pregnant women of childbearing age who do not have documentation of rubella vaccination, serologic evidence of rubella immunity, or laboratory confirmation of rubella disease. Women should be counselled to avoid pregnancy for 28 days after

administration of MMR vaccine. Women known to be pregnant should not receive MMR vaccine.[3]

Though these guidelines recommend universal vaccination of all non immunized women of child bearing age, the disease burden is still high especially in the developing world. In India CRS accounts for approximately 15% of congenital cataract and for 10-50% of cardiovascular defects. Since neurological sequelae are well known, it also contributes to a large proportion of preventable mental retardation in children.

Notable in our case were the presence of both classical and uncommon features. While cardiac involvement and growth retardation are the commonest, osseous changes are reported in 45% of patients. They include irregular trabecular pattern in long bones and irregularity with vertical translucencies at the metaphyseal ends. These features were observed in the fetal radiographs of our patient.

Genital abnormality like hypospadias has been reported only in a single case of CRS. We observed epispadis in the fetus and in the absence of any other contributing factor; we can attribute it to CRS.

Confirmation of CRS in our case was by cord blood IgM which was strongly positive. The rubella virus PCR on fetal blood was negative. This can be ascribed to two reasons: a reported PCR sensitivity of 83-95% and clearance of the virus at the time of testing.[4] It may also suggest testing for the virus in the affected tissue.

Conclusion

Our case highlights the importance of ascertainment of rubella immunization status in all child- bearing women and the severe phenotype when infection is contracted in the first trimester. Preconception care should include confirmation of the Rubella immune status and vaccination as indicated. Prenatal serologic screening in the first trimester is indicated for all pregnant women who lack acceptable evidence of rubella immunity. Testing for Rubella IgG antibodies only is recommended unless a suspicion exists of recent rubella exposure (contact with a person suspected or confirmed to have contracted rubella). Testing for rubella IgM can lead to detection of nonspecific IgM, resulting in a false positive test result and long-persisting IgM results that are difficult to interpret.[3,5] A low Rubella IgG avidity in suspected cases of infection contracted during pregnancy are helpful to evaluate for recent infection and associated risks to the fetus.

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Fragile-X Revisited- Novel Testing, Screening and Treatment Strategies

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Fragile X syndrome (FXS) (MIM #30955) is among the common human single gene disorders, and is the leading cause of inherited cognitive disability and also the second most common cause of genetically associated intellectual disability following trisomy 21. The name refers to a cytogenetic marker on X chromosome at Xq27.3, a "fragile site" in which the chromatin fails to condense properly during mitosis. FXS is caused by a dynamic mutation which involves an unstable expansion of a trinucleotide CGG repeat at the 5 - untranslated region (UTR) of the fragile X mental retardation[1] (FMR1) gene, located at Xq27.3. The silencing of the FMR1 gene causes deficiency of the fragile X mental retardation protein (FMRP), leading to the classical FXS phenotype. It is inherited as an X-linked dominant disorder with reduced penetrance. Important factors that influence disease expression are gender (completely penetrant in males and 50% penetrance in females), the number of CGG repeats and degree of methylation. The complex pattern of inheritance poses an extraordinary challenge for accurate diagnosis and genetic counseling of affected families.

Clinical Phenotype

The classical Fragile X syndrome is characterized by intellectual disability, speech and communication problems. Behavioral challenges include poor concentration, autistic-like behaviors such as an unusual fixed interest in some things and dislike for change in routine. People with Fragile X syndrome have a stronger likelihood of suffering from anxiety and hyperactivity. Physical features show marked heterogeneity. Characteristic features such as long face with prominent mandible, large ears and macro orchidism are seldom noticed in pre-pubertal children (Fig 1). Mental retardation varies from mild

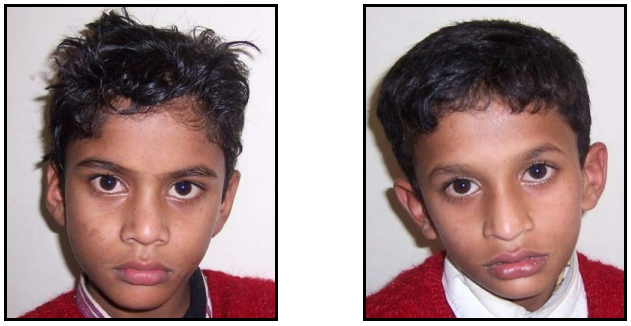

Fig 1: Two brothers with Fragile X syndrome. Note conspicuous absence of any facial dysmorphism (These pictures were provided by Dr Shubha Phadke, Department of Medical Genetics, SGPGIMS, Lucknow)

to profound retardation with males being more severely affected. Female carriers of full mutations may have long face and variable level of cognitive dysfunction (Fig 2). Male carriers of premutation

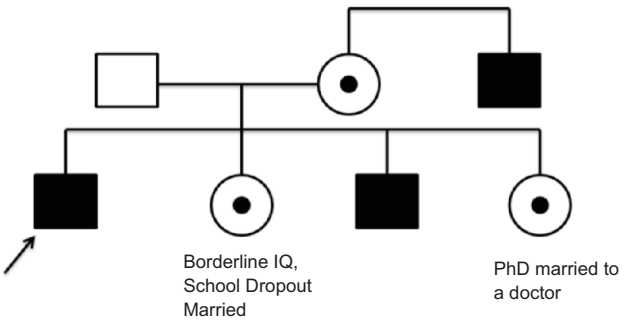

Fig 2: A pedigree with Fragile X syndrome showing X linked inheritance. Note variable intellectual abilities in sisters with full mutation.

may suffer from FXTAS (Fragile X-associated

Table 1: Table showing the CGG repeat size and associated phenotype

Category	CGG repeats	Methylation of FMR1	Female	Male
Stable	6 to ~45	Unmethylated	Not affected	Not affected
Gray zone	~45 to ~55	Unmethylated	Not affected	Not affected
Pre-mutation	~55 to ~200	Unmethylated	Usually not affected/FXPOI	Usually not affected/ FXTAS
Full mutation	>200	Completely methylated	~50% affected	All affected
Full mutation with mosaicism for repeat size	Different cells showing different number of repeats	Methylated in cells with full mutation, unmethylated in cells with premutation/ normal number of repeats	Variable presentation	All affected but higher functioning
Full mutation with mosaicism for methylation	>200	Variable methylation in different cells	Variable presentation	All affected but higher functioning
Full mutation unmethylated	>200	No methylation	Variable presentation	All affected but mild MR

FXPOI: Fragile X-associated primary ovarian insufficiency, FXTAS: Fragile X-associated tremor/ataxia syndrome

tremor/ataxia syndrome) which is a condition affecting balance, tremor and memory in males above the age of 50 years. Female premutation carriers may suffer from FXPOI (Fragile X-associated primary ovarian insufficiency) which is a problem which can lead to infertility and early menopause.

Genetics of Fragile X syndrome

The name of the syndrome comes from its location on the X chromosome. The gene for fragile X syndrome (the FMR1 gene) was identified in 1991. Under particular laboratory conditions (use of folic acid/ thymidine deficient cell-culture medium), the terminal end of the long arm of the X chromosome with the mutated allele can appear broken or fragile. The majority (>99%) of mutations in the FMR1 gene affect the length of a CGG repeat at the promoter region of the gene. On the basis of the size of the CGG repeat and its effect on gene expression, two classes of mutations have been identified in the FMR1 gene: 'premutations' and 'full mutations'. In normal unaffected individuals, the number of repeats ranges from 6 to 55 copies. In clinically unaffected premutation carriers, alleles may have between 55 and 200 CGG repeats (Fig 3).

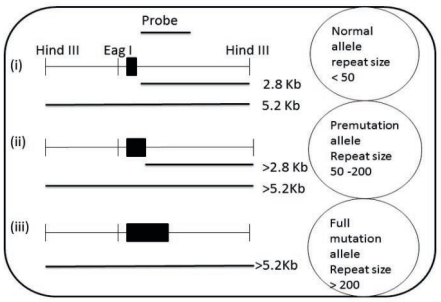

Fig 3a: Mutation, Premutation and normal allele size in Fragile X gene

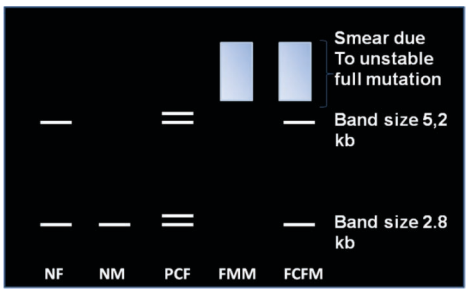

Fig 3b: Diagrammatic representation of Southern blot for Fragile X analysis
Note: NF –Normal female, NM –Normal male, PCF –Premutation carrier female, FMM –Full mutation in male, FCFM- Female carrier of full mutation

In individuals with full mutation, the repeat number ranges from 200 and above.

In affected individuals with a full mutation, the expanded repeats in the 5′ untranslated region of the FMR1 gene and the 5′ region surrounding the promoter of the FMR1 become de novo methylated rendering it transcriptionally inactive. This leads to deficiency of the gene product FMRP. FMRP (FMR protein) binds to specific mRNAs and has an important role in the regulation of protein synthesis at neuronal synapses. Its absence leads to disturbances in synaptic function and dendritic spine morphology, contributing to the clinical phenotype.

Testing for Fragile X syndrome

In view of great variability in the presentation, clinical diagnosis is extremely difficult. Hence testing for fragile X syndrome is indicated in all males with intellectual disability without an obvious cause. Numerous diagnostic methods have been developed for fragile X syndrome, including cytogenetic, Southern blot, polymerase chain reaction (PCR), methylation specific PCR (ms-PCR), and immunohistochemical analyses.

1. **Polymerase chain reaction (PCR)** specific for the CGG trinucleotide repeat region of FMR1 has high sensitivity for FMR1 repeats in the normal and lower premutation range (typically ≤ 100 to 120 repeats; varies by testing laboratory). However, traditional FMR1-specific PCR is less sensitive to larger premutations and fails to amplify full mutations.

2. **Southern blot analysis** detects all FMR1 alleles including normal, larger-sized premutations, full mutations and in addition determines methylation status of the FMR1 promoter region. Abnormal hypermethylation of FMR1 is the cause of transcriptional silencing and is critical to assess for premutation and full mutation alleles. Traditional PCR plus Southern blot analysis has been the "gold standard" for FMR1 molecular diagnosis.

3. **Detection by Antibody:** In past, an FMRP antibody test has been developed to measure expression in lymphocytes. This can detect the full mutation in males. It is not useful in mosaic males or females as some FMRP is still formed.

4. **Methylation status** can be assessed by PCR-based methods independent of measuring the number of CGG repeats.

5. **Sequence analysis.** Very few individuals (~1%) with fragile X syndrome have been identified with an intragenic FMR1 mutation.

6. **TP-PCR:** Triplet Repeat Primed PCR (TP-PCR) is a novel technique for rapid detection of Fragile X mutations, premutaions as well as methylation status. This technique uses a set of three primers to amplify the FMR1 promoter with CGG repeats. Capillary electrophoresis of PCR product shows presence of peaks beyond a threshold in cases with expanded repeats. This technique can distinguish between normal, intermediate size, premutation & full mutation alleles. However, the exact size of the repeats cannot be ascertained, which poses difficulty in prognostication during counseling. It is a rapid way which has been advocated to be useful for routine diagnostic use, carrier screening as well as newborn screening.

Treatment Strategies

There is currently no cure for Fragile X syndrome, but once it is diagnosed there are a number of management strategies that can help many children and adults.

Supportive care: A combination of educational, medical and behavioral management techniques have been demonstrated to improve the outcomes for affected individuals and their families. The child's educational program should include occupational, physical and speech therapies, introduced as early as possible. These therapies can be very effective when designed to meet the needs of an individual in an early intervention and school program. A multidisciplinary team, made up of parents, doctors, pediatricians, educational specialists, psychologists, speech therapists, geneticists, occupational therapists and nurses are

required for appropriate ongoing management for these patients.

Symptomatic Treatment: Specific manifestations like ADHD, aggressive behavior, anxiety, autistic symptoms, mood disorders and seizures can be managed using medications like CNS stimulants, antipsychotics, SSRIs (Selective serotonin reuptake inhibitors), anticonvulsants, etc. as per the symptoms. Use of medications for behavior should be judicious. Individuals with FXS have increased risk for seizures, with rates of 13% to 18% for boys and 5% for girls. Seizures in FXS are easily controlled with a single anticonvulsant. Treatment involves use of a range of anticonvulsant medications; however, because FXS is associated with hypotonia, loose connective tissue, and cognitive and behavioral problems, these issues should be taken into account while the choice of medication is being considered.

Novel Targeted Treatments

1. **mGluR5 antagonists:** FMRP is an RNA-binding protein that modulates dendritic maturation and synaptic plasticity through a mechanism involving particularly the inhibition of group 1 mGluR mediated dendritic protein synthesis. It has been suggested that mGluR5 antagonists would be an effective treatment for FXS. Early animal trials on mice models of Fragile X showed promising results following which human trials using various compounds with mGluR5 antagonistic actions are underway. Recently, a phase II clinical trial was completed, in which 12 adult patients with FXS received a single dose of fenobam to assess drug safety, pharmacokinetics, and a small number of cognitive and behavioral effects. In this trial, fenobam was reported to reduce behavioral abnormalities and improve cognitive performance. In a separate study, three young adult patients with FXS were treated with acamprosate, a drug with mGluR antagonist properties that is approved for maintenance of abstinence from alcohol. In all three patients, acamprosate was associated with improved linguistic communication and global clinical benefit. Other highly potent and selective mGluR5 negative allosteric modulators currently in clinical trials in FXS (see http://www.clinicaltrials.gov) include STX107 (Seaside Therapeutics, phase I trial initiated in the United States), AFQ056 (Novartis, phase II trial recently completed in France, Italy, and Switzerland), and RO4917523 (Hoffman-LaRoche, phase II trial initiated in the United States).
2. **AMPA receptor positive modulators (ampakine):** CX516 in a 4 week trial in adults with FXS did not show any associated improvement in memory, language, or attention/executive function (Berry et al, 2006). Similarly, trial of riluzole in 6 adults with FXS, did not show clinical benefit.
3. **GABA B receptor agonists:** Lithium targets multiple intracellular signaling pathways, which have been linked to Gp1 mGluR signaling. A pilot trial of Lithium treatment on 15 patients with FXS was found to have positive effects on behavioral adaptive skills and cognitive measures. GABA-B receptor agonists such as baclofen inhibit glutamate release, and subsequent postsynaptic mGluR5 activation. Baclofen has been shown to reduce audiogenic seizures in FMR1 knock out mice. A phase II study was conducted using arbaclofen (also known as STX209), the R-isomer of baclofen. This study has been completed and its results are awaited. Another GABA B antagonist, STX209 (Seaside Therapeutics), has shown benefit in a large Phase II trial comprising of 63 patients.
4. **Minocycline:** A tetracycline analog, which has shown beneficial effects on dendritic spine morphology and phenotypic benefits in mice models. It is believed to act by inhibiting matrix metalloproteinase-9 (MMP-9). A Phase II clinical trial is underway.
5. **Enhancement of cholinergic function** in the brain through administration of the acetylcholinesterase inhibitor donezepil was associated with improvement on measures of cognition and behavior in at least two Phase II trials.

Genetic Counseling

Genetic counseling for fragile X syndrome is complex and it is advisable to consult a geneticist to ensure correct interpretation of all positive fragile X test results and the provision of appropriate advice to the family. Mothers of all individuals with an FMR1 full mutation are carriers of an FMR1 mutation. Mothers and their female relatives who are premutation carriers are at increased risk for FXTAS and POI; those with a full mutation may have findings of fragile X syndrome. All carriers are at increased risk of having offspring with fragile X syndrome, FXTAS, and POI. Males with premutations are at increased risk for FXTAS. Males with FXTAS will transmit their FMR1 premutation expansion to none of their sons and to all of their daughters, who will be premutation carriers. Carrier testing for at-risk relatives and prenatal testing for pregnancies at increased risk are possible if the diagnosis of an FMR1-related disorder has been confirmed in a family member. The risk to offspring of a premutation carrier female varies as per the number of repeats and figures are available for predicting accurate recurrence risk. All male fetuses found to have full mutation on prenatal testing are likely to be affected. However, predicting the phenotype of a female fetus detected to have full mutation is difficult because of the highly variable clinical presentation.

Screening for Fragile X

A robust screening method is needed to accommodate future directions of early intervention and anticipation. Thus, to identify individuals with FXS and FMR1-associated disorders and to provide early intervention services for children, an FMR1 sensitive and specific screening method for both males and females is needed. Results of fragile X DNA testing allow accurate genetic counseling to be provided. Carrier testing for at-risk individuals and prenatal testing empowers families to make informed reproductive decisions. Newborn screening for Fragile X has recently been introduced in some populations and initial results show that it has been well accepted. The rationale for newborn screening is that it helps in providing early intervention for infants by the pediatrician or family care provider. In addition, this helps in identification of carrier females and provides opportunity for preventing birth of another affected child. Also premutation carriers can be afflicted with various phenotypes, and counseling and intervention for these can be provided. In addition, the health care provider should consider extended family DNA testing, with the help of a genetic counselor or geneticist, and follow-up care with a multidisciplinary treatment team. Some recent studies have also advocated population screening of reproductive age women for carrier status of Fragile X premutation/full mutation. This was based on reports on premutation carrier rates ranging from 1 in 100 to 1 in 250 in various populations. Offering carrier screening to women, preconceptionally or early during pregnancy would help in primary prevention of Fragile X syndrome. The psychosocial aspects, cost-effectiveness and feasibility of such a screening program are still being evaluated, and presently no guidelines are available for carrier screening of low risk pregnant/non-pregnant women.

Conclusions

Fragile X syndrome is the most common inherited cause of mental impairment among children. Confirmation of clinical diagnosis by genetic testing helps in providing early and appropriate clinical care, prenatal diagnosis services and extended carrier screening to the family. Although no definitive treatment for Fragile X syndrome is available at present, various new molecular therapies are under trial and raise hope for better management of these patients.

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S S Agarwal Sir will continue to guide us...

Dr S S Agarwal Sir left for his heavenly abode on 2nd Dec 2013. His sudden demise was a shock to us. My teacher and mentor and the Father of Indian Medical Genetics is no more. We, most of the medical geneticists and also clinical immunologists in India, are in these fields which were established and nurtured by Dr S S Agarwal. Twenty five years ago, medical genetics was a medical specialty unheard of by Indian doctors.

By a stroke of destiny, Dr Phadke and I came to SGPGI and I joined as a research officer while waiting for a faculty position in Pediatrics. But medical genetics hypnotized me and I continued in that direction. In addition to the fascinating aspects of genetics as a science, the intelligent, towering personality of Dr Agarwal was an equally important factor in attracting me towards medical genetics. His algorithmic approach to clinical diagnosis, devotion to research and vast up-to date knowledge of genetics and for that matter all frontiers of medicine, impressed me and everybody who came into contact with him. We admired his impressive talks and presentations. I learned not only genetics from him, but also several other important aspects such as a systematic clinical approach and the importance of research and laboratory work for clinicians. The department of medical genetics in SGPGI grew under his guidance.

Many of us earlier batches of DM students learned directly from him. I was the first DM student with him and had the wonderful opportunity to work closely with him for more than a decade. His case history writing in a file has to be shown to all students of every department. He would never compromise on the clinical details and would also do a thorough search of scientific literature for each case. He taught me to think big and plan big.

Dr Agarwal played a very important role in not just shaping the Department of Medical Genetics in SGPGI but also in planning out important details for the whole Institute relating to the academic programs, purchase procedures, etc. In his room there would be hundreds of files with his beautifully handwritten notes on various aspects of the institute including technical specifications of equipment for other departments as well. He established the medical genetics specialty in India and SGPGIMS has contributed almost 90% of the medical geneticists in India. Without his vision for DM in medical genetics, medical genetics would not have established its roots in India. Just a week before his untimely demise, he gave an inaugural address for a genetics workshop in the department. When he saw more than 10 of our department alumni as faculty for the course, he was very happy. He was enthused about the fact that most of the DM medical genetics students are now heading medical genetics centers all over India. He expressed his happiness at the success of the DM program and said a word of appreciation to me. These words I will remember forever.

Not only is he the father of medical genetics and medical genetics education in India, he has also been the steering force for research in genetics in India. During his lifetime, he headed various task force committees of ICMR, DBT and DST like the ones on thalassemia control, lysosomal storage disorders, newborn screening, genomics, stem cell research, etc and guided the younger generation. He wished to do much more. He was working with ICMR [and pushing me to make big documents] for establishing genetics centers in selected medical colleges in India. We still need his advice, vision and enthusiasm. With his demise, I feel the loss of fatherly support. More than 25 years of association with him have given me an idea about how he would think if faced with a given situation and felt many times that I know exactly what he would say or do. Still, I always feel the need for discussing scientific and other issues with him. I will miss him. His death is a big loss to biological sciences in India and has created a big vacuum which will not be filled for a long time.

He was excited when we floated the Indian Academy of Medical Genetics and was happy to see how the seeds of genetics he had sown have just started to grow all over the country. We have lost a visionary who architected medical genetics in the country. His words and wisdom will guide and inspire me and all his students for a long time to come.

I pray to God for peace of his soul.

Shubha Phadke, 3rd Dec 13

Approach to Intellectual Disability

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Definition

Intellectual disability or mental retardation is defined as significant sub-average intellectual function existing concurrently with deficits in adaptive behaviour and manifested during the developmental period.

Intellectual disability/ mental retardation (ID/ MR) has 3 components:

i. Significantly abnormal intellectual performance (determined by a test of intelligence)

ii. Impairment of the ability to adapt to the environment

iii. Onset during development before the age of 18 years

Developmental delay (DD) is a term usually used for young children, especially below 5 years of age, in whom formal IQ testing is not possible. It refers to delay in attainment of milestones and implies deficit in learning and adaptation. Global developmental delay (GDD) implies impairment of all spheres of development (gross & fine motor, social & adaptive, and language).

DD/ ID conditions include:

- Static non - progressive disorders with onset of cognitive impairment from birth/ early childhood
- Progressive disorders with onset of cognitive impairment in childhood
- Hereditary neurodegenerative and metabolic disorders with neuro-regression beginning some time after a period of normal development

ID/ MR is said to be present when the intelligence quotient (IQ) is less than 70. Classification of ID based on the IQ is as follows: mild - IQ 50 -70, moderate - IQ 35 – 50, severe - IQ 20 – 35 and profound - IQ < 20.

Etiology

Intellectual disability can result from a large number of conditions of both genetic and non-genetic origin. The broad etiological classification of DD/ ID is listed in Table 1.

Table 1: Etiological classification of intellectual disability

Genetic
Chromosomal anomalies (detectable by conventional cytogenetic techniques) • Down syndrome (most common) • Other numerical chromosomal anomalies (e.g. trisomy 13 & trisomy 18) • Structural chromosomal anomalies- deletion/ duplication/ unbalanced translocation/ ring chromosome • Mosaic chromosomal anomalies

Genetic
Submicroscopic chromosomal anomalies (not detectable by conventional cytogenetic techniques and detected by molecular cytogenetic techniques like FISH/ MLPA/ chromosomal microarray) • Microdeletion/ microduplication syndromes (e.g. Prader Willi syndrome, Angelman syndrome, Williams syndrome etc.) • Cryptic subtelomeric rearrangements • Cryptic interstitial rearrangements
Single gene disorders • Fragile X and other X linked monogenic disorders • Multiple malformation syndromes • Inborn errors of metabolism • Syndromic and non-syndromic monogenic disorders caused by mutations in genes required for neurogenesis, neuronal migration, neuronal and synaptic functions and transcription signalling cascades
Deregulation of imprinted genes (E.g. Prader-Willi syndrome, Angelman syndrome) • Uniparental disomy of imprinted gene • Deletion of non-imprinted gene • Imprinting centre defect
Multiple malformation syndromes without an identified genetic basis • Known sporadic syndromes • New/ private syndromes
Idiopathic (Familial or sporadic cases without a definite identified genetic aetiology)

Non-Genetic
CNS dysfunction due to identified prenatal, perinatal & postnatal acquired and environmental causes • Teratogenic exposures (e.g. fetal phenytoin syndrome, fetal alcohol syndrome) • Intrauterine fetal infections (e.g. congenital toxoplasmosis, congenital CMV infection) • Perinatal and postnatal asphyxia • Prematurity • Postnatal intracranial infections • Peri- and postnatal intracranial trauma and vascular accidents (hemorrhage or infarction)
Idiopathic (Cause not identified)

A precise cause is found only in about 50 - 70% of cases of severe ID; about 35 – 55% cases of mild ID remain idiopathic. Amongst the causes identified for severe ID in studies, genetic causes have been found to account for up to 25–50% of cases.

Down syndrome is the most common identified genetic cause of DD/ ID. It affects 1 in 700 – 800 live born children and accounts for up to 20 - 30% of all cases of MR. Trisomy 21 is found in 95% cases of Down syndrome, translocation involving chromosome 21 occurs in 3 – 4% cases and mosaic trisomy 21 occurs in ~1% of cases.

Fragile X syndrome is the most common monogenic disorder associated with ID and is the most common inherited cause of ID. It accounts for 1 – 2 % of all cases of ID and has an X – linked inheritance pattern.

Prevalence

The overall prevalence of DD/ ID in the general population world-wide is 2 – 3%. The prevalence of moderate and severe ID is 0.3–0.5% and of mild ID is 1– 3%. Mild intellectual disability is 7 – 10 times more common than the severe form. Most epidemiological studies show affected males to affected females ratio of around 1.3 – 1.9: 1; to a large extent the higher prevalence in males is because of X-linked causes of ID.

Diagnostic Approach

The approach to a child with DD/ ID includes a thorough clinical evaluation (clinical and family history, complete dysmorphologic examination, complete neurologic examination, and systemic examination) followed by a judicious use of laboratory tests, imaging studies and appropriate genetic tests.

A. Clinical history

A detailed clinical history that should be elicited from the parents/ guardians for every patient with DD/ ID should include the following:

- Antenatal period (maternal age at conception, history suggestive of teratogenic exposures [drugs and infections] especially in the first trimester, maternal illnesses, abnormal antenatal ultrasound findings)
- Perinatal period (prematurity, difficult labour, birth asphyxia, low birth weight, sepsis, hyperbilirubinemia)
- Postnatal period (seizures or abnormal posturing, history of intracranial infections/ trauma/ vascular accidents, spasticity, visual and hearing problems, exposure to lead, abnormal pattern of sleep)
- Detailed developmental history (time of attainment of milestones, any regression of milestones)
- Detailed behavioural history (changes in behavioural pattern, deterioration in school performance, self-injurious behaviour, repetitive behaviour, autistic behaviour, hyperactivity, attention deficit); behavioural phenotype can provide important clues to the diagnosis as listed in Table 2.

Table 2: Behavioural patterns associated with some intellectual disability conditions

Behavioural pattern	Intellectual disability condition
Autistic features, perseverative speech, problems in impulse control	Fragile X syndrome
Repetitive, stereotypic, purposeless hand movements, fits of screaming and inconsolable crying, autistic features, panic-like attacks	Rett syndrome
Self mutilation	Lesch Nyhan syndrome
Inappropriate happy behaviour and excitability	Angelman syndrome
Self mutilation, aggression and sleep disturbances	Smith Magenis syndrome
Overfriendliness, social disinhibition, attention deficit	Williams syndrome
Autistic features	15q duplication
Hyperphagia and sleep disturbances	Prader Willi syndrome

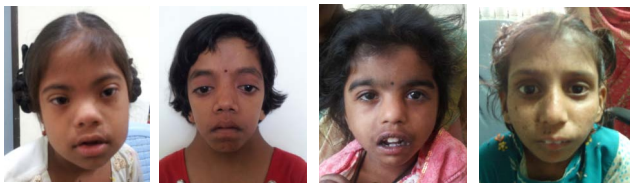
- History suggestive of metabolic disorders (failure to thrive, recurrent unexplained acute illness, seizures, ataxia, loss of psychomotor skills, recurrent somnolence/ coma, lethargy, abnormal body odour)

B. Family history

- Detailed minimum 3-generation family history with exact medical records wherever possible
- Particular emphasis on family members with developmental delay, intellectual disability, seizure disorders, psychiatric disorders, congenital malformations, miscarriages, stillbirths, and unexplained early childhood deaths
- Construction of a pedigree of 3 generations or more to ascertain the pattern of inheritance

C. Physical examination

- Complete anthropometric assessment (height, weight, head circumference, upper segment: lower segment ratio).
- Detailed dysmorphologic evaluation from head to toe should be done in every case of DD/ ID, followed by comparison of findings with available literature, OMIM and dysmorphology databases (such as London Dysmorphology DataBase, POSSUM). There are a large number of dysmorphic and multiple malformation syndromes associated with DD/ ID, which can be diagnosed through thorough physical examination. Figure 1 shows some common



A B C D

Figure 1: Some intellectual disability conditions diagnosable from the typical gestalt.
A. Down syndrome B. Noonan syndrome
C. Cornelia de Lange syndrome D. Cockayne syndrome

dysmorphic syndromes associated with intellectual disability.

- Detailed neurological evaluation must be done in every case and must include assessment of:
  - higher mental functions,
  - cranial nerve deficits,
  - motor and sensory system examination,
  - cerebellar signs,
  - involuntary/ abnormal movements,
  - persistence of neonatal reflexes
- Ophthalmological examination should be done in all cases of intellectual disability. This can provide important clues to the diagnosis (Table 3)

Table 3: Ophthalmological findings associated with some intellectual disability conditions

Ophthalmological finding	Associated intellectual disability conditions
Cataract	Galactosemia, congenital rubella syndrome, Lowe syndrome, congenital myotonic dystrophy, Cockayne syndrome, Warberg micro syndrome
Cherry red spot	Some lysosomal storage disorders: GM1 gangliosidosis, GM2 gangliosidosis (Tay Sachs and Sandhoff disease), Sialidosis, Galactosialidosis, Mucolipidosis, Niemann Pick disease
Corneal clouding	Mucopolysaccharidoses, Oligosaccharidoses, Mucolipidoses
Chorioretinitis	Congenital intrauterine infections
Lens dislocation	Homocystinuria, Sulfite oxidase deficiency
Retinal changes/ pigmentary degeneration	Norrie disease, Bardet Biedl syndrome, Mitochondrial disorders, Neuronal ceroid lipofuscinosis

D. Baseline Investigations:

- Complete hemogram including RBC indices (may provide clue to the diagnosis eg. alpha thalassemia – mental retardation syndrome, macrocytosis in abnormalities of vitamin B12 metabolism etc.)
- SGPT for gross liver function assessment
- Serum creatinine for gross renal function assessment
- Serum creatine phosphokinase must be checked in all young male patients with unexplained developmental delay as Duchenne muscular dystrophy may present in early childhood with developmental delay

E. Serum T4 & TSH:

- 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 is identifiable. Hypothyroidism may be associated with some genetic syndromes e.g. Down syndrome. It is very important to not miss hypothyroidism as it is a treatable cause of intellectual disability.

F. Neuroimaging:

- Neuroimaging is recommended in the work-up of intellectual disability cases particularly when at least one of the following is present:
  - abnormal head size: microcephaly/ macrocephaly
  - seizures
  - focal motor/ neurological findings on neurological examination
  - associated malformations esp. facial malformations
- In addition, it is preferable to do neuroimaging studies if no other aetiology is obvious; in conditions like neuronal migration anomalies and some cases of pre/ perinatal insult there may be no other clinical diagnostic clue.
- Neuroimaging helps to identify hypoxic ischemic sequelae, vascular insults, neuroectodermal syndromes, intracranial structural defects and neuronal migration abnormalities.
- MRI is generally preferable to CT scan for neuroimaging and is much more informative. However, a CT Scan is a better modality for certain situations such as documentation of intracranial calcifications associated with old hemorrhage, tuberous sclerosis complex and congenital CMV or toxoplasmosis infection, and for craniosynostosis.

G. Specific tests based on the clinical features in each individual case:

Based on the clinical findings in each individual case, the following tests may be additionally required in the work-up.

- Electrophysiological studies: Electroencephalogram (EEG)/ Audiometry/ Electromyogram (EMG) / Nerve conduction study (NCS)
- TORCH serology: for suspected congenital intrauterine infections in infants
- Ultrasonography to look for visceral malformations
- Echocardiography to look for cardiac malformations

H. Genetic Evaluation:

Genetic evaluation should be done in all cases of intellectual disability without a definitely proven environmental cause. The label of 'cerebral palsy due to adverse perinatal events' should not be given until there is definite history and evidence in neuroimaging.

a. Karyotyping:

- To confirm the diagnosis of a clinically suspected chromosomal disorder such as Down syndrome, Trisomy 13 or Trisomy 18.

- Should be done in all cases with intellectual disability, where a specific etiology has not been clinically identified.
- Conventional karyotyping (up to 550 band-level) yields a diagnosis in 2.5 – 3% of unexplained/ idiopathic ID cases.

b. Fluorescence in situ hybridization (FISH)/ Multiplex ligation-dependent probe amplification (MLPA) study:

- To confirm the diagnosis of clinically identified microdeletion syndromes such as Prader-Willi syndrome, Angelman syndrome, Di George syndrome, Williams syndrome etc.
- MLPA panels to check for multiple common microdeletion syndromes in one test are available commercially.
- MLPA panels to test for subtelomeric deletions and duplications on all chromosomes in a single test are also available commercially.

c. Testing for Fragile X syndrome:

- Fragile X screening can be done through PCR-based tests but confirmation requires Southern Blot testing.
- If fragile X molecular genetic testing is done using a phenotypic checklist of 7 features (proposed by de Vries et al, J Med Genet 1999) the diagnostic yield is ~7 – 8 % (long jaw, 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 behavior of initial shyness and lack of eye contact followed by friendliness and verbosity).
- Younger children have subtle dysmorphism which may be missed; therefore fragile X testing is recommended in all children especially males with idiopathic ID even in the absence of a family history.

d. Molecular genetic testing:

- If a specific monogenic disorder is suspected clinically, mutation analysis of the relevant gene should be done for confirmation and for identification of the exact disease-causing mutations in the family, wherever feasible. Identification of the mutations helps in offering accurate carrier screening and prenatal diagnosis for family members.

e. Metabolic testing:

- If a specific inborn error of metabolism (IEM) is suspected clinically, the relevant biochemical assay such as enzyme assay/ plasma amino acid HPLC/ urine GCMS for organic acids etc. is to be done for confirmation. Most IEMs present with neuroregression and have a progressive course.
- Routine metabolic evaluation in unexplained ID has an average diagnostic yield of only around 1%.

f. Cytogenetic microarray (CMA):

- Chromosomal microarrays have a diagnostic yield of around 12 – 20% in idiopathic ID.
- This has been recommended as the first line diagnostic test for idiopathic ID by the American College of Medical Genetics, but in India the test is still not being done as a first-line evaluation because the cost remains prohibitive.
- CMA detects genome-wide copy number variations (CNVs- deletions/ duplications).

g. Whole exome/ whole genome sequencing:

- The utility of newer genetic testing modalities such as whole exome and whole genome sequencing is now being explored for the evaluation of patients with idiopathic DD/ ID.

Management

- For most cases of DD/ ID, treatment is mainly symptomatic and supportive.
- Mainstay of therapy includes interventions such as early stimulation, physiotherapy, vocational training, special schooling and speech therapy.
- Institutionalization is to be discouraged and

integration into society is encouraged.

- A multidisciplinary approach is essential for appropriate management especially for multiple malformation syndromes and multi-systemic involvement.

Genetic counseling

Depends on the aetiology:

A. Chromosomal causes:

- Usually not familial except when one of the parents is a carrier of a balanced chromosomal rearrangement.
- 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:
  - 2 - 5% if the father is a carrier
  - 10 – 15 % if the mother is a carrier
  - 100% if either parent is a carrier of the 21; 21 translocation

B. Single gene disorders:

- Risk of recurrence depends on the mode of inheritance:
- For autosomal recessive disorders, the risk of recurrence in siblings is 25%.
- For autosomal dominant disorders, the risk of recurrence for the sibling is 50% if one of the parents is affected; in a sporadic case (de novo mutation), the risk of recurrence in the sibling is very low but the possibility of gonadal mosaicism in either parent cannot be ruled out.
- For X-linked recessive disorders, the risk of recurrence for male siblings is 50%, whereas females usually do not manifest the disorder.

C. Environmental causes:

- Recurrence is unlikely but can occur if the causative agent persists antenatally or postnatally in the environment of the next child also e.g. lead exposure, intrauterine exposure to teratogens like alcohol or specific drugs

D. When no etiology is identified:

- Empiric risks of recurrence can be predicted when no specific etiology is identified, but a specific prenatal diagnostic test cannot be offered for the next pregnancy.

Table 4: Empiric risks of recurrence of intellectual disability with accompanying features, without an identified specific etiology*

Accompanying feature with DD/ ID	Approximate risk of recurrence
Microcephaly alone	1 in 6 to 1 in 8 (10 – 15%)
Microcephaly with other features	1 in 30 (3%)
Infantile spasms	1 in 30 to 1 in 100 (1 – 3%)
Non – specific dysmorphic features	1 in 25 to 1 in 30 (3 – 4%)
Malformation	1 in 50 (2%)
No specific features; male proband	1 in 13 (8%)
No specific features; female proband	1 in 20 (5%)
Holoprosencephaly with normal karyotype & no forme fruste in parents	1 in 20 (5%)
Lissencephaly type 1	Very low (<1%)
Lissencephaly type 2	1 in 4 (25%)
Cerebellar hypoplasia	1 in 8 (12.5%)
Schizencephaly/ asymmetric porencephaly	Very low
Cerebral palsy (diplegia/ hemiplegia)	1 in 200 to 1 in 400
Symmetrical spasticity	1 in 8 to 1 in 9 (10 – 12%)
Asymmetric neurological signs	1 in 50 to 1 in 100 (1 – 2%)
Ataxic diplegia	1 in 24 (4%)
Congenital ataxia	1 in 8 (12.5%)

* Adapted from: Emery and Rimoin's Principles and Practice of Medical Genetics. Third Edition. Churchill Livingstone Elsevier.

Prenatal Diagnosis

A specific prenatal diagnostic test can be offered for future pregnancies in the family only if the exact aetiology is established in the index case with DD/ ID. The actual test offered depends on the nature of the genetic problem identified in the proband.

- Fetal karyotyping (preferably in amniotic fluid) is done if the proband has an identified chromosomal anomaly and/ or there is evidence of a balanced chromosomal rearrangement in either parent.
- FISH, MLPA or cytogenetic microarray analysis is done in the fetal sample (chorionic villus sample or amniocytes), if proband is identified to have a chromosomal microdeletion/ microduplication detected by FISH, MLPA or microarray.
- Targeted mutation analysis is done in the fetal sample (chorionic villus sample or cultured amniocytes) if the proband has a monogenic disorder with identified disease-causing gene mutations. In some cases, if the diagnosis of the monogenic disorder is confirmed in the proband but the exact disease-causing mutations are not identified, prenatal diagnosis may be done through linkage analysis, provided informative linkage markers are identified in the family (there is however ~5% chance of error because of the possibility of recombination).
- Relevant enzyme assay is done in fetal tissue (CVS/ cultured amniocytes) if the proband is confirmed to have a metabolic disorder such as a lysosomal storage disorder. Recent guidelines suggest that wherever possible, targeted mutation analysis must be combined with enzyme assay, for improving the reliability of the prenatal diagnostic test (to close to 100%). Metabolite assays in amniotic fluid, though conventionally done for prenatal diagnosis of inborn errors of metabolism such as organic acidurias, are not very reliable. For IEMs such as organic acidurias, aminoacidopathies, fatty acid oxidation disorders and urea cycle defects also, targeted mutation analysis and/ or the relevant enzyme assay provide reliable results.
- If the proband has a multiple malformation syndrome, prenatal diagnosis may be offered for the future pregnancies, through fetal targeted anomaly scan and fetal echocardiography at 18 to 20 weeks, followed, if required, by serial antenatal USG monitoring. Fetal MRI may also be done in some cases such as a CNS malformation in the proband. However, the family has to be counselled that fetal imaging cannot give information about intellectual functioning, it cannot detect all malformations and some anomalies such as microcephaly/ pachygyria etc. may not become apparent until later gestation.

Suggested Reading

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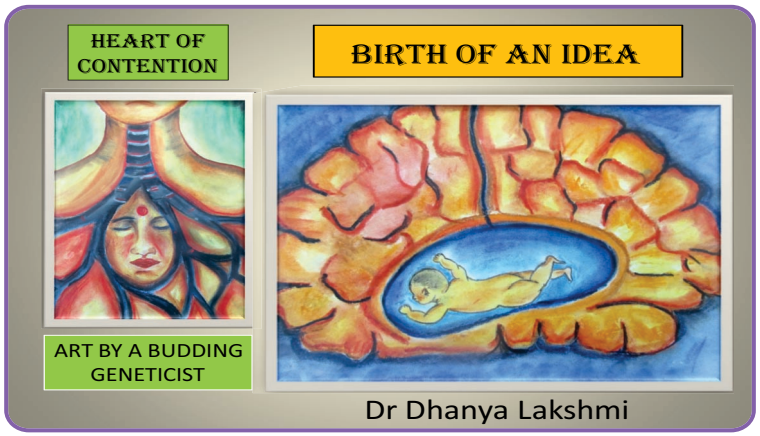

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Phenylketonuria: Past, Present and Future

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Phenylketonuria (PKU) is considered the epitome of inborn errors of metabolism. The history of phenylketonuria can be traced through the triumphs of medicine over inborn errors of metabolism in various fields like dietary management, newborn screening etc. It has been a fascinating journey for this disease right from its first description in 1930s to the present day era of newborn screening and dietary therapy. This article is an effort to apprise the practicing clinician regarding the past, present and future of this disease.

Table 1: History of Phenylketonuria[1]

- 1934- Asjbørn Følling demonstrated that intellectual disability was caused by elevated levels of phenylalanine in body fluids.
- 1940s- Lionel Penrose, described PKU as the first form of intellectual disability with a chemical explanation and introduced the idea that PKU could be treatable.
- Mid-1950s, PKU found to be caused due to deficiency of hepatic cytosolic phenylalanine hydroxylase (PAH) enzyme activity.
- 1960s- Guthrie microbial inhibition assay used for mass screening of newborns.
- Newborn screening for Hyperphenylalaninemia (HPA) has been routine throughout North America and the UK since the mid-1960s.
- 1970s- Discovered that not all HPA was PKU. Some forms of HPA found to be caused by disorders of synthesis and recycling of the cofactor (tetrahydrobiopterin).
- 1980s- Human PAH gene was mapped and cloned, and the first mutations identified.
- Research to improve the current treatment with restrictive phenylalanine diets, supplemented by medical formula, is ongoing

Phenylalanine is an essential amino acid in humans. Elevated levels of phenylalanine (hyperphenylalaninemia, HPA) is associated with brain toxicity and cognitive impairment. Hyperphenylalaninemia in humans is classified as:

- **Phenylketonuria (PKU)**
  - Most severe of the three types
  - Associated with plasma phenylalanine concentrations greater than 1000 µmol/L and a dietary phenylalanine tolerance of less than 500 mg/day
  - Associated with high risk of severe cognitive impairment.
- **Non-PKU hyperphenylalaninemia (non-PKU HPA)**
  - Associated with plasma phenylalanine concentrations consistently above normal (i.e., >120 µmol/L) but lower than 1000 µmol/L on a normal diet.
  - Lower risk for impaired cognitive development in the absence of treatment.
- **Variant PKU**
  - Individuals who do not fit the description for either PKU or non-PKU HPA.

Etiology

- Hyperphenylalaninemia is caused by deficiency of the phenylalanine hydroxylase enzyme caused due to mutations in the PAH gene.
- In about 2 % of patients, hyperphenylalaninemia may be caused due to impaired synthesis or recycling of tetrahydrobiopterin (BH4), a cofactor of phenylalanine hydroxylase enzyme.
- The genes/enzymes involved in metabolism of phenylalanine, perturbation of which can lead to different forms of hyperphenylalaninemia are shown in Figure 1 and Table 2.

Figure 1: Phenylalanine metabolism pathway showing various genes involved in the etiology of hyperphenylalaninemia.

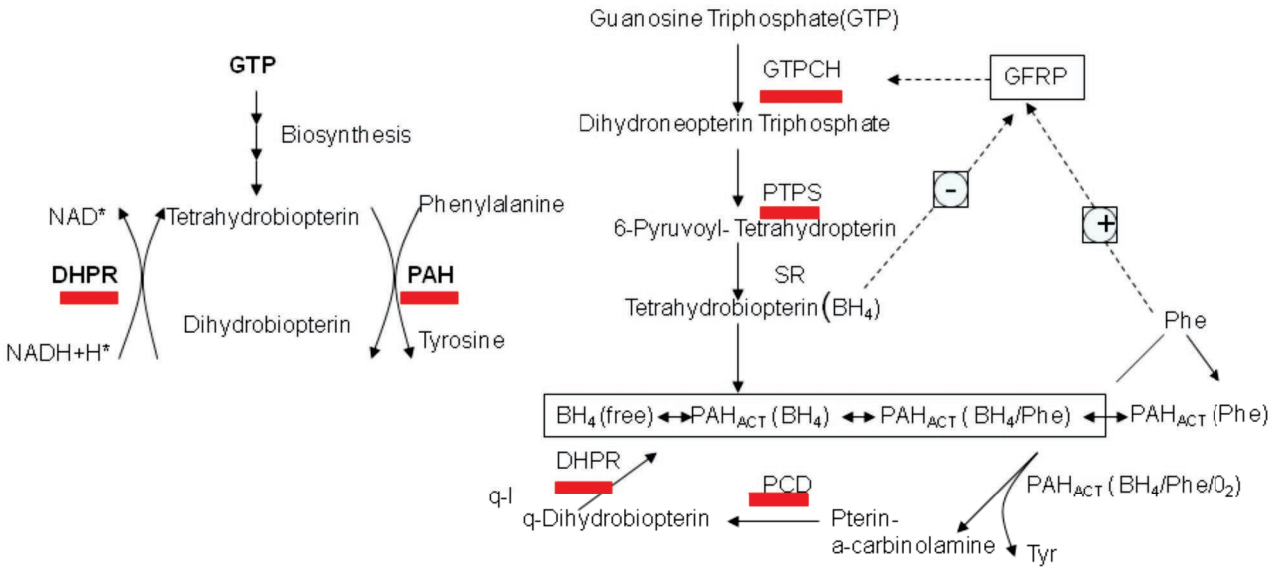

Table 2: Enzyme deficiency involved in the causation of hyperphenylalaninemia

Etiology of hyperphenylalaninemia:

- Deficiency of Phenylalanine hydroxylase (PAH) enzyme activity (98%)
- Impaired synthesis or recycling of tetrahydrobiopterin (2%):
  - Guanosine triphosphate cyclohydrolase (GCH1)
  - 6-pyruvoyl tetrahydrobiopterin synthase (PTS)
  - Dihydropteridine reductase (QDPR)
  - Pterin-4 acarbinolamine dehydratase (PCBD1)

Table 3: Clinical features of Phenylketonuria

- Severe intellectual disability
- Microcephaly
- Epilepsy
- Musty body odor
- Decreased skin and hair pigmentation
- Eczema
- Neurobehavioral problems

Clinical features

The clinical features associated with phenylketonuria are listed in Table 3. Based on the severity of clinical features and tolerance of dietary phenylalanine, PKU can be classified as[2]

- **Classic PKU**
  - Complete or near-complete deficiency of PAH activity
  - Tolerate less than 250-350 mg of dietary phenylalanine per day to keep plasma concentration of Phe at a safe level of no more than 300 µmol/L (5 mg/dL)
  - Most individuals develop profound, irreversible intellectual disability in absence of treatment
- **Moderate PKU**
  - Affected individuals tolerate 350-400 mg of dietary phenylalanine per day.
- **Mild PKU**
  - Affected individuals tolerate 400-600 mg of dietary phenylalanine per day.
- **Mild hyperphenylalaninemia (MHP)**
  - Affected infants have plasma phenylalanine concentrations lower than 600 µmol/L (10 mg/dL) on a normal diet.

Investigations

Amino acid analysis

Ferric chloride test in urine can be used as a screening test. However, quantitative plasma amino acid analysis is the standard method for confirmation of the diagnosis. This quantifies phenylalanine levels, levels of other amino acids and the PHE:TYR ratio which can help in confirmation of diagnosis.

Co-factor deficiency testing

Disorders of BH4 synthesis and regeneration can be evaluated in all patients with elevated phenylalanine since the treatment will differ significantly. Pterins can be measured in urine or blood and erythrocyte dihydropterin reductase (DHPR) is measured on whole blood spotted on filter paper. Reference values are available for different age groups.

Phenylalanine hydroxylase (PAH) activity

Enzymatic activity for PAH is detectable in hepatic and renal tissues only, and is not appropriate for either screening or diagnostic testing.

Mutation analysis

The human PAH gene is located at chromosome 12q23.1, spanning ~100kb, and is comprised of 13 exons. Over 500 mutations have been described and several approaches to mutation testing have been utilized like mutation screening, sequencing or detection of common mutations. Mutation analysis helps in planning for prenatal diagnosis in the next child and carrier screening within the family.

Management

Management of a child with phenylketonuria is multidisciplinary involving the treating paediatrician, dietician, metabolic specialist, clinical geneticist etc. The main goal of treatment is to reduce levels of phenylalanine in blood to tolerable levels.

Dietary therapy

Dietary therapy with restriction of dietary phenylalanine intake is the mainstay of therapy for phenylketonuria, requiring a decrease in the intake of natural protein and replacing it with a protein source deficient in phenylalanine. Patients diagnosed with PAH deficiency should be followed regularly and have blood levels monitored frequently until their levels have stabilized.[3] Diet modified for PAH deficiency should provide all other nutrients necessary for normal growth and development. Food products containing a phenylalanine-free amino acid mixture have been the mainstay of dietary therapy for PAH deficiency and are designed to meet established dietary requirements, as well as cater to individual needs. Multiple such formulas are available in market e.g. Periflex, Anamix, Phenylfree etc. Modified low-protein foods are also important to PAH deficiency diet management. Over the last decade, there have been many other modified low-protein foods

developed that mimic higher protein foods, as well as snacks that help to increase dietary variety and help to normalize the look of the low phenylalanine diet.[4] Access to these low protein foods remains a concern for the PAH deficiency patients as the cost of these products is high and they need to be imported from abroad. The goal of therapy is to maintain blood phenylalanine levels in the range of 120-360 µmol/L.[3,4] After one year of age and until 12 years of age, biweekly to monthly sampling is adequate. In adolescents and adults who are stable and well controlled, monthly testing may be adequate. In addition, plasma amino acids, transthyretin, albumin, complete blood count, ferritin, 25-OH vitamin D, vitamin B12, RBC essential fatty acids, trace minerals (Zn, Cu, Se) vitamin A, RBC essential fatty acids, comprehensive metabolic panel, and folic acid testing should be considered to look for other nutritional deficiencies.

It is important to note that lifelong dietary therapy is recommended for patients with untreated phenylalanine levels of more than 360 µmol/L

Pharmacotherapy

Tetrahydrobiopterin therapy

Sapropterin dihydrochloride, a synthetic form of the naturally occurring cofactor tetrahydrobiopterin was the first pharmacologic agent approved for treatment of PAH deficiency by the Food and Drug Administration in 2007.[5] It was observed that some patients with phenylketonuria respond to sapropterin with an increase in the metabolism of phenylalanine although they are not deficient in endogenous tetrahydrobiopterin. The mechanism of action is unclear, but tetrahydrobiopterin may act as a pharmacologic chaperone leading to improved folding and increased stability of the mutant protein. Clinical trials have demonstrated that approximately 40–50% of patients with PAH deficiency are sapropterin-responsive.[6] Patients at the mild end of the PAH deficiency spectrum are most likely to respond because some stable protein is required for sapropterin to function. Thus, every PAH deficient patient should be offered a trial of sapropterin therapy to assess responsiveness.

Sapropterin is given typically once a day at a dose of 5–20 mg/kg. The most commonly observed side effects include gastric distress, nausea, and diarrhea. Before routine treatment with sapropterin is initiated, a trial needs to be conducted to determine if the patient is responsive. Sapropterin-responsiveness is determined by obtaining a baseline blood phenylalanine level and then starting the patient on a single daily dose of sapropterin at 20 mg/kg.[5,6] Additional blood phenylalanine levels are obtained at regular intervals – usually at 24 hours, one week, two weeks, and in some cases, three or four weeks. A significant decline in blood PHE is expected in responders once treatment is initiated. Most sapropterin-responsive patients will show an almost immediate decline in the blood PHE level, but occasionally a delay of 2-4 weeks is seen.[6] Failure to observe a significant decrease in blood PHE level at 4 weeks suggests the patient is sapropterin-nonresponsive. Sapropterin is approved for use in conjunction with a PHE-restricted diet. In a small number of patients, typically those with the mildest forms of PAH deficiency, the blood PHE level can be maintained in the desired range with sapropterin therapy alone.[6]

Large neutral amino acids

Large neutral amino acids (LNAA) have been proposed as a therapy for PAH deficiency based on their ability to block uptake of phenylalanine (which is a large neutral amino acid) from the intestine and at the blood brain barrier.[1,7] Few clinical trials have shown efficacy but larger trials are necessary to demonstrate a positive effect and to determine long-term safety.[7]

Enzyme replacement therapy

Phenylalanine ammonia lyase is a bacterial enzyme that degrades phenylalanine to trans-cinnamic acid and ammonia; trans-cinnamic acid is rapidly converted to hippuric acid and excreted in the urine.[1,8] The compound put to clinical use is pegylated phenylalanine ammonia lyase (PEG-PAL), which will enter Phase 3 clinical trials in 2013. It is given by subcutaneous injection on a daily basis and has been shown to be very effective in lowering

blood phenylalanine levels, even in patients on a completely unrestricted diet.[8]

Treatment of Co-factor (Tetrahydrobiopterin) metabolism defects

Treatment of tetrahydrobipterin (BH4) metabolism defects requires the normalization of BH4 availability and of phenylalanine concentration in blood. This can be achieved by BH4 supplementation along with dietary modification, neurotransmitter precursor replacement therapy, and supplements of folinic acid in DHPR deficiency. The treatment should be initiated early and needs to be continued for life.

Maternal Phenylketonuria

Exposure of fetus to high levels of phenylalanine in utero, due to maternal phenylketonuria, can lead to various physical and cognitive teratogenic effects leading to microcephaly, poor fetal growth, congenital structural cardiac defects (CSHD), non-familial facial features, and intellectual disability. Intellectual disability is known to occur in almost 90% of foetuses born to mothers with uncontrolled phenylketonuria, especially when the exposure is before 8-10 weeks of pregnancy. Maternal phenylalanine levels should be maintained below 360 µmol/L during pregnancy using dietary restriction and Sapropterin. Mothers with phenylketonuria can safely breastfeed their babies.

Newborn Screening

It was possible to establish newborn screening (NBS) programs for PAH deficiency with development of the bacterial inhibition assay for phenylalanine using blood samples collected on filter paper cards. This was followed by adoption of tandem mass spectrometry (MS/MS) for newborn screening which has allowed NBS programs to screen for markers for multiple disorders on a single blood spot.[9] Increased phenylalanine concentrations in blood spots can be quantified as early as 24 hours after birth, and tyrosine concentrations can be used to calculate a PHE:TYR ratio. Although MS/MS based NBS is far more accurate compared to older screening methods, an elevated phenylalanine level is still non-specific and does not definitively indicate PAH deficiency.[9] Most newborn screening laboratories determine their own cut-off levels above which a test is considered to be positive and requires further evaluation. An international database of 133 contributing laboratories reports a mean cut-off for phenylalanine of 130 µmol/L (with a range of 65-234 µmol/L) and a PHE:TYR ratio >3 as abnormal.[10] Elevated levels need further evaluation for assessment of defects in BH4 synthesis or regeneration.

Although a number of successes have been achieved in patients with phenylketonuria, the journey is far from over. Universal newborn screening is still a dream in developing countries. Better biomarkers for early diagnosis and better dietary and pharmacologic agents need to be developed for effective management of patients with phenylketonuria.

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Same Genes, Opposite Phenotypes... & More

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Same genes, opposite phenotypes[1]

Disruption of 11p15 imprinted region, containing at least eight genes, results in two different fetal growth disorders, Beckwith-Wiedemann (BWS) and Silver-Russell (SRS) syndromes, with opposite phenotypes. Either gain of methylation of imprinting center (IC) or loss of methylation of IC2 will cause BWS whereas SRS is associated with loss of methylation of IC1 of BWS – SRS region in mouse models. The genes present in the BWS – SRS region show dosage-sensitive functions in developmental regulation of embryonic growth and placental function. Jacob et al suggest in their review that both SRS and BWS can be caused by the opposite imbalances in the levels of two groups of imprinted genes on 11p15.5. Thus BWS-SRS represents the opposite imprinted disorders, like Angelman and Prader-Willi syndromes.

Long awaited point mutations in Prader Willi syndrome identified[2]

Prader-Willi syndrome(PWS), caused due to the deletion of paternal copies of genes at 15q11-13, was so far believed to be a 'contiguous gene syndrome'. However Schaaf et al reported the first individuals with truncating mutations in MAGEL2, a protein coding gene in the PWS domain. After identifying the first mutation(p.Val551fs) by whole genome sequencing Schaaf et al ascertained three additional mutations (p.Pro601fs, p.Ile1061fs and p.Gln1024*) by reviewing the results of whole exome sequencing database of a clinical laboratory. The four subjects reported with these loss of function mutations had autism spectrum disorder, intellectual disability and varying degrees of PWS phenotype. In view of this report MAGEL2 sequencing or exome sequencing may be considered in cases of complex autism with a history of neonatal hypotonia, feeding difficulties and hypogonadism.

Exome sequencing: effective diagnostic strategy for small, non-consanguineous families with ID[3]

Schuurs-Hoeijmakers et al identified pathogenic variants in one novel, two known and five candidate genes for intellectual disability (ID) with the help of exome sequencing. All the families reported in this study are small non consanguineous families with two to five affected siblings.They identified three definite pathogenic mutations in SLC9A6, SLC6A8, and DDHD2 genes, out of which DDHD2 is surprisingly an autosomal gene. They also identified five potentially pathogenic variants in BCORL1, MCM3AP, PTPRT, SYNE1, and ZNF528 genes which so far not linked to the ID phenotypes. Thus exome sequencing can be used as a potential tool for identifying recessive mutations even in small non consanguineous families.

Ligand pair DCHS1 and FAT4 play a role in mammalian neurogenesis[4]

The study of Cappello et al on mouse models revealed key regulators in cerebral cortical development. Mutations in genes encoding cadherin receptor-ligand pair DCHS1 and FAT4 results in increased proliferation and impaired migration capability of neural progenitor cells. This causes periventricular neuronal heterotopia in the developing cerebral cortex and results in an autosomal-recessive multiple malformation syndrome, Van Maldergem syndrome (VMS). A reduction in Yap (a transcriptional activator) levels compensates the reduced expression of DCHS1 and FAT4 by accelerating the deposition of neurons in the cortical plate.

Kabuki syndrome: a genotype-phenotype study[5]

Kabuki syndrome (KS), a multiple congenital anomaly syndrome is characterized by typical facial features, skeletal anomalies, mild to moderate intellectual disability and postnatal growth deficiency. Mutations in MLL2 gene which encodes a histone – lysine N-methyltransferase of Trithorax group of proteins is the major cause of KS. Makrythanasis et al have published a study on the genotype-phenotype correlation of KS. The study also added 33 novel mutations to the MLL2 mutation spectrum. After detailed clinical investigations on 86 KS patients they concluded that individuals carrying loss of function mutations in MLL2 have a more severe KS phenotype than the MLL2 mutation negative group.

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genetic CLINICS

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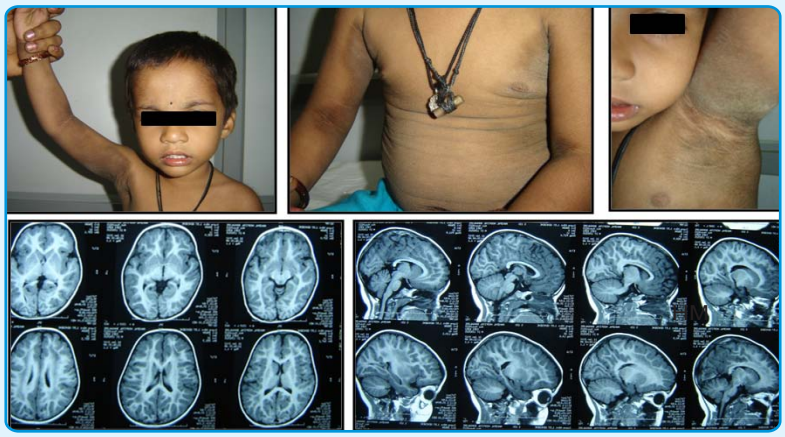
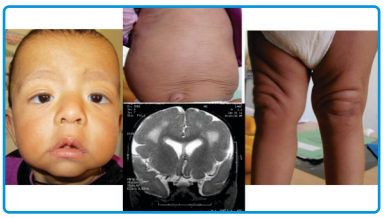
This three year old female child presented with global developmental delay and spasticity. Her MRI brain images are shown. Identify the condition.



Please send your answers by email to editor@iamg.in

Answer to PhotoQuiz 22 of the previous issue

CUTIS LAXA, AUTOSOMAL RECESSIVE, TYPE IIA; ARCL2A [OMIM #219200]



Cutis laxa is a heterogeneous group of disorders with variable phenotypes and inheritance patterns. Type II cutis laxa has features overlapping with wrinkly skin syndrome, as a result of which they are regarded as one disorder with a variable spectrum of severity by some authors. Due to the many phenotypic similarities in Gerodermia osteodysplastica and wrinkly skin syndrome, it has been proposed that even these two conditions, represent the same disorder. ATP6V0A2-related cutis laxa or ARCL cutis laxa is represented by a spectrum of clinical entities with variable severity of cutis laxa , abnormal growth, developmental delay, and associated skeletal abnormalities. Aside from cutis laxa, persistent wide fontanels, frontal bossing, slight oxycephaly, downward-slanted palpebral fissures, reversed-V eyebrows, and dental caries are characteristic. These patients can be divided into 2 major groups: ARCL2A, comprising those with a combined N- and O-linked glycosylation defect (CDG type II), and ARCL2B, those without a metabolic disorder. ARCL2A should be considered more of a multisystem disorder with cobblestone-like brain dysgenesis manifesting as developmental delay and an epileptic neurodegenerative syndrome rather than purely a dermatologic disorder. The occurrence of mutations in the same gene ATP6V0A2, indicates that wrinkly skin syndrome and some cases of autosomal recessive cutis laxa type IIA represent variable manifestations of the same genetic defect. This genetic defect is associated with abnormal glycosylation leading to a distinct combined disorder of the biosynthesis of N- and O-linked glycans. Interestingly, similar mutations have been found in patients with wrinkly skin syndrome, without the presence of severe skin symptoms of elastin deficiency.

Correct responses were given by:

- Prashant Verma, Jeddah, Saudi Arabia
- S Beena, Chennai
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