genetic CLINICS

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Editorial

Genes to Genome: Deciphering Complexities

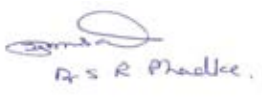
The American College of Medical Genetics has changed its name to American College of Medical Genetics and Genomics. This is the effect of revolution in molecular techniques. Everything is changing from Genetics to Genomics. Gone is the era of sequencing one gene after the other. One can now sequence the whole genome in one go. Identification of modifier genes and oligogenic disorders has blurred the distinction between monogenic disorders and multifactorial disorders. Now one wants to look at the whole genome or expression of all genes in one laboratory experiment. This has become possible due to microarray and next generation sequencing techniques. Next generation sequencing has made study of the entire genome possible and thereby changed the whole scenario. Mutation detection in big genes like the dystrophin gene is now easily possible. Major immediate clinical applications of the technique are many. Mutation detection for disorders with heterogeneous etiologies can be easily done by sequencing all causative genes in one chip. Such markedly heterogeneous disorders include deafness, cardiomyopathies, limb girdle myopathies, retinitis pigmentosa and congenital myopathies. Microarray can study expression of the whole genome in a tissue. Gene expression signatures of tumors are being used as diagnostic and prognostic markers. Gene expression profiles at different times and in different diseases are providing more insight into physiological states and disease pathology, thus deciphering some aspects of systems biology.

The most exciting aspect of next generation sequencing is the ease with which the whole genome of an individual can be sequenced. This can be done for various purposes. Sequencing the whole genome or preferably the whole coding region (exome) has been shown to be of great use for identifying the causative gene of a monogenic disorder. This has revolutionized gene identification for monogenic disorders in research settings. It has also been shown to be of significant use in clinical settings. In a child with an incurable, unknown disease whole exome sequencing identified the causative gene in the immunological pathway and based on the etiological diagnosis the child was cured by bone marrow transplantation. Comparing the genome of normal tissue with that of affected tissue has identified the gene for Proteus syndrome, a syndrome with mosaic pattern of involvement. Similarly comparison of the genome of tumor tissue with that of normal tissue can identify genetic defects in tumors and can help in developing personalized treatment for cancers.

But utility of exome sequencing or genome sequencing in individuals without any clinical problems is debatable and it opens up many scientific, ethical and psychological issues. The whole sequence of an individual can be studied to identify important genes responsible for monogenic disorders, cancer susceptibility as well as to study various polymorphisms to calculate risks for common disorders like ischemic heart disease, diabetes mellitus, psychiatric diseases, etc. However, it has been found that whole genome sequencing gives hardly any more information about risks of common disorders than what can be obtained by taking family history and other clinical details. On the contrary it may identify some causative mutations for late onset diseases or cancers leading to anxiety and problems with employment or insurance. Identification of such mutations is an equally difficult situation for geneticists, posing the dilemma of whether to disclose this information to the patient or not. Recent studies have shown that the variants of high clinical importance will be detected at an appreciable frequency in exomes and there is a need for developing efficient modes of interpretation and for dealing with such situations. Imagine a situation where the genome of a fetus is sequenced and some disease causing mutations, some variants increasing risk of multifactorial diseases and some variants of unknown significance are identified. Imagine the loads of complex

information for would-be parents to deal with and the decisions to be made. And this is not an imaginary situation. Sequencing of the whole genome is possible today and that too from the fetal DNA fragments extracted from mother's plasma. So the new technology has opened up a Pandora's Box. Scientists, social scientists and the public have to decide about how to use the available information in the best possible way.

Understanding the utility of whole genome and exome sequencing, the American College of Medical Genetics and Genomics has come out with a policy statement about when to use and how to transmit the information to the patient (For details see http://www.acmg.net/StaticContent/PPG/Clinical_Application_of_Genomic_Sequencing.pdf). The ACMG stresses the utility of whole genome sequencing for genetic phenotypes without a known causative gene. It also advocates its use for carrier screening to prevent affected offspring with serious disorders but is strictly against its use for prenatal testing and newborn screening. The policy statement also stresses the need for in-depth pretest counseling about what to expect from the test and what will be disclosed and what will not be disclosed. Also, distinction needs to be made about the use of whole genome sequencing in clinical and research settings. Though the technique, its interpretation and its use will evolve with time and experience, whole genome sequencing has come into clinical settings and needs physician- geneticists capable of dealing with the situation. As the jungle of genes gets completely mapped and more paths are made to enter inside, geneticists will be able to negotiate the genetic information in a better way for the patients, without getting lost in the side-roads and in the jungle of information and without confusing the patients.



Shubha Phadke

1st October, 2012

From Gene Mapping to Gene Therapy

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Direct to consumer genetic testing: Is it a two edged sword?[1]

In the modern era, direct to consumer genetic testing (DTCGT) has enabled lay persons to order genetic tests without the guidance of a health professional. This tendency has given rise to many uncertainties regarding which genetic tests can be directly offered to individuals and possible benefits and harms of such tests. Skirton et al have recently reviewed published literature of 9 years (2002-2011) regarding recommendations and policies of health professionals as well as consumers' view on DTCGT. Majority have discussed potential harms mainly in the form of inappropriate information regarding the clinical utility and validity of the test, cost involved, lack of scientific evidence, potential discrimination by insurance agencies and employers, and extra burden on health professionals to provide education and counselling regarding these tests. However, the possible benefits are individuals' autonomy and equitable access to genetic tests. The authors have suggested that there should be agreement on the basic code of practice. This includes the guidance of an experienced health professional, availability of proper genetic counselling and training of health professionals in providing advice to individuals regarding these tests. The other requirements suggested are individual's consent, accredited laboratories, trained staff to interpret the result, declaration of conflict of interest and adequate legislation acts.

Oral clefts: sub-categorisation is important prenatally[2]

Oral cleft is one of the commonest human malformations with a prevalence of 1:700 live births. Maarse et al have provided a systematic review on associated malformations and chromosomal abnormalities in prenatal and postnatal population with oral clefts. This review has analysed electronic data (1995 onwards) as well as data from the Dutch Oral Cleft Registry. The results from this study can help in taking proper informed decisions regarding prenatal invasive testing as well as prognosis and management of the affected child after birth. The authors have also provided an algorithm for management after antenatal diagnosis of oral cleft based upon sub-categorisation into cleft palate (CP) only, cleft lip only (CL) and cleft lip and palate (CLP). Presence of associated anomalies carries a higher risk of chromosomal abnormality (trisomy, and microdeletion, mainly 22q deletion) and microarray testing should be considered. Presumed isolated CL cases should not be offered prenatal invasive testing, while microarray testing can be considered in CP and CLP cases.

Centers of Mendelian genomics: Boon to clinical genetics[3]

Recent molecular techniques like cytogenetic microarray and exome sequencing have helped in identifying the genetic etiology in more than a dozen disorders with all modes of inheritance. Many of these Mendelian disorders are rare and have genetic heterogeneity. Collecting clinical information from all over the world can improve chances of finding and validating candidate genes and genetic variants. To achieve this goal, the National Institute of Health (NIH) has established 3 centers of Mendelian Genomics in the United States. These centers have been set up with the aim of providing the necessary collaborative framework, physical infrastructure, lab services and expert analysis for no cost. This will help in identification of genetic defects in as many Mendelian conditions as possible, in gaining insights into their

pathogenesis and in exploration of new therapeutic methods. This would require participation from the whole genetic community in identifying patients and families with rare Mendelian disorders and submitting their samples for molecular analysis.

Reading through stop codons: a long way to go[4]

Propionic acidemia (PA) is one of the most common organic acidemias with a varied spectrum of clinical variability ranging from lethal neonatal illness to chronic neurological impairment. Treatment is symptomatic and supportive with unsatisfactory outcome in most of the cases. The condition is caused by deficiency of propionyl coenzyme A carboxylase (PCC). As in other autosomal recessive disorders, patients with 10-15% of total enzyme activity have milder clinical manifestations. Sanchez-Alcudia et al have performed in vitro expression analysis on fibroblasts of patient with PA. They have used aminoglycosides for reading through premature termination codon (PTC) and have observed 10-50 fold increase in PCC activity leading to 10-15% enzyme activity in comparison to normal control. This study raises hope of treatment of PA and further clinical trials with read through drugs.

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OM: Resonance of Origin of Life

Contributed by: Dr Parag Mohan Tamhankar

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Om - Every geneticist in the world chants the sound at least once a day if not a hundred times. The origin of the suffix “ome” that wraps an entire world into a word is mysterious. The German anatomist Heinreich Wilhem Gottfried Waldeyer-Hartz coined the term “chromosome” for thread like structures in the nucleus to be stained by the dye fuchsin, “chromo” meaning colour and “soma” being Greek term for body. Therefore, when Hans Winkler coined the term “genome” in his book Verbreitung und Ursache der Parthenogenesis im Pflanzen-und Tierreiche (1920), the Oxford English Dictionary explains that Winkler probably derived it as gen + some from chromosome. But Joshua Lederberg and Alexa T McCray challenge this story since Winkler as a botanist would have surely heard about terms such as rhizome, biome, phyllome, thallome and tracheome which predated 1920. Bioinformaticians and molecular biologists lapped up the ome with glee. Thereby terms such as exome, transcriptome, metabolome all analogies of the previous ome terms have now been well defined and accepted. However deeply entrenched in this popularity is the feeling of oneness with the universe that the suffix provides. In Hinduism “Aum” or OM signifies the divine energy (Shakti) united in its three elementary aspects Bramha Shakti (creation), Vishnu Shakti (preservation) and Shiva Shakti (liberation and/or destruction). The revered words Hum (Buddhism), Amin (Islam), Amen (Christianity) are phonetically similar to OM. Please refer the website (http://www.genomicglossaries.com/content/omes.asp) maintained by Cambridge Healthtech Institute for an updated glossary and taxonomy of omes and omics.

Suggested Reading:

1. Lederberg J, McCray A. ‘Ome Sweet ‘Omics – A Genealogical Treasury of Words. The Scientist 15[7]: 8, Apr 2, 2001. Commentary
2. Paramhans Swami Maheshwarananda. The hidden power in humans. IberaVerlag, page 15, ISBN 3-85052-197-4.

Announcement

Indo-US Symposium on Disorders of the Developing Brain Manipal, India, Oct 27-28, 2012

Manipal University in association with Indo-US Science & Technology Forum announces Indo-US Symposium on Disorders of the Developing Brain in Manipal on October 27th and 28th 2012

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22q13.3 Deletion Syndrome (Phelan-McDermid Syndrome)

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Abstract

22q13.3 deletion syndrome also known as Phelan-McDermid syndrome is caused by subtelomeric deletion of the long arm of chromosome 22. The deletion could be a pure deletion or could be the result of an unbalanced structural chromosomal rearrangement. Clinical phenotype of 22q13.3 deletion syndrome is characterized by severe developmental delay, delayed/absent speech, normal to accelerated growth, hypotonia, facial dysmorphism and other minor malformations. Here we report a case of 22q13.3 deletion syndrome, due to de novo ring chromosome 22 with the characteristic clinical features, congenital heart malformation and hypermetropia.

Introduction

Structural chromosomal abnormalities are one of the important causes of multiple congenital anomalies with or without intellectual disabilities. Ring chromosomes are a type of structural chromosomal abnormality, with an incidence ranging from 1 in 27,225 to 1 in 62,279 in newborns and prenatal diagnosis studies.[1] Various mechanisms that result in the formation of ring chromosomes have been described, the most frequent mechanism being breakage in both arms of a chromosome, followed by fusion of both ends.[1] Usually formation of a ring chromosome results in terminal, loss of both arms of the chromosome (partial monosomy). Majority of them occur de novo, however there are few reports of ring chromosome showing parental inheritance.

Ring chromosome 22 that results in terminal deletion of long arm of chromosome 22 presents with characteristic syndromic manifestations described as 22q13.3 deletion syndrome/Phelan-McDermid syndrome (MIM 606232). Common clinical manifestations of the 22q13.3 deletion syndrome include variable intellectual disability (usually moderate to severe mental retardation), delayed or absent speech, facial dysmorphism, normal to accelerated growth and hypotonia.[2]

Here we report a case of 22q13.3 deletion syndrome due to ring chromosome 22 with typical findings of the 22q13.3 deletion syndrome – developmental delay, delayed speech, facial dysmorphism, and hypotonia. In addition she had congenital heart malformation (CHM), esotropia and hypermetropia.

Clinical Report

The propositus was a 1.6 year old female child who presented with congenital heart disease and developmental delay/delayed speech. She was the first born child of a non-consanguineous couple, delivered by Caesarean section, with a birth weight of 2,800 g.

Physical examination revealed a head circumference of 43.5 cm (-2SD to -3SD) and length of 80 cm (50th-60th centile). Facial features included a prominent metopic suture, upslant of eyes, periorbital fullness, squint, depressed nasal bridge, bulbous nasal tip, large and dysplastic ears, and pointed chin (Fig 1).

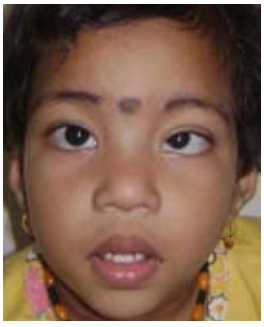

Fig 1. Face of the propositus showing upslanting eyes, periorbital fullness, squint, depressed nasal bridge, bulbous nasal tip, dysplastic ears and pointed chin

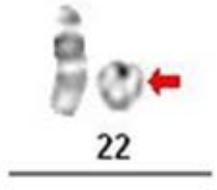

Fig 2. Partial karyotype of the propositus showing ring chromosome 22 (indicated by the black arrow), on the right side of the pair

Nails were small in both fingers and toes. Eye evaluation revealed esotropia and hypermetropia. Cardiac evaluation showed Tetralogy of Fallot (TOF). Neurological examination revealed hypotonia. Other systems were normal. Routine chromosome analysis (GTG banding) revealed ring chromosome 22 [46,XX,r(22)] (Fig 2). Simultaneous fluorescent in situ hybridization (FISH) test was ordered to rule out 22q11.2 microdeletion (DGS/VCF – DiGeorge Syndrome / Velocardiofacial syndrome) in view of TOF, periorbital fullness of eyes and bulbous nasal tip (subtle findings of 22q11.2 microdeletion). FISH test revealed presence both TUPLE1 probe signals (for region 22q11.2, probe of used for DGS/VCF genetic testing) and absence of one ARSA probe signal (for region 22q13.3, used as control probe for DGS/VCF genetic testing) (Fig 3) suggestive of deletion of region 22q13.3. Both parents' chromosome analyses were normal.

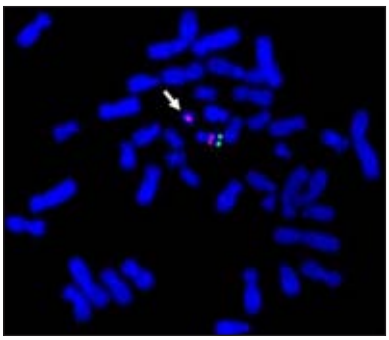

Fig 3. Fluorescent in situ hybridization (FISH) test using the Vysis DiGeorge probe kit (LSI TUPLE1/ARSA probe mix). Metaphase showing red signals representing TUPLE1 probe (for region 22q11.2, probe used for DGS/VCF genetic testing) seen on both chromosome 22 – normal and ring chromosome. Green signal representing ARSA probe signal (for region 22q13.3, used as control probe for DGS/VCF genetic testing) is seen on normal chromosome 22 and absent on ring chromosome 22 (indicated by the white arrow)

Discussion

22q13.3 deletion syndrome can result from pure terminal/interstitial deletion or could be the result of a structural chromosomal abnormality (unbalanced translocations or ring chromosome) involving chromosome 22. Clinical manifestations are highly variable; to a certain extent the deletion size correlates with the severity of the disease, but not in all cases..[3] Critical region delineation studies suggest three important genes for the 22q13.3 deletion syndrome clinical phenotype:SHANK3, ACR and RABL2B.[3,4,5] Haploinsufficiency of the SHANK3 (SH3 ankyrin domain containing protein) gene in particular has been suggested to be responsible for the clinical findings related to the central nervous system in patients with the 22q13.3 deletion syndrome.[3,4] As per different studies, around 37-80% of 22q13.3 deletions are pure terminal deletions, 14-52% are due to ring chromosomes, 7-20% are due to unbalanced translocations, and around 9% are interstitial deletions.[3,5,6] Majority of the 22q13.3 deletions are de novo (80%) and simple deletions.[7] The size of the deletion has been found to range from 130 kb to more than 9 kb.[3,5,6] Most of the de novo deletions have been found in the chromosome of paternal origin but the clinical features do not differ in severity based on the parental origin of deletion. The remaining 20% of deletion cases are familial, inherited from one of the parents with a balanced chromosomal rearrangement (translocation or pericentric inversion) and rarely from a parent mosaic for the deletion.[3,5,6,7]

The most common clinical features reported in various studies include severe global developmental delay, absent/severe speech delay, hypotonia, normal or accelerated growth and facial dysmorphism.[2,3,5,6,7] Autistic features are seen in around 50% of cases.[7] Facial dysmorphic features are often so subtle in some of the patients with significant developmental delay/delayed speech/absent speech that the facial features might go unnoticed. Common facial features described are epicanthic folds, depressed nasal bridge, bulbous nasal tip, large and dysplastic ears and pointed chin.[2] Unlike other syndromes associated with chromosome 22 (DGS/VCF, cat-eye-syndrome) facial dysmorphic features in 22q13.3

deletion syndrome are not very suggestive or striking. In our patient with developmental delay, delayed speech, hypotonia, TOF, and facial dysmorphism (subtle facial features of 22q11.2 microdeletion syndrome –DGS/VCF), routine chromosome analysis and FISH for DGS/VCF was investigated. Routine chromosome analysis revealed ring chromosome 22, which led us to think that this could be a case of 22q13.3 deletion syndrome. Our suspicion was further confirmed by FISH done with the DGS/VCF probes which showed absence of the control probe ARSA localized to 22q13.3 region. Similar coincidental diagnosis of 22q13.3 deletion with genetic testing done for DGS/VCF has been reported in few cases in literature.[7,8]

In addition our patient had congenital heart malformation (TOF), reported in few cases (VSD, PDA) although TOF has not been reported in patients with 22q13.3 deletion syndrome.[7] Usually growth in children with 22q13.3 deletion syndrome is normal or accelerated. Our patient's height was normal for age, but the head size measurement revealed microcephaly (-2SD to -3SD). Such growth failure is commonly reported in cases of 22q13.3 deletions occurring due to ring chromosome 22, as against normal or accelerated growth seen in cases of pure 22q13.3 deletions.[4,5,8] The other novel eye finding seen in our patient, which has not been reported earlier in 22q13.3 deletion syndrome, is hypermetropia. Variable clinical features to a certain extent can be explained by the size of the deletion, critical region involvement and coexisting deletions due to structural chromosomal abnormality.[3,4,5,6] However background genes and non-deleted homologous chromosome genes might also contribute to the phenotype.[4]

The facial features together with developmental delay, delayed/absent speech, autistic features and hypotonia, should lead to a suspicion of 22q13.3 deletion syndrome and further investigations. Routine chromosome analysis by GTG banding of high resolution can identify large deletions. However, the more appropriate tests in a case suspected to have 22q13.3 deletion would be FISH (using ARSA or D22S1726 probes) or array CGH along with routine chromosome analysis.

Acknowledgments: We are thankful to Dr Jayaram S Kadandale, Faculty Scientist, Centre for Human Genetics, Bangalore for doing FISH study in our patient.

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Inborn Errors of Metabolism with an acute presentation: Representative phenotypes

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With access to advanced technology along with greater awareness, pediatricians encounter situations necessitating knowledge of diagnosis and management of metabolic disorders. The main aim of this paper is to present a simplified approach through case studies to enable diagnosis of inborn errors of metabolism presenting as an acute illness.

Definition: Inborn errors of Metabolism (IEMs) are inherited disorders occurring due to deficiency of an enzyme or cofactor in a metabolic pathway. The phenotype results because of accumulation of intermediate toxic products or essential metabolite deficiency.

They present as

i) acute or progressive intoxication due to accumulation of toxic compounds proximal to the metabolic block ii) symptoms due to deficiency in energy production or utilization in tissues like liver, myocardium, muscle, brain etc. iii) as a storage syndrome

Age of presentation:

I) Neonatal presentation - Typically a neonate who rapidly deteriorates for no apparent reason after an initial symptom-free interval. Symptoms progress from nonspecific poor sucking and feeding, vomiting, abdominal distension, to rapidly progressive neurological abnormalities and encephalopathy.

ii) Later-onset, acute and recurrent attacks of coma, ataxia, vomiting and acidosis - The relapsing attacks are usually triggered by stressing events or excessive protein ingestion. Between attacks, patients may appear entirely normal.

iii) Chronic and progressive symptoms like failure to thrive, developmental delay, neurological deterioration, and psychiatric signs.

In a newborn presenting with acute encephalopathy, the five basic investigations listed in Table 1 are useful as a first step towards diagnosing IEMs presenting as acute encephalopathy. They are simple tests available in all laboratories and serve as a guide to further investigate for the type of IEM.

Table 1 : Metabolic derangements in different inborn errors of metabolism

Disorder	Acidosis	Ketosis	Lactate	Ammonia	Glucose
Maple syrup urine disease (MSUD)	N	++	N	N	N /↓
Organic acidemias	+	++	N/↑	N /↑	↓↓
Urea cycle disorders	N	N	N	↑↑	N
Lactic acidosis	+	+	++	N	N
Non ketotic hyperglycinemia, Sulfite oxidase/molybedenum cofactor def., Phenylketonuria, Peroxisomal, Galactosemia	N	N	N	N	↓in galactosemia
GSD I	+/-	+ (Postprandial)	+ (fasting)	N	↓↓

A case based approach to IEMs presenting with acute encephalopathy and the above five metabolic derangements is presented below.

Case 1: Acute encephalopathy with hyperammonemia

A third gravida was referred for prenatal diagnosis. Her first pregnancy was terminated for an intrauterine death. The second male neonate was

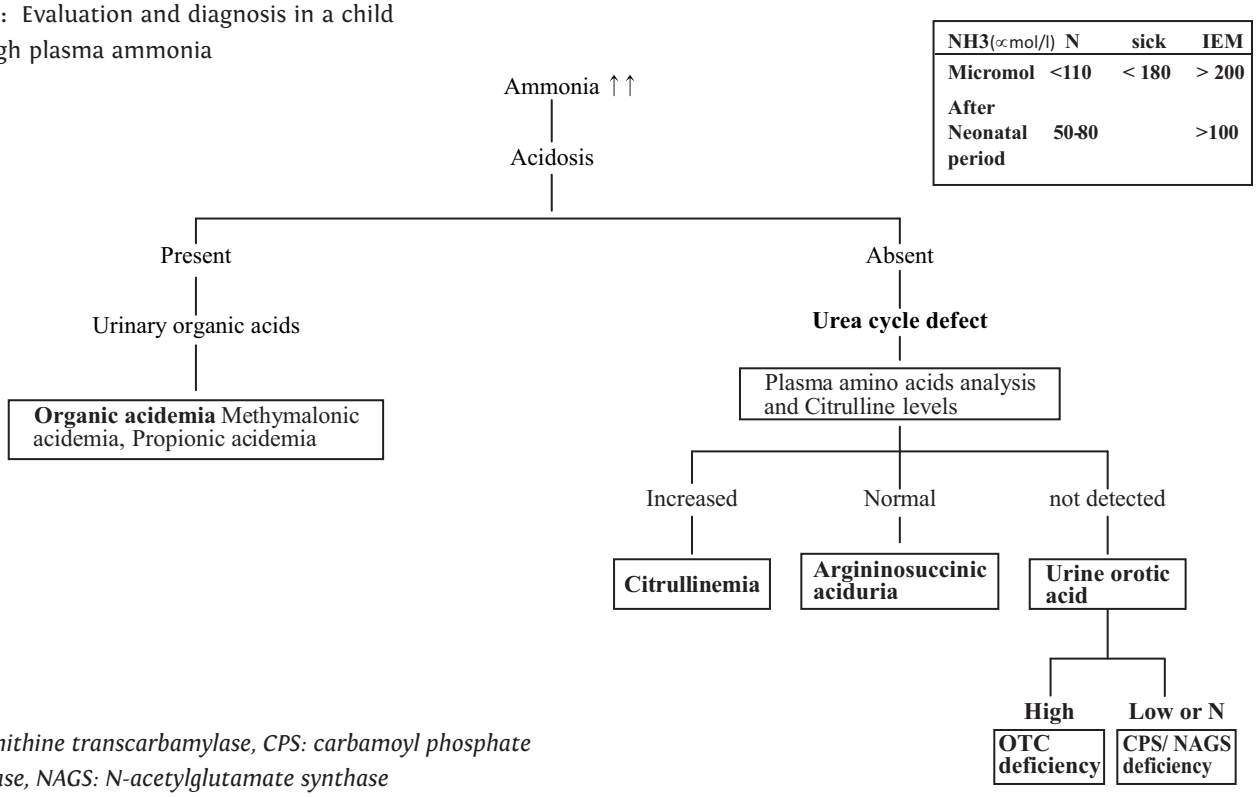
born at term and was appropriate for date. He fed well till day 3 of life when he developed vomiting, became lethargic and had seizures. The sepsis screen was negative and there was no hypo/hyperglycemia or dyselectrolytemia. On direct questioning a family history of a niece with similar symptoms was obtained.

There was no metabolic acidosis or ketosis. The plasma lactate was 12 mg /dl and normal. The ammonia was increased at 700 µmol/L. A urea cycle disorder was suspected and the neonate was investigated further. Quantitative analysis of plasma amino acids by high performance liquid chromatography (HPLC), tandem mass spectrometry (MS/MS), and urine organic acid estimation by Gas chromatography - mass spectrometry (GC-MS) were performed. The plasma citrulline levels were high (1344 nmol/l; ref: 17+/_ 5) and urine orotic acid was increased suggesting citrullinemia type I. Citrullinemia was confirmed in the neonate. He was managed with ammonia scavenging agents, dialysis, IV fluids, dextrose,and intralipid but continued to be encephalopathic and died on day 7 of life. Molecular analysis for citrullinemia on the stored DNA sample confirmed the neonate to be homozygous for a nonsense mutation in the ASS gene.

An elevated plasma ammonia level is an indicator of hepatocellular dysfunction due to any cause, the common causes being inborn errors of metabolism, infections or intoxications. Urea cycle disorders are the commonest metabolic disorders with high ammonia levels. The investigations and diagnosis of urea cycle defects is illustrated in figure 1.

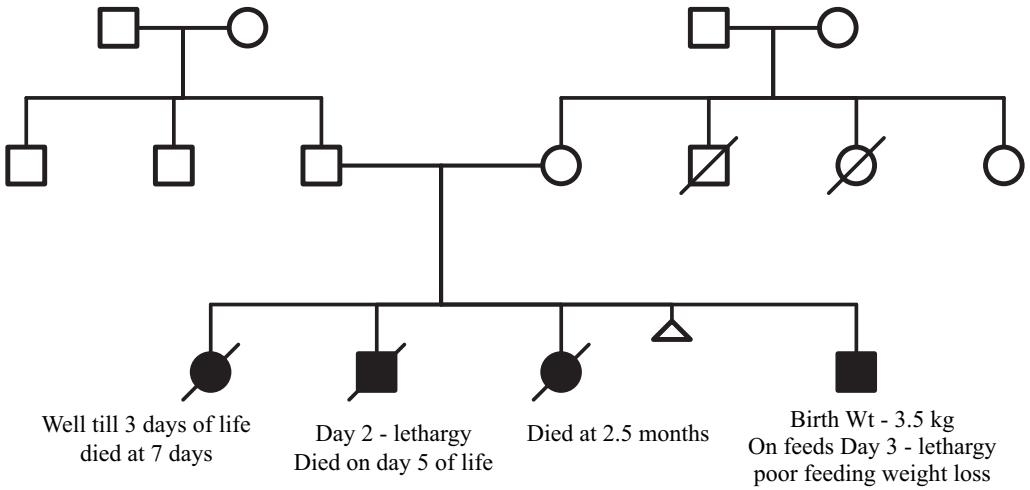
Central to this diagnostic algorithm is the quantitative analysis of amino acid levels in the blood and urinary orotic acid estimation by GC-MS. An important practical point is to collect a free flowing blood sample for ammonia estimation and transport this to the laboratory in ice for immediate estimation.

Figure 1: Evaluation and diagnosis in a child with high plasma ammonia



OTC: ornithine transcarbamylase, CPS: carbamoyl phosphate synthetase, NAGS: N-acetylglutamate synthase

Case 2: Acute encephalopathy with metabolic acidosis :



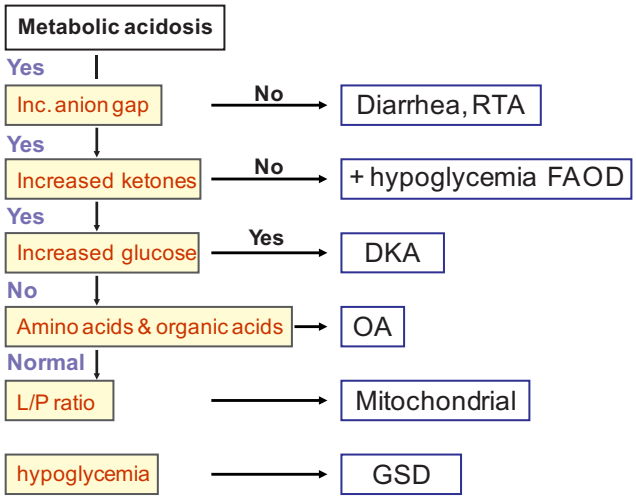
They were residents of Jharkhand and the neonate was transferred when he developed lethargy and poor feeding on Day 3 of life. At admission his sensorium was altered, and he was dehydrated and had metabolic acidosis which persisted even after he was stabilized. The anion gap was 26, plasma ammonia was 256 µmol/L and plasma lactate was normal. Sepsis screen was negative. There was history of 3 sibling deaths, two females and one male, all of who had presented similarly. Propionyl carnitine level was high on tandem mass spectrometry and 3 - OH propionate, propionyl glycine & methylcitrate were increased in the urine on GC-MS analysis. Propionic acidemia was diagnosed based on these reports. The neonate was started on protocol for management. However in view of a worsening encephalopathy and guarded prognosis, the parents took the child home. It is apparent from this family history that all the previous siblings had also died of Propionic acidemia. Unfortunately the possibility of a metabolic disorder had not been kept and appropriate investigations not done to prevent recurrence / initiate timely management before the onset of encephalopathy.

In contrast, timely diagnosis and appropriate management of Propionic acidemia is illustrated in this other case where a second born to non consanguineous parents was well till 14 months of age when he decompensated after a mild viral infection. He developed vomiting, lethargy and progressive encephalopathy. Investigations showed normal electrolytes, normoglycemia but persistent metabolic acidosis with an increased anion gap. His ammonia levels were 175 µmol/L. Urine was positive for ketones. Possibility of an organic academia was maintained and while awaiting the results of TMS and urine GC-MS analysis he was started on the emergency protocol management for organic acidemias with protein elimination and high caloric intake with carnitine supplementation. He improved on management and the diagnosis of Propionic acidemia was confirmed by MS-MS analysis which showed elevated levels of propionyl carnitine (8.0nmol/ml, ref: <3.5) and increased methylcitrate and 3 hydroxy propionate in urine. After stabilization, he was started on a diet restricted in isoleucine, valine, methionine and threonine with carnitine and metronidazole supplementation. He is now doing well, with normal developmental milestones. He had one minor episode of decompensation after a viral illness which was managed successfully. The second issue for this couple was to plan their next pregnancy. Propionic academia is an autosomal recessive disorder with a 25% risk of recurrence. Prenatal diagnosis is best performed at 11 weeks gestation by mutation

analysis in a chorionic villi sample. For this purpose the mutation in the proband in one of the two genes implicated in PA had to be identified. The mutation was found to be IVS1+5G>A and IVS3+2T>C in PCCB gene. Armed with this knowledge, we did prenatal testing in the next pregnancy and the fetus was not affected.

Metabolic acidosis is a common complication of almost any illness and is usually secondary to tissue hypoxia. However if there is associated ketosis, previous history of similar complaints or persistent acidosis after correction of tissue perfusion, metabolic disorders should be investigated for as per the approach represented in figure 2. The common organic acidurias prevalent in our country are maple syrup urine disease (MSUD), methyl malonic acidemia (MMA), propionic acidemia (PA), glutaric aciduria type I and multiple carboxylase deficiency (MCD).

Figure 2 : Approach to metabolic acidosis



RTA – renal tubular acidosis; FAOD – fatty acid oxidation defects; OA – organic acidurias; L/P ratio – lactate pyruvate ratio; DKA – diabetic ketoacidosis; GSD – glycogen storage disorders

Case 3: Acute encephalopathy with ketosis

A 2500 gms neonate was born at term and discharged home on day 2 of life. There were no adverse perinatal events. On day 5 he was readmitted with history of poor feeding and lethargy. He developed seizures, and dystonic posturing of limbs and progressed to encephalopathy. There was significant respiratory distress for which he required ventilation. Investigations for sepsis were negative. Urine ketones were positive and he had metabolic acidosis. The blood sugar was 78 mg/dl and electrolytes were normal. The DNPH test for ketones was positive. The quantitative amino acid analysis showed high levels of leucine - 2900 µmol/l (normal 65-220), isoleucine - 377 µmol/l (normal 26-100),valine - 384 µmol/l (normal 90-300) and alloisoleucine - 655 µmol/l (normal 0-5). This confirmed the diagnosis of Maple syrup Urine disease (MSUD) in the neonate. The neonate was managed with the specific protein restricted food for MSUD and the emergency protocol was initiated whenever he decompensated. The mother was trained to do the DNPH test at home when she felt the child to be unwell and initiate the emergency management at home. He performed well with good metabolic control and normal developmental milestones. At 22 months of age, a liver transplant was performed. This was the first ever liver transplant in India for MSUD. The boy, now 5 yrs old, is on no dietary restrictions and doing well. This case illustrates that early diagnosis and appropriate management of treatable metabolic disorders has a good outcome.

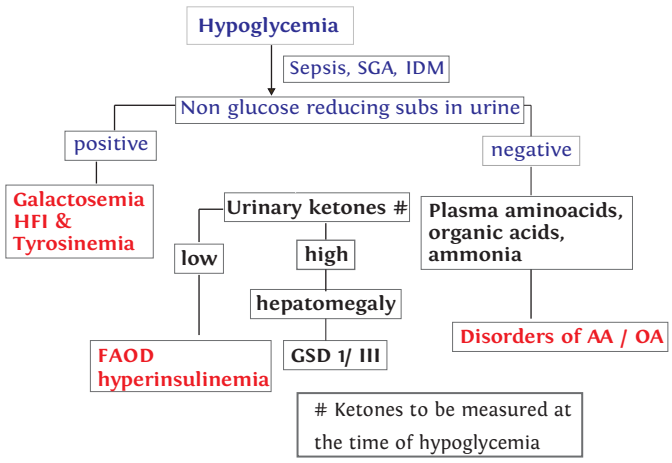
Case 4: A neonate with Hypoglycemia

In a child who presents with hypoglycaemia (defined as a blood sugar ‹45 g/dl at all ages) and ketone bodies in urine (ketotic hypoglycaemia), we investigate for abnormality in the pathway of glycolysis and gluconeogenesis. A 40 days neonate presented with abdominal distension and mild jaundice. On evaluation he had bilateral cataract, conjugated hyperbilirubinemia with mild elevation of SGOT and SGPT. INR was 1. The blood sugar was normal at 70 mg/dl. Urine succinylacetone was normal. Blood galactose and galactose -1- phosphate

levels were increased to 6032.9 (N. Range: ‹56 umol/L) and 4.5 (N. Range: ‹1mg/dL) respectively. Enzyme assay for Galatose-1-phosphatase transferase enzyme confirmed the diagnosis of galactosemia. Molecular analysis in GALT gene confirmed the presence of a homozygous mutation, CAT›CCT; c. A203C; p.H68P. The neonate was advised a galactose free diet. His cataract regressed spontaneously in 2-3 months. The metabolic parameters improved and on follow up the levels of galactose and galactose 1 phosphate decreased. His developmental milestones are normal and currently at 2 years age he is symptom free.

For the diagnosis of galactosemia it is imperative to take blood samples for assay prior to blood transfusion. It is best to combine analysis of the metabolites, galactose and galactose 1 phosphate along with with GALT assay. This helps to resolve borderline values of enzyme that may be obtained as well as diagnose epimerase and galactokinase deficiency.

Figure 3: Approach to hypoglycemia



SGA- small for gestational age;
IDM – infant of diabetic mother;
FAOD – fatty acid oxidation defects;
OA – organic acidurias;
HFI – hereditary fructose intolerance;
AA – aminoacidopathies;
GSD – glycogen storage disorders

To investigate a neonate /infant with hypoglycemia for a metabolic etiology, one can follow the algorithm in figure 3. Investigations should be done at the time of hypoglycemia and it is prudent to store a blood sample at the time of hypoglycemia.

Case 5: Acute encephalopathy with lactic acidosis

A male neonate, second offspring of a consanguineously married couple, developed poor feeding and lethargy with seizures 3 days after birth. The sepsis screen was negative. There was hypoglycemia and severe lactic acidosis. The serum ammonia was 78 micromol/litre. Urine ketones were positive. Blood pyruvate analysis was not available. There was progressive deterioration of sensorium with requirement of ventilator support. The MS/MS analysis for acylcarnitines was normal. The urine organic acid estimation by gas chromatography- mass spectrometry showed high lactate levels. A differential diagnosis of pyruvate carboxylase deficiency, pyruvate dehydrogenase (PDH) deficiency, disorders of gluconeogenesis like glucose 6 phosphatase deficiency, fructose 1-6 biphosphatase deficiency and phosphoenol pyruvate carboxykinase (PEPCK) deficiency was kept. There was history of death of a male sibling in the neonatal period with undiagnosed encephalopathy and acidosis.

No mutation was identified in the PDH gene. Gene sequencing of the pyruvate carboxylase gene showed a homozygous mutation in the PC gene confirming the diagnosis of Pyruvate carboxylase deficiency.

Case 6: Presentation with seizures

A four months female, born to consanguineously married Muslim parents, presented with history of seizure onset since 3 months age. There was no history of birth asphyxia and the girl had been apparently well for the first 3 months. On direct questioning, the mother stated that the infant's hair had not regrown after being shaved at 1 week of life. There was also some mild macular rash at the

nape of the neck. In view of resistant seizures she was on 3 anticonvulsants but with no response. With this classical triad of seizures, alopecia and rash, we clinically suspected biotinidase deficiency as the etiology of the phenotype. Blood samples were taken for biotinidase enzyme estimation and Tandem Mass Spectrometry analysis and she was started on tablet biotin, 5 mg thrice daily. The seizures stopped in 3 days and regrowth of her hair was noted. All anticonvulsants were tapered and currently she is doing well on tablet biotin replacement. Biotinidase deficiency was confirmed by enzyme estimation and further molecular testing showed a homozygous mutation in the BTD gene. This is another example which illustrates the good response to a treatable disorder if therapy is initiated timely and why metabolic disorders must be considered as an initial differential in the appropriate presentation.

Metabolic Autopsy: Very often the neonate / child is very sick and metabolic investigations may not be possible immediately. In this situation blood and urine samples must be preserved for diagnostic purposes. Pretransfusion blood sample in an EDTA and plain vacutainer as well as on the newborn screening filter paper must be stored. Freeze 15 ml urine at -80 & C as well as CSF/ tissue biopsy samples. Most of the IEMs have a 25 % risk of recurrence and prenatal diagnosis in a subsequent pregnancy is not possible in the absence of a definitive diagnosis in the proband.

Conclusion:

Neurometabolic disorders, especially those that present acutely are treatable and delay in diagnosis and initiation of treatment increases the morbidity and risk of mortality. A high index of suspicion is important to diagnose an IEM. The major hurdle in diagnosing an inborn error is the inability to consider a diagnosis. Once a differential diagnosis with the help of the basic investigations as detailed above is made, samples for special investigations can be sent to a reference laboratory as most of these tests are now available in India. It is helpful to liaise with a clinician involved in diagnosis and management of IEMs to discuss the useful investigations in a particular phenotype.

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Advances in the therapy of genetic disorders

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The common perception among pediatricians is that genetic disorders have no treatment. This is partially true, but so is the case for many disorders other than infectious, nutritional and parasitic disorders. With improved understanding of the pathogenesis of genetic disorders the situation is set to change. For example, a recent review listed 81 **'treatable inborn errors of metabolism'** presenting with intellectual disability as a major feature. This included disorders of amino acids (12), lysosomes (12), vitamins/co-factors (8), urea cycle (7), hyperhomo-cysteinemia (7), and others.[1] The diseases are presented on this website (www.treatable-id.org) as an interactive tool for the clinician and scientist. The information is presented in several different ways: ranging from the biochemical categories, signs & symptoms, diagnostic tests, to therapies and evidence. For each condition a disease page has been designed as an information portal with access to specific genetics, biochemistry, phenotype, diagnostic tests and therapeutic options. This is an extremely useful website.

Duchenne muscular dystrophy is an X-linked disorder caused by mutations in the dystrophin gene. Patients have severe, progressive muscle wasting, leading to death in the twenties. Almost 70 % of patients have deletions of one or more exons. These deletions result in disruption of the open reading frame, which prevents the full translation of its protein product dystrophin. Restoration of the open reading frame and dystrophin production can be achieved by exon skipping using antisense oligonucleotides that induce specific exon skipping during pre–messenger RNA (mRNA) splicing, aimed at reading-frame correction. This approach aims to transform the Duchenne muscular dystrophy phenotype to that of the milder disorder, Becker muscular dystrophy, typically caused by in-frame dystrophin deletions that allow the production of an internally deleted but partially functional dystrophin. Exon skipping provides a mutation-specific and thus potentially personalized therapeutic approach for patients with Duchenne muscular dystrophy. In a recent trial, weekly abdominal subcutaneous injections of PRO051 were given for 5 weeks to 12 patients. There was a dose-dependent molecular efficacy in patients with Duchenne muscular dystrophy, with a modest improvement in the 6-minute walk test after 12 weeks of extended treatment.[2] Other investgators have used different chemistries for the anti-sense oligonucleotides (phosphorodiamidate morpholino oligomers) with promising results. Cases without deletions but having a point mutation that is a stop codon have been targeted by use of PTC which reads through the stop codon. A recent trial did not meet with the endpoints set out in the study, but the results are promising. At the same time gene therapy has been successful in treating dystrophic mice. However trials in humans by introducing a transgene coding for full-length or a truncated version of dystrophin complementary DNA have not met with success.

Spinal muscular atrophy (SMA) is a genetic neuromuscular disease characterized by muscle atrophy and weakness. It is the second commonest single gene disorder in India, second only to thalassemia. One child out of approximately every 10,000 births is born with SMA in India. Most infants born with SMA Type I, the most severe form of SMA, usually die within their first two years of life. SMA is caused by a loss of, or defect in, the

survival motor neuron 1 (SMN1) gene. The SMN1 gene produces most of the SMN protein, which is critical to the health and survival of the nerve cells in the spinal cord responsible for muscle contractions. There is another gene - SMN2 gene - which is highly homologous to the SMN1 gene, but differs from it by the substitution of a single nucleotide (c.840C>T). This decreases the activity of an exonic splicing enhancer so that SMN2 gene produces a small fraction of functional SMN protein, which however is not enough to prevent disease if exon 7 of SMN 1 gene is deleted.

Antisense drugs have been designed to modulate the alternative splicing of the SMN2 gene to significantly increase the production of functional SMN protein. Researchers have shown that the anti-sense drugs increased production of functional SMN protein in multiple animal models of SMA, and resulted in an increase in the number of motor neurons and improvements in behavior and survival.[3] Antisense drugs do not cross the blood-brain barrier, and for treating neuro-degenerative diseases like SMA, antisense drugs need to be administered into the central nervous system (CNS). Researchers now plan to use this drug intrathecally in a single dose in patients with SMA, and will then move to multiple-doses in future studies. This represents a real breakthrough in the treatment of SMA. (http//www.isispharm.com).

Fragile X syndrome (FXS)

FXS is the leading cause of inherited intellectual disability, affecting approximately 1 in 2500-4000 males and 1 in 7000-8000 females. The disorder is caused by the elongation of CGG repeats above 200 units in the 5' untranslated region of FMR1 gene, leading to hypermethylation of the promoter region followed by inactivation of the gene. The patients have typical facial features such as an elongated face with prominent forehead, a protruding jaw, large ears and macro-orchidism. Patients may show behavioural problems including autistic-like behaviour, sleeping problems, anxiety, mood disorders, aggression, as well as neuronal hyperexcitability, manifested by hyperactivity, increased sensitivity to sensory stimuli and a high incidence of epileptic seizures

The FMR protein is a translator inhibitor. Therefore, as a consequence of the inactivation of the FMR gene, metabotropic glutamate receptor (mGluR) is stimulated, which leads to exaggerated internalization of an AMPA receptor. This in turn reduces the number of AMPA receptors in the synapses that are believed to be involved in memory and learning. This observation predicted that dampening the mGluR signalling would improve the clinical symptoms of FXS patients. Drugs like MPEP and AFQ056 were used. Phase II trial with AFQ056 caused a significant improvement on stereotypic behavior, hyperactivity and inappropriate speech, but only in those with the full mutation. Validation of these results is awaited. A phase II clinical trial with the selective receptor GABAB receptor agonist STX209, better known as arbaclofen, also showed promising results. A trial with minocycline, which leads to inhibition of matrix metalloproteinase (which is elevated in the hippocampus of fragile X mice), in 20 fragile X patients resulted in significant improvements across a range of behaviors without serious side-effects. However, a placebo-controlled trial is needed, especially as minocycline-induced autoimmunity could occur in patients. Lithium inhibits glycogen synthase kinase (GSK)3, which is affected by increased mGluR signaling, and treatment with this drug, which is already approved for use in patients with bipolar disorder, ameliorated the behavioral deficits in both fragile X mice and patients.[4]

Rett syndrome (RS)

This is an X-linked neuro-developmental disorder primarily affecting girls. Over 95% of typical RS cases are due to mutations in the gene encoding the MeCP2 (transcriptional modulator methyl-CpG-binding protein 2). This protein binds methylated CpG sites. It localizes primarily in the nucleus and activates histone deacetylases, resulting in chromatin compaction. These studies indicate that

expression of MeCP2 at optimum levels in as many cells as possible is critical for normal brain function. About 35% of pathogenic MECP2 mutations are nonsense mutations that may be amenable to pharmacologically induced 'read through' of premature stop codons by aminoglycosides or PTC. Increasing BDNF (Brain-derived neurotrophic factor) levels in Mecp2-deficient mice improved survival and partially reversed the RS-like phenotype. This has led to potential therapeutic strategies with ampakines that can increase BDNF expression. Another growth factor that, like BDNF, is widely expressed in the central nervous system is insulin-like growth factor 1 (IGF1). This work has led directly to Phase 2 studies of recombinant human IGF-1 (mecasermin, Increlex) in girls aged 2–12 years.[5]

Gene therapy The ultimate treatment in genetic disease is gene therapy. This may involve replacing a defective gene with a functional version, enhancing the baseline expression level of a gene or, contrastingly, suppressing the expression of genes that may contribute to the pathologic process. As the field of gene therapy continues its rapid advancement, it is likely that it will eventually become a standard clinical regimen. Gene therapy has been most successfully used to treat certain cases of primary immune deficiency disorders. Encouraging long-term follow-up data has been published for patients treated with retroviral vectors to correct adenosine deaminase-deficient severe combined immunodeficiency (SCID), X-linked SCID and chronic granulomatous disease, and a first-in-human study also reports clinical improvement for Wiskott–Aldrich syndrome.[6] Nathwani et al. in a recent remarkable study showed that a single intravenous injection of an adenovirus-associated virus (AAV) vector that expresses FIX can successfully treat patients with hemophilia B for more than a year.[7] AAV is a small (4.8 kb), nonpathogenic, single-stranded DNA virus from the parvovirus family. They generated a replication-incompetent vector that cannot propagate after gene transfer. This technology may soon translate into applications for other disorders, such as lysosomal storage diseases, alpha1-antitrypsin deficiency, and hyperlipidemias.[8]

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Congenital or traumatic deformity?

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genetic CLINICS

18

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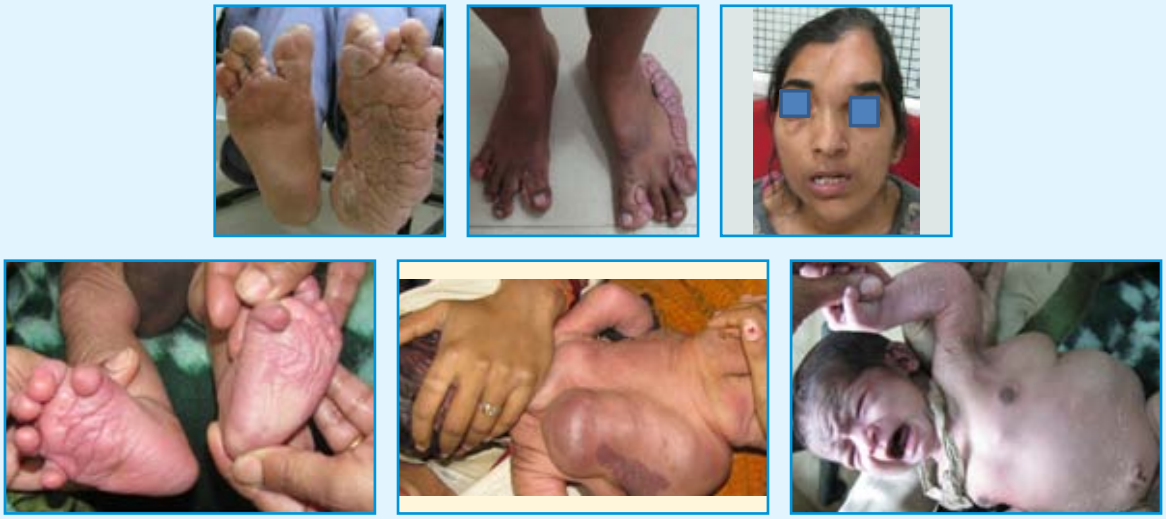
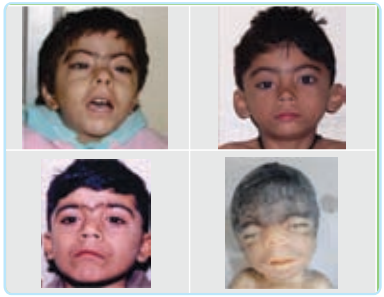
Identify this syndrome with lipomatous skin lesions, hyperplasia of plantar surface of feet and macrodactyly



Answer to PhotoQuiz 17 of the previous issue

Cornelia de Lange syndrome (OMIM # 122470, 300590, 610759, 614701)



Cornelia de Lange syndrome (CdLS) is characterized by distinctive craniofacial features, prenatal onset growth retardation, hirsuitism and upper limb reduction defects. The typical craniofacial features include synophrys, arched eyebrows, long eyelashes, small upturned nose, small widely spaced teeth, and microcephaly. Other frequently associated features are intellectual disability, cardiac septal defects, gastrointestinal dysfunction, hearing loss, myopia, and genital anomalies. Mutations in the NIPBL (5p13.1), SMC1A (Xp11.2), and SMC3 (10q25) genes have been reported to cause this condition and recently a fourth gene RAD21 (8q24.1) has also been found to be associated with this syndrome. NIPBL-related CdLS, SMC3-related CdLS and RAD21-related CdLS have an autosomal dominant inheritance pattern, while SMC1A-related CdLS is inherited in an X-linked manner.

Correct responses were given by:

1. Sunita Bijarnia Mahay, via email
2. Aruna G, Bangalore
3. Niby J Elackatt, via email
4. Abdul Mueed Bidchol, Manipal
5. Kalpana Gowrishankar, Chennai
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13. Saminathan D, Trichy
14. Leena Deshpande, via email
15. Mohandas Nair, Calicut
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