genetic CLINICS

Newsletter of Genetics Chapter of Indian Academy of Pediatrics

Volume: 2
Issue : 3 (July-Sept 2009)

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

The categories of article include:

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

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

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Lysosomal storage disorders: What is in store for the patients?

Dr Shubha R Phadke

Genetic diseases were seen to have a prognosis without hope and with cure nowhere in sight. The list of untreatable genetic disorders is long - malformations, mental retardation, neuromuscular disorders, disorders of bone and skin, cardiomyopathies, retinitis pigmentosa etc. But with advances in the understanding of molecular bases of genetic disorders the situation is changing.

Though the ultimate goal for treatment of genetic disorders i.e. gene therapy, still remains a challenge, other therapies based on knowledge of molecular pathogenesis and use of recombinant products have come into practice. Lyosomal storage disorders (LSDs) are one group of such disorders for which the twenty-first century has ushered in a great hope.

The metabolic disorders, for a long time, were considered to be amenable to treatment using strategies to intervene at various metabolic steps. The prototype of successful treatment of metabolic disorders is phenylketonuria (PKU). Equally good results are observed in some other metabolic disorders like biotinidase deficiency, galactosemia, congenital adrenal hyperplasia, MCAD etc. These disorders amenable to treatment have been selected for neonatal screening. The treatment for PKU has been in existence for more than 4 decades.

Over this period, success of similar degree has been achieved in only a very few disorders. Though the treatments based on diet modification, cofactor administration etc. have been developed to intervene in metabolic pathways over these years. But the number of disorders with complete response is still very small and for most of the disorders the response to treatment is only partial.

Lysosomal storage disorders (LSD) are a group of 40 or more conditions with abnormalities of metabolism of large molecules. These molecules are degraded in lysosomes by enzymes. The deficiency of various enzymes leads to disorders with varied manifestations due to accumulation of these complex molecules in various organs of the body .

The LSDs include various mucopolysaccharidoses (MPS) (Hurler syndrome, Hunter syndrome, Morquio syndrome, Sanfilippo syndrome, etc.), oligosaccharidoses (mannosidosis, fucosidosis, sialidosis, etc.), sphingolipidoses (Gaucher disease, Niemann Pick disease, metachromatic leocodystrophy, Tay Sachs disease, Sandhoff disease, generalized gangliosidosis), Fabry disease, Glycogen storage diseases (type II/Pompe disease), Mucolipidoses (I-cell disease, etc.) and lipid storage diseases (neuronal ceroid lipofuschinosis, Wolman disease etc). Most of the disorders have hepato-splenomegaly and coarse facial features due to storage of material which normally should have been degraded. Many of these disorders have involvement of the nervous system and a poor prognosis. There was no treatment for these disorders for ages and a continued downhill course, poor quality and short expectancy of life were the universal outcome.

Bone marrow transplantation with matched donors is successful in improving the outcome in some disorders, especially those without significant involvement of the central nervous system. The logical strategy of providing deficient enzyme was not successful for quite some time. Though the enzymes could be synthesized by recombinant DNA technology based methods, they could not be made to reach the site of action i.e., inside the lysosomes. The strategy to add mannose phosphate group to the enzyme so that the mannose receptor on the lysosomes can take up the enzyme was a milestone in the development of Enzyme Replacement Therapy (ERT) for lysosomal storage disorders. The first ERT became available in 1990s for Gaucher disease. Now worldwide more than 5000 patients of Gaucher disease type I are getting ERT which greatly improves the quality of life and length of survival. The same strategy has been successfully applied to others disorders like Hunter syndrome (MPS II), Hurler syndrome (MPS I), Maroteaux Lamy Syndrome (MPS VI), Pompe disease and Fabry disease. These treatments are very recent and have become available around 2003-2006.

The research for other lysosomal disorders is in the pipeline and the success story of Gaucher disease is likely to be repeated for other storage disorders in the recent future. One can get an idea about the utility of ERT from the few years of follow up of the cases on ERT. The liver and spleen regress in size markedly and hematological parameters normalize with ERT for Gaucher disease. Infants with Pompe disease who die by one year of age due to involvement of cardiac muscles and respiratory muscles achieve normal motor milestones if ERT is instituted early in the course of the disease. For mucopolysaccharidosis, there is great improvement in the joint contractures, skin thickening, coarse facies and hepatomegaly. At present ERT cannot help in improving neurological features. Hence for MPS, ERT is indicated only in cases without mental retardation and other features of central nervous system involvement.

Fabry disease is clinically very different from other storage disorders. The predominant symptom is neuralgic pain with or without angiokeratomas. These patients do not have mental retardation or organomegaly but are at risk of early onset ischemic heart disease and stroke and account for about 1% cases of end stage renal disease. Due to lack of characteristic clinical features, these patients are not diagnosed for decades and sometimes, due to their symptom of neuralgic pain, are

misdiagnosed as a psychiatric illness. ERT for Fabry disease not only helps in decreasing the neuralgic pain, but it helps to decrease the risk of renal failure, ischaemic heart disease and stroke by removing the ceramide deposits from blood vessels. Early diagnosis and treatment is essential to prevent the complications. This may be difficult as some cases may not have any symptoms in early life and renal and cardiac complications may be the presentation. As cardiac and renal disease can be of varied causes high level of suspicion is necessary for diagnosis of Fabry disease.

With the availability of treatment it is important to suspect these treatable disorders at the earliest and confirm the diagnosis by enzyme assay. These diagnostic tests are increasingly becoming available in India. It is essential to go beyond clinical diagnosis and get confirmatory tests done. Just putting all cases of hepatosplenomegaly, coarse facies and dysostosis multiplex in one bag of mucopolysaccharidosis is too inadequate to provide genetic counseling and prenatal diagnosis. It will also deprive the patient of treatment if he or she has a treatable type of mucoplysaccharidosis. Mucolipidosis and oligosacchariodosis also have clinical presentations similar to MPS and need to be suspected if the urine is negative for mucopolysaccharides. These disorders present commonly in early childhood but presentations at all ages including fetal hydrops are known.

Awareness about these disorders will go a long way in identifying these cases so that no patient is deprived of treatment for the lack of accurate diagnosis. For those disorders for which there is no ERT at present, recurrence in the family can be prevented by prenatal diagnosis. For lysosomal storage disorders prenatal diagnosis is possible by enzyme assay or DNA based tests on chorionic villi sample. To conclude, we are witnessing the beginning of a revolution in the field of lysosomal storage disorders. ERT for many other diseases is expected. ERT has proved its efficiency for some disorders and more therapies are on the horizon. Trials with other strategies of treatment like reducing substrate, chaperone therapy and many other drugs are ongoing. Cell mediated therapies and gene therapy trials are also underway. We expect more successes and this is supposed to lead us into a new era in the care of LSDs. There are still many challenges. Blood brain barrier renders ERT less effective in disorders with central nervous system involvement. Options like neonatal screening for presymptomatic diagnosis and early institution of therapy are being explored. Early treatment for Pompe disease is very effective and justifies neonatal screening. Similarly, early treatment is greatly beneficial in Fabry disease to prevent complications like stroke, renal failure and ischemic heart disease. Though thought to be rare, Fabry disease was found to be as frequent as 1 in 4500 in a study on neonates. World wide prevalence of all LSDs together is 1:1500 to 1:7000. Though there is no data of prevalence in India, experience of clinicians, large population and the high rate of consanguinity suggest that the absolute number of cases of LSDs in India is likely to be large and may be contributing more significantly to the burden of genetic disorders than elsewhere. Increasing awareness about LSDs, ERT, other upcoming treatments and all the latest developments in the field, will go a long way to help these patients and families.

Fig 1: All smiles – A patient of Fabry disease on ERT

Fig 2: Patients of Gaucher disease on ERT

Fig 3: A 4 year old girl with MPS ISH getting ERT

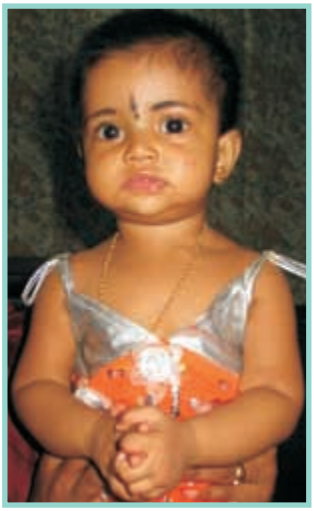

Fig 4: One year old girl with Pompe disease getting ERT since early infancy has normal motor milestones. (Thanks to Dr Sheela Nampoothiri for the image)

Fig 5: A 7 year old girl with MPS I SH has finger contractures, corneal clouding with normal IQ.

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1 July 2009

Spinal Muscular Atrophy in Clinical Practice

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INTRODUCTION

Spinal muscular atrophy (SMA) is a common inherited neuromuscular disease in children with a frequency of 1 in 10000 livebirths. It is a disease where spinal cord shows severe loss of motor neuron in the anterior horn region manifesting with muscle weakness of variable severity. Significant advances in understanding the molecular pathogenesis has resulted in better care of the families from the point of view of clinical genetics. This article discusses the practice of clinical genetics with respect to spinal muscular atrophy.

CLINICAL FEATURES

The classical features of spinal muscular atrophy are hypotonia and muscle weakness. Depending on the severity, the disease is divided into different types. The type 1 (Werdnig-Hoffman disease), the most common and the severest variety, accounts for about half of the patients with SMA. The disease usually manifests by 6 months of age and will never achieve independent sitting. Profound hypotonia and weakness hinders achievement of normal head control and they commonly present as floppy babies. They may demonstrate a bell shaped thorax with paradoxical respiration. Most succumb to the disease, mainly due to respiratory compromise by one to 2 years of age. Type 2 diseases has onset by 7-18 months of age and children usually sit but never stand and walk.

Figure 1. A floppy infant with SMA1

The life expectancy extends to late childhood. Type 3 (Kugelberg-Welander disease) is a mild form of the disease with onset beyond 2 years of age. These individuals are able to sit, stand and walk independently. However, adults have weakness of variable severity with some able to perform all activities independently whereas others require support for walking. Some may show calf hypertrophy. Most of them survive into adulthood with differing degrees of severity. A still milder form, type 4 has also been recognized. The other striking features are normal intellect and presence of fasciculations. Over long term, kyphoscoliosis and contractures increase the morbidity. It is important to realize the spectrum of clinical severity of SMA and consider this as a differential diagnosis in any individual demonstrating muscle weakness of lower motor neuron origin. Fasciculations, if clinically observed favors the diagnosis of SMA in a child with hypotonia and muscle weakness. The other diagnoses to be considered in a hypotonic infant are congenital myopathy, congenital muscular dystrophies and central hypotonia (especially Prader Willi syndrome). In milder forms of SMA, Becker muscular dystrophy and limb girdle myopathy may be considered in differential diagnosis.

INVESTIGATIONS

Creatinine kinase levels may be normal or mildly elevated. Earlier, electromyography and muscle biopsy were used to confirm the diagnosis. Electromyography shows features suggestive of denervation with spontaneous activity of positive, and sharp waves, fibrillation and occasional fasciculations. Motor unit action potential shows high amplitudes and long durations coupled with decreased recruitment. Skeletal muscle shows atrophic fibers with islands of group hypertrophy on biopsy[1]. Molecular testing now being easily available at several centers, the diagnosis can be quickly established by testing DNA obtained from peripheral blood. This obviates the need for not so easy to obtain electromyography and the invasive muscle biopsy. They still have their role when the mutation is not detected in a floppy child.

GENETIC BASIS

Survival motor neuron gene (SMN) was identified as the causative gene for spinal muscular atrophy[2]. SMN gene is located on the long arm of chromosome 5 (5q13). It is present in multiple copies in humans, one telomeric copy (SMN1) and several centromeric copies (SMN2). These genes contain nine exons and eight introns. These two genes differ in 5 nucleotides, with just one difference in the coding region. SMN1 gene codes for a protein containing 294 aminoacids. SMN is expressed in all tissues. All patients with SMA have at least one copy of SMN2. SMN2 has a C to T transition at position six of exon 7 (as compared to SMN1). This leads to alternate splicing of SMN2 and exclusion of exon 7 from the mRNA.

The resultant protein is non-functional and is rapidly degraded. Only about 10% of the product of SMN2 is properly spliced and leads to a normal SMN protein. This low level of expression leads to survival during embryonic life, but is not sufficient for the survival of motor neurons in the spinal cord. In milder forms of the disease, the degree of normal splicing afterwards may be up to 50% and may contribute to the mild phenotype of these individuals. But variation in the copy number of SMN2 alone does not completely explain the phenotypic diversity.

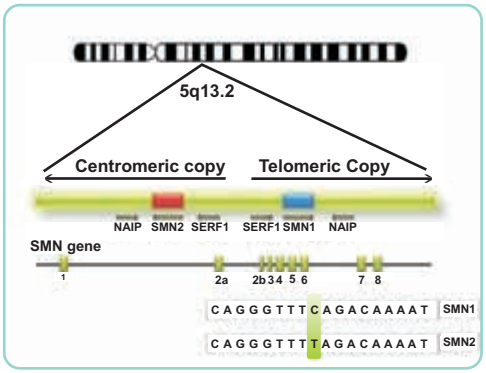

Figure 2. Diagram showing location of SMN genes on chromosome 5 and the difference of a single nucleotide on exon 7

MOLECULAR DIAGNOSIS

1. DIAGNOSIS IN THE PROBAND

In more than 98% of individuals with SMA, homozygous mutation in SMN1 can be identified.[2,3] The most common is homozygous deletion of SMN1 (the deletions vary in size but almost always includes exon 7) accounting for 95% of the cases and rearrangement or point mutation in the rest. The sensitivity of the test in individuals with clinical features of SMA for homozygous deletion is 95% and specificity is almost 100%[4]. This test is usually done by a polymerase chain reaction to amplify the exon 7 followed by differentiation of SMN1 and SMN2 by the susceptibility to digestion by a restriction endonuclease (Dra I) due to the single nucleotide difference. Homozygous deletion of exon 7 of SMN1 confirms the diagnosis of SMA. In the absence of homozygous deletion, the case needs review of clinical features. Myopathies (congenital and metabolic), muscular dystrophies and neuropathies (central or peripheral) causes need to be considered. In the presence of typical features of SMA and neurogenic electromyography, heterozygous deletion of exon 7 and a point mutation in the other SMN1 allele may be considered. The dosage of SMN1 may be estimated by allele specific PCR and quantification of the SMN1 and SMN2 alleles. Identification of intragenic mutation requires sequencing of the coding region.

INTERPRETATION OF THE TEST

Homozygous deletion of exon 7 confirms the diagnosis of SMA. In the absence of homozygous deletion, further testing for dosage of SMN1 and point mutations are complex. The availability of testing facility is also a limitation in this scenario. A strong clinical suspicion (with classical clinical features and electromyographic findings) is necessary to investigate further. The additional yield is only about 3% and hence cost-benefit ratio at present limits the further work up. It is strongly advisable to look into other conditions with similar features before these steps are undertaken.

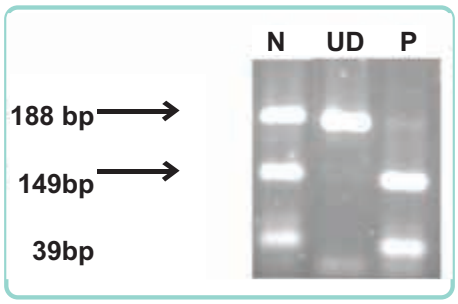

Figure 3. Molecular testing for detection of homozygous deletion of exon 7. The exon 7 of SMN gene is amplified using specific primers to give a product of 188bp size. Due to the similarity of sequences, exons of both SMN1 and SMN2 get amplified. The PCR product is then digested with the restriction enzyme Dra I and the products are run on an agarose gel. The single nucleotide difference between exon 7 of SMN1 and SMN2 acts as a restriction site for this enzyme and amplicon of exon 7 of SMN2 gene is cleaved by the restriction enzyme DraI into 149bp and 39bp products but not the exon 7 of SMN1. Hence, a patient (P) with homozygous deletion of exon 7 of SMN1 gives two bands (149bp and 39bp) corresponding to the exon 7 of SMN2. The normal person gives three bands (a band of 188bp corresponding to the undigested exon 7 of SMN1 and two bands of 149bp and 39bp corresponding to the digested exon 7 of SMN2).

2. CARRIER DETECTION

Carrier frequency of SMA is estimated to be 1 in 50. The PCR-RFLP technique used for diagnosis of patients with SMA cannot be used for carrier detection. Homology between SMN1 and SMN2 complicates this technique as the PCR for SMN1 frequently amplifies SMN2 as well. Hence the current strategies use real time quantitative PCR to quantify the SMN1 and SMN2 copies. Even multiplex ligation dependent probe amplification (MLPA) can be used for carrier detection. Carrier test for SMA is prone for errors as some carry both the copies of SMN1 on the same chromosome and no copy on the other

and point mutations are not detected resulting in false negative results.

3. PRENATAL DIAGNOSIS

Prenatal diagnosis in carrier couple is done by chorionic villi sampling at 10-12 weeks of gestation. The technique used for the diagnosis in the proband is applied here as well (usually the PCR-RFLP method of detection of homozygous deletions). It is essential to exclude the contamination of fetal sample by maternal cells during sampling.

TREATMENT AND PROGNOSIS

Currently the treatment is only supportive. Physiotherapy and respiratory therapy improves the outcome, especially in milder types of SMA. Older individuals with type 3 and 4 disease have slower progression and require psychological support in addition. At one end of the spectrum of the disease, the children with type 1 have relentless progression leading to death usually by their second birthday; those with minimal weakness can almost lead to normal life with no significant disability in carrying out the routine activities. As there is SMN2 gene in all patients with deletion of SMN1, interest is on enhancing the expression of SMN2 in tissues in patients with SMA. Several approaches are being studied towards this goal. These involve inclusion of exon 7 in transcripts of SMN2, enhancing the promoter activity of SMN2, modulation of protein translation and prevention of degradation of SMN2 gene product. Various drugs are under different stages of clinical trial.[5] Molecules like albuterol, phenylbutyrate and valproic acid have shown some improvement either at the cellular level or of muscle strength, but their clinical use is still far from reality .

GENETIC COUNSELING SCENARIOS

1. CHILD WITH SMA TYPE 1 & 2

When a child is suspected to have SMA type 1 or 2, the prognosis for good neuromuscular function and survival is poor. All cases need to have molecular testing. This requires about 2ml EDTA anti-coagulated blood. The sample can be sent to the laboratory at room temperature. If the child has a critical illness, it is essential to preserve the sample. The sample can be collected even postmortem and kept in refrigerator till it is shipped to the laboratory. When the diagnosis is confirmed by molecular testing (usually homozygous deletion of SMN1), it is implied that both the parents are carriers of the deletion. As the condition is inherited in autosomal recessive mode, the risk of recurrence in subsequent siblings is 25%. The parents are offered prenatal testing of chorionic villi sampling at 10-12 weeks of gestation in all subsequent pregnancies. Most families accept the prenatal diagnosis as the condition is very severe[1].

Figure 4a & 4b: A child with SMA2 at 2 years (4a) and another male with SMA2 at 20 years (4b) showing the phenotypic spectrum of SMN1 mutations

2.OLDER CHILDREN AND ADOLESCENTS WITH SMA 3 OR 4

The disease is less severe here. The phenotype varies widely in SMA3 and SMA4. Hence, predicting the course of the disease is difficult. They are advised to have good physiotherapy and occupational therapy to make the best use of existing muscle power. The confirmation of diagnosis by molecular testing raises several questions. Many of them have siblings who are asymptomatic. Presymptomatic diagnosis of the condition in them raises several ethical issues.

3. ADULTS WITH SMA

Adults with SMA1 may want to know if the condition would recur in their offspring. Here the risk of recurrence of the condition in the child of an adult with SMA2 is 1 in 20 (taking the carrier frequency to be 1 in 50). Again carrier testing of the spouse will be of great help. But direct prenatal testing for a 4% risk of disease is an option. But if the spouse carries a rare point mutation, the condition will go undetected in the fetus if tested by PCR-RFLP technique.

4.HISTORY OF PREVIOUS INFANT DEATH DUE TO SMA, NO MOLECULAR TESTS DONE

Carrier testing, when available, may be offered by quantitative measurement of exon 7 of SMN1 to the parents. This helps in offering the recurrence risk to the couple and to consider prenatal testing in subsequent pregnancies. In a situation where no carrier testing is feasible, the couple may be offered direct prenatal testing in the chorionic villi sample. It is essential to emphasize to the couple that many congenital myopathies and muscular dystrophies may mimic SMA1 and present as hypotonic infant. However, if fasciculations are documented in the earlier floppy infant or in the electromyogram, the diagnosis of SMA1 is more likely. Consanguineous marriage does not help much in considering SMA as many of the conditions with floppiness have autosomal recessive inheritance with the same 25% risk of recurrence.

POPULATION SCREENING FOR CARRIER STATUS

The American College of Medical Genetics has recently recommended offering carrier testing of all couples in all populations in view of the high frequency of the carrier state[6]. Such testing should be voluntary and be done only with informed consent with adequate support for genetic counseling. It is to be seen how this recommendation will be accepted by the physicians, general public and policy makers.

SUGGESTED FURTHER READING

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Thumb and great toe aplasia - Yunis Varon syndrome

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CASE REPORT

A 27-days -old female child was born as asphyxiated full term, low birth weight baby by caesarean section for polyhydroamnios to a nonconsanguinous young couple. She had multiple congenital anomalies. Antenatal ultrasound showed polyhydramnios. Anthropometric examination showed presence of microcephaly [head circumference =31 cm <-2SD]. She had light colored sparse hairs, epicanthic folds, anteverted nares, cupid bow like upper lip, labiogingival retraction, thin lower lip, hypoplastic alveolar, ridges, tight frenulum (figure 1a). She had hypoplastic finger nails, thumbs (Figure 1b), toe nails and great toe aplasia bilaterally (Figure 1c). Rest of the examination was normal. A written informed consent was obtained from the patient's father for publication of this case report and accompanying images. Skeletal survey showed an absent right clavicle (Figure 2) and hypoplastic left clavicles and agenesis of distal phalanges, middle phalanges of fingers and first metacarpals and metatarsals. Investigations showed normal CT scan and echocardiography. Thisphenotype is characterstic of Yunis varon syndrome.

DISCUSSION

Yunis varon syndrome is a rare autosomal recessive congenital malformation syndrome affecting skeletal, ectodermal and cardiorespiratory systems. Since 1980 when Yunis and varon described this syndrome around 17 cases have been described in literature.[1] It is characterized by pre and postnatal growth retardation, defective growth of cranial bones, with partial or complete absence of clavicles, with hypoplasia or aplasia of thumbs and big toes and distal phalanges and dysmorphic facial features like anteverted nostrils, short upper lip, and microcephaly. Other associations that have been reported are tetralogy of Fallot[2], deafness, pyloric stenosis[3], Dandy walker malformation, hydrocephalus hypertension[4], and liver abnormalities[5]. The possibility of the syndrome resulting from abnormal lysosomal storage has been suggested by several authors.[4,6] The overall prognosis for this disorder is poor. In a review only 3 of 13 patients with this syndrome survived first year of life but 2 of the survivors had severe physical and mental retardation.[2,5] Prenatal diagnosis of multiple congenital abnormalities by targeted second trimester ultrasound is important to provide adequate genetic counseling regarding long term prognosis, outcome and recurrence risk in next pregnancy.

LEGENDS TO FIGURES

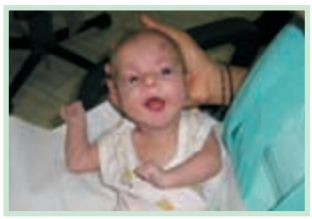
Fig 1a

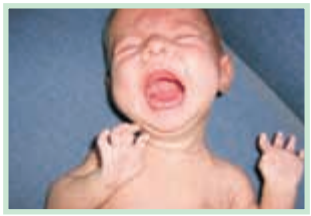
Fig 1b

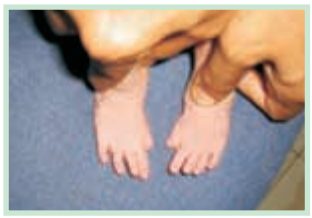
Fig 1c

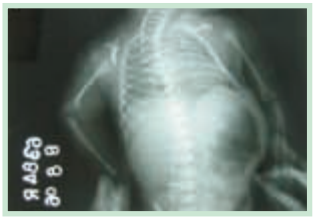
Fig 2

1) Figure 1a- Facial dysmorphism. Note the cupid bow like upper lip, labiogingival retraction
2) Figure 1b- Hypoplastic thumb
3) Figure 1c – Bilateral great toe aplasia and hypoplastic toe nails
4) Figure 2 - Xray showing absent right clavicle and left hypoplastic clavicle.

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Disorders Of The RAS-MAPK Pathway: Prototype of Pathway Disorders with Overlapping Phenotypes

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INTRODUCTION

A molecular pathway may be defined as a series of molecular interactions that generates a particular end product or leads to a certain cellular function. A signal transduction pathway is a molecular pathway that 'transduces' an extracellular signal into changes in gene transcription within the cell; the external signal in the form of an extracellular ligand binds to a surface receptor, resulting in the activation of intracellular signalling molecules to modify the transcription factor activity in the nucleus and alter transcription of specific genes. Many of the developmental processes in eukaryotic organisms including human beings are now known to be controlled by evolutionarily conserved signal transduction pathways. Disturbances in these pathways can affect these normal developmental processes and because a disturbance at any level of the pathway can lead to disruption of the common final end-product or function, defects in any of the involved molecules can produce similar or overlapping phenotypes. Many pathway disorders with overlapping phenotypes are known but perhaps the most extensively described are the disorders related to the RAS – MAPK pathway, also known as the neuro-cardio-facial cutaneous syndromes.[1] Ciliopathies and some of the disorders related to the sonic hedgehog pathway (SHH-GLI-PTCH), FGF pathway and WNT pathway are also known to have some similarity in their manifestations.

RAS-MAPK PATHWAY

The RAS – MAPK pathway is an important signal transduction pathway that controls the expression of genes involved in cell proliferation, cell survival, cell differentiation and apoptosis. RAS genes are homologues of rat sarcoma virus genes. Four main RAS genes are known: HRAS (Harvey rat sarcoma oncogene homologue), KRAS (Kirsten rat sarcoma oncogene homologue), ERAS (Embryonic stem cell expressed RAS) and NRAS (Neuroblastoma RAS oncogene). RAS genes encode the Ras proteins which are guanosine nucleotide bound proteins that cycle between an active GTP-bound and an inactive GDP-bound conformation. The Ras proteins are upstream members of the RAS – MAP kinase pathway. The RAS – MAPK pathway gets activated when an extracellular ligand such as a growth factor binds to a tyrosine kinase receptor such as the epidermal growth factor receptor. The receptor undergoes dimerisation and auto-phosphorylation and then binds to the SH2 domain of the adaptor protein GRB2 (growth factor receptor bound protein 2), through the SHP2 protein which is encoded by PTPN11 (protein tyrosine phosphatase non receptor type 11). This results in the recruitment of the GRB2-bound SOS (homologue of the Drosophila Son of Sevenless gene) protein to the plasma membrane. In this location, SOS comes into close proximity to the membrane bound Ras proteins, catalyses the conversion of the Ras- attached GDP to GTP and thereby activates the Ras proteins. Activation of Ras results in the sequential phosphorylation and activation of RAF, MEK (MAPK extracellularly regulated kinase and MAPK (mitogen activated protein kinase). The activated MAP kinase further acts on many nuclear and cytosolic substrates including transcription factors and other signalling molecules and thereby influences expression of many important genes involved in cell growth and proliferation. Signalling through this cascade is terminated when the Ras attached GTP is hydrolysed to GDP through GTPase activating proteins (GAPs) such as neurofibromin (NF1) and p120 GAP.[2] (Figure 1)

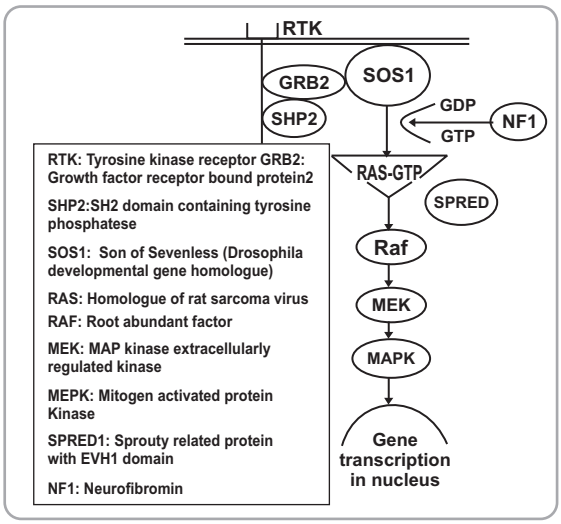

Figure 1: RAS – MAP kinase Pathway

Germline mutations involving any of the genes encoding any of the molecules involved in this pathway can affect normal developmental processes and many of the resulting syndromes are known to have overlapping phenotypes. In fact, the genetic basis of some of these disorders was traced to this pathway following perception of the similarity in their phenotypes to diseases already known to be related to this pathway. The diseases now known to be related to the RAS – MAP kinase pathway, also known as the neuro-cardio-facial cutaneous (NCFC) syndromes include Noonan syndrome, cardio-facio-cutaneous (CFC) syndrome, Costello syndrome, LEOPARD syndrome, neurofibromatosis type 1 and neurofibromatosis type 1 like syndrome.

Disorders related to the RAS – MAPK pathway

A) NOONAN SYNDROME:

Noonan syndrome (NS) is a syndrome characterised by short stature, mild mental retardation, typical mildly coarse facies (down-slanting palpebral fissures, hypertelorism, thick eyelids with ptosis, epicanthal folds, blue or bluish green irises, low set posteriorly rotated ears with fleshy helices), a broad webbed neck and a deformed chest (superior pectus carinatum and inferior pectus excavatum) with low set, widely spaced nipples (Figure 2). Fifty to eighty percent of cases have congenital heart disease of which pulmonary valvular stenosis is the commonest (20-50%), followed by hypertrophic cardiomyopathy (HOCM) (20-30%), atrial and ventricular septal defects, branch pulmonary artery stenosis and tetralogy of Fallot. Ocular abnormalities may be present in as many as 95% of cases and include strabismus, refractive errors and nystagmus. Associated abnormalities include cutaneous abnormalities such as follicular hyperkeratosis and increased cafe au lait spots, genitourinary abnormalities such as cryptorchidism, delayed puberty and mild renal anomalies, coagulopathy, lymphedema and cystic hygroma or hydrops in fetal life. Affected individuals also have a predisposition to malignancies especially myeloproliferative disorders and juvenile myelo-monocytic leukemia (JMML). Noonan syndrome has an autosomal dominant pattern of inheritance with a significant degree of variable expressivity. In 30 – 75% of cases it is found to be inherited from a parent and in the rest the mutations occur de novo. Four causal genes have now been identified: PTPN11 (~ 50%), SOS1 (10-13%), RAF1 (3-17%) and KRAS (<5%); the genetic basis in the rest remains to be identified. Most of the mutations in all the 4 genes are mis-sense, gain of function mutations. PTPN11 mutations were first identified in 2001 by Tartaglia et al; these mutations disrupt an auto-inhibitory interaction between the SH2 domain and the catalytic phosphatase domain resulting in enhanced phosphatase activity and activation of the RAS-MAPK pathway. After this initial identification of the involvement of the RAS – MAP kinase pathway in the causation of Noonan syndrome, other researchers started investigating the possibility of involvement of other molecules related to this pathway in cases of Noonan syndrome without PTPN11 mutations. Germline KRAS mutations were first reported by Schubbert et al in 2006 and have been found to cause disease by dysregulation of the RAS-MAPK signalling via two distinct pathogenic mechanisms: decreased GTP-ase activity and interaction of KRAS with the guanosine nucleotide ring resulting in increased GTP/GDP dissociation, both of which cause accumulation of the active (GTP bound) form of RAS.[3] Other genes involved in Noonan syndrome are the SOS1 gene and RAF1 gene, the mutations in which were first reported by Roberts et al (2007) and Pandit et al & Razzaque et al (2007) respectively[4,5]. The mutations reported in both genes are mainly missense gain – of – function mutations which cause enhanced activation of the RAS- MAPK pathway. A significant degree of genotype – phenotype correlation has been found for Noonan syndrome. Patients with PTPN11 mutations are more likely to have pulmonary stenosis (HOCM less prevalent), a greater degree of short stature, coagulopathy, pectus deformity & cryptorchidism. Specifically, mutations at codons 61, 71, 72 and 76 are associated with an increased risk of JMML. KRAS mutations produce a more atypical phenotype with more severe mental retardation. SOS1 mutations are associated with more ectodermal abnormalities (i.e. skin changes), more cardiac septal defects, less of short stature and no increased risk of malignancy. Noonan syndrome with RAF1 mutations have a significantly high association with HOCM.

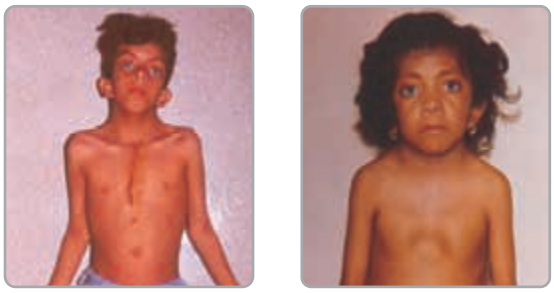

Figure 2: Two cases with Noonan syndrome with the typical facies, neck webbing and pectus deformity

B) CARDIO-FACIO-CUTANEOUS SYNDROME:

Cardio-facio-cutaneous (CFC) syndrome is characterised by typical cranio-facial features which are more coarse than in Noonan syndrome, cardiac defects and ectodermal changes. The typical facies includes a high forehead with bitemporal narrowing, hypoplasia of the supra-orbital ridges, hypertelorism, telecanthus, down-slanting palpebral fissures, epicanthal folds, ptosis, a short nose with a depressed nasal bridge and anteverted nares, low set, posteriorly rotated ears and a deep philtrum with cupid's bow lip. Cardiac anomalies in the CFC syndrome include pulmonic stenosis, atrial and ventricular septal defects, hypertrophic cardiomyopathy, valvular defects and rhythm disturbances. Dermatologic abnormalities typically present include xerosis, ichthyosis, hyperkeratosis of the arms, legs and face, eczema, hemangiomas, cafe-au-lait macules, erythema, lentigines and ulerythema ophryogenes (inflammatory keratotic facial papules) (Figure 3). Hair may be curly, woolly, brittle and sparse. The nails may be broad and dystrophic. Ocular complications include strabismus, nystagmus and refractive errors. Patients also usually have short stature and skeletal deformities such as a short neck, pectus deformity, kyphosis and scoliosis. Mild to severe cognitive delay and mental retardation may be present with or without seizure disorder, hydrocephalus, cortical atrophy and agenesis of the corpus callosum. Usually there is no association with malignancies.

CFC syndrome occurs due to an autosomal dominant de novo mutation; none of the cases reported so far were inherited from a parent. The genes involved are the BRAF gene (75-80%), MEK1 & MEK2 genes (10-15%) and KRAS gene (<5%). Mutations in

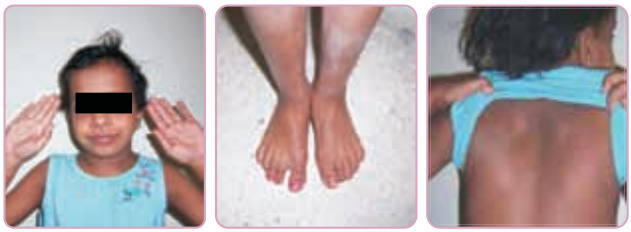

Figure 3: Child with cardio-facio-cutaneous syndrome with the typical facial changes, dry hyperkeratotic skin and sparse hair. Hyperkeratotic papules on back may be appreciable. She was operated for atrial septal defect and pulmonary stenosis.

the BRAF, MEK1 & MEK2 genes were first reported by Rodriguez – Viciana et al in 2006 and KRAS mutations by Niihori et al in 2006; both sets of workers discovered these genetic defects by studying the RAS pathway, which they suspected could be involved in view of the great degree of phenotypic similarity of the CFC syndrome with Noonan and Costello syndromes, both of which were known to be associated with germline mutations in the RAS pathway[6]. As in the case of Noonan syndrome, mutations in BRAF, MEK and KRAS genes in CFC syndrome cases are also all mis-sense gain of function mutations which cause increased activation of the MAP kinase in the RAS signalling pathway.

C) COSTELLO SYNDROME:

Costello syndrome (CS) is characterised by short stature, a typical cranio-facial appearance which includes coarse facies, relative macrocephaly, curly, sparse, fine hair, epicanthic folds, a wide nasal bridge, a short full nose and thick lips and cutaneous anomalies such as loose soft skin with increased pigmentation, facial and perianal papillomata. Cardiac anomalies are associated with CS, the most common being hypertrophic cardiomyopathy; other cardiac defects include valvular pulmonic stenosis and rhythm disturbances. Musculoskeletal anomalies in CS include diffuse hypotonia, joint laxity, ulnar deviation of wrists and fingers, spatulate finger pads, tight Achilles tendon, positional foot deformity, kyphoscoliosis and pectus deformity. Mild to severe developmental delay/mental retardation is usually present with or without seizures and hydrocephalus. Patients with CS have a predisposition to tumours especially solid tumours like rhabdomyosarcoma, neuroblastoma and transitional cell carcinoma of the bladder. Costello syndrome occurs due to an autosomal dominant mutation and almost all cases reported so far have been due to de novo mutations. Because of the phenotypic overlap between Noonan syndrome and CS, Aoki et al first investigated the genes downstream from PTPN11 in the MAP – ERK signalling pathway in CS cases. They sequenced the entire coding region of all the 4 RAS genes and found mutations in the HRAS gene.[7] Subsequently, HRAS mutations have been found in 85–90% of all cases of Costello syndrome. G12S is the most prevalent substitution and G12A is the second most common.[2] These mis-sense gain of function mutations derange the RAS GTP-ase activity and cause constitutive activation of the RAS-MAPK pathway.

D) LEOPARD SYNDROME:

LEOPARD syndrome consists of Lentigines, ECG abnormalities, Ocular hypertelorism, Pulmonic stenosis, Abnormal genitalia, Retardation of growth and sensorineural Deafness. Lentigines are present in thousands typically on the face, neck and upper trunk with sparing of the mucosa; cafe noir patches (large, darkly pigmented skin patches) may also be seen. Additional features are a variable degree of mental retardation, skeletal deformity and hypertrophic cardiomyopathy. Facies are similar to that of Noonan syndrome but milder. LEOPARD syndrome is inherited in an autosomal dominant manner and has variable expressivity. Mutations may occur de novo, may be inherited from an affected parent or there may be germline mosaicism. Mutations have been found in the PTPN11 gene (90%) and RAF1 gene (~ 3%). PTPN11 mutations in cases of LEOPARD syndrome were first reported by Digilio et al & Legius et al in 2002; they studied the NS gene PTPN11 in cases of LEOPARD syndrome because of the phenotypic similarity between the two.[8] However, the PTPN11 mutations associated with LEOPARD syndrome are loss of function mis-sense mutations which diminish the catalytic activity of SHP2, unlike in NS. How loss of function mutations result in a similar phenotype as gain of function mutations (NS) remains to be elucidated.[2] RAF1 mutations first reported by Pandit et al are gain of function mis-sense mutations.[5]

E) NEUROFIBROMATOSIS TYPE I:

Neurofibromatosis type 1 (NF1) is a neuro-cutaneous syndrome with some typical features that include pigmentation abnormalities of the skin such as cafe au lait spots and axillary and groin freckling, Lisch nodules in the iris and a predisposition for the development of nerve related tumours – especially cutaneous or subcutaneous benign neurofibromas, optic nerve gliomas, spinal cord tumours, neurosarcoma and malignant peripheral nerve sheath tumours. Additional features include an increased risk of solid tumours such as rhabdomyosarcoma, neuroblastoma and gastrointestinal stromal tumours, scoliosis and other bony anomalies like long bone bowing, pseudoarthrosis and sphenoid bone dysplasia, learning disability, mild to moderate mental retardation, vascular lesions like vascular ectasias and CNS aneurysms, short stature and macrocephaly. NF1 is an autosomal dominant condition. Mutations may be inherited from a parent, may occur through germline mosaicism or may arise de novo. The gene involved is NF1 which encodes neurofibromin, a GTPase activating protein, which closely interacts with the RAS – MAPK pathway by hydrolysing the Ras- attached GTP to GDP and terminating the signalling cascade. (Fig 1). While majority of patients have intragenic mutations within NF1, around 5% of cases have microdeletions involving the region of the NF1 gene.

F) NEUROFIBROMATOSIS TYPE 1 LIKE SYNDROME:

Neurofibromatosis type 1 like syndrome (NF1LS) consists of multiple cafe-au-lait spots, axillary freckling, macrocephaly, a Noonan-like facial appearance and learning difficulties. Although the phenotype closely resembles NF1, some typical NF1 features such as Lisch nodules in the iris, neurofibromas and CNS tumours are absent. These cases do not have NF1 or the NS mutations. Heterozygous loss-of function mutations in the SPRED1 gene (Sprouty Related Protein with EVH1 domain) have been identified in these cases. Spred-1 is a negative regulator of RAF and truncating mutations result in increased RAF1-kinase activity and increased phosphorylated levels of MEK and MAPK.

Thus, all the neuro-cardio-facial-cutaneous (NCFC) syndromes share considerable phenotypic similarity which includes a variable degree of mental retardation or learning disabilities, cardiac defects (particularly pulmonary valve stenosis and hypertrophic cardiomyopathy), facial dysmorphism, short stature, relative macrocephaly, skin abnormalities and an increased risk for malignancy (Table 1).

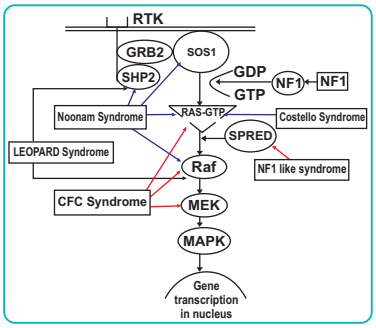

Figure 4: Disorders related to germ-line mutations in the RAS-MAP kinase pathway

This similarity is easily explained by their being related to molecular defects involving hyper-activation of a common pathway (Figure 4). However, the reasons for the significant phenotypic variation, inspite of the overlap, between these conditions are not fully understood. The possible mechanisms

Note: NS:Noonan syndrome, CFCS: Cardiofaciocutaneous syndrome, CS: Costello syndrome, LS: LEOPARD syndrome, NF1: Neurofibromatosis 1, NF1LS: NF1 like syndrome, PS: Pulmonary stenosis, ASD: Atrial septal defect, HOCM: Hypertrophic obstructive cardiomyopathy, PS: Pulmonic stenosis, MR: Mental retardation

FEATURE	NS	CFCS	CS	LS	NF1	NF1LS
Facies	Mildly coarse facies, hypertelorism ptosis, epicanthal folds, low set posteriorly rotated ears, clear blue irises	More coarse than in NS; 'Noonan-like' facies with bitemporal constriction	More coarse than in CFCS; wide forehead, depressed nasal bridge, full cheeks, low set posteriorly rotated ears with thick lobes	Mildly coarse: 'Noonan like'	Mildly coarse & 'Noonan like' in some cases	Mildly coarse & 'Noonan like' in some cases
Mental retardation	Normal IQ to mild MR	Moderate To severe MR	Mild To moderate MR	Normal IQ to mild MR	Learning difficulty, mild MR in some	Learning difficulty, mild MR in some
Cardiac defects	PS, HOCM, ASD, VSD	PS, ASD, VSD, HOCM, ECG changes	HOCM, PS, rhythm disturbances	PS, HOCM, ECG changes	PS in some	PS reported in 1 case
Cutaneous changes	Increased cafe – au – lait macules	Hyper-keratotic, dry skin; ulerythaema ophryogenes sparse, curly, friable hair	Deep palmar and plantar creases, loose & soft skin, facial & perianal papillomata	Multiple lentigines (in thousands) & café noir patches	Cafe-au-lait macules, axillary freckling, juvenile xantho-granuloma	Cafe-au-lait macules, axillary freckling
Short stature	Present	Present	Present	Present	Present	Present
Macrocephaly	Usually absent	Present	Present	Usually absent	Present in many	Present in many
Predisposition to mailgnancy	Present	Absent	Present	?	Present	?

Table 1: Clinical features of disorders related to the RAS – MAPK pathway

for the differences in phenotype could be the differential expression patterns of mutated genes, different mechanisms of disturbing RAS signalling through specific interactions between effector and regulatory proteins for different mutants, involvement of feedback mechanisms that affect only upstream molecules (like RAS) but not downstream molecules and the possible effects of the effector molecules on pathways other than the MAPK pathway.[2]

While germline mutations have been reported in the RAS genes in many of the NCFC syndromes, gain of function somatic mutations in RAS genes, especially KRAS, are found in 20 – 30% of all tumours. However, the somatic KRAS mutations reported in malignancies are different from the germline KRAS mutations found in Noonan and CFC syndromes. The explanation for this could be that the mutations associated with malignancies are so strongly activating that they are incompatible with normal embryogenesis and cause early embryonic lethality.

Knowledge of the pathway defects involved in the causation of these disorders provides potential molecular targets for therapy. Tyrosine kinase inhibitors are already being used successfully in cancer therapy e.g. the RAF-inhibitor sorafenib is beneficial in renal cell and hepatocellular carcinoma. In vitro studies have shown a response to MEK inhibition for different mutated proteins found in the CFC syndrome.[9] Mouse studies have shown that learning problems caused by NF1 knockout can be reversed by farnesyl-transferase inhibitors and statins that inhibit RAS hyperactivation by reducing the level of isoprenylated RAS. Clinical studies assessing the effect of statins on cognition in NF1 patients are underway. If these studies show any effect, this type of pharmacological treatment might also be studied in the larger group of RAS-MAPK syndromes.

OTHER GROUPS OF PATHWAYS DISORDERS

Ciliopathies, which are disorders of primary cilia, constitute another important example of pathway disorders with a significant degree of overlap in their phenotypes. Cilia are vital cellular components that play a significant role in diverse signal transduction pathways and thereby in the development of many tissue types including the kidney, brain, liver, eyes and bone. Many of the ciliopathies have hepato-renal cystic changes and retinal degeneration (Table 2)[10].

These prototypal pathway disorders demonstrate that 'lumping' of overlapping phenotypes can provide an important clue to the molecular etiology of diseases. This approach of analysing overlapping clinical phenotypes for a common etiological pathway can be used to study other syndromes that share similar clinical features.

DISEASE	GENE	INHERITANCE	PHENOTYPE
Alstrom Syndrome	ALMS1	AR	Early onset obesity, congenital retinal dystrophy, deafness, dilated cardiomyopathy, hyperinsulinaemia, hypertriglyceridemia
Bardet Biedl Syndrome	12 BBS loci	AR + oligogenic	Obesity, cone-rod dystrophy, polydactyly, hypogonadism, renal malformations and cognitive deficits
Joubert Syndrome	NPHP1, AHI1, CEP290 & TMEM67	AR	Cerebellar vermis hypoplasia, brainstem malformation, episodic hyperpnoea/ apnoea, ataxia, global developmental delay, polydactyly, retinitis pigmentosa, renal cysts
Meckel – Gruber Syndrome	MKS1, MKS3, FLJ20345	AR	CNS malformations especially occipital encephalocele, renal cysts, hepatic cysts, polydactyly
Oro-facial-digital syndrome type 1	OFD1	X linked	Malformations of face, oral cavity and digits, polycystic kidney disease
Nephronophthisis	8 NPHP loci	AR	Medullary cystic kidney disease
Senior Loken syndrome	NPHP1, NPHP4 & NPHP5	AR	Nephronophthisis and retinal degeneration
AD Polycystic kidney disease	PKD1 & PKD2	AR	Adult onset polycystic kidney disease
AD Polycystic kidney disease	PKHD	AR	Infantile onset polycystic kidney disease, polycystic liver disease
Leber congenital amaurosis	LCA5, CEP290, NPHP6	AR	Retinal dystrophy
Jeune Asphyxiating Thoracic Dystrophy	IFT80	AR	Narrow thorax, polydactyly, hepatorenal cystic changes

Table 2: Ciliopathies: note the common features namely polydactyly, renal cysts, retinal involvement and central nervous system malformations in varying combinations and autosomal recessive inheritance

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Contributed by:
Meenal Agarwal and Parag Mohan Tamhankar

COUNT THE GENES BEFORE THEY ARE HATCHED[1]

Prenatal diagnosis of cytogenetic abnormalities is primarily done for detection of aneuploidy in the fetuses after being identified as high risk in various screening procedures, but many unbalanced submicroscopic chromosomal aberrations below the resolution of a standard karyotype analysis(<4Mb) often remain undetected. Array-based comparative genomic hybridization (aCGH) allows for fast and accurate detection such chromosomal abnormalities. Van den Veyver et al described prenatal diagnosis on DNA extracted from CVS or AF using a targeted clinical CGH array on 300 pregnant women, who were undergoing amniocentesis or CVS for other reasons. Arrays were designed to provide redundancy with high sensitivity and specificity for detection of clinically significant unbalanced chromosomal abnormalities, while minimizing detection of copy number variations (CNVs) of uncertain clinical significance. Array CGH yielded new clinically relevant results in 7 (2.3%) analyses, which provided additional information for prenatal genetic counseling. Significant CNVs included four marker chromosomes, 9q34 deletion in an apparently balanced translocation, a case of TAR syndrome and a deletion of at least 800 kb in 15q26.3. Detection of CNVs of uncertain clinical significance remained at an acceptable low rate (1%). Proper information regarding benefits of aCGH and also of the potential detection of a CNV of uncertain significance should precede all aCGH testing.

TRIMMING THE STEM BEFORE IT TAKES ROOT[2]

Genetic diseases with intrauterine onset like skeletal dysplasias, inborn errors of metabolism and some muscular dystrophies can lead to permanent neural or musculoskeletal damage in early life. Intrauterine stem cell transplantation is a promising option. However, its utility is limited by the development of the fetal immune system after 14 weeks gestation. An ex vivo gene therapy approach targeting autologous first trimester stem cells to replace the missing or defective gene product should overcome this barrier. Chan and et al performed a phase 1 study to investigate the feasibility of harvesting circulating first trimester human fetal mesenchymal stem cells (hfMSCs) for ex vivo gene therapy. Thin-gauge embryo fetoscopy directed or ultrasound-guided fetal blood sampling was done. Harvested blood was plated for isolation of hfMSC and transduced by lentiviruses. These genetically manipulated cells can be infused back into the fetus where they should engraft and home to injured tissues.

AGCT IN COLOUR[3]

Recently developed of high-density, single-nucleotide polymorphism (SNP) arrays provide information on thousands of SNPs and structural variants. So software programs specially with visual data can provide useful information and assist in research. Sergio Barlati, Sergio Chiesa et al developed GenotypeColour™ as a new way of displaying and comparing SNP and CNV genomic data . Some impotant features are visibility of entire genome variability in a single screenshot, simultaneous display of the genotype and Copy Number state for thousands of SNPs, comparison of large amounts of samples by producing "consensus" images displaying regions of complete or partial identity and to show regions of potential uniparental disomy (UPD). All information can then be exported in a tabular format for analysis. The software is available free at http://www.med.unibs.it/~barlati/GenotypeColour and is especially useful for the analysis of multiple samples.

PRENATAL DIAGNOSIS OF SKELETAL DYSPLASIA: ACCURACY IS THE BONE OF CONTENTION[4]

The skeletal dysplasia comprises a group of disorders with generalized abnormalities of the skeleton, with many of them having onset in prenatal life. Diagnosis is mainly done post delivery by radiographs and autopsy. The molecular defect has been identified in almost half of the well-recognized skeletal dysplasias. However, the application of these findings to direct patient care is not yet possible for many of these disorders. Obtaining a precise diagnosis by prenatal ultrasound diagnosis is challenging because they are rare and many of the ultrasound findings are not necessarily pathognomonic for a specific disorder. Moreover misdiagnosis can lead to inaccurate recurrence risk information and suboptimal management of the patients. Krakow et al present ACMG Practice Guidelines for prenatal ultrasonographic diagnosis of skeletal dysplasia and provide an approach to a fetus suspected of manifesting a skeletal dysplasia along with common abnormal ultrasound findings and differential diagnosis, available resources and recommendations.

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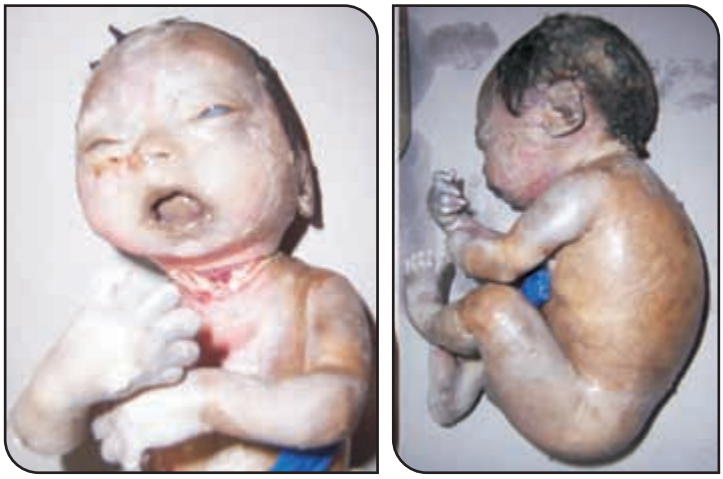
5

Contributed by: Shubha R Phadke, shubha@sgpgi.ac.in

This baby died immediately after birth due to respiratory distress. Baby was noted to have multiple joint contractures. Identify the condition



The response should be sent to geneticsiap@gmail.com

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

Answer to the PhotoQuiz 4 *of the previous issue:*

Ellis-van Creveld Syndrome (Chondroectodermal dysplasia) (OMIM No 225500)

Ellis-van Creveld syndrome is an autosomal recessive skeletal dysplasia with short limbs, short ribs, post-axial polydactyly and dysplastic nails and teeth. Congenital heart defects are also commonly seen. The images show disproportionate short stature with mesomelic shortening of limbs, deep-set hypoplastic nails, oral frenula and carpal synostosis. Defects in EVC1 and EVC2 genes are known to cause this condition.

Correct responses:

Chinmayee Ratha, *Hyderabad*
Mohandas Nair, *Calicut*
Saminathan D, *Trichy*
Kausik Mandal, *Lucknow*
Krithika MV, *Davangere*
Ravi Goyal, *Kota*
Sharafine Stephen, *via e-mail*
Aditi Dagli, *Gainesville*
Radhakrishna K, *Visakhapatnam*
Pramila G Menon, *Pune*

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