genetic CLINICS

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Amniotic cavity full..... LOVE!

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Two of my old patients turned up in the OPD today. I was seeing them in person for the first time! I had seen these patients 'in utero' while doing ultrasonographic examination. One was diagnosed as holoprosencephaly at 28 weeks of gestation and case two was diagnosed as tricuspid atresia at 18 weeks of gestation.

Case 1: A twenty one day baby was quietly sleeping in her grandmother's lap under the watchful eyes of the mother. After the baby was diagnosed to have holoprosencephaly at 28 weeks of gestation the father came home from Hong Kong. The mother wanted him with her to face the reality of the birth of a baby with such a major birth defect. The baby was born at 34 weeks of gestation. As advised, the couple brought the baby for karyotyping within the first few weeks of life. The holoprosencephaly was clearly seen in the CT scan done after birth. They re-discussed the issues about prognosis of holoprosencephaly. The situation was not very pleasant but they had not let their joy of child birth get dampened by it. The baby was named, new clothes were bought. The baby's small head was covered with a colorful cap. There was no undercurrent of blaming each other which we sense many a times when a child with a birth defect is born. I informed them that the karyotype was normal. The father told that soon the baby and the baby's mother would be joining him in Hong Kong. The parents and grandparents were not overtly disturbed or anxious. I am not saying that they were not sad but had accepted the situation and were ready to live with it. Not much was said. I reminded them about the risk of recurrence and early prenatal diagnosis. The father told that he may think about gene analysis at a later date. The grandmother had some queries about feeding. Some of her other questions she left unsaid. I understood that she wanted some positive news but she almost knew that the answers were not going to be very much hopeful. At last the grandmother asked me to measure the head circumference and whether it had grown over the last 3 weeks.

Was it complete acceptance that had brought peace in this family or was it a thin thread of hope or optimism? I do not know....

Case 2: She is an eleven month old chubby baby on the shoulders of the mother. She had undergone a surgery. Some more is planned for the future. Her mother is a nursing staff in our hospital. One afternoon after finishing her morning duty she had come for a routine malformation scan at 18 weeks of gestation. The USG showed tricuspid atresia. I myself was disturbed. She was alone. She was more than just a patient for me as she is working in our hospital. Rather than prolonging her anxiety for a day, I gave her the news. I called up the cardiologist and got her fetal Echocardiography done to confirm the diagnosis. The seriousness of malformation and the need of major cardiac surgeries after birth were discussed. As the pregnancy was less than 20 weeks she had an option of medical termination of pregnancy as well. She did not express her feelings at all.

Next day she told me that she had discussed the problem with her family in Kerala and nobody was in favor of termination. We did not get much follow up later. However, today she was here with the 11 month old baby. The baby was delivered in a tertiary care hospital in south India, received the care of specialists, underwent surgery and was doing well. Today I could see a proud and happy mother. My subtle attempts to understand the feelings of the mother were not successful. I did not get any idea about how she had felt during the rest of the pregnancy. Had she been apprehensive about child birth, the expected medical problems and surgeries? What was her source of strength to face all the ordeal and uncertainty which she could have easily avoided by termination? The only thing which I understood was that she was sure about her decision to continue the pregnancy and was ready to face any eventuality. And today she was happy....

These cases are not rare but a little uncommon in India where in people's minds the threshold for termination of pregnancy is a little low. Medical termination of pregnancy is not possible legally beyond 20 weeks of gestation. This made the situation difficult in case 1. In case 2, the decision 'not to terminate' was probably based on religious beliefs but the decision was firm and there was no dilemma. This gave the mother strength to face the problems. Similarly, in the first case, the family knew that there was no other option but to continue the pregnancy. This must have helped in acceptance of the situation. If any birth defect is diagnosed after birth or a neonate develops an illness, the mother and the family puts in all efforts to help the baby and provide the best possible treatment. The love tries to form a shield/a comfortable cocoon for the baby. Mother's love is unique. It accepts the child as it is. Can it not also accept the child in the womb as it is?

HearToHeARTalk: A new feature in GENETIC CLINICS

Human life encompasses a complex amalgamation of science, emotions, faith, beliefs, finances, ethics, law, society, religion, etc. A clinician faces all these aspects while dealing with the patient. It is not surprising that non medical issues come to the fore in case the disease is genetic in etiology. While providing genetic counseling many of these issues are surrounding the family in various layers. The counselor has to have sensitivity to perceive these and deal with them with care and respect the families' views and values as such. The genetic counselor often has to do the difficult task of giving bad news. Equally challenging is to help the family in organizing care of a seriously ill patient or handicapped person; which is not uncommon with genetic disorders. A good understanding of psychology and patience makes genetic counseling more fruitful. During the genetic counseling process all of us have touching experiences of families going through the ordeal or sad times. Each individual is different; each situation is different. Every body's reactions, ways of coping, are different. So are their own strengths and weaknesses! We wish to start this new feature to present emotional, social, psychological and ethical issues of the genetic counseling process. Sharing such experiences may help us to grow as human beings and genetic counselors.

Your experiences are welcome!

Editorial

Genetic Testing and Clinicians.... and Patients

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DNA is the basic molecule of life! No wonder it encompasses all fields of biology and medicine. Now there is a lot of information available about human DNA, mutations and their effect on health. With the recombinant DNA technology, medicine has reached a new 'molecular' level and entered an era of 'Molecular Medicine'. This has made a tremendous impact on the diagnostics and is resulting in new advancements in treatment strategies as well. Gene therapy and other treatments based on the information about molecular defects have shown great promise but most of them are still in research settings. But the diagnostics based on analysis of genes and chromosomes have already become a part of routine evaluation of genetic disorders.

These 'Genetic Tests' include mutation detection based on DNA analysis, chromosomal analysis and a recently developed group of investigations named 'molecular cytogenetic techniques'. Molecular cytogenetics includes Fluorescence in situ hybridization (FISH), quantitative fluorescence PCR (QF PCR) and microarray based cytogenetic analysis. This has increased the speed and resolution of detection of chromosomal anomalies. With FISH and QF PCR prenatal analysis for aneuploidies is possible within a day or two. FISH is also used for targeted analysis of small regions on chromosomes in cases with microdeletion syndromes; the diagnosis of which is not possible with traditional cytogenetics. Microarray chips for chromosomal analysis can detect deletions and duplications of minute regions of any part of the genome. These techniques are used for diagnosis, prenatal diagnosis, carrier detection and presymptomatic diagnosis of genetic disorders. The DNA based tests and cytogenetic tests also play a very important role in diagnosis and management of cancers. All of these tests are available in India in genetics centers and various diagnostic laboratories.

For appropriate use of the investigations there are many requisites other than good quality of the tests. The first requisite is suspecting or diagnosing a genetic disorder in an appropriate situation. The other requisite is a good understanding of the principle of the test and indications. Knowledge of the principle of the test helps clinicians to interpret the results and to know the limitations of the test. All clinicians may need to use the genetic tests depending on their area of work. Genetic disorders involving all systems of the body are known; these can have presentations similar to non - genetic disorders and will come to the clinics of every clinician. With the increasing awareness about genetic disorders and availability of diagnostic tests many genetic disorders are increasingly diagnosed in India and the number of published case reports of rare disorders is also increasing. For best utilization of available tests it is imperative that the present clinicians learn about molecular genetics, genetic testing and principles of genetic counseling. The need for clinicians of all specialties to learn the basics of clinical genetics is more in India as the number of clinical geneticists is still small and will continue to remain so for many more years to come.

The ways to learn and teach genetics to clinicians of today and medical students can be many and varied. The department of Medical Genetics at Sanjay Gandhi Postgraduate Institute of Medical Sciences (SGPGIMS), Lucknow has been conducting a two week-long course on Medical Genetics and Genetic Counseling on a regular basis, for the last decade. The Indian Council of Medical Research has been supporting this teaching course. More than 200 clinicians and budding geneticists have attended the course so far. Last month, the Ninth ICMR Course on Medical Genetics and Genetic Counseling was held in SGPGIMS. The course teaches basic genetics and introduces the participants to the concepts of molecular genetics and principles of genetic counseling. Clinical approach to common presentations of genetic disorders are discussed along with a large number of fully worked up cases of genetic disorders, each introducing some principle of genetics and counseling. Various laboratory techniques are demonstrated to the participating clinicians to teach them principles of the tests and interpretation of results. Stress is given to develop communication skills needed for genetic counseling. This course is an attempt to introduce the clinicians to the

fascinating world of medical genetics and give them a geneticist's perspective to look at the patients seen commonly by them. The course attempts to train the clinicians in diagnosis and counseling of common genetic problems, provides knowledge of the basics of genetics and leaves them with books and other resources to continue learning of genetics. Our experience shows that the awareness about genetics is increasing amongst clinicians and the ICMR Courses on Medical Genetics and Genetic Counseling have succeeded in creating enthusiasm about this medical specialty in them.

The course participants who are medical college teachers are encouraged to improve the genetics teaching of undergraduate and postgraduate medical students in their respective colleges. The Medical Council of India needs to update the genetics curriculum and also, make it mandatory for the teachers to undergo short term training in medical genetics, so that the teachers can teach the recent developments in genetics to the clinicians of the twenty-first century.

In the context of genetic testing, it is necessary to mention about 'Direct to consumer testing' or DTC. In this era of internet advertising and increasing awareness amongst lay persons, private companies are offering molecular tests for carcinoma breast, Alzheimer disease, and carrier status for many disorders like cystic fibrosis, spinal muscular atrophy, etc. Whole genome sequencing to identify susceptibility variants to common disorders like ischemic heart disease and psychiatric illnesses is also being marketed. If a consumer chooses to purchase a genetic test directly, the test kit is mailed to the consumer instead of being ordered through a doctor's office. A DNA sample is collected at home, often by swabbing the inside of the cheek, and mailed back to the laboratory. The result is sent by mail or over the telephone, or the results are posted online. Many of these tests may not be of proven utility or may not be validated. Though the direct to consumer test may be providing autonomy and a proactive role to the individual, it may be misleading sometimes. The results may be available to insurance companies or employers causing problems to the individual. Without guidance from a healthcare provider, individuals may make important decisions about treatment or prevention based on inaccurate, incomplete, or misunderstood information about their health. Undue anxiety may be created based on the uncertainty created by the results. No scientific or medical association is in favor of DTC. The statements of the American College of Medical Geneticists and American Society of Human Genetics about DTC are available on the web. The guidelines are that no genetic test should be done without the involvement of a trained medical geneticist or genetic counselor. The person should be provided with the information regarding the utility, limitations of the test and implications of the result for the individual.

It may not take much time for the fad of DTC to spread in India. Already many patients come to the clinic with the printouts of web based information about various diseases. Soon they may come with the advertisements of DNA tests or even with the reports of their DNA or genome sequence. So the doctors have to be prepared for the era of molecular genetics. Improving education about genetics for the clinicians is the need of the hour. The Indian Council of Medical Research has initiated a step in this direction about 10 years ago. We all need to contribute to it by teaching or learning, whatever role each of us can play.

Doctors need to know medical genetics.....& more than the patients!

Shubha Phadke
1st October, 2010

Participants of the Ninth ICMR Course on Medical Genetics and Genetic Counseling: Toast to the Era of Molecular Medicine

Cure for Duchenne Muscular Dystrophy: The Search continues

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Introduction

Duchenne muscular dystrophy (DMD) is a progressive, lethal, X-linked recessive disorder of skeletal and cardiac muscle affecting nearly 1 in 3500 live male births. DMD is caused by mutation of the dystrophin gene that provides a vulnerable target for new mutations. The cardiac and skeletal muscles of patients with DMD are deficient in dystrophin, a 427-kD protein located on the inside of the cell membrane. Without functional dystrophin, the muscle fiber is particularly vulnerable to damage from normal daily activities. As a result, damaged DMD muscle fibers eventually succumb to injury.

Affected individuals can have mildly delayed motor milestones and most are unable to run and jump properly due to proximal muscle weakness, which also results in the classic Gowers' manoeuvre (use of hands to climb up his own body while getting up from the sitting/squatting position). Most patients are diagnosed at approximately 5 years of age, when their physical ability diverges markedly from that of their peers and serum creatine kinase is markedly elevated. The muscle strength deteriorates, and boys require the use of a wheelchair before their teens. Respiratory, orthopaedic, and cardiac complications emerge, and without intervention, the mean age at death is around 19 years. Non-progressive cognitive dysfunction might also be present[1]. Boys with the allelic disorder Becker muscular dystrophy (BMD) have onset of symptoms later than DMD and slower progression. This is due to reduced amount of dystrophin that is also often shorter than normal but still can fulfill its role, though not as effectively as the normal version.

The search for a cure for DMD has been intense since the identification of the dystrophin gene in 1987 and the last decade has seen emergence of novel strategies. This review aims to summarize the recent findings and the progress of ongoing clinical trials in DMD. An overview of major therapeutic strategies and animal models in DMD are presented in Table 1 & 2 respectively[2].

TABLE 1: Overview of major therapeutic research strategies for Duchenne muscular dystrophy

Pharmacological agents	Corticosteroids[a] (prednisone, deflazacort)
	Myostatin inhibitors (myostatin antibodies, follistatin derivatives)
	Protease inhibitors (leupeptin)
	Others (Angiotensin-converting enzyme inhibitors[a], Idebenone, calcium channel blockers)
Cell therapy	Myoblasts, satellite cells, meso-angioblasts[b], mesenchymal stem cells[b]
Mutation specific therapy	Exon skipping agents[b] (Antisense oligonucleotides, Morpholinos) , Stop codon suppressing agents (PTC 124, Gentamicin)
Gene therapy	Micro or mini dystrophin[b] gene transfer, Utrophin upregulation

a =already in clinical use, b = most promising

TABLE 2: Commonly Used Animal Models In DMD

Model	Mutation in dystrophin gene	Effect	Pathology
mdx mouse	Nonsense mutation in exon 23	Stop codon induced, dystrophin synthesis aborted prematurely	Fiber degeneration (from 2-8 weeks), replacement by centrally nucleated regenerating fibers, less severe myopathy , life span nearly normal (23 months)
GRMD(Golden Retriever dogs)	3'splice-site point mutation in intron 6	Exon 7 skipped from transcript, frameshift, dystrophin synthesis aborted prematurely	Severely affected, massive muscle degeneration, closely resembles human DMD phenotype, life span 1-3 yrs
HFMD cats (hypertrophic feline muscular dystrophy)	Deletion of Dp427m and Dp427p promoter regions	No muscle dystrophin expression	Large areas of muscle fiber degeneration and regeneration, mononuclear infiltration, extensive muscle hypertrophy

PHARMACOLOGICAL THERAPY

Corticosteroids

Glucocorticoids are the only medication currently available that slows the decline in muscle strength and function in DMD and delays the 'time to wheel chair' by few years. Taking into account the well-described risks associated with chronic glucocorticoid administration, a balance needs to be established between positive and adverse effects (e.g., weight gain, cushingoid appearance, short stature, bone demineralization, acne, excessive hair growth, gastrointestinal symptoms, behavioral changes etc.).

The recommended starting dose for prednisone in ambulatory boys is 0•75 mg/kg daily, given in the morning. Deflazacort, if available, may be used at 0.9mg/kg/day as a first line therapy in the presence of pre-existing weight and behavioral issues. Daily administration is more effective than alternative regimes (i.e., alternate day, high-dose weekend, or a 10-day "on" cycling with 10 or 20 "off days"). The minimum effective dose that shows some benefit (albeit not to the maximum extent possible) is believed to be 0•3 mg/kg daily for prednisone. No generally accepted guidelines exist in the literature about the best time to initiate glucocorticoid therapy in an ambulatory boy with DMD, though it should be considered in the plateau or decline phase, usually at 4-8 yrs. A lower dose of 0•3–0•6 mg/kg might be an option after loss of ambulation. Long-term use of glucocorticoids requires much commitment on the part of the family. Essential issues for discussions should include potential side-effects, the obligation to closely monitor and manage any adverse issues that might arise, and the requirement to have the child followed closely by their primary-care physician and specialty health-care team. These decisions need to be made individually in partnership with the child and family, because tolerability of side-effects compared to perceived benefit is an individual judgment[1, 3].

Idebenone

Due to the loss of dystrophin, skeletal and heart muscle of DMD patient is under continuous oxidative stress, which leads to formation of scar tissue. Idebenone, an antioxidant like Coenzyme Q10, has been found to improve cardiac function in mdx mice. A 12 month phase II DELPHI (Duchenne Efficacy study in Long term Protocol of High dose Idebenone) trial has demonstrated efficacy on functional cardiac and respiratory parameters in 13 patients receiving 450mg/day Idebenone (SNT-MC 17) compared to 8 patients who received placebo. A multicentric phase III study named DELOS (DuchEnne muscular dystrophy Long term IdebenOne Study) in 240 DMD patients aged 10-18 yrs is ongoing.

Myostatin inhibition

Myostatin (GDF8), a member of transforming growth factor-β (TGF-β) superfamily, is a negative regulator of muscle growth. Mutations in myostatin gene lead to increased musculature, as seen in Belgium blue cattle. Attenuation of myostatin activity using blocking antibodies, peptides or pseudo-ligands has been demonstrated to increase muscle mass in mice in vivo. The recombinant human Antibody MYO-029 has been designed to bind to and inhibit the activity of myostatin, and was considered safe in healthy volunteers. However, it did not result in an increase in muscle mass in adult Becker dystrophy patients when used for a short period of 28 days. A new myostatin antibody that outperforms the old one is currently being tested in healthy volunteers. Follistatin, which is a powerful antagonist of myostatin, is also under evaluation[4].

Leupeptides: Leupeptides inhibit the protease calpain thus slowing down the muscle breakdown. A drug MYODUR which uses carnitine as the carrier molecule to target muscle cells and inhibits calpain by attaching a leupeptide analogue, has been effective in animal models.

Other drugs: Angiotensin-converting –enzyme (ACE) inhibitors should be considered in the management of Duchenne cardiomyopathy. Various calcium antagonists, including verapamil, diltiazem, nifedipine and flunarizine have been evaluated in small studies but did not show a significant beneficial effect on muscle function. The use of oxandrolone, an anabolic steroid, is not recommended. An expert opinion consensus panel has made no recommendations for the use of supplements such as coenzyme Q10, carnitine, amino acids (glutamine, arginine), anti-inflammatories/anti-oxidants (fish oil, vitamin E, green tea extracts) or for disease modifying drugs like pentoxiphylline.

Other drugs under research include albuterol, formoterol, L-arginine, losartan, cyclosporine etc.[1,5,6,7]

CELL THERAPY

Self-renewal and multipotency remain generally accepted as the defining features of stem cells. Stem cells give rise to progenitor cells, which can multiply but are unable to indefinitely self-renew or to completely reconstitute a tissue.

Satellite cells

Satellite cells reside beneath the basal lamina of muscle, closely juxtaposed to skeletal muscle fibers. They are normally mitotically quiescent, but are activated (i.e., enter the cell cycle) in response to stress induced by weight bearing or by trauma. The descendants of activated satellite cells, called myogenic precursor cells, or myoblasts, undergo multiple rounds of division before fusion and terminal differentiation. Activated satellite cells also generate progeny that restore the pool of quiescent satellite cells.

Myoblast transplantation as a therapy for DMD

The initial promising results of myoblast transplantation in mdx mice triggered a rapid series of clinical trials in DMD patients. However, these clinical trials produced either negative or very limited positive results. Table 3 provides a summary of issues related to myoblast transplant, which is also called “transplantation of myogenic cells”.

TABLE 3: Issues Related to Myoblast Transplantation

Problem identified	Proposed solution
Early death of transplanted myoblasts	Transplantation of high number of cells
Low migration distance from site of intramuscular injection	High number (100 injections per square centimeter) of adjacent intramuscular injections, modulation of MyoD expression, use of matrix metalloproteinase
Rejection if immunosuppression is not adequate, cyclosporine induces apoptosis of myoblasts	Use of tacrolimus, cyclophosphamide and treosulfan. Induction of specific immunological tolerance towards donor myoblasts, Transplantation of genetically modified autologous myoblast

Mesenchymal Stem Cells

These multipotent cells capable of forming bone, cartilage, fat, connective tissue, and muscle were first identified as a stromal population in the bone marrow, distinct from hematopoietic stem cells and new evidence indicates that mesenchymal stem cells (MSCs) are derived from perivascular stem cells associated with blood vessels. Multipotent stem cells with myogenic potential includes side population (SP) cells, muscle derived stem cells (MDSC), myoendothelial cells, pericytes, meso-angioblasts and CD 133+ progenitor cells.

After initial success in mdx mice, four Golden Retriever muscular dystrophy dogs were treated with meso-angioblasts from their own muscle tissue (autologous transplant) into which the gene for human micro-dystrophin was transferred. Also, six dogs were treated with cells from healthy dogs (heterologous treatment) which contained normal dystrophin, but which required immune suppression with cyclosporine. The results were much better after the heterologous treatment than after the autologous treatment. Torrente Y et al tested the safety of autologous transplantation of muscle-derived CD133+ cells in eight boys with Duchenne muscular dystrophy in a 7-month, double-blind phase I clinical trial. No local or systemic side effects were observed in all treated DMD patients and resulted in an increased ratio of capillary per muscle fiber with a switch from slow to fast myosin-positive myofibers[8,9,10]

MUTATION SPECIFIC THERAPY

In most cases of DMD, mutations are deletions of one or more exons (~60 %); however duplications (~6 %) and point mutations have also been found.

Exon skipping therapy

DMD gene on the short arm of X chromosome (Xp21) is one of the largest genes known, and is composed of 2,220,223 base pairs, which are grouped in 79 exons. These 79 exons code for 11,058 nucleotide bases in mRNA (0.5% of the gene) resulting in a 3,685 amino acids of Dystrophin protein. Remaining 99.5%of gene sequences (introns) are spliced by spliceosomes during processing of premature mRNA to mRNA.

Most of the mutations in Duchenne muscular dystrophy disrupt the reading frame sequence leading to an out of frame mutation whereas mutations in the less severe variant, Becker muscular dystrophy (BMD) usually maintain the reading frame (Reading Frame Rule) resulting in a shorter but functional protein. Exon skipping aims to restore the disrupted reading frame by converting an out of frame (Duchenne type) mutation to an in frame (Becker type) mutation. This production of partly functional dystrophin is achieved by antisense oligonucleotides (AONs) which hybridize to specific motifs involved in splicing and exon recognition in the pre-mRNA (Fig 1). This prevents normal spliceosome assembly and results in the failure of the splicing machinery to recognize the target exon, leading to exon skipping and reading frame restoration.

FIG 1: Principle of exon skipping strategy

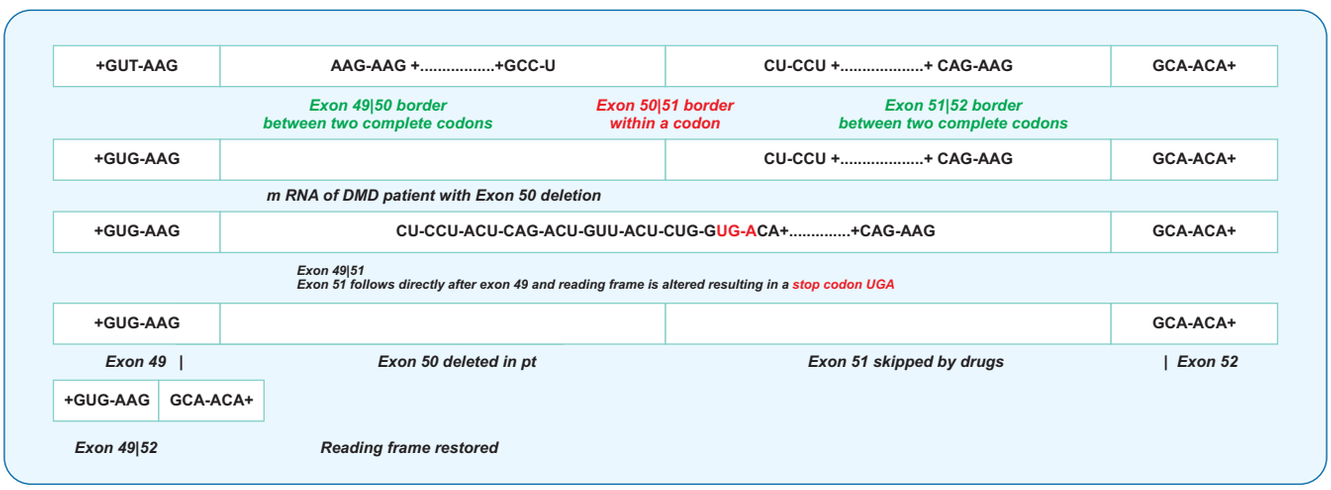

FIG 1 shows a part of dystrophin mRNA sequence corresponding to exons 49-52(each box represents an exon) emphasizing the fact that the border between exons 50 and 51 lies within a codon. In a patient of DMD with exon 50 deletion, exon 51 is read directly after exon 49. This alters the reading frame and results in a stop codon soon in exon 51, thus terminating dystrophin synthesis prematurely. Skipping of exon 51 during mRNA processing by Anti sense Oligonucleotide leads to exon 52 being read after exon 49, and as both these exons have borders between complete codons, reading frame is maintained resulting in the production of dystrophin , which lacks in 77 amino acids coded by exons 50 and 51, but results in a functional protein.

The two types of AONs are the 2'O-methyl-phosphorothioates (2'O-methyls or 2'OMePS) and the uncharged phosphorodiamidate morpholinos (PMO). They differ in the chemistry of backbone structure used to carry the nucleotide sequence to its desired site of action in a stable and non-toxic manner. Initial clinical trials used exon 51 AONs (PRO051) and morpholinos (AVI 4658) by intramuscular injections. Phase 2 trials of systemic delivery have also established safety of these drugs and shown dystrophin expression in a dose related manner. Preparations for a phase 3 study are underway.

Limitations of this approach include the fact that different oligos will be required for different mutations, repeated doses will be required depending upon the half life of oligos, skipped mRNA and the resulting protein; and long term toxicity issues at the doses required to induce efficient exon skipping. Newer modifications of the technique include exon skipping with U7snRNAs which contain the genetic information for the construction of AONs, or use of peptide nucleic acids (PNAs) where amino acids are used as a backbone to carry purine/pyrimidine bases rather than phosphate and ribose units in AON's,[7,11,12,13]

PTC 124 and Gentamicin

In about 13 to 15% of all DMD patients, the disease is caused by a nonsense mutation in the dystrophin gene, resulting in the introduction of a premature stop codon into the dystrophin mRNA. There are drugs that suppress the usage of these mutated stop codons without affecting the normal stop codons. Gentamicin has been tested in DMD patients but had low efficiency.

PTC 124 (Ataluren) is an orally active drug which is able to force the cells to ignore mutated stop codons. A randomized, double blind phase-IIb clinical trial was conducted to evaluate Ataluren for 48 weeks in 174 patients who were at least 5 years old and still ambulatory (able to walk >75 meters). Initial results have not detected any statistical difference in the primary outcome measure of distance covered in the 6 minute walk test. PTC 124 trials are also ongoing in Cystic Fibrosis and Haemophilia patients with nonsense mutations.

GENE THERAPY

Efforts to deliver a healthy gene to muscles of patients with DMD have faced many obstacles which include muscle targeting, immune modulation, systemic delivery and the large size of dystrophin gene. The delivery of "naked" DNA, a rudimentary form of gene therapy, proved to be inefficient and incapable of sustained transgene translation in DMD. In contrast, viruses are ideal vehicles for therapeutic gene transfer, having evolved to infect specific cell populations with high efficiency. The choice of virus is guided by factors including the target cell, immunogenicity, and required duration of transgene expression. Adenovirus was the early favorite for gene transfer due to its large capacity to carry as much as 35 kilobases (kb) of DNA, though concerns of immune response and emergence of replication competent virus remained. Adeno-associated virus (AAV) has become the vector of choice for gene therapy of muscular dystrophy in recent years. AAV is a small; nonenveloped single stranded DNA virus which requires co-infection with a second 'helper' virus for replication. It is efficient in transducing skeletal and cardiac muscle after intravenous injection, does not cause any known human disease, and induces a mild immune response. The small genome size carried by the AAV is a drawback overcome by use of minigenes which code for smaller but functional mini or microdystrophins, as is observed in Becker muscular dystrophy (BMD) patients. Trans-splicing strategies, in which a gene is split between two AAV vectors, can also be used to overcome the capacity limitations of AAV vectors. Recently developed serotypes such as AAV6, AAV8 and AAV9 have an improved efficiency of gene delivery to muscle.

Successful experiments with dystrophic mice and dogs led to a phase Ia double blind trial in which six boys with DMD received AAV Biostrophin into biceps muscle while the biceps of the other arm received only saline, and the procedure was well tolerated. There is a concern that the truncated dystrophin may not restore the phenotype to normal and the extent of correction remains to be established in clinical trials. Other approaches under development may augment the therapeutic effect of the small dystrophins. Such approaches include combinations of small dystrophins with overexpression of insulin growth factor-1, in particular, the muscle isoform (mIgf-1), or inhibition of the negative muscle growth regulatory factor myostatin by follistatin[4,14,15]

Boosting alternative proteins

Utrophin, a dystrophin homologue coded by the utrophin gene (UTRN) from chromosome 6q24, is structurally similar to dystrophin, consisting of an actin binding N-terminal domain, a central rod domain with spectrin-like repeats, and a cysteine rich C terminal which forms utrophin-glycoprotein complex. Utrophin is ubiquitously expressed in most tissues, including lungs, blood vessels and nervous system. In muscles, its expression is maturation dependant: in fetal muscle it is initially dispersed over the sarcolemma, during development it is gradually replaced by dystrophin, and in mature muscle, it is located only at the neuromuscular and myotendinous junctions. In the regenerating muscle of DMD patients, mdx mice and dystrophin deficient cats, utrophin has been found to be upregulated and redistributed to the sarcolemma. In view of the large size of the utrophin gene, consisting of 74 exons dispersed over 1Mb, truncated gene expression or protein delivery seems more feasible. As Utrophin is not a neo-antigen, it is unlikely to induce immune rejection. Adenoviral delivery of mini-utrophin restored the dystrophin-glycoprotein complex and rescued the dystrophic phenotypes in animal models. Drugs like biglycans, L-arginine, nitric oxide or hydroxyurea have enhanced the levels of utrophin at the sarcolemma in cultured myotubes or animal studies. A phase 1 clinical trial to assess the safety of BMN 195 has started in Jan 2010. BMN 195 aims to increase the levels of utrophin in muscle cells and thus compensate for the loss of dystrophin.

Other strategies to boost alternative proteins include α7β1-integrin, a laminin binding protein, which might also help to stabilize the link between sarcolemma and extracellular matrix, or direct administration of laminin-111.

Conclusions

Advancements in research in the treatments for Duchenne muscular dystrophy hold promise for modification of the disease course of this disabling and fatal disorder. Still no treatment modality has shown success good enough to be applicable to the patients. Though the strategies are very logical, innovative and have shown improvement in dystrophin expression in vitro, none of them have shown significant improvement in power in human studies. All the drugs and other treatment modalities are still in research mode. Efforts are also being made to combine them in order to overcome the intrinsic limitations of each of them. For example, gene therapy approaches have been used to deliver antisense oligos for exon skipping, and autologous transplantation of stem cells with lentiviral correction of genetic defect can avoid lifelong immunosuppression. However, the prospect of improved treatments should not distract us from what we can already achieve in medical management by giving attention to potential respiratory and cardiac complications. Implementation of core care standards, mandating for interventions such as wheel chair provision, non-invasive ventilation, pro-active management for cardiomyopathy, orthopaedic and rehabilitative interventions are effective in improving quality of life, health and longevity. Option of antenatal diagnosis is also available with a family history or previous child with DMD. We look forward to the time when these interventions are not our only approach to managing disabilities caused by Duchenne muscular dystrophy[1,14]

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Utility of Molecular studies in Skeletal Dysplasias

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Skeletal dysplasias or osteochondrodysplasias are a clinically heterogeneous group of genetic disorders characterized by the presence of generalized disordered bone growth and/or modeling. Overall incidence of skeletal dysplasias is approximately 1 case per 4,000 to 5,000 births while the overall frequency of skeletal dysplasias among perinatal deaths is about 9 per 1,000[1]. It has been suggested that the true incidence may be twice as high since many skeletal dysplasias are not diagnosed until short stature, joint symptoms, or other complications become obvious during childhood.

The classification of the skeletal dysplasias was initially based on clinical and radiographic criteria. The current classification of skeletal dysplasias is based on the genes responsible for the disorder [2]. In this 2006 revision of the International Nosology and Classification of Genetic Skeletal Disorders [3,4], 372 skeletal conditions are described, 215 of which are associated with 140 different genes.

The spectrum of skeletal dysplasias ranges from the perinatal lethal dysplasias to individuals with normal stature and survival but early onset osteoarthritis.

Prenatal Onset skeletal dysplasias - Approach:

Fetal skeletal dysplasias are difficult to diagnose in utero and an organized and comprehensive evaluation of the fetal skeleton is needed. Antenatal diagnosis presents in two clinical situations: i) with positive family history and precise description of the phenotype e.g. short rib polydactyly syndromes ii) where a skeletal dysplasia is suspected for the first time during routine ultrasound examination when a short bone / other skeletal findings are observed. Ultrasonographic evaluation of suspected skeletal dysplasia involves systematic imaging of the long bones, thorax, hands, feet, skull, spine and pelvis. Fetal tubular bones less than -3 SD, head circumference › 75th centile, and femur / foot length ratio ‹1 suggest a skeletal dysplasia. Possibility of lethality resulting from lung hypoplasia is important to identify. Chest-to-abdominal circumference ratio ‹ 0.618 and femur length-to-abdominal circumference ratios ‹ 0.16 are parameters suggestive of hypoplastic thorax. Additional features to look for are polydactyly, associated malformations like cardiac anomalies, cleft lip and brain malformations. Bone density, fractures and bent bones. also may give diagnostic clues.[5] Antenatal diagnosis of lethality is 95% sensitive, but prenatal diagnosis of the specific type of skeletal dysplasia reaches only 40%. To make a definitive diagnosis by post delivery examination, it is essential to obtain clinical photographs and radiographs of the baby after delivery or termination, in the antero-posterior and lateral views. It is useful to encourage for fetal autopsy and DNA storage of fetal blood / tissue for molecular diagnosis as applicable.

Non lethal skeletal dysplasias – Approach

These are a group of heterogeneous dysplasias with variable presentation, from late presentation in the third trimester (e.g. heterozygous achondroplasia, hypochondroplasia) to those presenting with disproportionate short stature in infancy or childhood to early onset osteoarthritis. Evaluation of these includes complete history and pedigree analysis for a possible mode of inheritance. Anthropometry includes height, upper segment: lower segment ratio, arm span and head circumference. Check for the presence of any associated anomalies like polydactyly, ambiguous genitalia, cleft palate, ophthalmological abnormalities, facial dysmorphism, etc. as these help in diagnosis of the specific dysplasia. The most essential part is radiographic evaluation of the tubular bones and vertebrae. Minimum radiographs required for evaluation are skull - PA and lateral, chest - PA view, pelvis - AP view with both femurs, left hand with wrist - AP view, thoracolumbar spine – AP and lateral views, long bones of upper and lower limbs. Based on all of the above a diagnosis of the type of dysplasia can often be established.

Molecular analysis:

Over the last decade understanding of the pathways of skeletal development has allowed identification of many genes for these conditions. This has led to revision of the classification as elucidated before and also allowed for confirmation of the diagnosis by analysis of the causal gene. Through some of the families counseled at the Genetic Centre in Sir Ganga Ram Hospital, we elucidate the utility of molecular studies in patients with skeletal dysplasias.

Since 1998 through 2007, the department of genetic medicine at Sir Gangaram Hospital counselled 200 families with suspected skeletal dysplasia at the genetic clinic. Detailed clinical, radiological, biochemical and molecular evaluations were carried out. The spectrum of skeletal dysplasias confirmed by molecular testing includes achondroplasia (ACH; n=64), pseudoachondroplasia (PACH; n=3), hypochondroplasia (HC; n=4), rhizomelic chondrodysplasia punctata (RCDP; n=1), hypophosphatasia (HP; n=1), osteogenesis imperfecta (OI; n=2), campomelic dysplasia (CD; n=1) and spondyloepiphyseal dysplasia tarda (SEDT; n=1).

Achondroplasia:

ACH, an autosomal dominant disorder, is characterized by

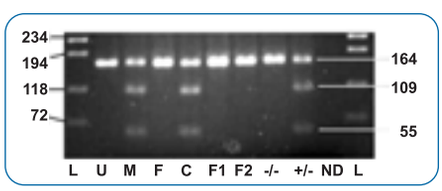

Figure 1 - **Achondroplasia - Gel electrophoresis of Bfml digested PCR products** [1138G>A]
Lane L: DNA ladder; U: uncut PCR product; ND: no-DNA; C: control; -/- : negative control, +/- : heterozygous control. In this family, mother (M) is achondroplasia heterozygote, father (F) is normal, their first child (C) has the mother's mutant allele, hence affected. The fetus (F1, F2) has inherited the normal allele from mother, hence not affected.

abnormal bone growth that results in short stature with rhizomelic shortening of the limbs, trident hand, a large head, and characteristic facial features. Intelligence and life span are usually normal, although compression of the spinal cord and/or upper airway obstruction increases the risk of death in infancy.

Mutations in the fibroblast growth factor receptor gene (FGFR3) are responsible for the phenotype with more than 99% patients having one of the two common mutations at nucleotide 1138. About 98% have G›A substitution and 1% have G›C substitution[6,7]. In our study, the G›A mutation was found in 94.7% (n=50), while the rare G›C substitution was found in 5.3% (n=3) [8] (Fig 1).

In majority of patients with ACH the parents have normal stature and disease results due to de novo mutations. The risk of recurrence for these families due to parental germline mosaicism has been reported to be as low as 0.02%[9]. In our series, nine families with sporadic achondroplasia opted for prenatal diagnosis. Of the nine fetuses, one was found to be affected, presumably due to parental germline mosaicism.

If one parent has achondroplasia, the risk of recurrence is 50% and prenatal diagnosis by chorionic villus sampling and molecular mutation analysis is preferred. One of the

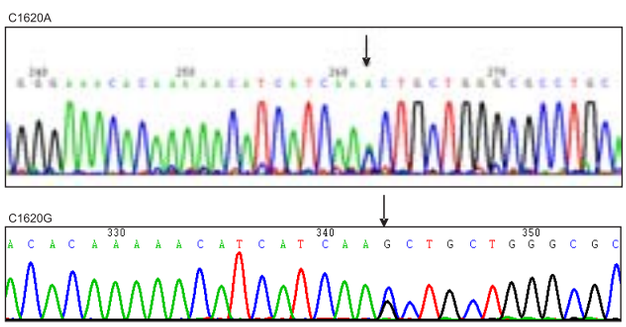

Figure 2 - **Sequencing of FGFR3 gene analysis of Hypochondroplasia common mutations** (FGFR3 exon 10 (codon 540) 1620 C>A (occurs in about 70%) or C>G (occurs in about 30%)

issues with ultrasound diagnosis for ACH is that short femur and large head circumference are not detected until about third trimester; hence prenatal ultrasound may not be very useful to provide prenatal diagnosis of ACH.

Hypochondroplasia

In another family an elderly primigravida was noted to have fetal long bone shortening at 26 – 28 weeks gestation in the antenatal ultrasound. In view of the advanced gestation no intervention was done antenatally. At birth, the neonate had near normal birth length, mild rhizomelia with subtle radiographic features. Targeted mutation analysis for ACH did not reveal an abnormality. We then performed FGFR3 gene sequencing and identified the common heterozygous mutation, C1620A that causes hypochondroplasia (Fig 2). This is a form of short-limbed dwarfism, similar to achondroplasia, but the features tend to be milder. Molecular diagnosis is required to confirm the diagnosis.

Pseudoachondroplasia

Many skeletal dysplasias go undiagnosed in infants and neonates because they do not manifest until short stature, joint symptoms, or other complications arise during childhood. It becomes important here to take a thorough family history and examine the child / adult for signs of bone abnormality. Pseudoachondroplasia is a skeletal dysplasia resembling achondroplasia but with normal head and face. It is characterized by late infancy to childhood onset of short stature, vertebral abnormalities in childhood radiographs, femoral head abnormalities, short tubular bones with changes in the epiphyses and metaphyses. We counseled a family with history of short stature and hip replacement who were considered to have a pathological disorder only after presentation of a young girl in the third generation with short stature, lumbar lordosis and short broad hands. Radiographs of the proband showed irregular epiphyses and metaphysis and pear-shaped vertebrae. A novel mutation was confirmed in the proband, her mother and uncle in the COMP gene. This family typically exemplifies the natural history of PACH with growth rate decline after two years age, progressive degenerative joint disease with requirement for hip replacement surgery.

Biochemical & molecular diagnosis: Rhizomelic chondrodysplasia punctata (RCDP)

RCDP is a rare, multisystem, developmental disorder, characterized by the presence of stippled foci of calcification in hyaline cartilage, coronal vertebral clefting, short stature, joint contractures, congenital cataract, ichthyosis, and severe mental retardation.

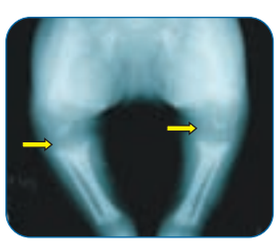

Figure 3: **Rhizomelic Chondrodysplasia Punctata**: Punctiform calcification – epiphyses, patella

A newborn with typical facial features, short limbs, mainly rhizomelia, ichthyosis, and history of respiratory distress at birth presented in the clinic. There was rhizomelic shortening, epiphyseal stippling and metaphyseal flaring (Fig 3). Biochemical investigations revealed high plasma phytanic acid and normal very long chain fatty acids suggestive of the diagnosis of chondrodysplasia punctata. The neonate was tested for mutations in the PEX7 gene known to cause autosomal recessive rhizomelic chondrodysplasia punctata. A homozygous two base-pair deletion was confirmed to cause the disease in the infant.[10]

Fig 4: **Infantile hypophosphatasia** Decreased skeletal mineralization Deformity and fractures of long bones

Prenatal diagnosis of Hypophosphatasia and Osteogenesis Imperfecta

Definitive prenatal diagnosis for all skeletal dysplasias is best done with molecular mutation analysis in fetal tissue at 11 – 13 weeks gestation after the disease causing mutation is identified in the proband. Lethal skeletal dysplasias like short rib polydactyly syndromes, can be identified prenatally by serial ultrasonographic examinations. However some dysplasias may not have antenatally identifiable ultrasonographic features till the late second and third trimester of gestation and in these families prenatal diagnosis by molecular methods is most useful. The following three prenatal cases exemplify the same.

A child with deformity and fractures of long bones, recurrent respiratory tract infections and severe osteopenia was evaluated in the clinic (Fig 4). Parents were consanguineous. The serum alkaline phosphatase level in the child was 10 IU/l (Normal range = 145-420IU/l). She was suspected to have autosomal recessive hypophosphatasia. A homozygous 5 base pair deletion in the TNSALP gene was detected in the child and parents were confirmed to be carriers of the mutation. The couple was offered prenatal diagnosis in the subsequent pregnancy due to a recurrence risk of 25%. The fetus had inherited a single copy of the mutation and was not affected.

Two adults with osteogenesis imperfecta were counselled for recurrence risk during pregnancy. In the first family, the lady had blue eyes and used hearing aid for deafness. She denied history of fractures. She had one normal child and was pregnant for the second time. With a clinical diagnosis of osteogenesis imperfecta, the COL1A1 gene was sequenced and a single nucleotide substitution, IVS 23+1

G>C in COL1A1 gene was detected in the mother. Chorionic villus sampling for prenatal diagnosis confirmed that the fetus had not inherited the disease causing mutation and was unaffected.

In the second family the husband had short stature, light-coloured eyes and history of bone fractures. Prenatal diagnosis was offered after detecting an amino acid substitution gly 862 ser in the COL1A1 gene in the father. The fetus had also inherited the same mutation and the parents opted for termination of the pregnancy.

Utility of stored DNA of proband

Campomelic dysplasia, an autosomal dominant dysplasia, is a severe disorder affecting the development of the skeleton and reproductive system. The condition is often life-threatening and is characterized by distinctive facies, Pierre Robin sequence with cleft palate, shortening and bowing of long bones and club feet. Other findings include respiratory compromise and ambiguous genitalia or female external genitalia in individuals with a 46,XY karyotype.

A non-consanguineous couple had lost two girls due to an undiagnosed condition in the neonatal period. Historically the first child had bowed legs while the second neonate had XY sex reversal with bowing of lower limbs. There was no other family history. The children were suspected to have campomelic dysplasia caused due to mutations in the SOX9 gene (SRY related gene). A splice acceptor mutation was identified in the stored DNA of both children, and 12-15% of somatic cells of the mother also had the mutant allele, making her a somatic and gonadal mosaic for the causative mutation. The mother had no clinical features of the disorder.

Role of molecular diagnosis for carrier analysis

A couple with one normal child approached us for genetic counselling and prenatal diagnosis regarding short stature in the wife's brother and her maternal uncle. These family members had disproportionate short stature and characteristic radiographic findings such as the humping of vertebrae. Since no females in the family were reported to be affected, an X-linked inheritance was suspected and molecular analysis for spondyloepiphyseal dysplasia tarda (SEDT) was performed. On testing the affected brother and maternal uncle, we found a novel frame-shift mutation in the TRAPPC2 (SEDL) gene on whole gene sequencing, making the consultand's mother an obligate carrier. The consultand tested negative for the same mutation, hence having a low risk of having an affected child making prenatal diagnosis unnecessary.

SEDT occurs almost exclusively in males. The name of the condition indicates that it affects the bones of the spine (spondylo-) and the ends (epiphyses) of long bones in the arms and legs. "Tarda" indicates that signs and symptoms of this condition are not present at birth, but appear later in childhood, typically between ages 6 and 10.

Conclusions:

Skeletal dysplasias are a heterogeneous group of disorders and pose a diagnostic challenge in the clinic. As elucidated, diagnosis is based on clinical and radiographic features with confirmation by molecular & biochemical analysis as applicable. The confirmation of the type of skeletal dysplasia is important for management and counselling the family. With a definitive diagnosis, an accurate risk of recurrence (sporadic/familial) can be offered and it also enables the family to opt for early prenatal diagnosis or carrier detection as illustrated.

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Nevoid Basal Cell Carcinoma Syndrome (Gorlin-Goltz syndrome)

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INTRODUCTION

Nevoid basal cell carcinoma syndrome (NBCCS) an autosomal dominant disorder is one of the hereditary cancer predisposition disorders. NBCCS is a single gene disorder caused by mutation in Patched (*PTCH1*) gene, localized to chromosome 9q22.3.[1] Major clinical manifestations are multiple basal cell carcinomas (BCCs), odontogenic keratocysts, palmar pits, and various skeletal features.[2] Clinical manifestations in NBCCS are highly variable. A family with NBCCS with highly variable clinical features among the affected family members is reported.

CASE REPORT

A two year and nine months old male child presented with polydactyly and left ventricle fibroma (operated). His developmental milestones were appropriate for age.

Family history revealed that the child's mother (age 30 years) was diagnosed to have skin malignancy (BCCs) and recurrent, histologically proven odontogenic keratocysts.

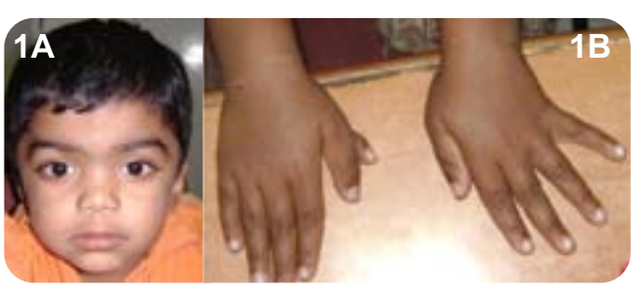

Fig 1A & 1B: Proband with flat facial profile, synophrys, and depressed nasal bridge. B – Preaxial polydactyly on right hand.

Maternal grandmother had similar problems.

Examination of the proband revealed: head circumference - 52.5 cm (› 97th centile), height – 94 cm (50th centile), two hair whorls, a flat facial profile, synophrys, depressed nasal bridge, short neck, right side preaxial polydactyly (Fig 1A & 1B), single café-au-lait spot over left thigh, hypopigmented skin lesion over chin and shawl scrotum.

Examination of mother revealed arched eyebrows, broad jaws and multiple pigmented nevi over face and sternal region (Fig 2A). Both hands showed numerous palmar pits

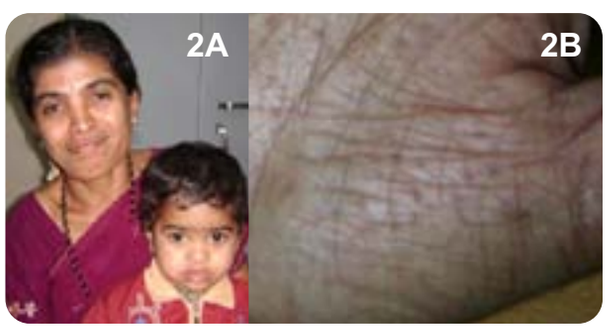

Fig 2A & 2B: Mother with broad jaw, flat facial profile and numerous palmar pits. Younger sibling with facial features similar to proband.

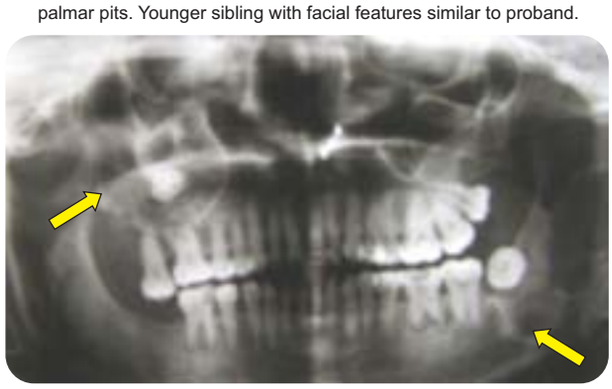

Fig 3: X- Ray oral region of mother showing cystic lesions (indicated by arrow)

(Fig 2B). Radiological investigations of oral region revealed cystic lesions (Fig 3)

Younger sibling's (age 1 year 4 months) facial features were similar to those of the elder affected brother (Fig 2A), however he didn't have polydactyly. Skin and cardiac evaluation was normal. According to the diagnostic criteria suggested by Kimonos et al, the family fulfills the criteria for NBCCS.[2]

DISCUSSION

NBCCS is known for interfamilial and intrafamilial variable clinical features.[2] Prevalence ranges from 1 in 30,827 to 1 in 256,000 in various studies worldwide.[3,4] Major clinical criteria features are BCCs, odontogenic cysts, palmar pits, rib abnormalities, calcification of falx

cerebri and an affected first degree relative. Minor criteria include macrocephaly, cleft lip, cleft palate, pectus deformity, Sprengel deformity, hand & foot anomalies, vertebral anomalies, medulloblastoma, cardiac or ovarian fibroma and others.[2,4] Diagnosis is considered in presence of two major or one major/two minor criteria. Further diagnosis can be confirmed by *PTCH1* gene mutation analysis.

PTCH1 gene encodes for a protein which acts as receptor in the Sonic Hedgehog signal (SHH) pathway. Mutation in *PTCH1* gene leads to uncontrolled transcription in specific cells of genes encoding members of the TGF-beta and Wnt families of signaling proteins through the SHH pathway. Mutations are widely distributed through out the gene. In 26-60% of the cases mutations in the *PTCH1* gene are de novo (sporadic cases).[3,4] DNA diagnosis is mainly useful in genetic counseling and in cases with atypical features or in sporadic cases - as a diagnostic test. If a mutation is identified in a proband , it can also be used for predictive genetic testing in children of familial cases. As some clinical features are age dependent, all the diagnostic criteria may not be fulfilled; in such cases it is recommended to look for other radiological features and/or DNA testing to confirm the diagnosis.[1,5] Cases with multiple clinical features (jaw keratocysts, palmar pits and BCCs) are more likely to screen positive for pathogenic mutation in PTCH1 gene.[1] Early diagnosis, especially in sporadic cases helps in optimum surveillance and management.

Clinical management includes surgical intervention, surveillance and preventive measures.[4,6] Surgical interventions are required for odontogenic keratocysts and BCCs. Both odontogenic keratocysts and BCCs show high recurrence rates. In view of high risk for BCCs, patients should avoid exposure to sunlight and radiations. Use of UV protective sunglasses, sunscreen lotions and proper body cover by clothing will help in preventing sunlight exposure. Vitamin A analogs are used by some to treat BCC which are superficial and useful as a preventive measure. Care should be taken to avoid vitamin A analogs in women of child bearing age, as it has teratogenic effects on the developing fetus. Radiotherapy for any type of malignancies is contraindicated. Other modalities to treat BCCs are electrodessication & curettage, photodynamic therapy and cryosurgery. Periodic surveillance should include examination of skin, growth and developmental assessment for children especially head circumference, screening for jaw keratocysts and other tumor surveillance (medulloblastoma, ovarian fibroma and cardiac fibroma). Medulloblastoma as a feature of NBCCS often presents in children younger than 3 years age and should raise the suspicion of NBCCS.[1,4] Life expectancy is generally normal with good prognosis for cases with NBCCS, except in cases with medulloblastoma.[4,6]

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Microarray for all...

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Array CGH as a first line test for MR – good value for money [1]

Array genomic hybridization (AGH) has a much better resolution and yield in detecting chromosomal imbalances causing unexplained mental retardation (MR) than conventional cytogenetic techniques such as karyotyping. However, the cost has always been presumed to be a prohibitive factor in using this technique as a first line test in the evaluation of MR. Regier et al tried to determine the cost – benefit profile of an AGH based testing strategy for MR, using a decision analytical model. The results of the statistical analytical tests showed that while a baseline AGH testing strategy lead to an average cost increase of $ 217 per patient, it also lead to an additional 8.2 diagnoses for every 100 patients tested, when compared to a karyotyping based strategy. The favourable cost effectiveness ratio has led the authors to suggest that using AGH as a baseline test for MR instead of karyotyping provides better value for money than using it as a second line investigation, after a karyotype has proved uninformative.

'CATCH - SCAN' – targeted capture and sequencing of the whole exome[2]

Whole genome sequencing through conventional/ first generation sequencing techniques is costly, time consuming and labour intensive. Ng et al therefore, sought to develop a cost – effective second generation method for targeted sequencing of all protein-coding regions (exomes) of the genome. They used the genome wide exon capture technique previously described by Hodges et al, which involves enrichment of all the exonic regions of the genome by array hybridization of test genomic DNA to exon specific oligonucleotide libraries. The exomes thus 'captured' were then subjected to shotgun sequencing. The feasibilty of the technique was first tested on the DNA samples of 8 HapMap individuals. Subsequently, its utility in mapping genes for Mendelian disorders was demonstrated by correctly identifying the gene (MYH3) already known to cause Freeman Sheldon syndrome, using this approach in 4 affected unrelated individuals.

Whole-exome sequencing for gene mapping – finding the needle in a haystack is possible after all! [3]

The whole- exome sequencing strategy developed by Ng et al for mapping genes underlying Mendelian disorders, as described above, was put to use by Ng, Bamshad and colleagues to identify the gene underlying a previously uncharacterised rare, autosomal recessive, multiple malformation disorder, Miller syndrome which comprises cleft palate, digital, ocular and other anomalies. Targeted exome capture followed by massively parallel short read sequencing was done in 4 affected individuals. Thereafter, a step-wise filtering approach based on exclusion of common and non damaging variants, was used to screen all the identified variants and select only those most likely to be pathogenic. The gene thus identified was DHODH, which encodes a key enzyme in the pyrimidine de novo biosynthesis pathway. Having thus proven its efficacy, the authors suggest that this technique is likely to revolutionize the genetic analysis of monogenic traits in the future.

Gene for Perrault syndrome identified – another addition to the peroxisomal disorders family [4]

Perrault syndrome is an autosomal recessive disorder characterised by sensorineural deafness, ovarian dysgenesis in females and some neurological manifestations, for which the causative gene was not known so far. Using the technique of whole-exome sequencing in 2 affected sisters of non-consanguineous parentage, Pierce et al found compound heterozygous mutations in the HSD17B4 gene that encodes 17 β hydroxysteroid dehydrogenase type 4 (also known as DBP - D-bifunctional protein) which is a peroxisomal enzyme involved in fatty acid beta oxidation and steroid metabolism. Six other families with Perrault syndrome tested, did not have any mutations in this gene, indicating that Perrault syndrome is a genetically heterogeneous condition. This study has yet again proven the utility of the whole-exome sequencing strategy in mapping genes for recessive disorders in small non-consanguineous families where neither linkage analysis nor homozygosity mapping would be useful.

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10

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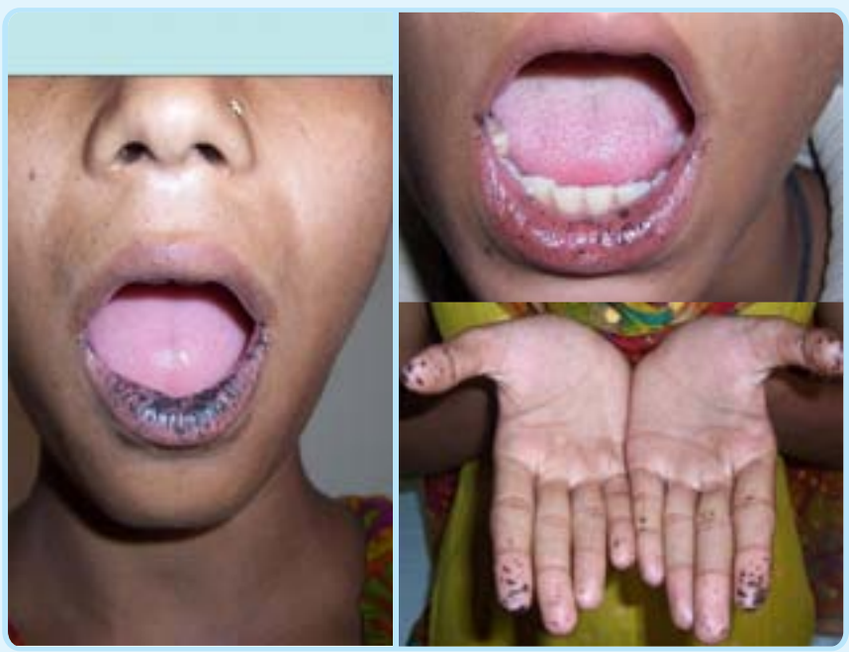
A girl and her brother (shown in the picture below) had come to see their father admitted in the gastroenterology ward for surgery of a tumor. Identify the condition.



Answer to the PhotoQuiz 9 of the previous issue

Meckel Gruber Syndrome (OMIM No. 249000)

This is a well known syndrome characterized by encephalocele, bilateral polycystic kidneys and polydactyly. Meckel Gruber syndrome (MGS) type 1 is caused by mutation in a gene encoding a component of the flagellar apparatus basal body proteome (MKS1). The other genes causing MGS are TMEM67 , CEP290 , RPGRIP1L and CC2D2A . There are some more loci for MGS. The mode of inheritance is autosomal recessive and the risk of recurrence in the sibs of a proband is 25%. As there are many genes causing MGS, mutation detection is a difficult task. Prenatal diagnosis by ultrasonography can be done by 14 to 16 weeks of pregnancy.

Correct responses to PhotoQuiz No. 9 were given by

1.Vishwanath Kulkarni, via e-mail
2.Anil B Jalan, Mumbai
3.Chinmayee Ratha, New Delhi
4.Seema Thakur, New Delhi
5.Soumya M, Davangere
6.Jayalakshmi KN, Davangere
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