THE INDIAN ACADEMY OF PEDIATRICS

genetic CLINICS

Newsletter of Genetics Chapter of Indian Academy of Pediatrics

Volume: 2
Issue: 2 (April-June 2009)

Table of contents:

Address for correspondence:
Dr Shubha R Phadke
Department of Medical Genetics
Sanjay Gandhi Postgraduate Institute of Medical Sciences, Raebareli Road, Lucknow-226 014
EPABX : 0522-2668005-8 | Extn. 2325, 2348 | E-mail : shubha@sgpgi.ac.in

genetic CLINICS

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

The categories of article include:

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

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

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

Originality and copyright: It is implied that the newsletter receives only the articles that have not been published elsewhere and is not being considered for publication by any other publisher and the authors transfer the copyright to the publishers.

The electronic version of articles should be sent by e-mail as an attachment to geneticsiap@gmail.com

Invited Editorial:

The Future of Genetics and Genomics in India

Ishwar C Verma
Chairman, Department of Genetic Medicine
Sir Ganga Ram Hospital, New Delhi
Email: dr_icverma@yahoo.com

February 12, 2009 marks the 200th Birth Anniversary of Charles Darwin (1809-1882), and 150th year since publication of his seminal work On the Origin of Species. The renowned 19th century naturalist made observations on plant and animal life that set science on a new course, introducing evolution as a unifying concept in all of genetics and biology. He was a timid scientist as he desisted from publishing his results for several years, as they were in serious conflict with the conventionally held views by the Church and Society. It was only when he received a manuscript propounding the same hypothesis from Douglas Wallace working in Borneo, that he published his observations to claim precedence.

An organism, on persistent exposure to a deleterious environment, undergoes changes. For evolution to occur, these changes have to be preserved and passed on to future generations. This is achieved through genes. From Darwin it has been a slow march to Mendel and many other scientists, to the exciting present. The last two decades has seen phenomenal advances in genetics, and the completion of the human genome project (HGP) in 2003 was a landmark year. As Francis Collins said after the HGP, the work of the geneticists has just begun. Witness the rapid march through the SNP project, the Hap Map project, Genome Wide Association Studies (GWAS), and leading on to Personal Genomes.

These rapid and revolutionary changes reflect in every day work of the geneticist. Let me take you on a detour of what we did in the first two weeks of January 2009. Of 194 cases that we counseled in the Department of Genetic Medicine, 39 (20.1%) were referred for evaluation and intervention for positive tests on biochemical screening – in the first as well as the 2nd trimester. Eighteen had abnormalities in ultrasound studies. From among these two groups, 37 required amniocentesis, or amnio infusion and other interventions. The amniotic fluid cells were subjected to 5-chromosome aneuploidy test, followed by culture. Prenatal diagnosis was carried out by chorionic villus sampling in 21 cases, for disorders such as thalassemia, Duchenne muscular dystrophy, congenital adrenal hyperplasia, oculo-cutaneous albinism, and cystic megalencephaly etc. All these required molecular techniques for diagnosis. Nineteen cases needed a genetic evaluation for recurrent spontaneous miscarriages. There were 30 cases of mental retardation with or without malformations. Of these, 18 had Down syndrome, while many others required metabolic screening by tandem mass spectrometry, HPLC for amino acid quantification, lysosomal enzyme assays, F.I.S.H. studies for microdeletion syndromes, while one required telomere deletion analysis. There were seven cases of Duchenne muscular dystrophy and we carried out deletion / duplication test for all the 79 exons. Although this is slightly more expensive, it does pick up deletions of exons not included in the 18 exon test. It also detects duplications in about 8 % of cases, that otherwise remain undiagnosed. It has become important to exclude deletions / duplications of all exons, because if the defect is due to a point mutation, and that happens to introduce a stop codon, treatment with PTC124 is feasible. There were five cases of spinal muscular atrophy (SMA) which we tested for deletion of exon 7. In one family we analyzed the siblings of the parents for carrier status by quantitative PCR, as they were concerned about having an offspring suffering from SMA. Most interesting were 26 cases of other single gene disorders, for which we had to search the literature to find out the causative gene, and how to go about finding the mutations in that gene. Witness the following:

A consanguineous couple from Kashmir had lost two babies at about 8 weeks and 6 months of age, respectively. The first daughter was suspected to have a congenital cyanotic heart disease but the diagnosis was never confirmed. The second son developed cyanosis and breathlessness and echocardiography confirmed the presence of primary pulmonary hypertension (PPHN). As two siblings were similarly affected we argued that there must be a genetic basis for this problem. My colleague Dr. Ratna Puri who looked into this, discovered that this susceptibility to PPHN arises from a polymorphism in carbamoyl phosphate synthetase 1 (CPS1) gene in the urea cycle pathway. This polymorphism slows down the cycle and results in reduction of arginine and citrulline. This finally leads to a low level of nitrous oxide resulting in pulmonary hypertension. Amazing isn't it. We are now setting up the molecular analysis of this polymorphism in CPS 1 gene.

A family had lost two children with cancer. Elder sib died at 3 years of age from osteosarcoma. The second sib had teratoid or rhabdoid tumors in the right parietal, occipital and temporal lobes and died at 4 years of age. There was no other family member with cancer. Dr. Seema Thakur, who evaluated the case, suspected Li Fraumeni syndrome. We have just completed analysis of p53 gene in the couple, and discovered a novel mutation in the mother. On this basis we will be able to test their fetus if he carries this mutation in p53 gene.

A four-year-old girl child presented with history of global developmental delay and seizures. She was born to non-consanguineous couple with uneventful antenatal period and birth. In the second year, she had speech delay, some autistic features and secondary microcephaly. An EEG showed

frequent diffuse independent single spike and wave discharges. On examination the child was fair in complexion and had an unusual happy affect. She had repetitive hand mouthing and toe walking. My colleague Dr. Sunita Bijarnia suspected Angelman syndrome. On a methylation study on DNA of the child there was failure to amplify the maternal allele, thus confirming this diagnosis.

The above examples show the power of molecular genetics. It has brought about a remarkable change in genetic counseling. We start everyday with anticipation of encountering something new, something that will challenge us. The pursuit does not end in finding the diagnosis, but in working out the gene involved, the number of exons it has, identifying the hot spot exons, tracing out the paper describing the primers for sequencing them, and eventually pinpointing the mutation. It is as exciting as getting to the top of Mount Everest.

I often receive emails from young students who wish to embark on a career in genetics, asking me whether it is worth it. I think of the advice that I received in 1961, when after my graduation in medicine I went to see Dr. Holmes at All India Institute of Medical Sciences. He was the Visiting Professor of Preventive Medicine and word had got around of the new community care approaches that he had introduced in the Rural Health Project of AIIMS. "Is there a future in Preventive Medicine" I asked him. He replied "You get out of a career proportionate to what you put in." This is certainly true. Genetic medicine offers great intellectual challenges, as Genetics can truly claim to be the central basic science of medicine in this century.

But the question that young people have uppermost in their mind is whether there is in enough money in the practice of genetic medicine. The pattern of disorders for which patients consult doctors has changed in the past decade, especially in the urban areas in India. I have witnessed increasing need for geneticists in the new hospitals that are coming up. I often receive requests for starting a genetics department in other hospitals, as they try to create a unique feature for themselves. The limiting factor is the lack of medical geneticists and genetic counselors. This change is apparent from the fact that five years ago doctors who had received training in DM Genetics at Sanjay Gandhi Postgraduate Medical Institute in Lucknow had difficulty in finding a placement after passing out. In the past three years they get a job as soon as they qualify. This is a refreshing departure form the past, and bodes well for the future of Medical Genetics. So I advise the youngsters looking for a career in genetics that they are headed in the right direction, and should keep going.

I also remind them to read Randy Pausch's Last Lecture: "Really Achieving Your Childhood Dreams" given at Carnegie Mellon University on Tuesday, September 18, 2007. He was terminally ill with prostate cancer, with secondaries in the liver. "If you had one last lecture to give before you died, what would it be?" Some of the core messages that he passed on to young students who had thronged the lecture hall to hear him were: (1) We cannot change the cards we are dealt, just how we play the hand. (2) The young look at the TV set, and see men landing on the moon, and feel anything's possible. Therefore dream big. (3) Fundamentals, fundamentals, fundamentals. You've got to get the fundamentals right, because otherwise the fancy stuff isn't going to work. (4) Experience is what you get when you didn't get what you wanted. (5) When you have a setback, remember, the brick walls are there for a reason. The brick walls are not there to keep us out. They are there to give us a chance to show how badly we want something. (6) I was told to go and get a Ph.D, and become a professor. And I asked, why? "Because you're such a good salesman that any company that gets you, is going to use you as a salesman. And you might as well be selling something worthwhile, like education. There are other important messages, but I would leave these for you to discover.

Good luck and enjoy the journey.

Stem cell therapy: Current status

Dr Ashwin Dalal
DM (Medical Genetics)
Head, Diagnostics Division
Centre for DNA Fingerprinting & Diagnostics, Hyderabad
Email: ashwindalal@gmail.com

Stem cells have always been fascinating for scientists due to their undifferentiated state that can give rise to a highly specialized cell type or organism and their seemingly endless self-renewal potential. Research on stem cells is advancing knowledge about how an organism develops from a single cell and how healthy cells replace damaged cells in adult organisms. This promising area of science is also leading scientists to investigate the possibility of cell-based therapies to treat disease, which is often referred to as regenerative or reparative medicine. Stem cells are one of the most fascinating areas of biology today. But like many expanding fields of scientific inquiry, research on stem cells raises scientific questions as rapidly as it generates new discoveries. The idea behind this review is to give an overview of the biology of stem cells and to discuss completed and ongoing clinical trials and current status of stem cell therapy.

STEM CELL

A stem cell has the ability to divide for indefinite periods often throughout the life of the organism. Under appropriate conditions, stem cells can give rise to the many different cell types that make up the organism. There are mainly two types of stems cells.

1. Embryonic stem cell
2. Adult stem cell

EMBRYONIC STEM CELL

Embryonic stem cells are derived from the inner cell mass of the blastocyst of the embryo. They are capable of undergoing an unlimited number of symmetrical divisions without differentiating (long-term self-renewal). They exhibit and maintain a stable, full (diploid), normal complement of chromosomes (karyotype). These cells are clonogenic, i.e. a single cell can give rise to a colony of genetically identical cells, or clones, which have the same properties as the original cell.

There is a lot of ethical debate on the use of embryonic stem cells for research and therapy. Since most of the trials do not use embryonic stem cells for therapy of human diseases, we will not discuss this topic further. Table I shows the potential uses of embryonic stem cells and dangers associated with their use.

Table I: Potential uses and dangers of Embryonic stem cell therapy[1]

POTENTIAL USES OF HUMAN EMBRYONIC STEM CELLS
• Transplantation of Embryonic stem cells for therapy
• Study early events in human development
• To explore the effects of chromosomal abnormalities in early development
• To test candidate therapeutic drugs
• Screening of potential toxins
• To develop new methods for genetic engineering
POTENTIAL DANGERS ASSOCIATED WITH USE OF HUMAN EMBRYONIC STEM CELLS
• Propensity of undifferentiated ES cells to induce the formation of tumors (teratomas)
• Immune rejection

ADULT STEM CELL

An adult stem cell is an undifferentiated cell found among differentiated cells in a tissue or organ, can renew itself, and can differentiate to yield the major specialized cell types of the tissue or organ. The primary roles of adult stem cells in a living organism are to maintain and repair the tissue in which they are found. Adult stem cells are rare. For example, only an estimated 1 in 10,000 to 15,000 cells in the bone marrow is a hematopoietic (blood forming) stem cell (HSC).

The list of adult tissues reported to contain stem cells is growing and includes bone marrow, peripheral blood, brain, spinal cord, dental pulp, blood vessels, skeletal muscle, epithelia of the skin and digestive system, cornea, retina, liver, and pancreas.

In a living animal, adult stem cells can divide for a long period and can give rise to mature cell types that have characteristic shapes and specialized structures and functions of a particular tissue[2].

Research on adult stem cells has recently generated a great deal of excitement. Scientists have found adult stem cells in many more tissues than they once thought possible. This finding has led scientists to ask whether adult stem cells could be used for transplants. In fact, adult blood forming stem cells from bone marrow have been used in transplants for more than 30 years[2].

Certain kinds of adult stem cells are known to have the ability to differentiate into a number of different cell types, given the right conditions. This ability of stem cells from one adult tissue to generate the differentiated cell types of another tissue is called plasticity. If this differentiation of

adult stem cells can be controlled in the laboratory, these cells may become the basis of therapies for many serious common diseases[1].

Due to the inability to efficiently and safely harvest or expand stem cells from most adult organs (eg, liver, gastrointestinal tract, heart, brain), the majority of human stem cell trials have focused on clinical applications for hematopoietic stem cells (HSCs), mesenchymal stem cells (MSCs), or both, which can be easily obtained in clinically sufficient numbers from peripheral blood, bone marrow, or umbilical cord blood and placenta.

HEMATOPOIETIC STEM CELL THERAPY

Hematopoietic stem cell (HSC) is a cell isolated from the blood or bone marrow that can renew itself, can differentiate to a variety of specialized cells, and can mobilize out of the bone marrow into circulating blood. The German pathologist Julius Cohnheim in 1867 was one of the first to realize that the bone marrow gives rise to circulating cells, including fibroblasts engaged in inflammatory wound healing processes. However, the clonogenicity of blood cell lineages and the concept of stem cell theory were only proven in 1961.

The sources of hematopoietic stem cells include:

- Bone marrow
- Peripheral blood
- Umbilical cord blood
- Embryonic stem cells

Of these, bone marrow derived cells have been used for last 30-40 years for treatment of various diseases like-

- Leukemias, lymphomas
- Inherited blood disorders
- Inborn errors of metabolism
- Stem cell rescue in cancer chemotherapy
- Graft-Versus-Tumor Treatment of Cancer
- Autoimmune diseases

Hematopoietic stem cell therapy for inherited and acquired blood disorders has been in clinical practice for a number of years. However, recently a few trials have tried the use of these cells for treatment of autoimmune diseases. Table 2 shows results of few of these trials.

Table 2: Clinical trials with HSCs in autoimmune diseases

SOURCE	DISEASE	RESPONSE
Burt et al, 2008[3]	Relapsing-remitting Multiple Sclerosis	0% progression at 2 years; 62% improved
Craig et al, 2008[4]	Crohn disease	100% remission; 33% relapse
Oyama et al, 2007[5]	Systemic sclerosis	70% progression-free survival
Statkute et al, 2007[6]	Vasculitis	Complete remission (n=3); partial response (n = 1)
Voltarelli et al, 2007[7]	Type 1 diabetes mellitus	13/15 patients remaining insulin-free
Vonk et al, 2007[8]	Systemic sclerosis	64% event-free survival at 5 years
Burt et al, 2006[9]	SLE	50% disease-free survival at 5 years
Snowden et al, 2004[10]	Rheumatoid arthritis	50% ACR criteria 50 or greater response at 12 months

UMBILICAL CORD BLOOD DERIVED STEM CELLS

In the late 1980s and early 1990s, physicians began to recognize that blood from the human umbilical cord and placenta was a rich source of HSCs. This tissue supports the developing fetus during pregnancy, is delivered along with the baby, and, is usually discarded. Since the first successful umbilical cord blood transplants in children with Fanconi anemia, the collection and therapeutic use of these cells has grown quickly and has been extended to other diseases like thalassemia etc. Umbilical cord blood recipients, typically children, have now lived in excess of eight years, relying on the HSCs from an umbilical cord blood transplant. A number of umbilical cord blood banks have started storing the umbilical cord blood of newborn children for future use. While umbilical cord blood represents a valuable resource for HSCs, research data have not yet conclusively shown their utility for use as stem cells to be used for differentiation into different type of cells for future use.

STEM CELL THERAPY FOR CORONARY ARTERY DISEASE

The possibility of using stem cell based therapies for people suffering an acute myocardial infarction or living with chronic heart failure has captured the imagination of both the medical community and lay persons. Since early reports in animal models 10 years ago, the stem cell field has made enormous advances in moving toward clinically applicable treatment options. An abundance of preclinical data demonstrates safety, feasibility, and efficacy, justifying the current entry into clinical trials of stem cell therapy in humans. This position, however, is extremely controversial, with some arguing that trials are premature because mechanistic insights are insufficiently addressed. The field of regenerative medicine will advance through the parallel conduct of in vitro/animal model studies and clinical trials.

If we look at results of studies of stem cell therapy for myocardial infarction then most of them report only a modest benefit compared to controls. The MAGIC (Myocardial Regeneration and Angiogenesis in Myocardial Infarction With G-CSF and Intracoronary Stem Cell Infusion) Cell 1 study compared intracoronary transplant of granulocyte colony–stimulating factor (G-CSF)– mobilized peripheral blood stem cells (PBSC) vs. treatment with G-CSF alone vs. an untreated control group[11]. Left ventricular ejection fraction (LVEF) improved in the PBSC group compared with the G-CSF-alone group, and there was an increase in restenosis in patients receiving G-CSF. The BOOST (Bone Marrow Transfer to Enhance ST-Elevation Infarct Regeneration) trial reported that Bone Marrow Mononuclear Cells (BMMCs) significantly improved LVEF 6 months after intracoronary transplantation[12]. Further the trial reported that the beneficial effect on LVEF was no longer significant after 12 months.

The ASTAMI (Autologous Stem Cell Transplantation in Acute Myocardial Infarction) trial found no significant beneficial effects from intracoronary transplantation of BMMCs on LVEF[13].

Taken as a whole, the results of intracoronary transplantation of progenitor cells following acute myocardial infarction show only a modest benefit. The benefit in these studies must

be compared with the fact that LVEF normally improves a few months after acute myocardial infarction, even without stem cell transplantation. Further the mechanism of action of stem cells is still not clear. Although at this time no data support either cell-based or specific stem cell therapies as standard clinical practice for cardiac applications, there is a wealth of preclinical and early clinical data showing safety, feasibility, and early efficacy of adult cell-based therapy. Evidence available suggests that further definitive clinical studies are necessary, and specifically, randomized controlled clinical trials before stem cell therapy can be used in routine clinical practice.

Apart from the diseases mentioned, stem cell therapy has been used for a variety of diseases with evidence of definite improvement in some of the studies. Table 3 shows a list of various diseases for which stem cell therapy has been tried in humans.

Stem cell therapies are showing promising results and a hope for future therapy for many disorders like cardiac diseases, neurodegenerative diseases, spinal cord injuries etc. However current research has shown many gaps in our understanding of biology of stem cells which must be filled till it becomes clinically applicable. There is a school of thought which says that most of the pharmacological agents used in the management of patients were tested in man without a full understanding of their mechanism of action. However this does not make scientific sense and unless the exact mechanism of action of stem cells is known, the experimental therapies may not be beneficial to the patients.

Stem cell therapy is not used in routine clinical practice as of now in any part of the world. Most of the work on stem cell therapies in humans has been done under research protocols which are scrutinized and then only approved by ethics committees of the respective institutes. Stem cell therapies are proceeding into randomized, placebo-controlled, double-blind clinical trials. These ongoing rigorously designed trials will contribute greatly to this emerging and exciting new therapeutic approach for human diseases.

Table 3: Clinical studies using stem cells for organ regeneration and tissue repair

ORGAN OR DISEASE	RESULTS	REFERENCE
Acute graft versus host disease (GvHD)	Complete remission of GvHD after stem cell infusion	Le Blanc et al, 2004[14]
GvHD	6 complete remission, 4 improvement	Le Blanc et al, 2005[15]
Osteogenesis imperfecta	Improved fracture rate and growth	Horwitz et al, 1999[16]
Breast cancer	No toxicity	Koc et al,[17]
Osteogenesis imperfecta	No toxicity, durable engraftment in 5 of 6 patients, acceleration of growth, decreased fracture rate compared to untreated controls	Horwitz et al, 2002[18]
Metachromatic leukodystrophy (MLD) and Hurler syndrome	No toxicity, low engraftment, no measurable clinical improvement	Koc et al, 2002[19]
Amyotrophic lateral sclerosis (ALS)	No toxicity	Mazzini et al, 2006[20]
Paraplegia	No toxicity	Moviglia et al, 2006[21]
Acute stroke	No toxicity	Bang et al, 2005[22]

References: (1) Stem Cells: Scientific Progress and Future Research Directions (2001) NIH Report. (2) Tögel F, et al. Dev Dyn 2007; 236: 3321-31. (3) Burt RK, et al. JAMA 2008; 299: 925-36. (4) Craig RM, et al 2001-2007, unpublished data, 2008. (5) Oyama Y, et al. Bone Marrow Transplant 2007; 40: 549-55. (6) Statkute L, et al. Ann Rheum Dis 2008; 67: 98-104. (7) Voltarelli JC, et al. JAMA 2007; 297: 1568-1576. (8) Vonk MC, et al. Ann Rheum Dis 2008; 67: 98-104. (9) Burt RK, et al. JAMA 2006; 295: 527-35. (10) Snowden JA, et al. J Rheumatol 2004; 31: 482-8. (11) Kang HJ, et al. Am Heart J 2007; 153: 237. e1-8. (12) Meyer GP, et al. Circulation 2006; 113: 1287-94. (13) Lunde K, et al. N Engl J Med 2006; 355: 1199-209. (14) Le Blanc K, et al. Lancet 2004; 363: 1439-41. (15) Le Blanc K, et al. Blood 2005; 106: a143. (16) Horwitz EM, et al. Nat Med 1999; 5: 309-13. (17) Koc ON, et al. J Clin Oncol 2000; 18: 307-16. (18) Horwitz EM, et al. Proc Natl Acad Sci 2002; 99: 8932-7. (19) Koc ON, et al. Bone Marrow Transplant 2002; 30: 215-22. (20) Mazzini L, et al. Neurol Res 2006; 28: 523-6. (21) Moviglia GA, et al. Cytotherapy 2006; 8: 202-9. (22) Bang OY, et al. Ann Neurol 2005; 57: 874-82.

Join Us!

The application form for membership of Genetics Specialty Chapter of Indian Academy of Pediatrics can be downloaded from the following link:

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

Multiplex Ligation-Dependent Probe Amplification (MLPA): A versatile technique in molecular diagnostics

Kausik Mandal, Shubha R Phadke
Department of Medical Genetics, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow
E mail: mandal.kausik@gmail.com

In the history of medical genetics, identification of correct number of chromosomes in mid 1950s was a major breakthrough. In the past three decades, DNA technology has revolutionized the diagnostics in medical genetics. Various cytogenetic as well as molecular genetic techniques like fluorescent in-situ hybridization (FISH) and its variations, polymerase chain reaction (PCR) and its modifications and comparative genomic hybridization (CGH) and its advanced version array-CGH, have been used to screen the genome or part of the genome for identification of mutations and loss or gain of DNA (copy number variations). Most of the known genetic disorders are known to be associated with hundreds of mutations and some with mutations involving more than one gene. Scientists are always in search of techniques which are fast, technically less demanding, cost effective yet sensitive enough to detect minutest variations in the genome. Multiplex Ligation-dependent Probe Amplification (MLPA) is a multiplex PCR method detecting abnormal copy numbers of up to 40 to 50 different genomic DNA or RNA sequences in a single reaction. For most hereditary conditions, gene (partial or whole) deletions or duplications account for less than 10 % of all disease-causing mutations, for some other disorders this is 10 to 30 % or even higher.[1,2] The inclusion of MLPA in clinical settings can therefore significantly increase the detection rate of many genetic disorders.

PRINCIPLE OF MLPA

There is a probe set (of two probes) for each target region. Using around 40 such probe sets, 40 different regions of the genome can be simultaneously amplified in one reaction. All the probe sets have common sequence (say F and R at each ends) which is similar in all probe sets used in a reaction (Figure1). These common sequences act as primers for PCR reaction. Each probe set for a target sequence has a stuffer sequence (attached to one of the probes). The length of the stuffer sequence is different for different probe sets, thus the amplification product of each probe set has a unique length. After the probes hybridize with the complementary sequences in the sample DNA, the two probes in a probe set are ligated by a thermostable ligase enzyme. If the target DNA does not have the complementary sequence, the probe will not hybridize and hence, will not get ligated. The ligation reaction is very specific; even a single base pair mismatch may hamper the ligation step. In the next step, all the ligated probe sets are simultaneously amplified by PCR, using a universal primer pair for the above mentioned F and R regions. In case of defect in ligation, further PCR amplification does not take place. The resulting amplification products of a commercially available MLPA kit range between 130 and 480 nucleotides in length. There is a difference of 7 to 8 base pairs between two neighboring PCR products. Hence, the PCR products can be separated by capillary electrophoresis. The peak height or peak area for each probe reflects the relative abundance of the target sequence in the DNA.

TECHNIQUE OF MLPA

The basic steps include DNA denaturation, addition of probe mix for overnight hybridization, ligation by a thermostable ligase, PCR of ligated products using a universal PCR primer pair and detection of products by capillary electrophoresis (figure 1). During each experiment of MLPA, at least five control samples are run along with around ten or more test samples. Interpretation of result can be done in two ways namely; visual examination of the peak profiles and comparing with a control, or statistical analysis by software specially designed for each probe mix. In the step 4 of the figure 1 the peaks representing subtelomeric regions of p arm of chromosome 5 are demarcated by arrows. The peak in upper panel (patient) is smaller as compared to the corresponding peak in the lower panel (control). The heights of other peaks in the upper and lower panel are comparable. This suggests that the patient has deletion involving the terminal part of p arm of one chromosome 5. The dosage quotient for each probe is calculated by dividing the normalized peak height in the patient by the mean of normalized peak heights of the controls. The result is depicted as mean ratio. If the value of ratio of the normalized peak height in the patient to the mean of normalized peak heights of the controls is 1.35 or more; it is taken as duplication and if it is 0.65 or less is taken as deletion. Relative likelihoods of the deletions and duplications is also calculated in the form of Odds ratios. High odds ratio of deletion: normal suggest high possibility of deletion and supports the results of peak height ratio. In general a ratio of normal to deletion less than 1:20 is taken as an indication for deletion. Same is true for duplication and if a ratio of normal: duplication is 1:20 or less then it is taken as suggestive of duplication, The representative data and its interpretation is given in step 5 of figure 1.

Figure 1: Principle and steps of MLPA

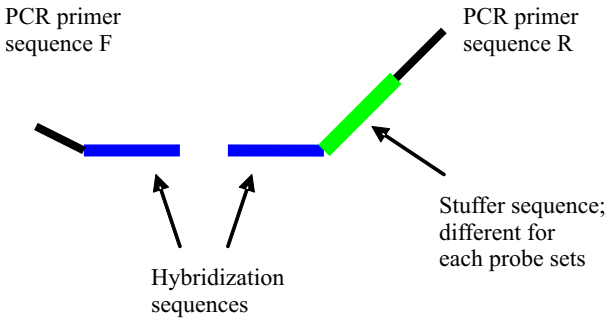
Step 1: Denaturation and hybridization – The probe will hybridize with complementary sequences in the sample DNA.

Step 2: Hybridized pairs of the probes will ligate with each other.

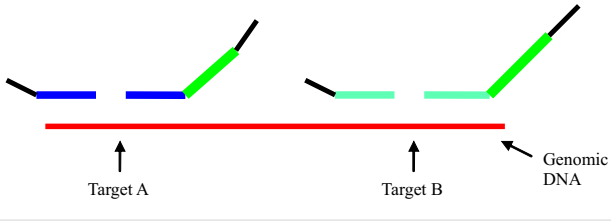
Step 3: PCR of the ligated products. Only ligated probes will be amplified.

Step 4: Capillary electrophoresis- The amplified products are separated on a sequencer

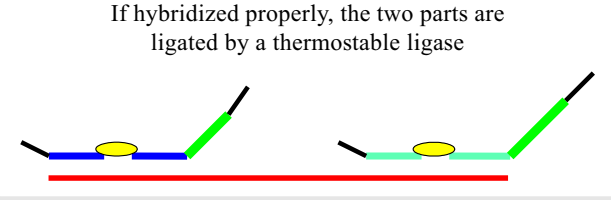
Step 5: Analysis



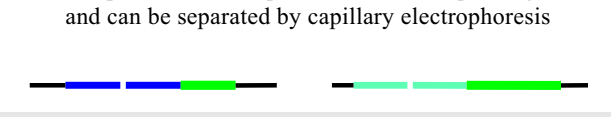
Probe



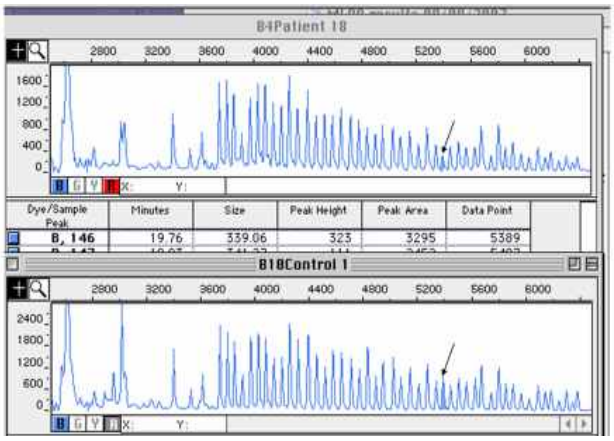
1. Denaturation and hybridization



2. Ligation



3. PCR of the ligated product



4. Capillary Electrophoresis

Probe mix used	Probe deleted or duplicated	Mean ratio	ODDS normal: deletion	ODDS normal: duplication	Result interpretation
Subtelomeric probes for all chromosomes	5p region	0.54	1:47	3:1	5p subtelomeric deletion
Probes for common microdeletion syndromes	CLDN5 (22q11.21)	0.41	1:20	2:1	DiGeorge syndrome
	GP1BB (22q11.21)	0.47	1:339	2:1	

5. Analysis of MLPA by calculation of mean ratio and odds with preformed excel sheet and interpretation of results: a representative example

STRENGTHS OF THE TECHNIQUE

- It requires a thermocycler (PCR machine) and a sequence type capillary electrophoresis (Sequencer), which are routinely available in most molecular biology laboratories.
- It is a multiplex technique, screening multiple areas of the genome simultaneously.
- Large number of samples can be handled simultaneously, with results being available within 24 hours.
- It is sensitive and requires very small amount of DNA (20 ng DNA).
- It does not require living cells as for karyotype.
- It identifies the frequent, single gene aberrations which are too small to be detected by FISH. It can be used to distinguish sequences differing in only one nucleotide by designing probes.[3]
- The technique is versatile, and can be designed for certain disorders and group of disorders with near similar phenotype.
- It is flexible. One can use few extra probes in addition to what is available commercially.
- It can be used to study RNA and epigenetic alterations (methylation) in the genome.
- It is reproducible (coefficient of variability of each probe

is 3% to 8 % when human DNA samples of 40-100 ng are analyzed).

- It is cost effective, when compared to other contemporary techniques[4].

WEAKNESSES OF MLPA

- It is a screening method, and is not as sensitive as sequencing or ARMS- PCR to detect point mutations. At present, MLPA probe sets have not yet been designed commercially, for detection of point mutations. Thus, as of now, this technique can detect only 10 to 15% of all mutations in single gene disorders in most cases (except in diseases like DMD where around 70% mutations are due to full exon deletions or duplications). Recently, new probe sets are being developed by scientists for some known point mutations for diseases like DMD and Marfan syndrome.
- It is not sensitive enough to differentiate between large and small deletions; since, whatever be the length of the deleted segment, the final analysis will detect it as a deletion of the segment for which the probe set was designed.
- Only known mutations can be detected. It is unlikely to precisely detect unknown mutations.
- It is not a direct method like FISH, where result can be visually interpreted. It requires statistical tools for interpretation of result.
- It cannot directly detect mosaicism; recently scientists are trying to make it possible.
- As this technique detects relative abundance of a specified segment of a genome as compared to other parts of the same genome, it does not detect triploidy or tetraploidy, where the DNA in the whole genome is proportionally increased.
- Some copy number variations are polymorphisms and it may be difficult to decide whether a particular deletion is a variation seen in normal people or a disease causing mutation.
- Sequence variations near the target site may influence the peak height.
- A detected copy number variation needs to be confirmed by another probe mix or other methods like FISH.

SCOPE OF MLPA

This technique is widely used for various disorders. The whole list of diseases is extensive. Each probe mix is a mixture of probe sets designed to detect copy number variations for diseases with similar manifestations or single gene disorders with known deletions and duplications.

It is being extensively used for mental retardation and other neurogenetic disorders. Identification of etiology of cases with mental retardation without obvious etiology or clinical clue to etiology is a challenge to clinicians and researchers. Small deletions and duplications of various parts of genome have been found to cause mental retardation. These deletions / duplications may be too small to be detected on traditional method of chromosomal analysis. The deletions / duplications of the ends of chromosomes (telomeres and subtelomeric regions) are found in about 5 to 7% cases of mental retardation without obvious cause. MLPA has been found a very useful technique to search for deletions / duplications of subtelomeric regions. For screening of subtelomeric regions of chromosomes in cases of mental retardation, more than one probe mixes are available commercially. Probe mixes are also available for common microdeletion syndromes and X-linked mental retardation. This makes MLPA an important modality of investigation in cases with mental retardation.

In addition to microdeletion syndromes, MLPA is also useful in identification of mutations for some monogenic conditions. Duchenne muscular dystrophy (DMD), Spinal Muscular Atrophy (SMA) and Limb-girdle muscular dystrophy (LGMD) are neuromuscular disorders, which can be screened by this technique. Deletions and duplications in Dystrophin gene account for more than 70% cases of DMD. Dystrophin is a very big gene and the deletions are found in all parts of the gene. MLPA being able to perform 40 PCRs (multiplex) in one test it can test for all areas of the gene in one test. Probe mixes are also being designed for diseases like retinitis pigmentosa, neurofibromatosis, Parkinsonism etc.

The other important use of the technique is for prenatal diagnosis of aneuploidy. Probe mix is commercially available for chromosomes 21, 18, 13, X and Y. Probes for subtelomeres and common microdeletion syndromes can also be used for rapid screening in prenatal samples. However, it is always advisable to use another method like FISH along with this technique for confirmation.

MLPA is also being used for cancers and hereditary cancer syndromes like neuroblastoma, retinoblastoma, breast cancer, Li-Fraumeni syndrome (LFS), ataxia-telangiectasia, etc.

The technique of MLPA is being modified to detect methylation defects in disorders like Beckwith-Wiedemann Syndrome, Russell-Silver Syndrome, Prader Willi syndrome and Angelman syndrome. It is also used for m-RNA analysis, pharmacogenetics, basic research (e.g. cytochrome P-450, repair genes) and animal experiments.

It is evident from the above list that the scope of this technique is immense and researchers are developing new probe sets for research into newer disorders. It is not far when this technique will be incorporated into routine diagnostics for most genetic disorders. However, it is to be emphasized that at present, MLPA is not useful to detect point mutations and for most single gene disorders this technique will detect only deletions and duplications, which is not more than 10 to 15% of all mutations.

References: (1) Aretz S, et al. J Med Genet 2007; 44: 702-9. (2) Redeker EJ, et al. Mol Vis 2008; 14: 836-40. (3) Schouten JP et al. Nucleic Acids Res 2002; 30(12): e57. (4) Palomares M et al. Clin Genet 2006; 69: 228-33

Methylmalonic Aciduria & Homocystinuria, Cobalamin C (cblC) type

Ratna D Puri, Sunita Bijarnia, Seema Thakur, Verma IC

Dept of Genetic Medicine,
Sir Ganga Ram Hospital, New Delhi.

Corresponding author: **Dr Ratna D Puri**, Email: ratnadpuri@yahoo.com

Abstract:

We report a child with methylmalonic aciduria and homocystinuria, cobalamin C type and the mutations in her.

CASE REPORT

A 3 years old girl, born to non consanguineous parents, presented to the emergency with history of rash of 25 days duration and altered sensorium since ten days. The rash was pruritic and involved the limbs. Fifteen days after the onset of rash there was history of progressively increasing unsteady gait and alteration of sensorium. There was no history of seizures or fever. Her developmental history revealed mild delay in both motor and language milestones.

On examination there was a faint maculo-papular rash on the limbs. She did not recognize her parents and her speech was severely affected. Hypotonia was present with preserved reflexes. While she was being investigated, she gradually improved with supportive therapy including vitamins but had resulting deficit in speech, behavior abnormalities and unsteady gait.

Investigations showed normal hemogram with no macrocytosis or anemia, peripheral smear, electrolytes, renal and liver function tests. There was no evidence of infection either bacterial or viral (including herpes simplex, Japanese encephalitis and enterovirus). CSF examination was normal. EEG showed generalized background slowing. On MRI there was dysmyelination in the parietal periventicular deep white matter bilaterally.

There was no acidosis and blood ammonia and lactate were normal. Autoimmune markers tests were normal. Skin biopsy did not show vasculitis. Echocardiography showed mild left ventricular hypertrophy. DMSA renal cortical scan was normal. Acylcarnitine profile was normal on mass spectrometry analysis. Urine Gas Chromatography Mass Spectrometry analysis showed increased excretion of methylmalonate (250 mmol/ml, normal: 0.3 - 3.6). Her serum homocysteine levels were high (167 micromol/l, normal: 3.36 - 20.44). Vit B 12 and folic acid levels were normal.

In view of the clinical profile of mild developmental delay and neurological decompensation with increased serum homocysteine and urine methylmalonate she was suspected to have cobalamin deficiency. Gene mutation analysis in the MMACHC gene revealed two heterozygous mutations in exon 3 of the gene (R111X and R132X) confirming the diagnosis of cobalamin C deficiency (cblC def).

She was treated with a low protein diet, methylcobalamine, folate, betaine, carnitine and vitamin E with follow up of serum homocysteine levels which have considerably decreased from the previous values. Hydroxycobalamin is the preferred preparation for treatment but this is not available in India.

DISCUSSION

Inborn errors of cobalamin (Vit B12) metabolism are rare autosomal recessive disorders. They are classified into nine complementation groups (cblA-cblH and mut). CblC is the commonest of these disorders and best understood. Cobalamin is an essential cofactor for two enzymes, methionine synthase (MTR) and methylmalonyl CoA mutase (MUT), (Fig1). The biochemical abnormalities in patients with cobalamin disorders are a reflection of the role of this cofactor in enzyme function. (Fig 1) Abnormalities in this pathway can cause three syndromes:

i) Defects of MUT or cofactor adenosylcobalamin (cblA, cblB) cause isolated methylmalonic aciduria.

ii) MTR and cblE, cblG, and cblD deficiency lead to elevated plasma homocysteine levels.

iii) Deficiency in the earlier steps of B12 metabolism where both MUT and MTR enzymes are affected. This results in methylmalonic aciduria and hyperhomocysteinemia (cblC, cblD, cblF).

While evaluating patients for these disorders nutritional deficiency of vitamin B12 must be excluded.

The triad of methylmalonicaciduria on urine analysis by gas chromatography – mass spectrometry (GC-MS), hyperhomocysteinemia and normal serum cobalamin levels, as was present in our patient, is required to diagnose cblC disorder. It differentiates patients with MUT enzyme deficiency with isolated methylmalonic aciduria and other

causes of isolated increased homocysteine levels.

Specialized assays involving incorporation of radiolabeled precursors into macromolecules in patient derived cell lines along with complementation group analysis can help establish the diagnosis of the specific complementation groups.

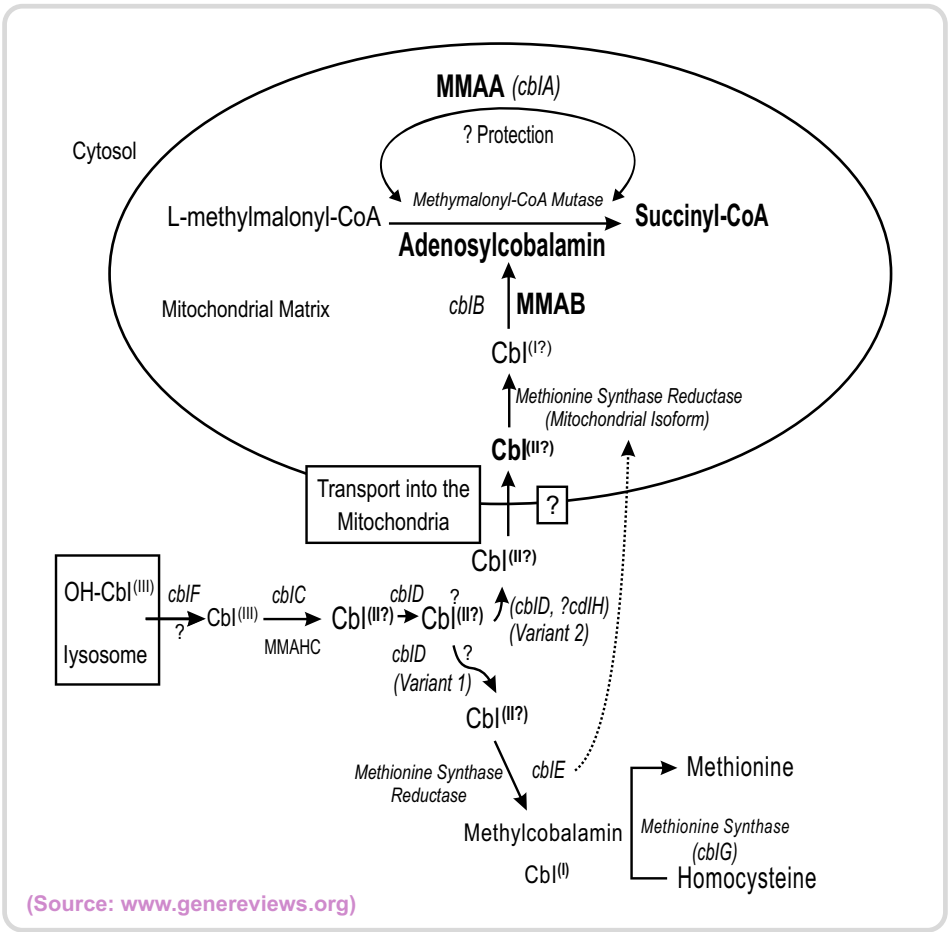
Clinical presentation of cblC deficiency is heterogeneous. Perinatal affection can manifest as small for date and microcephaly in the neonatal period. Some neonates present in an acute metabolic crisis with acidosis, poor feeding and failure to thrive. Other infantile manifestations are hemolytic uremic syndrome and infantile spasms. Failure to thrive, developmental delay, hypotonia, seizures, megaloblastic anemia and microcephaly are other presentations in the first few years of life. Late manifestations include insidious developmental delay with no metabolic deterioration or neuroregression with leukodystrophy on MRI in a previously normal child. Patients with isolated renal disease are also reported.

Cobalamin C deficiency occurs due to mutations in the MMACHC gene. Molecular analysis confirms the diagnosis in the proband, essential for prenatal diagnosis and is helpful for carrier ascertainment in other family members.

Some genotype phenotype correlation exists with nonsense and frameshift mutations having a severe phenotype. Our patient was heterozygous for a mild and severe mutation explaining the intermediate phenotype. The c.394C>T (p.R132X) mutation, primarily associated with late-onset disease, has also been reported in other Asiatic – Indian patients.

This case report reiterates the importance of evaluating for a metabolic disorder in children presenting with a sepsis like illness and encephalopathy. Baseline investigations for acidosis, serum ammonia, lactate, blood sugar, plasma homocysteine, plasma amino acid quantification along with acylcarnitine measurements by tandem mass spectrometry and organic acid estimation by GC- MS are the required panel for analysis of a suspected metabolic defect.

Fig – 1: Pathways utilizing cobalamin derivates



(Source: www.genereviews.org)

Contributed by:
Dr Shubha R Phadke
e-mail: shubha@sgpgi.ac.in

Genetic defect in Wilms tumor – Similar to Beckwith Wiedemann syndrome[1]

Beckwith - Wiedemann syndrome (BWS) is an overgrowth syndrome caused by abnormalities of imprinted region on chromosome 11p15. The maternal and paternal copies of genes in this region express differently. Individuals with BWS are at increased risk of Wilms tumor. Scott et al identified abnormalities of 11p15 in 3% of cases of sporadic Wilms tumor which were not associated with BWS. In most cases, the defects observed were paternal uniparental disomy or hypermethylation of the H19 differentially methylated region. Bilateral tumors were more common with an 11p15 defect.

Fragile X premutation and neuropsychological deficits: No impact before 50 years of age[2]

Fragile X mental retardation is the commonest cause of inherited mental retardation and accounts for 1 to 3% cases of mental retardation. The mutation in FMR1 gene is the cause of Fragile X mental retardation. The mutation is a dynamic mutation and there is increase in the triplets (CGG) in FMR1 gene. Normal individuals have <50 CGG repeats while individual with Fragile X mental retardation have more than 200 repeats. The individuals with 55 to 199 repeats are known as premutation carriers and were thought to be asymptomatic carriers. Now it is known 20% of females with premutation have premature ovarian failure and 30% of males over the age of 50 develop tremor/ataxia disorder associated with cognitive deficits, psychiatric symptoms and brain atrophy. Hence, Hunter et al studied premutation carriers (138 males and 506 females) in age group of 18 to 50 years for various neuropsychological functions like intelligence, memory and attention. No evidence for a difference in neuropsychological profile among carriers and non-carriers of the FMR1 premutation in adults under the age of 50 was noted in their study. This suggests that the premutation allele does not have a global impact on neuropsychological functioning of carrier adults under age of 50 year.

Down syndrome: paving the road to treatment[3]

Increased expression of genes on chromosome 21 has been demonstrated in Down syndrome. But it is not clear which genes are contributing to the phenotype of Down syndrome. DYRK1A is one of the genes on chromosome 21 and has been shown to have 1.5 fold expressions in brain of Down syndrome. Ortiz-Abalia et al studied mouse model of Down syndrome with over expression of Dyrk1A. They reduced the expression of Dyrk1A by gene therapy. The protein levels normalized after this gene therapy and there was reduction in the hyperactivity with improved coordination. This provides a ray of hope for the prevention/treatment of mental handicap in Down syndrome!

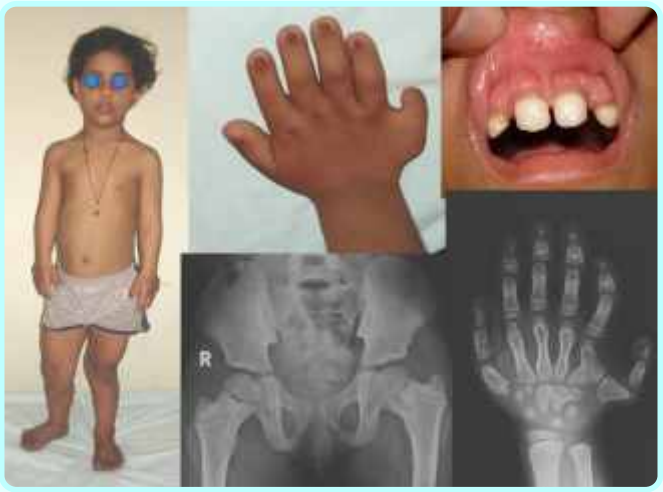
Definitions all the way[4]

American Journal of Medical Genetics has come out with a special issue 'Elements of Morphology: Standard Terminology' which comprises an editorial, introduction and six articles providing recommendations for the description and definition of human phenotypic variations. Every dysmorphologist would agree with the editor's claim that these pieces represent a timely and substantial contribution to the field of medical genetics. Over 30 clinicians working in the field of dysmorphology were involved in this work to standardize the terms and reach consensus regarding their definitions. The utility of this work is enhanced by the color illustrations and the descriptions of how each body part is measured. Do not miss this issue!

References: (1) Scott RH, et al. Nat Genet 2008; 40: 1329-34. (2) Hunter JE, et al. Am J Hum Genet 2008; 83: 692-702. (3) Ortiz-Abalia J, et al. Am J Hum Genet 2008; 83: 479-88. (4) Am J Med Genet Part A: 149A: 1-127.

4

This four year old girl presented with disproportionate short stature. 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 3

Pseudoarthrosis tibia in neurofibromatosis 1 (OMIM No 162200)

The child has pseudoarthrosis of tibia. In presence of café au lait spots, this finding confirms the diagnosis of neurofibromatosis 1. Five percent of individuals with neurofibromatosis type 1 present with congenital long bone pseudoarthrosis. In 50-80% of patients with congenital long bone pseudoarthrosis the etiology is neurofibromatosis. Tibia is the most common long bone involved. Pseudoarthrosis of tibia is congenital anterior tibial bowing which progresses to spontaneous fracture and subsequent fibrous nonunion. Tibial shaft shows narrowing towards the apex of the curve. (see ‹http://www.e-radiography.net/radpath/p/pseudoarthrosis.htm›)

Congenital anterolateral tibial bowing with polydactyly is rare disorder, which can be differentiated from NF1 by absence of neurocutaneous markers and presence of polydactyly

Correct responses:

Aditi Dagli, Gainesville, USA

Mohan Gupta, Agra

Mohandas Nair, Calicut

Neerja Gupta, New Delhi

Ravi Goyal, Kota

Yesodha Thani, via e-mail

4TH INTERNATIONAL CONFERENCE ON

BIRTH DEFECTS AND DISABILITIES IN THE DEVELOPING WORLD

Le Meridien Hotel, New Delhi

October 4-7, 2009

The major theme **"Translating Research into Cost-Effective Services for Care and Prevention"**

Early Bird Registration till **15th April, 2009**

Conference Indian Delegates	Accompanying Delegate	Student / Parent	CME/ Workshop: Delegate	Student / Parent	Banquet Dinner
Rs. 7,000	Rs. 5,000	Rs. 6,000	Rs. 2,000	Rs. 1,500	Rs. 3,000

Last date of submission of Abstracts: **15th April, 2009**
Financial support will be provided to those whose abstracts are selected for presentation.
For further details visit website: **www.icbd2009.com**

Address for Correspondence:
Organizing Chairman: **Dr Ishwar Verma**
Chairman & Head, Department of Genetic Medicine
Room No 25, Sir Ganga Ram Hospital, Rajinder Nagar, New Delhi –110060, INDIA
Tel: +91-11-2586 1767, 4225 1382, Fax: +91-11-4225 1034, E-mail: icverma@gmail.com, icbd@icbd2009.com

Bringing hope to those who think they have none.

One of the world's leading biotechnology companies, Genzyme is dedicated to making a major positive impact on the lives of people with serious diseases.

With many established products and services helping patients in nearly 100 countries, Genzyme is a leader in the effort to develop and apply the most advanced technologies in the life sciences. The company' products and services are focused on rare inherited disorders, kidney disease, orthopaedics, cancer, transplant and immune disease, and diagnostic testing.

genzyme

Genzyme India Pvt. Ltd., 1st Floor, Technopolis, Golf Course Road, Sector-54, Gurgaon 122001.

www.genzyme.com