genetic CLINICS

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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 : geneticsiap@gmail.com

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Instructions to authors:

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.
GeNefOcuS	These are usually invited commentaries on a specific topic by the experts in the field. If you have any idea, please contact the editor.
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.Only the author's name and email id will be published in the journal.
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. The reference is indicated in the text by the superscripted numbers after the full stop. List only the important references.

Cover page: The cover page should contain the category of article, title, names of the contributors, affiliation and e-mail address for correspondence. Full address for correspondence will not be published and is not necessary.

Generally the articles should include a summary (abstract), introduction, materials/patients and methods results and discussion. The case reports should include a summary, case report and discussion.

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Editorial

Research in Medical Genetics in India: The Road Ahead

Dr Shubha Phadke

Professor & Head, Department of Medical Genetics

Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, India

E mail: shubharaophadke@gmail.com

The last two decades have seen evolution of Medical Genetics as a medical sub-specialty. The pace of the discoveries in the field is really overwhelming. It can be said that despite India being one of the most populous countries, the specialty of Medical Genetics is still in its infancy. More than a dozen qualified clinical genetics centers are trying their best to provide medical care and diagnostic services at present. Research into clinical, pathophysiologic and therapeutic aspects of genetic diseases has taken center stage and in fact we can sense a fierce competition amongst the scientists. Molecular and computational facilities have enhanced the speed of research tremendously.

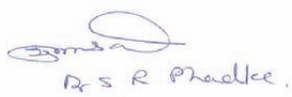
The efforts of scientists both in the clinic and in the laboratory contribute to the successful research in the field of medical genetics. In India, there are a sizeable number of centers and basic scientists working in the field of genetics. The younger generation appears to be fascinated by the exciting research developments in the field of medical genetics and appears to opt for a career in this field. I was very impressed by the number of young enthusiastic scientists who attended the recently held annual conference of Indian Society of Human Genetics in Lucknow. I may not be wrong if my estimate of young participants in the conference was more than 90% of the delegates. They presented their research work on varying aspects of genetics like cancer genetics, monogenic disorders, population genetics, multifactorial disorders, etc. The quality of research in the twenty-first century in India has definitely improved and a lot of the work of Indian scientists is being published in journals of international repute. All the research is intended to help the patients and their families by way of diagnosis, treatment and prevention directly or indirectly. But broadly, it can be divided into two greatly overlapping areas. One, with results of immediate clinical significance like identification of causative gene, spectrum of mutations in our population, trial of new therapies, screening programs, development of new diagnostic techniques and the other, with more in depth research to understand pathogenesis, to develop new therapeutic strategies, etc. Both are interrelated and equally important. But those with immediate applications in patient care are perceived to be important by the clinicians. Hence, most of medical research in India started with study of the spectrum of mutations of monogenic disorders like beta thalassemia, Duchenne muscular dystrophy, etc in Indian patients and establishment of DNA based diagnosis. The article by Padma and Dalal in this issue reviews and highlights the issue of development of PCR based diagnostics for clinical use. These PCR based tests are relatively easy, do not need radioactivity and can be modified in many ways to detect mutations of any kind.

The availability of DNA based diagnosis is expanding in the spectrum of disorders and centers providing these investigations. The data about mutations in Indian patients of common and rare genetic disorders is useful to devise the diagnostic strategies for Indian patients and also provides insight into population dynamics. The other important area of clinical related research is identification of causative genes for rare and new monogenic syndromes. India is a gold mine for rare autosomal recessive phenotypes of malformation syndromes, skeletal dysplasia and neurological disorders. Many new and rare syndromes have been reported from India. This is due to high prevalence of consanguinity and large population. But India had not contributed to gene mapping. Identification of STIL gene for autosomal recessive microcephaly (MCPH7) by Girimaji and his group, from Indian Institute of Science, Bangalore and a new locus DFNA59 for autosomal dominant non-syndromic deafness by Anand et al, at Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore, in the recent years need special mention in this regard.

With these successes in 2009 we may look forward to more activity in the direction of gene mapping in next few years. Out of about 5000 or more monogenic phenotypes, molecular basis of more than half is still unknown. With latest techniques based on microarray and exome sequencing, identification of causative gene has become simpler than before. Now small families can also be of use

for identification of the causative genes. Over the last two decades the number of clinicians trained in medical genetics and clinical genetics centers in India have increased. These centers have loads of patients with genetic disorders and with the expertise in clinical genetics can serve as a good source of clinical material of well delineated genetic syndromes. Close interaction between clinical and basic geneticists will be of great help in prospering research in this direction. Though most of the monogenic disorders are very rare as compared to common multifactorial disorders; understanding genetic bases of these is an important way to learn about developmental genetics and biology. Common variants in the genes of rare monogenic disorders are likely candidates for predisposing factors for common multifactorial disorders. Hence, I urge the funding agencies like Indian Council of Medical Research, Department of Biotechnology, Department of Science and Technology, etc to give priority to characterize mutations in Indian patients and mapping of genes for monogenic disorders.

Looking back at 1990s I feel that the research in medical genetics in India has come of age but we still have a long way to go. Collaborative efforts of clinical and basic geneticists with good support from funding agencies will produce novel results in research which in turn will help to improve care of patients and families with genetic disorders in India and the world over.



Shubha Phadke
1st July, 2010

Preconception Care

[1]Aditi Vian Varma, Girisha KM

1Medical student, Genetics Clinic, Department of Pediatrics, Kasturba Medical College, Manipal-576104

Email: girishkatta@gmail.com

INTRODUCTION

Interventions that aim to identify and modify biomedical, behavioral, and social risks to a woman's health or pregnancy outcome through prevention and management may be considered to encompass preconception care.[1] Elaborating this definition, this article hereby aims to address every woman of reproductive age irrespective of her intentions of pregnancy and every man who can father a child that he too does his part in this circle of care. What choices the mother makes in regard to her lifestyle and nutrition habits go a long way not only in affecting pregnancy outcome and fetal health but also the adult life of the offspring.

GOALS OF PRECONCEPTION CARE

A good preconception care should focus on the health of woman and man in all the aspects that optimize the outcome of the pregnancy, both for the baby and the to-be-mother.

WHEN DOES PRECONCEPTION CARE START?

Preconception care starts much before the pregnancy itself. Every adolescent girl, in fact, is included. Teenage girls are targeted for two main reasons: to optimize their weight and, to make sure of their nutrition intake. With the new era of super slim models that the media portrays, young women often suffer from underweight and eating disorders. Female athlete syndrome (low body mass index, menstrual irregularity and eating disorders), bulimic and anorexic nervosa disorders are on the rise. Women suffer not only from menstrual irregularities such as amenorrhea, but also low body mass index (BMI) which can lead to infertility. Low bone mass and nutritional deficiencies can lead to poor pregnancy outcomes. Overweight girls on the other hand, must be encouraged to exercise and regulate their dietary habits. We will see subsequently in this article why health promotion in adolescent age group is important in determining pregnancy outcome.

As most organs form 3-7 weeks after the last menstrual period, any teratogenic effect may occur by this time and as it is not always easy to predict the time of conception, it is ideal that every woman in the reproductive age group who comes to a primary health center receives due attention to the components of preconception care.

COMPONENTS OF PRECONCEPTION CARE

Age: Age of a woman during conception is important. Young girls below the age of 18 may not be prepared for pregnancy and raising up of children; they are also usually underweight leading to prematurity and low birth weight babies. Women at 35 years and above at the expected time of confinement, are at an increased risk for chromosomal disorders such as Down syndrome (most common) and also suffer from a more rigid pelvis which could pose a problem during labor. Women with advanced age may be sensitized regarding the risk of Down syndrome and options for prenatal diagnosis. Even the age of the father should be asked for as there is growing evidence showing that the older the father's age, the greater is the risk of new mutations of autosomal dominant disorders like Marfan syndrome and may be schizophrenia.[2]

Medical conditions: A thorough medical history should be an integral part of preconception care. Diabetes mellitus and hypertension are common problems. Gestational diabetes mellitus can complicate pregnancy by giving rise to hyperinsulinemia in the fetus, macrosomia and obstetric complications leading to cesarean deliveries. Hypertension complicating pregnancy may result in pre-eclampsia and rarely eclampsia which compromises the fetal outcome significantly. These conditions do not stop here and the child born to such a mother grows up with an increased risk of adult onset diseases such as diabetes, hypertension and hypercholesterolemia, especially if the mother is obese as well. In men, conditions such as diabetes mellitus, varicocele and epididymitis if left untreated can also reduce sperm quality and count. Appropriate interventions are needed to address the medical conditions in the preconception period itself.

Drugs: Several therapeutic and non-therapeutic agents are used by many women of reproductive age. Antiepileptics for seizure disorder, immunosuppressants (eg: cyclophosphamide is extremely teratogenic), anticoagulants (warfarin) and antitubercular agents are

the common medications that have a potential to adversely affect the fetal outcome by teratogenesis. Consideration should be given to use less teratogenic alternatives during the pregnancy and preconception period. However, some drugs are to be continued in the best interests of the woman (anti-epileptics, anti-tubercular agents). All non-steroidal anti-inflammatory drugs should be discontinued by 27 weeks of gestation. Methotrexate and leflunomide are extremely teratogenic and should be stopped both in men and women planning a pregnancy. Aspirin must be stopped if planning a pregnancy. Detailed history regarding use of any topical agents containing medications like retinoic acid (an ingredient in cosmetics) should be asked for, as these have potential teratogenic effects and should be stopped at least 3 months prior to planning a pregnancy. Some cosmetic applications should also be checked as they contain lead –a potential neurotoxin. Women who are survivors of cancer and who are receiving radiation or chemotherapy should be further evaluated before pregnancy. Often, it is advised that the couple plan their pregnancy at a later date after the completion of therapy with a teratogen (chemotherapy for cancers). A history of allergies is important so as to be aware of what medications are to be avoided during pregnancy. Every physician needs to be aware of the possible teratogenic effects of various drugs and the measures to be taken.

Family history: A family history, including consanguinity and diseases present in other members of the family should be taken. A complete three generation family medical history including ethnicity (e.g. Punjabis have a high incidence of beta thalassemia, French Canadians-Tay Sachs disease) must be asked for. Details from personal or family history that require further counseling such as history of chromosomal disorders, clotting disorders, developmental delay/mental retardation, early infant deaths or Sudden Infant Death Syndrome (SIDS), heart or neural tube defects, thalassemias, thrombophilias, orofacial clefts, sickle cell disease or trait (common in many tribes in several parts of India) and recurrent miscarriages in the family, should be asked for. Families with a medical condition may require specific tests and genetic counseling (carrier testing for hemophilia, Duchenne muscular dystrophy, fragile X mental retardation etc). The opportunity may also be used to inform the couple about possible prenatal testing for specific diseases whenever feasible. In several communities in India, especially the southern states of Karnataka, Tamil Nadu, Andhra Pradesh and Muslims it is common to find a high proportion of consanguineous marriages. Though a very few studies are available on the incidence of genetic abnormalities in such families, it is estimated that the risk of a birth defect increases to 3-5% from the background risk of 2-3%. However in such circumstances, a comprehensive pedigree analysis, screening for common conditions (thalassemia) and a malformation scan may be warranted.

Alcohol and substance abuse: Addictions such as alcohol and smoking should be enquired into. Prenatal alcohol use is considered a leading preventable cause of birth defects and developmental disabilities in the United States.[3] There is no established safe level for alcohol consumption during pregnancy. Spontaneous abortions, prenatal and postnatal growth restriction, birth defects, neurodevelopmental deficits, mental retardation are all part of the spectrum of fetal alcohol syndrome. Maternal smoking if eliminated during pregnancy would reduce infant deaths by 5% and reduce low birth weight singleton babies by 10%.[4,5] In men, nicotine and other chemicals in cigarettes can induce oxidative damage to sperm DNA. Substance abuse is a strict no for all the prospective parents.

Occupational hazards: Social and occupational history should be inquired both from man and woman. An ongoing exposure to metals, solvents, pesticides at work and any other hormonal or endocrine disruptors can impair sperm quality leading to infertility, miscarriage and birth defects. Women who have hobbies or work in artistic vocations exposing them to oil based paints, heavy metals such as lead used in stained glasswork, painting with non-latex based paints that are solvent based and contain metal and pigment, and use of heat guns to remove old paint and wall paper, jewelry making and metal tampering involving melting and soldering of metals are at risk as all these agents can be harmful to the developing fetus. Lead exposure can be harmful to the fetus. Lead being a potential neurotoxin; it can also be passed through breast milk to the feeding infant. It is also harmful to young children. If lead levels are elevated, dietary calcium supplements may minimize lead mobilization modestly, and increasing the amount of iron and calcium in the diet reduces the absorption of ingested lead. Gardening activities which involve exposure to pesticide, herbicides and rodenticides should also be avoided.

Diet and weight: Dietary history and weight assessment are very important in preconception care. As mentioned earlier, women who are underweight when entering the state of pregnancy are at a potential risk of having babies with intra-uterine and post-natal growth retardation, prematurity and low birth weight. A report suggests that infants born to underweight mothers (pre-pregnancy BMI ‹18.1kg/m^2) were 3 times more likely to have gastroschisis compared to infants born to normal weight mothers. [6] Maternal obesity on the other hand is associated with neural tube defects, low APGAR score, sometimes stillbirth, postpartum anemia in fetus, gestational diabetes, hypertensive and thromboembolic disorders in mother, shoulder dystocia during labor and increased incidence of cesarean deliveries.

Clinical studies have shown positive association between a healthy diet during preconception period and pregnancy and improved birth outcomes. All primary health care set-ups administer iron and folic acid, along with calcium tablets to all pregnant women. Iron deficiency is very prevalent in India. Recommended iron intake is 18 mg/day for women and 27 mg/day for pregnant women. The fetus may suffer from intra-uterine growth restriction and prematurity among others, in a mother who is iron deficient. In the mother, cardiac failure secondary to blood loss especially if she is predisposed to heart disease is the real danger of anemia. Neural tube defects are the second most common major congenital anomaly in the world. Dietary folate is known to reduce this risk. It is recommended that all women receive 400 mcg of folic acid daily in the peri-conceptional period. One way to time folic acid supplementation is that all the women who stop contraception to plan for a pregnancy start taking folic acid. Obese and diabetic women with poor glycemic control, women with seizure disorder on antiepileptic medication or those with prior neural tube defects are at increased risk for folic acid deficiency and are advised to have 4 mg of folic acid supplementation on a daily basis. Several countries have started fortification of wheat flour to decrease the incidence of neural tube defects. Some evidence shows it is much more beneficial to take multivitamins (especially in vegans and in women who drink alcohol-though they should not drink at all during pregnancy!) along with 400 mcg of folic acid –it reduces the incidence of malformations such as orofacial clefts, urinary tract defects and omphalocoele[7,8,9]

Iodine is an important dietary component in the peri-conception period and pregnancy. Recommended intake is 150 mcg for adults (› 12yrs of age) and 200 mcg for pregnant and lactating women. Inadequate iodine intake can lead to a spectrum of disorders from neonatal goiter and hypothyroidism, cretinism, mental retardation and can include stillbirth and abortion. Fetal thyroid concentrates iodine and synthesizes thyroid hormone by 10-12 weeks gestation, this being responsible for development of fetal central nervous system and myelination. Deficiency of iodine, hence, can lead to cognitive impairment, permanent mental retardation and cretinism.

Harmful exposure through diet: Methylmercury (potential neurotoxin to developing fetus) bioaccumulates through the food chain, exposure occurring primarily through consumption of seafood, freshwater fish, and shellfish.[10] Canned food packed in epoxy (white plastic container liners) are also best avoided.

Immunization: Vaccinations that are recommended in preconception care include hepatitis B and measles, mumps, and rubella vaccines. Hepatitis B can be transmitted to offspring vertically through the birth canal during delivery and can lead to chronic hepatitis and risk of hepatocellular carcinoma in the child. Limb atrophy, scarring of skin and extremities along with central nervous system and eye manifestations are complications of varicella in the first trimester. There is also risk of transmission of the disease to the fetus during the first and second trimesters. Congenital rubella syndrome, a complication of rubella virus infection is prevented by immunization of all adolescent girls. Measles during pregnancy with risk of spontaneous abortions, low birth weight and prematurity can be prevented by vaccination. In India all women receive 2 doses of tetanus toxoid 4 weeks apart as a preventive measure against neonatal tetanus.

Personal and psychosocial issues: Inquire in all women about their personal and psychosocial history. Psychiatric disorders during pregnancy are associated with poor obstetric outcomes, higher risk of postpartum psychiatric illness and increased rates of substance abuse. The mother pays less attention to her prenatal care and after child birth this tendency will lead to poor care of infant and subsequent adverse outcomes.

Some key issues to address in preconception care in Indian context

- Age of mother
- Evaluation of three generation pedigree
- Occupation
- Obstetric history
- Physical examination
- Maternal disease (diabetes mellitus)
- Rubella vaccination
- Drug intake (antiepileptics, anticoagulants, alcohol)
- Beta thalassemia screening
- History of mental retardation, stillbirth, malformations and recurrent abortions
- Enquire about common genetic disorders in the family: thalassemia, hemophilia, Duchenne muscular dystrophy, spinal muscular atrophy or other neuromuscular diseases
- Screening for diseases specific to the population and ethnicity

Summary and conclusions

A woman planning pregnancy needs to be advised on her lifestyle and diet. A comprehensive medical and family history including factors mentioned above should be taken. Necessary counseling should be given in case of positive genetic or family history. Examination and diagnostic tests to rule out anemia and other medical conditions, treatment of any infections in her and her partner and diet and lifestyle modifications if necessary, should be promoted in the couple, so that they may enter pregnancy in an optimum state. If a woman is not planning pregnancy, then contraceptive advice should be given, so that she may not have an unwanted pregnancy. Also disease preventive and health promoting measures should be encouraged, both in her and her partner, so that when they do plan a pregnancy, they will be in a good and healthy condition for it. It is envisaged that preconception care is an integral part of every woman's primary health care and also includes health care for men, so that the final outcome is a healthy mother, healthy baby and healthy family.

References:

1. Centers for Disease Control and Prevention. Morb Mortal Weekly Rep 2006; 55: 1-23.
2. Malaspina D, et al. Arch Gen Psychiatry 2001; 58: 361-7.
3. American Academy of Pediatrics. Pediatrics 2000; 106: 358-61.
4. Salihu HM, et al. Matern Child Health J 2003; 7: 219-27.
5. Ventura SJ, et al. Pediatrics 2003; 111: 1176-80.
6. Lam PK, et al. Epidemiology 1999; 10: 717-21.
7. Willett WC, et al. N Engl J Med 2001; 345: 1819-24.
8. Goh YI, et al. J Obstet Gynaecol Can 2006; 28: 680-9.
9. Itikala PR, et al. Teratol 2001; 63: 79-86.
10. National Academy of Sciences. Toxicological Effects of Methylmercury. Washington, DC: National Research Council: 2000.

Triplet Primed PCR (TP-PCR) – A Versatile Method For Molecular Diagnosis Of Triplet Repeat Disorders

Padma Priya T, Ashwin Dalal

Diagnostics Division
Centre for DNA Fingerprinting and Diagnostics, Hyderabad, Andhra Pradesh
E-mail: ashwindalal@gmail.com

INTRODUCTION

Trinucleotide repeats are highly polymorphic regions in the human genome which act as key players in a number of diseases known as triplet repeat disorders. Of these disorders, Friedreich ataxia (FA), Myotonic dystrophy type 1 (DM1) and Fragile X syndrome (FRAX) are caused by expansion of triplet repeats in the non-coding regions of corresponding genes. The other diseases caused due to triplet repeat expansion include Huntington disease, Spinocerebellar ataxias, etc. Here we describe application of a modified PCR assay for the diagnosis of Friedreich ataxia, Myotonic dystrophy and Fragile X syndrome.

OVERVIEW OF THE DISEASES

FA is an autosomal recessive disease caused by expansion of GAA repeats present in the first intron of frataxin or FRDA gene resulting in deficient frataxin mRNA and protein levels. Increased accumulation of mitochondrial iron due to loss of frataxin function and subsequent increased free radical generation and oxidative stress underlie the pathophysiology of FA. In normal individuals, the GAA repeats range from 7-22 and in affected cases these can be more than 66 to 1000. Myotonic dystrophies, also known as dystrophia myotonica, are a group of autosomal dominant disorders with highly variable phenotypes of which DM1 is caused by unstable expansion of CTG repeats in the 3' untranslated region of the myotonic dystrophy protein kinase (DMPK) gene located on chromosome 19. In normal population, the number of CTG repeats in the DMPK gene is between 4 and 37. The intermediate alleles with 35-49 triplets are not disease-causing but show instability while getting transmitted to successive generations. A repeat length of greater than 50 to 1000 results in the manifestation of DM1. The severity of the disorder and age of onset of symptoms correlate well with the number of repeats.

Another triplet repeat disorder is FRAX which is the most common cause of inherited mental retardation. It is caused by large CGG expansions in the 5'untranslated region of FMR1 gene, consequently resulting in hypermethylation of the CpG island present in the promoter of FMR1 further leading to the gene silencing. Normal individuals carry the repeats in the range of 5-54 while the affected individuals have more than 200 CGG repeats. Premutation carriers have repeats in the range 55-200.

There is an inverse correlation between repeat size and age of onset of the disease in FA and DM1. The triplet repeats tend to result in both somatic and germline instability and have a strong tendency to further expand in successive generations. This increasing number of repeats in subsequent generations explains the phenomenon known as anticipation. Owing to the broad spectrum of clinical presentations, handicapping nature of the disease phenotypes and unavailability of curative treatments, effective preventive measure is the only option available to help the families with these disorders. Molecular diagnostic tests are imperative for the early clinical diagnosis and timely prenatal diagnosis. The molecular diagnostic tests are also helpful in carrier detection and newborn screening especially in case of FRAX. Routine molecular diagnostics involves a traditional PCR, amplifying the repeat region followed by agarose gel electrophoresis whereas the gold standard is Southern blotting to determine the size of the repeats for these disorders. However, the larger expanded alleles get frequently missed by these traditional PCR approaches and Southern blotting is a very cumbersome and expensive method for routine diagnostics. Southern blot also needs radioactivity which most laboratories prefer to avoid. In this review we describe a new modified triplet primed PCR (TP-PCR) approach for the identification and characterization of expanded alleles and a complete molecular diagnosis of FA, DM1 and FRAX.

TRIPLET PRIMED PCR (TP-PCR)

TP PCR method has many advantages and suits well especially for rapid handling and testing of few samples as required in routine laboratory practice since the method is PCR-based, rapid, and not labor intensive. The analysis can be done using a small amount of DNA. It is a closed tube system that does not require post-PCR handling, and has a high sample throughput.[1]

The traditional PCR based approach is based on use of two primers (P1 and P2) flanking the repeats followed by agarose gel electrophoresis and sizing of number of repeats (Fig 1A).

Fig 1A. Illustration of binding of locus specific primers P1 and P2 during short PCR

The basic principle behind TP-PCR method is that this method involves two rounds of PCR reactions using 3 primers, P1, P3 and P4 (Fig 1B). The primer P1 is site specific and primer P4 consists of trinucleotide repeats and tail of primer P3. The properties of primer P3 are such that (a) it should contain little or no self complementarity (b) no homology to known human sequences. In the first round a locus specific primer P1 and a fluorescently labelled primer P4 which contains the specific repeat sequences is used. This pair of primers produces products of varying sizes based on site of binding of primer P4 (Fig 1C). However since a 10:1 molar ratio of P3 to P4 is maintained, primer P4 is exhausted in the early amplification cycles. In the latter cycles, primer P3 along with P4 undergoes the PCR reaction which results in PCR products of varying sizes and the extent of variation will depend on number of repeats present (Fig 1D). The products are then resolved by capillary electrophoresis on an automatic sequencer to interpret the results. In presence of expansion of triplet repeats beyond a threshold, the electropherogram shows a characteristic pattern of multiple peaks.[1]

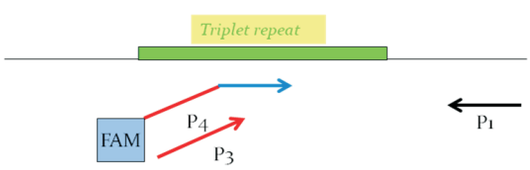


Fig 1B: Illustration of binding of primers P1, P3 and P4 during TP-PCR

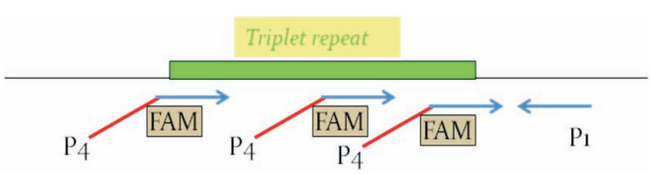


Fig 1C: Amplification during early PCR cycles by P1 and P4

Fig 1D: Amplication of the products of previous cycle by P1 and P3

Friedreich Ataxia:

TP-PCR being a highly sensitive technique, can be used as a first step in the molecular diagnosis of FA as it can accurately detect the expansion of large expanded alleles which can be seen as a characteristic ladder like pattern of the repeats in the electropherogram.[2] After TP-PCR if there is an expansion of the repeats in the pathogenic range (>250 bp) then to confirm, whether it is an affected case or a heterozygous carrier, a short PCR using locus specific primers P1 and P2 has to be performed, which will detect the normal alleles (Fig 2).

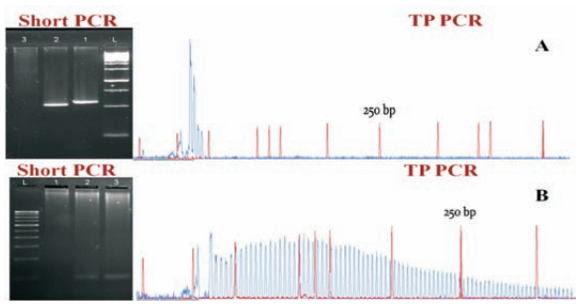


Fig 2: Upper panel A: Products of short PCR (2% agarose gel) L (1Kb ladder), lanes 1, 2 (normal cases showing single PCR product of approximately 200 bp), lane 3 (affected case showing absence of PCR product since the short PCR cannot amplify the region with large number of repeats) and electrophoregram of a normal case (TP-PCR) showing few peaks within 100 bp size.

Lower panel B: Short PCR of affected cases showing absence of PCR products due to large expansion (2% agarose gel) L (1Kb ladder), Lane 1(Homozygous expansion), Lanes 2 and 3 (Heterozygotes showing absence of PCR product from normal allele) and right panel – electrophoregram of an affected case (TP-PCR) showing peaks due to PCR products of varying sizes well beyond the threshold of 250 bp.

Although normal PCR with locus specific primers P1 and P2 detects the larger expanded allele, the results are not always consistent leading to a false negative diagnosis which can result in adverse repercussions in the concerned families. Hence, by TP-PCR in combination with short PCR, genotype of the affected patient, carrier and normal individuals can be accurately determined which will in turn help in the accurate diagnosis and prenatal diagnosis in the affected FA families.[2,3]

Myotonic dystrophy Type 1:

The CTG expansions in a DM1 affected individual can be very large i.e., even greater than 4-5 kb. So usual short PCR fails to amplify such large alleles and detects only the shorter allele. As DM1 is an autosomal dominant disorder, failure of amplification of the expanded allele in a short PCR poses quite a difficult situation.

TP-PCR efficiently detects the larger CTG expansions even though it cannot size them. A locus specific primer P1 is used in combination with a pair of primers P3 and fluorescently labelled P4 which have a common 5′ sequence (tail) for the TP-PCR amplification. The paired primer P4 has the sequence $(TGC)^5$ at its 3′ terminus, specific to the triplet repeat to be amplified.[2]

The TP-PCR electropherogram of an affected DM1 individual shows a typical ladder of 3 base pair periodicity with the peak height diminishing with increasing product

size (Fig 3). Role of TP-PCR is hence crucial in autosomal dominant diseases like DM1 where routine PCR most often fails to amplify the larger expanded alleles resulting in false negative results.

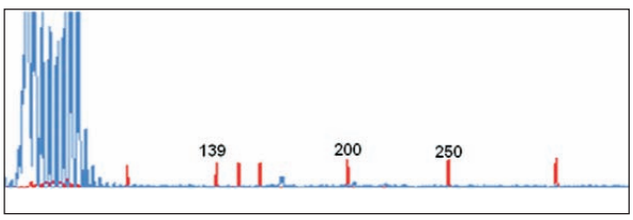


Fig: 3A: Electrophoregram of a normal individual (negative for Dm1) showing few peaks of less than 100 bp

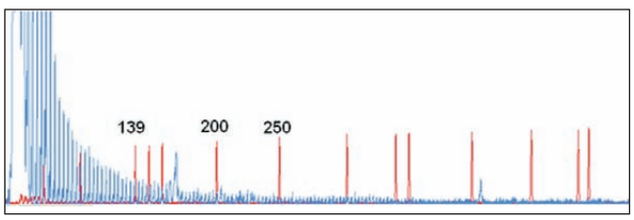


Fig: 3B: Electrophoregram of an affected DM1 case showing peaks of varying sizes beyond threshold

Fragile X syndrome:

Southern blotting has been considered as gold standard when it comes to determine the size of large expanded alleles in FRAX. However, it is a very time consuming, expensive and highly labor intensive procedure. Hence recently a fluorescent ms-PCR assay for FRAX has been described that classifies normal, premutation, and full mutation affected males and females according to their unique electropherogram patterns. [4]

Due to the difficulty in amplification of the GC rich CGG repeats in FMR1 the genomic DNA is modified according to published protocols. The bisulfite modified DNA is used in the mTP-PCR assay which employs three primers, a Ned-labelled forward primer upstream of the repeat (P1), an unlabeled tailed reverse primer annealing within the modified methylated repeat (P4) and a second unlabelled reverse primer specific to the tailed (P3) segment of the first reverse primer. PCR products following capillary electrophoresis detect all methylated alleles and produce a specific pattern in the presence of a premutation or full mutation (Fig 4).[4,5]

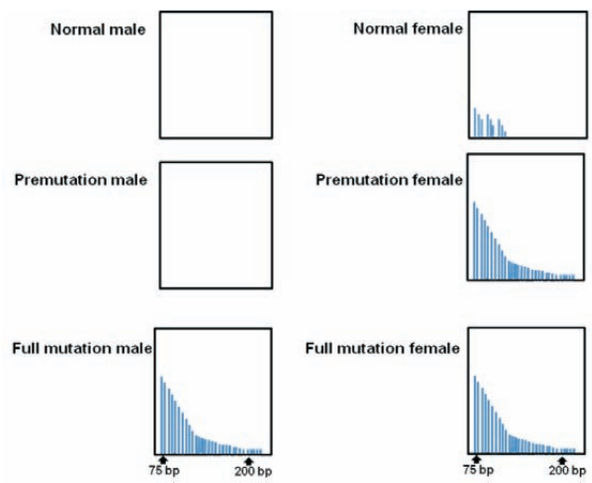


Fig 4: Representation of results of mTP-PCR assay of normal, premutation and full mutation males (Left panel) and females (right panel)

In conclusion, TP-PCR qualifies as a highly reliable, sensitive and robust technique which overcomes the shortcomings of a traditional PCR by detecting the very large expanded alleles of even more than 5 kb accurately. Southern blot is a very tedious, time consuming and expensive procedure which requires very large amount of DNA to detect the pathogenic expanded alleles. However, very small and poor quality DNA also gives results accurately in TP-PCR and it is a very rapid and fast process when compared with Southern blotting. TP-PCR cannot size the expanded alleles for which one has to proceed for Southern blotting. However, in routine diagnostics for patient diagnosis and prenatal diagnosis, TP-PCR can act as a versatile technique to detect the expanded alleles in patients with triplet repeat diseases.

References

1. *Warner JP, et al. J Med Genet 1996; 33: 1022-6.*
2. *Ciotti P, et al. J Mol Diag 2004; 6: 285-9.*
3. *Cossee M, et al. Eur J Hum Genet 2001; 9: 403.*
4. *Zhou Y, et al. Clin Chem 2006; 52: 1492-500.*
5. *Nygren AO, et al. J Mol Diag 2008; 10: 496-501.*

Double Trisomy In Two Boys With Down Syndrome

Seema Thakur, Meena Lall, IC Verma

Department of Genetic Medicine, Sir Ganga Ram Hospital, New Delhi

E-mail: seemat3030@sify.com

Introduction

Double trisomy is a rare event in live born babies. Majority of them are seen prenatally and end in miscarriage. Clinical features of the cases with double trisomy Down syndrome are variable and usually involve features of both Down syndrome and Klinefelter syndrome. Here we report two boys with double trisomy 21 and Klinefelter syndrome along with review of literature.

Case 1

This child (7 months old boy) was second in birth order and born to a non-consanguineous couple. The mother's age was 30 years and the father's age was 33 years. He was born at term and there was no history of birth asphyxia. He had upslant of palpebral fissures, a flat facial profile, depressed nasal bridge, hypertelorism, clinodactyly, and a wide sandal gap (Fig 1a). He had micropenis and the testis was not palpable on the right side (Fig 1b). At 6 months of age he developed pneumonia and echocardiography revealed a moderate sized PDA (patent ductus arteriosus, size 4.5 mm) and ASD (atrial septal defect, 8 mm) with left to right shunt. PDA ligation was done and ASD closure was planned at 2-3 years of age. He came for follow up at 2.5 years of age and had delayed developmental milestones.

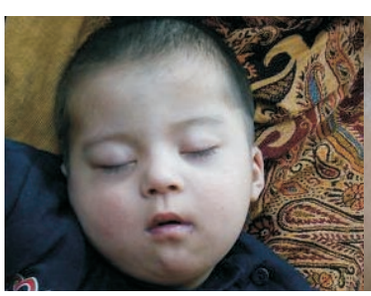


Fig 1a: Face of Case 1 showing features of Down syndrome

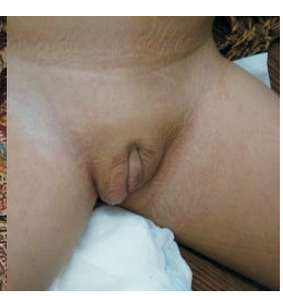


Fig 1 b: Micropenis

Case 2

This child (8 months old) with typical facial features of Down syndrome underwent colostomy at birth as he had high anorectal malformation. Pull through abdomino-perineal closure of sigmoid colon to the anal dimple was done at around 6 months of age. He had micropenis. Bilateral testes were palpable. There was imperforate anus. He had small scarred non-functioning left kidney. Echocardiography showed ASD (atrial septal defect). At 3 months of age he had delayed mile stones including speech delay.

Methods and Results

Whole blood cultures were set up to obtain metaphases, using modified Hungerford technique (1965).[1] Trypsin-Geimsa banding was done to identify and classify the chromosomes, using the international system for chromosome nomenclature (ISCN, 2009).[2] The karyotypes of both the patients showed 48,XXY,+21. Fifty metaphases were examined to rule out mosaicism. All fifty metaphases had an extra sex chromosome and an extra chromosome 21 (Fig 2). There was no evidence of mosaicism.

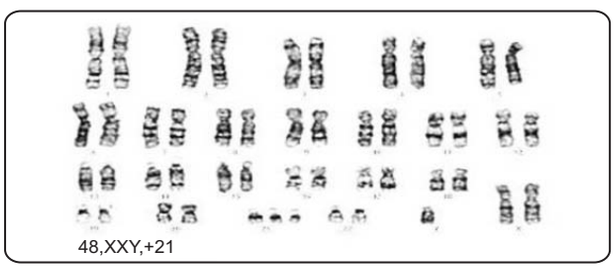


Fig 2: Karyotype of cases 2

This was further confirmed by fluorescence in situ hybridization (FISH) technique as described earlier.[3] Vysis DNA probes for chromosomes 21, X and Y, labeled with fluorescent dyes (spectrum orange, spectrum green and spectrum orange respectively) were hybridized with the patients' genome when the cells were at interphase. Signal enumeration was done in hundred nuclei, using the BX51 Olympus fluorescence microscope with motorized filter wheel. Locus Specific Indicator (LSI) probe for chromosome 21 showed three orange signals denoting the presence of trisomy 21 in all hundred cells enumerated (Fig 3). The centromere enumerating probes for the X-chromosome/spectrum green and the Y chromosome/spectrum orange showed the presence of two green signals and one orange signal denoting that the sex chromosome constitution was XXY in all hundred cells (Fig 3). There was no evidence of mosaicism. The same result was found in both the patients.

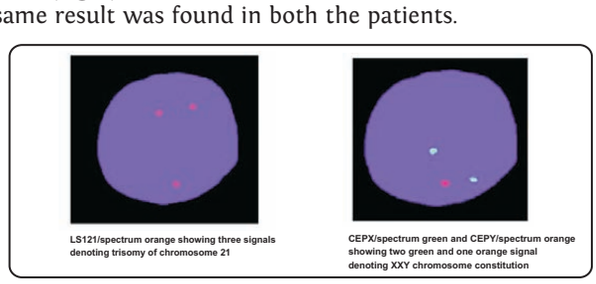


Fig 3: FISH for LSI 21 and CEP X and Y

Discussion

Ford et al were the first to describe a patient with double trisomy combining Down syndrome and Klinefelter syndrome in 1959.[4] This was the same year in which total numbers of chromosomes were identified in Down syndrome. Since then, approximately 62 cases have been reported in literature, and 17 have been diagnosed prenatally. [5]

All the cases reported in literature have typical facial features of Down syndrome. Both our cases had same dysmorphic features as typical Down syndrome. Both our cases had cardiac malformations. Case 2 also had high anorectal malformation. The other cases described in literature also had associated malformations with the same pattern of malformation as Down syndrome.

Presence of an extra X chromosome in case of Down–Klinefelter syndrome leads to a discernible overlap of features of both conditions. Moreover, in cases of double aneuploidy involving autosomes and sex chromosomes, clinical manifestations of the autosomal abnormality are usually predominant and those of the sex chromosomal abnormality tend to be missing. In patients with Down-Klinefelter syndrome, the Down syndrome phenotype often predominates. In patients with Klinefelter syndrome, the diagnosis is often made later in life, and they have a tall stature, absent or decreased facial and pubic hair, small hyalinized testes, a small penis, and feminine distribution of adipose tissue, including gynecomastia.[6] Signs that are sometimes noted in infants with Klinefelter syndrome are an underdeveloped phallus and scrotum, valviform hypospadias, hyperpigmentation of scrotal raphe, and small or ectopic testis.[7] Both our cases had micropenis. Case 1 also had undescended testes.

The severity of mental retardation in double trisomy 21 appears to be same as trisomy 21. At a follow up visit at 2-3 years of age both had speech delay.

Down syndrome(DS) and Klinefelter syndrome (KS) are relatively common chromosomal abnormalities, which occur in around 1/700 and 1/1000 newborns respectively, due to a chromosome non-disjunction during gametogenesis in one of the parents. The combined incidence of Down syndrome and Klinefelter syndrome is higher than expected.

The incidence of 48,XXY,+21 in the general population is 0.4 to 0.9 per 10,000 male births whereas the expected incidence is $0.27\text{-}0.7 \times 10^{-5}$. The frequency of the XXY among cases of Down syndrome is 11.7 per 10,000 Down syndrome patients i.e. 1.17/ 1000 Down syndrome.[5] There is evidence that nondisjunction is genetically determined, which suggests that both events arise from the same parent.[8] Although a number of cases of double trisomy have been reported, parental origin has been reported in only a few cases. Nondisjunction may be entirely maternal in origin, [8,9] entirely paternal in origin, and both maternal and paternal in origin.[10,11] Other double trisomies involving chromosome 21 and sex chromosomes are 48,XXX,+21, 48,XYY,+21. The frequency of these two trisomies is low as compared to 48,XXY,+21. This may be due to higher chance of fertilization by a Y bearing sperm if the ovum is disomic or a Y bearing sperm may promote nondisjunction.

Kovaleva and Mutton found that the risk for 48,XXY,+21 was age dependent, with a mean maternal age of 33 years and a mean paternal age of 38 years.[12] Parental age was advanced in Case 2.

Conclusion:

Down-Klinefelter syndrome is a rare occurrence with an estimated incidence in the general population of 0.4 to 0.9 per 10,000 male births and of 1.17 per 1,000 cases of trisomy 21. Cases of double trisomy reported in the literature have dysmorphic features similar to Down syndrome. Mental retardation is also of same severity as typical Down syndrome. Klinefelter syndrome is an incidental finding. The Down- Klinfelter cases have similar associated malformations as Down syndrome. Malformations of external genitalia appear to be a frequent association and clinically one can suspect Down-Klinfelter syndrome in the presence of genital malformations.

References

1. Hungerford DA. Stain Technol 1965; 40:333-8
2. ISCN (2009): An international System for Human Cytogenetic Nomenclature (2009). L G Shaffer, M I Slovak, L J Campbell (eds); Karger, Basel.
3. Van Stedum S, et al. In: Molecular Cytogenetics: Protocols and Applications. Fan YS (ed) 2002. Humana Press Inc., Totowa , NJ
4. Ford CE, et al. Lancet 1959; 1: 709-10
5. Jeanty C, et al. J Ultrasound Med 2009; 28: 673–81
6. Bojesen A, et al. J Clin Endocrinol Metab 2003; 88: 622–6.
7. Mozziconacci P, et al. Ann Pediatr (Paris) 1962; 38: 363–8.
8. Ikonen RS, et al. Hum Genet 1989; 83: 235–8
9. Shapiro A, et al. J Ment Defic Res 1960; 4: 48–50.
10. Schmidt R, et al. Am J Hum Genet 1978; 30: A93.
11. Saura R, et al. Ann Genet 1983; 26: 180–2
12. Kovaleva NV, et al. Am J Med Genet 2005; 134: 24–32.

Breaking Through Brittle Bone Disease

Contributed by:
Dr Parag M Tamhankar
E mail: paragt_md@rediffmail.com

BREAKING THROUGH BRITTLE BONE DISEASE...[1,2]

Osteogenesis imperfecta (OI) is an inherited connective tissue disease characterized by bone fragility and increased susceptibility to fractures. Autosomal dominant OI is caused by heterozygous mutations in either COL1A1 or COL1A2 which disrupt the stability of type 1 collagen, the major protein component of bone. Autosomal recessive (AR) OI is caused by faulty post-translational modification (PTM) [3 –hydroxylation of a single residue proline at position 986 (P986)in the alpha-1 procollagen chain]. PTM is important for protein-collagen interactions required for bone formation and carried out by a complex of three proteins CRTAP (encoded by CRTAP), P3H1 (encoded by LEPRE1) and CypPB (encoded by PPIB). Mutations in CRTAP and LEPRE1 have already been demonstrated in AR OI cases with rhizomelia. A recent breakthrough by two independent groups brought to light molecular intricacies of abnormalities in these three proteins. Van Dijk et al and Barnes et al investigated AR OI cases without rhizomelia and without mutations in CRTAP and LEPRE1, for mutations in PPIB. As a result, a total of three families have been found to carry missense or deletion mutations in PPIB. Clinically, the affected cases were indistinguishable from OI type IIB/III. They showed that immunohistochemistry of bone with antibodies to CRTAP, P3H1 and CypPB can aid differentiation. Cases with either CRTAP or LEPRE1 mutations show absent signal of both CRTAP and P3H1 but normal CypPB signal; whereas cases with PPIB mutations show absent CypPB but normal CRTAP and P3H1 signal. Levels of P986 3-hydroxylation were 33%, 22% and 16 % of control for patients with PPIB, LEPRE1 and CRTAP mutations respectively. P3H1 is responsible for the actual hydroxylation whereas mutations in the other two cause dysfunction of the complex. PPIB mutations also lead to defective cis-trans isomerization of peptide bonds and chaperone (procollagen export) functions in addition to defective hydroxylation.

NO SMOKE WITHOUT FIRE ...[3,4,5]

Smoking is traditionally thought to be an acquired behavioral trait. However, a series of three papers which present results of meta-analyses by three large consortia (Tobacco and Genetics Consortium (TAG), Oxford-GlaxoSmithKline study, ENGAGE Consortium) prove that smoking initiation, heaviness of smoking and smoking cessation were strongly linked to various genetic loci. Genome wide association was performed after more than 140,000 individuals were genotyped for approximately 2.5 million single nucleotide polymorphisms (SNP). The four smoking phenotypes studied included smoking initiation (ever vs. never smokers), age at onset of smoking, average number of cigarettes smoked per day (CPD) and smoking cessation (current vs. former smokers). The highest association was between CPD (heavy smoking) and SNPs in genes at the nicotinic acetylcholine receptor cluster on chromosome 15q25.1 (CHRNA5-CHRNA3-CHRNB4). Two newly associated loci 19q13 and 8p11 were found which harbor genes encoding nicotine-metabolizing enzymes (CYP2A6 and CYP2B6) and nicotinic acetylcholine receptor subunits (CHRNB3 and CHRNA6) respectively. These have been previously shown to be associated with nicotine dependence and cancer. Smoking initiation was most strongly associated with an SNP in BDNF on chromosome 11. Smoking cessation was most strongly associated with one SNP located near DBH on chromosome 9. More research is needed until these findings are inculcated in clinical practice. However, they may explain certain phenomena such as why certain people get a "buzz" (rush of pleasure) after their first puff while some others have nausea or a coughing fit. The former may certainly need to douse the fire within, lest it consumes them into an addiction.

Diluting the Purity of the First Step towards Life [6]

Phosphoribosylpyrophosphate synthetases (PRPS) catalyze the first step of de novo synthesis of purine, pyrimidine and pyridine nucleotides. Mutations in PRPS1 gene lead to four distinct syndromes viz., PRS-I superactivity (PRSIS), Charcot-Marie-Tooth disease-5 (CMTX5), Arts syndrome (AS) and X-linked non-syndromic sensorineural deafness (DFN2). Though above presentations are different, the underlying tissues affected are one or more of the following: neurological (ataxia, mental retardation, peripheral neuropathy, hearing loss), hematological (anemia) or immunological (recurrent infections). Gain of function mutations (like in PRSIS) and loss of function mutations (like in CMTX5, AS, DFN2) lead respectively to either elevation or reduction in levels of purines, pyrimidines and pyridines. But interestingly, purine rather than pyrimidine or pyridine imbalance is central to pathogenesis of most of the above disorders. Elevation of purines does not lead to elevated nucleosides but are degraded to produce excessive uric acid resulting in gout and renal stones. Decreased ATP and GTP (purines again), the key players of cellular energy and signaling are implicated in hitting the system where energy matters the most, i.e., the nervous system. Though myelin formation requires both purine (adenosine) and pyrimidine (CDP choline, CDP-neuraminic acid, CDP ethanolamine) nucleotides, demyelination occurs due to lack of purinosides simply because purine salvage depends on PRPS1 while pyrimidine salvage depends on kinases. Immunological disorders are caused due to abnormal accumulation of the deoxynucleotides deoxy-GTP or deoxy-ATP. Literally, ATP and GTP run and ruin the whole show. The key to treating the reduced purine levels lies in providing fodder for the purine production pathways that bypass PRPS1. Because dietary supplementation of purines won't work, the gut converts them into uric acid instead of absorbing them. S-adenosyl methionine may be just the right choice for this purpose. Would this strategy work ? Only time will tell !

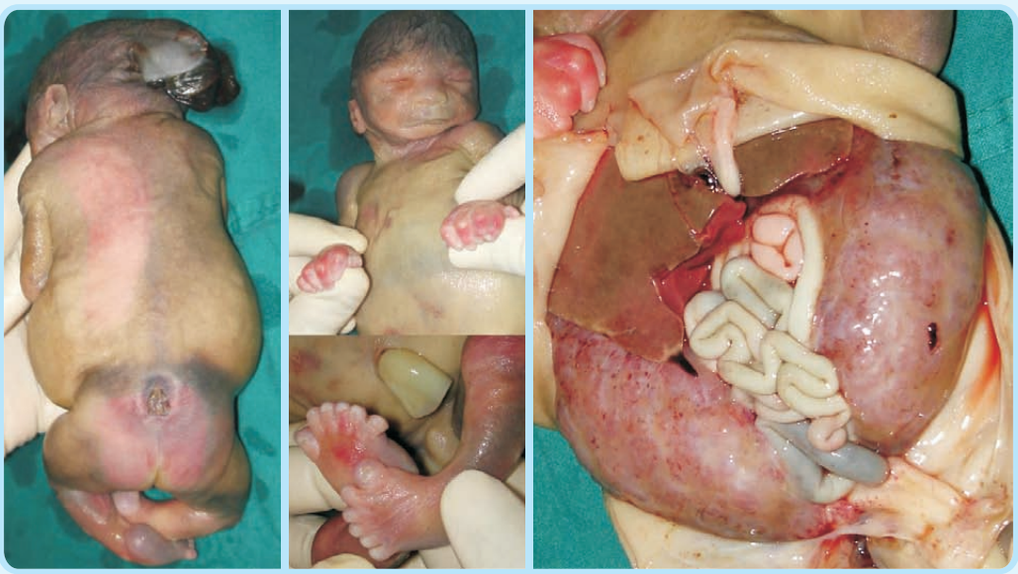
References

1. *Barnes AM. N Engl J Med. 2010; 362: 521-8.*
2. *van Dijk et al. Am J Hum Genet. 2009; 85: 521-7.*
3. *Amos et al. Nature Genetics 2010; 42: 436-40.*
4. *Furberg at al. Nature Genetics 2010; 42: 441-7.*
5. *Thorgeirsson et al. Nature Genetics 2010; 42: 448-53.*
6. *De Brouwer et al. Am J Hum Genet. 2010; 86: 506-18.*

9

Contributed by: Sreelakshmi Kodandapani, Muralidhar V Pai.
Department of Obstetrics and Gynecology, Kasturba Medical College, Manipal.
Email: laxmivinayaka@gmail.com

The fetus shown below had hepatic cysts as well. Identify the condition



Answer to the PhotoQuiz 8 of the previous issue

Langer-Giedion syndrome

(Tricho-rhino-phalangeal syndrome type II) (OMIM 150230)

Langer-Giedion syndrome is associated with multiple exostoses and distinctive facial features. Affected individuals may also have short stature and cone-shaped epiphyses. The characteristic appearance includes sparse scalp hair, a rounded nose, a long philtrum and a thin upper lip. It is a rare condition and its incidence is unknown. It is caused by the deletion or mutation of at least two genes on the q-arm of chromosome 8 (8q24). Researchers have determined that the loss of a functional EXT1 gene is responsible for the multiple exostoses and loss of a functional TRPS1 gene may cause the other bone and facial abnormalities. It is often described as a contiguous gene deletion syndrome because it results from the loss of several neighboring genes. Most cases are not inherited, but occur as random events. It is considered an autosomal dominant condition because one copy of the altered chromosome 8 in each cell is sufficient to cause the disorder.

Correct responses were given by:

1. Mohandas Nair, Calicut
2. Neerja Agarwal, New Delhi
3. Kalpana Gowrishankar, Chennai
4. Krithika MV, Davangere
5. Hemalatha S, Davangere
6. Kausik Mandal, Vellore
7. Yatheeshan KK, via e-mail

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