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Editorial

Ten classes in Medical Genetics and more to come...

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I am very happy to write this editorial for the eleventh issue of 'Genetic Clinics'. 'Genetics Clinics' came into existence in July 2008 and has been regularly published every three months. In each issue of the newsletter an attempt is made to cover various and latest topics which would interest clinicians and geneticists. The aim of starting the newsletter was to make clinicians aware about increasing applications of genetics knowledge and tests in clinical practice. We wish to reach the clinical geneticists, laboratory geneticists and clinicians interested in genetics. They are the ones who will be helping to dissipate the knowledge of genetics, identify patients and families with genetic disorders, and provide services like diagnosis, genetic counseling and prenatal diagnosis. As the number of clinical geneticists is very small in India, many clinicians including pediatricians, obstetricians and internists are taking a special interest in the specialty of clinical genetics and providing services to the patients. 'Genetic Clinics' provides a forum to these like minded people interested in medical genetics and helps them to learn and teach simultaneously.

Medical genetics is a very rapidly developing specialty and to keep oneself updated is a very difficult task. There is no dearth of available free information on various websites and databases and these are constantly updated. There are more than 5000 monogenic disorders and the list is ever-expanding. New genes and mutations are being identified at a very great speed due to technical advances like whole genome sequencing and microarray etc. Similarly, information about new investigative and therapeutic approaches is constantly increasing. With this explosion of knowledge it is impossible for anybody to keep abreast with the latest knowledge of genetic aspects of various diseases. 'Genetic Clinics' uses the input of various medical geneticists to put recent advances in the simplified form, so that clinicians with varying backgrounds can get a glimpse of recent developments in genetics and use the information for patient care and teaching.

The article and case reports are chosen to cover basic genetics, clinical aspects of genetic disorders and diagnostic tests. Various genetic investigations like cytogenetic tests, mutation detection for common disorders, sequencing and latest additions like microarray based cytogenetic analysis (cytogenetic microarray – CMA) are available in India. The clinician has to understand the principles of the test so that he/she can understand the result, its implications, the error rate, and reasons for it. This is essential for the appropriate use of genetic tests for genetic counseling. It is the responsibility of the clinician who is ordering the tests (which are usually costly) to discuss the utility and limitations of the test with the patient or family concerned. Equally important is post test counseling.

The article on molecular cytogenetics in this issue illustrates the principle and utility of in-depth evaluation using various techniques for better delineation of chromosomal abnormalities. The article on genetic disorders with increased bone density is an example to stress how much is known about the various genes causing genetic disorders and the importance of this knowledge in the management of patients. It also shows how the knowledge of genetic etiologies of various diseases with increased bone density has improved understanding of molecular events of bone physiology. Similar is the situation in many diseases where knowledge of the causative genes of phenotypic abnormalities has improved the understanding of normal development and other physiological processes of the body. This is bound to lead to development of new treatment strategies in the coming decades. Various strategies in the process of exploration as in the case of Duchenne muscular dystrophy and success stories in enzyme replacement therapies for Lysosomal storage disorders have been presented before the readers in previous issues. The views and perspectives of experienced clinical geneticists on some areas of clinical importance are presented under 'Genefocus'. Judging by the feedback, the readers seem to enjoy the regular columns like 'Genexpress' and 'Photoquiz'. The newly started 'Heartoheartalk' which is a platform to share different emotional and psychosocial experiences commonly experienced during the genetic counseling process has also been appreciated by many of

the readers. 'Genetic Clinics' has published cartoons and poems to add a little fun and art to the science.

I feel that this endeavor of the newsletter has been successful in achieving the aim of reaching clinicians and creating excitement about this field, and in turn helping patients and families with genetic disorders in India. To keep this activity going is possible because of the support of many contributors and I request all to send articles and case reports to add variety to each coming issue.

The quality design and printing has contributed greatly to the success of the newsletter. The colorful pictures and diagrams make the articles appealing and add to the ease of reading and understanding. At this juncture of the eleventh issue I thank Genzyme India Private Limited and its team for helping us to bring out the newsletter and to send it to you all. Hope you continue to enjoy reading the newsletter, learn and teach!

Happy New Year!

Shubha Phadke
1st January, 2011

ANNOUNCEMENT
ISHG 2011

Theme : International Conference on "Genomics, Genetic Diseases and Diagnostics" and 36th Annual conference of the Indian Society of Human Genetics

Place : Manipal Life Sciences Centre, Manipal University, Manipal 576104, Karnataka

Date : 14-16, February 2011

Contact: Dr. K. Satyamoorthy
Director, Manipal Life Sciences Centre, Manipal University, Manipal 576 104, Karnataka, India
Email: dbt@manipal.edu; ishg2011@gmail.com | Website: http://ishg2011.manipal.edu

ANNOUNCEMENT

The First Indo-US Symposium on Skeletal Dysplasia

Diagnosis, Radiology, Pathogenesis, Molecular Genetics, Management,
Surgical Interventions, Genetic Counseling, Prenatal Diagnosis and Recent Advances

Date : 12th and 13th Feb 2011

Place : Department of Medical Genetics,
Sanjay Gandhi Postgraduate Institute of Medical Sciences,
Lucknow

Faculty :

Dr David Rimoin	Dr Ralph Lachman	Dr William Horton
Dr Robert Bernstein	Dr William McKenzie	Dr John Graham
Dr Moise Danielpour	Dr Uwe Kornak	And Eminent Indian Faculty

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Molecular Cytogenetics Illustrated: SKY and FISH

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INTRODUCTION

Traditional chromosomal analysis was the mainstay of cytogenetic investigations until the advent of molecular cytogenetics. The molecular cytogenetic techniques have revolutionized the practice of cytogenetics. The resolution of analysis has increased from 10 Mb for chromosomal analysis to just a few base pairs with newer methods like array comparative genomic hybridization (aCGH). Application of these modern cytogenetic techniques has improved the detection of chromosomal aberrations. In this article, we discuss the methods and applications of two molecular cytogenetic techniques, namely Fluorescent In Situ Hybridization (FISH) and Spectral Karyotyping (SKY). The principles and utility of these techniques have been explained by taking an illustrative case of balanced reciprocal translocation.

I. FLUORESCENCE IN SITU HYBRIDIZATION (FISH)

In situ hybridization refers to procedures that demonstrate DNA sequences directly on chromosome preparations. Since resolution is relatively better than conventional cytogenetics, the exact regional localization of a sequence on its corresponding chromosome can be determined. The chromosomal rearrangements and deletions of very small size which can not be detected by traditional cytogenetics can also be detected by FISH. The other advantage of FISH is that it does not need live dividing cells and can be done on interphase nuclei as well, hence reducing the reporting time to one or two days. FISH can be used on old samples from paraffin blocks and/ or on cells which are difficult to culture. FISH is based on the use of DNA probes labeled with fluorescent dyes, which hybridize to their complementary sequences on the chromosomes, where they produce a fluorescent signal.

METHODOLOGY OF FISH

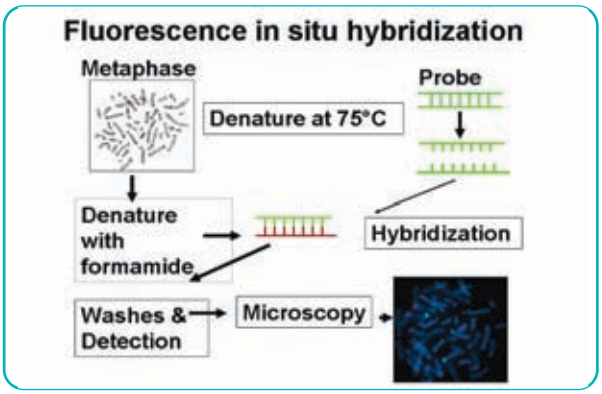

Fig 1. The flow chart for FISH methodology

FISH PROBES

The probes are made by labeling the required DNA by nick translation, random priming or by PCR methods. Generally, probes less than 40 bases long are synthetically manufactured and probes that range from 80 kb to 1 Mb are manufactured with molecular biology methods using cosmids, Yeast Artificial Chromosomes (YACs), P1-derived artificial chromosomes (PACs) or Bacterial Artificial Chromosomes (BACs).

FISH probes are highly specific for their target DNA and divided into four main types.

1. *Gene or locus specific probes:* These probes target specific nucleic acid sequences. e.g., BACs, YACs, cosmids. They are mainly useful for mapping genes on chromosomes and for detection of microdeletions e.g. probes for Prader Willi syndrome / Angelman syndrome, Williams syndrome etc.
2. *Centromere probes:* These are repetitive-sequence probes, which bind to repetitive regions of the

centromere, which are AT-rich regions. Centromeric probes have applications in identification of marker chromosomes and numerical chromosomal abnormalities e.g. detection of trisomy 13, 18, 21 in prenatal samples.

3. *Telomeric probes:* These are also repetitive probes, which bind to telomeric regions TTAGGG. Telomeric and subtelomeric probes help in identifying cryptic chromosomal deletions in cases of idiopathic mental retardation.[1]

   Locus specific, telomeric and centromeric probes can be used for interphase FISH as well.

4. *Paint probes:* These probes contain sequences specific to either a single chromosome (Whole Chromosome Paint i.e., WCP) or an arm of a chromosome. These are particularly useful for identification of chromosomes involved in translocation, marker chromosome and ring chromosome. [2]

There are two types of FISH techniques based on labeling patterns:

i) *Direct FISH*: In this technique, the pure DNA is isolated from the region of interest and fluorochromes like fluorescein isothiocyanate (FITC) are directly incorporated by labeling the DNA. This labeled DNA is then used as a probe for hybridization to chromosomes. Most of the commercially available probes for detection of microdeletions, aneuploidy etc are of this type. The disadvantage of this method is that these probes are expensive since a lot of fluorescent dye is needed.

ii) *Indirect FISH*: This method of FISH is mostly used in research set up since the cost of this type of labeling is much less than direct labeling of DNA. The indirect FISH labeling is done by enzymatic methods such as nick translation. In an indirect labeling reaction, reporter molecules such as biotin or digoxigenin are incorporated into the DNA. Such indirect labels require a detection step in which the reporter molecule is labeled with an agent such as avidin or antidigoxigenin that is conjugated to a fluorochrome. The detection methods for biotinylated probes employ avidin-fluorochrome conjugates, whereas those for digoxigenin-labeled probes employ anti-digoxigenin-fluorochrome conjugates.

FISH MICROSCOPY

FISH signals are visualized by fluorescence microscopy using a light source that lights the fluorescently labeled specimens. Mercury vapor lamps and Xenon lamps are used to excite the fluorochromes, which in turn emit light at a different wavelength.

CLINICAL APPLICATIONS OF FISH

1. Aberrations as small as 0.5 kb can be identified, which is not possible by conventional karyotyping e.g microdeletion syndromes, subtelomeric deletions.
2. Confirmation or detection of numerical and structural chromosomal aberrations e.g. aneuploidy screening for chromosome 13, 18, 21 in amniotic fluid. The advantage of using FISH for aneuploidy detection is the rapidity of results. FISH results will be ready within 48 hours while traditional karyotyping from amniotic fluid cells need cell culture and will take at least 2 weeks.
3. Determination of origin of marker chromosomes.

II SPECTRAL KARYOTYPING (SKY)

SKY is a molecular cytogenetic technique used for characterization of numeric and structural chromosomal aberrations. SKY shows the chromosomes in 24 different colors. SKY involves a combination of epifluorescence microscopy, charge-coupled device (CCD) imaging and Fourier spectroscopy to measure the complete emission spectra at all image points.[3, 4] As opposed to FISH, which limits the analysis to specific chromosomes or regions of chromosomes, SKY permits the visualization of all chromosomes at one time, painting each pair of chromosomes with a different fluorescent color. An interferometer is used to assess the spectrum of fluorescent wave lengths for each pixel of the CCD chip. Identification of the components of this spectrum is then achieved by a dedicated computer program applying a classification algorithm which then assigns a pseudo-color to each component.

METHODOLOGY OF SKY

The SKY probes are generated based on flow-sorting of chromosomes which are then individually labeled with different fluorophores. A Degenerative Oligonucleotide Primed Polymerase Chain Reaction (DOP-PCR) labeling is used to incorporate the fluorochromes, suppressed with Cot-1 DNA and hybridized on metaphase slides, washed and visualized.

APPLICATIONS OF SKY

1. Detection of complex chromosomal rearrangements, which cannot be identified by conventional cytogenetics.
2. Identification of origin of marker chromosomes
3. Detection of telomeric translocations that are difficult to detect by conventional techniques.

CASE REPORT

Reciprocal chromosomal translocations provide a good opportunity for identification of genes associated with disease traits. Many genes associated with mental retardation and other monogenic diseases have been mapped using individuals with such translocations. These studies have used the methods of FISH, SKY etc as described above to identify the gene interrupted by the translocation. Most of the reciprocal translocations are balanced and do not disrupt any gene at the breakpoint but can lead to unbalanced gametes and hence poor reproductive outcome in the form of recurrent spontaneous abortions or birth of a child with malformations and mental retardation. Some of the reciprocal translocations are difficult to interpret using conventional cytogenetics, especially when they involve more than two chromosomes. In this paper we describe a patient with balanced reciprocal translocation (BRT) involving chromosome 3 and 14, and confirmed by chromosome painting. Other molecular cytogenetic techniques were also applied to this case to demonstrate the utility of molecular cytogenetic techniques to confirm the chromosomal regions involved in this chromosomal aberration.

CASE DETAILS

A couple was referred to us with a history of previous two children with birth defects. The first pregnancy had resulted in an anencephalic child and second gestation resulted in maternal polyhydramnios associated with oesophageal atresia in the fetus. There were no phenotypic abnormalities recorded in this couple.

Chromosomal analysis using GTG-banding (G-banding using Trypsin and Giemsa) was carried out on cultured peripheral lymphocytes on metaphase chromosomes using standard laboratory techniques which revealed a karyotype of 46,XY,t(3;14)(p12;q12~13) in the husband (Fig. 2). The exact breakpoint on the chromosome 14 was not clear. The chromosomal analysis of his wife showed a normal karyotype of 46,XX.

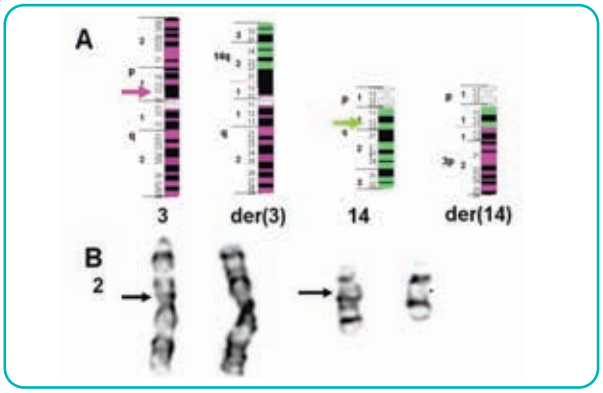

Fig 2: Ideogram and partial karyotype of the translocation in the proband. A. From left to right are shown normal chromosome 3, derivative chromosome 3, normal chromosome 14 and derivative chromosome 14. Material from chromosome 3 is shown in pink/black and material from chromosome 14 in green/black. The pink and green arrows show the location of breakpoints on the normal chromosomes. B. The GTG-banded partial karyotype showing the normal and derivative chromosomes. Translocation breakpoints are shown by arrows on the normal chromosomes

In order to confirm the chromosomes involved in the translocation, a SKY was performed with the SKY Paint© Kit-Human (Applied Spectral Imaging). This confirmed the translocation and it also showed absence of involvement of other chromosomes in the translocation (Fig. 3).

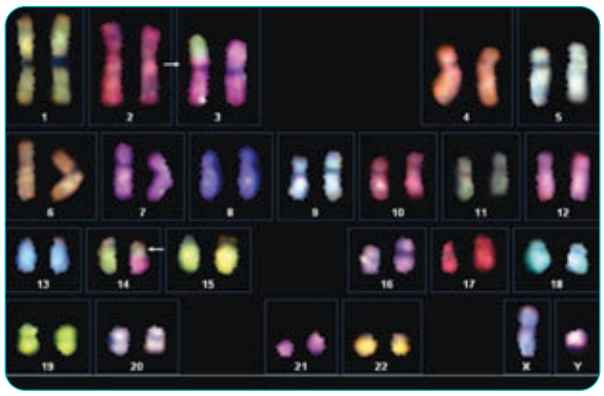

Fig. 3: SKY on the lymphocyte metaphase of the proband. Representative karyotype illustrating the display color. The derived chromosomes 3 and 14 are seen in both pink and green color (arrows).

The other method which can be used to confirm the chromosomes involved in the translocation is chromosome painting which was also applied to this case. FISH using whole chromosome paint probes (WCP) for chromosomes 3 and 14 (Poseidon probes from Kreatech, Netherlands) was done according to the manufacturer's instructions. WCP FISH was carried out with paint probe 3 labeled with TRITC [Tetramethylrhodamine-5- (and 6)

isothiocyanate] (red color) and paint probe 14 labelled with FITC (Fluorescein isothiocyanate) (green color). Both the normal chromosomes 3 and 14 were seen to be colored red and green respectively. However, the derived chromosomes of 3 and 14 showed both red and green colors confirming the translocation involving the two chromosomes (Fig. 4).

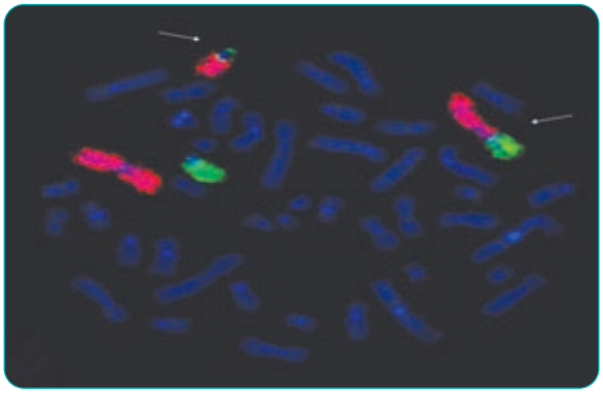

Fig. 4: WCP FISH on the lymphocyte metaphase spreads of the proband. The paint 3 was labeled by TRITC (red signals) and paint 14 by FITC (green signals). The arrows show the derived chromosome 14 and derived chromosome 3.

Further fine mapping was performed by selecting two BAC clones RP11-291P10 on 3p12.3 and RP11-170D09 on 14q12 according to the current human NCBI Reference Sequence[5] utilizing the UCSC Genome browser[6] and obtained from imaGenes, Berlin, Germany. The international standard nomenclature was used for clone names. BAC DNA was isolated using NucleoBond Plasmid Midi kit (Macherey-Nagel, Dueren, Germany) according to manufacturer's instructions. The isolated BAC DNA was labelled with biotin-16-dUTP (Roche Diagnostics, Mannheim, Germany) by nick translation and FISH analysis was done on the metaphase slides as described by the standard protocols.[7] The BAC clone RP11-291P10 showed signals on normal as well as derivate chromosomes 3 (Fig. 5A) and RP11-170D09 showed signals on normal as well as derivative chromosomes 14 (Fig. 5B). Thus the break points were confirmed to be present distal to the locations of these probes, namely 3p12 and 14q12.

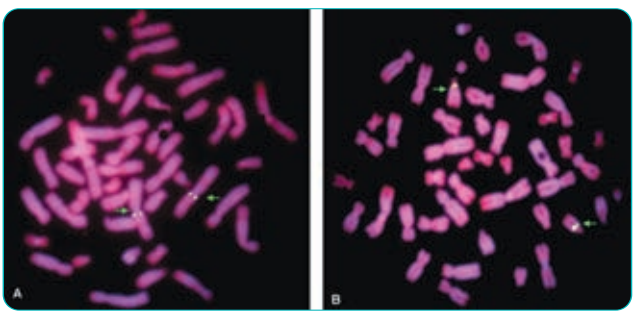

Fig. 5: FISH with BAC clones on lymphocyte metaphase spreads . A. FISH with RP11-291P10 (located on 3p12) showing signals on normal 3 and derivative 3. B. FISH with RP11-170D09 (located on 14q12) showing signals on normal 14 and derivative 14.

The proband described in the current work has a translocation involving chromosomes 3 and 14. By using the modern molecular cytogenetic techniques we could confirm the breakpoint regions. The WCP helped to confirm the chromosomes involved in the translocation. SKY confirmed the reciprocal translocation and also ruled out the involvement of other chromosomes. Using the two BAC clones, we could demonstrate the indirect FISH technique that helped us to characterize the breakpoint region distal to the 3p12 and 14q12 region.

This high level of breakpoint analysis is not required for clinical purposes of genetic counseling and prenatal diagnosis; especially if the origin of chromosomes, breakpoints and balanced nature of translocation is obvious from the traditional karyotype as was the situation in this case. The detailed evaluation of this case by various types of molecular cytogenetic techniques illustrates application of different techniques and their utility for in-depth analysis of cytogenetic rearrangements. The molecular cytogenetic techniques however, are helpful in identifying the origin of a marker chromosome or cases wherein, either small segments of chromosomes are involved or more than two chromosomes are involved in the translocation. The molecular cytogenetic evaluation is also required if the cytogenetic analysis shows apparently balanced translocation but there is some phenotypic abnormality suggesting submicroscopic loss or gain of genetic material at the breakpoints or a possibility of disruption of some gene at the breakpoint.

Cytogenetic research and diagnostics have greatly benefited by molecular cytogenetic techniques. Together all these techniques have revolutionized the field of molecular cytogenetics.

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Too dense bone: Too many genes!

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Bone is a vital tissue which not only provides support to our bodies, but is also important for hematopoiesis and maintenance of mineral homeostasis. The density of bones is an exquisitely regulated phenomenon. Various hormones, local trophic factors and enzymes govern bone formation and breakdown by their actions through osteoblasts i.e., the bone forming cells and osteoclasts which are the bone resorbing cells. The osteoblasts perform the function of bone formation, which involves the deposition of collagen fibre network and formation of calcium hydroxyapatite crystals. The process of bone degradation is carried out by osteoclasts which are derived from mononuclear precursors of myeloid lineage. They play an important role in bone remodelling and maintenance of mineral homeostasis. They can degrade the mineral as well as organic bone matrix[1,2]

The dissolution of bone mineral involves the process of cell polarization, formation of the ruffled border and sealing zone and finally formation of resorption lacuna where hydrochloric acid is actively secreted resulting in the dissolution of bone mineral hydroxyapatite. The carbonic anhydrase enzyme mediates the formation of H+ ions for secretion into the resorption lacunae and the H+ATPase channel transports these protons into the resorption lacunae. Degradation of bone organic matrix is carried out by a specialised enzyme Cathepsin K, which is a collagenase[1,2] The understanding of the formation & differentiation of osteoclasts and their functioning is integral to understand the pathogenesis of various disorders of increased bone density. Increased bone density may occur due to:

1. Excessive bone formation by the osteoblasts: as occurs in Sclerosteosis, Van Buchem disease, Craniometaphyseal dysplasia and one form of osteopetrosis.

2. Decreased bone resorption by the osteoclasts: as occurs in various forms of osteopetrosis and other disorders.

TABLE 1 : GENES INVOLVED IN OSTEOBLAST AND OSTEOCLAST FUNCTIONING AND THE DISEASES ARISING BY THEIR DEFECTS

GENE	FUNCTION	DISEASE
IMPORTANT FOR OSTEOBLASTS		
SOST	Promotes differentiation, matrix formation, mineralisation	Sclerosteosis, Van Buchem disease
ANKH	Decreases matrix formation, mineralisation	Craniometaphyseal dysplasia
LRP5	increases bone formation by wnt pathway	AD osteopetrosis
IMPORTANT FOR OSTEOCLASTS		
(I) Important for formation & differentiation of Osteoclasts		
RANK and RANKL	RANK , a transmembrane receptor, interacts with RANKL to induce NF kappa B leading to osteoclast differentiation	AR osteopetrosis
OPG	Decoy receptor for RANKL, inhibits NF Kappa B signalling	Juvenile Paget disease
TGF beta	Decreases RANKL and increases OPG	Camurati Engelmann disease
LEMD3	BMP and TGF beta signalling	Osteopoikilosis, Melorheostosis
PORCN, WTX	Wnt signalling	Osteopathica Striata
NEMO/IKBKB	Inhibits NF Kappa B signalling	AR osteopetrosis

(ii) Important for acidification machinery		
ATP6i/TCIRG	H+K+ATPase for transport of H+ into resorption lacuna	AR osteopetrosis
CLCN7	Chloride channel for transport of Cl- into lacuna	AD and AR osteopetrosis
CA1	Carbonic anhydrase for generation of H+ in osteoclasts	AR osteopetrosis
OSTM1	Associated with CLCN7	AR osteopetrosis
PLEKHM1	Important for vesicular trafficking & acidification machinery	AR osteopetrosis
(iii) Important for organic matrix degradation		
CTSK	Collagenase secreted into lacunae by osteoclasts	Pyknodysostosis

Note: AR- Autosomal recessive, AD- Autosomal dominant

CLASSIFICATION

Disorders of increased bone density may be divided into the following categories on the basis of underlying pathophysiology[1]:

1. Disorders of osteoclast differentiation
2. Disorders of NF kappa B signaling
3. Disorders of osteoclast function
4. Disorders of acidification machinery

The first two group of disorders lead to osteoclast poor forms of osteopetrosis whereas the others lead to osteoclast rich osteopetrosis, the more common form of osteopetrosis.

The other classification of these disorders based on a clinical basis as per the **Nosology and Classification of Genetic Skeletal Disorders, 2006 Revision** is as follows[3]:

i. Increased bone density without modification of bone shape
ii. Neonatal osteosclerotic disorders
iii. Increased bone density group with diaphyseal /metaphyseal involvement

As per this classification, there are at least 30 disorders with increased bone density.

This article is going to discuss some common disorders and their genetic bases.

I. INCREASED BONE DENSITY WITHOUT MODIFICATION OF BONE SHAPE

1. OSTEOPETROSIS: This disorder is associated with a generalized increase in bone density without any modification of bone shape. It is also known as marble bone disease. It has both autosomal recessive and autosomal dominant forms with an incidence of 1 in 250,000 and 1 in 20,000 respectively.[1,2] It can arise due to mutations in the following genes:

i. TCIRG1: Encodes the V0 subunit of H+ATPase (ATP6V0A3) which functions to transport the H+ ions into the resorption lacunae of osteoclasts. Mutations in this gene account for >50% of cases of osteopetrosis and this form of osteopetrosis is inherited in an autosomal recessive manner[1,2,4]

ii. CLCN7: Mutations account for 75% of adult onset autosomal dominant form, which is mild and may be asymptomatic. However, homozygous mutations lead to severe neuropathic forms with associated neurodegeneration and neuronal ceroid lipofuscinosis like illness.

iii. OSTM1: Leads to malignant infantile osteopetrosis; shows autosomal recessive inheritance.

iv. PLEKHM1: Leads to intermediate type osteopetrosis; shows autosomal recessive inheritance.

v. LRP5: Autosomal dominant inheritance leading to adult form of osteopetrosis.

vi. CA 1: Deficiency of carbonic anhydrase in the osteoclasts as well as renal tubular cells leading to decreased H+ ion production. It shows autosomal recessive inheritance and presents as a mild childhood form with associated renal tubular acidosis

vii. RANK & RANKL mutations: These lead to osteoclast poor forms of osteopetrosis and show autosomal recessive inheritance. RANKL mutations are associated with hypogammaglobinemia.

viii. NEMO: X linked forms of osteopetrosis due to mutations in NEMO (NF-Kappa-B Essential Modulator) gene which is an inhibitor of NF kappa B pathway. The disorder is called OL-EDA-ID (osteopetrosis, lymphedema, anhidrotic ectodermal dysplasia and immunodeficiency).

Clinical features of osteopetrotic conditions:[1,2]

The disease can have three presentations:

I. **Malignant/ Severe forms:** These have onset in infancy and death in childhood (10 yrs). Presents with bone marrow failure, hepatosplenomegaly due to extramedullary hematopoiesis, signs of cranial nerve compression like hearing loss (78%), facial palsy, blindness and craniofacial changes like macrocephaly and frontal bossing (Fig 1). There is predisposition to fractures and osteomyelitis. Radiographs show diffuse increase in bone density, loss of corticomedullary differentiation and 'bone-in-bone changes' in metacarpals (Fig 2). Age of onset, inheritance pattern and the presence of associated features, such as neurodegeneration, mental retardation, skin and immune system involvement, or renal tubular acidosis may point to particular subtypes of osteopetrosis.

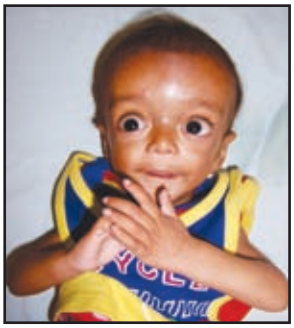

Figure 1: Face of a patient with infantile osteopetrosis showing prominent forehead

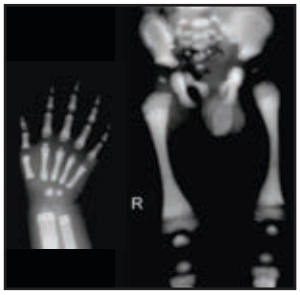

Figure 2: Radiographs showing increased bonedensity and bone in bone appearance in osteopetrosis

Due to the autosomal recessive mode of inheritance, there is a 25% risk of recurrence of the malignant form of osteopetrosis.

Increased bone density cannot be detected prenatally by ultrasonography or radiography. Hence, prenatal diagnosis can only be provided by detection of mutation in the chorionic villus sample if the mutation has been identified in the proband (affected child) or carrier parents.

ii. **Intermediate forms:** This group presents in childhood with mild anemia and tendency to fractures after minor trauma. Optic nerve compression may occur. Radiographs show increased bone density.

iii. **Mild forms:** are of adult onset, usually detected after a fracture (femur neck, cervical spine hangman fracture), osteoarthritis or osteomyelitis especially of the mandible. Cranial nerve compression is rare. Radiographs show increased bone density, Erlenmeyer flask deformity of long bones and sandwich vertebrae.

Differential diagnoses

Other conditions with diffuse increase in bone density and bone marrow failure which need to be considered in the differential diagnosis of osteopetrosis are fluorosis, poisoning with beryllium, lead or bismuth, myelofibrosis and Paget's disease (sclerosing form).

Management[2]

i. Bone marrow transplantation (BMT): For cases with severe autosomal recessive osteopetrosis, it offers a curative treatment. This measure shows a 5 year disease free survival of 75%, but cannot reverse all the complications like optic neuropathy and neurological involvement. Some forms like the osteoclast poor osteopetrosis and the ones with primary neurologic deterioration respond poorly. Benefit is better if transplant is carried out before the age of 3 months.

ii. Supportive and symptomatic measures:

- Hypocalcaemic seizures: Calcium and vitamin D supplementation
- Bone marrow failure: red blood cell and platelet transfusions as required
- Surgical decompression of the optic nerve and other cranial nerves when involved
- Immunoglobulins in setting of hypogammaglobulinemia
- In osteopetrosis with renal tubular acidosis: Potassium supplements, no bicarbonates

iii. Surveillance measures:

- Ophthalmic evaluation: Yearly study of visual evoked potentials (VEPs), visual field and acuity testing, baseline X ray of optic foramina
- Yearly audiometry
- Routine dental surveillance and maintenance of oral hygiene
- Yearly blood counts
- Avoidance of activities with fracture risk

iv. Interferon gamma 1b (IFNγ1b) treatment: May be useful in cases which are unlikely to respond to BMT or as a bridge to BMT. It acts by increasing bone marrow

space and marrow resorption and improving immune function.

Other diseases belonging to this group of increased bone density without change in shape of bones are as follows:[1,5]

2. PYKNODYSOSTOSIS: This disorder is characterized by short stature, increased bone fragility, persistent open anterior fontanel and acro-osteolysis of the terminal phalanges. Patients have a typical 'open mouth outline' facial appearance due to frontal bossing, micrognathia, loss of the mandibular angle and dental anomalies including persistence of deciduous teeth resulting in a double row of teeth. It is an autosomal recessive disorder caused by mutations in Cathepsin K. Radiographs show generalised increase in bone density, loss of mandibular angle and acro-osteolysis.
3. OSTEOPOIKILOSIS: This is a benign, usually asymptomatic condition. It is diagnosed radiographically by the presence of multiple symmetrical circular/ovoid sclerotic opacities of the ischiae, pubic bones and the epimetaphysial regions of the short tubular bones (Figure 3). It may be isolated or can occur in association with elastic or collagen connective tissue naevi of the skin, a condition termed Buschke- Ollendorff syndrome (BOS). Its inheritance is autosomal dominant with mutation in LEMD3 gene.

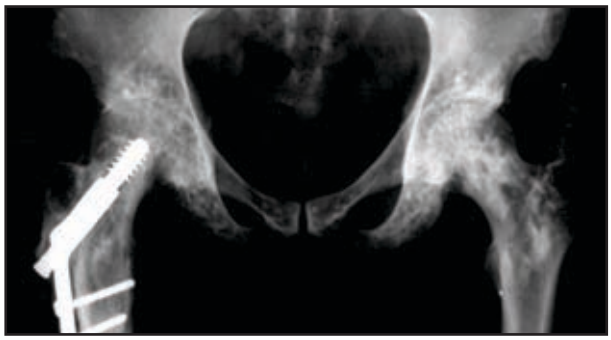

Figure 3 : Punctate oval opacities in the pubic bones and femoral head in a patient with osteopoikilosis

4. MELORHEOSTOSIS: Usually a sporadic sclerosing bone condition manifesting in a sclerotomal distribution, frequently affecting one limb. There is cortical hyperostosis with thickening, which resembles dripping candle wax on radiographs. It is due to heterozygous mutations in LEMD3 gene.
5. OSTEOPATHIA STRIATA: It is an X-linked dominant condition due to mutations in WTX gene. It is characterized by longitudinal striation of the metaphyses of the long bones. It may be an isolated condition or may occur with cranial sclerosis which is a clinically heterogeneous condition, ranging from mild skeletal manifestations to multisystem organ involvement in the form of macrocephaly, hearing loss, cleft palate, developmental delay and cataracts.

II. NEONATAL OSTEOSCLEROSIS GROUP[1,5]

1. BLOMSTRAND DYSPLASIA: An autosomal recessive disorder due to inactivating mutations in PTHR1 (Parathyroid hormone receptor) gene, which is characterized by advanced bone age and dentition. The neonate presents with short limbs, polyhydramnios, hydrops fetalis, facial anomalies and preductal aortic coarctation.
2. DESMOSTEOSCLEROSIS: An autosomal recessive disorder which is characterized by short limbs, multiple congenital anomalies and diffuse increase in bone density. Elevated levels of the cholesterol precursor desmosterol and relative deficiency of cholesterol are seen. It occurs due to mutations in the gene encoding 3 alpha hydroxysterol delta 24 reductase which converts desmosterol to cholesterol.
3. RAINE DYSPLASIA: A lethal disorder with diffuse increase in bone density, characteristic fish like facies, choanal atresia and protrusion of brain through fontanelles. Mutation in FAMC20 gene have recently been found in 6 patients.
4. CAFFEY DISEASE: The classic form of the disease is characterized by painful, warm, bony swellings in a child less than 5 months age and subsequent spontaneous resolution with normal looking bones (Fig 4a and b); it has an autosomal dominant inheritance and is due to mutation in the COL1A1 gene. The prenatal lethal form presents like osteogenesis imperfecta type II and is perhaps caused by autosomal recessive mutations.

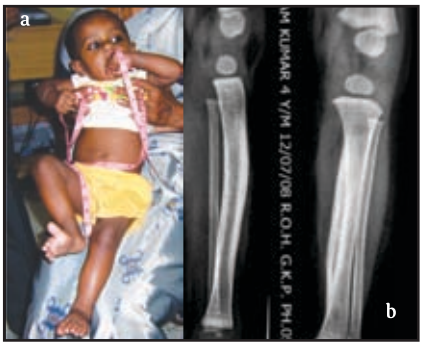

Figure 4 : Caffey disease showing (a) Soft tissue swelling of leg (b) Hyperostosis of tibia

III. INCREASE BONE DENSITY WITH PREDOMINANT METAPHYSEAL OR DIAPHYSEAL INVOLVEMENT[1,5]

1. CRANIOMETAPHYSEAL DYSPLASIA: This disorder is characterized by metaphyseal involvement with Erlenmeyer flask like deformity and sclerosis of cranial bones leading to optic nerve and auditory nerve compression (Fig 5). Both autosomal recessive and autosomal dominant forms exist. The dominant forms are caused by mutations in the ANKH gene.

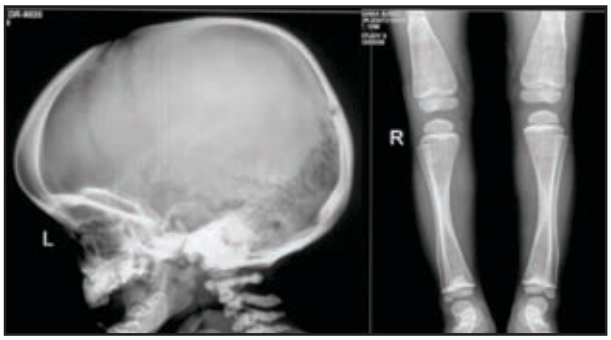

Figure 5 : Sclerosis of the base of skull and Erlenmeyer flask deformity of long bones of leg in Craniometaphyseal dysplasia

2. DIAPHYSEAL DYSPLASIA / CAMURATI ENGELMANN DISEASE: It is an autosomal recessive disorder due to mutations in TGF beta gene. It presents with painful bony swellings in legs with diaphyseal bone thickening, muscular hypotonia and wasting and systemic signs.
3. JUVENILE PAGET DISEASE: It is caused due to mutations in OPG (Osteoprogerin), which leads to increased bone turnover. It presents as painful deformity and expansion of limbs and cranial enlargement in childhood. Increased fragility of bones, premature loss of teeth and dwarfism are noted. In adulthood, massive increase in cranial sclerosis is seen.
4. SCLEROSTEOSIS: Homozygous mutations in the SOST gene cause massive sclerosis of all bones. Patient has gross facial distortion, proptosis, a prominent mandible and features of cranial nerve compression.
5. VAN BUCHEM DISEASE/ ENDOSTEAL HYPEROSTOSIS: This disorder is due to recessive mutations in SOST leading to moderate cranial sclerosis, prominent mandible and occasional cranial nerve compression. Sclerosis of mandible, clavicle and diaphyses of long bones also occur.
6. CRANIODIAPHYSEAL DYSPLASIA: Autosomal dominant and recessive forms are described. There is cranial and facial osteosclerosis. Marked distortion of face , obstruction of paranasal sinuses and cranial nerve compression occur. Asymmetric thickening of diaphyses of long bones is characteristic. The gene is not yet known.
7. FRONTOMETAPHYSEAL DYSPLASIA: This X linked dominant disorder which is allelic to Melnick - Needles

Messages

- Impaired function of osteoclasts and disturbance of osteoblast-osteoclast balance lead to various disorders with increase bone density.
- These disorders lead to brittle bones prone to fractures, encroachment of bone marrow and compression of the nerves passing through bone foramina, especially various cranial nerves.
- The severity of these disorders can range from asymptomatic (as in osteopoikilosis and mild forms of osteopetrosis) to potentially lethal conditions (as in malignant forms of osteopetrosis and neonatal lethal disorders).
- Treatment of these disorders remains primarily symptomatic and supportive, although bone marrow transplantation is curative for some forms of osteopetrosis and decompression surgeries can be useful in cases with cranial nerve compression.
- Evaluation of family members can recognize apparently asymptomatic relatives. Genetic counseling is essential to give an accurate recurrence risk estimate. If causative mutation is identified in a patient, prenatal diagnosis can be offered for pregnancies at risk.
- The study of the molecular basis of these disorders provides insight into the mechanisms involved in maintaining homeostasis of the bones. This provides hope for designing novel therapies for various monogenic as well as multi-factorial bone disorders.

and oto-palato-digital syndromes, is caused by mutations in the filamin A gene. The disorder is characterized by supraorbital prominence and sclerosis of base of skull. Patients also have deafness and urogenital defects. Long bones show Erlenmeyer flask deformity.

8. Various other disorders like Pyle metaphyseal dysplasia, oculodentoosseous dysplasia, trichodentoosseous dysplasia and many other rare conditions are also associated with increased bone density.

Management: These conditions cause morbidity primarily by compression of cranial nerves and may be amenable to treatment by decompression craniectomy. Dental care is also an important measure. The Camurati Engelmann disease responds to immunosuppression. Treatment for other complications is primarily symptomatic.

Conclusion

These numerous disorders of excessive bone density presenting in various forms indicate the delicate homeostasis between bone formation and bone resorption. An insight into their molecular pathogenesis is important to realize the complex mechanisms by which bone formation and breakdown are governed. This would help us in designing better treatment strategies which may be extrapolated to various other conditions of altered bone density, especially common disorders like osteoporosis.

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International Rare Disease Day

February 28, 2011

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Long term efficacy of gene therapy.....

Contributed by:
Girisha KM
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Long term efficacy of gene therapy[1]

Abina et al have reviewed the long-term outcome after gene therapy in nine patients with X-linked severe combined immunodeficiency (SCID-X). Nine patients who underwent ex vivo retrovirus-mediated transfer of gamma chain to autologous CD34+ bone marrow cells between 1999 and 2002 were assessed. Of these, 8 patients were alive after a median follow up period of 9 years (range 8 to 11). Gene therapy was initially successful in eight of them at correcting immune dysfunction. Four patients developed acute leukemia, a major concern. Seven patients including three survivors of leukemia had sustained immune reconstitution. Three patients required immunoglobin replacement therapy. Sustained thymopoiesis was established by the persistent presence of naive T cells in three patients even after chemotherapy. The authors conclude that gene therapy is an option for patients who do not have an HLA-identical donor for hematopoietic stem cell transplantation and for whom the risks, which includes acute leukemia, are acceptable.

Gene for Kabuki syndrome[2]

An autosomal dominant condition with multiple malformations and mental retardation, Kabuki syndrome is now found to be caused by mutations in MLL2. Using exome sequencing, Ng et al have done massive parallel sequencing of 10 affected individuals. After filtering against existing SNP databases, they did not find a candidate gene containing previously unknown variants in all affected individuals. Using less stringent filtering criteria, allowing for genetic heterogeneity or missing data, they identified multiple candidate genes. However, genotypic and phenotypic stratification highlighted MLL2, which encodes a Trithorax-group histone methyltransferase. Seven probands had newly identified nonsense or frameshift mutations in this gene. Sanger sequencing of MLL2 gene detected mutations in two of the three remaining individuals with Kabuki syndrome and in 26 of 43 additional cases. In families where parental DNA was available, the mutation was confirmed to be de novo (n = 12) or transmitted (n = 2) in concordance with the phenotype. Their results suggest that mutations in MLL2 are a major cause of Kabuki syndrome; however, genetic heterogeneity, less stringent selection of patients and mutations in the non-coding region of the gene are possible explanations for absence of detectable mutations in some patients diagnosed to have this condition.

Susceptibility locus for tuberculosis[3]

Tuberculosis, an infectious disease has a susceptibility locus! Thye et al, have done a genome wide association study on 11,425 African patients with tuberculosis. rs4331426, located in a gene-poor region on chromosome 18q11.2, was associated with disease (combined P = 6.8 × 10−9, odds ratio = 1.19, 95% CI = 1.13–1.27). Their study demonstrates that genome-wide association studies can identify new susceptibility loci for infectious diseases like tuberculosis, still a major health problem in India. The day is not far when all medical geneticists will be found to have some common susceptibility loci Too!

Treatment for Vascular Ehlers-Danlos syndrome[4]

Ehlers-Danlos syndrome, vascular type, due to mutations in the COL3A1 gene can be treated by celiprolol, a cardioselective β blocker with β2 agonist vasodilatory properties. Fifty three patients with this condition were recruited into a randomised, open trial with blinded assessment of clinical events in eight centers; 25 patients were given celiprolol and 28 patients were not given the drug. The trial initially planned for 5 years, was stopped after about 4 years for treatment benefit. While 14 (50%)of the patients in the control group had vascular events such as arterial rupture or dissection in the 4 year follow up period, only 5 (20%) in the celiprolol group had these primary endpoints. Ong et al, suggest that celiprolol might be the treatment of choice for physicians aiming to prevent major complications in patients with vascular Ehlers-Danlos syndrome.

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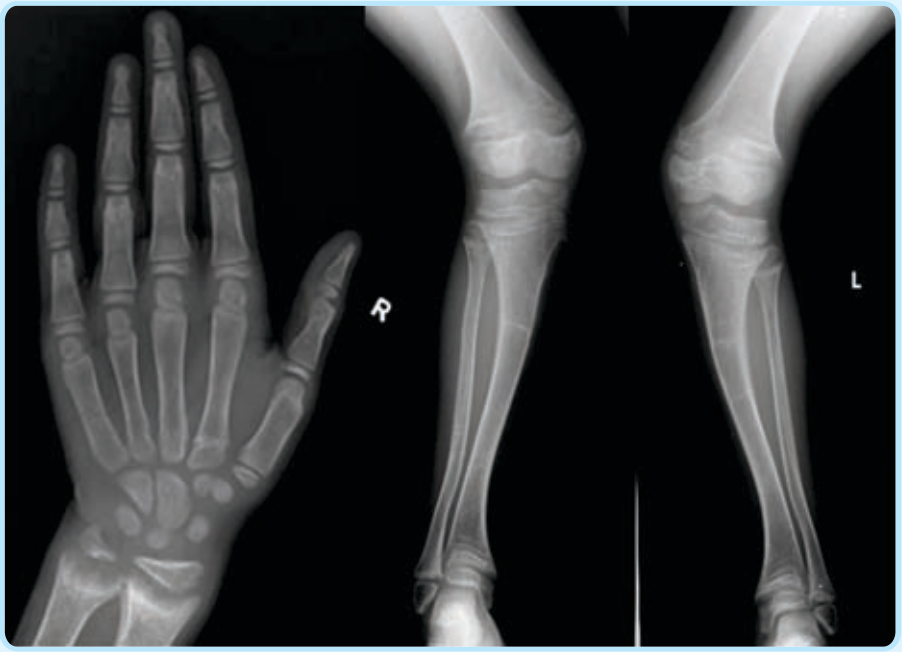
11

Contributed by: Dr Hitesh Shah, Department of Orthopedics, Kasturba Medical College, Manipal. Email: hiteshshah12@gmail.com

These are the radiographs of a child with short stature and knock knees. She has a similarly affected sister and father. Her calcium, phosphorous and alkaline phosphatase levels were normal. There was no metabolic acidosis. Identify the condition.



Correct responses to this quiz will receive a cash prize of Rs 1000/- and a certificate at the First Indo-US symposium on skeletal dysplasia to be held at Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow during February 12-13, 2011. To be eligible for the award, the respondent has to be a registered delegate for the conference. Send your answers to geneticsiap@gmail.com before 31st January 2011.

Answer to the PhotoQuiz 10 of the previous issue

Peutz Jeghers syndrome (OMIM 175200)

Peutz Jeghers syndrome is characterized by pigmented macules of the skin and mucous membranes, and gastrointestinal polyposis. The mucocutaneous pigmentation appears in childhood as brown-black or blue-black freckles around the mouth, on the buccal mucosa, and on the hands. Intestinal polyps occur characteristically in the jejunum, but also in other parts of the gastrointestinal tract. It is inherited as an autosomal dominant trait

Correct responses to PhotoQuiz No. 10 were given by

1.Lalit Bharadia, Jaipur
2.Narendra Chaudhary, Manipal
3.Kuldeep Singh, via email
4.Mohandas Nair K, Calicut
5.Chinmayee Ratha, New Delhi
6.Anoop Verma, Raipur
7.Anil Jalan, Mumbai
8.Ravi Goyal, Kota
9.Kalpana Gowrishankar, Chennai
10.Yatheeshan KK, Kasaragod
11.Pramila Gopal Menon, Pune
12. Yesodha Thanikachalam, via email

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