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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

Editorial

# Indo US Symposium on Skeletal Dysplasia

## *A reflection of the Current Clinical Genetics Scenario in India*

**Dr Shubha Phadke**

Professor & Head, Department of Medical Genetics

Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, India

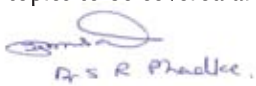
E mail: shubharaophadke@gmail.com

Diagnosis and management of genetic disorders has evolved as a medical specialty. Common genetic disorders like thalassemia, hemophilia, Duchenne muscular dystrophy are usually taken care of by pediatricians, internists or respective specialists. But many genetic disorders are very rare and the improved ability to provide them with diagnosis, mutation detection, specialized and new treatments has led to the need for clinicians specially trained to deal with these rare genetic disorders. Management of genetic disorders needs a unique genetic perspective which includes family history, genetic counseling and prenatal diagnosis. Though the clinical genetics specialty came into existence in India more than two decades ago, it is only during the last decade that it is gaining prominence. This is because of improved diagnostic facilities, establishment of several clinical genetics centers across the country and increasing awareness about genetic counseling and prenatal diagnosis amongst pediatricians and obstetricians.

In the current scenario, the need for conferences and symposia in the field of clinical genetics has become pertinent. This was the general opinion of the participants of the recently held Indo-US Symposium on Skeletal Dysplasia at Lucknow. This symposium was organized with the aim of learning from experts in the field, from the USA. The event was supported by the Indo US Science and Technology Forum with additional assistance from the DBT, ICMR, CSIR and MCI. The topic chosen was rather specialized with focus on just the skeletal dysplasias, a group of genetic disorders of the bone, and the organizers were quite apprehensive that it might not garner much interest especially amongst the non – geneticist clinicians. But there was a tremendous response and senior as well as budding geneticists from all over India were there to participate; the topic attracted radiologists as well. The scientific program of the symposium was planned in a way to cover all aspects of skeletal dysplasia and included lectures on clinical and radiological evaluation, molecular diagnosis, molecular pathophysiology, orthopedic and neuro- surgical treatments, prenatal diagnosis and recent advances. There was a galaxy of speakers including world renowned experts in the field such as Drs Rimoin, Lachman, Horton, Graham, Danielpour and Mackenzie from the USA. The Indian scenario was presented by Drs Agarwal, Verma and many young geneticists from India. It was an academic feast to listen to the stalwarts. Such opportunities to interact with clinicians and geneticists from US will definitely prove to be fruitful. The poster presentations gave an overview of the work on skeletal dysplasia being done in different centers in India and were quite impressive. The symposium gave an opportunity for like minded clinical geneticists (still a rare species in India!) to come together. Such opportunities are very important for the growth of the specialty in India. Overall, the symposium on skeletal dysplasia affirmed that Clinical Genetics in India has come of age.

In the same vein, it needs to be mentioned that clinical geneticists in India are so small in numbers that other specialists need to partly take up the responsibility of diagnosis, management and counseling for genetic disorders. With this purpose of improving the knowledge base about clinical genetics amongst all practitioners, we try to give updates and guidelines for common genetic disorders, through this newsletter 'GENETIC CLINICS'. Two articles in this issue, namely, 'Neonate with Down syndrome' and 'QF PCR', are written with this aim. Facing a neonate with Down syndrome is not a rare moment and every neonatologist, pediatrician and obstetrician will need to do the difficult task of breaking the news of Down syndrome in a neonate, to the family. Counseling for positive screening tests is also equally challenging as the concept of probability involved in screening tests is difficult for many laypersons to understand. Positive screening tests open up the options of various confirmatory tests. The obstetrician, armed with knowledge of the principles and limitations of traditional karyotyping, QF PCR and Fluorescence in situ Hybridization (FISH) techniques used for chromosomal analysis of prenatal samples would be able to guide his/her patients appropriately.

We request your feedbacks which will surely help us to improve the content of 'GENETIC CLINICS'! Ideas about topics to be covered are also welcome.



Shubha Phadke

1st April, 2011

# THE FIRST INDO US SYMPOSIUM ON SKELETAL DYSPLASIA

## Feb 12-13, 2011 at
## Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow

Participants of the First Indo US Symposium on Skeletal Dysplasia

Poster presentations


Buddding Geneticist

Cultural program by special friends: Shruti and Gore

Women Power!

Dr David Rimoin

Dr William Mackenzie

Dr John Graham

Dr Sharma and Dr Agarwal

Looking forward to collaborate...

# Fetal Valproate Syndrome: Report of Three Cases with Congenital Heart Disease and Facial Dysmorphism

**Siddram J Patil [1], Viralam S Kiran [2], Sandeep Kudale [3]**

[1] Centre for Molecular and Metabolic Diagnostics & Research, [2] Department of Pediatric cardiology ,
[3] Department of Pediatrics, Narayana Hrudayalaya Institute of Medical Sciences, Bangalore, India
E-mail: drsjpatil@gmail.com

## INTRODUCTION

Children exposed to intrauterine teratogens are at risk of birth defects and deficit in cognitive functions. Maternal epilepsy and use of antiepileptic drugs (AEDs) is one of the important teratogens associated with variable clinical features in offspring. In majority, maternal epilepsy and AEDs are safe during pregnancy with no untoward effect on offspring.[1] Based on current recommendations, effect of maternal epilepsy and AEDs on fetus can be minimized, but cannot be entirely ruled out. Reported here are three children born to mothers on sodium valproate (VPA). One mother in addition to valproate also received clonazepam during antenatal period. These cases presented with congenital heart disease (CHD) and facial dysmorphism.

## CASE REPORTS

### Case 1

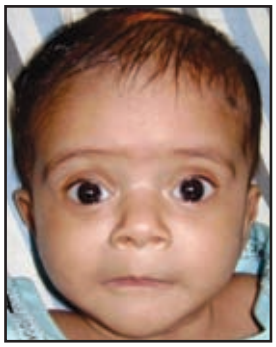


Fig 1. Case 1: Close up of the face – note the upslanting eyes,thin eyebrows, depressed nasal bridge, anteverted nostrils, long smooth philtrum, thin upper lip

Five months old, second born, female child presented with congenital heart disease. The mother was known to suffer from epilepsy. The mother was on VPA 1000 mg in divided doses during pregnancy. Before conception she was supplemented with folic acid 5 mg once a day (preconception). The antenatal history was uneventful. The baby was born at term through a normal vaginal delivery and weighed 2.5 Kg at birth'. The elder male sibling of the proband is 3 years 6 months old and the mother was on the same drug during his pregnancy.

Examination of the proband revealed dysmorphic facial features including upslanting palpebral fissures, long smooth philtrum, thin upper lip, depressed nasal bridge, anteverted nostrils and an open anterior fontanel (Fig 1). Cardiac evaluation revealed Fallot's tetralogy. In addition she had hypoplastic nails. On follow-up at age 1 year, her anthropometric measurements were as follows: head circumference 42.3 cm (- 2SD to - 3SD) and length 70 cm (5th – 10th centile). Chromosomal analysis was normal.

Elder male sibling's cardiac evaluation was normal and his developmental milestones were appropriate for age. His facial features however, were suggestive of fetal valproate syndrome and the mother also complained that he had hyperactive behavior.

### Case 2

Eleven months old, second born, female proband presented with congenital heart defect and developmental delay. Maternal history revealed that the mother is a known case of epilepsy and was on sodium valproate 1200

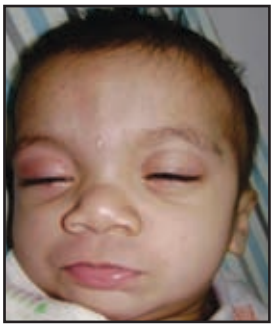


Fig 2. Case 2: Close up of the face - refer text for description

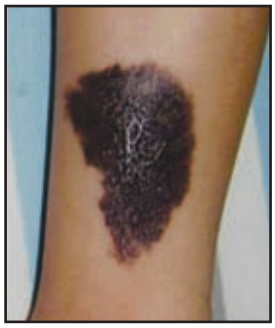


Fig 3. Case 2: Hyperpigmented skin patch over the left leg

mg / day in divided doses with clonazepam 0.5 mg once a day, before and during the antenatal period. Folic acid was supplemented after 3-4 months of conception. During the first pregnancy the mother was on AEDs (exact details not known) but from the given history, the first offspring does not appear to be having any significant health problems. Proband's examination revealed a broad/high forehead, prominent metopic ridge, groove below the lower eyelids on both sides, depressed nasal bridge, broad nasal tip, anteverted nostrils, low set posteriorly rotated ears, thick lower lip, thin and longer upper lip, long smooth philtrum, micrognathia, fifth finger clinodactyly

on both sides and a hyperpigmented skin patch over the left leg 3.5 x 2 cm (Fig 2 & 3). Her anthropometric measurements at age 12 months were as follows : head circumference 41 cm (-2SD to -3SD) and length 68 cm (< 3rd centile). Cardiac evaluation revealed a moderate sized, secundum atrial septal defect. Chromosomal analysis was normal. Examination of the elder sibling was not done, as parents refused to come for follow-up.

**Case 3**

A 7-day old male baby was referred from intensive care unit with congenital heart disease, facial dysmorphism, hypocalcaemia and with suspicion of 22q11 microdeletion. Mother was a known case of epilepsy on VPA 750 mg per day in divided doses. She was on periconceptional folic acid, 5 mg once a day. Antenatal ultrasound and maternal AFP done at 19 weeks of gestation were normal. The baby was delivered by cesarean section with a birth weight of 3.75 kg. Examination revealed a hairy forehead, generalized increased body hair, broad nasal root & nasal tip, thin eye brows, groove under lower lids on both sides, and low set ears with overfolded helix (Fig 4). Cardiac evaluation revealed hemitruncus with small patent ductus arteriosus. Baby died in the neonatal period before surgery for CHD. Chromosomal analysis was not done.

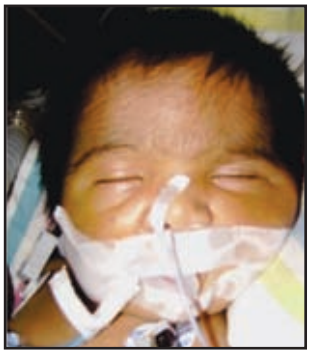


Fig 4. Case 3: close up of the face - refer text for description

**DISCUSSION**

Epilepsy is a common neurological disorder affecting 1% of adults.[2] Implications of epilepsy and use of AEDs for adult females of reproductive age are different from adult males of reproductive age.[3] Women of childbearing age on AEDs with or without epilepsy (as AEDs are also used for treating mood disorders etc) are at increased risk of having offspring with congenital malformations, cognitive deficits, intrauterine growth retardation, stillbirth and neonatal morbidity (hemorrhage, withdrawal symptoms etc).[4] This makes preconceptional counseling very important for mothers on AEDs with or without epilepsy. Current recommendations suggest the following: (i) advise preconceptional counseling, to curtail the risk to the offspring (ii) switch from polytherapy to monotherapy if possible, (iii) informed choice of a safer single drug, preferable use of sustained release preparations, dosage adjusted to clinical response (seizure free) irrespective of therapeutic range, not to exceed beyond per day safe limits of dosage, (iv) periconceptional folic acid supplementation and (v) fetal anomaly scan.[4] Commonly used AEDs are VPA, carbamazepine, phenytoin and phenobarbital. There are many newer drugs which are being used to treat epilepsy. The overall risk of congenital malformations in children exposed to AEDs is 4-6% against 2-3% in the general population.[1] The risk of congenital malformations increases in cases with AEDs polytherapy in comparison to AED monotherapy.[3] Among the various AEDs, VPA is widely used and is accepted because of its broad spectrum efficacy and non-sedative feature.[5] Current recommendations and studies suggest that in pregnant mothers VPA should be used as monotherapy, not more than 1000 mg / day and in divided doses to minimize the risk to the growing fetus.[3,6] Clinical features among fetuses exposed to VPA monotherapy include neural tube defect, cleft palate, congenital heart disease (CHD), psychomotor retardation, craniofacial dysmorphism, skin defects (including pigmentary lesions) and other organ malformations (eye, brain, genitourinary and skeletal system etc).[5,6] Reported risk of congenital malformations due to exposure to VPA monotherapy during the first trimester of pregnancy is 7.3 -15.1%, which is higher in comparison to any other AED given as monotherapy.[7,8] The risk of fetal malformation increases dramatically with VPA dosage more than 1000 mg – 1400 mg.[3,8] Periconceptional folic acid seems to have a protective effect in preventing fetal malformations but not in all cases.[9]

Children exposed in-utero to VPA show dose dependent, variable features of psychomotor dysfunction ranging from normal development to developmental delay (20%), mental retardation (10%), seizures and behavioral issues.[6] Reported literature suggests that minor craniofacial anomalies are seen in both treated and untreated maternal epilepsy.[5,6] DiLiberti et al first described craniofacial features with or without major anomalies after VPA exposure in the year 1984 and coined the term "Fetal Valproate Syndrome" (FVS).[10] Craniofacial features (facial gestalt) seen in FVS cases might be peculiar in constellation and they include a small broad nose, anteverted nares, depressed nasal bridge, long hypoplastic philtrum, thin upper lip, thick upper lip and grooves below lower eyelids (infraorbital creases). Other facial features include epicanthic folds, thin eyebrows, hypertelorism, broad forehead, low set ears, micrognathia and microcephaly.[5,6] All three cases reported here had facial features suggestive of FVS along with CHD. None of them had any other major internal organ malformations. Together, the facial features are useful in the clinical diagnosis of FVS. Cardiac malformations are seen in 26% (18/70) of described and documented cases of FVS.[6] Cardiac lesions can be variable and common lesions reported are ventricular septal defect, aortic stenosis, pulmonary

stenosis and patent ductus arteriosus.[6] Thomas et al studied the risk of cardiac malformations in infants born to mothers with epilepsy and on AEDs.[11] Risk of cardiac malformations in cases with polytherapy was 10.3% and in cases with monotherapy 6.5%. In addition, they observed that cardiac malformations were seen more often in cases on VPA monotherapy, but this finding was not statistically significant. Cardiac malformations were found to be unrelated to other variables such as maternal age, folic acid supplementation, epilepsy syndrome etc.[11]

In case 1, the mother was on VPA 1000 mg per day and case 3, on 750 mg per day in divided doses. Both the mothers were taking VPA within the recommended dosage limits and were on periconceptional folic acid (5 mg) supplementation. In case 1, the elder male sibling had facial features suggestive of FVS with hyperactive behavior without any internal organ malformations. Similar cases have been reported with variable intra/inter familial clinical expression in children born to mothers on recommended dosage of VPA with or without folic acid supplementation. [5,7,9]

Case 3 had facial dysmorphism, CHD and hypocalcemia. The facial features were suggestive of FVS. Although there was suspicion of 22q11 microdeletion (DiGeorge / Velocardiofacial syndrome), facial features on examination were not suggestive of 22q11 microdeletion. However it is important to rule out chromosomal cause in all cases of CHD with facial dysmorphism. Genetic counseling issues will differ in cases with CHD of chromosomal causes.

In case 2, the mother's VPA dosage was higher than the recommended dosage level and in addition she was on clonazepam. The child had more characteristic facial features of FVS than the other two cases. Clonazepam is also known to increase the risk of congenital malformations, when given as monotherapy or when added along with other AEDs.[3,12]

The cause for inter/intra familial variability of FVS, VPA/AED dosage dependent & independent clinical features and the variable protective role of folic acid could be explained by complex gene-environment interactions involving the maternal and fetal genotype/metabolism.[12] Ornoy provides a good review of the mechanism through which VPA can cause teratogenicity by using complex different pathways.[12] These cases further illustrate that no particular level of VPA can be considered safe and the clinical features due to valproate teratogenicity are highly variable. All mothers (if VPA cannot be avoided) should be counseled preconceptionally in every pregnancy regarding the risks to the fetus, even if their intake is within the recommended safe drug levels and the previous child has no health-issues. With all the recommendations for the use of VPA during pregnancy, follow up scan for fetal anomalies including fetal cardiac evaluation by echocardiography is necessary as well as postnatal evaluation for neonatal morbidity and follow-up assessment of psychomotor functions.

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# Progressive Diaphyseal Dysplasia

**Shamsi Abdul Hameed, Hitesh Shah**
Pediatric Orthopedic Service, Department of Orthopedics, Kasturba Medical College, MANIPAL 576 104, Karnataka State, INDIA
Email: hiteshshah12@gmail.com

### Introduction

Progressive diaphyseal dysplasia [(Camurati-Engelmann disease (CED); (OMIM 131300)] is characterized by progressive expansion and sclerosis predominantly affecting the diaphyses of the long bones. It typically presents in childhood with generalized muscle weakness, lower limb pains and waddling gait.[1,2] It is a rare autosomal dominant skeletal dysplasia. We report a sporadic case of progressive diaphyseal dysplasia presenting with non specific limping and waddling gait.

### Case Report

A 10–year-old girl presented with insidious onset gradually progressive bilateral symmetrical weakness of lower limbs with non-specific pain since the age of 3 years. Weakness was involving proximal muscles of the upper and lower

limbs. She also complained of pain in her legs (Fig 1). She was born of a non-consanguineous marriage with no affected family members. Developmental milestones were normal. On examination, she had normal higher mental functions. She had progressive lumbar lordosis. There was a generalized loss of muscle bulk. Muscle tone was normal and reflexes were preserved. Gait was waddling type. Laboratory studies including erythrocyte sedimentation rate, calcium, phosphorus, alkaline phosphatase, TSH and creatine kinase were within normal limits. Muscle biopsy was reported to be normal. Radiological examination revealed bilaterally symmetric diaphyseal cortical thickening with increased density of long bones (Fig 2-5). Radiographs of spine and skull were normal, and appropriate maturation of bones was observed. Based on the history, physical examination, laboratory tests and radiographic studies, the diagnosis of the progressive diaphyseal dysplasia (Camurati-Engelmann disease) was made.

Fig 1. Clinical photograph showing bilateral symmetrical long slender thighs and legs

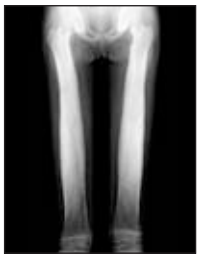

Fig 2. Anteroposterior radiograph of the thighs shows bilateral widening and increased density of the femurs.

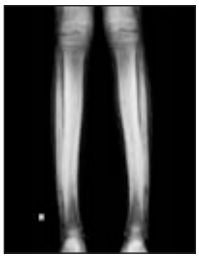

Fig 3. Radiograph of both legs shows deformation of the tibia and fibula that are hyperostotic, with cystic changes in a thickened cortex

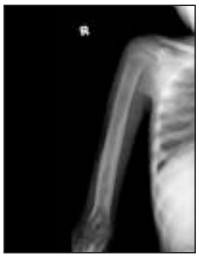

Fig 4. Anteroposterior and lateral radiographs of right humerus show bilateral widening and increased density of the humerus

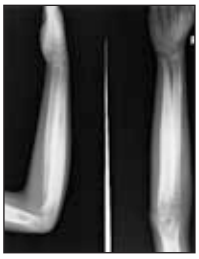

Fig 5. Radiograph of radius and ulna shows periostal and endosteal sclerosis. The medullary cavity is narrowed and in some places obliterated with cystic changes in the cortex

## Discussion

Camurati-Engelmann disease (CED) is a rare genetic disorder that affects the diaphyses of the long bones, skull, clavicle, or in rare cases, the facial bones. The reported incidence of the disorder (also known as progressive diaphyseal dysplasia or Engelmann's disease) is one in 1,000,000. The first and second cases reported in the literature were published by Cockyane and Camurati in 1920 and 1922. Many authors however credit the first description to Engelmann because of the case report published in 1929. The diagnosis of progressive diaphyseal dysplasia is based on clinical and radiological features. The variability shows up in the clinical and radiographic features of the disease. The disease affects the diaphyses of the long bones but in rare cases can affect the metaphyses. The reported bones of involvement in decreasing order are the femur, tibia, fibula, humerus, ulna, and radius. The progression also may include the skull, facial bones, vertebrae, pelvis, and distal extremities.[1,2] The disease is characterized by widening of the periosteal and endosteal surfaces. Symmetric bone growth is a cardinal feature of the disorder. The severity of the disease varies among patients, from those who are asymptomatic and are diagnosed only incidentally by radiographs showing bilateral increased density of long bones to babies who are unable to walk because of severe leg pain, underdevelopment, and abnormal gait. Clinical & radiological features progress with age.[1] Therefore, progression of the disease is highly variable and difficult to predict. The other manifestations such as anemia, hepatosplenomegaly and cranial nerve compression due to involvement of the base of the skull are less common.[3] Cranial base hyperostosis has been described in less than quarter of the patients reported.[4] Hearing loss and headache are the most frequent findings where as ophthalmopathy, facial nerve weakness, trigeminal neuropathy are less common. Infants have feeding problems, inability to gain weight and a wide based waddling gait. Common skeletal abnormalities are flat feet, valgus deformity of the ankle, bow legs and knock knees.[5]

The mutations causing this disease lie in the transforming growth factor-beta 1 (TGFb1) gene.[6] It is inherited in autosomal dominant fashion. It is believed the TGFb1 gene is directly related to the balance of osteoblast and osteoclast function, among other biologic processes. TGFb1 is a known inhibitor of myogenesis and adipogenesis, causing reduction in fat and muscle mass, characteristic muscle wasting, and easy fatiguability. The musculoskeletal involvement can cause varying abnormalities including lordosis, kyphosis, coxa valga, genu valga, pes planus, and frontal bossing. Corticosteroids are useful in the treatment of symptoms of this disease. There is no indication of shortened lifespan or predisposition to other chronic or life threatening illnesses.

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# Newborn with Down Syndrome: Care and Counseling

**Shubha R Phadke, Rekha Gupta**

Department of Medical Genetics

Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow

E mail: shubharaophadke@gmail.com

Birth of a child in a family is a very exciting, enjoyable and happy moment for the parents. Everybody expects a normal, healthy child. Parents dream about the child and make plans for the future throughout the pregnancy. With such expectations, when a child with a malformation or a birth defect is born, it shatters the lives of the parents. Of course, the family wants to treat and help the baby to the best of their ability. Birth defects like cleft lip, meningocele, and encephalocele are externally obvious and the parents get a fair idea of the problem. In case of internal defects like cardiac anomalies or duodenal atresia the symptoms of the baby make the parents realize that there is something wrong with the baby. But what about Down syndrome? Most of the neonates with Down syndrome do not have external malformations. The characteristic facial dysmorphism which is obvious to a pediatrician may not raise any concern in the parents' minds. Hence, breaking the news that the neonate has a serious handicapping condition is a very difficult task to the neonatologist and the obstetrician.

Some clinicians think that it may not be proper to tell the family immediately that the baby has a problem. Nobody is comfortable with the challenging task of breaking the bad news to the unexpectant and happy family. The justification of such clinicians is that let the family enjoy the child birth and over the next few months some other pediatrician may disclose the diagnosis of Down syndrome to the family or the parents themselves may notice the developmental delay and other problems and may consult a doctor.

But there are many valid reasons to disclose the diagnosis of Down syndrome in the neonatal period.

The most important reason is that it provides an opportunity to investigate the baby for major malformations like tracheo-esophageal fistula, cardiac malformation, cataract, duodenal atresia, gastrointestinal malformation, etc. Secondly, most of the parents feel hurt if they realize that the diagnosis was kept hidden from them. Delaying diagnosis also leads to loss of an opportunity to get karyotype done and offer genetic counseling, especially as some babies with Down syndrome may die during the neonatal period.

**1. When to disclose the diagnosis of Down syndrome in a neonate?**

The best time is as early as possible. Usually, it should be done within the first 2-3 days of life. If the neonate develops any problems due to some associated malformation, then the possibility needs to be discussed immediately with the family.

**2. Who should talk to the family?**

A senior experienced consultant should take the responsibility of breaking the news as it is a major and serious problem for the family. It is important as the news should be given with great sensitivity and the clinician should be confident and comfortable in answering questions of the family and should also be capable of dealing with the disturbed psychological situation. A pediatrician or a neonatologist and the obstetrician should sit together with the parents and tell the diagnosis.

**3. To whom should the news be told?**

Preferably both the parents should be told together that their newborn baby has Down syndrome. If there is some doubt in the diagnosis as in cases of premature babies, then the possibility of Down syndrome may be conveyed to the family and detailed counseling can wait till confirmation by karyotyping or other quicker methods like fluroscence in situ hybridization or QF-PCR.

**4. How to break the bad news?**

As mentioned earlier, breaking the news that the almost normal looking neonate is likely to have mental handicap is a great challenge to the pediatrician. Most pediatricians may face this situation some time in their lives. Even to an experienced clinical geneticist this task is an ordeal that he or she would like to avoid. However, though it is a difficult task, it has to be done by somebody. There is no right way to break the bad news, but some ways are worse than others. Care has to be taken to understand the state of mind of the parents. Most of the parents remember very well the moment and the words of the doctor when they were told that the baby has Down syndrome. The family should be given prior intimation that the clinician wants to discuss some issues about the baby.

Sit in privacy with both the parents and preferably the baby as well. In India, more often than not, grandparents or other relatives are also involved. They can be a good source of support to the family during such a stressful period. The language should be simple. The conversation should start on a personal note, like enquiring the name of the baby. During the course of discussion the baby should be referred by name as "your baby" and not words like "Mongol babies" or "these mentally retarded children". There are a few recommendations on how best to deliver the diagnosis of Down syndrome to the parents (see suggested reading).

The physician should be caring and empathetic. He or she should assess and acknowledge the reaction of the parents to stress and help them accordingly. There should be enough time for questions. Follow up visits are needed to reinforce information and help the family to cope up with the situation and slowly accept the reality.

**5. What should be told?**

The information about Down syndrome should include chromosomal etiology, associated major problems like mental handicap and malformations in some cases. Genetic etiology and chromosomal nature may need some explanation in the layman's language. It needs to be made clear that even though Down syndrome is genetic in nature, in most of the cases it is not inherited and usually there is no similarly affected member in the family.

Information about the general population prevalence may help to avoid guilt in the parents due to the birth of a child with Down syndrome. The severity of mental handicap needs more explanation. Most of the children with Down syndrome are moderately retarded. They learn to talk, walk and do self care and some simple repetitive jobs. It is reassuring to parents that children with Down syndrome can lead a happy and useful life. But most of them need lifelong supervision at home and work. There should be a truthful but positive approach.

The immediate need to investigate for internal anomalies like cardiac defects, cataract, etc should be clearly told; absence of any malformation may relieve the parents' anxiety to some extent. Positive aspects like no immediate risk to life, no physical handicap are usually helpful. Need for regular surveillance for hypothyroidism and hearing problems can be mentioned. Rare possibilities like leukemia, atlantoaxial dislocation, and Alzheimer disease need not be told. Information about early infant stimulation programs, patient support groups and relevant literature for parents should be given.

**6. Risk of recurrence in sibs**

These issues are discussed in subsequent visits, after the karyotype report is available. Information about prenatal screening and prenatal diagnosis is useful. If the baby has free trisomy 21 (47 chromosomes with 3 copies of chromosome 21) then the risk of recurrence in the next pregnancy of the mother is usually 1%. And in this situation the karyotypes of the parents are not needed. If the baby has Down syndrome due to translocation of chromosome 21 to the other chromosome 21 or any other chromosome, then the parental karyotypes from peripheral blood are need to give the risk of recurrence. If one of the parents is a carrier of a balanced translocation involving chromosome 21, the risk of recurrence varies from 5% to 100% depending upon the type of translocation and the parent involved. The other situation when the karyotypes of the parents may be needed for genetic counseling is when a child with Down syndrome dies before karyotyping could be done.

**Conclusions**

Facing a neonate with Down syndrome is an experience by itself as is the first meeting with his/her parents,

which every obstetrician, neonatologist and pediatrician will have to undergo a few times in life. This article may help the physicians to prepare for the situation and to deal with it with confidence. In this era of prenatal screening for Down syndrome, birth of children with Down syndrome may decrease. However, seeing a neonate with Down syndrome after negative results of prenatal screening is much more devastating for the family. Counseling such a family is a more difficult task. Empathy, good communication and adequate time are the important requisites for the success of counseling.

A similar situation needs mention here and that is prenatally detected case of Down syndrome. Breaking the news of a fetus being affected with Down syndrome after a prenatal diagnostic test is just as difficult as disclosing the diagnosis to the family of an affected neonate. Though usually the pregnancy is terminated in case Down syndrome is prenatally detected, the decision of termination is painful for the family and leads to a great sense of loss. The counseling should be done with senstivity and care should be taken to minimize emotional trauma to the family.

It should always be remembered that not only does the child with any congenital defect need medical management; the parents also need a lot of emotional support along with all the possible and latest information about the nature of the abnormality, etiology, prognosis, possible treatment and genetic counseling.

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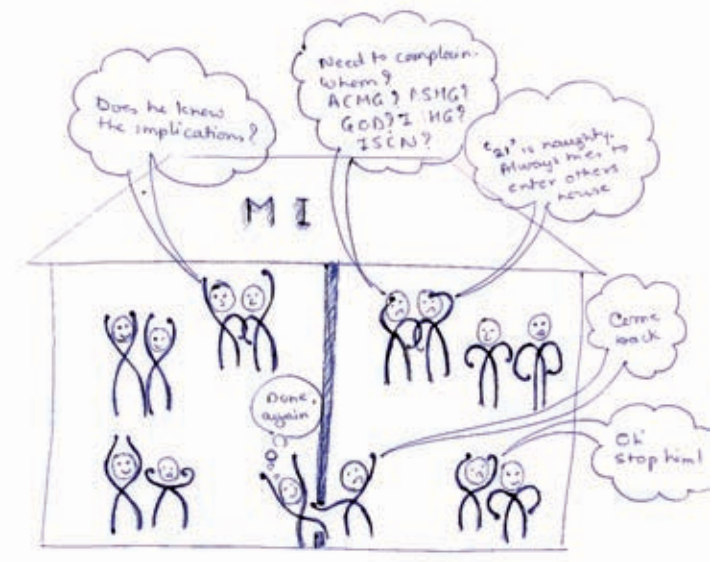
## GeNeTooN




**The Great Escape**
Contributed by Shubha R Phadke

# Preimplantation Genetic Diagnosis

**Dr Seema Thakur**

Sr. Consultant, Dept. of Genetics and Fetal Medicine
Fortis Hospital, New Delhi
E-mail id: seemat3030@sify.com

Preimplantation genetic diagnosis (PGD) is an alternative to prenatal diagnosis and is applicable to couples who are at risk of genetic disease in the offspring and want to avoid pregnancy termination. PGD involves the diagnosis of a genetic disorder in embryos obtained by in vitro fertilization (IVF), selection of healthy embryos, and transferring them to the mother. The first application of PGD was in 1988 by Handyside et al., at the Hammersmith IVF unit in London, in a case for sex determination for a couple at risk of an X-linked disorder. It is estimated that PGD has been performed in more than 50 different centers in more than 3,000 cycles. PGD has become an important technique for couples at risk for a large number of monogenic disorders.

## PGD AND IVF UNIT

For an optimal PGD center, the requirements are:

1. An excellent IVF program
2. An excellent genetic diagnostic center

The success depends upon the collaboration of services between the units, rigorous quality control, and follow-up of PGD couples and babies.

## CLINICAL AND LABORATORY METHODS

The steps involved are

- Genetic work up
- Ovarian hyperstimulation
- Fertilization by intracytoplasmic sperm injection (ICSI) technique
- Transfer of embryo in the culture medium so as to produce cleavage stage embryo (day 3). PGD on day 3 by fluorescence in situ hybridization (FISH) for chromosomal abnormalities or single cell polymerase chain reaction (PCR) for monogenic disorders after taking out one or two blastomeres.
- Embryo transfer on day 3 or 4

## EMBRYO BIOPSY

Cell biopsy involves two steps: the puncture of the zona pellucida surrounding the oocyte or embryo and the removal of a cell or cells. This can be achieved by Polar body (PB) biopsy, cleavage stage biopsy, or blastocyst biopsy.

### Polar Body Biopsy

Polar body biopsy is indicated only for female chromosomal disorders. The adult egg produces 2 small cells called polar bodies. One of these cells can be removed and tested, providing information on only the chromosomal content of the egg. This technique is infrequently used.

### Cleavage Stage Biopsy

The most common approach is to biopsy single blastomeres from day 3 embryos. The human zygote undergoes mitotic division every 24 h before compacting to form the morula on day 4. On day 3, when the embryo is at the 6- to 8-cell stage, two blastomeres can be removed (Fig 1). Embryo biopsy requires zona drilling and

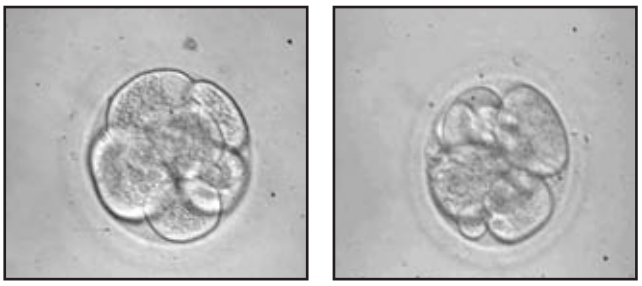


Fig 1: Human embryos 8 cell stage

blastomere aspiration using a micromanipulator. The limitation of cleavage stage biopsy is that only 1–2 cells can be removed, and that time is limited to 24 hours to complete the analysis to transfer the embryo on day 4.

### Blastocyst Biopsy

Blastocyst biopsy can be performed on day 5 or 6 post insemination, and 10–30 trophectoderm cells can be removed without harming the inner cell mass. Blastocyst formation begins on day 5 post-egg retrieval and is defined by the presence of an inner cell mass and the outer cell mass or trophectoderm. A hole is drilled into the zona pellucida and cells are removed from the trophectoderm using a fine biopsy pipette. Genetic analysis is performed via FISH or PCR analysis as described below. The problems associated with single-cell PCR such as allele dropout (ADO) or amplification failure (AF) virtually disappears, as 10–20 cells are available for testing.

### GENETIC TESTS IN PGD:

### FISH in PGD for aneuploidies (Fig. 2)

Older mothers who consider IVF because of difficulty in conceiving due to declining ovarian function or those with history of miscarriage are candidates for PGD-AS (PGD for Aneuploidy Screening). The success rate (Pregnancy per transfer) in these women is 36%. The probes used for aneuploidy screening are either 5- color FISH or 9 or 12-color FISH. In 5- color FISH, aneuploidy of chromosome 13, 18, 21 and X & Y is tested. In 9- color FISH, in addition to above, aneuploidy of 15, 16, 17 and 22 is used. The detection rate varies between 60-80% depending upon number of probes used. Error rate reported is about 7%.

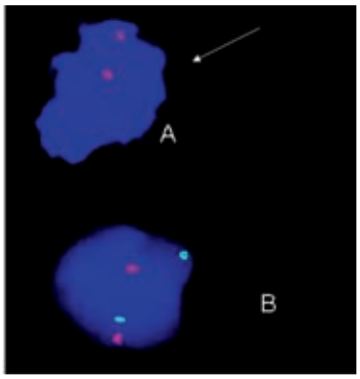



Fig 2: A. Fluorescence in-situ Hybridization (FISH) signals on blastomere showing disomy of Chromosome 18 (2 spectrum orange signals), B. FISH signals showing disomy for chromosome 21 ( 2 spectrum orange signals) and chromosome 18 ( 2 spectrum green signals) on blastomeres of 2 cell stage embryos)

### FISH in translocation carriers

There is a high risk of aneuploidy in carriers of balanced translocation (0-30%). For such couples PGD is an impressive and alternative option to prenatal diagnosis and termination when the fetus is affected. Munne et al demonstrated that translocation carriers who undergo PGD have better outcome of pregnancy than those who don't opt for PGD (92% VS 13%). The pregnancy rates per embryo transfer in such couples were comparable to IVF in general (approximately 25%).

PGD for chromosomal abnormalities has been mainly done by FISH technology. Recently CGH (Comparative Genomic Hybridization) has been started. The FISH probes commonly used are subtelomeric probes and whole chromosome paint probes. PGD for Robertsonian translocation is simple and dual color FISH can be used, one for each chromosome involved. PGD for reciprocal translocation is more difficult as each family has a personal translocation. In reciprocal translocation, total 4 probes are used. One each for the translocated region and one each for a point on the two chromosomes involved. So, a normal or balanced cell will produce 4 signals whereas unbalanced cell will produce 5 or 3 signals. Mosaicism for both normal and abnormal chromosomes in the same cell is a major concern and this can lead to misdiagnosis.

### PGD for Single gene disorders

In PGD, PCR is used on a single cell obtained after embryo biopsy. However, it is still challenging to obtain a reliable diagnosis on a single cell.

### Single cell PCR

Different molecular strategies have been incorporated in PGD to help prevent misdiagnosis. This includes nested PCR, multiplex PCR, fluorescent PCR, and the use of microsatellites and other polymorphic markers. Nested PCR enhances the specificity of amplification, as well as reducing the risk of carryover contamination. It requires two serial amplification reactions. The first PCR amplifies the sample template, using an external set of primers to produce a DNA fragment encompassing the entire mutation site. This then becomes the template for the second round of PCR amplification, which uses specific internal primers, situated within the first external primers (nested PCR), or by using one of the previous external primers with one internal primer (heminested PCR).

Major challenges to a single cell PCR include

1. Amplification failure
2. Allele drop out (ADO)
3. Contamination – maternal/ paternal

Paternal contamination can be avoided by using single sperm fertilization as in ICSI. Maternal contamination can be checked by polymorphic markers.

Allele drop out can be seen when one of the alleles fails to amplify leading to misdiagnosis and various methods have been adopted to overcome this which includes direct mutation testing as well as by linkage analysis.

A list of monogenic disorders for which PGD is commonly carried out is given in Table 1. These 10 disorders comprise about 80% of all cycles performed for single gene disorders using PCR.

Table 1: Single gene disorders for which Preimplantation Genetic Diagnosis is commonly done

**Autosomal Dominant Disorders**

- Huntington chorea
- Myotonic dystrophy
- Charcot-Marie-Tooth disease

**Autosomal Recessive Disorders**

- β-thalassaemia
- Cystic fibrosis
- Spinal muscular atrophy
- Sickle cell disease

**X-linked Disorders**

- Fragile X syndrome
- Duchenne muscular dystrophy
- Hemophilia

**ETHICAL ISSUES IN PGD**

There are certain issues, which need ethical consideration. Two important ones include:

1) PGD for sex selection.
2) For selecting a HLA- matched child through PGD so as to provide a cure for the affected child.

**CONCLUSIONS**

PGD is an alternative to prenatal diagnosis for couples with risk of genetic disorders in the child. PGD is a relatively new procedure, and much ongoing research is being performed to expand and improve it. The chances of having normal pregnancy in couples with monogenic disorders (autosomal recessive, sex linked) is about 75% and 50% (autosomal dominant) whereas pregnancy rate for PGD and IVF is about 30%. Hence, prenatal diagnosis still is a good option for monogenic disorders in India and worldwide especially taking into consideration the lack of easy availability, error rate (7%), cost, ease of procedure and the chance of getting an unaffected baby.

PGD becomes very important when –

1) The family is having recurrent abortions due to unbalanced chromosomal abnormalities in conceptuses - in a family with a balanced rearrangement
2) There are recurrent affected babies of autosomal recessive disorders leading to low moral of the family.
3) Prenatal diagnosis is not an option because termination of pregnancy is not an option due to religious reasons.
4) IVF is done for subfertility

**Acknowledgment:** I would like to acknowledge Dr. Aashish Fauzdar, PhD. Molecular Biology & Immunology Lab, Indraprastha Apollo Hospitals, New Delhi for providing figures for PGD.

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# Quantitative Fluorescent Polymerase Chain Reaction (QF-PCR) for Aneuploidy Detection

**Vasudevan TG, Vahab SA, Girisha KM[1], Gopinath PM, Satyamoorthy K**

Manipal Life Sciences Center and [1]Genetics Clinic, Kasturba Medical College, Manipal University, Manipal-576104

Address for Correspondence:

Dr. K. Satyamoorthy, Manipal Life Sciences Centre, Manipal University, Manipal-576104, Email: dbt@manipal.edu

## INTRODUCTION

Prenatal testing for aneuploidy (changes in the chromosome number) has undergone a major change from the time the first prenatal diagnosis of Down syndrome using amniotic cells was reported. Over the past four decades, different invasive techniques have been developed, which involve collection of amniotic fluid, chorionic villi or fetal blood and culturing the cells in vitro for several days before metaphase chromosomes can be analysed by using staining techniques.[1] These techniques have ensured early and accurate detection of chromosomal abnormalities – both structural and numerical. It was reported that without prenatal diagnosis (and selective termination), trisomy 21 would account for approximately one-quarter of all cases of mental disability in children.[2] However, the delay in getting the results due to the time needed for culturing the cells and finalizing the report (about two to three weeks) could be an anxious long wait for the couple concerned. This particular drawback has been addressed by the use of quantitative fluorescent polymerase chain reaction (QF-PCR) and Fluorescent in situ Hybridisation (FISH) techniques.[3] These methods are thought to be able to replace or, in some cases, act as an adjunct for conventional cytogenetic and/or biochemical testing of several pathologic and genetic disorders. Both are selective procedures and allow detection of only a few of the congenital disorders.

Various diagnostic tools based on isolation of nucleic acids have been developed with the advent of polymerase chain reaction (PCR). The inherent nature of PCR to amplify even small amounts of DNA (~ ng levels) confers a great advantage for clinical diagnostic purposes. Thus, during the past decade, several variations and modifications to the PCR process have helped to fine-tune its evolution as a diagnostic tool. The advent of real-time PCR analysis coupled with results from the Human Genome Project (HGP) and the subsequent haplotype analysis (HapMap project) has resulted in remarkable changes in the field of clinical diagnostics. As more information is culled out from the human genome sequence, it is becoming possible to diagnose several diseases based on the nucleotide changes that are present in the genome. Techniques such as PCR are well-equipped to amplify such contrasting regions of the genome and are becoming useful tools for diagnosis.

## PRINCIPLE OR QF-PCR

QF-PCR is one such technique for diagnosis, where the short tandem repeat (STR) regions of the genome are targeted for amplification. These STR regions have repeats of small sequences present as multiple copies one after the other. As the number of repeats present on any chromosome and individuals can vary, the STRs act as genetic markers and the copy number of each marker is indicative of the copy number of the chromosome. The variability in the length of a STR marker helps to identify each chromosome separately and can be used to know the number of copies of the chromosome in concern. For example if there are 3 copies of an STR marker on chromosome 21 then the inference is that the sample studied has 3 copies of the chromosome 21. Fluorescently labelled marker-specific primers are used for PCR amplification of individual markers. The resulting PCR products are analyzed and quantified using an automated genetic analyser. Generally, the markers are selected in such a way that they are located along the length of each chromosome to increase the chance of detecting unbalanced chromosome rearrangements. At least two markers are necessary for each one of the chromosomes studied, as homozygosity at one locus could affect the diagnosis of aneuploidy. While in theory this method can be used to detect variations in any chromosome, in practice, markers have been designed and used to detect changes in chromosomes 13, 18, 21, X and Y (Figures 1 & 2).

The relative copy number of each allele is determined by

calculating the ratio of the peak areas or peak heights detected for each marker. A normal diploid sample has the contribution of two of each of the investigated chromosomes. In case of heterozygous markers, two alleles of a chromosome-specific marker are detected as two peaks in a 1:1 ratio and as one peak in the case of homozygous (have alleles of same length) markers. The detection of an additional allele as three peaks in a 1:1:1 ratio or as two peaks in a 2:1 or 1:2 ratio indicates the presence of an additional marker copy possibly corresponding to an additional chromosome (Figure 3).[4] Subjects who are homozygous or monosomic for a specific marker will display only one peak.

**Figure 1: Genotype of a normal individual, obtained using four different genetic markers specific for three chromosomes (13, 18 and 21) – D13S628, D18S386, D21S11 and D21S1270. Two copies of each are seen.**

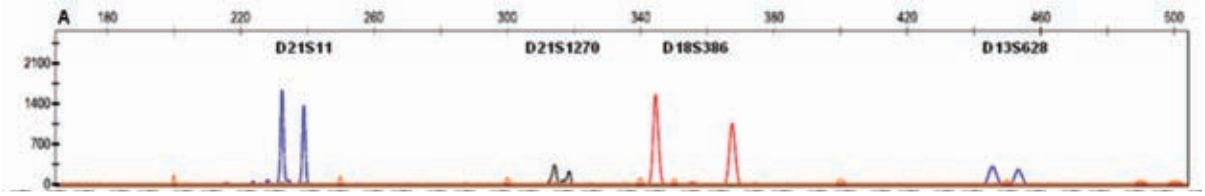



**Figure 2: Enumeration of X and Y chromosomes in pre-natal samples by QFPCR using two gender-specific markers – DXS996 (X chromosome) and SRY (Y chromosome). The presence of a peak corresponding to SRY marker, specific for chromosome Y in the top panel (A) in a male fetus and the absence of peak corresponding to SRY in the bottom panel (B) in a female fetus. The X-chromosome marker DXS996 is present in both the samples.**

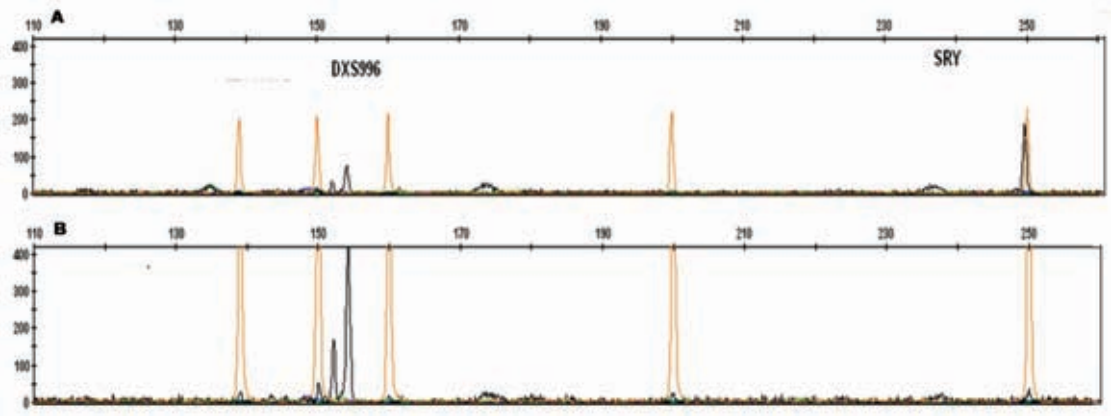
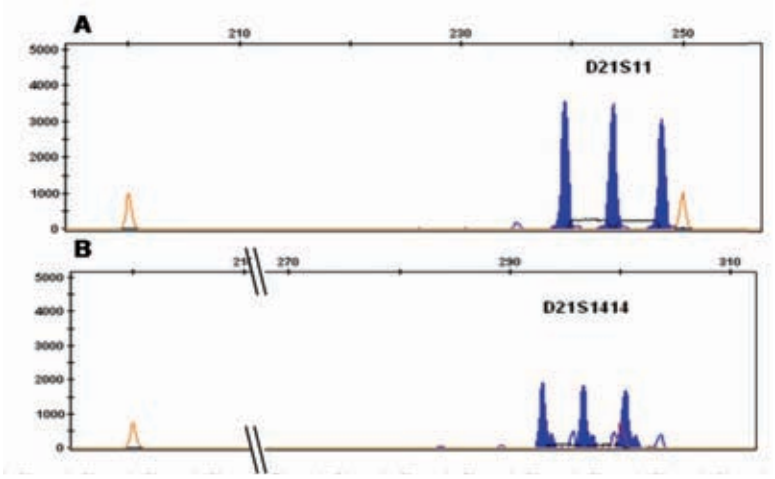



**Figure 3: Genotype of a 'trisomy 21' sample, obtained by QF-PCR using markers specific for chromosome 21 – D21S11 (panel A) and D21S1414 (panel B) (Black peaks).**



In other words, where a microsatellite marker is heterozygous, the ratio of its allele peak areas represents a disomic (1:1) or trisomic (2:1, 1:2 or 1:1:1) chromosome complement. However, a marker is uninformative if only a single peak is observed. The amplification of a single marker can sometimes vary greatly when compared to that of the other markers due to allele size heterogeneity and differences in sample type and quality. Thus, a comparison of allele peak areas of different markers, as an indicator of chromosome copy number, is not recommended. Furthermore, because only peak areas within a single locus are compared, allele dosage ratios are more resilient to the effects of the plateau phase of the PCR than other dosage assays.

Some of the chromosome-specific markers that are widely used are listed in table 1.

### USES AND ADVANTAGES

The most important application of QF-PCR is in the test for aneuploidy of chromosomes, especially in case of prenatal diagnosis, as the whole process is rapid and simple when compared to the conventional cytogenetic method of prenatal diagnosis. It is also equally accurate. QF-PCR can be used to test for the number of copies of a given gene (gene dosage) present in a sample. One of the advantages is its feasibility with a small sample or a very few cells. Since the analysis can easily be automated, many samples can be processed at the same time.[5] The automation of part of the procedure allows high throughput of samples at a very low cost, which makes rapid prenatal diagnosis available to all pregnancies either as a tool in reducing parental anxiety during the wait for completion of fetal karyotype or in improving pregnancy management in case of abnormal result. The high sensitivity of the method ensures that only a small amount of the sample is required. Since highly polymorphic STR markers are used, testing samples suspected of being contaminated with maternal cells as well as assessing zygosity in multiple pregnancies is possible. The use of several highly polymorphic and chromosome-specific STRs also makes it possible to detect partial trisomies. Aneuploidy testing for several chromosomes such as 13, 18, 21, X and Y is possible and in almost all cases, the interpretation is straightforward, just after a few hours of sampling – in contrast to the number of days it takes for cytogenetic methods to diagnose chromosomal abnormalities.

**QF-PCR: Salient Features**

- Makes use of variable short tandem repeat regions to detect chromosomal variations
- Rapid diagnosis means short waiting period for parents (reduced anxiety)
- Feasible using fewer cells
- Highly sensitive and straight forward interpretation
- Easy automation; high-throughput possible
- Low cost method

### LIMITATIONS

One possible disadvantage of the method is that it can detect only selected chromosomal disorders, as is the case with the cytogenetic methods such as Fluorescent In-Situ Hybridization (FISH). In prenatal diagnosis by QF-PCR, some of the potential problems that can arise are: maternal cell contamination, mosaicism, primer site polymorphisms, and somatic microsatellite mutations. Evidence of a second genotype, as shown by inconsistent dosage ratios for each chromosome, extra allele peaks, or both, usually indicates contamination of the sample by maternal cells, although it may represent a chimera or twins. Maternal cell contamination is usually associated with blood-stained amniotic fluid samples. Mosaicism leads to discrepancies between QF-PCR and karyotype results.

### CURRENT STATUS

Some reports suggest that this method can be used as a stand-alone test for women suspected to carry a fetus with an increased risk of Down syndrome.[6] Ogilvie et al presented data with no misdiagnoses for non-mosaic trisomy or triploidy.[7] Lau et al conducted a study on discrepancies between QF-PCR results and routine karyotype in long-term cultures.[8] Most of the discrepancies between the results (usually <1%) were found to be due to mosaicism. Based on this and earlier reports it is suggested that QF-PCR results should be

confirmed with routine cytogenetic analysis.[9] Partial chromosome duplication may be identified by QF-PCR analysis by the presence of both normal and abnormal results with two different markers on one chromosome. This pattern may indicate a cytogenetically visible abnormality or one that is submicroscopic. However, inherited submicroscopic duplications are less likely to be clinically significant, and they can be categorized as copy number variants. Primer site polymorphisms can result in complete or partial allele dropout (ADO) due to reduced or absent hybridization of the primers to genomic DNA. Partial ADO in a normal sample can either give an abnormal diallelic ratio consistent with trisomy for that region, or an inconclusive ratio whereas complete ADO in an abnormal sample can result in a normal diallelic ratio at that locus. Somatic changes in the length of a microsatellite sequence, due to DNA replication and proof- reading errors, may be visible as an unequal triallelic result, where the areas of the two lowest alleles combine to equal the highest allele, or skewed diallelic ratios.

**Table 1. Markers used to detect trisomies and other disorders – specific for chr. 13, 18, 21, X and Y are listed (adapted from Mann et al. 2008)[10]**

| Marker Name | Location | Size Range (bp) | Marker Name | Location | Size Range (bp) |
|---|---|---|---|---|---|
| D13S252-F D13S252-R | 13q12.1 | 260-330 | D21S1409-F D21S1409-R | 21q21.2 | 160-220 |
| D13S305-F D13S305-R | 13q13.3 | 418-482 | D21S1411-F D21S1411-R | 21q22.3 | 256-345 |
| D13S628-F D13S628-R | 13q31.1 | 425-474 | D21S1435-F D21S1435-R | 21q21.3 | 160-200 |
| D13S634-F D13S634-R | 13q21.33 | 355-440 | DXS6807-F DXS6807-R | Xp22.3 | 300-380 |
| D13S325-F D13S325-R | 13q12.12 | 235-315 | DXS1283-F DXS1283-R | Xp22.3 | 295-340 |
| D18S386-F D18S386-R | 18q22.1 | 320-417 | DXS981-F DXS981-R | Xq13.1 | 225-260 |
| D18S390-F D18S390-R | 18q22.3 | 340-415 | DXS1187-F DXS1187-R | Xq26.2 | 125-170 |
| D18S391-F D18S391-R | 18p11.31 | 190-235 | XHPRT-F XHPRT-R | Xq26.2 | 260-300 |
| D18S535-F D18S535-R | 18q12.3 | 450-500 | DXS7423-F DXS7423-R | Xq28 | 350-420 |
| D18S819-F D18S819-R | 18q11.2 | 370-450 | DXYS267-F DXYS267-R | Xq21.31Yp11.31 | 240-280 |
| D18S978-F D18S978-R | 18q12.3 | 180-230 | AMEL-F AMEL-R | Xp22.2/Yp11.2 | 106/112 |
| D21S11-F D21S11-R | 21q21.1 | 220-283 | SRY-F SRY-R | Yp11.31 | 248 |
| D21S1437-F D21S1437-R | 21q21.1 | 283-351 | DYS448-F DYS448-R | Yq11.223 | 323–370 |

## LABORATORY PRACTICES

The processing of a number of prenatal samples at one time, and the risk of sample mix-up, necessitates stringent quality control procedures. In addition, care must be taken, as with all PCR-based tests, to avoid contamination of tested material with amplified products of previous reactions and external DNA. It is preferable to carry out sample and DNA preparation procedures in a class II

biological containment cabinet. Samples, depending on whether they are amniotic fluid, chorionic villus or tissue or fetal blood cells, need to be processed differently and are stored at 4°C. DNA preparation should be a quick and simple procedure especially when dealing with a large amount of samples. Care is taken to ensure the prepared DNA does not contain any residual contaminants or PCR inhibitors that can interfere with the diagnostic processes. Prepared DNA is usually stored at -20°C until further analysis. In case of high-throughput analysis, batches of PCR assays can be prepared in advance, tested and stored at –20°C. These are 20-µl aliquots of a master mix that contains all components except DNA, which is added immediately before temperature cycling, to give a total volume of 25 µl. The analysis can be carried out in any standard genetic analyser. The fluorescent labels may have to be substituted depending on the filter sets of each analyser. Post-PCR cleanup to remove excess primers and free dye molecules, need not be carried out, due to time constraints. Although there are now several published studies describing the use of QF-PCR as a diagnostic test, it is important to validate the QF-PCR strategy in the laboratory in which it is to be used. Control samples are required, and a pilot study is recommended before the implementation of a QF-PCR based aneuploidy diagnostic service, especially if primer sets are used that are not described in the published literature.

## CONCLUDING REMARKS

Nicolini et al have reviewed the role of QF-PCR in diagnosing prenatal aneuploidies.[5] In Western countries, prenatal diagnosis has become much more common than it was a couple of decades ago. This could be attributed to the development of new diagnostic methods such as QF-PCR and FISH, in addition to the routine karyotype. It is also due to increased safety of prenatal sample collection technique namely amniocentesis. Quick and early knowledge (usually carried out in the first or second trimester of pregnancy) of any aneuploidal changes in the foetus gives the parents time to plan their course of action. This choice was unavailable a few decades ago. Rapidity of the test results can be termed as one of the biggest advantages of a method such as QF-PCR. There are reports that discrepancies are bound to give either false positive or false negative results, which are shown by the karyotype analysis albeit in a small percentage of tests (<1%). In some cases, as mentioned above, the authors feel the method is fool-proof – especially in the case of detection of trisomy 21 and sex chromosome aneuploidies; while there an equal number of reports stating that anomalies in QF-PCR necessitates confirmatory tests by routine cytogenetic mechanisms – especially in cases of maternal blood contamination, mosaicism, or structural changes in chromosomes. Thus, it could come down to making the choice on an individual basis, depending on the women's condition [advanced maternal age or positive maternal serum screening or to reduce anxiety] as the physician sees it. For low risk patients, the conventional cytogenetic method alone could be the option.

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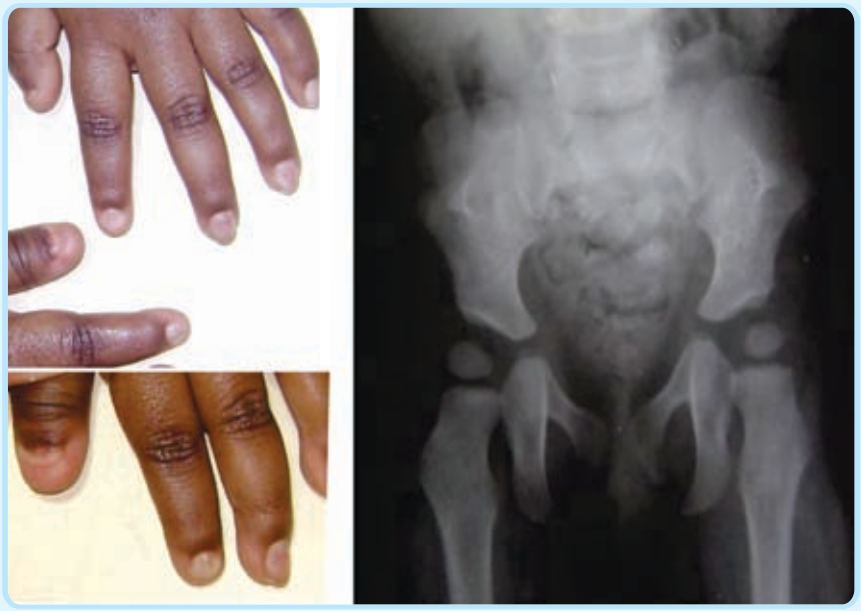
## Messages

1. Traditional karyotyping gives info about numerical and structural abnormalities of all chromosomes but reporting time is about 2 weeks.
2. FISH and QF-PCR can detect only numerical abnormalities of the chromosome or chromosomes tested.
3. Both these techniques have error rates as low as traditional karyotyping. And hence, can be used as standalone test.
4. QF-PCR and FISH have very short reporting time of one and 2 days respectively. Advantages and limitations of both the tests are similar. But QF PCR is technically easy and can be used to handle a large number of samples. So the laboratory may choose either of the tests as per the convenience of the laboratory.
5. If QF-PCR or FISH is used as the only test for prenatal diagnosis then it should be conveyed to the patient that the numerical abnormalities of chromosomes other than tested and structural abnormality of any chromosome cannot be detected by these tests and with normal results of any of these tests the residual risk of chromosomal abnormality is 1 in 160.
6. Traditional karyotyping may be performed in addition to FISH or QF PCR in all cases. Both the tests can be done from the same prenatal sample of amniotic fluid or chorionic villi.

## 12

Contributed by: Dr SJ Patil, Centre for Molecular and Metabolic Diagnostics & Research, Narayana Hrudayalaya Hospitals, Bangalore. Email: drsjpatil@gmail.com

A 2-years-3-months old girl presented with the features shown in the photographs. She later developed nephrotic syndrome. Identify the condition.



Answer to the PhotoQuiz 11 of the previous issue

## Schmid type metaphyseal chondrodysplasia (OMIM 156500)

Schmid type metaphyseal chondrodysplasia is characterized by mild to moderate short stature, irregularities of the metaphyseal ends of long bones (marked in distal femur), widening of physis, bow legs, genu valgum and coxa vara. It may be associated with mild platyspondyly, vertebral body abnormalities and end-plate irregularity. It may be associated with normal hand radiographic pictures or subtle abnormal shortening of the tubular bones and metaphyseal cupping of the proximal phalanges and metacarpals. Hand and spine involvement improve with age. It is caused by heterozygous mutation in the COL10A1 gene on chromosome 6q21-q22.3 and is inherited in an autosomal dominant fashion. It should be considered in the differential diagnosis for various forms of rickets.

Correct responses to PhotoQuiz No. 11 were given by

Chinmayee Ratha, chinmayee3@gmail.com - has won the award during the First Indo-US symposium on skeletal dysplasia

Yatheeshan KK, via email

Anoop Verma, via email

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