IAMG
Test Treat Teach

genetic CLINICS

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Editorial

Prenatal Diagnosis – Over the Counter / Direct to Consumer Testing

Prenatal diagnosis was a very revolutionary concept in the prevention of a disease. Though it appears a very radical method, it got accepted almost worldwide and now has become an integral part of obstetric care. Availability of screening of all pregnant women for relatively common disorders like neural tube defects, thalassemia major and Down syndrome was another milestone in the prevention of genetic disorders. These being offered to all pregnant women who are at low risk of genetic disorders has wider implications. In one way it gives an option of preventing disorders with poor outcome and saves the family from having to cope up with the lifelong struggle of bringing up a child with a physical or mental handicap or a lifelong disease. On the other hand, it makes every family face the burden of undergoing screening tests, waiting for the results and invasive testing and results in procedure-related risks to many. With all these tests and results, probabilities are associated with a lot of uncertainties making pregnancy a period of dilemmas and tensions rather than a pleasurable experience of awaiting a new bundle of joy!

Starting from maternal age as a screening test and then, triple marker test, Down syndrome screening has witnessed a variety of transitions. First trimester biochemical screening with additional use of ultrasonographic markers like nasal bone, ductus venosus flow, and tricuspid regurgitation has increased sensitivity to 90% or more. Addition of second trimester screening in a contingent or sequential manner has increased sensitivity to as high as 98%. With these applications birth of babies with Down syndrome is becoming extremely rare especially in countries that do not have limitations of resources. Even though the risk associated with amniocentesis has decreased so much that it can be offered to low risk women as well, this has not come into routine practice yet.

With this background, there has been an ongoing search for an accurate prenatal diagnostic test which can be offered to all pregnant women without the risk of procedure related loss. The search has lead to development of non-invasive prenatal diagnosis from maternal blood. Extraction of fetal cells from maternal blood and using them for fetal diagnosis is being attempted for more than a decade. The recent shift is to the free fetal DNA molecules in maternal plasma. The maternal plasma has fragments of DNA and a very small fraction of that is from the fetus. Using these free fetal DNA fragments from maternal plasma, the scientists have successfully diagnosed fetal Rh type in Rh negative mothers. This is being accepted in clinical practice for a long time. Over the last few years various scientists have shown success in the diagnosis of trisomy 21 using free fetal DNA in maternal plasma. A success rate of 97% and accuracy of 98.8% has been achieved. The nucleotide differences in the mother and father, use of genes expressing exclusively in the placenta, digital PCR, and differences in methylation status of maternal and fetal genes are some of the strategies used for fetal diagnosis from cell-free fetal DNA in maternal plasma. Over the last eighteen months more than one lakh cases of prenatal testing for trisomy 21 were done using free fetal DNA from maternal plasma. It is important to discuss use of cell-free fetal DNA as a noninvasive test for prenatal screening v/s for prenatal diagnosis. The American College of Medical Genetics and Genomics and the International Society of Prenatal Diagnosis have issued guidelines about use of aneuploidy test on free fetal DNA in maternal blood as a screening test. This being a noninvasive way of obtaining the fetal sample, it is definitely getting acceptance by the patients and obstetricians worldwide. However, at present, cost-effectiveness analysis supports the role of noninvasive prenatal testing as a screening tool,

but not a diagnostic tool. The cost analysis done by Ohno and Caughey published in the June 2013 issue of Prenatal Diagnosis has shown that noninvasive prenatal testing as a screening tool that requires a confirmatory amniocentesis is cost effective compared with its use as a diagnostic tool and leads to far fewer losses of normal pregnancies. The calculations show that the number of abortions due to false positive diagnosis due to non-invasive prenatal diagnosis is likely to be much more than the number of amniocentesis related abortions if non-invasive test using free fetal DNA in maternal plasma is used as a screening test and each screen positive woman is offered a confirmatory test by amniocentesis.

The next step to noninvasive prenatal aneuploidy screening is to use massively parallel sequencing of free fetal DNA in maternal plasma. A Chinese laboratory has used this method and shown almost 100% sensitivity and specificity for detection of aneuploidy of any of the chromosomes. They detected not only trisomies of 13, 18 and 21 but also of chromosomes 16 and 22. Only one trisomy 18 was missed out of 101 cases of all trisomies. With these results it appears prenatal screening of aneuploidies is reaching a stage where the uncertainties and error rates are reduced markedly. Of course, for these screening tests the sample needed is maternal blood which can be taken easily in a most noninvasive way; therefore, there is a fear that this may become a direct-to-consumer test. It must be noted that at present noninvasive prenatal screening using free fetal DNA in maternal blood needs pretest and post-test counseling and most importantly confirmation of abnormal results should be done by invasive testing.

The noninvasive prenatal diagnosis of aneuploidy can be done as early as 4 weeks and may provide an option of antenatal treatment rather than termination of an affected pregnancy. This is not only a dreamy future today! A recently published article in Nature has beautifully shown successful treatment of cell lines with trisomy 21. The group introduced the XIST gene in one of the three copies of chromosomes 21. XIST gene expression from one of the two X chromosomes in females is responsible for Lyonization and switching off genes on the Lyonized X chromosome. In a similar way, the XIST expressing copy of chromosome 21 got Lyonized and only the genes from other two copies of chromosomes 21 were expressing. This led to correction of trisomy 21 phenotype at the cellular level. The scientists also showed that the modified trisomy 21 cell line with one chromosome 21 Lyonized, when transformed into neural cells, grew rapidly as compared to neural cells originating from the trisomy 21 cell line. Fascinating times lie ahead for sure!

The ease of chromosomal analysis from maternal blood also frightens me as an Indian and makes me wonder whether it will be misused for fetal sex determination and add to the already unfavorable female to male ratio in the Indian population and pose more problems for our legal system that is unable to prevent fetal sex determination and female feticide. Measures at emergency level are needed to stop the growing menace of female feticide. All of us need to think and act to prevent misuse of such scientific developments, before the situation gets out of hand!

Shubha Phadke
1st October, 2013

Announcement

UCSC Genome Browser and Human Genome Bioinformatics Workshop

A hands-on training on how to use UCSC Genome Browser and more!
Dates: 16-17 November 2013
Kasturba Medical College, Manipal University, Manipal

Contact: Dr Girisha KM, Email: genetics.clinic@manipal.edu

For more details, please visit:
http://www.manipal.edu/documents/ucsc%20genome%20browser%20and%20human%20genome%20bioinformatics.pdf

Mesomelic limb defects: a handle for prenatal diagnosis of Trisomy 18 syndrome

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Introduction

Trisomy 18 has been recognised as a definitive syndrome since 1960 when it was first described by Edwards et al. and is the second most common multiple malformation syndrome. The prevalence is approximately 1/6,000-1/8,000 live births and the overall prevalence, taking into account fetal losses and pregnancy terminations after prenatal diagnosis, is 1/2500-1/2600.[1]

Trisomy 18 is suspected in newborns with growth retardation, craniofacial and limb abnormalities and various major malformations. In the prenatal period, these cases are diagnosed on the basis of screening by maternal age, maternal serum marker screening or detection of sonographic abnormalities during the first and second trimester. The major sonographic abnormalities include increased nuchal translucency, growth retardation, choroid plexus cyst, overlapping of fingers, and congenital heart defects. Here we discuss USG and autopsy finding of three foetuses with trisomy 18 syndrome with variable mesomelic limb defects especially radial ray defects.

Case 1

A thirty year old female patient was referred to the Medical Genetics outpatient department in view of a positive first trimester screening for trisomy 13/18 (risk of >1:50) and positive triple test for trisomy 21 (risk of 1:213). Antenatal ultrasound showed a 17 weeks 5 days fetus with prominent lateral ventricles, bilateral talipo-equinovarus (TEV), non-visualisation of stomach and complex congenital heart disease. Amniocentesis was done and fetal karyotype showed trisomy 18.

Fetus was brought for autopsy which showed mild dysmorphic features in the form of depressed nasal bridge, low set ears and micrognathia (Fig 1). There was presence of bilateral TEV and left club hand on external examination. Abnormal internal findings included esophageal atresia, hypoplastic left heart and hypoplastic right lung. Skeletal survey revealed hypoplastic ulna and consequent medial deviation of the wrist joint on the left side.

Fig 1: Fetus 1 with medial deviation of left wrist and bilateral talipes equinovarus

Case 2

Antenatal ultrasonographic examination of a thirty four year old female patient showed a single live fetus of gestational age 18 weeks 3 days, with short and bent femur of left side and bilateral malposition of hands in flexed position. There was presence of hypoplastic left heart anomaly, brachycephaly and choroid plexus cysts. The family was advised amniocentesis and fetal karyotyping in view of possibility of chromosomal anomaly. Fetal karyotype revealed trisomy 18. Pregnancy was terminated and the baby was brought for autopsy.

On external examination, there was mild brachycephaly with anteverted nares. There were gross limb anomalies: the right upper limb had a

flexed wrist with a rudimentary thumb attached by a thin pedicle, and in the left upper limb, the forearm was short with the wrist in radial deviation and absent thumb suggestive of radial ray hypoplasia (Fig 2). The right lower limb showed oligodactyly with ectrodactyly and the left foot had presence of polydactyly with syndactyly between first and second, and also third and fourth digits. Internal examination revealed hypoplastic left ventricle and horseshoe shaped kidneys with presence of duplicated ureter on the left side.

Fig 2: Fetus 2 with radial ray defects, polydactyly and ectrodactyly in limbs.

Case 3

A twenty nine year old primigravida at 23 weeks of gestation was referred to the medical genetics outpatient department in view of the antenatal ultrasound showing single umbilical artery, absent radius, hypoplastic ulna, dextrocardia, dilated right renal pelvis with non-visualization of left kidney. Ultrasonography done at our centre showed fetus with diaphragmatic hernia, bilateral hypoplastic forearms, strawberry shaped skull, bilateral renal pylectasis with single umbilical artery and retrognathia.

Fetus on autopsy had findings of facial dysmorphism in the form of hypertelorism, short upturned nose, low set ears, micrognathia and prominent philtrum. There was bilateral mesomelia with absent thumbs and bilateral talipes equinovarus (Fig 3). Both lungs were hypoplastic. Lobulation of lungs was normal. Heart was shifted to right but was structurally normal. There was presence of left sided diaphragmatic hernia. Part of the liver, stomach and small bowel loops were herniated in the thorax. Two-vessel cord was present. Radiography revealed bilateral absent radius and thumb. Ulna was small and curved. Rest of the skeleton was normal.

Fig 3: Fetus 3 - note forearm shortening with absent thumbs

Discussion

Trisomy 18 syndrome has been extensively reported and reviewed. There were more than 130 major and minor abnormalities known to occur in this syndrome earlier but recent studies have reported more than 160 ultrasonographically detectable anomalies at various gestation periods. One or more anomalies are detected in over 90% of foetuses and two or more abnormalities are present in 55% of cases. Here, we have presented 3 fetuses with ultrasonographic and autopsy findings suggestive of trisomy 18, especially with radial/ ulnar ray anomalies.

Postnatal outcome in trisomy 18 syndrome is poor. Most recent studies report a median survival of 3-14.5 days. Approximately 50% of babies with trisomy 18 live longer than 1 week and only 5-10% of children survive beyond the first year. The trisomy 18 phenotype results from full, mosaic, or partial trisomy 18q but complete or full trisomy 18 is the most common form.[2]

With the help of maternal serum screening, high resolution ultrasound equipments and skilled ultrasound operators, majority of the trisomy 18 cases can be diagnosed prenatally. Knowledge of the various ultrasonographic findings, especially limb abnormalities helps in the prenatal detection of these syndromes. The sensitivity of antenatal ultrasound in detecting trisomy 18 before cytogenetic results has been found to be as high as 91.5%.

Common major structural malformations in the trisomy 18 syndrome include those of the heart including septal defects, patent ductus arteriosus, and polyvalvular disease and genitourinary abnormalities including horseshoe kidney which is the most common defect. Other anomalies like omphalocele, esophageal atresia with tracheo-

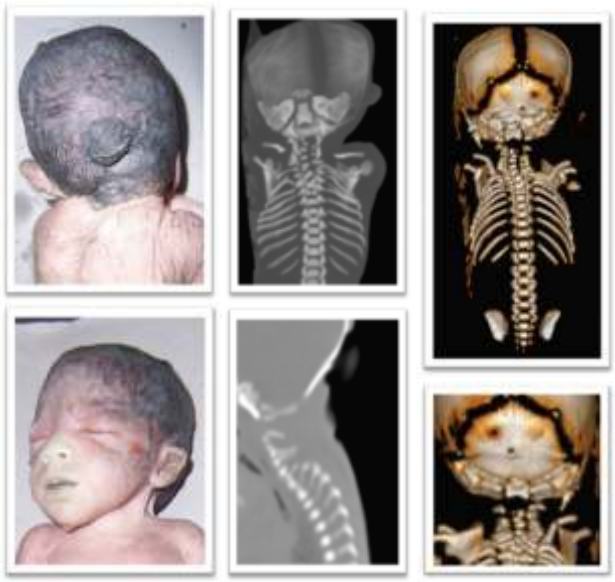
esophageal fistula, pyloric stenosis, Meckel diverticulum, cerebellar hypoplasia, agenesis of corpus callosum, polymicrogyria, spina bifida, orofacial clefts, microphthalmia, coloboma, cataract, corneal opacities and in the limbs, radial aplasia/hypoplasia have been described and occur in 5 to 25% cases. In the group diagnosed before 16 weeks gestation, the most frequent findings are increased nuchal translucency (>95th centile) or cystic hygroma. In the group diagnosed after 16 weeks of gestation, the most frequent ultrasound feature are abnormalities of extremities, present in upto 70% cases.[3]

Though radial ray defect is a feature of various syndromes like Holt Oram syndrome, Thrombocytopenia Absent Radius (TAR), Fanconi anemia, Leri Weill dysostosis, VATER syndrome etc, trisomy 18 syndrome should be strongly considered in the differential diagnosis of radial ray defects due to its highest prevalence and poor outcome. In approximately 35% cases of trisomy 18, only one major anomaly is detected. Hence, even isolated limb abnormalities should alert one to look for other anomalies and the possibility of trisomy 18 syndrome. Keeping in mind the prognosis, limited survival and the morbidities associated with the syndrome, prenatal diagnosis helps in appropriate and timely genetic counselling of families.

References

1. Crider KS et al. Am J Med Genet 2008, 146A: 820–6.
2. Cereda and Carey. Orphanet Journal of Rare Diseases 2012, 7:81.
3. Viora E et al. Am J Med Genet 2007, 143A: 553–7.

GeNeImage



Prenatal USG showed short neck with fusion defects of cervical vertebrae and a mass outside skull in the occipital region suggestive of a lipoma.
Prenatal MRI suggested intracranial communication of the mass. Postnatal CT scan confirmed cervical vertebral defects & demonstrated a small whole in the occipital bone.
Histology of the mass showed hemangiomatous anomaly.

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Cytogenetic Microarray: A revolution in Cytogenetics

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Introduction

Intellectual disability (ID) is the most common clinical situation encountered in medical genetics for counseling. Confirmation of diagnosis is the first step to provide prognosis, genetic counseling and option of prenatal diagnosis to the family. Despite the exhaustive investigations, etiology remains unidentified in 30-50% of the cases.[1] With the advent of molecular cytogenetic techniques i.e. fluorescence in situ hybridization (FISH), multiplex ligation probe dependent amplification (MLPA) and now cytogenetic microarray (CMA) in the evaluation of ID, the diagnostic yield has been increasing. CMA is a technique to study chromosomes at a very high level of resolution. It can detect gains or losses of sizes as small as 10 kb. FISH and MLPA techniques provide higher resolution than traditional karyotype but they only analyze the few targeted regions (up to 45 regions in MLPA) in the genome. In contrast to this, CMA provides whole genome coverage like a karyotype but at an extremely high resolution. That is the reason for higher diagnostic yield of this technique in the evaluation of developmental disabilities, multiple congenital anomalies (MCA) and/or autistic spectrum disorders (ASDs). Moreover, physicians do not need to have a clinical diagnosis before ordering CMA. With an experience of CMA in a very large number of cases with developmental disabilities it is clear that CMA detects abnormalities in about 20% of cases with intellectual disability.[2] Now, CMA is recommended as first tier test in the investigation of ID/MCA/ASDs.[3]

Principle of CMA and platforms

CMA is based on the principle of complementary hybridization of nucleotides. The probes for regions are spotted on a slide. There is extreme miniaturization as the small chip has about 3 million probes corresponding to various regions of the whole genome on it. The probes are fluorescently labelled. Test DNA is hybridized to the probes on the chip. The excess of un-hybridized probes are washed and the slide is scanned by the scanner which quantifies the fluorescence signal intensity at each of the millions spots. Scanning and interpretation is done by computerized software.. The signal intensity of each probe is compared with reference data and analysis is done by platform specific software. The results are provided in the form of copy number losses i.e. deletions or copy number gains i.e. duplications. The results not only give the exact size of the area deleted or duplicated but also the exact location in the form of nucleotide numbers of the ends of the region and hence one knows the gene content of the region. Some CMA platforms use comparative hybridization technique in which patient and control DNA samples tagged with differentially coloured fluorochromes are co-hybridized with the probes on the chip. The signals of different fluorochromes at a spot are evaluated by the relative comparison of strength of the signals.

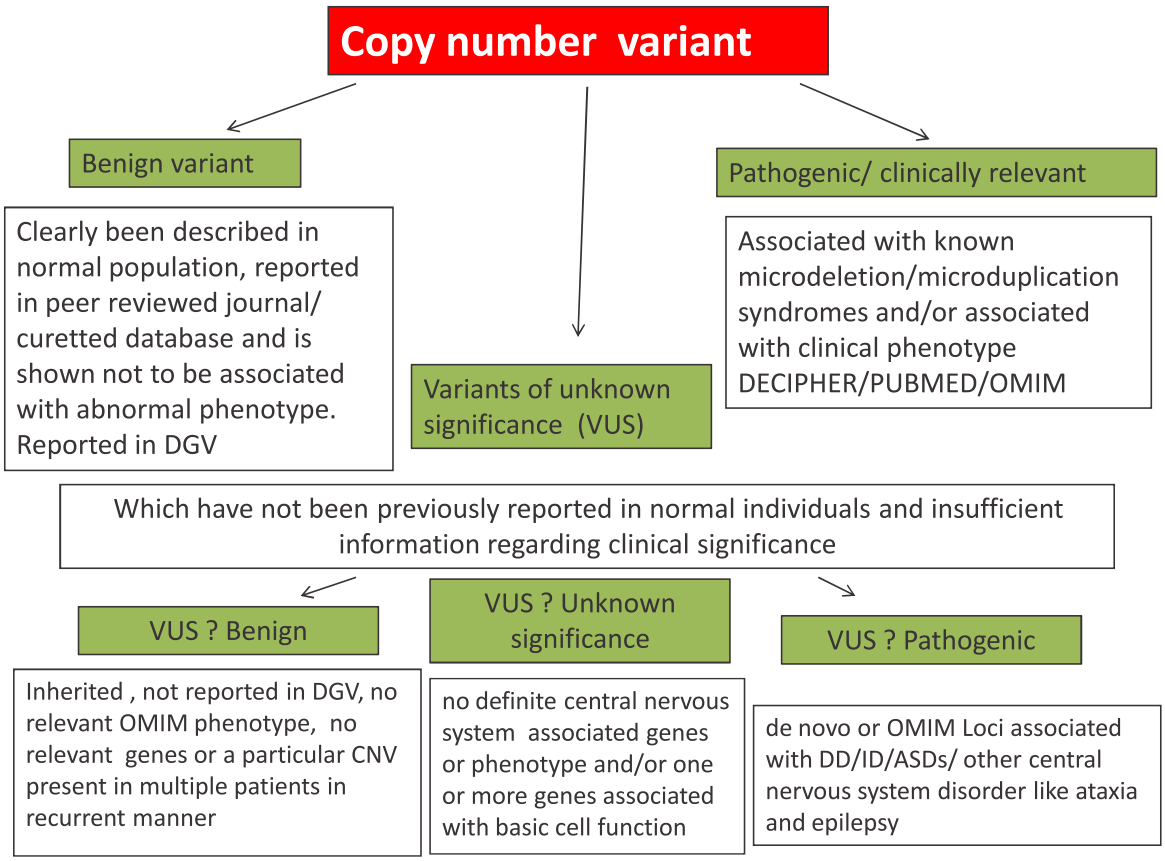
There are various CMA platforms available (Affymetrix, Illumina, etc) and recently a few guidelines have also been published regarding the use of array platforms, their probe density and

overall genome coverage.[4] Almost all of these platforms use combination of probes for copy number variants (CNV) and single nucleotide polymorphisms (SNP). Usually the probe density is higher in the areas for known abnormalities but in addition the whole genome is covered by a backbone of probes.

Because of use of SNP probes in the platforms, in addition to copy number variations, these microarray platforms also detect areas of homozygosity which are important to identify uniparental disomy and help to locate autosomal recessive loci in consanguineous families.

Analysis of variations identified in CMA

Each variant identified by the software is analysed on the basis of its size (no guidelines are available about size cut off, but most cytogenetic laboratories use 200 kb as a size limit for reporting a variant), gene content, published case reports/ series and database search.[5] The various databases which are used for such bioinformatic analysis are PUBMED (ncbi.nlm.nih.gov/pubmed), Online Mendelian inheritance in Man (omim.org, OMIM), DECIPHER (http://www.sanger.ac.uk/PostGenomics/decip), Database of genomic variation (DGV), UC Santa Cruz Browser (http://www.genome.uscs.edu,UCSC), ISCA (https://isca.genetics.emory.edu/iscaBrowser/) and European Cytogeneticists' Association Register of Unbalanced Chromosome Aberrations (http://umcecaruca01.extern.umcn.nl:8080/ecaruca/ecaruca.jsp, ECARUCA).[6] On the basis of this analysis each CNV is classified into benign, pathogenic or variants of unknown significance (VUS), as presented in figure 1. These VUS are further divided into probably benign, probably pathogenic or of unknown significance on the basis of available information (Fig 1). [5,6]



Illustrative cases

A few cases which illustrate the utility of the chromosomal microarray technique are described here.

Case 1

- 8 months old female child with developmental delay, facial dysmorphism (hypertelorism, straight eye brows and deep set eyes) and post axial polydactyly in the left foot (Fig 2a).
- CMA identified 3 Mb deletion in the 1p36 region (Fig 2b)

Fig 2a. Photograph of patient showing facial dysmorphism (hypertelorism, deep set eyes, straight eyebrows and deep set eyes)

Fig 2b. Cytogenetic microarray results (Affymetrix 2.7 M array) showing deletion at 1p36 region

- Interpretation : Pathogenic
- Comments – 1p36 microdeletion is a known microdeletion syndrome and clinical features of this patient were consistent with the characteristic features of this syndrome described in literature.

Case 2

- 3 years old female child with global developmental delay, subtle facial dysmorphism and postaxial polydactyly (Fig 3a).
- CMA showed presence of 3 Mb deletion in the 22q11.2 region (Fig 3b).
- Interpretation : Pathogenic
- Comments: Though 22q11.2 microdeletion is a known syndrome, the facial phenotype was not characteristic and congenital heart disease which is a common feature of 22q deletion syndrome was not present. The postaxial polydactyly which was present in this case is seen only in 15% of cases of 22q11 microdeletion. Therefore, in this case clinical suspicion of this syndrome was not possible. (Fig 3)

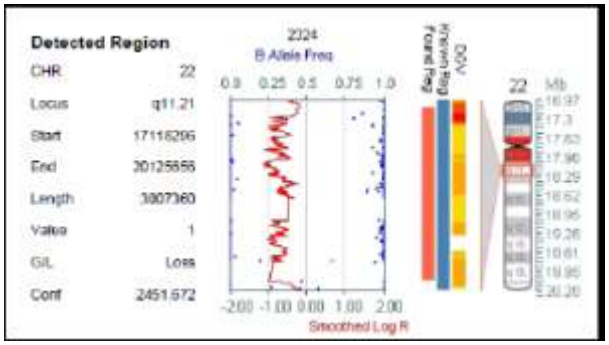


Fig 3b. Cytogenetic microarray (Illumina Cytochip) result showing 3 Mb deletion in 22q11.2 region.

Fig 3a. Photograph of a 2 year old girl with subtle facial dysmorphism

Case 3

- 17 years old boy with intellectual disability, subtle facial dysmorphism and post axial polydactyly.

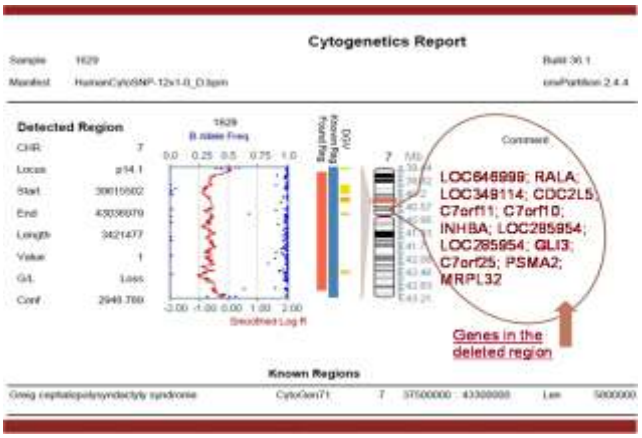


Fig 4. Cytogenetic microarray (Illumina Cytochip) results in case 3, showing 3.4 Mb deletion in the 7p14.1 region, harbouring the GLI3 gene.

- CMA identified 3.4 Mb deletion in the region of 7q12 (Fig 4).
- Interpretation : Pathogenic
- Comments: On bioinformatic analysis, GLI3 gene which is known to be associated with polydactyly was found to be situated in this region.. Hence there was a genotype phenotype correlation. (Fig 4)

Case 4

- 14 years old boy, with ID, facial dysmorphism, and brachydactyly.
- CMA showed the presence of 14 Mb deletion in 10q21.11.
- Interpretation : Pathogenic
- Comments: This region has not been not known to be associated with ID previously. Because of the presence of a de novo large microdeletion (CMA of the parents was normal), this variant was interpreted as pathogenic.

Case 5

- 4 months old female child with failure to thrive and microretrognathia
- CMA showed 9 Mb deletion in 7q36.1 and 13 Mb duplication in 11q24.1.
- Interpretation: Pathogenic
- Comments: These double segment imbalances may be due to separate chromosomal events or an inherited/de novo chromosomal rearrangement. Extended pedigree analysis showed that 2 relatives on the paternal side were having varying degrees of ID. Her paternal first cousin, who was 4 years old with GDD, facial dysmorphism and congenital heart disease, was found to have 9 Mb duplication at 7q36.1 and 13 Mb deletion on 11q24.1. In this family, familial chromosomal rearrangement is the most likely diagnosis. Fluorescent in situ hybridisation (FISH) for the involved chromosomal segments will be the investigation of choice to diagnose the presence of balanced chromosomal rearrangements in this family.

Case 6

- Child with global developmental delay and polydactyly
- CMA showed 1.1 Mb loss at 6q12.3 region.
- This pericentromeric region variant has been reported in DGV and no pathogenic phenotypes were reported to be associated with it in DECIPHER/OMIM/PUBMED.
- Interpretation and comment: CMA report did not show any probable pathogenic gain/loss. However, this does not rule out single gene disorders or imbalances of regions not represented on the microarray platform.

Case 7

- 10 months old boy with global developmental delay.
- CMA showed 424 Kb copy number gain on 3p26.3, and 518 kb copy number gain on 18p23.
- Bioinformatic analysis: Both these regions were partially reported in DGV and were not associated with any pathogenic phenotype in DECIPHER/OMIM/PUBMED. CHL1 gene, which is a member of the L1 gene family of neural cell adhesion molecules, is situated in the 3p26.3 region. It is a neural recognition molecule that may be involved in signal transduction pathways. The deletion of one copy of this gene may be responsible for mental defects in patients with the 3p- syndrome. Thus this variant was interpreted as a variant of unknown significance probably pathogenic. The other copy number gain at 18p23 was not very well reported in DGV and had the associated OMIM phenotype of the 18q deletion syndrome. This region had no genes and was interpreted as VUS of unknown significance.

Application of CMA as an adjunct to traditional karyotyping

Traditional karyotypes detect abnormalities but in some cases the exact delineation of the cytogenetic abnormality may not be possible due to the low level of resolution of the technique. In such a situation, chromosomal microarray may be able to provide additional information which can be interpreted in the context of the cytogenetic abnormality observed and thus give a clear picture.[7,8] In the following situations CMA can be a useful adjunct to the karyotype:

- To identify cryptic imbalances at the breakpoints in cases with cytogenetically apparent balanced translocations.
- To identify extra material on a chromosome.
- To identify an extra chromosome of unknown origin (a marker chromosome).

Limitations of CMA

Like any other diagnostic technique, CMA has its own limitations. These are as follows:

- Availability of various CMA platforms which differ in their probe density and overall coverage.
- No stringent guidelines regarding interpretation of copy number gains/ losses.
- Difficulty in interpretation of variants of unknown significance.
- Incomplete penetrance/variable expressivity/ interaction between many variants.
- Rapid addition of new data implies that interpretation of variants is likely to change in the future. This needs continued interpretation of a CMA report and puts a huge burden on clinical cytogeneticists and the family as well.
- Cost of CMA is a special issue especially in the Indian scenario where the family has to pay for the test. Many a times, parental CMA is also required for the analysis of variants. This further adds to the cost. MLPA using probes of subtelomeric and common microdeletion regions still can be considered as the first tier test in Indian patients with ID.
- Does not identify origin of marker chromosome in some cases / low level of mosaicism / balanced chromosomal rearrangements.
- Does not identify monogenic causes of intellectual disability.

CMA in prenatal diagnosis

CMA is now being increasingly recommended in prenatal samples with or without fetal malformations. In various studies the overall yield of CMA in prenatal diagnosis is said to be in the range of 2-3%. The yield can be as high as 8-10% in prenatal samples where antenatal ultrasonography has detected one or more malformations.[9] The department of Medical Genetics, Sanjay Gandhi Post Graduate Institute of Medical Sciences has recently started offering CMA for prenatal diagnosis on a research basis.

Conclusions

CMA has proven to be of significant utility in the evaluation of patients with ID/MCA/ASDs. The American College of Medical Genetics has advocated CMA as a first tier investigation in the evaluation of children with intellectual disability, autism and malformation syndromes. Now with the use in prenatal diagnosis, it is likely to revolutionize the diagnostic yield in fetuses as well. However physicians and concerned families need to understand its limitations and the need for genetic counseling both before and after the test results.

References

1. Moeschler JB, Shevell M and the Committee on Genetics. Pediatrics 2006; 117; 230416.
2. Manning M, Hudgins L and the Professional Practice and Guidelines Committee. Genet Med 2010; 12: 742-5.

3. Miller DT, et al. Am J Hum Genet 2010 ;86:749-64.
4. Kearney HM, et al. Genet Med 2011 ;13:676-9.
5. Kearney HM, et al. Genet Med 2011 ;13:680-5.
6. de Leeuw N, et al. Hum Mutat 2012
7. De Gregori M, et al. J Med Genet 2007; 44: 750-62.
8. Gribble SM, et al. J Med Genet 2005 ; 42:8-16.
9. Yatsenko S, et al. Clin Genet 2012. doi: 10.1111/cge.12027. [Epub ahead of print]

Announcement

SIAMG-Genzyme Fellowship in Clinical Genetics

Eligibility:

A postgraduate degree (MD/MS) in Pediatrics, Obstetrics and Gynaecology or Medicine. Candidates with super specialization (DM) are also encouraged to apply.

Venue:

Training will be provided in a Medical Genetics centre with clinical and laboratory genetics facilities. The first program will be organized at the Department of Medical Genetics, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow.

Award Support:

Financial support to cover boarding and lodging/ daily expenses, outstation travel expenses and institute fees (About Rs 50,000/- all inclusive per candidate, per month).

July 2013 – April 2014 Fellowship Schedule:

Batch 1 : July 1, 2013 – September 30, 2013
Batch 2 : October 1, 2013 – December 31, 2013
Batch 3 : February 1, 2014 – April 30, 2014

For details, please visit: http://www.iamg.in/ or write to Dr. Ashwin Dalal at info@iamg.in

Announcement

International Conference on Human Genetics and 39th Annual Meeting of Indian Society of Human Genetics [ISHG-2014]

Dates and Venue: 21st to 25th January, 2014
Ahmedabad Management Association [AMA], IIM Road, Ahmedabad, Gujarat, INDIA

Secretariat Contact: Dr. Jayesh Sheth/Dr. Alok Dhawan/Dr. Frenny Sheth
FRIGE's Institute of human Genetics
FRIGE House, Jodhpur Gam Road, Satellite, Ahmedabad - 380 015.
Ph.: 079-26921414, Fax: 079-26921415
Email: secretariat@ishg2014.org, ishg2014@gmail.com

For more details visit conference website: www.ishg2014.org

Fetal Therapy- Current approaches and future possibilities

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Introduction

Strictly speaking, fetal therapy is defined as a therapeutic intervention for the purpose of correcting or treating a fetal anomaly or condition. However, in a broader sense, it encompasses any prenatal treatment administered to the mother with the primary indication to improve perinatal or long-term outcomes for the fetus or newborn. This implies that even conventional practices like folic acid supplementation, management of maternal thyroid disorders, steroid therapy for lung maturation, management of polyhydramnios and therapy for fetal infections, all fall under the gamut of fetal therapy. The need for fetal therapy arises when a fetus is found to be affected with a condition in-utero which, if left unattended, would lead to fetal demise, irreversible injury to the fetus or obstetric complications.

Guidelines[1]

The International Fetal Medicine and Surgery Society guidelines for fetal therapy are as follows:

- The natural history of the disease should be at least partly understood.
- The condition should be lethal or could result in severe morbidity if not treated in utero.
- The fetal intervention should be at least partly corrective and the results should compare favourably with those obtained following postnatal treatment.

Medical therapies[2]

The therapeutic approach to the fetus can be medical or surgical. Medical approach is most commonly by the transplacental route, i.e. by administration of medication to the mother orally, intramuscularly or intravenously, which then passes onto the fetus through the uteroplacental circulation. Various indications and routes of fetal medical therapies are mentioned in Table 1.

Administration of thyroxine by intra-amniotic route in cases of hypothyroid fetal goitre, maternal administration of dexamethasone for prevention of genital ambiguity in female fetuses affected with congenital adrenal hyperplasia and management of polyhydramnios by maternal NSAID therapy are some of the relatively well established therapies of documented benefit. The same cannot be said about newer approaches like administration of fresh frozen plasma to fetuses with Smith Lemli Opitz syndrome, where doubtful benefit was achieved in a single case report only, and many other similar rare conditions. Medical therapy of the fetus is non-invasive or at the most minimally invasive in nature, hence newer studies and data are bound to emerge at a fast pace and this field is likely to evolve with promising results in the future. Some obvious drawbacks are the non-curative nature requiring continuous and repeated medication during pregnancy followed by postnatal therapy, which again is likely to be long term and specific adverse effects associated with the particular therapy.

Table 1: Fetal medical therapies

Fetal condition	Therapy	Route of administration	Evidence of use
Congenital Adrenal Hyperplasia	Dexamethasone 20ug/Kg/day	Maternal oral	Effectively prevents genital ambiguity in female foetuses in 85% cases
Polyhydramnios	Indomethacin(300mg/day)Sulindac (400mg/day)	Maternal oral	Efficacy 80-100%;Problems of premature ductus closure
Fetal hypothyroid goitre	Levothyroxine 150-600ug	Intra-amniotic instillation Intra-muscular	Oral route not found useful
Fetal thyrotoxicosis due to maternal Grave's disease	Propylthiouracil 100-600mg/day	Maternal oral	Fetal teratogenic effects
Fetal tachyarrhythmias	Anti-arrythmic drugs-Digoxin, Sotalol, amiodarone, Flecainide	Maternal oral, i.v. Fetal i.v, i.m.	Proven efficacy
Congenital heart block	Immunoglobulin Corticosteroids	Maternal i.v. Maternal oral	Ineffective in 3rd degree block. May prevent progression of 2nd degree block
Congenital cystic adenomatoid malformation of lung	Corticosteroids	Maternal i.m.	Few reports of efficacy. Trial underway
Fetal biotinidase deficiency	Biotin	Maternal oral	Few case reports
Fetal methylmalonic acidemia	Vit B12	Maternal oral/i.m.	Few case reports
Fetal pyridoxine dependent seizures	Pyridoxine	Maternal oral	Few case reports
Fetal Smith Lemli Optiz syndrome	Fresh Frozen Plasma	Fetal intravascular , intraperitoneal	Few case reports

Surgical therapies [3,4]

When compared to medical therapies, fetal surgical interventions are more definitive and may lead to almost complete correction of the fetal defect. However, they are invasive and associated with all operative complications like infection, preterm labour, anaesthetic hazards, etc. As of now, only very limited conditions are amenable to surgical therapy and only few centres in the world have experts who can perform these procedures. Before operating on a structural fetal defect, it is essential to ensure that the defect is isolated and not part of a multiple malformations syndrome. Hence, fetal MRI and fetal karyotyping are absolute pre-requisites before performing fetal surgery. In addition the following principles need to be followed: a) Condition should be severe enough to warrant intervention, b) Prenatal intervention is associated with better prognosis and c) Condition should not be severe enough to be irreversible already. The surgical approach can be through the following routes:

- **Open surgery**: This requires a hysterotomy and general anaesthesia. It has been used for correction of lesions like congenital diaphragmatic hernia, congenital cystic adenomatoid malformation of lung, myelomeningocoele and sacrococcygeal teratomas. However, the highly invasive nature and associated high incidence of complications

limit its utility.

- **Fetoscopy guided/FETENDO:** This procedure is done through an endoscopic instrument which is inserted through a small abdominal & uterine incision. It also requires general/regional anaesthesia and uterine tocolysis. Conditions which are amenable to therapy by this approach are twin-to-twin transfusion syndrome, congenital diaphragmatic hernia, posterior urethral valves, amniotic bands and selective fetal termination of the anomalous twin.
- **Ultrasound guided:** This is a minimally invasive approach requiring local/regional anaesthesia. It can be used for radiofrequency ablation, shunt placement in bladder outlet obstruction & cystic thorax lesions, endovascular/cardiac procedures, and fetal blood sampling as well as transfusion.

The following is an overview of the commonly used fetal therapeutic procedures:

- **Fetal blood transfusion:** This was the first minimally invasive fetal therapy procedure used by Liley et al in 1963 for management of Rh haemolytic disease. Initial transfusions were given intra-peritoneally under fetoscopic guidance. Later, USG-guided intravascular transfusion became the preferred technique. Besides Rh haemolytic disease, other causes of fetal anemia like parvovirus infection and feto-maternal hemorrhage are also amenable to this intervention. Presently management of Rh haemolytic disease involves serial monitoring of middle cerebral artery peak systolic velocity for evidence of fetal anemia, followed by ultrasound-guided fetal umbilical vein puncture, estimation of fetal hematocrit and subsequent infusion of packed RBCs if hematocrit is ‹30%. The RBCs infused have to be O negative, compatible with maternal blood, washed, filtered, gamma irradiated, with a hematocrit 80%. The amount to be transfused is calculated on basis of the fetal weight, fetal hematocrit and the blood unit hematocrit. The target hematocrit is usually 40-50%. The process of transfusion involves ultrasound guided puncture of the umbilical vein, confirmation of fetal blood, infusion of a fetal paralytic agent followed by slow infusion of the PRBC under continuous ultrasound visualisation. At end of infusion of the calculated volume, fetal blood is collected and the hematocrit checked to confirm that the transfusion has been successful. Subsequent transfusions are usually done at intervals of 15-20 days and delivery can be planned after lung maturity is attained. Various procedural complications can arise like fetal demise, bradycardia, cord bleeding, preterm labour and infection. The fetal survival rate is 88% and in the absence of hydrops has been reported to be as high as 96%.[5]
- **Myelomeningocele repair:** Myelomeningocele gives rise to motor & sensory defects, orthopedic problems involving lower limbs, bowel & bladder dysfunction, hydrocephalus secondary to hindbrain compression (requiring shunt placement in 80% of cases), and death due to posterior fossa syndrome in 15-30% individuals. The pathophysiology is believed to be damage to an exposed neural tube and cerebrospinal fluid leak leading to Chiari malformation and hydrocephalus. Attempts to prenatally repair the defect have been made on the rationale that closure of the exposed spinal cord would help in preventing damage to the neural tissues and stop the CSF leak, thereby reversing brainstem herniation & hydrocephalus. Various animal models of prenatal surgery showed positive results, following which the first fetoscopic repair was attempted in 1997 and the first open surgery in 1998. Presently, the specific pre-requisites for myelomeningocele repair are a) defect at level S1 or below, b) Normal leg movements and bladder emptying c) Arnold Chiari malformation d) Mild to moderate ventriculomegaly. Data from previous procedures at CHOP (Children's Hospital of Philadelphia) (n=51) and Vanderbilt University (n=178) showed

reversal of hindbrain herniation in 38%, decreased the need for shunting in 43-59%, improved leg and bladder function and improved later cognitive function in selected fetuses. Recently MOMS (Management of Meningomyelocele Study), a multicentric long duration trial (n=183) in USA, has shown definite benefit of open meningomyelocele repair in terms of survival, shunt requirement and motor function. However, there was no cognitive benefit and the procedure was associated with a significant risk of prematurity and scar dehiscence.[6,7]

- **Lower Urinary tract obstruction:** The most common etiology is posterior urethral valves. Other possible causes can be urethral atresia and cloacal abnormalities. The perinatal mortality can be as high as 90%, primarily due to pulmonary hypoplasia. 50% of survivors develop renal impairment. The rationale of in-utero treatment is to increase amniotic fluid volume, prevent pulmonary hypoplasia and restore bladder & renal function. The candidates for intervention are fetuses with severe disease, decreased amniotic fluid, but preserved renal function. Various parameters have been used to ensure adequate renal functioning, like urinary biochemical parameters and echogenicity of kidneys on ultrasound; however, none are known to be reliable. Therapeutic approaches are:

  a) Vesico-amniotic shunt placement- this is done with ultrasound guidance, under local anaesthesia. A double pigtailed catheter is introduced connecting the bladder with the amniotic cavity. More than 300 shunts have been placed at various centres across the world in the last 25 years. Forty percent neonatal survival has been reported in past studies, but up to 50% cases develop renal impairment later in life. Recent studies have raised questions about the methodologies of previous reports. Presently a randomised control trial PLUTO (Percutaneous Shunting for Lower Urinary Tract Obstruction) is underway to assess the efficacy of this approach.[8]

  b) Fetal cystoscopy- this involves direct visualisation of the bladder for identifying the cause of obstruction. If posterior urethral valves are causative, these can be disrupted using laser fulguration, guidewire perforation and hydroablation. Although initial reports have shown good neonatal survival rates, more trials are required to assess the effectiveness of this approach.[8]

- **Congenital diaphragmatic hernia repair-** This condition is associated with a neonatal mortality of 50-70%, resulting from pulmonary hypoplasia and pulmonary hypertension. In utero therapy is proposed to be useful by reducing the pressure effects on thoracic organs. Selection criteria for fetal surgery are a) liver in thorax, b) gestation less than 25 weeks, and c) low lung: head ratio. The surgical approach was initially through hysterotomy, followed by correction of the diaphragmatic defect. This was not shown to show any survival benefit in randomised trials. However, minimally invasive approach using a fetoscope, FETO (Fetal Endoscopic Tracheal Occlusion), has shown promising results in recent times. This involves fetoscopic insertion of a balloon/clip/plug to occlude the trachea, which helps in growth of the lungs by preventing egress of the lung fluid. Although initial reports from CHOP (Children's Hospital of Philadelphia) and UCSF (University of California, San Fransisco) did not show significant benefit, recent results from the Eurofetus trial and a large Belgium trial of 210 patients (using in-utero balloon removal late in gestation) have been promising. Survival for left CDH is reported to improve from 25% to 49% and right sided CDH 0% to 35%. Efforts are underway to conduct a multicenter randomised trial (TOTAL- Tracheal Occlusion to Accelerate Lung Growth). The removal of the intratracheal balloon can be done through an EXIT procedure

(Ex-utero intrapartum therapy), which involves an intrapartum procedure to secure fetal airway, while the baby maintains perfusion through utero-placental circulation or through ultrasound/fetoscopic guided removal in late third trimester.[9,10,11]

- **Fetoscopic selective laser coagulation of placental vessels (SLPC)** – This procedure is used primarily for treatment of twin-to-twin transfusion syndrome (TTTS). TTTS occurs in 10-15% of mononchorionic twin pregnancies due to abnormal placental communications between the circulation of the twins. This leads to one of the twins acting as a donor twin, whereby it starts pumping blood into circulation of the co-twin, and the other twin becomes the recipient. This leads to growth restriction & oligohydramnios in the donor, and overgrowth & polyhydramnios in the recipient. The donor is at risk of hypovolemia & renal failure, while the recipient is at risk of heart failure. Overall mortality is 80-100% and only 40-50% of survivors are neurologically normal. The therapeutic approach to this condition is to interrupt the abnormal placental communicating vessels. A fetoscope is inserted into the uterine cavity, a laser fibre (Nd:Yag or diode) is introduced through the operative channel and the abnormal placental vessels coagulated. The Eurofetus trial concluded in 2004 that this procedure is the treatment of choice in TTTS

Table 2: Fetal surgical therapies

Fetal condition	Pathophysiology	Rationale for in utero therapy	Therapeutic approach	Evidence of use
Congenital diaphragmatic hernia	Pulmonary hypoplasia and pulmonary hypertension	Timely reversal of pulmonary hypoplasia and prevention of pulmonary hypertension	*Open surgery *Fetoscopic tracheal occlusion	*Not useful *Large trials show survival benefit, randomised trial commenced
Lower urinary tract obstruction	Progressive renal damage by obstruction Pulmonary hypoplasia by oligohydramnios	Urinary diversion prevents obstructive uropathy and restores amniotic fluid volume	*Vesico - Amniotic shunt *Cystoscopic posterior urethral valve ablation	*Variable results, randomised trial underway *Novel technique, requires further study
Neural tube defects	Damage to exposed neural tube; CSF leak, leading to Chiari malformation and hydrocephalus	Covering exposed spinal cord, cessation of leakage preventing hydrocephaly and reversing cerebellar herniation	Open surgery - Closure of spinal defect	Large trial showed survival and long term benefit
Twin-to-twin transfusion	Intertwin transfusion leads to	Bichorionization stops intertwin	Fetoscopic Selective Laser Fulgration of	Large trials have shown definite

	oligopolyhydramnios sequence, hemodynamic changes; obstetric complications	transfusion, reverses cardiac failure, delays delivery	Placental Vessels	survival benefit, treatment of choice for 18-26 weeks, ›stage I TTTS Long term outcome needs evaluation
Sacrococcygeal teratoma	High output cardiac failure by arteriovenous shunting Fetal anemia by tumor growth and/or bleeding within a tumor	Cessation of steal phenomenon Reversal of cardiac failure; Prevention of polyhydramnios	*Open or fetoscopic excision of tumor *Ablation or embolisation of feeding vessels	Few case reports
Thoracic space - occupying lesions	Pulmonary hypoplasia (space-occupying mass) Hydrops by impaired venous return (mediastinal compression)	Prevention of pulmonary hypoplasia and cardiac failure	* Open surgery- Excision of solid lesions * Ultrasound guided placement of thoraco-amniotic shunts for cystic lesions	* Few case reports 60% survival rate * Few case series 70-85% survival rate
Cardiac malformations	Critical lesions causing irreversible hypoplasia or damage	Prevention of hypoplasia or arrest of progression of damage	Ultrasound guided valvuloplasty	Technical success of 60-70% in aortic valvuloplasty. Further trials required
Amniotic bands	Progressive constrictions causing irreversible neurological or vascular damage	Pr evention of limb deformities and function loss	Fetoscope guided excision of bands- scissors or laser or electric current	Few case reports
Fetus acardiacus and discordant anomalies	Discordant anomalies: where one fetus can be a threat to the other, or to avoid termination of entire pregnancy	Selective feticide to improve chances of the other fetus; avoidance of termination of entire pregnancy	Selective feticide of anomalous fetus- Intracadiac saline/potassium chloride injection under ultrasound guidance Coagulation/ablation/ligation of umbilical cord vessels	Many case series show survival benefit for co-twin Results better for dichorionic twins

(Stage 2 or beyond in severity) detected between 18-26 weeks gestation. The procedure is reported to have neonatal survival rates of 75-85% and normal neurological outcome in 75-94% of survivors. Presently more data is needed to draw conclusions regarding the long term outcome of the survivors.[12]

- **Others:** Fetal lung lesions are amenable to in utero therapy by resection for solid lesions or shunt placement for cystic lesions.[13] Cardiac lesions esp. outflow tract obstruction can be approached by valvuloplasty of aortic & pulmonary valves.[14] Fetal tumors like sacrococcygeal teratomas can be excised by open surgery or their blood supply can be occluded by ablation or embolisation. Table 2 shows the pathophysiology and therapeutic approaches for surgically correctable fetal conditions.[3]

Novel therapies in the pipeline[15]

- **In Utero Stem cell therapy**- Due to the relative immunodeficient state of the fetus, introduction of stem cells may lead to better engraftment. This formed the basis of various IUHSCT (In Utero Hematopoietic Stem Cell Transplantation) trials in mouse models. However, most achieved only low level chimerism, and significant host response was seen. Few human trials showed benefit in Severe Combined Immunodeficiency syndrome. However, for all other disorders, this approach is in the pre-clinical stage.
- **In Utero Gene Therapy**- The introduction of a deficient gene has the potential to prevent disease prior to the onset of irreversible organ damage. In view of relative immunodeficient state and small size of fetus, the success of gene uptake and integration into large proportion of cells is also likely to be better. The procedure involves ultrasound guided introduction of the transgene into a fetal cavity or organ of interest. Presently, this approach has been tried in rodent models of various metabolic, central nervous system & musculoskeletal diseases. However, though transient gene expression has been attained, sustained benefit has not been found. Due to the potential for teratogenic effects & germline transmission, presently, human trials are not considered ethical.

Conclusions

The development of various fetal treatment strategies offers hope for in utero management of fetal disorders and birth of a healthy baby for families who are confronted with the scenario of an anomalous fetus. However, this field is still in its infancy, and more experience from multiple centres is needed before it can be hailed as standard of care for fetal pathological states.

References

1. Ville Y. Prenat Diagn 2011; 31: 621–7.
2. Hui L, et al. Prenat Diagn 2011; 31: 735-43.
3. Deprest JA, et al. Semin Fetal Neonatal Med 2010; 15: 58–67.
4. Van Mieghem T, et al. Best Pract Res Clin Obstet Gynaecol 2012; 26: 711-25.
5. Moise KJ Jr. Obstet Gynecol 2008; 112:164-76.
6. Adzick NS, et al. N Engl J Med 2011; 364: 993-1004.
7. Bebbington MW, et al. Prenat Diagn 2011; 31: 689–94.
8. Ruano R. Prenat Diagn 2011; 31: 667-74.
9. Hedrick HL. Semin Pediatr Surg 2013; 22: 37-43.
10. Jani JC, et al. Ultrasound Obstet Gynecol 2012; 39: 7–9.
11. Dekoninck P, et al. Early Hum Dev 2011; 87: 619-24.
12. Khalek N, et al. Semin Pediatr Surg 2013; 22; 18–23.
13. Khalek N, et al. Semin Pediatr Surg 2013; 22: 24–29.
14. Arzt W, et al. Prenat Diagn 2011; 31: 695-8.
15. Pearson EG, et al. Semin Pediatr Surg 2013; 22: 56–61.

Short – on sensitivity!

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Jayant and Rashmi (names changed) were married for a year now. It was a dream come true for Rashmi's three brothers- Anil, Anuj and Vimal (names changed). It was a perfect marriage and the couple looked beautiful together. Rashmi was young, slim, tall and pretty as a 'picture'.Jayant was a handsome young man and well settled financially. The brothers were so content in having found the "perfect" match for their only sister. They gave a lavish reception to the boy's family at the wedding, gave expensive gifts, and did everything they could do, just to make sure that their princess would not have to listen to anything hostile in the family which was much more well to do than their own.

Everything went well and one year after the marriage, Rashmi had conceived. Everything was going fine till 10 weeks of pregnancy when her gynaecologist took the family history. She counseled the couple regarding the probability of a hereditary disorder running in Rashmi's family, the possibility of the fetus being affected by this genetic disorder and referred the couple to our outpatient department.

The couple did not waste any time in visiting our OPD. Jayant looked very upset and anxious. Rashmi maintained her calm. As we started taking the history, we came to know that the genetic disorder for which the gynaecologist had referred was short stature in Rashmi's three brothers. Jayant did most of the talking. He said that because Rashmi was so tall and beautiful, he and his family did not think that there could even be the slightest possibility of the disorder getting transmitted to his children. We counseled them that any further opinion from our side could be given only after examining at least one of the brothers. The three brothers were staying in different parts of the country, so we asked them to call the eldest one Anil, who was staying closest to Lucknow.

After 3 days, to our utter surprise, the couple walked into the OPD with all the three brothers- Anil, Anuj and Vimal who had come from different cities. All of them were short in height but proportionate. The eldest, Anil, was 38 years old, married, had 2 children and was a teacher by profession. The younger one, Anuj, was 34 years old, had completed a master's degree, was soon to be married and was the principal of a Hindi medium school. The youngest, Vimal, was 28-years old, unmarried, and had started his own business after completing his graduation. All three seemed hardworking, well settled adults, with no physical or mental health problems. They were very pleasant to talk to with very amiable personalities and were well adjusted to their lives and heights. Outside of the clinical setting in which we found ourselves, no normal person would have given them a second look or thought of them as being "defective" in any way.

Jayant was aghast at the idea of having his baby look like his brother-in-laws. He curtly stated again and again without any respect towards his in-laws' sensibilities that he would not want his child to carry forward the uncle's genetic defects. He was so obsessed with the idea of bringing his "perfect" child into this world, that he appeared blind to all other people and their feelings. Rashmi maintained a stoic silence through this. Human beings are such wonderful creatures with such myriad ways of reacting to the same emotions and thoughts. What could be poison for one man, could be different for another. All this talk instead of bringing about any negative reaction from Rashmi's brothers, made them feel as "guilty as hell". They were shattered and stood there with their heads down, although they were in no way responsible for the couples' predicament. Looking at them being tortured and humiliated by Jayant's endless verbal rant made us feel sorry for the poor folks.

Now regarding their reason for having come to us. We evaluated them in detail. Anil's height was 138cms, just about half a feet shorter than the lower limit of normal of an average Indian adult male. Anuj's height was 144 cms and Vimal's height was 153 cms. Apart from this, there were no skeletal deformities and no systemic abnormalities leading to any physical, sexual or mental disability. Since the short stature was proportionate with no deformities and no associated features, along with a history of Anuj and Vimal having received few Growth hormone injections at 18 years of

age, we took the opinion of our endocrinology consultant for the possibility of Growth hormone deficiency. A clinical diagnosis of Isolated Growth hormone deficiency was made. It is a genetically heterogeneous condition known to have autosomal dominant, autosomal recessive and X-linked forms of inheritance. Known genes involved in the genetic etiology of isolated growth hormone deficiency include those that encode growth hormone (GH1), growth-hormone-releasing hormone receptor (GHRHR) and transcription factor SOX3 (leading to XR inheritance). However, mutations are identified in a relatively small percentage of patients, which suggests that other, yet unidentified, genetic factors are involved.

Genetic counseling was provided for the family. The couple was told that the chances of their baby having short stature due to growth hormone deficiency would range from 0-25%. Prenatal diagnosis could be provided only if the exact genetic basis was identified in affected members of the family. The chances of detection of a mutation in the family and the cost were also explained to them. The possibility of growth hormone therapy after birth if indicated was also discussed.

The case illustrated and emphasized the unwillingness of the society to accept disability even in its most subtle form and the taboo associated with it. With the advent of prenatal diagnosis, we now have the 'right to know' and are implored to make 'informed choices' about our health and of those we love. Because of the emphasis on responsibility towards one's own health, within high modernity, as well as notions of perfection, parents are rapidly being faced with an obligation to use these technologies for not only the future of their child but also to secure their own future. Perfection which earlier happened laboriously over generations through the process of Darwin's "natural selection" has now been put in our hands. We now have the tools to choose what constitutes "perfect" and what would only be "good" or worse "bad". Whom to give life to and whom to wither away? Such power in human hands needs to be tempered with virtue and grace.

The other issue which needs to be addressed is the sensitivity towards the proband. Diagnosis of the proband is of paramount importance for genetic counseling. The proband may not be the sib or offspring of the consultand and his / her consent is important before getting access to his/ her medical records and samples. He/ she or the guardian should give consent for detailed evaluation. Sometimes this is not possible due to lack of co-operation of the affected individual or his/ her guardians. In this family the affected brothers were very co-operative and came all the way on the request of their sister and her husband. But what we observed was that the young brother-in-law was so engrossed with the possibility of having a 'suboptimal' or an 'abnormal' child like his brother-in-laws that he did not realize that many of his words were hurting and humiliating to the intelligent, successful and well-adjusted brothers- in- law. Respect towards other human beings is the basic quality of civilized and good 'human' beings. Jayant crossed his limits a number of times while talking about 'short people like his brothers- in- law' and was not apologetic about it. He did not realize that each of them had some points better than him. His approach to the situation lacked the sensitivity the situation demanded. Autonomy should not be the most valuable principle of bioethics, even if it is the most dominant feature of human behavior (e.g. selfishness). If it is, we arrive at a society with lack of concern for the poor and sick. Instead, we need to balance selfishness with sufficient altruism, to make a true loving society. We can refocus our concerns to consider the best interests of the individual and the family and not just the autonomy of the individual. Genetic freedom is not unconditional freedom, because part of the concept of autonomy must be recognition of other people's autonomy, or values.

I would like to end with a quote that sums up the high aspirations and zest for perfection of parents regarding their unborn baby in the modern society with the availability of modern technologies.

"Dear Designer Baby, Your mother and I created you but then decided to give you a little help by inserting some desired genes. We thought you should look as nice as possible, so you're quite handsome now. We thought it might help if you were a little smarter than others, and so you are. And you should be slim, not fat. We love you, so we made you a better person. Hope you like yourself. Love, Dad." Austin E. Sakong (Ref. Time Magazine, Feb. 1, 1999)

This case reminds all of us to talk with great sensitivity to and about 'different' people whom we geneticists see every day as a part of our clinical work!

Inactivating the extra chromosome in Down syndrome

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Shutting DOWN the extra chromosome 21[1]

Down syndrome is the leading genetic cause of intellectual disability and millions of Down syndrome patients across the world also face multiple other health issues. Jiang et al tried the first chromosome therapy for Down syndrome with the idea of dosage compensation correction. They inserted the XIST gene in to the DYRK1A locus on chromosome 21 of induced pluripotent stem (iPS) cells derived from a Down syndrome patient. The XIST non-coding RNA coats chromosome 21 and triggers stable heterochromatin modifications, chromosome-wide transcriptional silencing and DNA methylation to form a 'chromosome 21 Barr body'. The trisomy silencing of chromosome 21 may define the poorly understood cellular pathways deregulated in Down syndrome, and therapy of Down syndrome. This work might also help in studying human chromosome silencing.

60-yr-old riddle of Vel blood group solved[2-4]

The Vel-negative blood group phenotype was first described in 1952, named after Mrs. Vel, by Sussman & Miller. However the underlying gene is unknown and transfusion services have had difficulty finding compatible blood for Vel-negative patients. Three research groups successfully unravelled the molecular basis of the Vel blood group antigen. A 17-nucleotide frameshift deletion in the gene encoding the small integral membrane protein 1(SMIM1) defines the Vel-negative blood group phenotype. The results of these three research groups establish SMIM1 as a new erythroid gene and Vel as a new blood group system. This discovery enables the identification of rare Vel-negative blood donors by genotyping.

New genes responsible for malformations in cortical development (MCD)[5]

Malformations of cortical development are an important cause of epilepsy and severe intellectual disability and the genetic causes of MCD remain largely unknown. With the whole-exome sequencing and genome-wide SNP genotyping approach Poirier et al identified the mutations in KIF5C, KIF2A, DYNC1H1 and TUBG1 genes. The mutations in these genes are involved in a wide range of cortical and gyral pattern abnormalities associated with microcephaly, callosal, cerebellum and/or brainstem dysgenesis. The study suggests that centrosomal and microtubule-related proteins are important in proper cortical development and the microtubule-dependent mitotic and post mitotic processes are major contributors to the pathogenesis of MCD. This study also highlights the power of next generation sequencing in identifying several genes in a group of disorders with similar phenotypes. Time to bank DNA!

Recessive gene for HUS[6]

Lemaire et al with the help of exome sequencing identified the loss-of-function mutations in DGKE (encoding diacylglycerol kinase ε) in patients with atypical hemolytic-uremic syndrome (aHUS). DGKE is the first gene implicated in aHUS that is not an integral component of the complement cascade (CFB, CFH, CFI, C3, MCP and THBD). DGKE transmits as an autosomal recessive trait with high penetrance like CFH and MCP. These findings suggest the chance of new pathophysiologic mechanism of aHUS.

Genomic medicine for DMD[7]

We are yet to find a definitive cure for Duchenne muscular dystrophy (DMD). In a review, Fairclough et al summarizes several promising genetic approaches like viral gene therapy, termination codon read-through, exon skipping and increasing levels of Utrophin. Viral gene therapy with its chequered history is coming of age as the understanding of viral genome has been improving and yet facing challenges like large size RNA and difficulty in targeting all muscles. Usage of drugs for promoting the read-through of translation stop codons has to be improved in efficacy. Exon skipping with RNaseH-independent antisense oligonucleotides (AONs) is a correction strategy with promising progress and increasing levels of Utrophin, a dystrophin related protein is another assuring pharmacological treatment strategy. But for both of these clinical trials are underway. A timely update on a difficult to treat condition.

References

1. Jiang, J. et al. Nature 2013; 500: 296-300.
2. Ballif, B.A. et al. EMBO Mol Med 2013: 5, 751-61.
3. Cvejic, A. et al.. Nat Genet 2013: 45: 542-5.
4. Storry, J.R. et al. Nat Genet 2013: 45: 537-41.
5. Poirier, K. et al. Nat Genet 2013; 45: 639-47.
6. Lemaire, M. et al. Nat Genet 2013: 45, 531-6.
7. Fairclough, R.J., Wood, M.J. & Davies, K.E. Nat Rev Genet 2013: 14, 373-8.

genetic CLINICS

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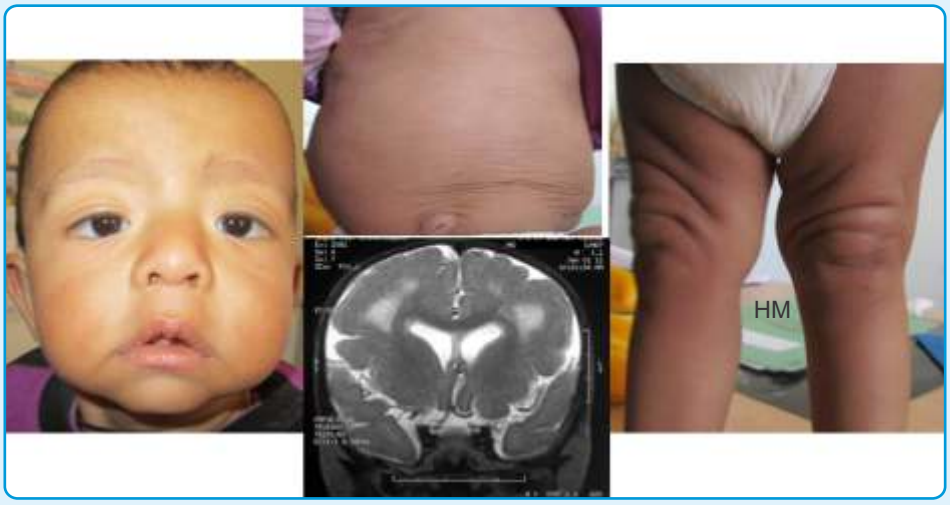
This 14 months male child, born to consanguineous parents, presented with developmental delay, seizures and joint laxity. Identify the condition.



Please send your answers by email to editor@iamg.in

Answer to PhotoQuiz 21 of the previous issue

Yunis-Varon syndrome (OMIM # 216340)

Yunis-Varon syndrome is a rare autosomal recessive disorder characterized by cleidocranial dysplasia (dysostosis of skull, hypoplastic facial bones, agenesis of clavicles), digital anomalies, and severe neurologic impairment. The digital anomalies typically include aplasia/ severe hypoplasia of thumbs and halluces, and absence of distal phalanges of fingers. In a majority of cases the disorder is lethal in infancy. Yunis-Varon syndrome is caused by mutations in the FIG4 gene on chromosome 6q21.

Correct responses were given by:

- Divya P, Vellore
- Sameer Bhatia, Jalandhar
- M L Kulkarni,Davangere
- D Saminathan, Trichy
- Aravind, Chennai
- Subapriya K, Chennai
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