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Abstracts - Invited Papers (Speakers)

Importance of Marker Chromosome Characterization in Prenatal Diagnosis

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Presence of a marker chromosome at Prenatal Cytogenetic analysis leads to very sensitive diagnostic and counseling issues. The marker chromosome denotes presence of missing or extra DNA, however, it does not ALWAYS translate into morphological or functional abnormality in the fetus / new born. Routine G-banding fails to characterize most small marker chromosomes due to its size and a lack of characteristic banding patterns. Additional molecular Cytogenetic tests are necessary and available to identify and characterize the marker chromosome. The information collected helps to delineate the possible clinical consequences of the marker chromosome. This information is important and vital to the parents facing an important decision and helps the obstetrician in pregnancy management.
Chromosome 15 derived markers are the most common and majority are of bi-satellited morphology. The clinical outcomes in such cases depend upon presence of material from bands 15q11.2 and beyond, which contains important genes responsible for brain development and those associated with Prader-Willi or Angelman syndrome.
X chromosome derived markers are also common. They may represent missing X/Y chromosome or represent the 47th chromosome. Clinical outcomes heavily depend on presence or absence of X-inactivation center (XIC) in these marker chromosomes.
The possibilities and limitation of such analyses will be presented in details with help of cases from our laboratory and a literature review

Early Anomaly Scan at 11-13 Weeks of Pregnancy Including Nuchal Translucency Measurement

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To evaluate feasibility and effectiveness of early malformation scan including fetal echocardiography and nuchal translucency measurement at 11 to 13 weeks. The study group consisted of 6489 singleton "medium"-risk pregnancies examined between 1998-2002 with a well known outcome of pregnancy. Examination included screening for somatic anomalies, fetal echocardiography and the assessment of nuchal translucency for risk assessment of trisomy 21,13 and 18 following the FMF criteria. All examinations were done by four experienced gynecologists specialized in prenatal diagnosis of at least ten years.
The prevalence of major anomalies was 3.5% with a 1.3% frequency of major cardiac anomalies and a

2.1% frequency of chromosomal abnormalities. The detection rate of major anomalies up to 13.6 weeks was 77%, of major cardiac anomalies 88% (95/108). TOP following the diagnosis of severe anomalies was performed in 185 cases with 73% of these being done before 15.6 weeks. Women older than 34 years in 73% decided not to have invasive procedures.
Examination of the fetus at 11 to 13 weeks including early malformation scan, fetal echocardiography and the assessment of the nuchal translucency has the capacity to detect and also exclude a major part of severe abnormalities at an early stage of pregnancy, if properly done.

First and Second Trimester Biochemical Markers for Prenatal Diagnosis

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Screening for Down syndrome and other serious birth defects in the early second trimester (15-20 weeks) using maternal serum markers measured has been done since the mid-1980s, beginning with AFP and later including hCG, estriol (uE3) and, more recently, inhibin A. Screening performance with these four serum markers in combination is an 80% detection rate (DR) for a 5% false positive rate (FPR). Inhibin A is a powerful second trimester marker, equivalent to hCG in performance. In the past few years, new screening methods that involve testing a few weeks earlier and the integration of first and second trimester markers have been proposed and implemented. Serum markers in the late first trimester (10-13 weeks), such as PAPP-A together with free beta-hCG (or total hCG and Inhibin-A) can be combined with the first trimester ultrasound measurement, nuchal translucency thickness (NT), to produce an earlier screening test that has a DR of about 85% for a 5% FPR, similar to four marker testing in the second trimester. However, if the best first trimester markers, PAPP-A and NT, are combined with the four markers in the second trimester, the resulting integrated test has a DR of 85% for a 1% FPR. The 80% reduction in FPR (from 5% to 1%) means an 80% lower amniocentesis rate and an 80% reduction in the number of procedure-related miscarriages. The integrated test is a major improvement in the safety of prenatal screening and is the best prenatal screening test currently available.

Gene Therapy of Hemophilias-Successes and Pitfalls

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The hemophilias are X-linked inherited disorders characterised by deficiency of factor VIII (Hemophilia-A) or factor IX (Hemophilia-B). Although recent advances in its diagnosis and development of safe & effective treatment, has brightened the vision of affected individuals to look towards a normal life expectancy, the need for a permanent cure has always been felt. The hemophilias were recognized early on as an ideal candidate for a gene transfer approach to therapy because their clinical manifestations are attributable to the lack of a single protein that circulates in minute amounts in the plasma. The goal of gene therapy for hemophilias is to provide stable insertion and expression of specific gene with long-term therapeutic production of the coagulant protein without stimulating an immune response to the transgene product or the vector. An ideal gene transfer approach to ahcieve this goal would also require a minimally invasive procedure for gene delivery with minimal associated morbidity.

Sustained therapeutic expression of factors VIII and IX has been achieved in preclinical studies using a wide range of gene transfer technologies targeted at different tissues leading to six different phase I/II clinical trials that resulted in limited efficacy but minimal toxicity. These advances parallel the development of improved gene delivery systems. Long-term therapeutic levels of factor FVIII and FIX have been achieved in adult FVIII- and FIX-deficient mice and in adult hemophiliac dogs using adeno-associated viral (AAV) vectors, high-capacity adenoviral vectors (HC-Ad) and lentiviral vectors. In mouse models, some of the highest FVIII or FIX expression levels were achieved using HC-Ad vectors with no or only limited adverse effects. Recombinant adeno-associated viral vectors appear most promising for hemophilia gene therapy. Non-viral *ex vivo* gene therapy approaches have also led to long-term therapeutic levels of coagulation factors in animal models. Nevertheless, the induction of neutralizing antibodies (inhibitors) to FVIII or FIX sometimes precludes stable phenotypic correction following gene therapy and is one of the major challenges. Further improvement of the various gene delivery systems is warranted to bring a permanent cure for hemophilia one step closer to reality. The ethical implications of the perception of gene therapy as an inevitable therapeutic goal in light of its anticipated benefits, acceptable risk, perceived consumer need and the unknown cost of this intervention also need to be discussed.

Implications of Profile of Mutations in the β-Globin Gene for Prenatal Diagnosis of Thalassemia Syzndromes in India

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Hemoglobinopathies are a major group of single gene disorders in India and about 40 β-thalassemia mutations have been identified. HbS and HbE are also prevalent in some communities. With growing awareness on these disorders, the requests for early prenatal diagnosis are increasing.
We characterized the mutations in 611 at risk couples(β-thalassemia major-546, Sickle cell anemia-47, Hb E-thalassemia-10, Hb S-thalassemia-8, Hb D Agri-Thalassemia-1) referred for prenatal diagnosis. 19 b-thalassemia mutations and 3 abnormal hemoglobins were detected. 13 b-thalassemia alleles remained uncharacterized. In 4 heterozygotes, no mutation was detected after scanning the entire β globin gene. Both partners had identical mutations in 53% of couples and the fetuses were at risk for 17 different homozygous genotypes. In 47% of couples, both partners had different mutations and fetuses were at risk for 65 different double heterozygous genotypes.
A stepwise approach helped to offer diagnosis to all families in a cost effective way. Reverse dot blot hybridization was first used to screen for 6 common Indian β-thalassemia mutations[IVS1-5(G→C), IVS1-1(G→T), Codon 8/9 (+G), Codon 41/42(-CTTT), Codon 15 (G→A), Codon 30 (G→C)] with HbS and HbE simultaneously. Then, screening for the 619bp deletion was done by PCR and electrophoresis. Diagnosis was possible in 84% of families. ARMS, DGGE and framework linkage analysis was used for the remaining families. Few couples were called again for second trimester fetal blood analysis.

Optimal and Practical Down's Syndrome Screening Strategies

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Antenatal screening for NTDs, initially using maternal serum AFP at 15-19 weeks gestation and later routine ultrasound anomaly scanning at 18-20 weeks, has led to a massive fall in birth prevalence over the last 20 years. Experience gained from this has stimulated a parallel development in antenatal screening for Down's syndrome.

Second Trimester: Statistical models predict for multiple maternal serum marker combinations, including AFP: (1) free β-hCG yields a higher detection rate (DR), for a 5% false-positive rate, than hCG; (2) uE_3 increases the DR; (3) inhibin A increases the DR further, and (4) for a 5% false-positive rate the maximum DR is 72%. Prospective studies have confirmed these predictions.
First Trimester: Earlier testing has obvious advantages: earlier reassurance and if termination required it could be completed before fetal movements are felt and less traumatically. The only disadvantage is that NTD detection necessitates either a separate AFP test after 15 weeks (it is invalid earlier) or reliance on the ultrasound anomaly scan. Statistical models predict that the DR, using combination of PAPP-A and free β-hCG at 10-13 weeks, is similar to that of 2-3 marker second trimester screening. The addition of further serum markers yields a DR comparable with 4 markers in the second trimester. Ultrasound NT alone has a predicted DR of a same magnitude. But by far the best results are predicted for the combination of serum markers and NT where the DR is 90%. Prospective studies are consistent with these predictions.
Both First and Second Trimester: Another approach is to combine NT with markers from two serum samples, PAPP-A at 10-13 weeks and 4 markers at 15-19 weeks. The model predicted DR is just 3% greater than for first trimester screening alone. And even this small increase may not be achievable in practice as it requires 'non-disclosure' of the first trimester markers, which is particularly difficult for NT.
Second Trimester Reconsidered: Despite the obvious advantages of screening in the first trimester not all centres are in a position to benefit from this approach because ultrasound is of insufficient quality or they don't have access to CVS. Such centres might consider combining the serum markers with ultrasound nuchal skin-fold measurement. This is a weaker marker than NT but when results are expressed in multiples of the median and risks are estimated the model predicted detection rate is as high as 82%.
Future Developments: New ultrasound markers, particularly nasal bone determination, could lead to an even higher DR. Whilst there is not yet sufficient experience with this to accurate predictions, the DR is expected to exceed 95%.

Fetal Imaging by Color Doppler and 3D Power Doppler

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In recent years, two significant developments have taken place in diagnostic ultrasound techniques. The first of these is Power Doppler Imaging and the other is multiplanner imaging with three-dimensional (3D) reconstruction.
Initially, 3D reconstruction was applied to surface rendering of anatomical features. Technological progress has lead to almost photographic quality of images including detailed multiplanner illustration of blood vessels in 3D.
In Color Doppler principle is applied to enable vascular flow to be identified in a color-coded display which indicates the direction of flow. In simple terms, blood flowing towards the ultrasound transducer is conventionally depicted in a band of colors ranging from deep red (low velocity) to bright yellow (high velocity). Similarly, flow in a direction away from the transducer is indicated by a a band of colors ranging progressively from deep blue (blue velocity) to cyan (high velocity).
Thus Color Doppler Imaging illustrates only the direction of flow, color-coded mean velocities and the range of the mean velocities.
Power Doppler adds a 4^{th} parameter-amplitude or energy. In this case, the amplitude of reflected signals from each "clump" of red blood cells within the vessels are recorded and displayed in a different color scale. The higher the clump density, the higher the signal amplitude and the brighter the displayed color.

Thus power Doppler Imaging detects amplitude which is related to density. Power Doppler Imaging therefore gives high detectablitity of low velocity-flow and therefore gives better representation of vascularity in any defined region of the scanned area.
The development of 3D scanning apparatus has introduced the possibility of visualizing vascularity in three dimensions. Whether an abdominal or an interactivity ultrasonic transducer assembly is used, it is now possible to generate within a few seconds, a complete 3D volume data set compossed of soft tissues and blood vessels which can be displayed simultaneously in 3 orthogonal planes. The vessels are clearly defined in color against a background of soft tissue gray-scale echoes and this can be manually rotated to reveal the total vascular network.
The applications in fetal imaging will be amply illustrated in the presentation.

Inborn Error of Metabolism-Post and Prenatal Diagnosis by GC/MC: Cremere Experience

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Most inborn errors of metabolism (IEM) cause severe pathological sequelae like mental retardation, sudden infantile death or other irreversible conditions. In the developed countries newborn or mass screening programs are being implemented to channelise the quality of population by reducing the burden imposed by genetic disorders.
The study reports 5 years of experience in simultaneous diagnosis of about 100 IEM by GC/MS using specific urinary metabolite markers. The easily accessible urine, its transport on the air-dried filter, and GC/MS diagnosis helped the clinicians having limited health infrastructure and lack of advanced laboratory facilities at their fingertips.
Total 1517 high risk children with some features of seizures, movement disorders, vomiting, poor feeding, metabolic acidosis, lethargy, mental and / or motor delay were screened by GC/MS analysis; out of which 320 (21.80%) were diagnosed to have metabolic disorders. The higher incidence of 26 % (78 out of 299) in neonates below 30 days of age emphasized the need of screening in at least NICU babies in the absence of NBS program in India. The most common IEM found were MMA (N= 19), MSUD (N= 8), Glutaric aciduria type I & II (N= 11), Propionic acidemia (N= 8), OTC (N= 5), Urea cycle disorders (N= 7), Galactosemia (N= 7), FDPD (N= 8) and Canavan disease (N= 6).
Low birth weight (34.6 %), convulsions (33 %), prematurity (27%), acidosis, respiratory distress and refusal to feed (each 13 %) were the most common clinical indications in NICU babies. The other associated high-risk genetic factors were consanguinity, history of mental retardation and death of earlier sibs in the family. The prenatal diagnosis of some organic acidemias was possible using amniotic fluid when the diagnosis in the index case was confirmed. The pregnancy was continued when the absence of abnormal metabolites was indicated by GC/MS. A few cases will be presented illustrating the mangement and genetic counseling done with preventive approach.
Many developing countries are making fast progress in setting up biochemical and genetic services for newborn and mass screening. CREMERE study has offered the important information to health policy makers while selecting the IEM for NBS program in India, and thereby generating the genetic epidemiological data.

Periconceptional Care

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During the last decade, there has been a movement to expand the definition of prenatal care to encompass preconceptional counseling. It helps to minimize risk factors for adverse pregnancy outcome amongst high risk women with medical diseases like diabetes mellitus, epilepsy or poor obstetric history by providing extensive advice as well as by means of the early detection and treatment of diagnosed abnormalities. Identification of risk factors for adverse pregnancy outcome is a main component of preconception care, but requires adequate time and knowledge. Preconceptional care has to be followed with immediate post conceptional (hence the term "Peri-conceptional") and antenatal care. Preconceptional counseling is preventive medicine for obstetrics. All factors that could potentially affect perinatal outcome are identified, the couple is advised of the risks and a strategy is provided to reduce or eliminate the pathological influences made evident by her family, medical, obstetrical history and/or specific testing. The preconceptional visit may be the single most important health care visit when viewed in the context of its effect on pregnancy.

Although there are many potential benefits of the pre conceptional health care models, barriers to its implementation remain. The concept, method and outcome of preconceptional care in terms of referrals, referral indications, treatment policy and impact on possible future reproductive benefits have not been studied in India.

Randomized trials of the efficacy of preconceptional counseling are scarce. Maternal and perinatal outcomes also are dependent upon the interaction of maternal fetal and environmental factors, and it is difficult to ascribe pregnancy outcome to one specific intervention. Nevertheless, there have been several prospective and case control trials that clearly demonstrate that preconceptional counseling improves pregnancy outcome. Diabetes mellitus is the prototype of a medical disorder for which preconceptional counseling is beneficial, since most of the maternal and fetal risks can be avoided if conception occurs when glucose control is regulated. Preconceptional counseling in epilepsy usually includes the recommendation to switch to the least teratogenic drug regimen or possibly even discontinue medication before conception. The American Academy of Neurology (1998) recommends that reproductive age epileptic women undergo preconceptional counseling and they conceive while taking folic acid and the least teratogenic monotherapeutic antiseizure medication.

Pregnancy outcomes for women with hypertension, renal disease, thyroid disease, asthma and heart disease were significantly improved if they had received counseling.

Birth Defects, which are now the third common cause of neonatal and infant mortality and morbidity can be avoided with primary, secondary or tertiary prevention strategies. Congenital conditions clearly benefiting from counseling are neural tube defects, thalassemias, cystic fibrosis, rubella. Folic Acid supplement in first pregnancy also reduces 'a priori' risk (Czeizel and Dudas 1992).

Identification of risk factors for adverse pregnancy outcome is a main component of preconception care. Questionnaires with variables pertaining to social, nutritional, medical, infections disease, medication, reproductive and family history are an accurate screening tool and their implementation facilitates the provision of preconception care. PC care is effective for the introduction of periconceptional folic acid/multivitamin supplementation and for the reduction of smoking and consumption of alcohol resulting in significant reduction than expected. In an appropriately timed and planned pregnancy successful pregnancies with little risk can be achieved.

Screening for Genetic Disorders: Indian Scenario

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Newborn Screening and Prenatal screening for various genetic disorders is well established in public health programs of most of the western countries and some of the developing countries for considerable years. The utility of these programs is beyond doubt now. Each country has to tailor its program according to its own needs and resources.

The success of any screening program depends upon its relevance and acceptance. These factors in turn depend on the participation of the public at large, the medical fraternity and the Government. Recently we have started a State of Art facility for Newborn and first and Second trimester prenatal screening in Pune. We encountered many interesting facts. There are a lot of misconceptions in the minds of clinicians caring for women and children regarding screening. Confusion between screening and disease diagnosis was very common. More than half of them believed in high risk screening in spite of concrete evidence on the contrary. Majority were worried about the introduction of routine screening because of the cost involved even after understanding the importance of screening. The same trends were observed in the postgraduates in OBGY and Pediatrics.
The newborn and prenatal screening is the logical next step in public health programs of our country as the infectious diseases are now controlled. Government may be skeptical of implementing it because of the cost. In these times of small family norm private participation is therefore most important. Education of public as well as the medical fraternity is therefore extremely essential for introduction of the screening program.

Genetic Screening Programme, Preventive Genetics

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Genetic disorders have become more important public health issue as with the improved medical care causes like infectious diseases, malnutrition, prematurity have been controlled to a great extent. With the completion of Human Genome Project our understanding of impact of our genes on human body in health and disease is undergoing a sea change. Chromosomal anomalies have an incidence of 0.7-1% while single gene disorders are encountered in 1% births. Multi-factorial disorders and somatic cell genetic disorders also cause considerable morbidity, mortality adding to the burden of genetic diseases. Although we can not change our genetic blueprint our genetic destiny is not totally preordained. By implementing multi-specialty, holistic approach at all stages of our lives we can make a lot of difference in the incidence of genetic disorders, their severity, chances of survival and the quality of life of the affected individuals. To be really effective this preventive genetic initiative has to be introduced at macro level to cover the entire population of the country. For achieving this, following steps are needed,

- Baseline epidemiological studies in the target population.
- Campaign for increased awareness in the population about the target disorder & the program.
- Establish/strengthen efficient two-way referral system to cover the entire population and integrate preventive genetics in healthcare system.
- Organise genetic centres of excellence.
- Devise & conduct appropriate genetic screening programs.
- Networking of all tertiary level institutions, genetic centres in the area as well as globally.

It is also essential to make the program equally effective at micro level (individual patients, families). A high degree of awareness about genetic disorders as well as health seeking behaviour is needed in general population. The preventive genetic program has to be targeted at all stages of life. The important components of this program include,

A) Prior to Conception:

- Evaluate ethnic background, family history
- Pre-marital counseling and management
- Pre-conception evaluation and counseling of the couple
- Control of medical disorders like diabetes, hypertension,
- Pre conception therapy with folic acid, zinc etc,

B) Post Conception:

- Avoiding high risk factors/behavior

- Pre-Implantation Genetic Diagnosis (PGD),
- Study of Foetal cells in maternal blood,
- 1st-trimester Pre-natal genetic screening and diagnosis,
- 2nd trimester genetic screening and diagnosis.
- Intensive, multi-specialty management of high risk patient, foetal therapy

Once a genetic anomaly/disorder is diagnosed, it is advisable to counsel the patient and the family in a sympathetic manner and make them aware about the impact of the disorder on the affected individual as well as other family members (present as well as future!). The counseling should not be directed/ coercive after giving all information about the problem the decision making should be left to the couple.

Management of children with significantly severe genetic anomaly requires a team of clinicians, social workers and counselors. Special facilities, schools special diet and medications are to be advised in many disorders, if done in timely fashion they can be of great help. Family liabilities, financial burden should be sympathetically discussed and all possible help should be given. These couples should be asked to come for counseling, specific advice before they think of going for another conception in future.

Thus by implementing the Preventive Genetics campaign at all strata of the society at all stages of life we can hope to make a meaningful impact on the incidence, morbidity, mortality and social burden due to genetic, congenital disorders.

Control of Sickle Cell Disease: Field Based Screening and Prenatal Diagnosis in Tribal Communities

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Sickle cell disease (SCD) is an important medicogenetic problem among tribal communities which constitutes about 8% of total population of India. A program for effective control of SCD is initiated in Wayanad district of Kerala. The major components of the program being (i) stopping the spread of the disorder by identifying sickle cell status through mass screening covering the total tribal population of the district and marriage counseling, (ii) prevention of birth of cases with the disease by prenatal diagnosis, and (iii) bringing down the morbidity and mortality due to the disease by initiating penicillin prophylaxsis and special vaccination in homozygotes at an early age.

Subjects numbering 74,750 have been screened by sickling test. Of these 6,825 were detected to be carriers of sickle hemoglobin (HbAS), while 1,292 were homozygotes having sickle cell disease (HbSS). Two hundred forty five target couples where both partners are carriers were given option of prenatal diagnosis during next pregnancy.

Prenatal diagnosis has already been carried out using ARMS technique in four such couples. The fetus in three cases (75%) revealed carrier status and one was normal (25%) with absence of beta globin sickle gene. All were advised to continue with the pregnancy. The results were confirmed in two after the delivery, one ended up in spontaneous abortion and another is still continuing pregnancy.

Spectrum of Mutations in Indian Patients with Cystic Fibrosis: Implications in Prenatal Diagnosis

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101 children with confirmed CF (based on clinical features and sweat chloride testing), were referred for molecular genetics studies. The genomic DNA was extracted from peripheral blood and was screened first for Delta F508 mutation and 3849+10kb C→T mutation. If both these were negative, then 17 exons of CFTR gene were screened by SSCP and sequencing, if altered confirmation was observed. The work is ongoing and all exons have not been studied. Out of 101 patients 21 (20.7%) were homozygous for Delta F508 mutation and 14 (13.8%) were heterozygous. Four patients (4%) carried 3849 + 10 kb C→T mutations. In addition 5 rare and 4 new mutations were identified. So far the mutations have been identified in 48 (47.5%) patients. 20 couples were screened for Delta F508 mutation as the history suggested that their children died of suspected Cystic Fibrosis. Interestingly 4 couples were detected to be heterozygous for Delta F508.

As the mutation analysis was negative for common mutations in significant proportion of cases, dinueleotide repeat analysis was also standardized for linkage studies. Prenatal diagnosis was carried out in 11 cases. Out of these, in 5 of the cases in which affected child was homozygous for delta F508 mutation, 2 fetuses were normal and 3 were heterozygous. In one couple where mutation of only one parent was identified, fetus was negative for the identified mutation. In 5 families, prenatal test was done using linkage studies with dinucleotide repeats, all fetuses tested were unlikely to be affected.

In conclusion the mutational spectrum of CF patients in India is different from the West and the profile is highly heterogenous. Delta F508 mutation has been reported to be as high as 70% in caucassian population, whereas in our study is quite low. This has clear implications in counseling and prenatal diagnosis, as alternative strategies like linkage analysis come for rescue.

Ultrasound Markers of Chromosomal Anomalies

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Major chromosomal abnormalities are often associated with multiple fetal defects that can be detected by ultrasound examination. The overall risk for chromosomal abnormalities increases with the total number of defects that are identified. It is therefore recommended that, whenever defect/marker is detected at routine ultrasound examination, a through check is made for the other features of the chromosomal abnormality known to be associated with that marker; should additional defects be identified, the risk is dramatically increased.

If the mid-trimester scan demonstrates major defects like hydrocephalus, holoprosencephaly, multicystic renal dyplasia and severe hydrops, it is advisable to offer fetal karyo-typing, even if these defects are apparently isolated.

Minor defects or markers, like nuchal edema, short femur, choroid plexus cyst, hyperechogenic bowel etc. are common and they are not usually associated with any handicap, unless there is an associated chromosomal abnormality. Routine karyotyping of all pregnancies with these markers would have major implications, both in terms of miscarriage and in economic costs. The estimated risk can be derived by multiplying the background risk (based on maternal age, gestational age, history of previously affected pregnancies and, where appropriate, the results of previous screening by nuchal translucency and / or biochemistry in the current pregnancy) by the likelihood ratio of the specific defect.

Recent Advances in Prenatal Diagnosis of Aneuploidies

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Chromosomal aneuploidies account for 95% of liveborn chromosomal abnormalities resulting in increased morbidity and mortality. The present study aimed at integrating prenatal diagnosis of aneuploidies with management of increased-risk pregnancies. Prenatal diagnosis was done to screen aneuploidies of chromosomes 13, 18, 21, X and Y using Fluorescence In Situ Hybridization (FISH) on dividing and non-dividing (interphase) cells obtained from amniotic fluid and chorionic villi samples in increased-risk pregnancies and spontaneous abortuses material. Interphase FISH detected aneuploidies in high-risk pregnancies that were later confirmed using conventional cytogenetics. In spontaneous abortion samples, FISH analysis was successful in all cases while conventional cytogenetics was not informative in some. Aneuploidies detected were mainly trisomies 21, 16 and 13 followed by triploidy and monosomy X. Further, interphase FISH analysis could provide a result where culture failed. Usually when maternal serum screening (done at 14-16 weeks of pregnancy) is positive, patients are informed about the increased risk and suggested to undergo amniocentesis. By the time patient opts for amniocentesis and cytogenetic results are available, the pregnancy is quite advanced (about 23-24 weeks) especially in case it needs termination. In such cases, interphase FISH is a boon that provides result within 24 hours giving the couples time to make decisions regarding the ongoing pregnancy.

Genetics of Recurrent Spontaneous Abortions

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Chromosome abnormalities appear to be the main cause for pregnancy loss in 50% to 80% of case of recurrent spontaneous abortion. Both maternal and paternal factors have been proposed, including maternal age and gestational age at time of the loss, as well as significant increases in sperm chromosome aneuploidy, apoptosis, and abnormal sperm morphology. Monosomy X and triploidy account for approximately 20-25% of all chromosomal anomalies. In cases of spontaneous abortion, following assisted reproductive techniques, monosomy of the X chromosome is the most frequent chromosomal abnormality. In the majority of cases of monosomy X, the remaining X chromosome has been found to be of maternal origin in around 75% of cases. In human triploidy, the additional genome has been found to be of paternal origin in approximately 70% of cases. These data suggest that paternal factors are important in the development of these two conditions. I will propose models that seek to explain these conditions at a molecular level by (i) the association of Y chromosome microdeletions leading to the loss of the entire Y chromosome and a 45,X cell line and (ii) familial male-linked twinning tendency associated with female infertility.

Prenatal Diagnosis of Bleeding Disorders – A Solution for Control of Such Disorders in India

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In developing countries like India where there is legalisation of abortion prenatal diagnosis is the best method of preventing the birth of affected male child in hemophila families.
We have performed carrier detection in 380 females from 233 families by RFLP using the intra and extra-genic polymorphic markers of factor VIII and IX genes. The polymorphisms used were intron 18 Bcl 1, intron 19 Hind III, intron 22 X ba 1 and D x 552/st 14 of the factor VIII gene and intron 1 Dde 1, intron 4 Taq 1, 3 Hha 1, Residue 148 Mnl 1 of the factor IX gene. Forty three families subsequently underwent antenatal diagnosis by chorionic villus sampling in first trimester of pregnancy. Of the 78

severe hemophilia A patients screened for intron 22 inversions, 21 were positive. The prevalence of intron 1 inversion was relatively low (<2%).
Second trimester antenatal diagnosis was also performed during weeks 18-20 of the gestation in eight families. These were women who were refered late in the pregnancy or families in which the key female was not informative for the markers used or where the index cases were deceased. Four of the fetuses were affected and four were found to be unaffected. The diagnosis was offered by performing clotting and antigen assay by ELISA in the blood obtained by cordocentesis.
Our laboratory has also offered prenatal diagnosis in the first trimester of pregnancy in 2 cases of type III VWD (severe) by VWF intron 40 VNTR analysis. So, we believe if few more centres can be established in India for prenatal diagnosis in hemophilia families, the disease load can be reduced to large extent.

Genetics of Hereditary Ataxias

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The hereditary ataxias, which are a group of clinically heterogenous neurodegenerative disorders have been shown to be caused by instability and expansion of trinucleotide repeats in the coding as well as noncoding regions of about a dozen distinct genes. These repeats which are normally polymorphic in normal individuals and restricted to a particular range become unstable once they cross this threshold. A wide heterogeneity in the prevalence of various ataxias in the different populations is observed. This has been attributed to the ethnicity and inherent genetic differences between populations. We have carried out an extensive analysis of ataxia in over 300 families in the Indian population. There is a wide difference in prevalence even between populations of northern and southern India. In the north India, SCA 2 is maximally present followed by SCA1, SCA12, MJD and SCA7 whereas in the South India the frequency of the SCA1 is the highest followed by the others. Using single nucleotide polymorphisms (SNPs) as well as microsatellite markers we have been able to trace the founder predisposed haplotype in the case of SCA2, SCA1 and SCA12. There are certain SCAs like SCA8, SCA10, DRPLA which have not been so far reported in the Indian population. Moreover, nearly 47% of the cases of ataxia cannot be assigned to known loci. These could be due to mutations in novel unidentified loci unique to the heterogenous Indian population. Additionally, between families there is a wide variability in clinical severity in the presence of similar mutations which indicates the presence of modifier genes/mutations. The availability of complete human genome sequence and associated polymorphisms allows us to identify novel genes and modifiers, using a combination of computational and molecular genetics approaches. Our efforts in this direction will be discussed.

Gene Therapy: A Promising Future Medicine

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With the rapid progress in the field of molecular biology and recombinant DNA technology, genome

technologies are benefiting mankind specially in the clinics. Newer and more sensitive techniques for molecular diagnostics are coming into the market. So also, this technology has contributed to treatment of diseases. Gene Therapy is a novel mode of treatment where genetic material is used in treating diseases. This involves transferring a gene to the target cell and efficiently expressing the new gene. Extensive research has gone into designing ideal vectors for gene delivery and testing them in animal models and phase I/II clinical trials. At present viral vectors are the most efficient means of delivering genes to target cells. For ethical and social reasons so far, human gene therapy is restricted to somatic cells.

Monogenic disorders, with single gene mutations were initially thought to be ideal candidates for gene therapy, where the defective gene would be replaced by the good gene. However, restoring long-term function in target cells has become a major challenge, although there are some recent success reports. Most clinical trials are now aimed at cancer or cardiovascular diseases or HIV infection. In cancer, one aims at destroying the malignant cell, which is less daunting than maintaining long-term expression of transgene to cure the disease.

Learning from the clinical trials and from the technological advances in the field, gene therapy appears to be a promising way of treating some of the dreadful diseases.

Clinical Relevance of CGH Microarrays

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The field of cytogenetics has already entered the molecular era and a rapid expansion of its contribution is seen in the genomic disease management. Fluorescence In Situ Hybridization (FISH), Quantitative Real-Time Polymerase Chain Reaction (PCR) and Comparative Genomic Hybridization Microarrays (array CGH) are on the forefront of the development of molecular diagnostics. Clinical utilities with FISH and PCR range from disease screening, diagnosis, determination of therapy selection and response, monitoring minimal residual disease to an early detection of disease relapse. Array CGH is a forward-looking revolution in the field of molecular cytogenetics. With its robustness and sensitivity, a clinical embracing of this new technological platform can easily be visualized in the near future. As the human genome sequence is ascertained, genome-wide screening with microarray technology will gain eminence in the clinical scenario, yield better solutions and bring the concept of personalized medicine in cancer closer to reality than ever before. Significant implications of array CGH are also seen in many prenatal/postnatal conditions.

Thalassemia – Prevention Through Prenatal Diagnosis

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To achieve optimal treatment for children with thalassemia major is a battle. The addition, of 8000 or more children with the problem annually to the existing numbers makes that battle into a loosing struggle. Controlling the constant addition, is an acceptable solution to the problem.

Preventing marriages between at risk couples or the use of Artificial insemination through donor sperm are not practical solutions in our country.

Identifying at risk pregnancies early and offering the couple, through prenatal diagnosis, the option

to make an informed choice is the method adopted by most societies worldwide. This is a very simple and practical approach. Obstetricians have a responsibility in this preventive approach and can play a vital role in avoiding the suffering of thousands of children and their families.
This talk offers practising Obstetricians an approach to this prevention through antenatal carrier detection, and counselling. It will explain how the further steps like assessment of pregnancies, family gene studies, choosing the right prenatal diagnostic procedures, and interpreting the results are carried out

Analysis of Male Factor Infertility

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The revolutionary techniques of IVF originating in the 70's have given life to more than half a million children. The advent of intracytoplasmic sperm injection (ICSI) allows the possibility of biological fatherhood to many couples with male factor infertility. Assisted Reproduction Techniques (ART) like TESA, PESA, MESA and open biopsies have helped to obtain sperm required for ICSI even from azoospermic males and enabled them to father normal children.
There is evidence that 60% cases of male infertility have an underlying genetic basis. We have analyzed these genetic causes using different parameters. Karyotyping was done to detect structural and numerical chromosome anomalies. Sperm FISH in some cases was done to determine the percentage of chromosomally normal sperm. Y chromosome microdeletions were screened using multiplex PCR technique for 18 loci belonging to AZFa, AZFb and AZFc regions including the DAZ gene. Cystic Fibrosis mutation DF508 was checked in cases of patients showing absence of vas deferens. We also carry out Preimplantation Genetic Diagnosis (PGD) in patients with structural and numerical chromosomal anomalies so that only chromosomally normal embryos can be transferred to the mother during IVF.

Medicolegal and Ethical Aspects of Prenatal Diagnosis

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Prenatal diagnosis is based on certain screening tests done routinely or on clinical suspicion. They are meant either to exclude or confirm a condition or to compute the probability of that condition occurring in a particular pregnancy. The various tests can be grouped in to (i) Non invasive examination like sonography (ii) Tests performed on blood and (iii) invasive examination like chorion biopsy or amniocentesis to obtain tissues for testing. Medicolegal problems can arise out of any of these. PNDT Act strictly prohibits performance of these tests at centers and by persons not registered as competent with the authorities. Vitally important is the fact that communicating to the patient or her relatives the sex of the fetus revealed by these tests is absolutely banned. Very strict prohibitive penalties are prescribed for any person flouting PNDT Act. Other important areas wherein medicolegal problems arise are performance of procedures without valid consent, complications of diagnostic procedures and wrong or missed diagnosis. Any invasive procedure requires informed written consent? In practice at least, the consent is implied and not put down in black and white. But the general rule that a female person can be examined by a male doctor only in the presence of a female nurse must be meticulously

followed. Invasive procedures could lead a complication eg. abortion or limb defects may result from chorion biopsy or amnionitis may follow amniocentesis. These could result in medicolegal problems in litigation. The most important aspect of prenatal diagnosis as in any field of medicine is proper counseling. The need for the various tests, their possible complications, their limitations, implications of the test reports and possibility of false positive or false negative tests must be discussed in details with the patient and her relatives and all her doubts and reservations adequately clarified before undertaking the tests. Such thorough and proper counseling and meticulous performance of the tests by a competent person would prevent most of the medicolegal problems.

The ethics of carrying out prenatal diagnosis is sometimes debated. The aim of prenatal diagnosis is to spare the unborn, the unfortunate parents and the society at large the avoidable miseries resulting from the birth of the babies born with severe anomalies not compatible with reasonable life. This concept is being challenged by those who believe that every unborn child has a right to life however handicapped or unhealthy it may be. It may also be debated whether prenatal diagnosis tests with their possible, though rare, complications be performed for a couple who is not willing to abort the fetus if found to be so abnormal or defective that it would end up in grossly incompatible or subnormal life. In all such debates the advantages accruing to the fetus, prenatally diagnosed with certain defects in the form of intrauterine corrections of defects, resorting to premature delivery to minimize the damage caused by the defects and enabling prompt and immediate treatment right at birth should not be ignored. In the end all ethical issues should be left to the concerned couple to decide upon after adequate counseling.

Karyotype and FISH When to Use / What is the Real Application in Genetic Workup

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A new technique of molecular cytogenesis FISH, mainly Fluorescent insitu hybridization is a technique where DNA probes are attached to metaphase chromosomes or interphase nuclei. Basic steps involved are labeling of DNA probes, preparing interphase and metaphase spreads, insitu hybridization and visualization with specific filters. Techniques are used for diagnosis or investigation purpose in many areas of medicine like Pediatrics, Medical genetics, Maternal fetal medicine, reproductive medicine and pathology. Several hundred probes are now available for the same.

Procedure of FISH varies from case to case. Sufficient knowledge of cytogenetics is essential in order to interpret FISH results and co relate with clinical diagnosis, treatment, prognosis. Sensitivity limitation and pitfalls need to be evaluated thoroughly before application. Now newer techniques are introduced like PRINS (primed insitu labelling), SKY (Spectral Karyotyping), CGHC (Comparative Genomic Hybridization).

Applications of FISH include detection of structural abnormality, microdeletion syndrome, detection of subtelomeric aberrations in patients with unexplained mental retardation, preimplantation diagnosis of common aneuploidies, study of mosaicism and effect on development, study of specific translocations and gene rearrangement in human cancer as well as identification of unknown material in the genome.

There are certain guidelines for clinical application of FISH. In disorders in which cytogenetic testing cannot provide results FISH is considered important for diagnosis and where Cytogenetic studies are available for diagnosis FISH should be supplemented by karyotyping for final diagnosis.

Genetics of Developmental Abnormalities

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Hereditary developmental anomalies of the upper and/or lower extremities in humans are easily recognizable phenotypes that can be used in the mapping and cloning of genes important for normal human development. Limb malformations occur as isolated malformations or as part of syndromes. In general approximately 1:500 to 1:1000 children are born with congenital limb defects. Of all limb defects, alterations in the digital number and/or disruption of normal development (i.e. polydactyly, ectrodactyly, syndactyly, synpolydactyly, brachydactyly) are the most frequently observed congenital anomalies. Mendelian segregation can be identified in families with these phenotypes, with the majority showing an autosomal dominant mode of inheritance. Sporadic cases have also been described. Of all limb malformations polydactyly of hand and/or foot is the most frequently observed with estimated prevalence of 0.37 to 1.2 in 1000 live births. The major causes of these limb malformations are abnormal genetic programming and intra-uterine disruption to development. To date, mutations in only GLI3, HOXD13 and p63 have been associated with several clinically distinct phenotypes. For example, 33 GLI3 mutations have been detected in individuals associated with six distinct phenotypes from various ethnic groups. The delineation of these developmental limb anomalies and identification of novel genes has enormous impact on genetic counseling and also reveal pathophysiological mechanisms that control limb development.

Multifetal Pregnancy and Prenatal Diagnosis

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Multifetal pregnancy should be clinically managed as a high-risk pregnancy. The incidence of congenital structural malformations is two to three times higher in fetuses of multiple gestations when compared with those of singleton gestations.
Prenatal genetic diagnosis can be effectively and safely performed as long as the unique requirement of multiple gestations are appreciated.
Genetic counseling must address issues such as calculation of specific genetic risks for multiples, the unique procedural risks imposed by multiples, and the options available should fetal abnormalities be detected.
Sonographers must be able to assess amnionicity, chorionicity and zygosity, proficient in fetal anatomic scanning and in guiding invasive diagnostic procedures.
Operators need to be aware of the special challenges presented by multiples and should have a wide breadth of experience in the performance of both amniocentesis and chorion villus sampling in singleton gestations. They should also be experienced in the performance of these procedures for multiple gestations, which is technically more demanding. These procedures need to be performed in such a way as to ensure that both fetuses are reliably sampled and documented so in follow up care any abnormalities can be correctly identified. Discovery of discordant abnormal results including the consideration of selective termination, require psychological impact of a multiple pregnancy complicated with genetic abnormality.

Molecular Characterization, Prenatal Diagnosis and Carrier Screening of Muscular Dystrophy

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Duchenne Muscular Dystrophy (DMD) and Becker's muscular Dystrophy (BMD) are pan ethnic progressive X-linked recessive neuromuscular disorders caused by deletions, duplications or point mutations in the dystrophin gene located on Xp21 where the clinical picture is variable depending upon whether the DNA defect disturbs the reading frame of the gene.
Molecular diagnosis of DMD / BMD is done by multiplex PCR to look for deletions in the dystrophin gene as about 70% of cases show deletions. Such information also has therapeutic implications, as the use of oligonucleotides to bypass a single deleted exon, or use of gentamycin in cases of non-functional dystrophin due to a stop codon. This knowledge also helps carrier screening and prenatal diagnosis for prevention of this distressing disease.
Carrier screening of high-risk female relatives is done by linkage analysis of polymorphic markers or by dosage analysis if the deletion in the family is known.
Prenatal diagnosis can be done at 10-12 weeks of pregnancy where the analysis involves determining the sex of the fetus, looking for deletion in the gene (if known in the affected child) or linkage analysis to determine whether the fetus has inherited the high risk or low risk X-chromosome. For linkage analysis, the sample of the affected child plays a key role and it is important to store the DNA of the affected child specially if there is no deletion in the gene.
Thus, advances in molecular genetics can help in confirming the diagnosis, carrier screening and prenatal diagnosis to reduce the burden of muscular dystrophy in future generations.

Clinical Findings in Chromosome Aberrations

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Since the first chromosome aberrations (trisomy 21) were demonstrated in 1959, the number of detectable aberrations has increased to several thousand. The increase in detection of chromosome aberrations and the increasing demand for especially prenatal chromosome examination has increased the number of examinations to nowadays about 2.000 per year per million population. The standard cytogenetic laboratory requires facilities not only for cell culture and banding techniques, but also for fluorescent in situ hybridisation (FISH), comparative genomic hybridisation (CGH) and chromosome dissection, including the appropriate software. The classical clinical picture of chromosome aberrations is characterised by mental retardation, a pattern of multiple minor and major anomalies and growth retardation. In submicroscopic aberrations, the patterns of clinical findings are often less distinct, and many cases lack striking physical abnormalities. The most consistent finding in autosomal aberration is mental deficiency while the most useful finding for clinical recognition of a chromosome aberration is a pattern of minor anomalies. Congenital major malformation can be very specific for a given aberration, however, they are by far less consistent and there is a high rate of discordance not only in siblings, but even in monozygotic twins with the same aberration. In general, frequent isolated malformations are also frequent in chromosome aberrations and vice versa. Chromosome aberrations constitute a very valuable tool for gene mapping. Useful observation are balanced rearrangements associated (and especially co-segregating) with a defect which mostly is dominantly or X-linked inherited in patients with no additional findings, and occurrence of a defect which is rare in chromosome

aberration in a patient with additional mental retardation and other findings and a small autosomal or X-chromosome deletion or (rarely) duplication. Attempts should be made to extend the screening for submicroscopic chromosome aberrations to segments apart from the telomeres in a fashion which makes if affordable, and to find better criteria and less invasive techniques for prenatal chromosome examinations.

There is a vigorous prenatal selection against chromosome aberrations. Data from spontaneous abortions led to the conclusion that not much less than every second conceptus in humans has an unbalanced chromosome aberration. In early spontaneous abortions, more than 1/2 are chromosomally unbalanced. Numerical and ploidy aberrations prevail much more than later in life, and the 3 most frequent abnormalities are 45,X, trisomy 16 and triploidy. Trisomy 16 is lethal before the 12th week of gestation, and by the age of 10 postnatal months, triploidy is lethal as well. It is estimated that about 90 – 98% of 45,X embryos die prenatally, predominantly during the first 12 weeks of gestation and due to severe dysplasia of the lymphatic system. The incidence at 40 weeks gestation of chromosome aberrations can be estimated as about 1:100 – 1:200; the incidence in stillborns is at least 4 times higher.

As to the **parental origin**, there is a strong bias for most chromosome aberrations. While trisomies occur predominantly during female meiosis and are age - dependant, terminal deletions and ring chromosomes are to their majority paternal in origin and occur independent of parental ages. Interstitial deletions show an almost equal distribution with respect to parental origin. Additional isochromosomes behave similar to trisomies from which they originate while isochromosomes replacing a normal homologue show an almost equal distribution. More complex aberrations, especially in a mosaic state, often have unexpectedly complex ways of formation, not infrequently with additional segmental uniparental disomy. In triploids, about 3/4 have an additional maternal haploid set due to either meiosis 1 or meiosis 2 failure, and the 1/4 paternal ones almost always are the result of dispermy.

Karyotype-phenotype correlations: For most chromosomal segments, deletions have a more adverse impact on the phenotype than duplications; triplications, viable for only a few segments, in severity stand between the 2 former. The number of aberrations and possible combinations is almost unlimited. For many segments, deletion leads to early prenatal demise while duplication leads to multiply malformed liveborn offspring. Many chromosome aberrations can be clinically recognized on the basis of a unique pattern of minor and major anomalies. If findings occur which are otherwise rare in chromosomal aneuploidy, this mostly gives a hint towards involvement of a single gene included in a deleted segment and thus for haploinsufficiency. This leads to the phenotype of a dominant gene disorder with the additional findings of growth and mental retardation and sometimes other anomalies. In some instances, several genes with specific phenotypes can be deleted, causing “contiguous gene syndromes”, i.e. phenotypes including two or more distinct genetic disorders. Examples include the WAGR syndrome with aniridia and Wilms tumour (deletion of 11p13) and the Giedion-Langer syndrome with multiple cartilagineous exostoses and cone shaped epiphyses due to deletion of 2 genes mapping to 8q24.1. For duplications and deletions it can be shown that the critical segment for the major dysmorphic phenotype is quite narrow and close to the telomere; if the deletion/duplication extends more proximally, growth and mental retardation as well as incidence and severity of major malformation become more pronounced without much of aggravation of the pattern of multiple minor anomalies.

During the last years, fluorescence in situ hybridisation (FISH) has allowed to extent the detectability of chromosome aberrations to the submicroscopic level, especially for deletions. Previously defined clinical phenotypes were shown to be caused by submicroscopic deletions, e.g. the Prader-Willi, Angelman, DiGeorge and velo-cardio-facial syndromes. These interstitial deletions have to be specifically searched for which requires initial clinical recognition or, at least, suspicion in order to initiate the appropriate FISH and other examinations. By contrast it is possible to investigate patients with a “chromosomal phenotype” for submicroscopic aberrations in a screening fashion without a specific suspicion. There exist easy to handle sets of probes for the subtelomeric regions which are particularly frequently involved in aberrations. A screening for the entire chromosomes would be a very time consuming and expensive task, however, first attempts for this have already been made, and the next step would be automatisation and establishing strict criteria for clinical selection. Another option would be to increase the resolution of comparative genomic hybridisation (CGH), which now

is at about 12 mB, to the level of 3 – 4 mB. All these efforts will be a challenge for clinical geneticists and require a good collaboration between clinicians and laboratory.

Genomic Imprinting and Uniparental Disomy in Human

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Genomic imprinting is defined as "the differential modification of the maternal amd paternal genetic contributions to the zygote, resulting in the differential expression of parental alleles during development and in the adult". The term stems from the German word Prägung and was initially created by the later Nobel Prize laureate Konrad Lorenz for observations in geese: newborns follow the first moving object they see (usually the mother). In nowadays medical genetics, the imprinted allele is the one specifically switched off, either the maternal or the paternal. Abnormal phenotypes can arise from mutation, absence, or duplication of the allele which is active, thus leading either to absence or increased amount of a gene product which in turn causes abnormal development.
Especially clinically relevant are imprinted segments on chromosomes 6, 7, 11, 14 and 15. For some imprinted regions which mostly contain clusters of genes there exist control elements, and mutations may occur in the genes or one of the genes themselves as well as in the "imprinting center", thus releasing imprinting. Imprinting is released during gametogenesis and is set again in early embryogenesis. Imprinting is usually followed by methylation of the switched off gene or chromosomal segment, and this is one way of a quick diagnostic test.
Evidence for imprinting comes from abnormal phenotypes in patients with uniparental disomy (UPD) for one chromosome; rarely, only segmental UPD is found, and these cases present hints towards a finger mapping of imprinted regions. It can be distinguished between maternal and paternal UPD, between UPD of an entire chromosome versus segmental UPD, and between heterodisomy and isodisomy. Heterodisomy starts from meiotic nondisjunction and is therefore almost always maternal. One will almost always find segments in which, due to recombination, reduction to homozygosity is found. Heterodisomy versus isodisomy at the centromeric region give evidence for meiosis I versus meiosis II nondisjunction while complete isodisomy is considered to be the consequence of a mitotic error. Hence, maternal UPD is predominantly heterodisomy and is associated with increased mean maternal age while isodisomy is predominantly paternal UPD and occurs independant from paternal (but not from maternal!) age.
The Wiedemann-Beckwith or EMG (exomphalos-macroglossia-gigantism) syndrome is caused by a double dose of the paternal 11p15 gene which contains the IGF2 (insulin-like growth factor, only paternal gene active) and the H19 (only maternal gene active) genes. Paternal non-mosaic UPD of the entire 11 is probably lethal as it was never found in a liveborn; most patients with the EMG syndrome with UPD show both mosaic and segmental (11p15) paternal UPD. They make up about 20% of all EMG patients. About 50% show abnormal methylation in another gene, the LIT1 or KCNQ10T1 gene which is also maternally imprinted, another 10% reveal abnormal methylation at either IGF2 or H19, and a minority show either paternal duplication of the 11p15 segment or a maternal translocation presumably disrupting an imprinting controlling locus. In about 5%, none of the above-mentioned abnormalities can be demonstrated. While methylation is normally complete in the critical CpG islands of the maternal LIT1 allele, it can be either fully absent or imcomplete in EMG cases. Some patients with segmental mosaic UPD 11p15 or abnormal LIT1 methylation do not present with the full EMG phenotype, but rather with advanced growth and hemihypertrophy, but without macroglossa and omphalocele/umbilical hernia and also without organomegaly and earlobe creases characteristic for this syndrome. The risk of developing Wilms tumour is highly depending on the result of the molecular tests: it is 50% for the patients with abnormal H19 methylation, 27% in UPD cases, 20% for the cases with all examinations showing normal results, and 0% for the patients with abnormal LIT1 methylation.
The Prader-Willi syndrome (PWS) is caused by loss, mutation or imprinting of a gene or, more likely, several genes at close proximity at 15q11.2-q12 whose paternal alleles are active and the maternal ones

switched off. Thus, the syndrome can arise through maternal uniparental disomy, paternal deletion or an imprinting mutation. The critical region is the same as that for Angelman syndrome (see below), but imprinting is different. The critical region (PWS/ASCR) is flanked by similar repeats which tend to associate and to recombine. Other unbalanced recombination products are the frequent additional inv dup(15) chromosome and a triplication or, rarer, duplication in tandem formation. In contrast to the Angelman syndrome, UPD, with a proportion of about 30%, is relative frequent in PWS which reflects the tendency to meiotic nondisjunction in female gametes. The SNRPN (small nuclear ribosomal protein N) gene obviously plays a role in the formation of the syndrome, but cannot be solely responsible for the latter. Presumably, an array of genes in that region plays a role for the clinical features which include: severely diminished intrauterine activity, severe neonatal hypotonia, inappropriate appetite and tendency to obesity starting with about 9 months of age, hypogenitalism and hypogonadism, small and delicate hands and feet, moderate mental and growth retardation. Secondary findings include early onset of diabetes, scars from scatching, early cardiovascular insufficiency due to overweight. The first diagnostic test is a methylation test which, if positive, must be followed by either FISH with the SNRPN probe of microsatellite marker analysis and chromosome examination. A few percent of deletions are de novo or familial unbalanced translocations inherited from the father, and in these families, Angelman cases can be present if the unbalanced translocation stems from the mother. Rarely, an additional i(15p) chromosome is found in UPD cases: then, both normal 15s are maternal, and the isochromosome is paternal in origin. Since maternal heterodisomy mostly is secondary to maternal trisomy 15, and since fetal trisomy 15 is lethal, but placental trisomy 15 is not, trisomy or mosaic trisomy 15 can be found at chorionic villus chromosome examination with a subsequent normal karyotype in amniocytes. It can be expected that about 1/3 of these instances should have maternal UPD15, and this has to be determined following the trisomy at CVS.

Angelman syndrome (AS) is characterized by severe to profound mental deficiency, seizures which are difficult to control, ataxia, unmotivated outbursts of laughter, microcephaly, and a broad mouth opening. The deleted region in most cases is similar to that in PWS, however, origin is from the mother. The paternally imprinted gene(s) map a little bit more distal within the commonly deleted segment. They include the ubiquitin 3A (UBE3A) gene. In contrast to PWS in which there is no single gene known whose mutation may cause the full pattern of the syndrome, mutations in the latter gene may cause AS. About 75% of AS cases have a 15q11.2-q12 deletion; paternal UPD (almost always isodisomy) is found in only 5% as expected considering the low incidence of meiotic nondisjunction in sperms. UBE3A mutations can be demonstrated in about 5%, and very rare are imprinting mutations. Same as in PWS, UPD cases with an additional i(15p) – here of maternal origin – and others with de novo or familial unbalanced translocations are occasionally found. Another parallel to PWS is that UPD cases, in contrast to almost all deletion cases, are not hypopigmented for hair and iris colour. The reason for the latter is that the „p“ gene which maps inside of the commonly deleted region and whose haploinsufficiency causes hypopigmentation (homozygous deletion/mutation leads to one type of albinism) is not imprinted and thus both copies are active in the UPD cases.

Primordial growth retardation or Silver-Russell syndrome (SRS) goes along with pre-and postnatal growth retardation (birth weight around term about 2000g, adult height about 1.50m), no microcephaly, normal intelligence, a triangular face with high frontal hairline and small mandible, clinodactyly of little fingers and often asymmetry, especially of limbs, and areas of aberrant skin pigmentation. About 10 percent of SRS patients show maternal UPD 7, and, parallel to PWS with maternal UPD 15, increased mean maternal age at conception and a proportion of cases with placental (mosaic-) trisomy are found. Patients with segmental UPD and studies on imprinted segments within chromosome 7 showed that there are 2 candidate regions for imprinted genes, their loss causing SRS, one at 7q32 and the other at 7p11.2-p13. No gene, however, is yet known whose mutations or deletion would cause the syndrome in a mechanism parallel to PWS and AS for chromosome 15. UPD cases tend to have mild developmental delay in contrast no non-UPD cases.

Paternal disomy 6 leads to benign neonatal diabetes mellitus which spontaneously resolves after a few years. Observation of one case with segmental UPD narrowed down the critical region to a smaller segment in 6q. Maternal disomy 14 causes, as the minimal consequences, pre- and postnatal growth retardation and early puberty. The facial characteristics of SRS are not seen in these patients. Many patients have additional findings, however, as mosaic trisomy 14 is not lethal and may cause a quite variable clinical picture, it is not clear whether these additional findings are due to UPD or to hidden

mosaic trisomy. The same is the case for the clinical findings in cases with paternal UPD 14, the findings in different reported cases being very dissimilar, not allowing to define even a minimal clinical phenotype.
Strategies to detect UPD cases include the search for UPD in patients with unclassified malformation syndromes born to "older" mothers and the investigation in instances of translocations between homologous chromosomes. The latter may lead only to repeated spontaneous miscarriages, but otherwise a normal phenotype, indicating that there are probably no imprinted genes on the chromosome involved in the rearrangement, such as on chromosomes 13 and 21. If e.g. only growth retardation is found (in cases of maternal UPD 16 and 22), it remains unclear if the latter is caused by imprinting or by malfunction of the trisomic placenta. Despite the frequent occurrence of trisomy 18, no instance of maternal (or paternal) UPD 18 has so far been reported; thus, it might be that at least maternal UPD 18 is lethal to the embryo and the same could be the case with e.g. UPD 19.
Recent case reports have suggested that imprinting mutations (which cause a minority of cases with Prader-Willi and Angelman syndrome and a distinct proportion of cases with Wiedemann-Beckwith syndrome) might be more frequent in pregnancies achieved through intracytoplasmatic sperm injection (ICSI). If this turns out to be true, than ICSI pregnancies should be especially carefully monitored e.g. for fetal activity (grossly reduced in Prader-Willi syndrome), fetal growth, tongue size and omphalocele (all characteristic for WBS). Ther is no such ultrasound marker for Angelman syndrome.

Microdeletion Syndromes

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Microdeletions are chromosome deletions which are, at least mostly, not visible under the microscope. As many of them were detected because of a specific phenotype, and many phenotypes had been defined clinically long before it was detected that a small chromosome deletion was the cause, they often include genes whose haploinsufficiency causes an abnormal phenotype. For this reason, the term "contiguous gene syndromes" has been proposed for instances with more than one deleted gene in close proximity. Microdeletions constitute a powerful tool for mapping of genes whose mutations / deletions cause phenotypes uncommon to chromosome aneuploidy. The most well – known examples for microdeletions include the 22q11.22 microdeletion causing the DiGeorge or velo-cardio-facial syndrome, the 7q11.23 microdeletion causing the Williams-Beuren syndrome, 15q 11.2-q12 with Prader-Willi and Angelman syndromes, 17p13.3 with Miller-Dieker syndrome, 11p13 with WAGR syndrome, 8q24.1 with Giedion Langer syndrome, and 4p16.3 with Pitt-Rogers-Danks and Wolf syndromes. Some interstitial microdeletions seem to occur with increased frequency at sites of identical repeats through pairing between chromosomes at meiosis as evidenced by microsatellite marker analysis at flanking regions which show a recombination at the deletion breakpoints. Prominent examples for deletion and thus haploinsufficiency of more than one gene include the WAGR (Wilms tumour-aniridia-genito-urinary malformations-retardation) syndrome with deletion of the PAX6 gene causing aniridia and a Wilms tumour gene at 11p13, the Giedion-Langer syndrome with deletion of a gene for multiple cartilagineous exostoses (EXT1) and one for the tricho-rhino-phalangeal syndrome (TRP1) at 8q24.1, and up to 8 different X-linked diseases in males with deletions at Xp21. Terminal microdeletions, -duplications and deletion-duplication with less specific phenotypes are recognised with increasing frequency. The determination of genes involved in microdeletions, especially those for abnormal brain development and function, is still at the beginning. A higher resolution of comparative genomic hybridisation (CGH) and / or the more widely use of microsatellite genome - wide screening in patients with MCA / MR syndromes is likely to detect many more interstitial microdeletions. Microsatellite marker analysis shows that terminal microdeletions mostly occur in the paternal homologue while interstitial microdeletions show a small preponderance of maternal origin. In the latter, unbalanced recombination between chromatids of two homologues is more frequent than

recombination between the chromatids of one homologue.

Prenatal Diagnosis of Lysosomal Storage Diseases

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Lysosomal storage disorders are a heterogeneous group of about 40 genetic disorders. The clinical phenotype is very much variable depending on the tissue distribution of the storage material & degree of enzyme deficiency. Though individually they are rare, in combination the incidences are about 1:5000 and displayed considerable clinical and biochemical heterogeneity. The majority are inherited as autosomal recessive condition accept for MPS II and Fabry's disease, which are X linked. Clinical diagnosis of these disorders is often difficult because the features lack specificity and histopathological techniques are limited because they will not usually distinguish between different types of storage material.
They can be divided in to three groups according to the major organ system pathology :

(1) Primary involvement of the central nervous system without significant somatic or skeletal pathology, disorders of gray matter (Gangliosidosis) and disorders of white matter (leucodystrophy)
(2) Primary involvement of the reticuloendothelial system with or without neuropathology e.g. Niemann Pick disease & Gaucher disease
(3) Multisystem involvement in which skeletal manifestations are prominent features (Mucopolysaccharidosis & Mucolipidoses)

Prenatal diagnosis of LSD's is possible provided the confirmed diagnosis is known in an index case. Prenatal diagnosis can be achieved by a variety of technique including enzyme activities in chorionic villus study, cultured chorionic trophoblastic cells (CT) and cultured amniotic fluid cells (Fibroblasts). Ultra structural examination of chorionic villus often gives an independent mean of diagnosis and in some cases molecular genetic studies for mutation deletion or linkage analysis are appropriate.
During prenatal study, the common concern is about pseudodeficiency of Arylsulfatase A in metachromatic leucodystrophy and other enzymes as well for different LSD's. Therefore other enzyme assay is necessary not only in the index cases but also in the parents.
A large number of LSD's may present as fetal hydrops and the diagnosis can be established by fetal blood sampling microscopic examination and biochemical assay of the appropriate enzyme. Though prenatal diagnosis of LSD's are cumbersome, require skills and thorough knowledge of the enzyme activities, the diagnosis in which an affected fetus is indicated should have confirmation of the diagnosis by cultured CT and fibroblast cells & wherever possible molecular genetic and morphological studies are recommended.

Birth Defects Registry – The Need, Scope and Limitations
Birth Defects Registry (BDR) - The Need

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As per the statistics from the developed countries, about 2-3% of births are associated with major

congenital anomalies. In India, twenty five million births occur every year. If we assume the birth prevalence of congenital malformations in India is 2% - likely to be more - then in absolute numbers it could be 500,000; i.e. every year there are 500,000 babies born with some form of birth defect in India, which is equal to the number of new cases of leprosy diagnosed in India in a given year. It shows that birth defects, pose a great challenge to the Indian health care delivery system. Fortunately, it has been proved that many of the major anomalies such as neural tube defects, (NTD), conotruncal anomalies of the heart such as double outlet right ventricle (DORV), anterior abdominal wall defects such as omphalocele and oral clefts are preventable by supplementation of periconceptional folic acid alone. Thus, data on the magnitude of birth defects is essential to not only to plan preventive strategies, but also to organize methods of supportive care for the affected individuals and families. This can be achieved only through an organized system like a registry for birth defects.

Congenital anomalies are one of the leading causes of perinatal mortality in developed countries. Until a few years ago, sepsis and neonatal infection were contributing to a huge proportion of perinatal and neonatal mortality in India. But due to the evolution of better medical facilities, our nation is undergoing an epidemiological transition; i.e. many neonatal infections are better controlled today, which eventually increased the relative proportion of congenital anomalies as a cause of perinatal mortality.

Though several hospital-based studies published statistics on the prevalence of congenital anomalies, it has not been estimated at the population level in India. As per the published statistics, the prevalence varied from 13.7 to 52 per 1000 births. This wide variation is rather due to various methods adopted by each study than due to varying geographical distribution of BD, because none of them was population based. Hence, the scope of BDR is to ascertain nationwide population level prevalence of birth defects.

An ideal BDR should be population-based (based on the residence of the mother) but poor track record of registration of vital events in India[2] is the major impediment in setting up a population-based BDR. An alternative is the hospital-based registry, (based on place of birth) which can become a population-based registry once it collects data from all hospitals that conduct deliveries in a defined geographic area provided the home based deliveries are less in that area. Until this can be achieved we will not get the true magnitude of birth defects. The next thing is the man power, in a country like India where trained professionals in the field of public health and in epidemiology is another obstacle. The third is the money to be spent in surveillance. Since, birth defects is not recognized as a public health problem by government of India, private sector has to spearhead this process, which eventually depends on external funding.

Data from our three years experience in the Chennai Birth Defects Registry and one years data from the Salem/Erode Registry will be presented.

Prenatal Interventional Procedures

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Ultrasound has provided a window to the developing fetus. A better understanding of fetal structure and function has helped in unraveling many mysteries of the unborn. With developments in technology, excellent anatomical and pathological details can be obtained. Added to this was the frustration of the neonatologist and neonatal surgeon who were often mute spectators when a new born with full blown disease was handed over to them. The logical progression of this situation was to make an attempt at early diagnosis and understand the natural history of the disease which would facilitate early institution of treatment to the fetus or newborn. A variety of prenatal procedures are available for diagnosis and treatment of the unborn patient.

Therapeutic Interventions

Interventions can be done for treating an in-utero condition. The two major conditions for which

therapy can be done are Rh isoimmune disease and obstructive uropathy.
Rh Isoimmune Disease: Fetal anaemia due to Rh incompatibility can be corrected in-utero by intra-uterine transfusions. Diagnosis of fetal anaemia can be made by various ultrasound features and by fetal blood sampling. The presence of a dilated umbilical vein, ascites and oedema indicates severe anaemia. An increased velocity of flow in the umbilical vein and a pulsatile wave form of the umbilical vein on doppler also indicate fetal anaemia. However, the best way to diagnose fetal anaemia is by drawing a sample of fetal blood and estimating the haematocrit and haemoglobin.
If the haematocrit falls below 25%, an intra-uterine transfusion is indicated. Fresh O-ve cells, packed to a haematocrit of 70 - 80 is given intra peritoneally, through the intrahepatic umbilical vein or through the umbilical vein at the placental insertion site. Under continuous ultrasound monitoring, the packed cells are given at the rate of 2 - 5ml / min into the fetal circulation. Periodic monitoring of the fetal heart is done during the procedure. The volume of cells to be transfused is calculated by taking into consideration the donor hematocrit, fetal hematocrit and the feto-placental blood volume at that period of gestation. Fetal blood samples are drawn for estimation of hematocrit during the procedure. The transfusion is given till a fetal hematocrit of above 45% is acheived. However, during the procedure, if the fetus develops bradycardia or a tachyarrythmia, caution has to be exercised and the procedure may have to be deferred. A fetal salvage rate of above 90% can be achieved by repeated intra-uterine transfusions.
The major complication which causes concern is cord hematoma. It can occur when the transfusion is given in the umbilical vein near the placental insertion site. The complication rate is lower if the intrahepatic portion of the umbilical vein is chosen. Additionally, if the needle is dislodged in this site, the cells will spill into the fetal peritoneal cavity which is quite safe. Umbilical vein thrombosis is another complication which can occur with intravascular transfusion. Repeat transfusions are based on the rate of drop of hematocrit which is estimated by doing a another fetal blood sampling two weeks after the first transfusion. Generally the rate of drop will be about 1% per day.
After the second transfusion, the rate of drop of hematocrit is usually slower, probably because the fetal bone marrow is suppressed and fetal red cell production is less. The pregnancy is managed in a similar fashion till the time safe delivery can take place.

Obstructive Uropathy

Obstruction to the fetal renal tract can be unilateral or bilateral. Unilateral obstructive uropathy does not require any prenatal intervention as long as the other kidney is functioning. Bilateral obstructive uropathy may be due to PUJ obstruction or PUV. Obstruction due to post. urethral valves may require prenatal vesico-amniotic shunting in selected cases. Owing to the wide spectrum of presentation of the disease, it is difficult to choose the appropriate case for shunting. Hence, this procedure has a high failure rate.
Prenatal detection of fetal renal function can be achieved by aspiration of a sample of urine from the bladder and estimation of Na, Cl, and osmolality. Estimation of urinary b 2 macroglobulin appears to be a better predictor of residual renal function and may be required to plan further management. In selected cases, a double pig tailed shunt is introduced into the fetal bladder with the distal end of the shunt being placed in the amniotic cavity. Good decompression of the fetal urinary tract can be expected immediately after the shunt placement. However, there is always the risk of the shunt being displaced as the fetus may pull it out. Repeat shunting may have to be done in such cases. At this point, we still do not have a good method to predict the prognosis for long term renal function in fetuses with obstructive uropathy. The natural history of the disease has to be understood better to plan prenatal treatment of such cases.

Amnioinfusion

The five major causes of oligohydramnios are:

- Premature rupture of membranes
- Bilateral absent or non-functioning kidneys
- Fetal hypoxia

- IUGR
- Post dated pregnancy

Of these causes, premature rupture of membranes and bilateral absent or non-functioning kidneys can lead to near total absence of amniotic fluid. In hypoxia, IUGR and post dated pregnancy, there is a gradual dimunition of amniotic fluid. Absence of amniotic fluid early in pregnancy can lead to a number of sequelae to the developing fetus. Between 16 - 24 weeks of gestation, loss of amniotic fluid can cause lung hypoplasia.

Although the mechanism of lung hypoplasia has not been well understood, it has been proved that replacing fluid in the amniotic cavity in early pregnancy prevents lung hypoplasia. Secondly, in the absence of amniotic fluid, visualization of fetal anatomy is very difficult. Replacement of amniotic fluid helps in better evaluation of fetal anatomy and diagnosis of structural malformations which may not be obvious in the absence a good acoustic window in oligohydramnios.

Technique: Amnioinfusion can be done as a diagnostic procedure or for therapeutic purposes. The most common indication is for diagnostic purposes in the second trimester. Under strict aseptic precautions, a 20 gauge needle is introduced into the amniotic cavity. Care shoud be taken, as, in severe oligohydramnios, it may be difficult to place the needle precisely in the amniotic cavity. About 5ml of normal saline is injected to check the position of the needle. The turbulence caused by the passage of saline into the amniotic cavity can be visualized as fine echogenic moving particles. Loops of cord may appear as clear spaces and may be mistaken for a pool of amniotic fluid. Colour doppler helps in identifying loops of umbilical cord easily. Once the position of the needle is satisfactory, normal saline warmed to 37 degrees Centigrade is pushed through the needle at a rate of about 10 - 20 ml per minute. Continuous monitoring of the passage of fluid into the amniotic cavity is essential. The quantity of fluid to be instilled varies. the end point is to achieve good visualization of fetal anatomy, with the deepest pool measuring at least 2 cm. Quantitatively, enough fluid is injected to create an amniotic fluid index of about 8 - 10. Saline is also infused into the fetal peritoneal cavity to confirm the absence of fetal kidneys. Serial amnioinfusions as therapy for premature rupture of membranes have been attempted. The success rates are limited, as, if there is a large rent in the amniotic membrane, the instilled fluid tends to leak out immediately. In the author's own series two cases have been delivered after serial amioinfusions done for early rupture of membranes.

Multifetal Reduction

Improvement in ovulation induction and assisted reproduction techniques have resulted in an increased number of higher order multifetal gestations. The perinatal mortality and morbidity increases with increasing number of fetuses. Multifetal reduction is a term applied to describe the technique of reduction of fetus or fetuses in pregnancies with three or more embryos. The final number of viable fetuses left behind is at least two. A careful survey of all the sacs and embryos is done to map the topography. Selection of the embryo/s to be reduced is based on the following criteria :

- Choose the sac/s closer to the fundus.
- It is preferable to choose embryos which are smaller.

The placentae of the sacs chosen should be separate from each other. Fusion or vascular communications between the placentae may result in damage to the other fetuses.

Timing: It is preferable to wait till the embryonic cardiac activity is well established. Though a precise timing is difficult to advocate, it may be judicious to perform the procedure between 9 -12 weeks. The incidence of miscarriage increases with increasing gestational age. Prior to eight weeks, spontaneous resorption of an embryo can occur.

Technique: The procedure can be done transabdominally or transvaginally. In the transabdominal technique, a 22 gauge needle is passed through the sac into,the fetal heart. 2 - 4 mEq of potassium chloride is injected into the fetal heart. After confirming cardiac asystole, the needle is withdrawn. Absence of cardiac activity should be ascertained by a scan done 15 minutes after the procedure. Though potassium chloride is the method of choice, various other methods which have been tried are:

- Air embolisation
- Exsanguination

· Cardiac tamponade using ice cold ringer lactate solution into the pericardium or thorax.

Miscarriage rates after multifetal reduction range between 10-20% Selective Reduction is a term applied to reduce one abnormal fetus in a multiple pregnancy. This is most often done in the second trimester and should only be done for dichorionic pregnancies without fusion of the placenta. The method adopted is the same as for multifetal reduction. Selective reduction should never be done for monochorionic pregnancies, as, the the other fetus may also die.

Risks of Multifetal Reduction

Miscarriage: rates range from 10-20%. As mentioned earlier it also depends on the period of gestation and fetal number. Operator experience has a definite bearing on the outcome. Generally antibiotics are not given after the procedure. The risk of infection is rare if strict aseptic precautions are followed has been reported after the death of a co-twin. However after mulifetal reduction the incidence of DIC appears to be very rare. The most dreaded complication in selective feticide is ablation of the wrong twin. Care should be exercised in identifying the twin which is to undergo the procedure. If placental vascular communications exist between the fetuses, the normal twin may also die. Hence, selective reduction should only be done for dichorionic twins with non-fused placentae. A variety of prenatal diagnostic and therapeutic procedures are available for management of structural, hematological and biochemical problems in the developing fetus. Adequate care should be taken for selection of cases for prenatal diagnostic procedures. Fetal treatment schedules should be based on information available regarding the natural history of the disease and the risk / benefit ratio. A good example would be to compare the results after treatment for Rh disease and Fetal obstructive uropathy. Whereas in Rh disease, the benefits to the fetus far outweigh the risks involved, in the latter, it may be very difficult to predict long term renal function and a conservative approach may be in the best interests of the fetus and parents. Before embarking on any invasive therapy to the fetus, a karyotype of the fetus must be done. The parents should be counseled regarding the details of the procedure and the possible outcomes.

Pattern of Genetic Counseling for Hemoglobinopathies – A Laboratory Scientist's Viewpoint

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Hemoglobinopathies are common disorders in India. Due to high prevalence in certain communities, social practices of consanguinity and endogamy, clinical severity of the disease prenatal diagnosis is demanded foe Beta Thalassemia, Hb S anemia, Hb E thalassemia etc. Genetic counseling is an important aspect of prenatal testing.

Our hospital is a referral center where prenatal diagnosis is a paid service. Till date 1350 diagnoses are done at various gestations by mutation screening, RFLP, globin chain synthesis and HPLC methods using DNA from CVS, amniocytes and or Fetal blood cells.

Genetic counseling is offered procedures and laboratory diagnosis are necessarily explained while having the consent. The counseling is non-directive and includes genetic, medicine and therapeutic, laboratory work aspects of prenatal diagnosis. The information is well received when given in mother language. Conventional counseling is sufficient in 95% cases; mostly there is history of an affected child. In about 5% cases more that one session may be required. They include cases of 1st pregnancy diagnosis, laboratory related problems, insufficient sample and multiple fetal pregnancies. The post diagnosis counseling is done mostly when there is difficulty in accepting the results eg consecutive fetuses are affected. The referring professionals also consult us in complicated cases.

To avoid the repeated visits of distant couples we follow certain pattern that is adapted from our database of mutation analysis and the methods for safe sampling and diagnosis.

Along with the laboratory analysis, coping with the emotional, psychological and economic consequences together is a tough job. However this kind of bridging with the clinets is felt helpful in smooth running of the service and assuring the reliance on our results. The liability and creditability of the laboratory is better understood.

Genetic Causes of Male Infertility: Indian Scenario

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Genetic factors have been found to play a role in about 10% of male infertility. Although male infertility found to be common all over the world, the causative factors are different among infertile men belonging to various countries. We have undertaken a study on infertile men to establish possible genetic causes of male infertility among Indian populations.
We have analyzed 340 azoospermic men, of which 29 (8.5%) showed Y chromosome deletion. Deletion in *AZF*c region was the most common (82.8%) followed by *AZF*b (55.2%) and *AZF*a (24.1%). In another study, we have analysed the complete mitochondrial sequence of an individual with low sperm motility (~10%) and found 9 missense and 27 silent mutations in mitochondrial genes of sperm but not of the blood cells. There was a 2-nucleotide deletion in the mitochondrial COII genes, introducing a stop codon, which might be responsible for low sperm motility. We have also analyzed the CAG repeat motif in the androgen receptor (AR) gene, which has been reported to be associated with male infertility, in 280 azoospermic and 201 fertile men. We do not find any association of CAG repeat expansion in AR gene with male infertility in Indian populations. This suggests that what is true for one population may not be true for other populations.

Fetal Therapy: *In Utero* Hematopoietic Stem Cell Transplant for Beta-Thalassemia

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In maternal-fetal medicine the scientific approach is gradually but progressively shifting from the diagnostic side to the therapeutic side. Fetal therapy can nowadays be achieved , with good clinical outcomes, in many prenatal pathological conditions, both medical and surgical. The authors illustrate their experience with "In Utero Haematopoietic Stem Cell Transplant" ("IUHSCT"), particularly in betaThalassemic fetuses. IUHSCT has been reported by some authors, with good results in Immunodeficiency Syndromes ("SCID") and some metabolic diseases (osteogenesis Imperfect, Lysosomal Storage Diseases) but poor results in beta-Thalassemia, in which the donor's hemopoiesis has no evaluative advantage over the recipient's one. Our guidelines for IUHSCT in BetaThalassemia are :
1)-The donor is the father ("aploidentical HLA donor)
2)-Earlier is better: IUHSCT should be carried out before 12 weeks of pregnancy
3)-High dose of Hematopoietic Stem cell ("Megadose") : 10 times more the pro/kg dose used in a post-natal setting.
4)-High grade of T-Lymphocyte depletion , in order to avoid the GVHD in the fetus
5)-Intraperitoneal route of infusion
The authors describe also their method non-invasive evaluation of the grade of engrafment : fetal cells in maternal circulation are collected after 26 weeks, and they are investigated through specifically

designed DNA-probes in order to identify their genetic origin : the percentage of donor-derived fetal erythroblasts can represent the percentage of engrafment.

Preimplantation Genetic Diagnosis: Scientific and Ethical Issues in Embryo Selection for HLA-Matching

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Preimplantation Genetic Diagnosis (PGD) has been gaining increasing consensus in IVF contests in order to karyotype embryos before "in utero" trasfer: exclusion of aneuploid embryos from further transfer highly increases success rates in medically assisted conception . Using PGD we can also diagnose "Single Gene Disorders" at embryonic stage, avoiding to transfer affected embryos and, if possible, also eterozigous carrier embryos : this can avoid further therapeutic terminations of pregnancy. Recently PGD has been used to select HLA-compatible embryos with a previous sibling affected by haematological diseases , which can be treated with Bone Marrow Transplant (BMT) or Cord Blood Transplant (CBT).
This use of PGD seems particularly interesting in inherited hemoglobinopathies and hemopoiesis disorders . The authors discuss their experience concerning the use of PGD for HLA-matching with a previous affected sibling: The choice of PGD should be thoroughly balanced, examinating both scientific and ethical aspects of the problem. Since, especially in developing countries where the incidence of inherited disorders of hemopoiesis is already high, the risks of traditional transfusion therapies are already high , and the quality and expectancy of life poor, PGD for HLA-matching seems the new strategy to definitively treat these diseases. Accurate policy should be implemented to optimise cost/benefit ratio of PGD for HLA-matching.

A Clinical View of Neurometabolic Disorders

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There are over 250 known neurometabolic diseases and the list is growing everyday with advances in biochemistry, genetics and molecular biology. Though individual metabolic disorders are rare, together they constitute a significant number of the patients attending genetic or neurological clinics. The age of molecular genetics has seen an explosion of gene mapping and identification of the mutations responsible for the disease. Finally the abnormal gene products i.e. receptors, neurotransmitters, cytoskeletal and signaling proteins etc, responsible for the diseased state are being identified and should help in understanding the pathophysiology paving the way for rational therapy
Autosomal recessive (AR) inheritance is by far the most common mode of inheritance. Autosomal dominant (AD) inheritance is much less common in childhood. Many of these are caused by expansion of normal triplet repeats leading to neurological dysfunction. Mitochondrial inheritance (MI) is recognized since the last decade.
There are several ways to classify these diseases - gray vs white matter, type of subcellular organelle affected, disorders of intermediary or metal metabolism, acute vs. chronic symptoms and finally the usual age of onset. We have used the last classification for its simplicity.
Tandem mass spectroscopy, MRI / MR spectroscopy and molecular diagnosis has made many of these disorders easily diagnosable. Histology of affected tissues is now rarely needed.
The most prominent clinical symptoms along with age of onset helps in narrowing down the diagnosis

and ordering appropriate tests. For instance in the neonate acute encephalopathies are often due to disorders of organic / amino acid or of the urea cycle. In infancy many present as psychomotor delay or regression with other manifestations e.g. peripheral neuropathy in the leucodystrophies or visceromagaly in the lysosomal disorders. In later childhood dementia and seizures are often seen in disorders of the grey matter like in the different neuronal ceroid lipofuscinosis. Specific symptoms like a movement disorder or strokes are seen in disorders like Wilsons disease or MELAS.
A working clinical diagnosis is useful to direct investigations.
In most of these disorders specific treatment is unavailable and diagnosis is important more for counseling, for planning future pregnancies and prenatal diagnoses. Certain disorders do respond to replacement of specific deficiencies (e.g. biotin in biotinidase deficiency) or to removal of toxic products with intensive dietary regimens (e.g. dietary therapy for PKU, MSUD) or chelating agents (e.g. penicillamine in Wilson's disease). Occasionally responses are seen after bone marrow transplant (e.g. X-linked ALD) or enzyme replacemnt (e.g. Gaucher's diseases).
Unique to India are some conditions like the megelencephalic leucoencephalopathy with subcortical cysts.

Adult Neuronal Stem Cells: Relevance to the Clinic

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The fact that the adult mammalian brain can generate new neurons, at least in discrete regions, throughout the lifespan of the organism has generated immense excitement for both basic neurobiologists and clinicians interested in the promise that this discovery holds. In distinct neurogenic zones in the mammalian nervous system, stem cells/ progenitors divide to give birth to immature neurons that then migrate and integrate functionally in the neuronal circuitry. The process of adult neurogenesis has been observed in a number of mammalian species including rodents, marmoset and macaque monkeys, as well as most recently in humans. In addition to observing new neuron birth *in vivo*, these neuronal stem cells have also been isolated from living tissue and maintained *in vitro*. These neuronal stem cells in vitro not only generate neuronal and glial cells but can also give rise to cells from other lineages such as the haematopoetic lineage under suitable conditions. The ability to regulate the process of neurogenesis *in vivo* holds immense promise in the repair of neuronal damage and indeed several studies suggest that the adult mammalian brain is not as restricted in it's ability to repair damage as had been previously thought. The idea that transplantation of these stem cells may be of potent use to repair brain regions that are inherently non-neurogenic is also a tantalizing possibility that is the focus of considerable research interest.
Amongst the major neurogenic regions is the hippocampus, which can generate as many as 250,000 new neurons each month in the dentate gyrus region of the rat brain. It is as yet unclear what is the exact functional role of adult neurogenesis, but there are exciting preliminary findings that suggest a possible function in complex processes such as learning, memory and mood. Understanding the regulation of this complex process is likely to yield insights of relevance to both the basic function of adult neurogenesis and in the discovery of therapeutic targets that can control this process.

Molecular Methods in Prenatal Diagnosis

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The high socio-economic burden of genetic disease in India induces people to accept prenatal diagnosis (PND) to avoid having an affected child. Molecular diagnostics has revolutionized this

field.
Beta thalassemia is common in India, and has proved to be the prototype for using molecular technology for prenatal diagnosis - find the mutations and develop a simple method for their analysis. In Duchenne muscular dystrophy PND is done by testing for deletions in the dystrophin gene, and linkage studies in cases with no deletions. Spinal muscle atrophy is also common in India, and can be definitely diagnosed by testing for deletion of exon 7, while linkage is still necessary for about 5 % of cases without a deletion. Linkage is also used for PND of hemophilia, because the mutations in different families are different.
Molecular techniques are available in India for prenatal diagnosis of cystic fibrosis, Wilson disease, achondroplasia, Apert syndrome, Huntington disease, myotonic dystrophy, spinocerebellar ataxias, congenital adrenal hyperplasia, oculocutaneous albinism and steroid sulfatase deficiency. There is literally no limit to the disorders for which prenatal diagnosis can be performed provided the gene is known, and there is no heterogeneity in the disorder. However it is essential to obtain a good family history and analyze it properly to ascertain risk and to identify the gene involved. Amnio-PCR is invaluable for PND diagnosis of chromosomal disease. Clinicians should know about availability of PND in various disorders, and learn to store the DNA of an affected child. Networking of the various molecular labs is also desirable.

Chorionic Villi Analysis – Pitfalls By Discrepancies Between The Extraembryonic Tissue And The Fetus Proper

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Chorionic villi analysis (CVS) is a reliable method in prenatal diagnosis. However, in rare cases placenta confined mosaicism causes problems in predicting immediately the true fetal karyotype. Here we present data on false normal or false pathological cytogenetic results in short term cultures (STC) of chorionic villi:
(1) In a case of a fetal translocation trisomy 21 all analysed cells of the STC showed only a normal karyotype. In contrast, all cells of the long term culture (LTC) presented with a 46,XY,t(21;21),+21 chromosome constitution. Molecular studies were performed to reveal the genetic composition of the aberrant chromosome.
Moreover, this cytogenetic constellation we took as granted to examine the tissue composition seen on slides prepared after STC using FISH with LSI 21 probes (Vysis).
(2) In the second case, a 45,X karyotype was seen in STC. LTC yielded only cells with a normal male karyotype. Both cases were complicated by the presence of a twin pregnancy.
The origin of feto-placentar cytogenetic discrepancies will be discussed with respect to early embryonic development. The impact of a high quality sonographic examination is emphasized to avoid an erroneous cytogenetic diagnosis. Proposals for an optimal CVS analysis are given.

Molecular Diagnostics in Genetics: Techniques and Difficulties

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Although the human genome project is nearly completed, and all human genes are known, molecular diagnostics for genetic diseases remains a challenging problem.
Whereas the disease gene for more than 1000 genetic disorders has been identified, molecular diagnostics are available for only a minority of these in most countries. Furthermore, the analysis is often incomplete, slow and expensive. The most important bottlenecks for cost-effective molecular diagnostics are the nature of the mutation and the rareness of genetic diseases in general.
Disorders caused by a single mutation (eg. fragile X), or few mutations (haemochromatosis) can be easily diagnosed by molecular methods, but the vast majority of genetic diseases are caused by private mutations specific for a single family . Consequently, the whole gene has to be analysed by complete sequencing, or screening methods such as WAVE, DGGE or SSCP followed be sequencing of the abnormal fragments. This laborious methodology is only cost-effective when enough samples are available. Due to the rareness of most of the genetic disorders this can not be set up cost-effectively on a regional or even national level.
To facilitate genetic diagnostics an international network of diagnostic labs was organised, existing of many referral labs worldwide, 20 expert test labs, and 1 central lab that accepts all samples and issues all results. This network is called GENDIA (for GENetic DIAgnostics). GENDIA now offers diagnostic tests for more than 400 genetic diseases (www.GENDIA.net).

Confined Placental Mosaicism in CVS and the Effects of Uniparental Disomy on Pregnancy Outcome

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Mosaic chromosome abnormalities are detected in about 2% of diagnostic CVS. In the majority of affected pregnancies the abnormal cell line is the minority cell line and is restricted to the extra-embryonic tissues. Such pregnancies usually proceed uneventfully to term. However, a proportion of affected pregnancies have adverse outcomes. The three main considerations when attempting to predict prenatally which cases are at risk of adverse outcome, are mosaicism in the fetus, altered placental function due to the presence of a significant load of aneuploid cells and uniparental disomy in the normal cell line. Most adverse outcomes appear to be associated with mosaicism arising from correction of trisomic conceptions, although this may reflect the relative ease of establishing the link for these cases, rather than the full picture.
Unfortunately, experience with one mosaic chromosome abnormality cannot simply be extrapolated to others. Each abnormality has its own characteristic frequency and pattern of generation and it own associated risks. For trisomies 13,18 and 21 and the sex chromosome abnormalities, the risk of mosaicism in the fetal tissues is the major concern. For trisomies 2 and 16, the primary consideration is the effect of placental trisomy on its function, although fetal mosaic trisomy may be much more important than we have previously thought. For trisomy 15 and a minority of cases of trisomy 7, the effects of uniparental disomy also need to be considered.
The study of cytogenetic abnormalities in early embryos is now helping us to understand the origins of chromosome mosaicism. Combining this knowledge with quality data regarding pregnancy outcome following prenatal finding of mosaicism, will eventually lead to much better prediction of 'at risk' pregnancies.