genetic CLINICS

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Genetic Clinics is a quarterly newsletter published by the Department of Medical Genetics, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow on behalf of Genetics Specialty Chapter of Indian Academy of Pediatrics. The newsletter aims to provide a forum that enhances the practice and education of medical genetics in India. Articles of interest to the medical professionals in the field of medical genetics are welcome. The broad topics include: Genetic bases of diseases, chromosomal disorders, dysmorphic syndromes, malformations, Mendelian disorders, genetics of complex diseases, genetic testing, prenatal diagnosis, perinatal autopsy, teratogenesis, genetic counseling, laboratory practices, professional issues, psychological aspects, social aspects and legal aspects in the practice of medical genetics. The articles undergo limited peer-review at present and editing of content and style.

The categories of article include:

DeNoVo	Original articles with new findings and development in the field of medical genetics are considered. Word limit is 2000. Restrict the number of references to 15.
GeNeViSTA	Review articles, approach to common genetic problems and opinions from experts in the field are considered. Word limit is 1500-2500. Number of references should not exceed 10.
Clinical Vignettes	Brief case reports not exceeding 1000 words. Limit the number of references to 5.
GeNeXprESS	This is intended to serve as a guide to further reading. Articles of interest to clinicians published recently in leading journals are covered. One paragraph should describe the article.
PhotoQuiz	Good quality photographs of a typical genetic disease or clinical sign. Three to four sentences should describe the condition followed by a question asking the readers to identify the condition. There should be preferably one answer to the query which is unambiguous. The answer should also be provided with one paragraph giving crisp information on the condition.
gEne Mails	Letters to the editor discussing the contents of previous issues, comments and suggestions to the editorial board are considered. The section does not ask the response of the author to the comments.
GeneQueries	Clinical case scenarios in practice can be posted and the opinions of experts are sought by the editorial team on further management. The query needs to be precise and unambiguous. Both the question and the answer are published in the same issue.
EvEnTs	Conferences, workshops and continuing medical education programs related to the field of medical genetics are published free of cost. They should be as brief as possible. They are subject to editing of content and style.
GeNeToONs	Cartoons, jokes, humor related to the field of medical genetics are welcome.

Style of references: The articles should conform to Vancouver style of referencing. Only one author is listed.

Photographs of patients: It is the responsibility of the authors to take written consent from the patient or guardian for publication of photographs.

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Invited Editorial

Studies on Human Genetic Variations: Paving the Way to Molecular Medicine

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There is an enormous variation in the sequences of DNA between two randomly chosen healthy humans. These variations are seen in coding as well as in the non-coding regions. Not all changes in the DNA sequence lead to abnormal protein or a disease. The variations in the genome can be single base pair substitutions known as Single Nucleotide Polymorphisms (SNPs) or Variable Number of Tandem Repeats (VNTRs). There is one SNP for every 500 nucleotides. These genetic changes that do not cause disease are known as neutral variations. These variations are responsible for variations amongst individuals like physical characteristics, biochemical variability, disease susceptibility and variations in the response to drugs and environmental agents. Post 'Human Genome Project', there is a sharp increase in studies on human genetic variations all over the world. As a result, the scientific literature has been flooded with the latest molecular genetic data portraying the pattern, distribution and structuring of genetic variation in different human groups. The last decade has seen 'Human Genome Diversity Project (HGDP) and 'Hap Map' project providing a new dimension to the study of human genetic variations and its role in biomedical research.

Different population groups based on geo-ethnic, socio-cultural and language characteristics are genetically distinct though there may be admixture to a variable degree. Hence, individuals in a group are more likely to be genetically similar than the individuals between two different population groups. The studies on genetic variations face the challenge of developing a detailed understanding of the heritable variations in the human genome and target the objectives of reconstructing human evolution and determining the amount, pattern, distribution and structuring of genetic diversity across different geo-ethnic, socio-cultural and linguistic human groups. These studies are necessary to gain a better understanding of disparity in human susceptibility to diseases and differential response to pharmacological agents. The studies will also give insight into the multifaceted interactions of genetic and environmental factors in producing varied phenotypes based on the information of the genetic structure or ancestry of a population or an individual. Such studies involve research in varied disciplines such as medical genetics, population genetics, anthropology and pharmacology, with the aim of profiling the genotype of an individual or a population. Population based genetic information requires a perspective correlation with the past human movements for the historical content, with the climatic and environmental changes for the geographical aspect, with the fossil and paleontological information for the anthropological information and, with the rate and pattern of mating for the socio-cultural factors and above all it requires a strong interpretation based on more reliable statistical approaches.

Genetic variations, both neutral polymorphisms as well as disease related mutations get simultaneously affected by the evolutionary processes of mutation, migration, random genetic drift and selection resulting in their differential distribution amongst different population groups. Therefore, an understanding of the structure of neutral human genetic variations is highly valuable for understanding genetics of health and diseases. The understanding of neutral genetic variations aid biomedical research in at least three ways:

(I) Molecular sub-classification of the diseases can be done on the basis of genetic profile. The distinctive examples include the parallel presence of high frequency of hemoglobin HbS allele and sickle-cell anemia among sub-Saharan Africans. However, the same disease is also found in Hispanics and inhabitants of northwestern India and central Greece. The level of clinical consequences differs in different individuals as well as different populations though the disease causing mutation may be same in all patients. This depends partly on environmental factors and also the genetic background. Information about genetic markers modifying the disease will help in sub-classifying the disease in a better way. This may be more helpful in prognostication and use of therapeutic strategies than labeling the disease only on the basis of ethnic affiliation or phenotype. The concept of genetic ancestry is a much better indicator than race or ethnicity

to determine whether one carries the marker of a genetic disease. The population specific high prevalence of characteristic diseases has been observed though these diseases are seen all over the world. There is a high prevalence of Tay Sachs disease, Torsion Dystonia, Breast cancer and Gaucher disease among Jewish populations and not among Arabs, probably as a result of founder effect or inbreeding despite belonging to the same geographical area. A similar example is the absence of factor V Leiden mutation (A2086G) and prothrombin gene mutation (C10965T) among north Indians despite frequent occurrence of thrombotic events and various reports of possible Caucasian genetic ancestry.

(ii) Information about an individual's genetic ancestry can improve medical diagnosis and treatment on an individualized basis. The genetic differences among ethnic groups often are reported to cause differences in responses to drugs. An important example is that of β-1-adrenoreceptor variant (Arg 389 Gly) associated with an increased response to beta-blockers. Frequency of this variant varies significantly between European Americans -0.723 and African Americans -0.575. Another example is CYP2D6 which is a drug-metabolizing enzyme (DME) that encodes a member of cytochrome P450 family. Its null allele renders the gene product inactive to an extent that individuals with homozygous state for null allele experience little or no analgesic effect for the prodrug codeine. This null allele of CYP2D6 is reported in a frequency of 10% among north European ancestry. On the contrary, about 98% Arabs are able to transform codeine into the active form morphine.

(iii) Large scale studies on genetic aspects of multifactorial diseases are being done over the last decade with the expectation of development of personalized, predictive medicine and development of new strategies for treatment of common disorders like diabetes, hypertension, ischemic heart disease, psychiatric diseases, etc. It appears a mammoth task and has not resulted into anything that is clinically applicable. One of the difficulties in research in this field is due to the great population variability. One strategy for studying the genetics of multifactorial disorders is to study one or many polymorphisms in a candidate gene or all over the genome in a group of patients and a group of normal individuals. Various polymorphisms have different frequencies in different populations. Therefore if all the individuals in patient group and control group are not of the same genetic ancestry, the results may not be reliable. This is one reason for commonly seen inconsistency of results in two or more studies done on the same polymorphism and in the same disease. Incorporation of population genetic structuring in study designs of association studies can improve the outcomes several folds. Genetic knowledge of population sub-structuring and stratification is an essential requirement for proper selection of controls and for identifying disease pre-disposing alleles that may differ across ethnic groups. It has also been reported that risk allele may have different effects in people with different genetic ancestry. Individuals homozygous for APO E4 allele of APO E gene in Asian individuals has ~5 fold higher risk of developing Alzheimer's disease than heterozygous individuals. Similarly, three important variants of CARD15 or NOD2- R702W, G908R and 1007fs have been associated with an inflammatory bowel disorder- Crohn's disease in European-Americans but not in Europeans or Asians.

The information generated by the genetic variation studies has facilitated the possibility of replacing the proxy tags of geography, ethnicity, race or caste by an accurate genetic profile of an individual or a population sub-group. This possibility of replacement and knowledge about population sub-structuring and stratification based on the human genome sequence has potentially changed the approach to biomedical research. However, search for more reliable and resolving genetic markers that can infer the correct ancestries and resolute the genetic structuring accurately is still in progress. The more such ancestry specific marker become available, the more accurate our approach towards different diseases will become.

The knowledge of human variations will help in classifying people based on their genetic makeup rather than the current available ways like geoethnic, racial, language or religion based. The information about genetic variations will help in studying various multifactorial diseases, pharmacogenomics, and phenotype variability and thus help in individualizing medicine for best results. However, search for such genetic tags requires information about the genetic ancestry and genetic structure of contemporary populations, more knowledge about various evolutionary and socio-cultural factors that have shaped the present day genetic diversity and therefore more genetic studies revealing population specific private markers, alleles or haplotypes. The genetic variation studies have exhibited an early promise but still there is a long way to go and many populations are still waiting to get genetically explored.

‘Genomic Medicine’
Where is the ‘Evidence’?

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The practice of any form of medicine is based on evidence. The evidence may be available or collected from different sources including traditional beliefs, socio-cultural practices, personal observations, anecdotal annotations, case-controlled studies, double-blind randomised trials, and recent scientific developments. The philosophy behind ‘evidence-based medicine’ is not new and to a large extent reflects basic human instincts. Essentially ‘evidence-based medicine’ aims at the conscientious, explicit and judicious use of the current best evidence in making decisions about the care of individual patients. It is in fact the ‘personalized medicine’ in practice. The ‘evidence-based’ clinical practice is now pivotal in planning, funding and in delivering the health care. Clinicians, public health practitioners, health commissioners/purchasers, health planners, politicians and public seek formal ‘evidence’ in approving any form of health care provision.

Since the completion of the human genome project and the rapid accumulation of huge amount of data, scientists and physicians alike are excited on the prospect of ‘personalized health care’ based on individual’s genotype and phenotype. The first decade of the new millennium now witnesses the application of genomics in the practice of ‘evidence-based medicine’. The practice of medicine, including health promotion and prevention of disease, stands now at a wide open road as the scientific and medical community embraces itself with the rapidly expanding and revolutionising field of genomic medicine. This brief commentary highlights some fundamental aspects of genomics-based ‘evidence’ in the practice of modern medicine that is rapidly evolving into ‘genomic medicine’.

One of the fundamental principles of ‘evidence-based medicine’ includes scientific understanding of anatomy and physiology in both holistic terms and as well as in individual parts. The human body is organized into organ systems, tissues, cells, and cell components that are reduced to genetic and genomic profile. The structure-function relationship in biological terms is ultimately dependent upon the genotype. The molecular dissection at the genome or gene level is thus fundamental to understanding the morbid variation in terms of anatomy, physiology and biochemistry. The scope of molecular and cell biology in medicine is unlimited as this encompasses practically whole of genetics and genomics. Genetics conventionally relates to specific genes in relation to a number of different traits and characteristics whilst genomics encompasses the whole genome including all genes, DNA polymorphisms, RNA and its varied forms, and all other polymorphisms that might have current or evolutionary biological relationships. Thus it is not surprising to encounter plenty of evidence around in support of the role of genetics and genomics in the understanding of both normal structure and pathologic changes in relation to practically all aspects of clinical medicine ranging from the most uncommon disorders to the most common medical diseases that afflict the humans.

Recent developments in genome science and technology have led to a number of scientific fields ending with ‘-omics’. The suffix ‘omics’ is fast becoming fashionable. Even well before the completion of the Human Genome Project in 2003, some new scientific ‘-OMIC’ fields emerged within cell and molecular biology. Apart from genomics, we now have a plethora of fields ending with omics. Examples include transcriptomics, functional genomics, proteomics, pathogenomics, toxicogenomics, nutrigenomics, pharmacogenomics and the list is expanding rapidly. However, from biological perspectives, essentially only three of these matter- genomics, proteomics and metabolomics[1].

Genomics is about studying all genes in their entirety including all related non-coding sequences and polymorphisms. Proteomics is the study of all proteins, and metabolomics is the study of all molecules derived from metabolism (metabolites) in any living organism. Metabolomics is one of the many tools in personalized medicine, the core objective of genomic medicine. The National Human Genome Research Institute (NHGRI) defines metabolomics as the evaluation of tissues and body fluids, such as urine, plasma, blood, saliva and cerebrospinal fluid, for metabolite changes that may result from physiological responses or pathological changes. The term is interchangeably used with "metabonomics", which is an older term that usually refers to principles or rules that govern the generation and regulation of metabolites. However, increasingly the latter term "metabolomics" is accepted as this encompasses both theoretical and practical aspects.

So far human and medical genetics has largely concentrated on well-defined genetic diseases and some complex medical diseases with possible genetic bases. Apart from some diagnostic applications, there have been extremely limited therapeutic benefits. The ultimate goal of clinical medicine is to offer treatment based on individual's genetic make up or genomic profile. This is what is meant by personalized medicine. Development of new drugs based up on individual's favourable or unfavourable drug response would become the gold standard. Several academic and medical institutions and pharmaceutical companies worldwide are all working to learn more about fundamental cellular components, which can lead to more effective treatments for people based on their genetic structures and acquired differences.

While genomics researchers are searching for variations in genes that cause disease and proteomics researchers are seeking out abnormal protein patterns in different pathological conditions, metabolomic researchers focus on studying abnormal metabolite patterns. Numerous metabolites are generated through different chemical reactions in several metabolic pathways that are essential in maintaining homeostasis and regulate various body functions. It is estimated that there are probably 3,000 metabolites that are essential for normal growth and development (primary metabolites) and thousands more unidentified ones that are not essential for growth and development (secondary metabolites) but may help fight off infection and other forms of physiological stresses. Among these very small metabolites, known as low-molecular-weight metabolites, are of major importance. These include amino acids, sugars and lipids. The metabolomic studies are conducted using predominantly two methods- nuclear magnetic resonance (NMR) and mass spectrometry (MS). NMR can identify and quantify hundreds of metabolites in a sample of body fluid. MS complements NMR in that it can display quantity and generate profiles of thousands of metabolites with more sensitivity than NMR. The profiles are then run through powerful computers that process, store, and generate data in a form for scientists and clinicians to visualize and interpret.

Researchers and potential future users are both optimistic and cautious about predicting the potential of metabolomics that is still in infancy. However, it is likely that the application of metabolomics in clinical practice would be cheaper and faster compared to other –omic sciences and technologies. More importantly the collection of biological samples for metabolomic studies shall become routine through relatively non-invasive or minimally invasive procedures, for example vene-puncture or lumbar puncture. Any abnormal result could then be confirmed with genomics or proteomics based tests, which would require highly sophisticated equipments and expertise.

Metabolomics is a relatively new field with tremendous potential. It will help in profiling the chemical phenotype of the individual or organism under study. In a crude way, this is part of the clinical medicine in the form of

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several routine or specialised biochemical tests. However, these have often limited power in predicting the current disease state and therapeutic outcomes. Metabolomics is essentially combining this approach with genomics or proteomics. Researchers and scientists working in the field of metabolomics believe that the field has enormous potential to improve human health in a number of ways:

1. To make safer drugs by predicting the potential for adverse effects (or toxicity) earlier.
2. To target specific groups of people most likely to benefit from a drug, while excluding its use by those who may be harmed by it.
3. To speed the discovery and development of drugs
4. To diagnose disease and predict the risk of disease
5. To determine whether a treatment is working or not
6. To monitor healthy people to detect early signs of disease.

In brief, metabolomics is " a molecular way to do what physicians have done for thousands of years", which is to diagnose and treat patients based on a combination of symptoms and signs aided by a battery of tests. Same thing is done at molecular level. The challenge of metabolomics is to identify molecules or patterns of molecules, out of tens of thousands of metabolites that are specific enough to use as disease markers.

On-line information on metabolomics is available: www.metabolomicssociety.org or www.fda.gov / nctr / science/ centers/ metabolomics/

The major burden of mutant genes or pathogenic influences of genetic or genomic variation is reflected in predisposition or protection for multitude of complex disorders affecting all body systems. Essentially, predisposition or protection for a complex phenotype is based on gene-environment interactions. In most cases, environmental factors include dietary factors, microbial infection or infestation and toxic exposure. Specialist fields of nutrigenomics, pathogenomics and toxicogenomics have emerged respectively dealing with these major environmental factors.

It is widely recognized that there is marked inter-individual variation that modulates the true effect of dietary intervention or modification. This variation is fundamentally related to the genetic makeup of the individual. The individual genetic make up and variation is reflected either in the form of genetic predisposition or protection. This is perhaps a major factor influencing dietary effects in cancer risk through the genomic-nutrient and metabolic-phenotype interactions.[2] However, an individual's overall phenotype, including health status, is achieved and maintained by the combination of metabolic activities under differing circumstances at different stages of the life cycle and the complex interactions among genotype, metabolic phenotype, and the environment. This approach and concept are likely to receive a major boost in the current phase of rapid high-throughput technology developments in genomics, proteomics, and metabolomics that analyse DNA sequences, RNA transcripts, proteins, and nutrient-metabolic pathways. These advances have transformed biological studies on nutrient-gene interactions that are crucial in our holistic understanding of complex metabolic processes through functional genomic and metabolic profiling. Perhaps one of the major benefits of the gene-nutrient-metabolism approach could be the development of individualized dietary recommendations to reduce cancer risk. The genetic/genomic profiling can be harnessed in the future in developing individualized nutrition in the prevention of a wide range of chronic diseases (Figure 1.).

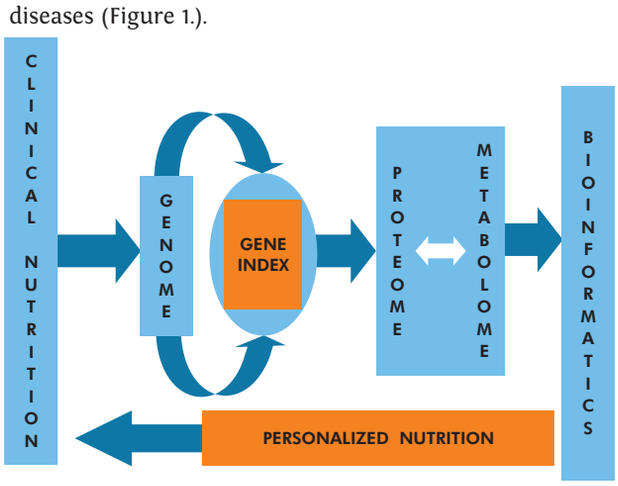

Fig 1. Personalized nutrition

The collection of evidence in nutritional genomics is based on two separate approaches. Firstly, the traditional hypothesis approach that specific nutrient influences the expression of certain genes and proteins through its effect at a particular point in the biochemical pathway following the accepted steps of DNA to mRNA and protein. Secondly, a thorough understanding of functioning of all the inter-related systems that either depends upon or is influenced by the particular nutrient. This approach is now discussed under the broad term of systems biology. This approach allows examination of the evidence starting from genes, proteins, and metabolites that together form the functional metabolic unit influenced by the specific nutrient. Various terms are being used for this- nutritional genetics, nutrigenetics and nutrigenomics. The latter is preferred by the majority as individual genomic signatures are the final determinants in the outcome of genotype-nutrient-metabolic inter-relationships. It is argued that nutrigenomics is by far the best model of genomic medicine as it satisfies all the criteria for holistic style of 'evidence-based' clinical medicine.

In conclusion, the 'evidence-based medicine', an established concept in the practice of modern clinical medicine is on the verge of rapid transformation due to exponential growth of scientific evidence following the completion of human and other genomes. The new genome-based technologies and bioinformatics tools offer tremendous power for revolutionising the diagnosis and therapy in a number of human diseases. The genome-based evidence, made accessible to clinicians and health professionals, is robust, accurate and individualized or narrowed down to the small patient population groups. The future of medicine and public/population health looks promising as new opportunities shall emerge from powerful genomic technologies and development of personalized nutritional, anti-microbial and pharmacological agents. The future clinicians and health professionals will need to be equipped with knowledge and skills in applying broad range of genomic-based diagnostic and therapeutic tools. The transition from the present day 'evidence-based' approach to 'genomic-based' approach is in process leading to Genomic Medicine.[3]

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Foetal Tissue Sampling For Prenatal Diagnosis

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INTRODUCTION:

According to the basic tenets of clinical practice, confirmation of any disease or disorder is possible only after study of target tissue or organ by laboratory tests like histology, biochemistry or genetic tests. The same is true for fetal diseases, hence fetal tissue sampling and laboratory studies is a core activity in clinical prenatal genetics.

With the advent of imaging modalities like high resolution ultrasound, colour doppler, 3-D ultrasonography and magnetic resonance imaging (MRI), our ability to visualize the fetus has increased tremendously. Genetic screening programs are also improving with the advances in DNA technology. In spite of these advances, direct access to foetal tissues is still needed in large number of disorders for confirmation of diagnosis, prognostication and fetal therapy.

To make the optimal utilization of fetal tissue sampling it is necessary to do a complete work-up, this should include,

- Careful history and physical examination covering family and individuals from a genetic point of view
- Genetic counseling, pre-marital and pre-conception counselling
- Use of genetic screening tests,
- Comprehensive and early antenatal care
- Anomaly scans at 11-14 & 18-20 weeks of pregnancy

When this ground work has been done, only then, the fetal tissue sampling can be undertaken. It is also very important to consider some important factors before going ahead, these include:

- Indication for the foetal sampling technique,
- Appropriate timing and method of the sampling,
- Competence of the clinician (as many of these procedures have a long learning curve), ability of the laboratory to give reliable diagnosis,
- Risk of recurrence of the disease, risk of the tissue sampling-both to the mother and the fetus, limitations of laboratory tests,
- Reliability of the sampling procedure, laboratory results,
- Detailed counseling, proper consent prior to undertaking the tests.

It is very vital to have a close co-operation between the obstetrician, imaging specialist and the geneticist. The post-procedure counseling should be also done as teamwork to avoid confusion & improve patient-compliance.

VARIOUS FETAL TISSUE SAMPLING TECHNIQUES:

- Non-invasive sampling viz. isolation of foetal cells from maternal blood,
- Pre-implantation genetic diagnosis (PGD) this includes polar body biopsy and blastomere /trophectoderm biopsy,
- First trimester tests like Chorion Villous Sampling (CVS), early Amniocentesis,
- Second trimester samplings like Amniocentesis, late CVS, Cordocentesis, foetal skin biopsy etc.

ISOLATION OF FOETAL CELLS FROM MATERNAL BLOOD:

The maternal blood contains foetal cells practically all throughout the pregnancy though they are more at 6-10 weeks of pregnancy. These can be picked up and studied for direct, non-invasive & safe prenatal diagnosis. This has an advantage over the genetic screening programs by its specificity, sensitivity. But there are many technical difficulties.

a) Very low density of foetal cells (1:140,000 i.e 1-2 foetal cells in 3ml of maternal blood), this increases two folds by second trimester. This can be overcome by sorting of fetal cells by Flow-cytometry (FACS), magnetic column sorting (MACS), Avidin-biotin columns, immuno-magnetic beads or by micromanipulation-pickup from enriched maternal blood.[1] But this is not as yet enough to give sufficient yield of foetal cells to give easy, reliable diagnosis by conventional techniques.

b) Residual cells from previous pregnancy can give false results, especially when trophoblasts are targeted. Even slightest contamination from laboratory person or external source can cause serious problems in diagnosis.

c) As the cell yield is low and difficult to culture, genetic diagnosis is done by multiplex PCR, multi-colour FISH; and these methods have their own limitations. Nucleated foetal RBCs are the most useful, reliable foetal cells among the different cell types. In an interesting observation Ganshirt-Ahlert et al and Simpson and Elias found that the number of fetal cells in the maternal sample increased in pregnancies with aneuploidy foetuses.[2,3]

With refinements of technology, most of these problems are being overcome and successful prenatal diagnosis has been done for chromosomal aneuploidies as well as single gene disorders like thalassemia, Duchenne muscular dystrophy, etc.

A new aspect of clinical research involves study of cell free DNA and RNA of fetal origin in the maternal blood. With this new method it is possible to find the Rh(D) status of the fetus as well as DNA diagnosis of single gene disorders.

PRE-IMPLANTATION GENETIC DIAGNOSIS (PGD):

Prenatal diagnosis done at oocyte stage or pre-implantation blastomere/blastocyst stage is the most exciting advance in the field of genetic diagnosis. It helps the artificial reproductive techniques (ART) by avoiding implantation of potentially defective embryos, thus improving the success rate as well as avoiding the need for medical termination of pregnancies with genetic disorders at a later date. To setup a PGD program, we must have a well conducted, busy in vitro fertilization (IVF) setup with high success rate, technical capability to do a safe, reliable embryo biopsy and a state of the art molecular genetic laboratory capable of reliable single cell diagnosis. It is very labor intensive investigation with very high costs.

The PGD is done by,

- Polar body biopsy: (only maternal genetic studies are possible),
- Blastomere biopsy: One or two blastomeres are biopsied at 4-8 cell stage
- Trophectoderm biopsy: this is done at the blastocyst stage, the sample size is more and hence prenatal diagnosis is much more reliable.

Well over 1000 births have been recorded after successful PGD till now, all over the world, for chromosomal aneuploidies as well as many single gene disorders. The long-term effects of PGD on the health of the progeny are yet to be understood as this method has been used only over the last decade.[4]

CHORION VILLUS SAMPLING (CVS):

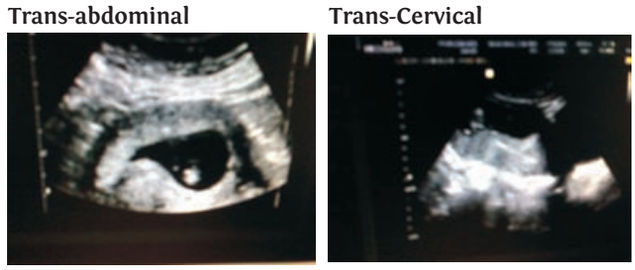
This sampling technique was first used in 1968. It has advantage of early, rapid and more private diagnosis with the option of safer first trimester termination. The indications include:

- Chromosomal aneuploidy
- Single gene disorders
- Advanced maternal age
- Multiple congenital anomalies
- Biochemical study.

CVS is done between 10-12 weeks of pregnancy though it can also be done in second trimester (up to 22-28 weeks) as an alternative to amniocentesis, with good results. Ultrasound guided trans-abdominal or trans-cervical routes can be used with comparable safety and efficacy. The techniques are illustrated in figure 1.

Fig 1. Techniques of chorionic villus sampling

Trans-abdominal **Trans-Cervical**



The success & safety of the procedure depends upon the experience of the operator. In our experience of over 10,000 cases, our success rate and pregnancy loss rates have been 98.8% and 2.1% respectively.[5] Main difficulties of the laboratory procedure include maternal cell contamination and placental confined mosaicism. After the publication of report by Firth et al in 1991, scare was created about limb reduction defects (LRD) following CVS. But after large population studies about the background incidence of LRD & WHO studies about the safety of CVS, it has been conclusively proved that there is no increase of LRD following CVS.[6] The WHO guidelines for safety of CVS include avoiding CVS before completion of 10 weeks of pregnancy, avoiding excessive manipulations and compression of uterus and only properly trained operators should do the procedure.

With the advent of first trimester screening by nuchal translucency and biochemical screening (PAPP-A, free Beta HCG), chorion villous sampling has come in to prominence once again.

AMNIOCENTESIS:

Amniocentesis was the first tissue sampling technique used for prenatal diagnosis in 1956 and is still used widely. Technically it is the easiest sampling technique though in very obese patients and in cases with oligohydramnios, it can be very difficult. It is performed between 15-22 weeks of gestation for culture, while it is done between 26-34 weeks for lung maturity or Rh-isoimmunisation cases. Early amniocentesis as an alternative to CVS was tried between 11-15 weeks but failed to show any advantages and had higher complications. Amniocentesis is done by inserting a lumbar puncture needle (gauge 20 or 22) in a fluid pocket under ultrasonographic guidence.

Indications for amniocentesis are, chromosomal studies on cultured/uncultured amniocytes, single gene disorders, biochemical studies for metabolic diseases, microbiological immunological studies in congenital infections, bilirubin studies in Rh-iso-immunisation and for fetal lung maturity evaluation. In our multi-centric study the success rate and the pregnancy loss rates were 99.5% and 0.8% respectively.[7] Clinical complications include amniotic fluid leakage, preterm labor, infection, feto-maternal transfusion, fetal injury, amniotic band syndrome and other fetal disruptive lesions. These can be kept to a minimum by uniform clinical technique protocols, experienced operators only to perform the procedure and full aseptic precautions. Many a times, the complications are more related to the underlying condition for which the procedure was done rather than the amniocentesis itself.

The other problems include acellular / bloody taps, failure of the cells to grow in culture and contamination of the samples

FOETAL BLOOD SAMPLING BY CORDOCENTESIS:

As foetal blood is the most ideal tissue for genetic studies, cordocentesis is an important technique, it also gives direct access to fetal circulation for diagnostic as well as therapeutic purposes. Initially cordocentesis was done through the fetoscope. After the advent of high resolution USG, it is being done by percutaneous direct needling. The indications for cordocentesis include,

- Genetic study : cytogenetic, molecular genetic testing.
- Congenital infections like Toxoplasmosis, CMV etc.
- Immune, non-immune hydrops
- Intrauterine growth retardation, twin-to-twin transfusion syndrome
- Foetal therapy like intra uterine transfusions, fetal medication, fetal surgery.

Placental end of the umbilical cord is the most common site, though umbilical end or free loop of cord can also be sampled. Intra-hepatic umbilical vein is another useful site with no chance of maternal contamination. In difficult cases with severe oligohydramnios direct tapping of the heart has been done without much problem. Cordocentesis has a long learning curve and should be done by experienced operators. In our multi-centre experience of over 1000 cases the success rate was 93% with pregnancy loss rate of 2.85%.[8]

CONCLUSIONS:

Foetal tissue sampling is a vital component of fetal medicine program. To make it safe, effective and acceptable to the patients, it is essential to have well organised multi-discipline programs with good cooperation between all specialities, have good training programs for clinicians to perform the samplings. It is also necessary to have good networking of local and distant genetic centres to share information, technology and patient material so as to make the optimum use of the high-tech, expensive facilities. That will also make these facilities available to masses so as to make meaningful contribution to the patient care.

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Wolcott Rallison syndrome: A novel mutation in a South Indian family

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SUMMARY:

We report on a child with Wolcott Rallison syndrome (WRS) and the novel mutation in the pancreatic eukaryotic initiation factor 2 (eIF2) kinase gene.

CASE REPORT

A mother with third degree consanguineous marriage was referred with an index girl child at age 10 months for evaluation of Neonatal Diabetes Mellitus (NDM) and pre-pregnancy counseling. On evaluating she had an uneventful antenatal period with no history of Gestational diabetes or pregnancy induced hypertension during pregnancy. The baby had an uneventful neonatal period until 3rd month when the baby was reported to have unprovoked seizures and was evaluated. The child had documented episode of prerenal azotemia and DKA during the admission. Investigation revealed increased blood glucose – 278 mg%. Initial HbA1c was noticed to be 23.2% (4.4 -6.7) with a normal lipase and amylase study and a low C peptide (0.3). Lipid profile was within normal range. With prerenal azotemia, diabetic ketoacidosis, seizures, and hyperglycemia the child was managed as NDM with insulin, glucose monitoring and seizure control. At 10 months the child was further evaluated. Physical growth and development were normal for her age (Fig 1). At presentation she was completely asymptomatic with no further episodes of seizures or other systemic manifestations. Bones were normal on radiographs, except for mild osteopenia (Fig 2). There were no epiphyseal changes present at the time of presentation. However a slight suspicion of a monogenic single gene disorder was entertained particularly in the context of consanguinity and to explore the possibility of prenatal testing. Mutation testing for neonatal diabetes was sought for. The initial screen for common mutations in PNDM such Kir6.2 KCNJ11 Exon 1, SUR1 ABCC8 Exons 1-38 and INS Exons 2-3 were negative. Subsequently EIF2AK3 was screened for WRS; sequence analysis showed a novel frame shift mutation (c.2304_2305delTG), a deletion of TG at nucleotides 2304_2305 in exon 13 of EIF2AK3 was identified in the proband. The child was diagnosed to have Wolcott Rallison Syndrome. This paved a way to plan monitoring in index child as well as offer prenatal diagnosis in subsequent pregnancy

DISCUSSION

Diagnosis of "early-onset" diabetes can occur within the first days or months of life usually with presentation of hyperglycemia. The time of presentation is variable and a potential diagnostic problem is the differentiation of a monogenic cause vs. autoimmune type 1 diabetes in these early-onset children. In the last decade, explosion of work in nonimmunological forms of diabetes, diagnosed most frequently within the first 6 months of life has produced insight into the multiple hereditary causes. Because the neonatal period is defined as the first 4 wk of life, whereas diagnosis of these cases extends through 6 months of age the term "congenital diabetes mellitus" and "neonatal diabetes mellitus" are used interchangeably. NDM is rare, variously quoted as one case per 300,000 to 500,000 live births.[1, 2] Permanent diabetes mellitus diagnosed within the first 6 months of life (permanent neonatal diabetes, PNDM) is a rare disorder likely to be monogenic rather than autoimmune. In PNDM, Wolcott-Rallison syndrome (WRS) is identified to be an autosomal-recessive disorder with less than 20 cases described in the world literature. It is characterized by the association of permanent neonatal or early-infancy insulin-dependent diabetes, multiple epiphyseal dysplasia and growth retardation, and other variable multisystemic clinical manifestations.[3,4] Skeletal abnormalities such as epiphyseal or metaphyseal dysplasia, and its association in virtually all patients with WRS is considered a cornerstone for diagnosing this rare clinical entity. Mental retardation, hepatic and kidney dysfunction, cardiac abnormalities, exocrine pancreatic dysfunction and neutropenia are the other presentations noted in WRS. The gene responsible for this disorder is EIF2AK3, the pancreatic eukaryotic initiation factor 2 (eIF2)kinase.[3,5] Environmental stress and modifier genes contribute a large extent to the clinical variability of WRS. Though the incidence of WRS has not been studied, in a regional study in South India, 6 cases of WRS among a series of 12 infants with IODM (infant onset Diabetes Mellitus) has been reported from January 2003 to December 2007. In that two children with WRS died.[6] Index child is being nutritionally supported and followed-up. Mother has been advised to bring the unaffected fetus for a postnatal follow up.

A diagnosis of WRS should be considered in patients presenting with insulin-dependent diabetes starting in early infancy, in case of an association with epiphyseal dysplasia and EIF2AK3 gene screened for mutations in these patients.

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Fig 1:Photograph of the index child

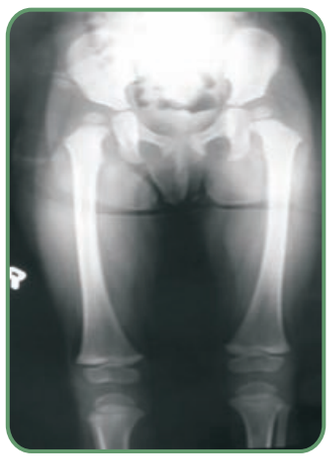

Fig 2:Radiograph showing normal femora

ACKNOWLEDGEMENTS:

Molcecular diagnostic laboratory, University of Exeter, UK for diagnostic support

Gimme Hope Ma !!

As I woke up in the mornin
I had a great yearnin
that as you lay in the sun baskin
One day I would feel the very warmth on my skin

Gimme hope Ma !
That also one day my hands would feed
the birds that were so busy outside chirpin

Gimme hope Ma !
that also one day my eyes and ears would be takin
all the beautiful sights and sounds that your wonderful world is makin

Gimme hope Ma
that also one day I would see your hands clappin
for the accolades that I would be rakin

Gimme hope Ma !
that also one day my lips would be kissin
your bosom when you would be close to me huggin

Yesterday as your Messenger came in
I hoped that it would be an apostle of joy
and not “otherwise” in the makin

Gimme hope Ma !
that tomorrow I would be proud to be havin
two of ‘those’ that you and pa are possessin

Gimme hope Ma !
Today as I lie within, my heart next to yours beatin
and it shan’t be forever I am knowin
and that tomorrow for me brings in
More than ‘just-a-useful’ life !!

Prenatal diagnosis results.....Parents' anxious wait.....Fetus also waits..... May be fetus thinks differently .....
When would the time come when the prenatal diagnosis would realize the fetal hopes and aspirations.....

(Dedicated to those sons and daughters who walked away to give their parents a better life)

Contributed by -

Parag M Tamhankar ‹*paragt_md@rediffmail.com*›

Contributed by:
Dr Shagun Aggarwal & Dr Prajnya R

EXPANDING LIST OF GENES FOR AN EXPANDING WAISTLINE: NEW LOCI FOR OBESITY[1]

Obesity is fast emerging as a global epidemic affecting not only the developed but also the developing countries. Apart from lifestyle factors, genes affecting body mass index (BMI) are being found to contribute significantly to the causation of obesity. FTO and MC4R genes were initially discovered to be linked to obesity. Based on their review of recent genome-wide association studies, Hofker and Wijmenga have compiled a list of 15 additional loci influencing BMI and hence contributing to obesity. Many of the proposed candidate genes linked to these loci have been shown to have a high expression in the central nervous system especially the hypothalamus, suggesting their role in controlling satiety responsiveness and energy expenditure. Identification of the causal genes at these loci would provide valuable insights into the molecular etiology of obesity and perhaps, may even pave the way for gene therapy for this growing health menace!

UNRAVELLING THE MYSTERIOUS TANGLES: PET SCAN FOR PREDICTING ALZHEIMER'S DISEASE[2]

Alzheimer's disease is one of the most common causes of dementia in the elderly. ApoE is one of the genes found to be involved in the causation of this disease. Studies have shown that carriers of the $\varepsilon 4$ allelle of this gene have an increased risk of developing the disease. Reiman et al have studied the use of PET scan with a radioligand binding to fibrillar Aβ (which has been found in neuropathology specimens of brains of Alzheimer's disease patients), for identifying individuals with the ApoE $\varepsilon 4$ allelle who are likely to develop the disease. They have proposed that this modality may be used as a predictor of Alzheimer's disease and would help in commencing preventive therapy in such individuals. However, larger and longitudinal studies may be needed to evaluate the clinical application of this technique.

BETTER SAFE THAN SORRY: ANTIBIOTIC PROPHYLAXIS REDUCES RISK OF POST AMNIOCENTESIS FETAL LOSS[3]

The risk of fetal loss after second trimester amniocentesis has been variably reported to be 0.06 to 2.9%. Infection following the procedure may be one of the factors contributing to abortion, but very few studies have investigated this. Giorlandino et al conducted an open randomised control trial comparing the post- procedure outcome of a large cohort given prophylactic oral Azithromycin (500mg/day in the 3 days preceding amniocentesis) with that of the control group not given any prophylaxis. They have reported an abortion rate of 0.03% in those receiving antibiotics as compared to 0.28% in controls. Also, only 0.06% of women in the prophylaxis group were found to develop preterm premature rupture of membranes as compared to 1.12% in the control group. Thus, this simple measure of pre-procedure antibiotic prophylaxis may go a long way in significantly reducing the risk figures for post – amniocentesis fetal loss.

NOW SHORT RIB POLYDACTYLY IS A CILIOPATHY TOO![4]

The list of ciliopathies (disorders of primary cilia) is becoming longer. Bardet-Biedl syndrome, Meckel Gruber syndrome, Joubert syndrome, autosomal recessive and autosomal dominant polycystic kidney disease, Alstrom syndrome and nephronophthisis are some of the conditions that have already been discovered to be ciliopathies. The latest addition to this growing group is the short rib polydactyly type III syndrome. Short rib polydactyly syndromes are autosomal recessive osteochondrodysplasias presenting with short long bones, short & narrow thorax and polydactyly. Merrill et al have identified mutations in the DYNC2H1 gene in three families of SRP type III. DYNC2H1 encodes a protein responsible for retrograde transport in cilia. The authors have shown through functional studies on chondrocytes that mutation of this gene leads to abnormal morphology and functioning of cilia. Cilia are thought to mediate matrix signals for chondrocyte differentiation and hence their disruption leads to impaired osteogenesis.

THROWING LIGHT ON DARKNESS: GENE DISCOVERED FOR FAMILIAL HYPERPIGMENTATION[5]

Familial progressive hyperpigmentation is a rare genetic disease presenting with hyperpigmented skin and mucosal patches at birth. With increasing age, there is progressive increase in pigmentation with extensive involvement of conjunctivae, face, neck, trunk, lips, oral mucosa, palms and soles. Wang et al have performed genome wide scan in a six generation Chinese family with this condition and using two point linkage, have identified mutations in a gene called KIT ligand (KITLG) in the affected individuals. Using functional studies, they have shown that this mutation results in gain of function and increases melanin production by melanocytes.

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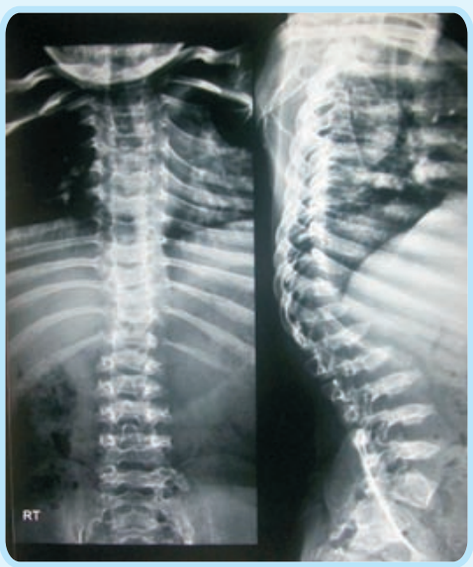
Contributed by: Dr Mohandas Nair, Calicut , mohandas_nair68@yahoo.com

These are the radiographs of spine of an 11- year- old- girl admitted with quadriplegia.

What is the diagnosis?



The response should be sent to geneticsiap@gmail.com

The names of responders with the correct diagnosis will be published in the next issue.

Answer to the PhotoQuiz 5 of the previous issue:

Restrictive Dermopathy (OMIM 257210)

Restrictive dermopathy is a lethal condition with a tense skin with congenital contractures. The face may be dysmorphic with a small pinched nose, hypertelorism, micrognathia and low set ears. Histologically the skin shows hyperkeratosis with dermal hypoplasia. Mutations in Lamin A and Zinc metalloproteinase STE24 have been shown to cause this condition.

Correct Response by:

Patil SJ, Bangalore

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