Chromosome rearrangements are a notable cause of embryonic lethality and birth defects. Identifying the genes that underlie the pathogenesis of chromosome deletion and duplication syndromes is a challenge because the affected chromosomal segment can contain several detrimental genes. The identification of these genes that are relevant to these disorders often requires the analysis of individuals that carry rare, small deletions (microdeletions), translocations or single gene mutations. An attempt has been made in the present study to optimize a methodology for answering the etiology of congenital heart diseases (CHDs), which encompasses DiGeorge Syndrome (DGS), Velo Cardio Facial Syndrome (VCFS), and Cono Truncal Anomaly Face Syndrome (CTAFS) with special emphasis on del22q11.

Congenital Heart Disease

A congenital defect of heart is the second most common malformation when the heart or blood vessels near the heart do not develop normally before birth. It has been reported that of all live births 15-30 children per 1000 have a major congenital malformation and congenital malformations of the heart are the second most common of all birth defects occurring in 5-8 per 1000 live births. Further, the rate of severe heart defects in spontaneously aborted pregnancies may be up to 10 fold higher.

Congenital heart defects, being a complex trait, are thought to result from a variety to genetic and/or environmental factors acting independently, additively or synergistically. Risk factors contributing to CHDs include abnormal levels of retinoic acid, infections such as rubella, smoking and consumption of alcohol and intake of certain drugs like acutane (acne medication), lithium (used to treat certain forms of mental illness), and possibly certain anti-seizure medications during pregnancy. These factors either act directly on the embryo or alter the placental function or molecular dynamics of the cell.

The 22q11.2 deletion has been identified in most patients with the DGS, VCFS and CTAFS or Takao Syndrome. The list of findings associated with the 22q11.2 deletion is extensive and varies from patient to patient.

The DGS was first described in 1965 in an infant who showed congenital absence of the thymus and the parathyroid along with abnormal levels of serum calcium and impaired cellular immunit. It was suggested that all infants with congenital hypoparathyroidism should
be studied for defects in cellular immunity. In 1976, a group of scientists reported certain characteristics of patients having cardiac abnormalities such as peculiar faces including hypertelorism, short palpebral fissures, broad root of the nose, abnormal ears and nasal voice with or without velopharyngeal deficiency.

This syndrome was called as Cono Truncal Anomaly Face Syndrome (CTAFS) or Takao Syndrome. Shprintzen reported a similar syndrome but added cleft palate and learning disabilities and named it as Velo Cardio Facial Syndrome (VCFS). Clinically, although the phenotype is highly variable, these syndromes are typically characterized by aplasia or hypoplasia of the parathyroid glands, conotruncal cardiac defects and mildly dysmorphic facial features. All these clinical features can either be seen independently or in conjunction with each other. The most common cardiac defects in DGS include truncus arteriosus, interrupted aortic arch and tetralogy of fallot. Common cardiac defects in VCFS include ventricular septal defects, tetralogy of fallot and right aortic arch defects. These cardiac defects can be isolated or seen in conjunction with each other, which decides the severity of the disease.

In the early 1990s, it was found cytogenetically that one and the same chromosome aberration, 22q11 deletion, caused all the above three syndromes. These were found to be different manifestations of the same chromosome deletion. In 1993, a group of British researchers proposed an entirely new name for the syndrome, CATCH 22, an acronym in which C stands for cardiac anomaly, A for anomalous face (characteristic appearance), T for thymic hypoplasia (underdeveloped thymus gland), C for cleft palate, H for hypocalcaemia (low blood calcium levels) and 22 for chromosome number 22 which is now being commonly referred to as Catch 22.

Clinical features of this included hypocalcaemia, craniofacial anomalies (cleft palate, external ear anomalies, abnormally small jaw, wide spaced eyes, broad nasal root), psychiatric disorders, behavioural defects and cardiovascular defects. The cardiovascular defects included tetralogy of fallot (TOF), interrupted aortic arch (IAA), Truncus Arteriosus (TA), ventricular septal defect (VSD), right aortic arch (RAA), overriding aorta, transposition of great arteries (TGA), coarctation of aorta (COA) and aortic valve stenosis. Delay in growth, minor skeletal and renal defects were other symptoms included in the syndrome.

Thus, the symptoms of del22q11 syndrome are many and diverse with all symptoms may or may not be present in an affected individual and, if present, symptoms can occur with varying degrees of severity. Therefore, distinct clinical features of del22q11 syndrome can show variable expressivity and incomplete penetrance.

Cytogenetic Review of CHDs

Cytogenetically del22q11 syndrome as described earlier is characterized by a microdeletion on chromosome 22q11.2. Affected individuals carry the deletion on only one of the chromosome 22, so it is presumed to be gene haploinsufficiency syndrome. In most cases, the deletion occurs de novo, but in about 10% of cases it is inherited from a mildly affected parent. A predominantly maternal genetic transmission has been observed. Cytogenetically the frequency of 22q11.2 deletions in this group of cardiac malformations varies from 0 to 65%. However, other abnormalities such as haploid deletions involving chromosome 10p and balanced translocation between chromosome 2 and 22 e.g. t(2;22), t(2;6) or t(11;22), t(15;22), t(8;12) have been reported which are rare. Involvement of chromosome 8, -15, -14, der(22), der(13), der(14), follow(22), +21, dup(22), +21, dup(4) has also been noted. The del22q11 genetic lesion is remarkably homogenous in affected individuals with only a handful of exceptions. Molecular biology studies revealed that approximately 90% of patients have a typically selected region of 3Mb, which encompasses an estimated 30 genes, whereas about 8% of patients have a smaller nested deletion of 1.5Mb, which encompasses 24 genes. Understanding the etiology of del22q11.2 syndrome has been confounded by several observations including the fact that the severity of the phenotype is not related to the size of the deletion. The deletion on chromosome 22 can be detected through classical cytogenetics. However, the standard karyotypic analysis is insensitive and even with high resolution banding (HRB) techniques only 10 – 20% of 22q11 deletion can be detected. This low frequency of detection of del22q11 is linked to certain inherent limitations of cytogenetics.

Limitations of Cytogenetic Techniques in Detection of Microdeletion of 22q11

The technique of karyotyping requires relatively larger volume of blood. In order to avoid failures due to probable contamination, cells are needed to
be cultured in duplicates or triplicates. Sufficient numbers of metaphases are required for analysis. Sometimes due to failure of culture, very few or no metaphases are obtained. Chromosomes of poor morphology may result in poor or no banding at all leading to incorrect analysis. The final analysis gets affected due to poor spread of chromosomes or poor banding resolution. The karyotyping procedure on metaphase spreads can give specific information on chromosome number and structural chromosomal changes, but analysis is restricted to a limited number of chromosome spreads. Finally, due to insensitivity of the technique to detect smaller deletions, a proportion of patients with genetic alterations in 22q11 may be missed. In spite of these shortcomings, cytogenetics is recommended for testing, as it will detect other chromosomal aberrations, which are likely to be present.

With the advent of sensitive and specific technique of fluorescence in situ hybridization (FISH) recently, the diagnosis of microdeletion has become simpler, easier and more accurate which has resulted in significant increase in diagnosis of patients. Currently, FISH is considered as the method of choice for detection of microdeletions. FISH technique helps in analyzing relatively large number of cells (metaphase as well as interphase) and specific chromosomal changes such as microdeletion. The important aspect of FISH technique is the application of different probes designed to detect genetic changes in a cell. Labeled probes hybridized to target DNA are detected by immunocytochemical reaction using fluorescence microscopy.

Advantages of FISH over Conventional Cytogenetics

The volume of blood required is lesser as compared to classical cytogenetics. The long and time consuming process of blood cells culturing is not required as cells can be fixed on the slide after treating with hypotonic solution. Analysis can be done on non-dividing interphase cells and metaphases are not required. As compared to conventional cytogenetics, FISH is less time consuming requiring 2 days and the analysis and interpretation of results is easier. However, the FISH technique needs to be standardized optimally as it involved many pre-treatment steps on slides, which further determines the signal quality and quantity per cell. These pre-treatment steps should be critically optimized with appropriate time exposure by using proteases such as pepsin or proteinase K. An optimum time period is required for the probes to enter as cells are surrounded by excess amount of proteins. Longer pre-treatment carries a risk of losing cells on the slides whereas pretreatment for shorter time period will emit weak signals that will not be visible as the probe will not enter into the cell due to presence of excess amount of proteins around the cells. Washing procedure following hybridization is another critical step as higher temperature of the washing solutions or vigorous shaking of the coupling jars containing slides will result in the loss of bound probe thus ultimately reducing the signal quality and quantity.

Indian Scenario

Isolated and regional statistics with regards to the incidence of the 22q11 deletion syndrome in Indian scenario is available. However, these studies are based on clinical data. According to the international medical literature, it has been estimated that approximately one of 4000 children is born with a 22q11 deletion. The use of FISH technology in the diagnosis of the syndrome is still relatively recent in India; therefore a large number of cases are likely to be remained undiagnosed.

It is concluded that to achieve Millennium Development Goals no later than 2015, there is a need to identify approaches and means to translate knowledge into effective intervention. This means better utilization of existing tools, development of new tools for diagnosis, treatment and prevention of birth defects.

The ICMR Genetic Research Centre has been screening families with congenital heart disease for chromosome 22 microdeletion. Of the 105 patients screened 6 had microdeletion. This tool was used for prenatal diagnosis of this defect successfully. Preconceptional administration of 4 mg folic acid will further reduce the recurrence of congenital heart disease thus reducing the burden of this disorder in families, society and the nation.

It can be added that the completion of the human genome project has provided a wealth of information on molecular basis of genetic disorders unraveling the mystery of etiological factor in congenital heart disease.
References


This write-up has been contributed by Mr. Harshvardhan Gowde, Research Assistant and Dr. Z.M. Patel, Dy. Director, Genetic Research Centre, Mumbai.
Dermatological manifestations in HIV infection and its correlation with CD4 Count.

This prospective, controlled study was conducted on 781 confirmed HIV infected individuals in the age group of 19 to 54 years (average age 32.2 years) along with 149 asymptomatic HIV positive individuals (Control group) to find out the incidence of various types of mucocutaneous lesions in HIV positive patients in the presence of HIV infection alone, in the presence of coexisting opportunistic infections, other sexually transmitted diseases and correlate their CD4 and CD8 counts.

The clinical profile of patients revealed that the cutaneous manifestations e.g. face involvement in molluscum contagiosum by themselves may be a pointer to the diagnosis. The pattern and severity of face involvement points to the degree of immunosuppression as was corroborated by the CD4 counts. The difference in CD4 cell count in herpes simplex infection, oral candidiasis, dermatophytosis, scabies, xerosis and popular urticaria as compared to the asymptomatic population was highly significant. A statistically significant difference was not seen in bacterial infections, candidial balanitis, pityriasis versicolor, drug reactions, half nail and cases of hyperpigmentation.

The study concludes that certain dermatological disorders are very important markers for the diagnosis of HIV infection. Same will define dermatoses and correlate positively with advancing HIV disease and falling CD4 counts.

Assessment of AgNOR technique as tumour marker in cervical carcinogenesis

The study aimed at investigating the diagnostic importance of AgNOR counts in the cervical smears in the process of cervical carcinogenesis and also discriminating the different grades of squamous infra epithelial lesions of cervix (SIL). Silver nitrate staining for AgNOR counts was performed in 50 cervical smears of cytologically diagnosed normal, inflammatory, LSIL (mild dysplasia), HSIL (moderate and severe dysplasia) and squamous cell carcinoma. These women were derived from the ongoing routine out patient cytological screening in progress at Queen Mary's Hospital of the University. A progressive rise in AgNOR counts was noticed when the severity of the pathological lesions of cervix increased. The study pointed out great diagnostic values of AgNOR counts in discriminating the LSIL and HSIL cases of cervix particularly the borderline cases.

The potentiality of AgNOR counts as tumour marker in cervical carcinogenesis was tested by follow up of 52 cases of mild and 10 cases of moderate dysplasia to observe biological behaviour of dysplasia with initial low and high AgNOR counts. The follow up study revealed that dysplasia cases with low AgNOR counts mostly regressed to normal while those with high counts persisted or progressed to a higher grade. There was a definite correlation between higher AgNOR counts and persistence or progression of dysplasia cases, however, the number of cases followed has been not very large to reach a definite conclusion regarding the capability of the AgNOR counts as tumour marker in cervical carcinogenesis.

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The following meetings of various technical committees/groups of the Council were held:

Meeting of Scientific Advisory Group (SAG)

SAG of the Division of Non-Communicable Diseases
August 7-8, 2007

Meetings of Project Review Committees (PRCs)/Task Forces (TFs)/Project Review Groups (PRGs)/Expert Groups (EGs) held at New Delhi

PRC on Neurology
June 13, 2007

PRC on Oncology
June 18, 2007

PRC on ICMR-INSERM Project in the Field of Neurosciences
June 25, 2007

PRC on Cellular and Molecular Biology
July 2, 2007

PRC on Oral Health
July 9, 2007

PRC on Experimental Medicine and Anaesthesiology
July 19, 2007

EG on TF Project on Suicide Behaviour
July 20, 2007

PRC on Otorhinolaryngology
July 24, 2007

TF on Urban Mental Health Problems and their Service Needs
August 10, 2007

TF on Urban Mental Health Needs Assessments and Service Delivery Models in Tsunami affected Population of Coastal Tamil Nadu
August 10, 2007

EG for Setting a Centre for Advanced Research on Pancreatic Diseases
August 20, 2007

TF on Global Environmental Change and Respiratory Diseases
August 22, 2007

PRC on Gastroenterology
August 23, 2007

Participation of ICMR Scientists in Scientific Events:

Dr. C.P. Puri, Director, National Institute for Research in Reproductive Health (NIIRRH), Mumbai, visited National Primate Centre at Singapore (June 3-6, 2007).

Dr. K.D. Ramaiah, Deputy Director, Vector Control Research Centre (VCRC), Pondicherry, participated in the VIII meeting of WHO Implementation Research Steering Committee at Geneva (June 4-7, 2007).

Dr. Mausumi Bharadwaj, Assistant Director, Institute of Cytology and Preventive Oncology (ICPO), NOIDA, participated in the WHO Workshop on Practical Course on HPV Genotyping and HPV 16/18 Serology at Lousiane (June 4-8, 2007).

Dr. A.C. Mishra, Director, National Institute of Virology (NIV), Pune, participated in a discussion for Requirement of Automated Sample Management and Retrieval System for Biorepository Applications at CA San Frascisco (June 7-13, 2007).

Dr. A.C. Mishra along with Dr. V.A. Arankale, Deputy Director (Sr. Grade), Dr. M.S. Chadha, Deputy Director, Dr. Sarah S. Cherian, Assistant Director and Dr. Konika Ray, Senior Research Officer, NIV, Pune, participated in the International Conference on Options for the Control of Influenza VI at Toronto (June 17-23, 2007).

Dr. T. Hussain, Assistant Director, National JALMA Institute for Leprosy and Other Mycobacterial Diseases (NJIL&OMD), Agra, participated in the International Symposium on Genetic and Immune Correlates of HIV Infection and Vaccine Induced Immunity at Budapest (June 10-13, 2007).

Dr. P.R. Narayanan, Director, Tuberculosis Research Centre (TRC), Chennai, participated in the meeting of the WHO Strategic and Technical Advisory Group on Tuberculosis at Geneva (June 11-13, 2007).

Dr. N. Arunachalam, Deputy Director (Sr. Grade), Centre for Research in Medical Entomology (CRME), Madurai, participated in the I Community of Practice Workshop for Research Teams Participating in the WHO/ TDR Research Initiative on Eco-Biosocial Research on Dengue in Asia at Bangkok (June 11-15, 2007).
Dr. Geetanjali Sachdeva, Senior Research Officer, NIRRH, Mumbai, participated in X Annual Frontiers in Reproduction Symposium at Massachusetts (June 13-17, 2007).

Dr. N.S. Wairagkar, Deputy Director, NIV, Pune, participated in the Asia-Pacific Dengue Prevention Board Meeting at Colombo (June 21-23, 2007).

Dr. J.M. Deshpande, Director, Enterovirus Research Centre, Mumbai, participated in XIII Informal Consultation on the Global Polio Laboratory Network and Ad-hoc Working Group at Geneva (June 27-29, 2007).

Dr. B.K. Tyagi, Deputy Director (Sr. Grade), CRME, Madurai, participated in a meeting to discuss future plans of collaboration between CRME, Madurai and the Department of International Health, Immunology and Microbiology, University of Copenhagen on Molecular Assay of Insecticide Resistance Development in Major Vectors of Malaria in India and Urban Malaria at Copenhagen (June 28 – July 3, 2007).

Dr. A.C. Mishra, Director, NIV, Pune, participated in the I meeting of Regional Advisory Group on Dengue at Phuket (July 2-3, 2007). He also participated in the Briefing of Delegates to the Interdisciplinary Working Group on Avian Influenza at Singapore (July 31 – August 4, 2007).

Dr. K. Ghosh, Director, Institute of Immunohaematology, Mumbai, participated in the XXI Congress of the International Society of Thrombosis and Haemostasis at Geneva (July 6-12, 2007).

Dr. N. Balkrishna, Senior Research officer, National Institute of Nutrition (NIN), Hyderabad, participated in the X European Nutrition Conference at Paris (July 10-13, 2007).

Dr. Soumya Swaminathan, Deputy Director (Sr. Grade), TRC, Chennai, participated in the Steering Committee Meeting on Nutrition and HIV/AIDS at Vienna (July 11-13, 2007). She also participated in IV IAS Conference on HIV Pathogenesis and Prevention at Sydney (July 22-25, 2007).

Dr. N. Selvakumar, Deputy Director (Sr. Grade), TRC, Chennai, participated in the meeting on Policy Guidance on Drug Susceptibility Testing of Second Line Drugs at Geneva (July 16-17, 2007).

Dr. Ashwini Shete, Research Officer, National AIDS Research Institute (NARI), Pune, participated in the GCLP Implementation Meeting at Johannesburg (July 18-19, 2007).

Dr. S.K. Niyogi, Deputy Director (Sr. Grade), National Institute of Cholera and Enteric Diseases (NICED), Kolkata, participated in the Discussion Meeting and Testing of Certain Laboratory Samples as part of the Haemophilus influenzae Type B Pneumonia and Meningitis Surveillance Study at Dhaka (August 5-11, 2007).

Dr. Anil Prakash, Deputy Director, Regional Medical Research Centre, Dibrugarh, participated in the Workshop on Advanced Techniques in Anopheles Culture at Atlanta (August 6-10, 2007).

Prof. A.P. Dash, Director, National Institute of Malaria Research, Delhi, visited Vector Control Research Unit at Universiti Sains, Malaysia (August 13-17, 2007).

Prof. Arvind Pandey, Director, National Institute of Medical Statistics, New Delhi, Dr. Soumya Swaminathan, Deputy Director (Sr. Grade),TRC, Chennai; and Dr. Kamalesh Sarkar, Asstt. Director NICED, Kolkata, participated in the VIII International Congress on AIDS in Asia and Pacific at Colombo (August 19-23, 2007).

Dr. P.R. Narayanan, Director, and Dr. V.D. Ramanathan, Deputy Director (Sr. Grade), TRC, Chennai, and Dr. R.S. Paranjape, Director; Dr. Seema Sahay, Assistant Director; and Dr. M.R. Thakkar, Senior Research Officer, NARI, Pune, participated in the AIDS Vaccine 2007 Conference at Seattle (August 20-23, 2007).

Dr. C. Dayaraj, Assistant Director, NIV, Pune, participated in the XIII International Congress of Immunology at Rio de Janeiro (August 21-25, 2007).

Dr. Ashwini Kumar Mishra, Research Officer, Institute of Pathology, New Delhi, participated in the LV Session of the International Statistical Institute Conference at Lisbon (August 22-29, 2007).

Appointment

Dr. G.B. Nair took over as Director of the Council’s National Institute of Cholera and Enteric Diseases, Kolkata w.e.f. August 23, 2007.
Trainings/Fellowships/Associateships

Dr. H.G. Sadhu, Deputy Director, National Institute of Occupational Health, Ahmedabad, availed WHO Fellowship for Introductory Course on Field Epidemiology at Bangkok (June 4-29, 2007).

Dr. A.H. Bandivedekar, Assistant Director, NIRRH, Mumbai, availed Training on Rhesus Monkey Semen Cryobank at California National Primate Research Centre (July 1-14, 2007).

Mr. K. Rajendran, Research officer, NICED, Kolkata, availed WHO fellowship for Training on Epidemiology and Biostatistics at Khon Kaen University, Thailand (July 2- August 10, 2007).

Dr. G. Bhanuprakash Reddy, NIN, Hyderabad, availed Short-Term DBT Overseas Associateship 2006-07 at University of Michigan (July 2 – September 30, 2007).

Dr. R. Harikumar, Senior Research Officer, NIN, Hyderabad, availed Training on Bioethics and Ethics Committee Administration at Seattle (July 9 –December 14, 2007).

Dr. Mamta Chawla Sarkar, Senior Research officer, NICED, Kolkata, availed Training on Collaborative Research on Rotavirus at Sapporo (July 16 – August 16, 2007).

Dr. Sandipan Ganguly, Senior Research Officer, NICED, Kolkata, proceeded to avail counterpart Training in Japan under JICA-NICED project entitled Prevention of Emerging Diarrhoeal Diseases –Phase II, for a period of 6 months (w.e.f. July 2007).

Dr. Avninder Pal Singh, Research Officer, IOP, New Delhi, availed UICC International Cancer Technology Transfer Fellowship at the National Cancer Institute Bethesda (August 20 –September 20, 2007).

Dr. Geeta Ramachandran, Senior Research Officer, TRC, Chennai, availed Training under Fogarty AIDS International Training and Research Programme for 4 months and 25 days at Tufts University, Boston (w.e.f. August 27, 2007).