PROCEEDINGS OF THE 3rd NATIONAL RESEARCH WORKSHOP

FETAL ORIGIN OF CHILDHOOD AND ADULT DISEASES: GENETIC, EPIGENETIC AND ENVIRONMENTAL PERSPECTIVES

SDUAHER

24th & 25th January 2012

SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION AND RESEARCH

A DEEMED TO BE UNIVERSITY

TAMAKA, KOLAR-563101.

Sri Devaraj Urs Academy of Higher Education & Research, Kolar 563 101
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THIRD NATIONAL RESEARCH WORKSHOP

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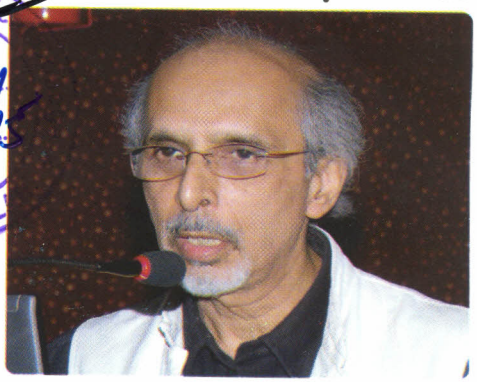
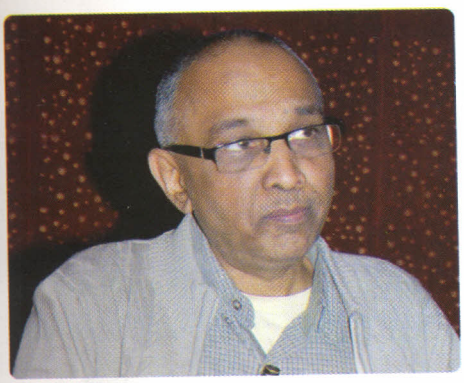


Prof. C S Yagnik



Prof. Sudhakar M Rao

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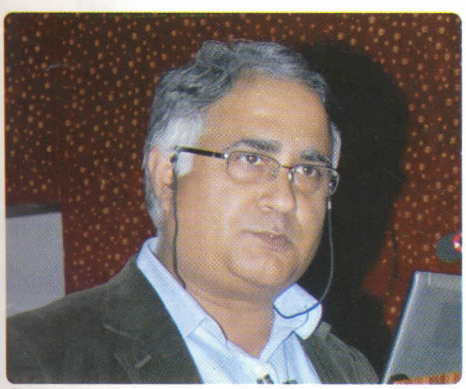

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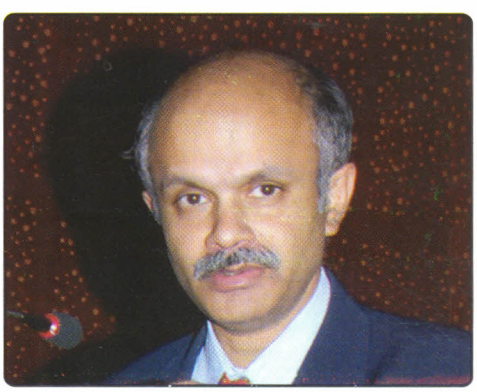

Dr.Arvind Shenoi

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Dr. Nalini Menon

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Dr. Kalyani R

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Sri Devaraj Urs Academy of Higher Education and Research
Third National Research Workshop

Sri Devaraj Urs Medical College is an ISO certified and NAAC accredited, 25 Years old Medical College situated in the semi rural environs of Kolar, Karnataka. In 2007 it was granted a Deemed to be University status by the Ministry of Human Resources Development, Government of India, under the name Sri Devaraj Urs Academy of Higher Education and Research.

The thrust areas of The Academy are imparting excellent medical education, giving quality patient care and encouraging and engaging in research, especially in clinical and basic medical sciences as well as community/environment related topics.

The academy conducts several departmental research project and also collaboration with centers of excellence like CCMB Hyderabad; Indian Institute of Science, Bangalore; St. John's Medical College, Bangalore; Public Health Foundation of India, Hyderabad and Shankar Nethralaya, Chennai apart from international collaboration with University of Minnesota, USA.

The 3rd National Research Workshop of SDUAHER was held on 24th and 25th January 2012. The theme of the workshop was **"Fetal origin of Childhood and Adult Diseases: Genetic, Epigenetic and Environmental Perspectives"**. A total of 270 delegates (120 faculty, 110 PG students, 30 interns and 10 external delegates) attended the workshop. Several undergraduate students also availed the opportunity of attending many of the talks.

In keeping with the theme of the workshop, on 24th January 2012 the inaugural lamp was lit by a 7 year old girl, a patient of Fanconi's anaemia, in the presence of Shri. R.L. Jalappa, President of SDUAHER, the Vice chancellor Dr. S. Chandrashekar Shetty and the invited speakers. The thought provoking and motivating talks were spread over two days.

On 25th January 2012, Dr. Eric Hoffman, the discoverer of the gene causing Duchenne Muscular dystrophy, conducted a 'Grand Rounds' in the Pediatric Dept. of SDUMC. Later, he delivered a lecture " Genomic Medicine, Muscular Dystrophy and Toxicogenomics"

This brief 'Proceedings' of the workshop has been compiled to serve as a reference of the talks by eminent faculty, as well as to record the scientific activity of the Academy.

GENOMIC MEDICINE, MUSCULAR DYSTROPHY AND TOXICOGENOMICS

Dr Eric Hoffmann

I have worked before in India at Chennai and Coimbatore regarding some public health programmes for children suffering from Duchenne Muscular Dystrophy. I hope to build on that experience gained from that programme, which was quite successful to do some work with people in the Kolar region. I will be talking today about Duchenne muscular dystrophy, particularly about two drug development programmes that have the potential to change the life of these patients; they can serve as a model for other diseases. And then I will speak about Genomics and public health looking at the toxicogenomics of PCB exposure, a study which was done in Slovakia; the genomics, intellectual and developmental delay and autism and then about the Kolar toxicogenomics project that we are currently working on.

The first part of my presentation will be on Duchenne Muscular Dystrophy (DMD) which must be quite familiar to the clinicians gathered here. It is the most common single-gene disorder in all world populations. Very briefly, it is X-linked recessive so its predominantly males who are affected; they are born normal but then begin to show progressive weakness of the proximal muscles around the age of 5 years; and then the muscles gradually deteriorate ending up with inability to carry out actions of daily living with an early death. About the history of Duchenne dystrophy, it was in the 1800s that it was identified as a clinical entity. In the 1950s it served as a model for population genetics; in the 1970s there was a disease-focussed research by the Muscular Dystrophy Association. In the 1990s, the gene for the disease was identified, cloned and the protein was identified. I was fortunate to be a part of the group that identified the gene. And in the last two decades till 2010, the focus has been on therapeutics development. It was initially thought that identification of the protein and the gene could magically give rise to molecular therapies, stem cell therapies etc. This was actually because everyone had an overoptimistic approach as to the therapeutics of this disease; there are now 70 trials on Duchenne dystrophy and 23 clinical trials currently recruiting in DMD.

The early best bets were stem cell therapy, gene therapy; early in-vitro cellular and in-vivo murine proof-of-principle studies that looked promising. But since then, it has only been 25 years of challenging barriers to clinical applications. What was less-expected was other approaches that have been started subsequently; a kind of personalized medicine where the idea was to target specific mutations; and a detailed knowledge of what exactly happens at the muscle level-trying to understand the molecular pathology.

The first drug programme is about a small molecule drug that is used to skip over mutations/repair mutations. This is called as exon skipping and this approach required us to precisely define the exon/intron boundaries and also what are the precise mutations that caused Duchenne. With that knowledge, we could design drugs that could overcome the mutations

Dr. Eric P Hoffman, Ph.D is the Director, Research Centre for Genetic Medicine at the Children's National Medical Centre, Washington D C and Professor at George Washington University, USA

themselves.

The second drug programme is that which will help the drug to remodel the muscle; here, the protein that is missing is absent from fetal life in these patients. But it takes 5 years to show symptoms and another decade to worsen. So we need to know why it is progressive and why muscle loses the ability to remodel. With an understanding of that process, we can help muscle to remodel and help it to deal with dystrophin deficiency.

First we need to understand why a muscle cell needs dystrophin. This dystrophin gene is the largest gene in the human body. It has 79 exons; while all other genes are around 30,000bp, this dystrophin gene is around 2 ½ million bp long. The dystrophin protein is a large cytoskeletal protein that attaches to the plasma membrane (alongwith other proteins) and stabilizes the plasma membrane. Muscle are usually involved in physical activities and hence they need to have great strength and with this comes the requirement of them having very stable plasma membranes. So dystrophin serves as a reinforcement. This is in normal muscle. In a patient with a deletion of a single exon, the gene is unable to put together a continuous reading frame for the normal protein and hence it produces a truncated protein with loss of function. There is a frameshift, a stop codon and termination of protein formation. Hence, no dystrophin implies no stability. What we do in exon skipping is that we introduce the anti-sense drug that is designed to go into the bloodstream, into the muscle, into the nucleus and attach to the exon next to the deleted one. What then happens is that, the mRNA splices the exon previous to and one exon away from the deletion. This restores the reading frame and makes a semi-functional dystrophin that can still, if not very effectively, stabilize the muscle plasma membrane. Hence, this approach is called as exon skipping as it takes out an additional exon above the mutation.

Though there have been studies on anti-sense drugs for 20years with more than 90 clinical trials and more than 2000 patients recruited, only one anti-sense drug has been approved so far. And this too is no longer marketed now. The barriers are providing sufficient intracellular drug for efficacy and the problem of innate immunity acting against these. But in Duchenne, there is a 100-fold increase in target efficacy of anti-sense drugs. One is because drug entry to cells is facilitated by overt breaches in myofiber cell membrane. The second is because, unlike other approaches where the goal was to knock-down 90% of mRNA transcripts, here, the goal is to rescue atleast 10% of the mRNA transcripts which is far easier than knocking down. So drug entry increases efficacy by about 10-fold and mRNA rescue increases by another 10-fold both of which give approx imately 100-fold increase in target efficacy.

We conducted a proof-of-principle study in a dog model to check this. DMD in dogs is similar, but more rapid leading to death by 6 months. The mutation also was challenging because we had to deliver 2 drugs for 2 different mutations leading to skipping of 2 exons. These were tested with drugs prepared according to the morpholino chemistry. So we used drug in a very high dosage, 40-60mg/kg weekly doses with a very high cumulative dose. After treatment, the histology showed much improvement towards normal muscle histology. Hence, it was possible to bring back dystrophin systemically through these injections. This was tested by a 15m

running test before and after morpholino injection. So the drugs with the older chemistry had been through more clinical trials but they were limited because of their toxicity to a dosage of 6mg/kg whereas the newer morpholino chemistry had fewer clinical trials but could attain higher levels in vivo.

In the 2nd type of drug development program, we use glucocorticoids. While with most drugs we want to attain a sustained serum dose, for the glucocorticoids, it is very short-lived because of rapid metabolism. So daily transient dosing is needed. Novel glucocorticoid analogs are used like VBP15. The studies done on mice showed several effects in comparison to prednisolone.

Now, the second part of the talk about toxicogenomics. We studied Polychlorinated biphenyls (PCB) exposure in Slovakia. The exposure biology program had environmental exposures, psychosocial stress, nutrition/ physical activity and indicators of biological response. We collected biological samples, phenotype and epidemiological data in Slovakia; identified key PCB congeners in samples and used normal college student volunteers. There were 2 subsets of children those with high PCB concentration and those with low PCB concentration. The types found were PCB 138 and PCB 153. Endocrine disruption was also seen. Genomic signatures show significant similarities in cell and tissue response. We need to extend epidemiological studies on PCB exposure.

Regarding, Genomics, intellectual disability and autism; intellectual disability is a limitation in functioning and adaptive behavior, begins at less than 18 years and affects 1.5 to 2% of population. Most are persons identified early in childhood due to developmental delays like motor, cognitive and speech problems. Autism spectrum disorders affect 1 in 100 to 1 in 150 children. Many children with autism spectrum disorders also have intellectual disability. There have been genome-wide discovery of chromosomal copy-number changes and single nucleotide changes. Technological advances like Array-CGH have also helped. Exome sequencing also helps in identification of de novo mutations.

Lastly, to talk about our proposed project in Kolar, we intend to work on the environmental factors and epidemiology of dysmorphia and developmental disabilities in Kolar district with Dr Ranganath, Dr Krishnappa and Dr Krishnaswamy. There is a high incidence of unusual dysmorphic conditions like cardiac, congenital malformations and rare developmental disorders. Gold mining in the region can lead to toxic environmental by products; also, the people of Kolar mainly use untreated groundwater for drinking. A collaborative effort has been planned between the biomedical, environmental and chemical experts in India and genetic experts from Washington DC. Two workshops will be held in Kolar and initial submission of the project plan was done in March 2011; a resubmission with revisions will be done this year.

MATERNAL NUTRITION AND FETAL PROGRAMMING OF DIABETES AND CARDIO VASCULAR DISEASE

Prof CS Yagnik

Diabetes Mellitus is a growing epidemic in the world. Once considered to be prevalent in the higher socio economic class and developed countries, it is now seen to co exist with poverty and under nutrition and the young and the poor are increasingly affected. This is a huge economic burden for the affected persons and countries more so when they are developing countries.

Diabetic research has brought to light many mechanisms of development of disease and treatment. But the diabetic dogma continues to be prevention. Obesity, diet, physical inactivity and stress are important precipitating factors in people susceptible to the disease. Much work and strategies focus on these factors in the prevention and treatment of the disease. This brings up the question of genetic susceptibility and whether it is modifiable.

The earliest observation between gene and diabetes was made from the Dutch famine birth Cohort study that showed that children born to pregnant mothers of that time showed an increased risk of developing diabetes, obesity, cardiovascular diseases, microalbuminuria and other health problems. Famine in the early and mid part of pregnancy was associated with obesity while a famine at late part showed less obesity. People now in their 60s conceived during the great famine showed different molecular setting for a gene that influenced growth. It appears that limited food intake during pregnancy altered genetic material in the embryos in the early stages of development. These alterations are not changes in genetic codes but in the setting of code that switches on or off the gene. This is known as epigenetics and one of the main process is in the methylation of DNA. Researchers compared the degree of methylation of the IGF2 gene in those conceived during the famine with their siblings and found a decrease in methylation. This did not apply to those affected by famine during their later stages of pregnancy showing that epigenetic information is particularly vulnerable during early foetal life.

Maternal undernutrition results in foetal undernutrition. The foetus adapts to this by inadequate development of organs like in kidney where there is a decrease in nephron numbers, decrease zonation of liver, decrease in βcells in the pancreas, decrease in muscle and bone mass with increase in fat mass. All these changes thus leads to decreased insulin sensitivity, secretion, decreased IGF and insulin secretion, altered appetite programming leading to leptin resistance and increase in corstisol. All these predispose to diabetes which worsens with over nutrition after birth. India has the highest number of maternal malnutrition and also undernutrition in the under five year olds, thus increasing the incidence of diabetes . The concept of "Thin fat Indian" highlights the fact that Indians with comparable BMI to western

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population have more adiposity.

This growing realization that even a mild change in the intrauterine environment influences the baby's prospects not only in the perinatal period but over the entire length of its life is the central issue of the study of Developmental Origins of Health and Disease (DOHaD)). The process influencing long-term fetal outcomes is called fetal programming.

Researchers in India have focused their attention on maternal micronutrient nutrition and offspring body composition. Indian babies are "'thin-fat," part of this phenotype results from a maternal imbalance of vitamin B12 and folate nutrition. They happen to be co factors and co substrates in methylation. Maternal homocysteine concentrations predicted fetal growth restriction, and low maternal vitamin B12 and high folate predicted higher insulin resistance in the child, thus contributing to the concept of nutrient-mediated teratogenesis. Vitamin B12 deficiency was associated with gestational glucose intolerance and babies born to diabetic mothers had heightened risk of adiposity and glucose intolerance as early as 5 years of age, suggesting that a dual teratogenesis (due to simultaneous occurrence of micronutrient deficiencies and hyperglycemia) could make a substantial contribution to the escalating epidemic of diabetes in India. Subsequent studies revealed that the real shape of this relationship was U shaped, i.e., both low and high birth weight increased risk of type 2 diabetes. It is important to understand that the story is not about birth weight but about fetal programming, and that intergenerational prevention of type 2 diabetes (primordial prevention) will need to target maternal nutrition and metabolism.

GROUND WATER CONTAMINATION: IMPLICATIONS FOR HUMAN HEALTH

Prof. Sudhakar M. Rao

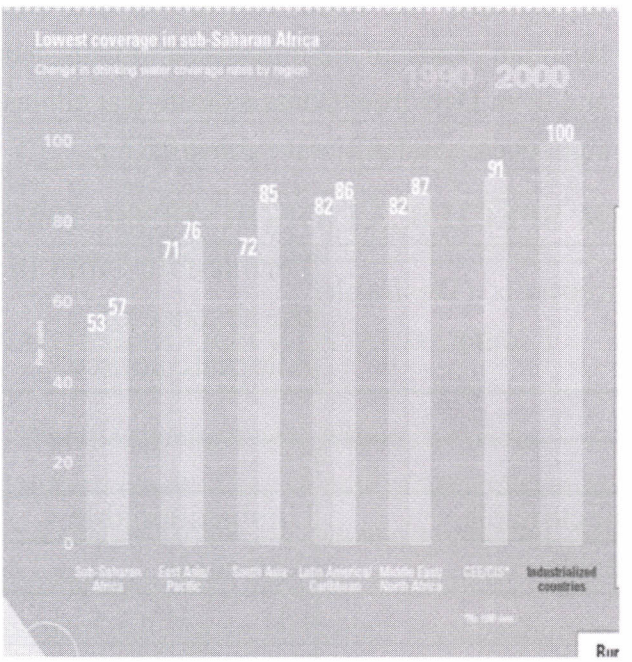
Problem Statement

Groundwater serves as a decentralized source of "safe drinking water" for millions of rural and urban people. It accounts for nearly 80 per cent of the rural domestic water needs, and 50 per cent of the urban water needs of the country. Utilization of drinking water is shifted from surface water to ground water because of industrial and environmental contamination. Hence from 1950 onwards government has taken measures to provide safe drinking ground water.

Water Quality Issues

Water quality issues can happen because of Natural sources like fluoride, arsenic, iron and anthropogenic Sources. Anthropogenic sources causes much more stress in a very wide range of parameters and to a large magnitude in water.

Access to safe drinking water is determined by percentage of population using improved sources. Improved sources include household connection, public standpipe, borehole, protected dug well, protected spring, rainwater collection. Not improved water source include unprotected well, unprotected spring, river, pond, vendor provided water, tanker truck water.



South Asia	
Maldives (-)	100
Bangladesh (94)	97
Pakistan (83)	90
Nepal (67)	88
Regional average (72)	85
India (68)	84
Sri Lanka (68)	77
Bhutan (-)	62
Afghanistan (-)	13

Prof. Sudhakar M Rao, Ph.D, Dept. of Civil Engineering, Indian Institute of Science, Bangalore has also been a visiting Professor at McGill University and Ryerson University, Canada, and Cardiff University, U.K

Chemical Standards

Arsenic

Inorganic arsenic is a documented human carcinogen . A relatively high incidence of skin and possibly other cancers that increase with dose and age has been observed in populations ingesting water containing high concentrations of arsenic. First observed in west Bengal.

Aluminum

Aluminum has appeared to be associated with the brain lesions characteristic of Alzheimer disease, and in several ecological epidemiological studies the incidence of Alzheimer disease has been associated with aluminum in drinking-water.

Chromium

In epidemiological studies, an association has been found between exposure to chromium(VI) by the inhalation route and lung cancer. IARC has classified chromium(VI) in Group 1 (human carcinogen) and chromium(III) in Group 3.

Hardness

Although a number of ecological and analytical epidemiological studies have shown a statistically significant inverse relationship between hardness of drinking-water and cardiovascular disease, the available data are inadequate to permit a conclusion that the association is causal.

Lead

Lead is toxic to both central and peripheral nervous systems, inducing subencephalopathic neurological and behavioural effects.

Selenium

In humans, the toxic effects of long-term selenium exposure are manifested in nails, hair and liver. Data from China indicate that clinical signs occur at a daily intake above 0.8 mg.

INDIAN STANDARD DRINKING WATER SPECIFICATION(BIS10500: 1991)

Sl.No	Substance or Characteristic	Requirement (Desirable Limit)	Permissible Limit in the absence of Alternate source
Essential characteristics			
1.	Colour, (Hazen units, Max)	5	25
2.	Odour	Unobjectonable	Unobjectionable
3.	Taste	Agreeable	Agreeable
4.	Turbidity (NTU, Max)	5	10
5.	pH Value	6.5 to 8.5	No Relaxsation
6.	Total Hardness (as $CaCo_3$) mg/lit.,Max	300	600
7.	Iron (as Fe) mg/lit,Max	0.3	1.0
8.	Chlorides (as Cl) mg/lit,Max.	250	1000
9.	Residual,free chlorine,mg/lit,Min	0.2	--

Desirable Characteristics			
10.	Dissolved solids mg/lit,Max	500	2000
11.	Calcium (as Ca) mg/lit,Max	75	200
12.	Copper (as Cu) mg/lit,Max	0.05	1.5
13	Manganese (as Mn)mg/lit,Max	0.10	0.3
14	Sulfate (as SO_4) mg/lit,Max	200	400
15	Nitrate (as NO_3) mg/lit,Max	45	100
16	Fluoride (as F) mg/lit,Max	1.9	1.5
17	Phenolic Compounds (as C_6H_5OH)mg/lit, Max.	0.001	0.002
18	Mercury (as Hg)mg/lit,Max	0.001	No relaxation
19	Cadmiun (as Cd)mg/lit,Max	0.01	No relaxation
20	Selenium (as Se)mg/lit,Max	0.01	No relaxation
21	Arsenic (as As) mg/lit,Max	0.05	No relaxation
22	Cyanide (as CN) mg/lit,Max	0.05	No relaxation
23	Lead (as Pb) mg/lit,Max	0.05	No relaxation
24	Zinc (as Zn) mg/lit,Max	5	15
25	Anionic detergents (as MBAS) mg/lit,Max	0.2	1.0
26	Chromium (as Cr^{6+})mg/lit,Max	0.05	No relaxation
27	Polynuclear aromatic hydro carbons (as PAH) g/lit,Max	--	--

BACTERIOLOGICAL STANDARDS

I. Water entering the Distribution system

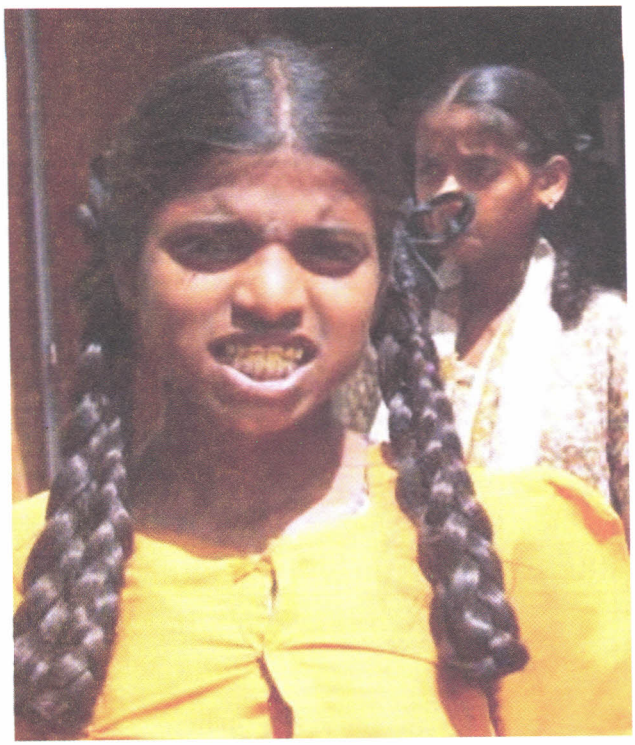
Coliform count in any sample of 100 ml should be Zero. A sample of the water entering the distribution system that does not conform to this standard calls for an immediate investigation in to both the efficacy of the purification process and any two consecutive samples or more than 5% of the samples collected for the year. the method of sampling.

II.Water in the distribution system

1. E.coli count in 100ml of any sample should be zero.
2. Coliform organisms not more than 10 per 100 ml in any sample.
3. Coliform organisms should not be present in 100 ml of

Fluorosis:

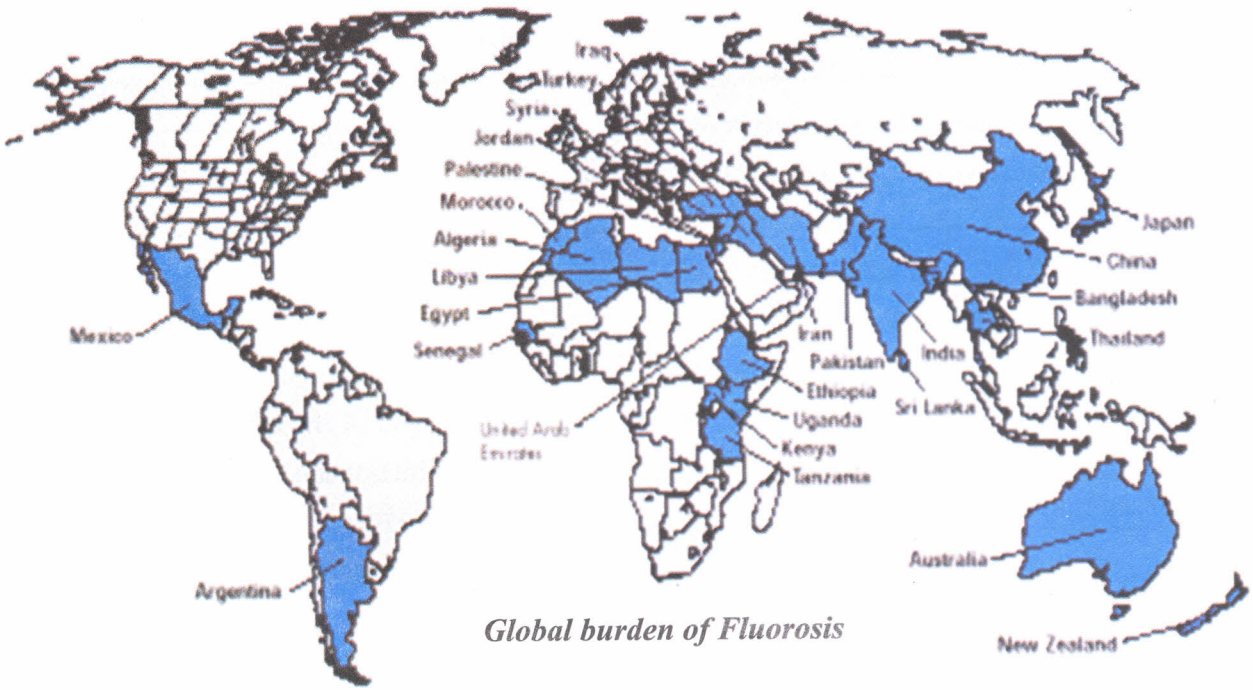
Fluoride in drinking water is an environmental problem because of the effects caused on long term use. Health impacts include dental fluorosis at serum fluride levels of 1.5 to 4.0 mg/L, dental and skeletal fluorosis > 4.0 mg/L and crippling fluorosis > 10 mg/L.



Picture showing manifestations of skeletal and Dental fluorosis

Fluoride occurrence in ground water:

Fluorides in ground water derived mainly from dissolution of natural minerals in the rocks and soils with which water interacts. Most common fluoride bearing minerals are **Fluorspar / Calcium fluoride (CaF_2), Apatite / Rock phosphate [$Ca_5F(PO_4)_3$],Cryolite / Sodium aluminum fluoride (Na_3AlF_6).** Dominant factors influencing fluoride build up in water are geology, contact time with fluoride minerals, ground water chemical composition and climate.



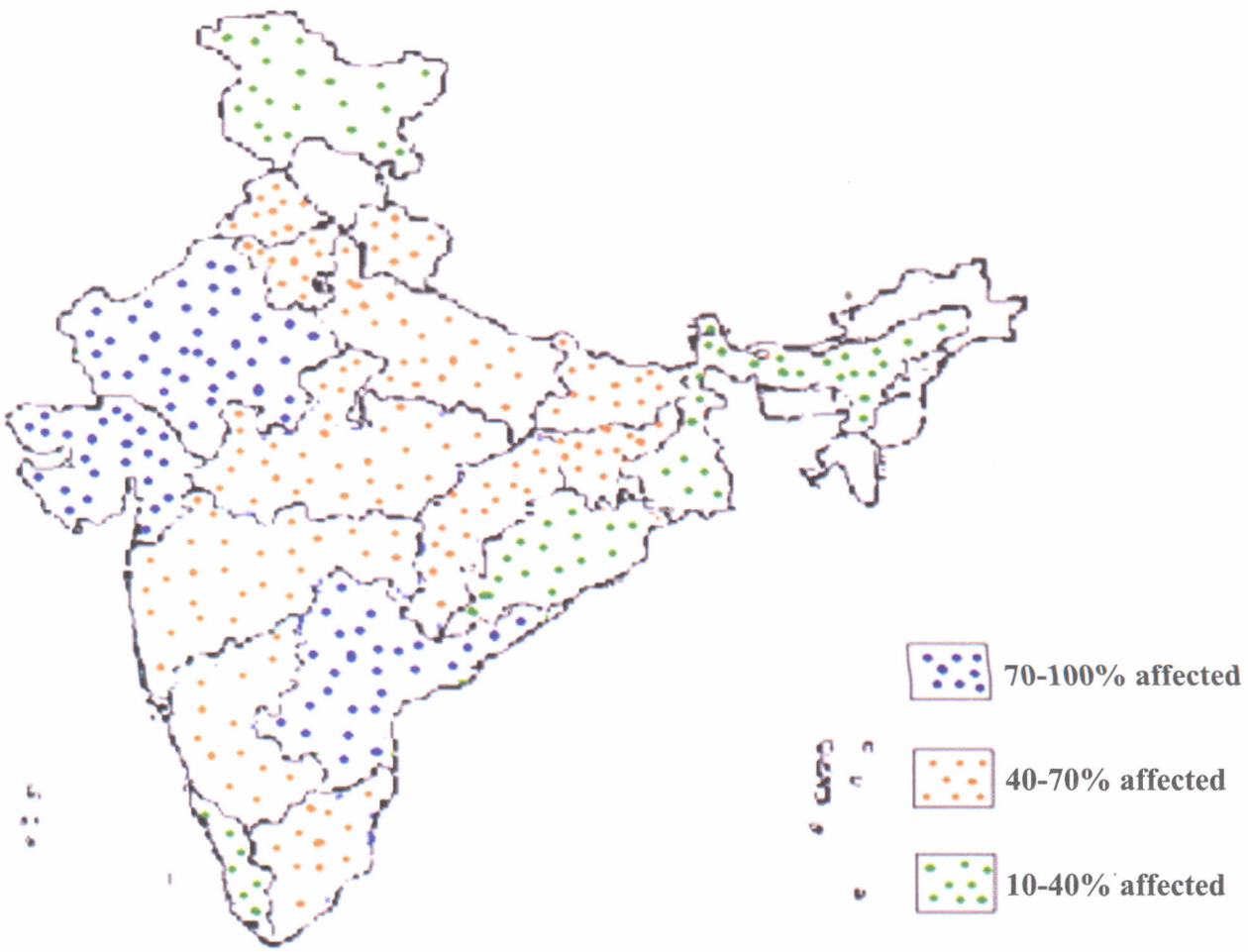
Global burden of Fluorosis

Magnitude of the problem : World

According to WHO estimate 260 million people world wide in 30 countries are drinking water with more than 1.0 mg/L of fluoride.Worst affected areas are arid parts of northern China (inner Mongolia), India, Sri Lanka, West Africa (Ghana, Ivory Coast, Senegal), North Africa (Algeria), South Africa, East Africa (Kenya, Uganda, Tanzania, Ethiopia), Northern Mexico and Central Argentina.Countries With Endemic Fluorosis Due to Excess Fluoride in Drinking Water (UNICEF - WES Repot)

India:

Endemic fluorosis is a public problem in India. Almost 60-65 million people drink fluoride-contaminated groundwater. The number affected by fluorosis is estimated at 2.5-3.0 million. Atleast 20 states of India, including the new creations Uttaranchal, Jharkhand and Chattisgarh are endemic to fluorosis. Andhra Pradesh, Gujarat, Rajasthan 70 100 % districts are affected .Bihar, Punjab, Haryana, Karnataka, Maharashtra, Madhya Pradesh, Tamil Nadu, Uttar Pradesh, some parts of Delhi 40 70 % districts are affected. Assam, Kerala, Orissa, West Bengal, Jammu & Kashmir 10 40 % districts are affected.Fluoride concentration in 15 districts of Karnataka



Picture showing flourosis endemic areas in India

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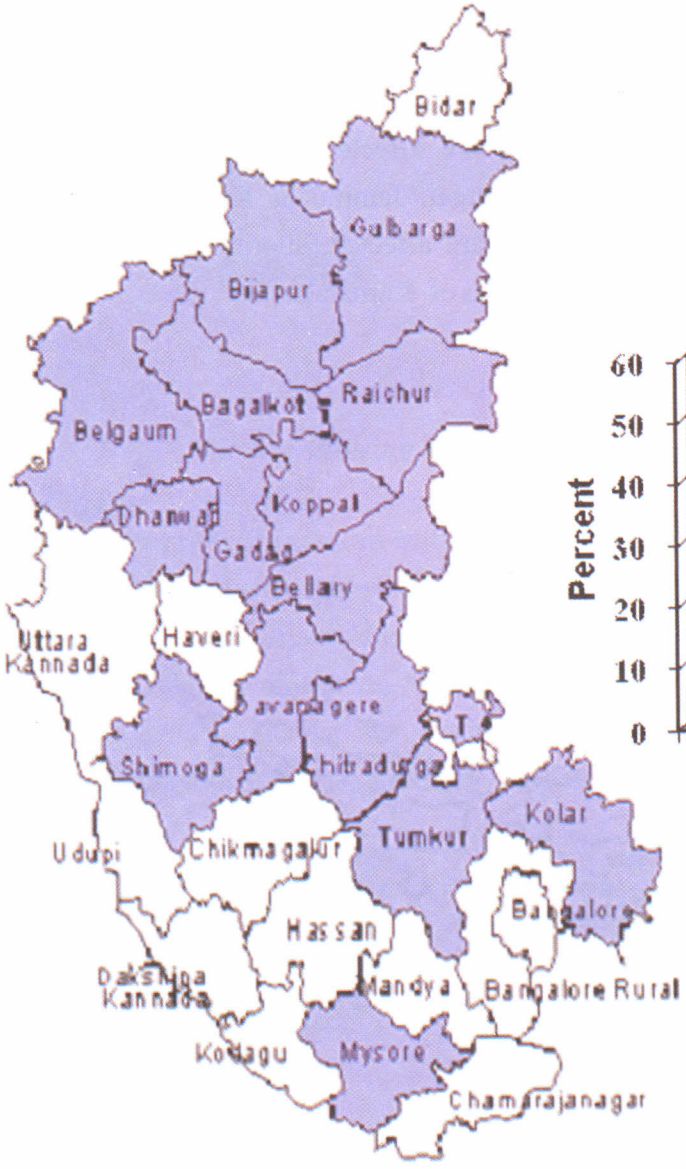

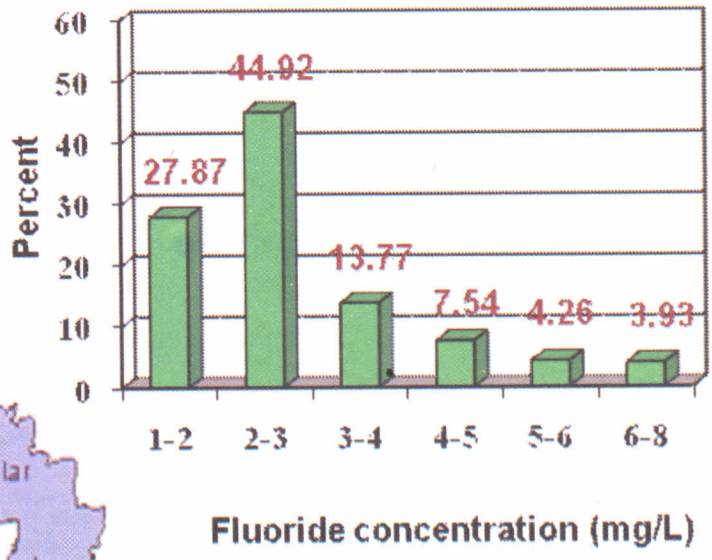

Fluorosis burden in Karnataka

Geochemistry as to why flurides are higher only few areas

Low rainfall is the reason for increased fluoridecontent in soil. On geochemical saturation of CaCo3 (calcide),hence to make up the deficiency CaF2 is dissolved hence fluride conc increases.

Water quality improvement using Magnisim oxide in Shola village, Rajasthan

Sanitary situation in India

63% of the urban population in **India** has got access to sewerage and sanitation facilities (47% from sewer and 53% from low cost sanitation) as on March 2004. As a consequence, open defecation is prevalent widely in rural areas but also significantly in urban areas too. Most nitrogen is excreted as urea, which readily degrades to ammonium. Nitrate is formed by the sequential, microbially-catalysed oxidation of ammonia to nitrite and then to nitrate.

Table 1. Chemical dosages for IISc method

Initial fluoride concentration, mg/L	Volume of 7.5 % calcium chloride solution to be added, ml/L	Magnesium oxide dosage, g/L	Initial bi carbonate concentration, mg/L	Calcium hydroxide dosage, g/L	Volume of 5 % sodium bisulfate solution to be added to treated water, ml/L
1.5 - 7	4	1-1.5	250 - 400	0.3	1-3
1.5 - 7	4	1-1.5	>400 - 500	0.4	1-3
1.5-7	4	1-1.5	> 500-700	0.50	3-6

Sample Parameters was as follows:

pH	8.91
EC(µS)	2861
TDS(ppm)	1857
Carbonate(ppm)	89
Bicarbonate(ppm)	1068
Fluoride(ppm)	18.4

Magnesium oxide was added to water in the below mentioned concentrations thereby decreasing fluoride levels to acceptable levels.

Pathogen movement and removal by soil

Bacteria travel depends on velocity of groundwater flow. During travel, fraction die or retained (adsorbed or screened) on soil matrix. Key factor for removal of bacteria and viruses from groundwater: effluent residence time between contamination source and point of water abstraction. Unsaturated zone is most important line of defence against faecal pollution of aquifer as it is less permeable. Probable survival time for coliforms in anaerobic groundwater environment is 4-7 days. One log removal of E Coli needed 4 m of decline

in groundwater level.Probable survival time for coliforms in anaerobic groundwater environment is 4-7 days: Average say 5.5 days.

Case study at Mulbagal Taluk, Kolar

Along with an NGO calle 'Argyam' the following study was conducted. Bore wells within and outside Mulabagal town were analysed for geochemical and biological characteristics.Key findings of the study were as follows:

- Nitrate concentration of borewell waters inside the town was significantly higher than that of the outer borewells.
- Borewells situated inside the town had full E coli organism, where as borewells situated outside were pathogen free.
- Contamination during water transmission was significantly more. Water which was free of pathogens at the source had organisms at the point of use.

Questions emerging from the studies

1. Can bore-well water be considered free of pathogen presence?

Contamination from leachates of on-site sanitation systems and improper well-protection tend to render bore-well water contaminated with pathogens.

2. Should alternative forms of sanitation be adopted?

Wherever possible off-site sewerage systems be implemented. However in country like India, where the proposed alternative requires a great deal more water or a substantially increased cost, on-site sanitation system remains a viable choice

Possible solutions to reduce pathogen contamination of bore-wells

In pathogen contaminated aquifers, guided by the site hydro-geology, engineer the vadose zone thickness to ensure 7 log removal of pathogen in infiltrating leachate. Improve well construction & protection to minimize groundwater contamination.As studies have identified transport, storage, and user practice in the home, as contamination source, end-of-use point treatment be implemented.

PEDIATRICIAN'S VIEW ON FETAL ORIGIN OF ADULT DISEASES

Dr. Y.K. Amdekar

Evolution is the basis of human survival. It involves genetic and epigenetic adaptation. Species select genetic constitution based on demands of environmental situation through genetic mutations. Two additional mechanisms come into play for individual survival 1.Accommodation -temporary 2. Plasticity which is irreversible interlinked with early life programming. Prof Alan Lucas coined the term "programming" which refers to "setting" of physiological system by early stimulus during critical period. There is no widely accepted definition of critical period? Intrauterine period ? 1000 days.

• Prof Peter Gluckman proposed "match-mismatch theory"- explains early nutritional deprivation followed by abundance of subsequent nutrition. Thrifty gene probably started when periods of famines repeatedly led to period of starvation and there could be some period of good nutrition. But in today's better economic situation there may not be such famines where we should get genetically ready. Therefore today with better economy state "Thrifty" gene is an evolutionary disadvantage. Change in the function of gene is epigenetic and we know individual's disease risk is determined by epigenetic events and there may be epigenetic inheritance.Fat metabolism is well regulated in all other species. They remain lean even when adequate food is supplied. It is not environment alone but there should be genetic and epigenetic basis for the diseases. "Thrifty" genotype predicts continuation of high level of disease unless life style is changed. Therefore there is a reason to intervene in those patients with thrifty genotype.

• Epigenetic and gene-environment interactions make it imperative not to consider any disease as being either genetic or environmental. As a clinician we should look at which one to modify for better result genetic or environmental factor. Relative contribution of genes and environment is difficult to determine. Gene-environment interaction may affect expression of genes eg. diet low in phenylalanine suppresses PKU manifestation. Gene-environment correlation individuals with certain genotypes more likely to be found in certain environments. Genes can shape creation or selection of environment

• As a clinician we should know what is the critical period of development, is it gestation period or postnatal 2 years? Fewtrell and Lucas study showed that upward centile crossing from 18 months to 9 years is more significant. If I see a growth chart of a baby born with small birth weight with a proportionate height, I consider that there is no fetal malnutrition. Therefore low birth weight is not necessarily fetal malnutrition. In fact Weight for length is a realistic parameter. Weight alone is not a good parameter. In the

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Indian scenario a thin baby but proportionate height gets into adulthood without any disease. IUGR is better seen as weight and length correlation. When head size is small we know that there is chronic malnutrition during fetal life. It is the change in the life style like lack of physical activity in child hood with abundance of food that leads to overweight and obesity rather than fetal malnutrition alone. Breast fed babes show good growth rate and we know that is not harmful in their future life. Monozygotic twins with the same availability of food may show different growth. Therefore it is not just genetics or food or environment, there may be some more factors which we should find out.

- "One-size-fits-all" in nutrition is a paradox unlike in immunization, salt iodization or water fluorination where it fits all. Now, we know that Folate excess in pregnancy is known to result in colorectal malignancy in few women. High zinc supplementation is also harmful.

- Exposure to environmental toxins, air pollutants and drugs as well as maternal nutritional imbalance have been implicated in causation of several diseases such as neurodegenerative diseases, heart defects, learning disability, cancer, thyroid disorders and autoimmune diseases.

- Diabetes is linked to viruses, nutrition, toxins and socio-economic factors. Therefore every disease is multifactorial. In such a complex situation should we respect adaptation and nurture it rather than intervene? Should we manipulate fetal growth or postnatal growth?. I feel health of girl child through early life to adulthood would decide the outcome of healthy next generation.

- The following factors viz.,birth weight, current size, degree of postnatal change in centiles are important factors and may have a role. I feel a growth chart is more helpful in nutrition intervention rather than one single measurement like birth weight. Fetal growth chart should be implemented for the correct intervention.

Summary

- There is no agreement on critical period of development.
- Consider weight for length / height as a parameter to assess nutritional status
- Monitor growth chart through childhood and intervene at the onset of upward centile crossing
- Life style plays important role
- Long term goal (for posterity) to ensure adequate nutrition to girl child from birth to motherhood

GENETIC COMPLEXITIES OF A SIMPLE HEREDITARY DISORDER

Dr Anuranjan Anan

What does it take for the sense of hearing to develop? About 1% of our genome of 40000 express messages. That is, 400 genes or molecular protein express exclusively in cochlea for functioning. In addition the complexity in these genes also contribute.

Deafness is a highly heterogenous disorder. There are several reasons for it(Fig1). 80% of the disorder are autosomal recessive, 20% autosomal dominant and 1-2% X-linked. The autosomal dominant are the cases that account for a large burden where deafness manifests with other clinical issues.

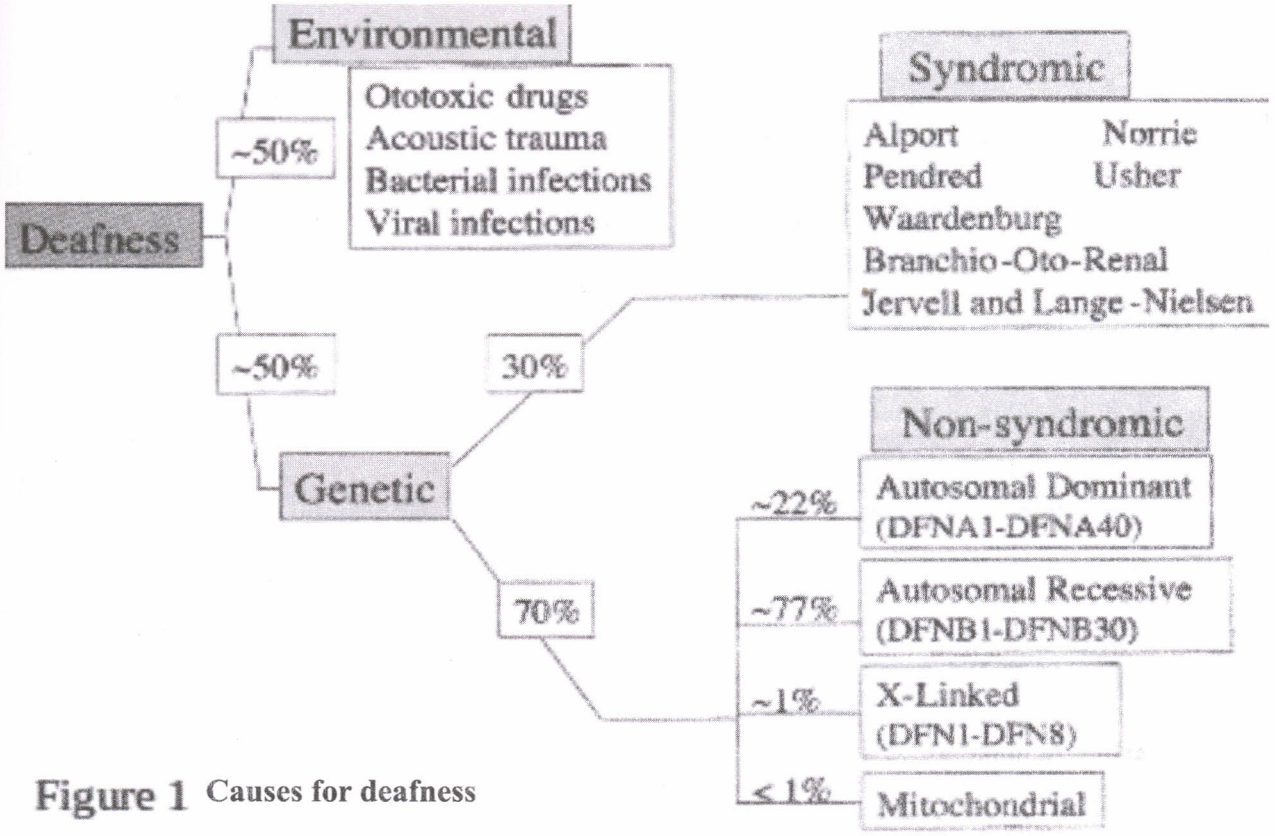

Figure 1 **Causes for deafness**

Many genes identified, yet, the effect of mutations or on functional contribution to cochlea or hearing physiology is not known. What is certain is their precise location from chromosome 1 to 22 and the chromosome X and Y(Fig 2). DFNA stands for dominant variety and DFNB for recessive variety. The genes on chromosomes X is indicated without A or B. With so many genes, there is parallel complexity with respect to what they code for. Therefore molecules of whole variety are involved when genes get mutated. These protein affected are involved in various parts of cochlea, both structural and functional, eg: channel component, ion transporter, motor molecule,

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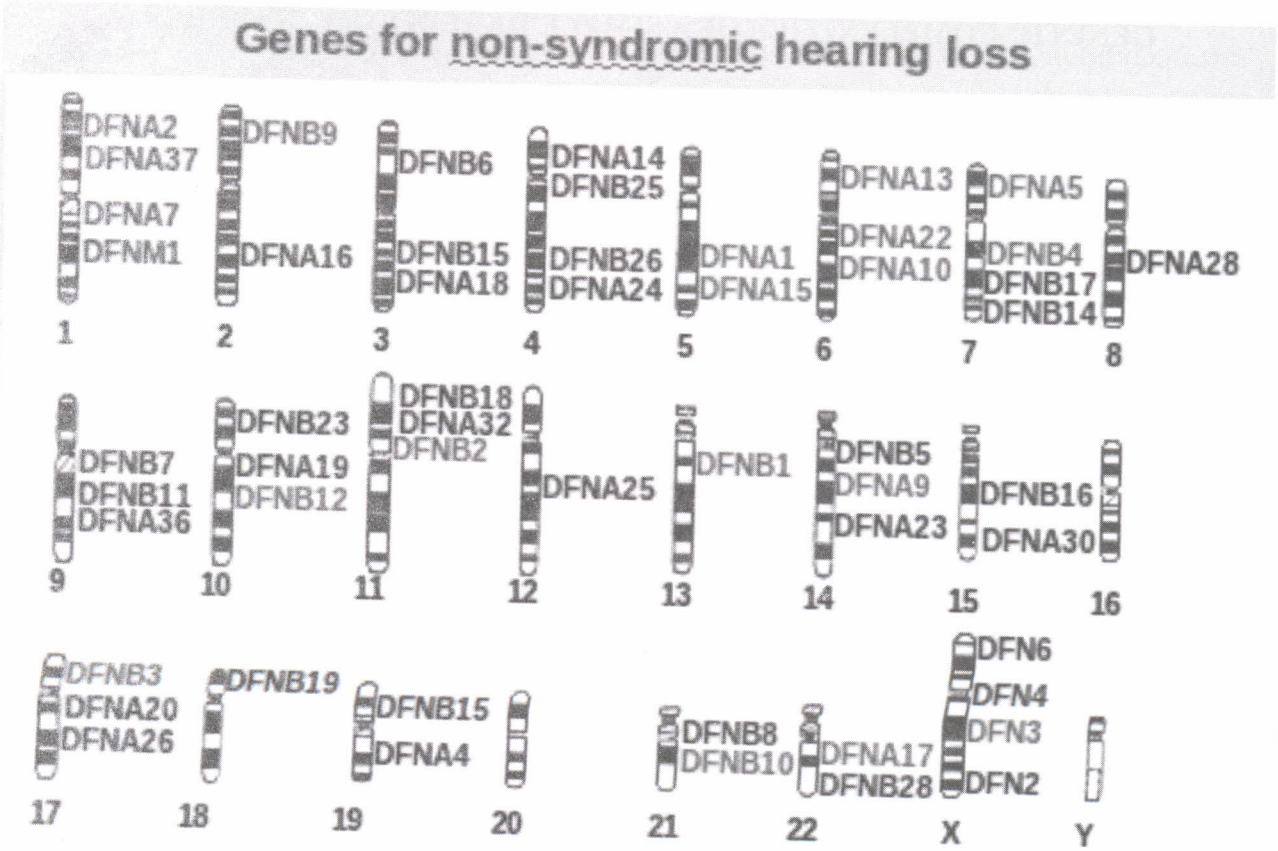


Figure 2 **Genes for non-syndromic hearing loss**

The work at JNCASR involved utilizing the extensive knowledge of 50 years and 80 locations to identifiy the contribution of these genes in causing deafness in our population. The initial work was to get the data about these genes from various laboratories around the world where emigrant Indians have been evaluated for deafness or have tested the samples from deaf Indians. Then to set up molecular epidemiology to derive proper way of diagnosis and help these families with proper intervention or disorder management.

The demographic profile and presenting symptoms of this type of deafness was included. Then prenatal and perinatal history was taken including developmental milestones and educational history. Clinical evaluation was done to rule out syndrome and non-syndromic hearing loss(NSHL). Also detailed audiologic assessment was done to know type, severity, progression, onset of deafness along with frequencies involved and the involvement of vestibular system. The study included 503 families with more than 2 involved members. The reason to choose this was to reach these mutations as quickly as possible and to utilise them practically.

The Connexin-26(Cx-26), one of the gap junctions molecules found in one of the tribes is one of the well characterised genes in population of world where there is extensive epidemiologic data this gene accounts for 20-50% of NSHL hence this was the first gene to be studied. This gene is also easy to work upon without much complexity of sequence content. The Cx-26 was examined in one affected member of each of the families. If the person has Cx-26 mutation, then by use of functional cell biologic assay it was found if the change found in DNA was pathogenic or non-pathogenic or benign type.

This is an important outcome when one finds a sequence variant in any DNA. If there is no Cx-26 mutation then a linkage exclusion by marker discordance/concordance test is carried out. For this test a family with at least two affected members is important. In this test genes are so chosen that the markers are closely linked to them. There are at least two markers on either side of genes which are so closely linked that there is less chance of these markers combining away from the genes.

If there is marker concordance , then that particular gene of interest is examined. This is a good test for exclusion but has some false positivity. If mutations are not found in the set of genes that were studied, provided structure of family was, right then complete genome study is carried out without specifically concentrating on the particular gene, hoping to find something new. The whole algorithm is built up to begin with gathering knowledge about what is known and also getting more knowledge. In the end families are found having new molecules. The set of genes chosen right from Cx-26 to stereocillin are shown in Fig 3.

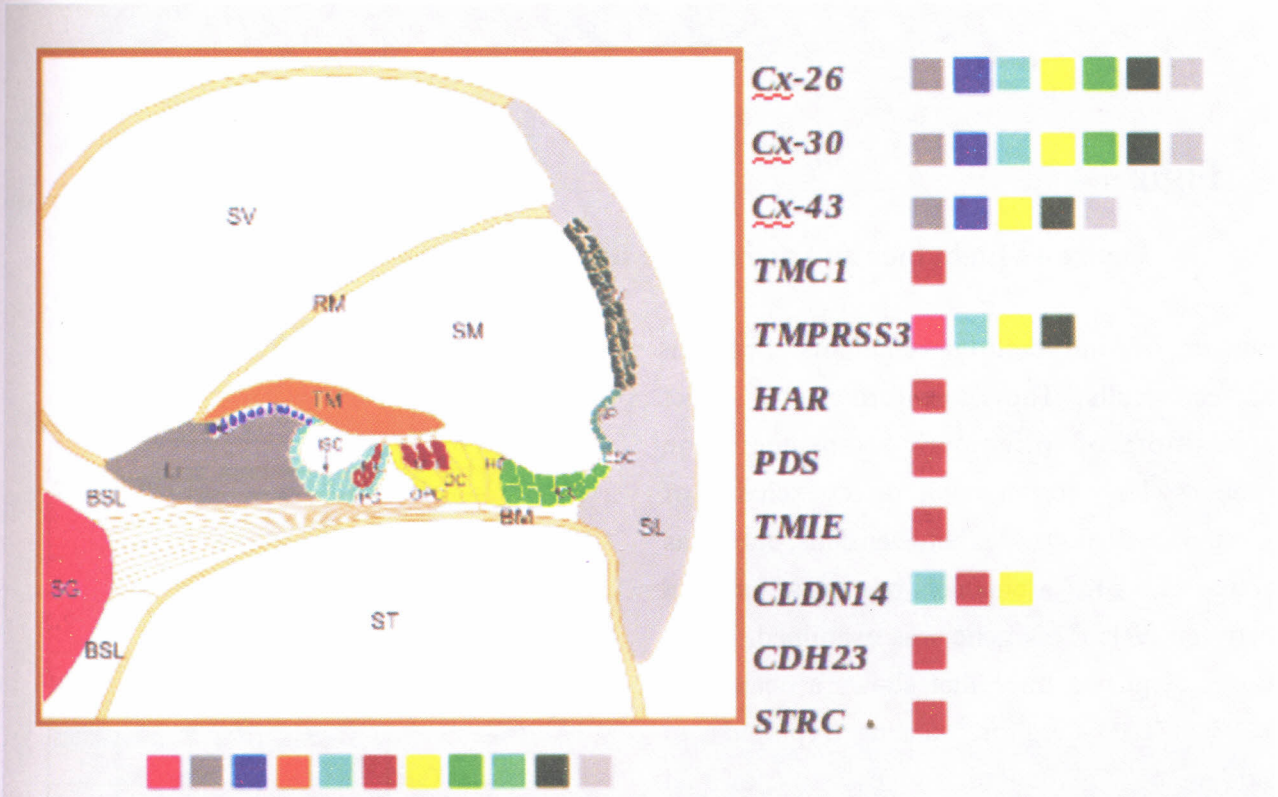


Figure 3 **Mutational analysis of deafness genes**

The various location of expression of genes can be correlated. What expression patterns in these cell types may mean when these genes are not present and those cells are affected with respect to deafness.

Firstly coming to Cx-26, it has been hypothesised that Cx-26 is responsible for gap junction, that is recycling back important ions which are important for continuation of electrical signal generation in the cochlea. (Fig4)

Those are essentially gap junction which are

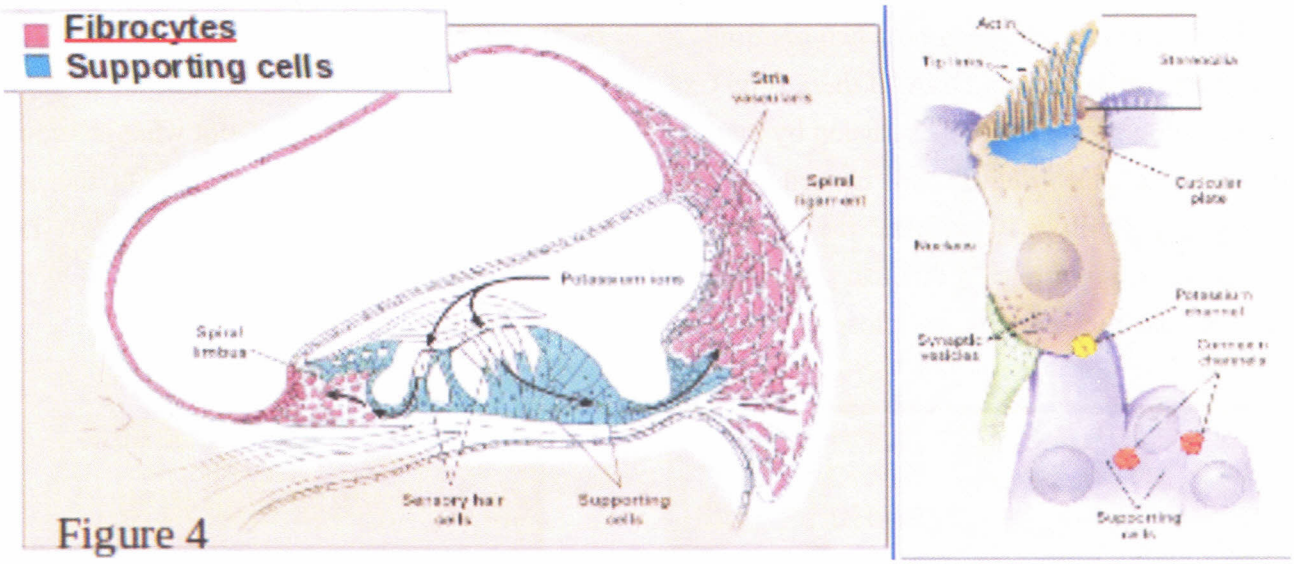


Figure 4

Figure 4 : Light blue and maroon are the places where Cx-26 is expressed.

cluster of intracellular channels between adjacent cells. They are formed by direct apposition of oligomeric transmembrane proteins. They permit small, direct exchange of ion and small molecules between cells, but not as active as Na,K,Ca channels but crude junction between cells. This gene was examined. There was a sequence trace that shows a change of TGG to TAG therefore causing tryptophan to stop codon. This mutation gives us a truncated protein which is not capable of forming a subunit of that functional channel. This led to an audiological profile showing SNHL(Fig 5).

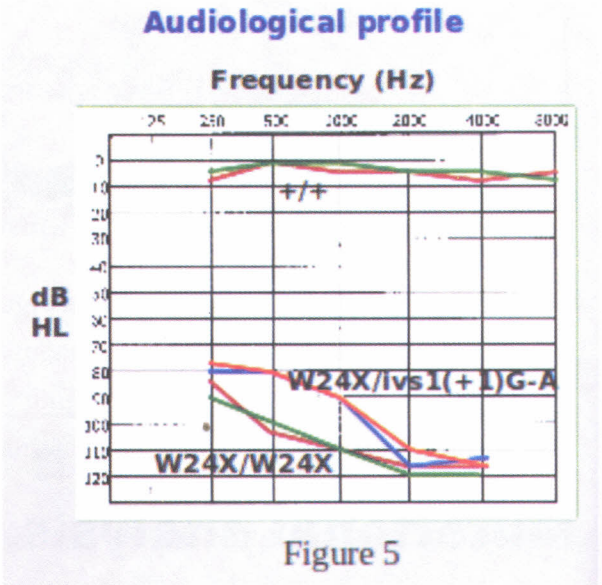


Figure 5

There are a number of such mutation, 14 of them in Cx-26 gene. It has coding and non-coding part. Both were examined and mutation found in both parts. These are either tryptophan changing to stop codon or to another amino acid, ie mis sense mutation which doesn't let protein function effectively or regulatory kind of mutation in splice intron exon junction. Most of the mutation are 1-2 % except W24X which was 80% in the samples examined. This is the most prevalent mutation in this gene . Cx-26 is responsible for almost 25% of deafness cases in the sample of 500 examined which is 125 cases. Of these cases 80% of them are due to W24X mutation. Rest are due to other mutation. If a matrix of genes generated with respected to its nature of being variable or conserved and place these 26 Cx molecules then one notices that most of these mutation fall in conserved part.

Since W24X was found in the study frequently, it was studied to see if it was a recent mutation or one that occurred long ago and carried down. All chromosome containing this mutation were examined. Also several markers

located close to this mutation. The message of the study was that, this mutation has entered our population 394 generations ago, ie approximately 7880 years back. This is more in South India. There are similar genes with founder effect across the world (Fig 6)

mutation such as I35S protein is spread throughout and doesn't locate on the membrane to form tight junction. Also is the case with R184P or R75W. Therefore localising is defective to a great extent.

Sometimes one doesn't pick up functional

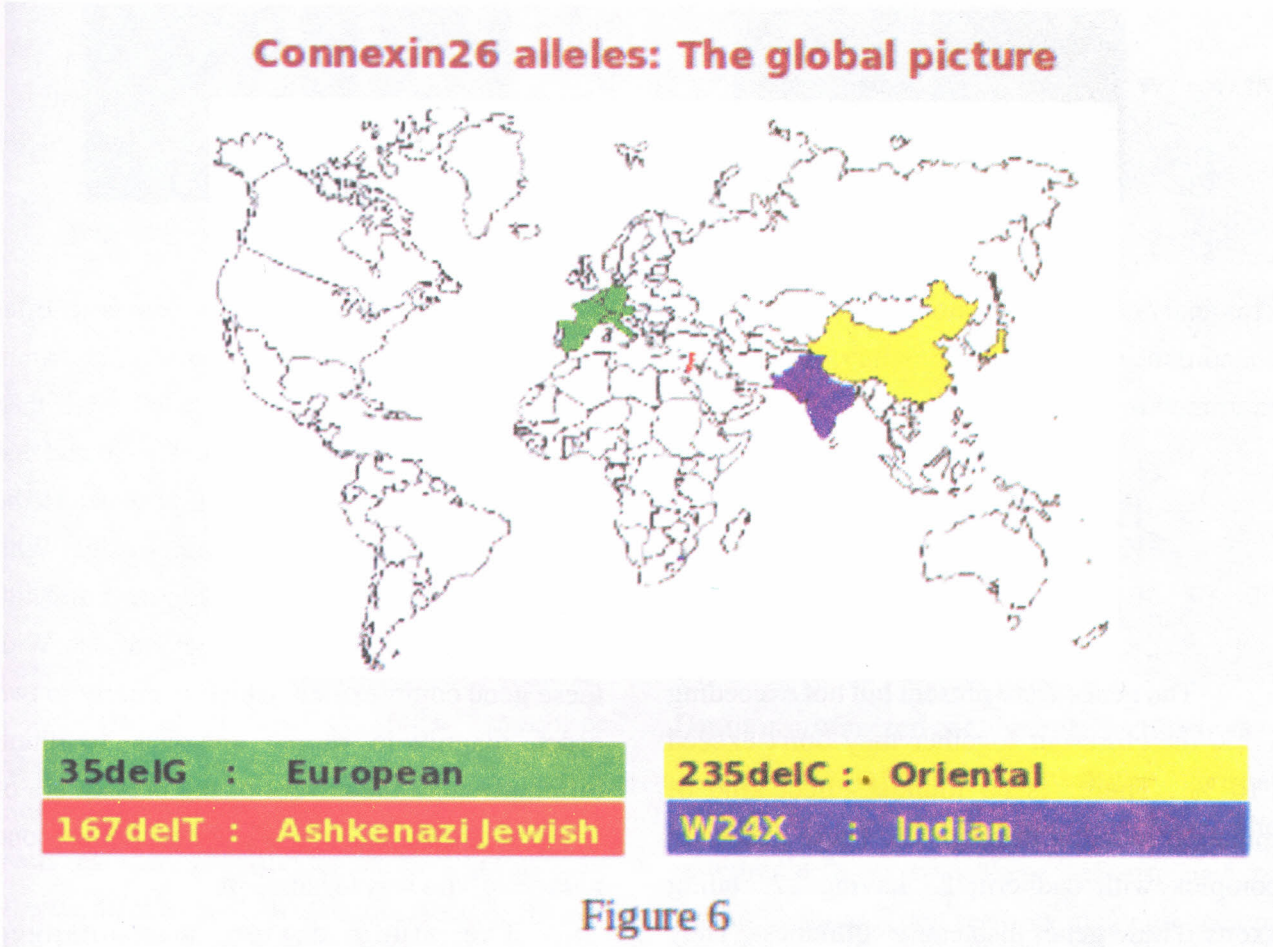


Figure 6

Further study was to look at how mutant protein locates in cell type. A variant of HeL a cell lines was used. This cell line is chosen by sub culturing again and again largely devoid of gap junction. So one can use this clean slate background of cell line to put in the gap junction that needs examination and see now mutant protein locate and whether they have ability to do what actually the gap junction do, ie transfer of ion between cells. In normal situation one finds tight buttons in between cells. W24X has no protein so one does not see anything. Other entity which reside on the membrane very well so another test was done which involved injecting a dye called Lucifer yellow. A cluster of cells is chosen and the dye is injected into one of the cells. This dye then moves from cell to cell. This test is done in normal Cx-26 and mutant Cx-26 to see dye transfer taking place between cells in mutant and wild type condition. This transfer is mediated through the connexin gap junctions. In a wild type the dye essentially percolates through to neighbouring cells giving greenish hue to all cells whereas in a mutant it stays and

gives a bright yellow hue(Fig 7&8).

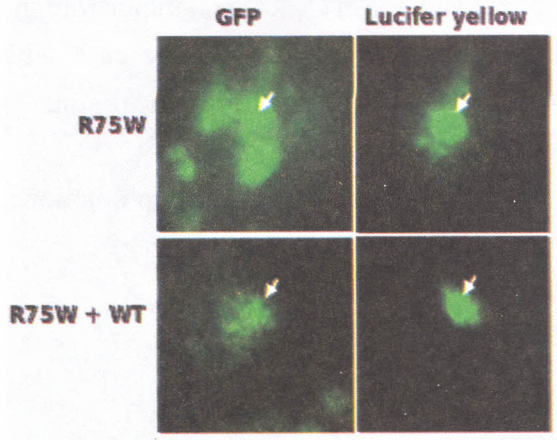


Figure 7 **Wild gene**

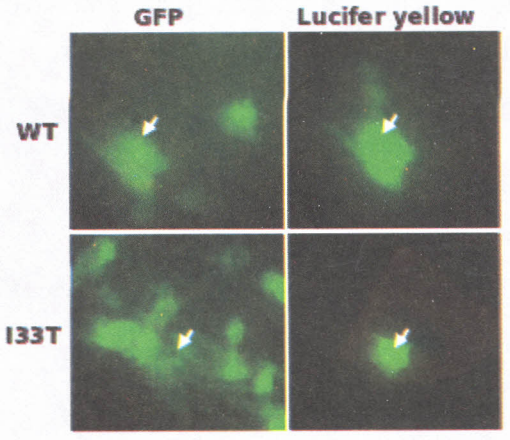


Figure 8 **Mutant gene**

This analysis was also done in 5 other genes after concordance/ discordance study. The genes examined were:

- TMC-1
- Hormonin
- Cadherin
- TMPRESS
- TMIE

The genes were present but not exceeding 1-2% therefore put together they don't exceed >10 % . There is no founder effect kind of mutation found at these genes. These genes are complex with cadherin 23 having 22 coding axons. These genes also cause blindness. They cause Usher syndrome but also a large number of NSHL without any blindness. The message of this exercise is that, put together, these 5 genes along with connexin account for about 45%of sample size. There are very limited number of mutation that account for majority of cases, so disorder per se is not only genetically heterogenous, but has heterogeneity at allelic level because even within a gene there is a single mutation anywhere in the world but lot of private mutations. Nearly 45 mutations are found in Indian population with these 6 genes put together.

So by this study of about 6 genes, the situation is very complex. If it comes to offer these as a diagnostic test or to create an algorithm of what should be tested first what next, then only gene that can be tested is for Cx-26 and that too for W24X mutation. Once all gene need to be tested the situation turns too complicated. With only these 6 genes, let alone 14 known and 400 other speculated genes over 45 generations. With these gene complexities, which is nearly to two order magnitude more, one has to think differenty with the advent of the present era of genomics. The problem are multifold and one has to evolve a way to handle it.

The study design was changed afterwards, from going gene after gene , which are not mapping to existing gene to see if it is possible to identify all 66 genes in 1-2 days. Today there are 103 loci known, these are locations in genome where we know genes are present, but not always been found. Today 66 genes for NSHL are known.

Of the families with Cx-26 excluded one analysis gave the following result.

Next level is the medical and genetic counselling once the families are identified. Also to enrol remaining 500 families to get better statistics. The study led to a certain village

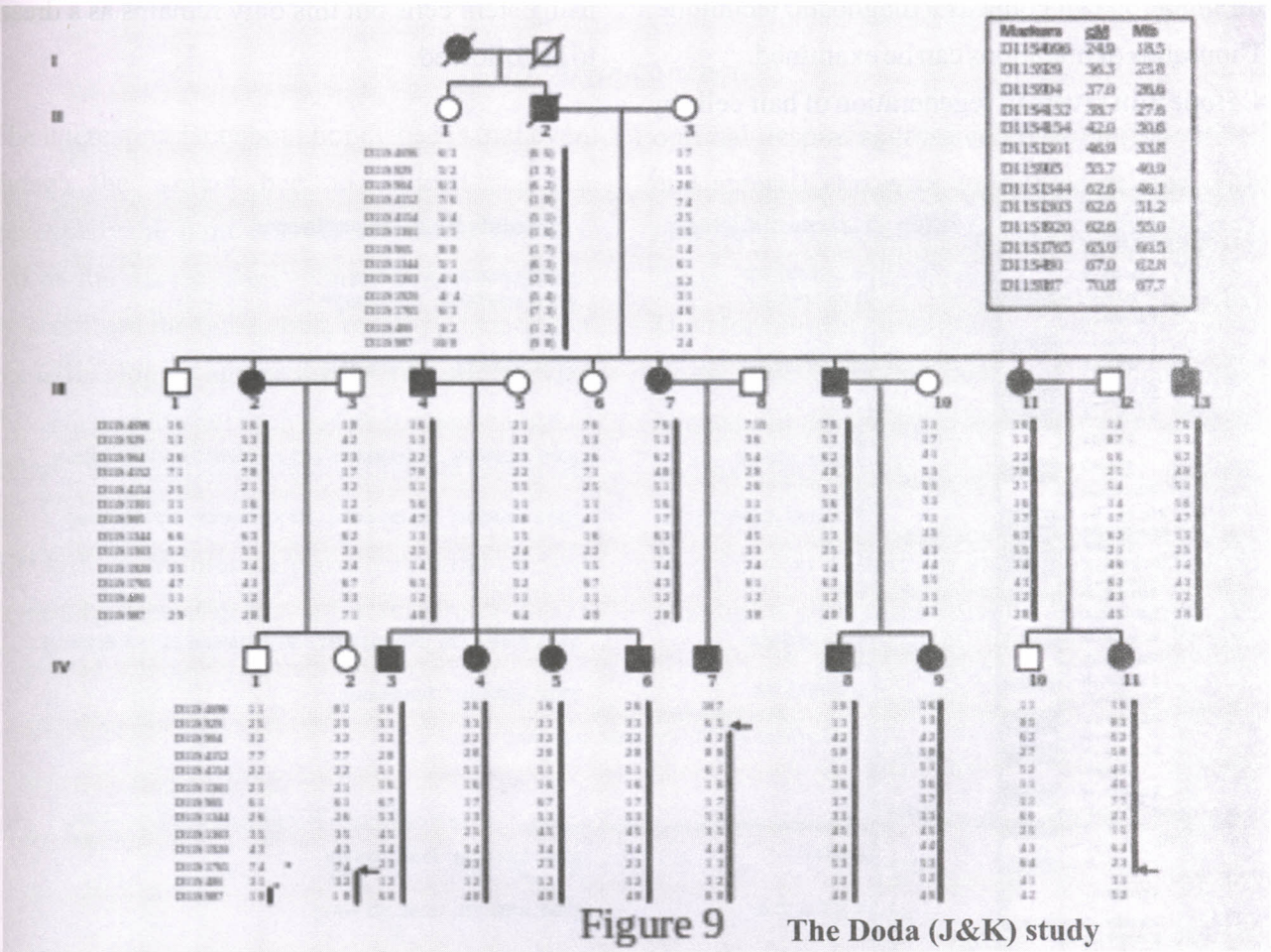


Figure 9 The Doda (J&K) study

called' village of silence', Doda, Jammu & Kashmir where of the 300 families, 50% have mostly deaf members. The data collection over 2 years revealed, 70 individuals from a family (Fig 9) were affected and 50 other unaffected. 70% of the affected were due to pendrin gene and 30% were due to otoferlin gene. This is because the community has evolved to be a deaf community with deaf-deaf marriages and migration of deaf people from surrounding area. In another study on a family in Maharashtra about 500 markers were used. This led to a new loci which had not yet been reported. This was DFNA-59 (Fig 10) on chromosome 11.

The study is promising on target sequencing and whole exon sequencing in gene DFNA 59.

Human subtracted cochlear cDNA library

There are 425 messages almost exclusively present in human cochlea. The experiment to put back these 425 msg back to human genome. Then see how many go back to genes in known locations and to unknown location. The summary of this study was that the loci was 96 and the result were as given in (Fig11).

Opportunities in deafness genetic research

1. More genes are waiting to be identified. May affect other diseases like otosclerosis.
2. Multifactorial causes like age related loss, noise induced hearing loss and its interction with epigenomes is still a black box.
3. DNA chips (Otochips) wherein one does not examine gene by gene, instead all known genes

for mutation can come as a diagnostic technique. Thousands of mutations can be examined.

4. Hope is in reversal/ regeneration of hair cells by using stem cells but this only remains as a dream to be followed.

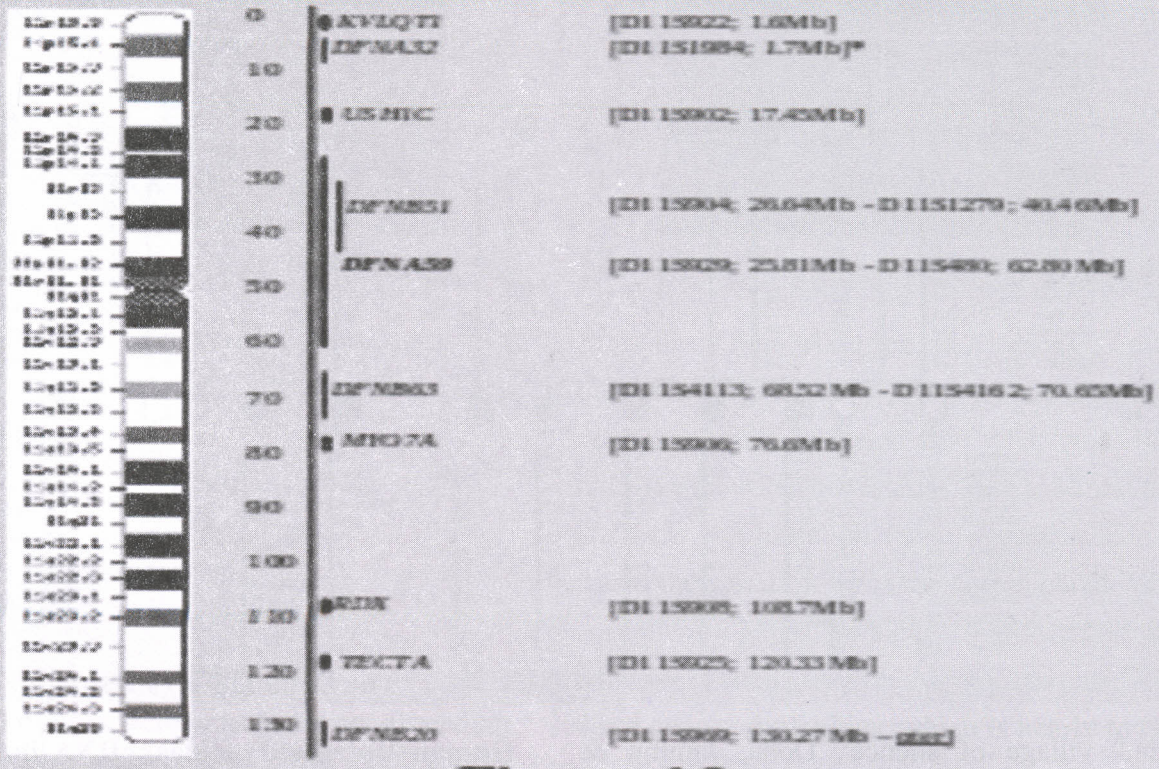


Figure 10 DFNA 59 gene

Non-syndromic deafness genes

Total loci known: 96

Autosomal dominant loci: 51
- Loci with genes known: 21
- Loci with genes unknown: 18
- Reserved loci: 12

Autosomal recessive loci: 39
- Loci with genes known: 20
- Loci with genes unknown: 16
- Reserved loci: 3

X-linked loci: 6
- Loci with genes known: 2
- Loci with genes unknown: 3
- Reserved loci: 1

cDNAs mapping to the known deafness loci
198

cDNA mapping to the unassigned genomic regions
215

Figure 11

EXTRA-UTERINE GROWTH RETARDATION

Dr. Arvind Shenoi

The interesting fact about honey bee is that even though the worker bee and queen bee are genetically the same it is the nectar that they are fed at the larval stage which differentiates a worker from the queen bee, resulting in a queen bee to live longer and be fertile. The inference of this is that early nutrition has an immense impact in the growth and development for the rest of the life.

The term extra-uterine growth retardation was coined by Reese et al. after screening 24371 preterms (23-34 weeks gestation), they found 28% to be growth restricted at discharge, Risk factors being smaller gestational age (SGA), male, and severity of illness.

Magnitude of the problem: Data from our country shows about 31.3% neonates are born LBW (<2500g), 3.4% VLBW (<1500g), 0.7% ELBW (<1000g), Post natal growth is sub-optimal despite aggressive nutrition (Parenteral and enteral).This poor growth associated with nutritional deficiencies has an implication on future neuro-development.

Developmental outcome-preterm SGA

They have a higher incidence of neurologic abnormalities compared to Preterm AGA. Due to additive effect of prematurity and growth retardation.

In a 12 year follow up study done at Manipal Hospital it was noticed that height & weight and mean IQ at 12 years were significantly decreased compared to AGA babies.

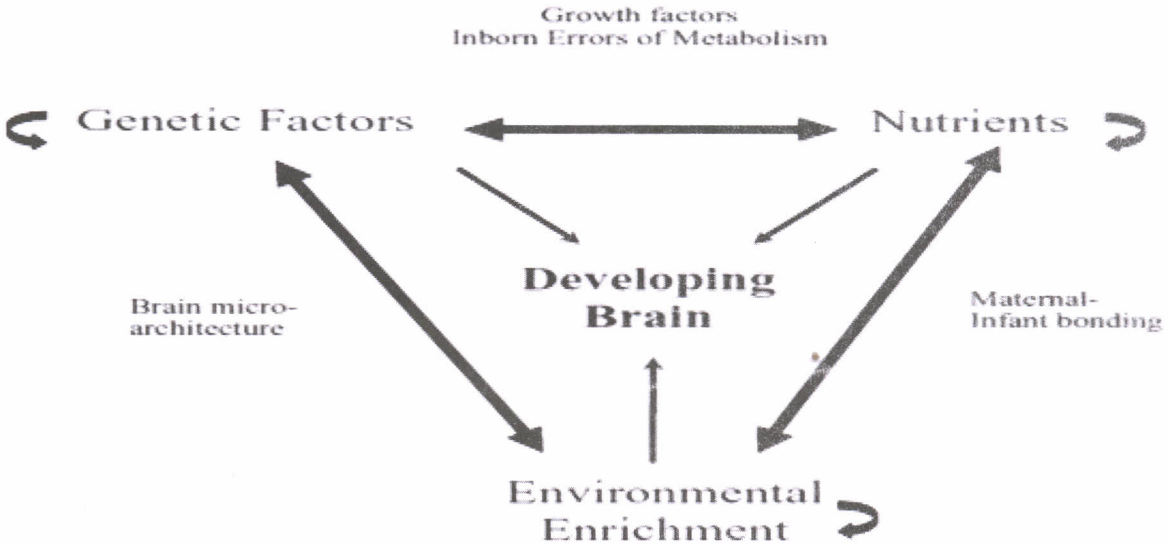


Figure 1. Shows Factors effecting the development of brain.

Factors effecting the development of brain.

What is happening in the brain during fetal and early postnatal life?

Nutrients with particularly large effects on early brain development and behavior

Dr. Arvind Shenoi, is the consultant Neonatologist and medical director of Cloud Nine Hospital Bangalore, and Adjunct Professor of Pediatrics, Manipal University

nucleic acid biochemistry, Decreased embryonic/fetal brain DNA, RNA and protein content, decreased brain IGF-I and GH receptor gene expression.

Biochemistry/Neurochemistry: *Zn deficiency inhibits* GABA stimulated Cl influx into hippocampal neurons, Zn deficiency inhibits opioid receptor function in cerebral cortex, Zn released from presynaptic boutons.

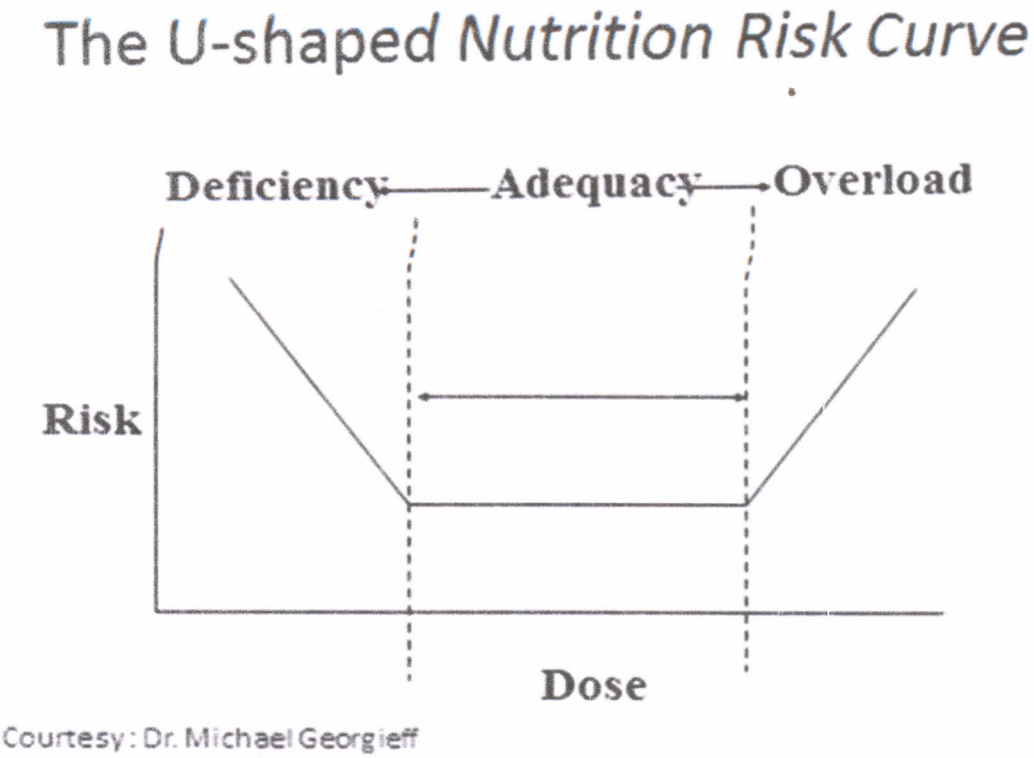
Candidates for "Brain Enhancement":

- Choline,
- Oligosaccharides,
- Neurotrophic factors (growth factors),Brain Derived Neurotrophic Factor,
- Docosohexaenoic acid,As supplementation rather than repletion of deficit (current formulas)

Risk of toxicity

- Iron: Infants with high normal Hb had lower IQs (Lozoff et al, 2008)
- Zinc toxicity cause Cu deficiency which in turn leads to Iron deficiency and anemia as Cu is necessary for iron absorption. Cu also modulates *dopamine metabolism.*

The U-shaped Nutrition Risk Curve



Courtesy: Dr. Michael Georgieff

PRENATAL AND NEONATAL DIAGNOSIS OF GENETIC DISEASES BY MOLECULAR CYTOGENETICS

Dr Jayarama Kadandale

Cytogenetic analysis is at present the basic element of the diagnostic process of genetic disorders which are caused by chromosomal abnormalities. Numerical or structural chromosome abnormalities such as an increase or reduction in the number of chromosomes, or a translocation of part of one chromosome to another, produce a variety of clinical phenotypes including malformation and mental retardation. I will be talking about the laboratory techniques used to do genetic diagnostics for identifying abnormalities. These diseases could be prenatal, neonatal and adult diseases including cancer, but I will be limiting my talk here today to prenatal and neonatal genetic diseases that are diagnosed by Molecular Cytogenetics. Before that, I will just talk a few words about the conventional cytogenetic techniques that we carry out in the lab. These can be confirmed by using DNA probes.

There are different kinds of tests Cytogenetic diagnosis, DNA diagnosis and diagnosis of metabolic disorders. I will be concentrating only on Cytogenetic methods of diagnosis. These are called as Clinical Cytogenetics where we do testing by chromosome analysis. Prenatal diagnosis is indicated in cases where there is a family history of birth defects, or where already a child with a birth defect is born or someone has one or more relatives with a birth defect. Samples that are used or accepted for prenatal diagnosis are peripheral blood, amniotic fluid, chorionic villi, cord blood, skin or bone marrow. The turnaround time depending on the type of sample varies from 6 days to 3 weeks. What we basically look for is the presence of any aneuploidies or structural abnormalities. Once we culture the cells, after 3 days we fix the cells and put it on a slide, stain them and do banding which gives a characteristic banding pattern to each chromosome that ultimately helps in identification. We capture a metaphase from a slide and analyse the karyotype. A normal male karyotype is 46, XY and a normal female karyotype is 46, XX. One set of chromosomes comes from one parent and the other set comes from another parent.

The commonest abnormality is Down's syndrome which was first seen in 1866 and identified by Dr Down. It was not known then that this was a chromosomal abnormality but only later in 1956, the abnormality was identified. In 1957, an extra chromosome was detected in these patients; in 1959, other cases were also confirmed to have an extra chromosome 21. There are typical features and a typical appearance of people with Down's syndrome. This extra chromosome need not always be a free trisomy 21. Based on the type of trisomy, counseling is offered that includes the potential risk of Down's for the next child as well. For eg:- the recurrence risk for a free trisomy 21 is just 1%. The other type is called as Robertsonian translocation where an extra 21

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sits on one of the acrocentric chromosomes. The risk here is around 50% for the next baby. This happens because one of the parents is a carrier. Another type of translocation is the extra 21 sitting on another 21; in this case the risk is 100%.

Another type is a case of a mosaic, where there is existence of more than one kind of a cell line in a person. One example is a mosaic with 45, X and with extra 21st chromosome. This is an abnormal female karyotype with double aneuploidy.

In Molecular Cytogenetics, the most commonly used method is called as FISH (Fluorescent In-Situ Hybridization). This serves as an adjunct to conventional karyotyping as it can detect aneuploidies much faster (within 24hrs). Another use is that it can detect microdeletions and microduplications that cannot be detected by a routine karyotype report. Here, the deletion is too small to be seen. This is required by pregnant women with abnormal ultrasound findings, elderly primi and those women with a family history of a particular genetic disorder. Thus, FISH is faster, specific, accurate and reliable. It can be applied to nondividing cells, uncultured cells, amniotic cells, cells from chorionic villi and the results can be expected within 24hrs of the sample reaching the lab. It can also be used on cultured cells or chromosomes by using DNA probes to detect translocations.

For the basics of the procedure, we initially denature the ds-DNA and attach a signal to one of the strands. This makes it a probe. This is then applied onto a slide containing the chromosomes which will then go and bind/hybridize to the target region and it can be visualized the next day by detecting the signal. There are different kinds of probes available like centromeric probes, telomeric probes, NOR probes, arm-specific paint, band-specific paint, single-copy genes and whole chromosome paint probes. These are available commercially for some well-known diseases. Interphase FISH is when we directly collect the cells from a sample like amniotic fluid, put it on a slide and react it with a probe and give the report. Even mosaicism cases can be studied. The use of probes and FISH can be used to confirm doubtful cases where the question arises whether to continue a pregnancy or not. The most commonly used probes are for X, Y, 13, 18 and 21. They are usually combined with a control so as to help differentiate from triploidy or tetraploidy.

With regard to the microdeletions, these are defined as those that have deletions less than 5 MB in size (that cannot be detected by karyotyping). Examples are Wolf-Hirschorn syndrome with deletion of 4p.16.3 , Williams syndrome with 7q.11.23 deletion, Prader willi/Angelman syndrome with deletion of 15q.11.23, DiGeorge syndrome with 22q.11.23 deletion. Most of these are located next to the centromeric regions and are hence, very fragile. As a rule, these have to be checked on chromosomes/cultured amniotic cells.

Not only for microdeletion, FISH probes have to be used for other abnormalities as well that cannot be definitively reported by karyotyping. Karyotypes with additional material of unknown origin is one such example. This will have to be done to counsel the patient and also regarding the next pregnancy. Particular chromosome probes are used in such cases. We can prove cases of insertion and also the origin of such unknown material/segment.

Additional techniques of Molecular cytogenetics are being used nowadays, which are Comparative Genomic Hybridization

(CGH), M-FISH, Spectral karyotyping (SKY), FIBER-FISH etc. Among these, M-FISH and SKY are the most widely used. Both these techniques are essentially whole chromosome paints where a different colour is imparted to each of the 23 chromosomes so as to be able to make out the breakage/transfer of segments onto other fellow chromosomes or just deletions. All such detection is done by the capturing of fluorescent signals and hence, it goes without saying that we do these procedures with sophisticated fluorescent microscopes.

NUTRI-GENETICS & FETAL DEVELOPMENT NEURAL TUBE DEFECT (NTD) AS A MODEL

Dr. Koumudi Godbole

Nutritional genomics is a science studying the relationship between human genome, nutrition and health. It can be divided into two disciplines:

a) Nutrigenomics: studies the effect of nutrients on health through altering genome, proteome, metabolome and the resulting changes in physiology.

b) Nutrigenetics: studies the effect of genetic variations on the interaction between diet and health with implications to susceptible subgroups. More specifically, nutrigenomics studies how individual differences in genes influence the body's response to diet and nutrition. For example, people with an enzyme deficiency caused by mutations in the enzyme phenylalanine hydroxylase cannot metabolize foods containing the amino acid phenylalanine and must modify their diets to minimize consumption. With modern genomic data, severe gene mutations with less severe effects are being explored to determine whether dietary practices can be more closely personalized to individual genetic profiles. However, there have been few validated studies for these kinds of

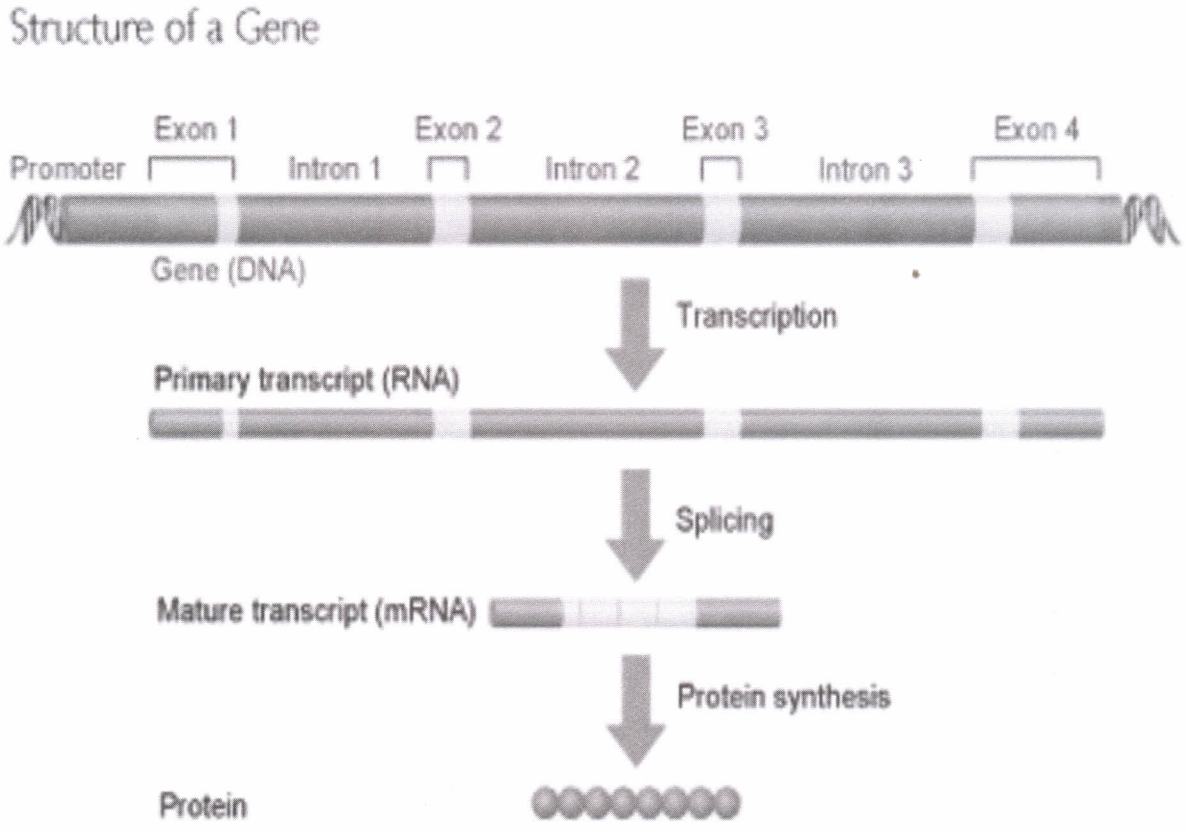


Figure -1 showing the structure of the gene

Dr.(Mrs.) Koumudi G Godbole, a pediatrician is a Fellow of the Canadian College Clinical Geneticist. Presently she is a consulting clinical Geneticist at DeenanathMangeshkar Hospital and a Research Associate at the Diabetes unit of the KEM Hospital Research Centre, Pune.

classical gene mutation effects.

***Structure* of a gene**

There are two general types of gene in the human genome: **non-coding RNA genes** and **protein-coding genes.**

Non-coding RNA genes represent 2-5 per cent of the total and encode functional RNA molecules. Many of these RNAs are involved in the control of gene expression, particularly protein synthesis. They have no overall conserved structure.

Protein-coding genes represent the majority of the total and are expressed in two stages: transcription and translation

The boundaries of a protein-encoding gene are defined as the points at which transcription begins and ends. The core of the gene is the coding region, which contains the nucleotide sequence that is eventually translated into the sequence of amino acids in the protein. The coding region begins with the initiation codon, which is normally ATG. It ends with one of three termination codons: TAA, TAG or TGA. On either side of the coding region are DNA sequences that are transcribed but are not translated. These untranslated regions or non-coding regions often contain regulatory elements that control protein synthesis.

Both the coding region and the untranslated regions may be interrupted by introns. Most human genes are divided into exons and introns. The **exons** are the sections that are found in the mature transcript *(messenger RNA), while the* **introns** are removed from the primary transcript by a process called **splicing**.

"Central Dogma" of Biology

The majority of genes are expressed as the proteins they encode. The process occurs in two steps:

- **Transcription = DNA → RNA**
- **Translation = RNA → protein**

Transcription is the process of creating a complementary RNA copy of a sequence of DNA. Both RNA and DNA are nucleic acids, which use base pairs of nucleotides as a complementary language that can be converted back and forth from DNA to RNA by the action of the correct enzymes. During transcription, a DNA sequence is read by an RNA polymerase, which produces a complementary, antiparallel RNA strand. As opposed to DNA replication, transcription results in an RNA complement that includes uracil (U) in all instances where thymine (T) would have occurred in a DNA complement.

Transcription is explained easily in 4 or 5 steps, each moving like a wave along the DNA.

1. RNA Polymerase moves the transcription bubble, a stretch of unpaired nucleotides, by breaking the hydrogen bonds between complementary nucleotides.

2. RNA Polymerase adds matching RNA nucleotides that are paired with complementary DNA bases.

3. RNA sugar-phosphate backbone forms with assistance from RNA polymerase.

4. Hydrogen bonds of the untwisted RNA+DNA helix break, freeing the newly *synthesized RNA strand.*

5. *If the cell has a nucleus, the RNA is* further processed (addition of a 3' poly-A tail and a 5' cap) and exits through to the cytoplasm through the nuclear pore complex.

***Transcription** is the first step leading to gene*

expression. The stretch of DNA transcribed into an RNA molecule is called a *transcription unit* and encodes at least one gene. If the gene transcribed encodes a protein, the result of transcription is messenger RNA (mRNA), which will then be used to create that protein via the process of **translation**

Mutation

In molecular biology and genetics, **mutations** are changes in a genomic sequence: the DNA sequence of a cell's genome or the DNA or RNA sequence of a virus. They can be defined as sudden and spontaneous changes in the cell. Mutations are caused by radiation, viruses, transposons and mutagenic chemicals, as well as errors that occur during meiosis or DNA replication. They can also be induced by the organism itself, by cellular processes such as **hypermutation.**

Mutation can result in several different types of change in sequences; these can either have no effect, alter the product of a gene, or prevent the gene from functioning properly or completely.

Classification of mutation types

1) By effect on structure

The sequence of a gene can be altered in a number of ways. Gene mutations have varying effects on health depending on where they occur and whether they alter the function of essential proteins. Mutations in the structure of genes can be classified as:

Small-scale mutations, such as those affecting a small gene in one or a few nucleotides, including:

Point mutations, often caused by chemicals or malfunction of DNA replication, exchange a single nucleotide for another. A point mutation can be reversed by another point mutation, in which the nucleotide is changed back to its original state (true reversion) or by second-site reversion (a complementary mutation elsewhere that results in regained gene functionality). Point mutations that occur within the protein coding region of a gene may be classified into three kinds, depending upon what the erroneous codon codes for:

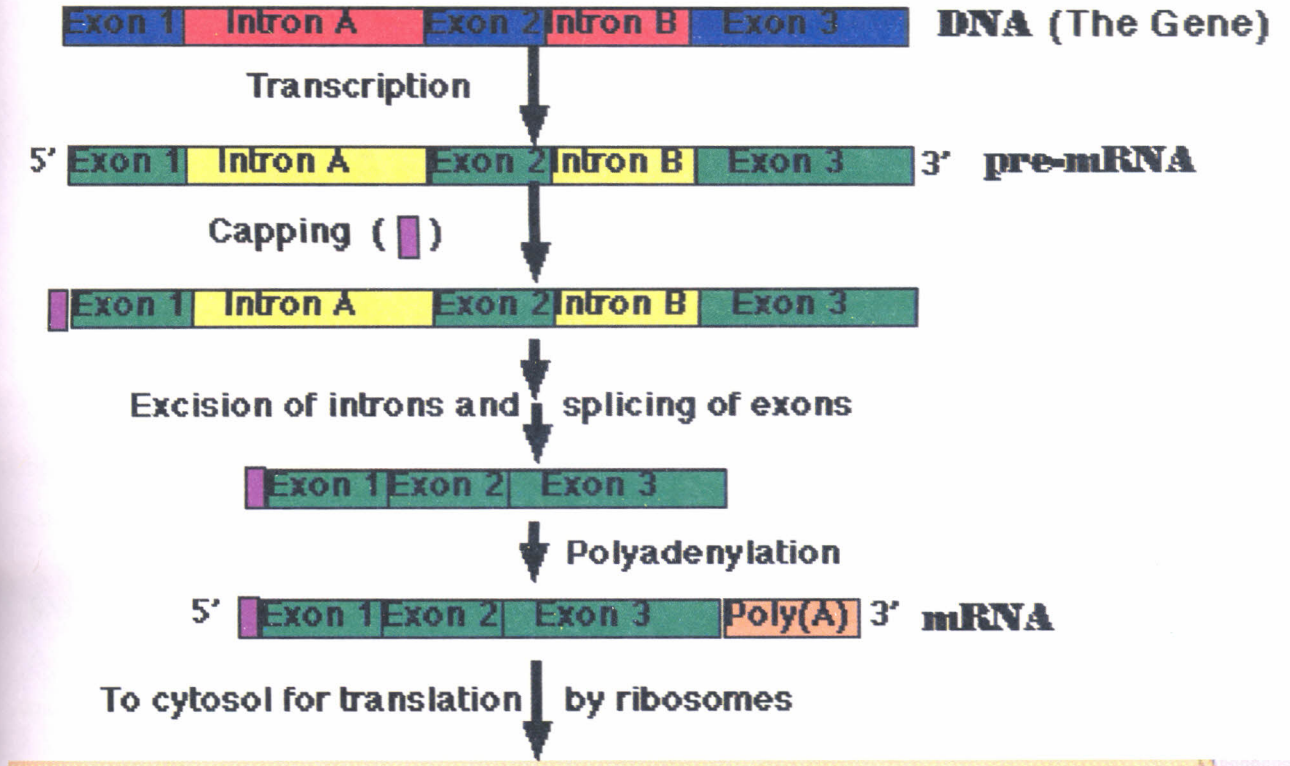
- **Silent mutations**: which code for the same amino acid.
- **Missense mutations**: which code for a different amino acid.
- **Nonsense mutations**: which code for a stop and can truncate the protein.

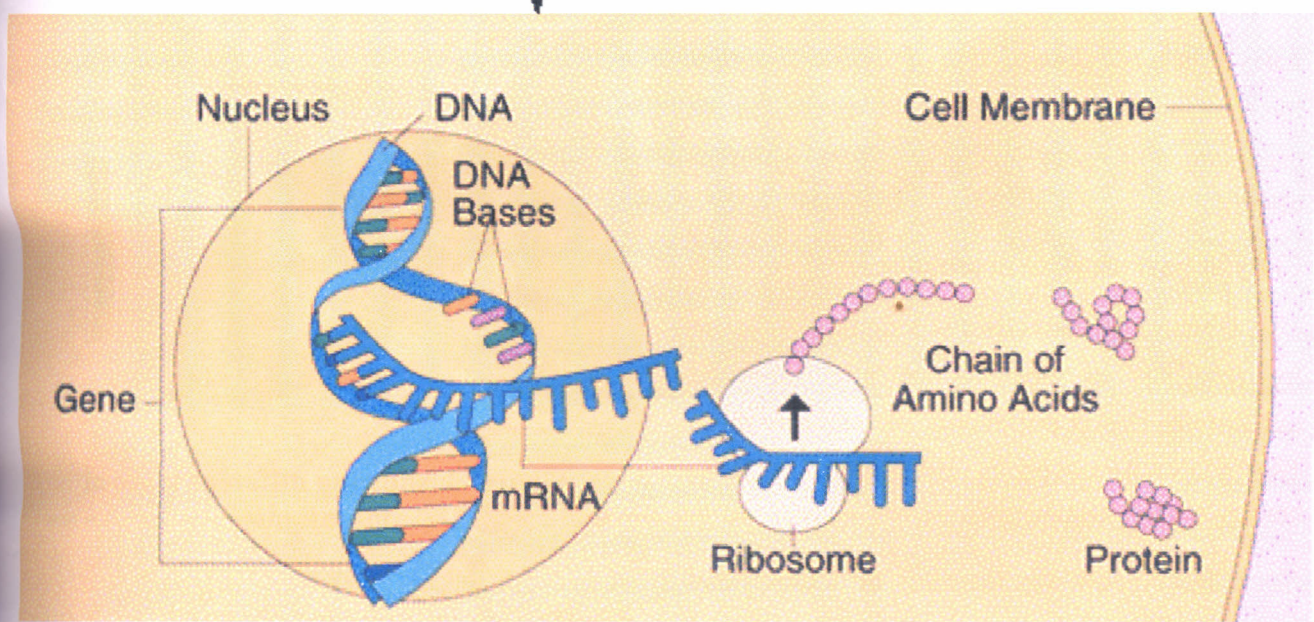
Insertions add one or more extra nucleotides into the DNA. They are usually caused by transposable elements, or errors during replication of repeating elements. Insertions in the coding region of a gene may alter splicing of the mRNA (**splice site mutation**), or cause a shift in the reading frame (**frameshift**), both of which can significantly alter the gene product. Insertions can be reverted by excision of the transposable element.

Deletions remove one or more nucleotides from the DNA. Like insertions, these mutations can alter the reading frame of the gene. They are generally irreversible

Large-scale mutations in chromosomal structure, including:

- **Amplifications** (or gene duplications) leading to multiple copies of all chromosomal regions, increasing the dosage of the genes located within them.
- **Deletions** of large chromosomal regions,





5' ...A T G G C C T G G A C T T C A... 3' Sense strand of DNA

3' ...T A C C G G A C C T G A A G T... 5' Antisense strand of DNA

Transcription of antisense strand

5' ...A U G G C C U G G A C U U C A... 3' mRNA

Translation of mRNA

Met — Ala — Trp — Thr — Ser — Peptide

Figure 2 showing the process of transcription and translation

leading to loss of the genes within those regions.

o Mutations whose effect is to juxtapose previously separate pieces of DNA, potentially bringing together separate genes to form functionally distinct fusion genes (e.g. bcr-abl). These include:

- **Chromosomal translocations**: interchange of genetic parts from nonhomologous chromosomes.

□ **Interstitial deletions**: an intra-chromosomal deletion that removes a segment of DNA from a single chromosome, thereby apposing previously distant genes.

□ **Chromosomal inversions**: reversing the orientation of a chromosomal segment.

Loss of heterozygosity: loss of one allele, either by a deletion or recombination event, in an organism that previously had two different alleles.

2) **By impact on protein sequence**

□ A **frameshift mutation** is a mutation caused by insertion or deletion of a number of nucleotides that is not evenly divisible by three from a DNA sequence. Ex: BAT ATEF ATR----------deletion (frame shift)

□ In contrast, any insertion or deletion that is evenly divisible by three is termed an *in-frame mutation.Ex:* BAT … FAT RAT--------deletion in frame)

□ A **nonsense mutation** is a point mutation in a sequence of DNA that results in a premature stop codon, or a *nonsense codon* in the transcribed mRNA, and possibly a truncated, and often nonfunctional protein product.

□ **Missense mutations** or *nonsynonymous mutations* are types of point mutations where a

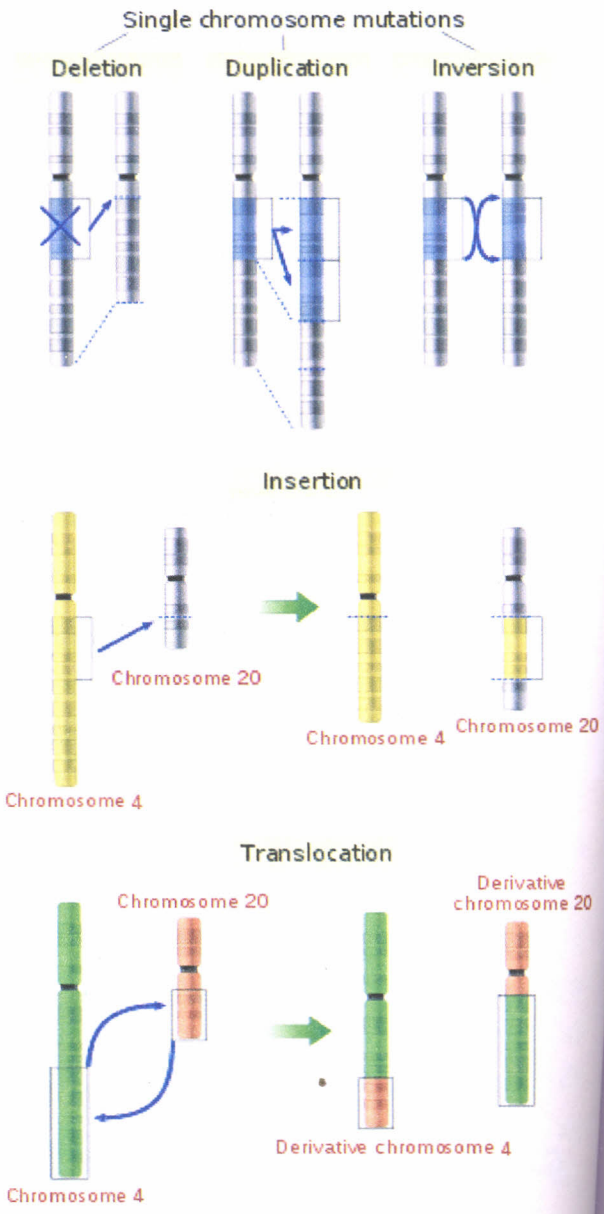


Figure -3 showing different types of mutations

single nucleotide is changed to cause substitution of a different amino acid. This in turn can render the resulting protein nonfunctional. Ex: **B**AT ATE FAT RAT------- mis-sense

□ A **neutral mutation** is a mutation that occurs in an amino acid codon which results in the use of a different, but chemically similar, amino acid. The similarity between the two is enough that little or no change is often rendered

in the protein. For example, a change from AAA to AGA will encode arginine, a chemically similar molecule to the intended lysine.

- **Silent mutations** are mutations that do not result in a change to the amino acid sequence of a protein. They may occur in a region that does not code for a protein, or they may occur within a codon in a manner that does not alter the final amino acid sequence. Silent mutations occur because of the degenerate nature of the genetic code.

SNP: Single Nucleotide Polymorphism

A **single-nucleotide polymorphism** (**SNP**, pronounced *snip*) is a DNA sequence variation occurring when a single nucleotide A, T, C or G in the genome (or other shared sequence) differs between members of a biological species or paired chromosomes in an individual. For example, two sequenced DNA fragments from different individuals, AAGCCTA to AAGCTTA, contain a difference in a single nucleotide. In this case we say that there are two *alleles*: C and T. Almost all common SNPs have only two alleles. The genomic distribution of SNPs is not homogenous, SNPs usually occur in non-coding regions more frequently than in coding regions or, in general, where natural selection is acting and fixating the allele of the SNP that constitutes the most favorable genetic

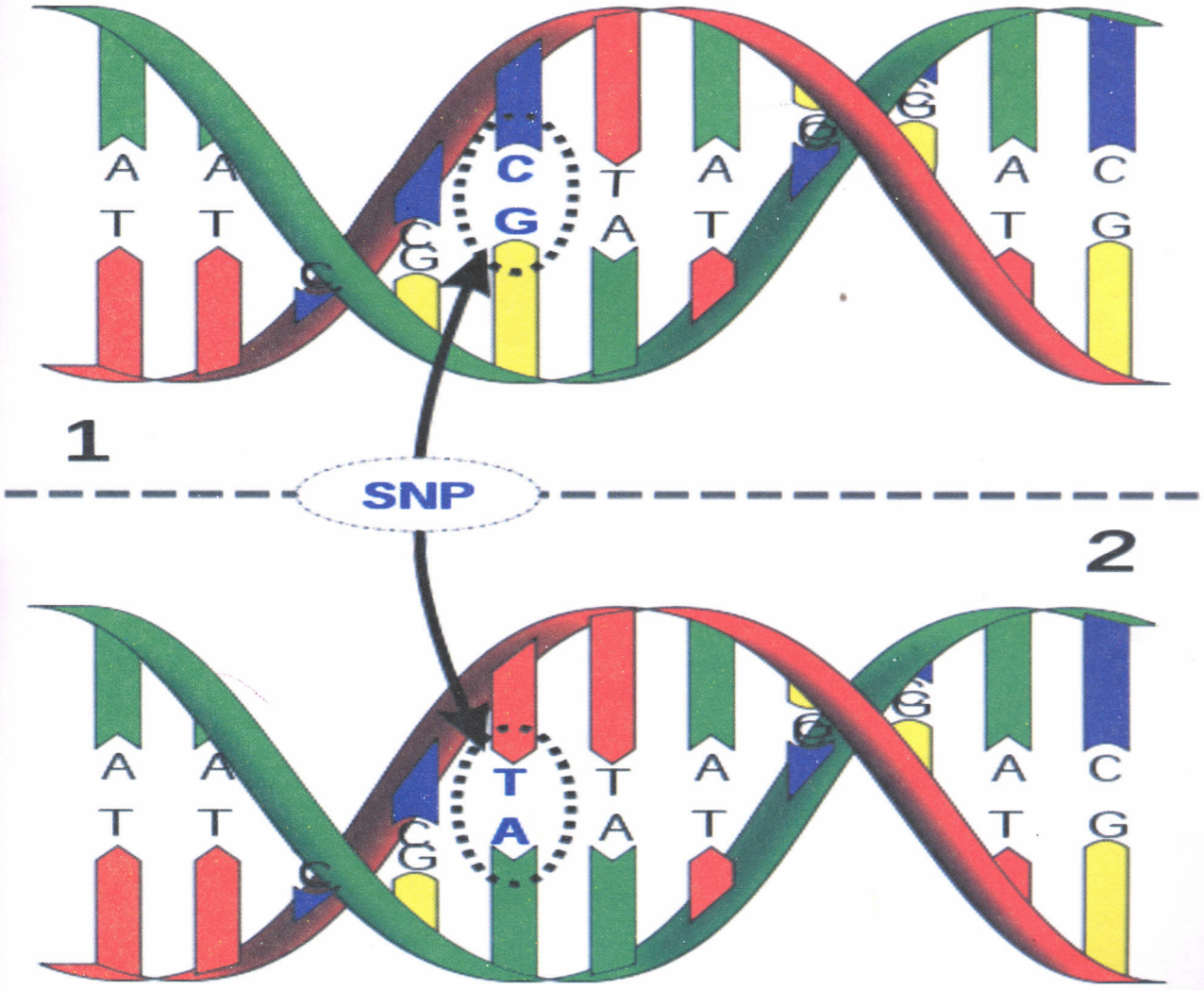


Figure -4 showing single nucleotide polymorphism

adaptation.

Within a population, SNPs can be assigned a minor allele frequency the lowest allele frequency at a locus that is observed in a particular population. This is simply the lesser of the two allele frequencies for single-nucleotide polymorphisms. There are variations between human populations, so as a SNP allele that is common in one geographical or ethnic group may be as much rarer in another.

These genetic variations between the individuals (particularly in the non-coding parts of genome) are exploited in DNA fingerprinting, which is used in forensic science. Also, these genetic variations underlie differences in our susceptibility to, or protection from all kinds of diseases.

Epigenetics

Epigenetics is one of the most rapidly expanding fields in biology. The recent characterization of a human DNA methylome at single nucleotide resolution, the discovery of the CpG island shores, the finding of new histone variants and modifications, and the unveiling of genome-wide nucleosome positioning maps highlight the accelerating speed of discovery over the past two years. Increasing interest in epigenetics has been accompanied by technological breakthroughs that now make it possible to undertake large-scale epigenomic studies. These allow the mapping of epigenetic marks, such as DNA methylation, histone modifications and nucleosome positioning, which are critical for regulating gene and noncoding RNA expression. In turn, we are learning how aberrant placement of these epigenetic marks and mutations in the

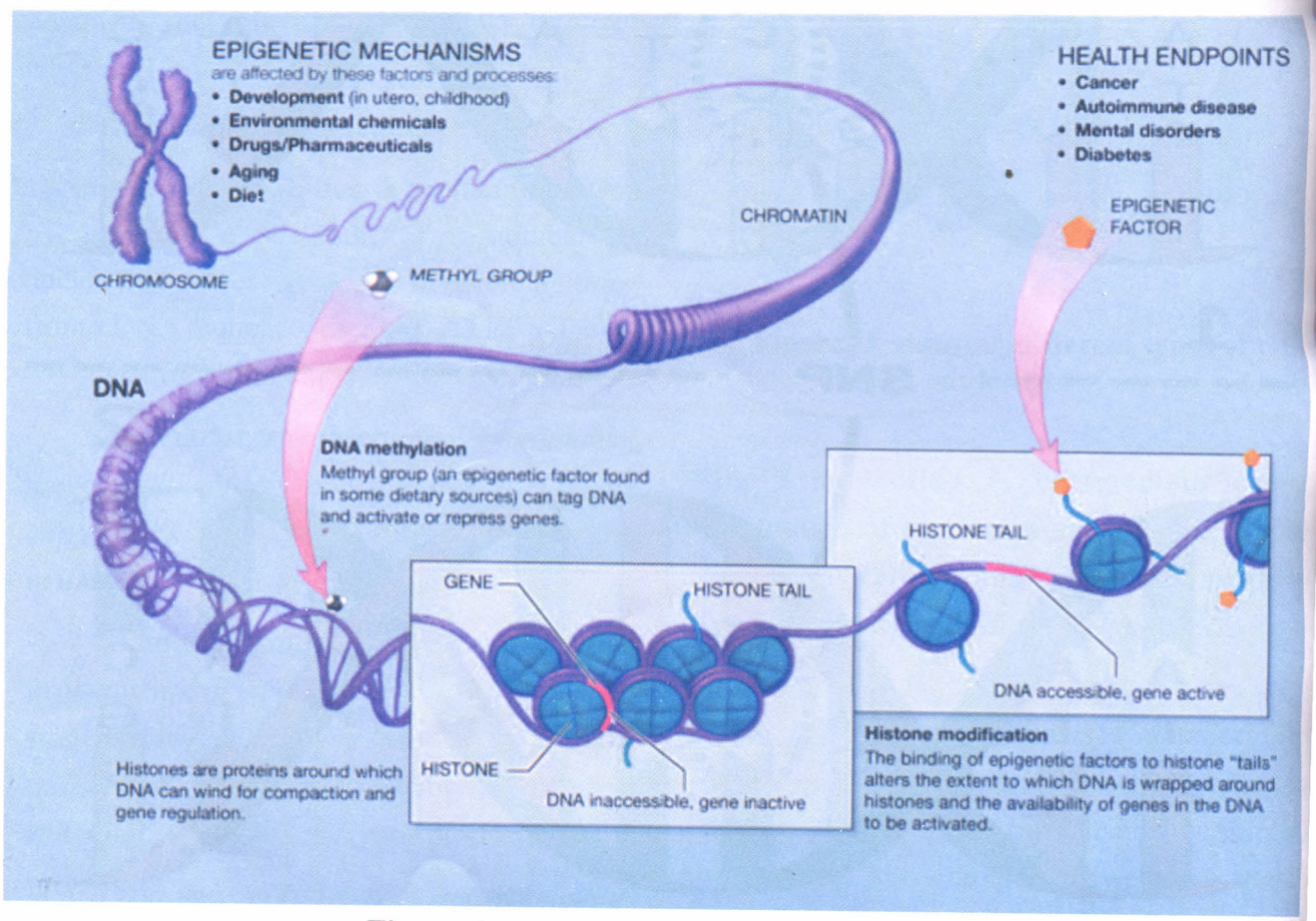


Figure -5 showing epigenetic modifications

epigenetic machinery is involved in disease. Thus, a comprehensive understanding of epigenetic mechanisms, their interactions and alterations in health and disease, has become a priority in biomedical research.

Epigenetic Modification

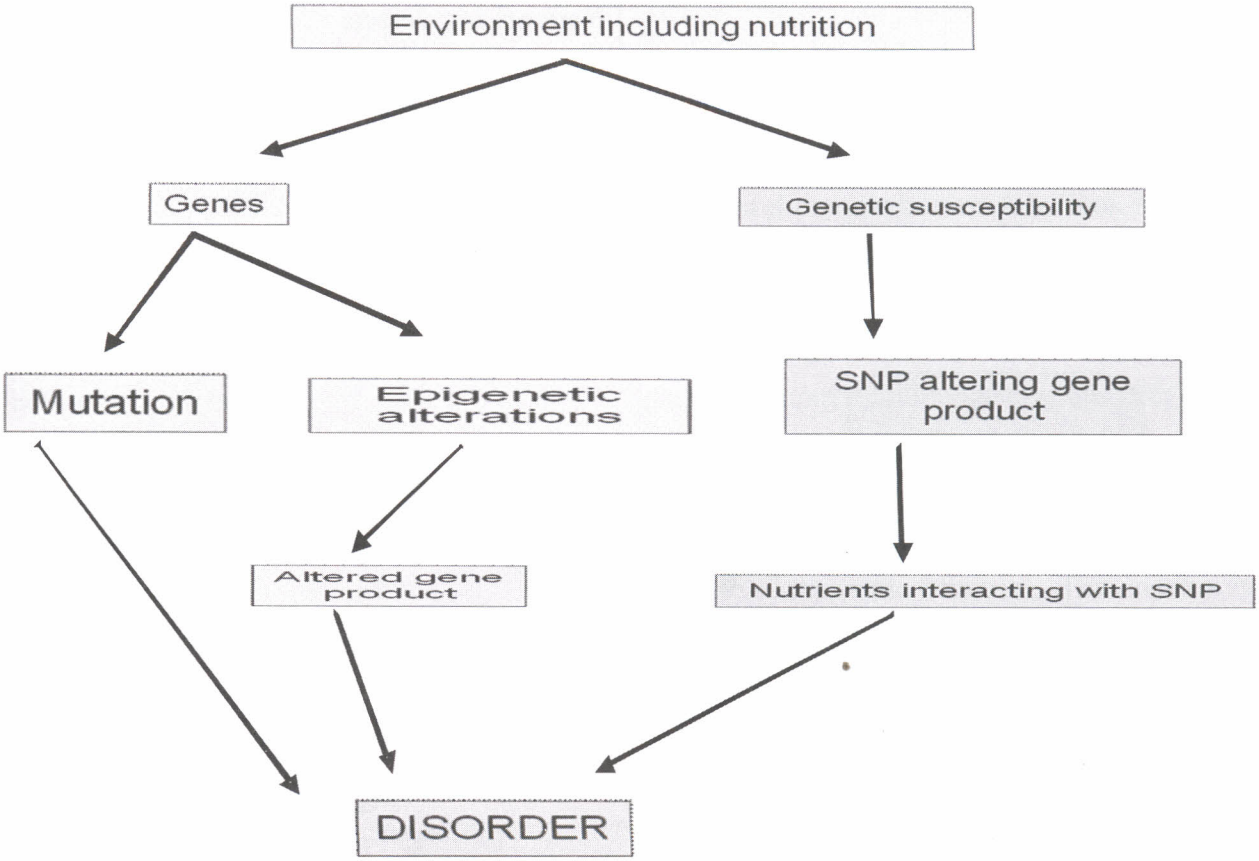
This includes alteration in gene function without altering DNA sequence. Some of the genes are not inert and in fact interact with the environment. Such changes are dynamic and can occur across lifetime. The changes may be generalized or tissue specific and are often related to developmental stage.

Gene Silencing by Histone Deacetylation

Deacetylation removes acetyl groups from histone tails, causing the DNA to wrap more tightly around the histones and blocks access by to transcription factors. The overall result of histone deacetylation is a reduction in gene expression.

Gene Silencing by DNA methylation at CpG islands

CpG" stands for cytosine and guanine separated by a phosphate which links the two nucleosides together in DNA. Cytosines in CpG dinucleotides are methylated by DNA methyltransferase to 5, methyl-cytosine. Methylation suppresses while demethylation allows gene expression.



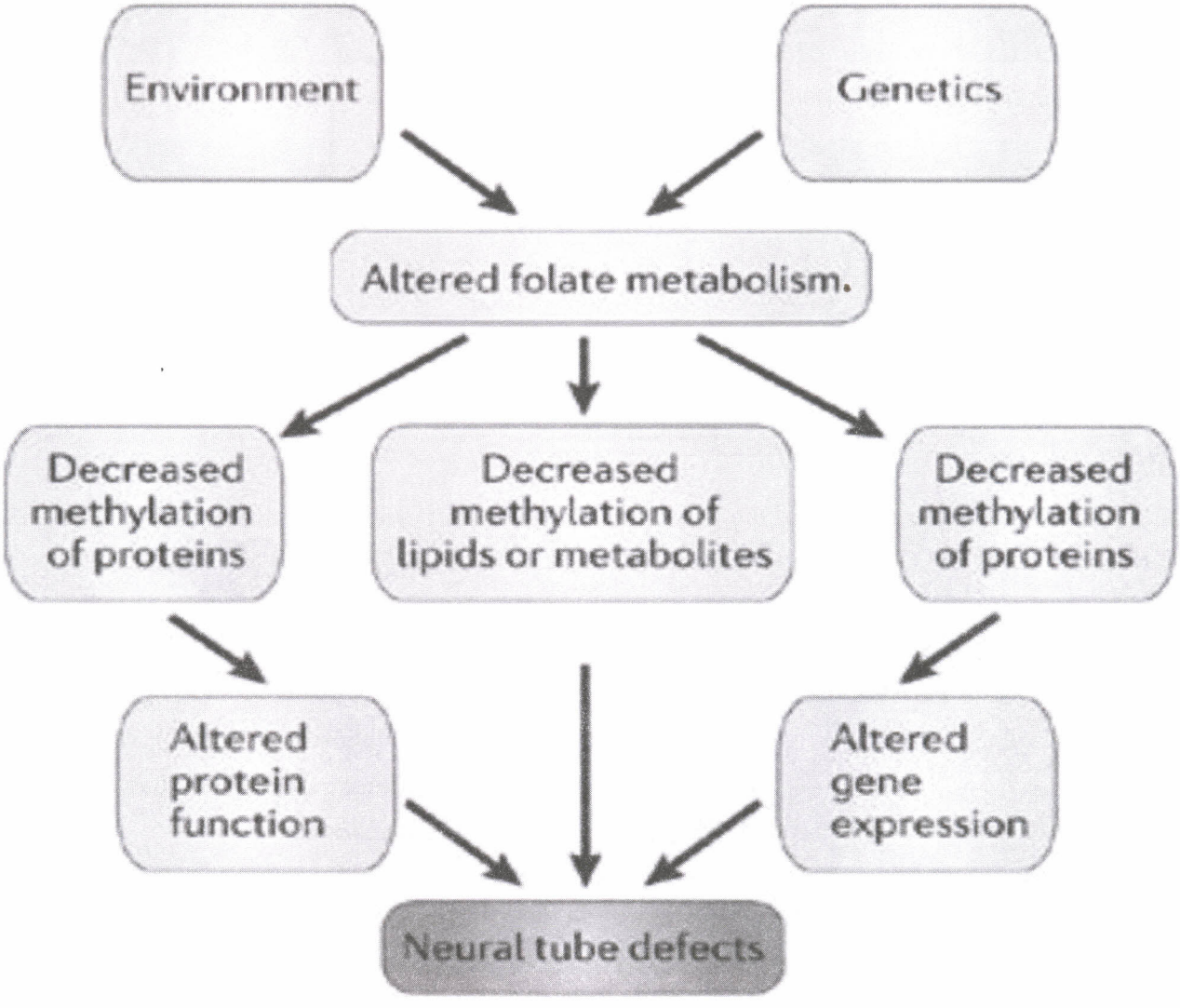
Flow chart showing how environmental factors can cause genetic disorders

Birth defects: Mechanisms

- Malformation: genetic origin
  - Neural tube defect
- Deformation: modification of a normally growing structure, often by mechanical

Developmental Aberrations

Gametes	Embryo	Foetus	Post natal Adults
Gametogenesis	Organogenesis Hyperplasia	Hyperplasia Hypertrophy	Growth and maturation
Infertility Early pregnancy loss	Miscarriage Birth defects	Birth defects IUGR Networking defects	Altered growth and development Lifestyle disorders Fertility issues Malignancies



Flow chart depicting factors causing neural tube defects

factors

- Congenital Talipes Equino Varis

• Disruption: modification of a normally growing structure, often by vascular accidents

- Radial ray defect

• Sequence: chain of events following one basic abnormality

- Pierre-Robin sequence: micrognathia-cleft palate

Neural tube defects

They are of 3 types

1) Open defects Primary neural tube closure defects. Ex: Anencephaly, Myelomeningocele, craniorachischisis.

2) Herniation defects Primary axial mesodermal defects with herniation of neural tissue. Ex:Encephalocele, Meningocele.

Closed defects Tail bud (secondary neurulation defects). Ex:Spina bidfida occulta, diastematomyelia, Tethered cord.

Environmental factors

a) Nutritional deficiency vit B12/ Folate/ Choline/ Zinc

b) Maternal diabetes, drugs, infections

Genetic factors

a) Single gene defects Meckel Gruber syndrome

b) Genetic susceptibility- MTHFR, TCN2

Background

• The reported incidence of neural tube defects (NTDs) in India is high, up to 11.4/1000 births in some states.

• No systematic study to explore etiology including micronutrient deficiencies including folate, vitamin B_{12}.

• No national policy on periconceptional vitamin (folate) supplementation.

• Available data suggests lower prevalence of folate deficiency and higher prevalence of B_{12} deficiency, possibly due to predominant vegetarian dietary habits.

• Inadequate data on polymorphisms in 1-carbon metabolism genes including MTHFR in Indian population.

A study conducted by Godbole et al titled **"Maternal one-carbon metabolism, MTHFR and TCN2 genotypes and neural tube defects in India" showed** significant association of high maternal

plasma homocysteine concentrations with NTDs in the offspring. There was no association of maternal folate or B12 levels with NTDs but low maternal holo-transcobalamin predicted strong risk of NTDs in the offspring. The commonly associated maternal polymorphism 677C>T in the MTHFR gene did not predict risk of NTDs in the offspring. 1298A>C and 1781G>A polymorphisms in MTHFR were protective. Maternal 776C>G polymorphism in TCN2 was strongly predictive of NTD in the offspring.

So they concluded that maternal B12 deficiency played a possible role in the etiology of NTDs in India over and above the well-established role of folate deficiency.

Prevention of Neural Tube Defects

• Creating awareness about role of micronutrients

- Especially during peri-conception period
- In public and professionals alike

• Defining magnitude of micronutrient deficiencies across the country

DIAGNOSIS, ASSESSMENT AND INTERVENTION STRATEGIES FOR AUTISM SPECTRUM DISORDERS AT SPASTICS SOCIETY OF KARNATAKA, BANGALORE

Mrs.R.Krishnaswamy, Mrs.Mamtha, Mrs.Sarita Alexander and Dr.Nalini Menon

The Historical beginning of Spastic Society of Karnataka dealing with Autism Spectrum Disorders dates back to late 1980s.

Definition:

AUTISM is a behaviourally defined developmental disorder in children below 3 years of age affecting communication skills, social skills, thought and behaviour and processing and integrating sensory information. Autism Spectrum Disorder (ASD) is an umbrella term used to indicate a wide ranging severity and manifestation of impairments in 3 basic areas which is defined as a triad of social interaction & understanding, all aspects of communication (verbal and non-verbal) and flexibility of thinking and behaviour, including problems with imagination.

The different conditions that get included in ASD are:

Asperger Syndrome: This is the high functioning side of the spectrum with the children having near-normal speech and cognitive skills along with poor social skills, narrow, but specialized interests, inflexible adherence to non-functional routines and rituals. Many of them may have motor clumsiness.

Atypical Autism (PDD-NOS): This is a milder version of autism where the child does not show all the features to qualify for a diagnosis of autism or the age of onset may be more than 3 years.

Autism: This refers to children who satisfy the full criterion for diagnosis of autism.

Rett Syndrome: It is a degenerative disorder which affects mostly females and generally develops between 6 and 18 months of age. Some of their features are loss of speech, repetitive hand wringing, body rocking and social withdrawal. This may be accompanied by significant mental retardation.

Childhood Disintegrative Disorder (also called Regressive Autism): The child will have a clear evidence of apparently normal development in the areas of communication, social relationship, play and adaptive behaviour for at least the first 2 years of life. Subsequently, the child will show all the features of autism.

Incidence of ASD: A disorder which was considered a rare one in the 70s and 80s is recording incidence figures of 1 in 150 in countries like the USA and the UK. Figures in India could range between 1 in 250 - 500. A variety of factors like a high degree of awareness and fluidity of the diagnostic criteria may be partly responsible. ASD has a prevalence of is 60 /10,000 including Autism (20 /10,000) and

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Dr. NaliniMenon, Ph.D, has been working in the field of special education, especially autism for 23 years. Currently, she heads the Rainbow Centre for Autism at Spastics Society of Karnataka, Bangalore.

Asperger/ PDDNO (40 /10,000) which is much more common than Down syndrome. Male to female ratio of Autism and Asperger Syndrome is 3-4:1 and 10-15:1 respectively.

Early concerns of autism in children are:

Communication concerns:

- Does not respond to name, unable to tell what he wants.
- Language is delayeddoes not follow instructions -- appears deaf at times.
- Does not point or wave bye.
- Used to say a few words, but tend to be mute.

Social Concerns:

- Does not smile socially.
- Seems to prefer to play alone.
- Gets things for himself/herself -is very independent (due to inability to seek help).
- Has poor eye contact.
- Is in his own world.
- Is not interested in other children.

Behavioural concerns:

- Temper tantrums
- Hyperactivity
- Unco-operative and oppositional
- Do not know how to play with toys
- Toe walking
- Unusual attachment to objects
- Tendency to line things up in a particular way
- Play is stereotypical and repetitive
- Oversensitive to certain textures, sounds, taste and smell
- Odd movement patterns: body rocking, finger play, hand flapping, jumping etc

Intelligence: Ranges from severe retardation to giftedness

Splinter skills:

- May be hyperlexic
- Very good at decoding skills
- Extremely good with puzzles
- Amazing calendar skills
- Good with calculations
- Amazing visual memory
- Exceptional talent in language and music

Mood swings: Laughing and crying spells for no apparent reason

Attention problem: Ranges from extreme distractibility to perseveration

Sleeping and eating disturbances are present.

Sensory problems:

- Sensory defensiveness: avoid sensory stimuli from environment
- Sensory seeking: Seeking different sensory experiences

Seizures occur in early childhood and adolescence in >30%

Absolute Indications for Immediate Further Evaluation -**"Red Flag Signs":**

- No babbling by 1 year.
- No gesturing (pointing, waving bye) by 1 year.
- No single word by 16 months.
- No 2 word spontaneous (not just echolalia) by 2 years.
- No joint attention by 18 months.
- No imitation/ pretend play by 18 months.
- ANY loss of ANY language or social skills.

Diagnostic criteria for Autistic Disorder: DSM IV (Diagnostic and Statistical Manual)*A. A total of six (or more) items from (1), (2), and (3), with at least two from (1), and one each from (2) and (3):*

(1) Qualitative impairment in social interaction, as manifested by at least two of the following:

(a) Marked impairment in the use of multiple nonverbal behaviors such as eye-to-eye gaze, facial expression, body postures, and gestures to regulate social interaction

(b) Failure to develop peer relationships appropriate to developmental level

(c) A lack of spontaneous seeking to share enjoyment, interests, or achievements with other people (e.g., by a lack of showing, bringing, or pointing out objects of interest)

(d) Lack of social or emotional reciprocity

(2) Qualitative impairments in communication as manifested by at least one of the following:

(a) Delay in or total lack of, the development of spoken language (not accompanied by an attempt to compensate through alternative modes of communication such as gesture or mime)

(b) In individuals with adequate speech, marked impairment in the ability to initiate or sustain a conversation with others

(c) Stereotyped and repetitive use of language or idiosyncratic language

(d) Lack of varied, spontaneous make-believe play or social imitative play appropriate to developmental level

(3) Restricted repetitive and stereotyped patterns of behavior, interests, and activities, as manifested by at least one of the following:

(a) Encompassing preoccupation with one or more stereotyped and restricted patterns of interest that is abnormal either in intensity or focus

(b) Apparently inflexible adherence to specific, nonfunctional routines or rituals

(c) Stereotyped and repetitive motor mannerisms (e.g., hand or finger flapping or twisting, or complex whole-body movements)

(d) Persistent preoccupation with parts of objects

B. Delays or abnormal functioning in at least one of the following areas, with onset prior to age 3 years:

- Social interaction,
- Language as used in social communication,
- Symbolic or imaginative play.

C. The disturbance is not better accounted for by Rett's Disorder or Childhood Disintegrative Disorder.

About DEC: Spastic Society of Karnataka dealing with Autism Spectrum Disorders and is also associated with a sister organization called Developmental Centre for Exceptional Children (DEC) which was established in 1986 with Dr.M.S.Mahadeviah, Mrs.Rukmini Krishnaswamy and Dr.Nandini Mundkur as founder members. 1st child with Autism was seen in the centre in a 5 year old girl in the late 1980's. During 1991, DEC organized perhaps the first scientific seminar in India on autism. Over a period of 10 years (1991-2000), around 100 children with autism were seen at DEC. The small sporadic number of cases was perhaps due

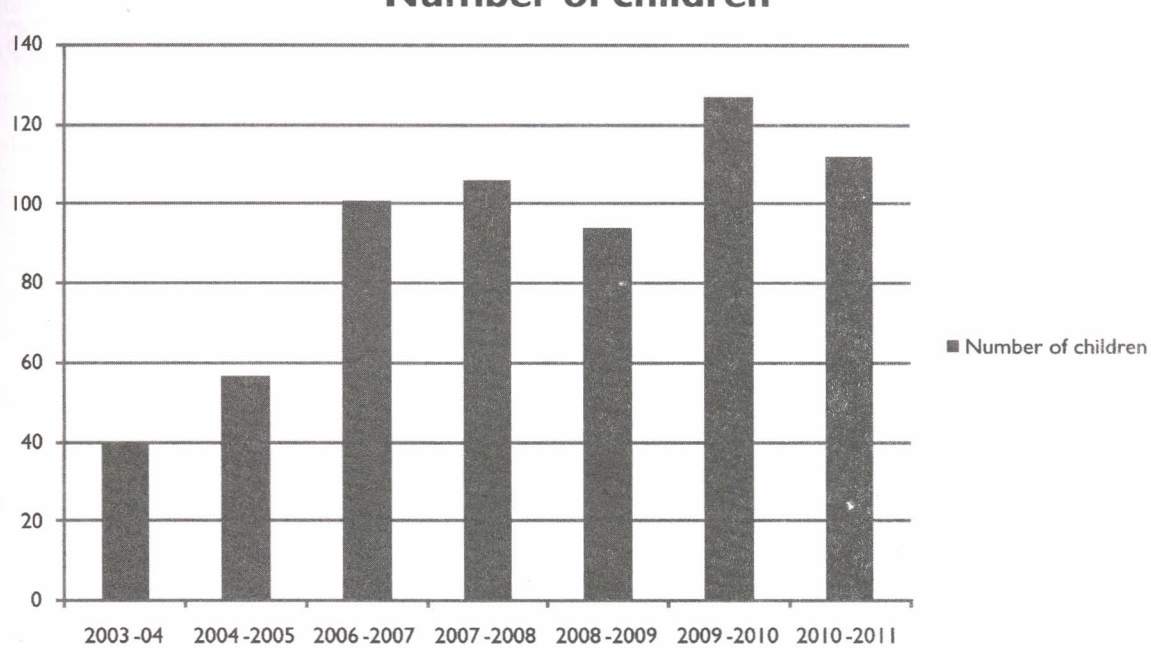

Figure 1: Prevalence of children with autism at DEC

	< 5 yrs of age	< 10 yrs of age	< 15 yrs of age	< 16 yrs of age	Total no. of cases
2011	48	57	15	6	126
2010	22	79	18	5	124
2009	2	67	24	5	98

to gross unawareness of the condition of autism amongst the medical fraternity and lack of awareness of the existence of the centre.

Diagnostic Tools used are the following:
Childhood Autism Rating Scale (CARS)
DSM IV R. (Diagnostic and Statistical Manual)
Prevalence of Autism at DEC: The maximum number of cases seen over a period of 2 years (from 2003-2005) was 97. There was a significant increase during the subsequent years to an average of 110-120 cases from the year 2007 onwards (Fig: 1).

There was a big jump in the number of children below the age of 5 years who were being referred to **DEC** indicating an enhancement of awareness among medical professionals as well as general public (Table 1)

An apparent higher prevalence among the higher income groups and parents with technical educational background was observed (Fig 2).

A brief account of system Diagnostics, Assessment and Intervention:

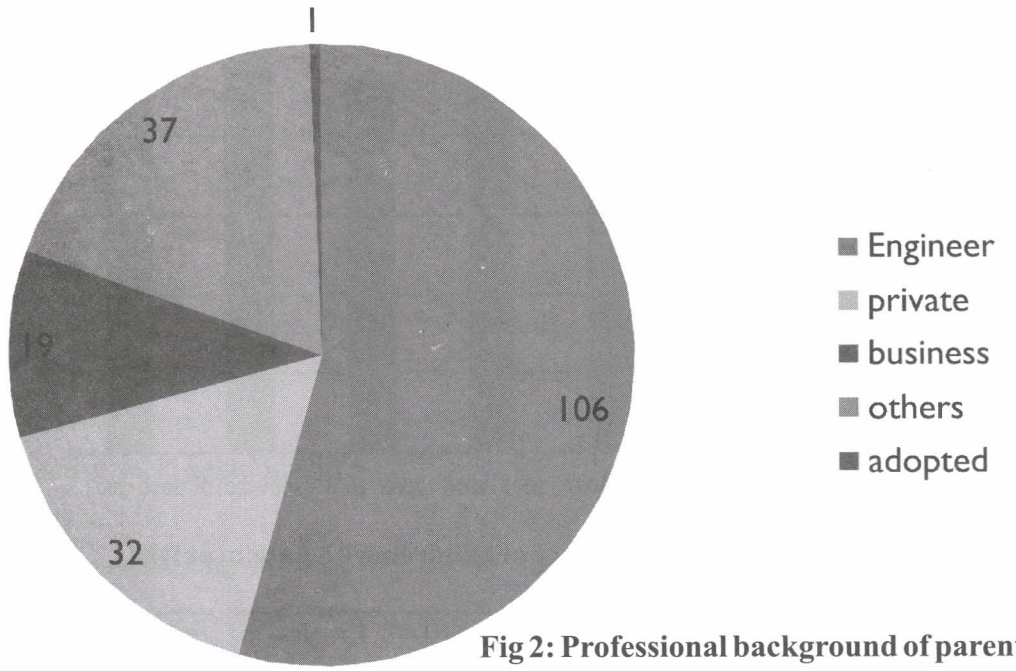

Fig 2: Professional background of parents

The team comprises of medico social workers, pediatric neurologist, child psychiatrist, speech therapists, occupational therapists, clinical psychologist and special educators. After a detailed interview with parents to collect all relevant information regarding child's personal information, family medical history, presenting complaints in different areas like vision, hearing, motor skills, communication skills, behaviour etc., the child is referred to the pediatric neurologist and/or the child psychiatrist for a correct diagnosis. Then the child is referred for various evaluations of behaviour, sensory issues, speech, communication and cognitive issues. After the evaluation, parents are counseled regarding the child's problems and the course of interventions that would benefit the child.

At Rainbow Centre, the following services are currently offered:

- Parent training programme for early intervention for children who have been just diagnosed.
- Group programme for school age and adolescent children.
- Individual therapies for behaviour modification, speech and sensory integration.

Some of the western countries have been reporting alarming increases in the incidence of ASD around 1 in 100 live births. In India also, there is a significant increase in the numbers, but there is lack of documentation to prove it unambiguously. Many professionals who have worked in the rural areas have noticed extremely low rates of incidence of ASD compared to other developmental disabilities. The increasing numbers seem to have serious long term implications in terms of the economic and social responsibilities to the community. Current trends stress the need for prevention in addition to cure. It has been fairly established that autism has a strong genetic origin along with environmental influences. In the light of this it is

important to substantiate or rule out some of the trends like rural urban bias, regional and socio-economic bias.

The following studies need to be done in a very systematic and scientific manner:

- Epidemiological data collection in both urban and rural areas.
- Adaptation and validation of existing screening and diagnostic tools.

Any inferences and insights emerging from these studies should go a long way in identifying probable environmental factors triggering autism in genetically vulnerable groups.

DISEASE BURDEN DUE TO ARSENIC AND FLUORIDE CONTAMINATION IN DRINKING WATER: ENVIRONMENTAL PERSPECTIVES AND POSSIBLE REMEDIATION

Dr. Samiran Mahapatra

Providing pure uncontaminated drinking water to the community is of utmost importance. Drinking water can have different types of impurities like microbes, organic chemicals, pesticides, chlorine, inorganic common salts, particulate matter, arsenic & fluoride. The consequences of drinking such water can have mild to serious effects on health. The probability of these being present can vary from rare for arsenic and fluoride to high for microbes and particulate matter in water.

The contaminants in drinking water can be classified into dissolved ions/ compounds and insoluble matters. The dissolved ions are further classified into 5 classes and insoluble matters into 2 classes.

To make water palatable it has to be free from microbes and chemicals. The microbial contaminants in drinking water are bacteria (E.coli, salmonella, V.cholera) , viruses (polio, rota, hepatitis A) and parasites (cryptosporidium parvum, Giardia lamblia). The normal BIS standard is no fecal coliforms/ 100ml, but it was present in the samples obtained from different states of India.

The drinking water from South Indian metros had a viral load of entero virus ranging from 10^2-10^5 per L with MS2 phage (80%), hepatitis E (78%), hepatitis A(66%) and rota virus (66%) respectively. Even the presence of entamoeba histolytica and Giardia lamblia was very high in various cities of India.

The BIS specification for safe drinking water (microbial contamination) is absence of coliforms. But viruses and cysts are found in treated drinking water even in the absence of coliforms which are not picked up.

Hence USEPA established that any unknown water could be turned as microbiologically safe drinking water if log 6, log4 and log3 bacteria , viruses and parasites respectively are removed by any purifier which is the toughest global drinking water standard. WHO thinks that provision of safe water alone will reduce diarrhoeal and enteric diseases by upto 50% even in the absence of improved sanitation or other hygiene measures in people of all ages in both the developed and developing world.

To meet all these Hindustan developed the Pureit water purifier which gives water that is 'as safe as boiled water' without the use of eletricity and running piped water. Pureit works by having (1) microfibre mesh (2) compact carbon trap (3) germ kill processor (4) polisher and (5) battery life indicator which meets the germ kill standards of the toughest drinking water regulatory agency in the USA, the environmental protection agency (EPA).

The other aspect of drinking water is safety from chemicals. The commonest chemical being Flouride which represents about 0.06-0.09% of earths crust and occurs in minerals like flurospar, rock phosphate, cryolite, apalite, mica etc. Fluorite (CaF_2) is a common fluoride source

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Classification of Contaminants in Drinking Water

Dissolved ions / compounds					Insoluble matters	
Class 1 Primary constituent (>5 mg/L)	Class 2 Secondary constituent (>0.1 mg/L)	Class 3 Tertiary constituent (>0.01 mg/L)	Class 4 Trace constituent (<0.01 mg/L)	Class 5 Transient constituent	Class 6 Solids	Class 7 Microbes
Bicarbonate, Magnesium, Sodium, Calcium, Organic matter, Sulfate, Chloride, Silica	Ammonia, Iron, Potassium, Borate, Nitrate, Strontium, Fluoride	Aluminum, Manganese, Copper, Phosphate, Arsenic, Lead, Zinc, Barium, Lithium, Bromide	Antimony, cobalt, Tin, Cadmium, mercury, Titanium, Chromium, Nickel	Acidity / Alkalinity constituents from C, N, O, S cycles; Treatment residues-Cl_2, CrO_4^{2-}, SO_2, SO_3^{2-}; Radionucleids	Floating, Settable & Suspended	Algae, Bacteria, Fungi, Viruses, Spores

Prevalence of Bacteria

Widespread contamination in Urban India*

Location	Fecal coliforms / 100 ml	% FC positive samples
Maharastra- Mumbai	7-320	68% (C) 39%(S)
Thane	8-213	44% (C), 31% (S)
Gujrat- Ahmedabad	11-200	55% (C), 38% (S)
W. Bengal-Calcutta	3-260	38% (C), 63% (S)
TN- Chennai	2-2500	43% (C), 100% (S)
Kerala-Cochin	137-2000	70% (C), 83% (S)
AP- Hyderabad	6700-380000	63% (C), 71% (S)
UP-Kanpur	8-132	38% (C), 63% (S)
Rajasthan-Jaipur	22-200	31% (C), 50% (S)
BIS standard	0	Nil

NEERI survey, 2000: (C) = City, (S) = Suburbs

Prevalence of Bacteria

Widespread contamination in Rural India also

Location	Fecal coliforms / 100 ml	% FC positive samples
Maharastra- Ex Mumbai	4-50	33%
Thane district	23-520	60%
Gujrat-Ex Ahmedabad	11-200	38%
W. Bengal- Ex Calcutta	5-13	100%
TN- Ex Chennai	4-300	75%
Kerala-Ex Cohin	55-450	88%
AP- Ex Hyderabad	18-108	100%
UP-Ex Kanpur	8-132	60%
Rajasthan-Ex Jaipur	39-200	67%
BIS standard	0	Nil

NEERI survey, 2000

Prevalence of Protozoans

City	Entamoeba hystolytica Number of samples +ve/samples processed	Giardia lamblia Number of samples +ve/samples processed
Ahmedabad	6/18	5/18
Kolkata	3/9	0/9
Chennai	3/18	1/18
Cohin	5/12	1/12
Delhi	4/11	1/11
Jaipur	4/11	1/11
Kanpur	1/10	0/10
Hyderabad	2/10	1/10
Mumbai	9/24	1/24
Thane and new Mumbai	7/17	1/17

NEERI survey, 2000

occurs in both igneous and sedimentary rocks. When consumed below 1.5 ppm provides dental health but when exposure to more than 1.5 ppm occurs over a period of time leads to dental fluorosis (1.5-4ppm) (irregular calcification disorder of enamel forming cells) , skeletal fluorosis(>4ppm) (abnormal increase in bone density) , premature birth, low IQ in children born from mothers consuming high levels of fluoride and crippling fluorosis (>10ppm).

The symptoms of skeletal fluorosis include chronic joint pain (arthritis). Fluoride also depresses the thyroid gland causing hypothyroidism and obesity. Hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$ stucture is a prototype for the stucture of bone mineral. Fluoride ion substitutes the hydroxyl ion giving rise to fluroapatite $Ca_{10}(PO_4)F_2$. This fluroapatite is denser than hydroxyapatite but very brittle leading to the deformation of bones causing fractures.

The normal limit of fluoride in drinking water is WHO -1.5 mg/l, in India-1.2mg/l, (<1.5ppm).

A person may have exposure to fluoride by,

1. Drinking water
2. Foods (fish, rice, tea)
3. Dental products (tooth paste)
4. Inhalation with aluminium and phosphate fertilizer products.

High fluoride content in ground water is present in various parts of the world and India.

The contributing factors to high fluoride concentrate are from rocks and soils bearing minerals like fluorite, apatite and mica, regions having an arid climatic condition and in use of aluminium, phosphate fertilizers.

The various methods available to remove fluoride are,

Most Affected Geographies

- **South Asia (India, Sri Lanka, Afghanistan)**
  (India Rajasthan, Gujarat, Karnataka and Andhra Pradesh and many other states totaling ~20)
- **North China (Inner mongolia)**
- **West Africa (Ghana, Ivory Coast, Senegal)**
- **North Africa (Algeria, Libiya, Egypt, Sudan)**
- **South Africa**
- **East Africa (Kenya, Uganda, Tanzania, Ethiopia**
  F level more than 20 mg/L)
- **Northern Mexico (6% of the population suffers from fluorosis)**
- **Central Argentina**

High Fluoride content in ground water

Sources :

Water quality Fact sheet, British Geological Survey

Journal of Colloid and Interface Science 348 (2010) 579–584.

CARBON 48 (2010) 333-343.

Advantages and Disadvantages

Technology	Advantages	Disadvantages
Ion Exchange/ Adsorption	Simple, economic, appropriate for drinking water in small communities or point of use applications Selective removal of fluoride , local availability	Low adsorption capacity at high pH values in most adsorbents like alumina, need for frequent regeneration, social non acceptance of bone char etc.
Membranes	High capacity of removal (up to 95%) , works in high salinity conditions	Low economic viability, high maintenance cost, fouling, scaling, and membrane degradation.
Precipitation	Low cost, simple, Established	Waste disposal issues, unwanted chemicals in water

International Groundwater Resources Assessment Centre Report nr. SP 2007-1

Drinking water limits for arsenic

Standards / Guidelines	WHO	EU	NL	TVO-D	DVGW	BIS
Arsenic in µg/L	10	50	10-60	10	10-30	50

WHO: World Health Organization Drinking water Guidelines (DWG)

EU: European Union DWG

NL: Dutch standards for groundwater

TVO-D: German drinking water standards

DVGW: German surface water (raw water) guidelines.

BIS: Bureau of Indian standards

The newer emerging technologies are

1. Capacitative deionization
2. Electrocoagulation.

Arsenic contamination in drinking water affects about 100 million people in Bangladesh, India, China and Mexico. It is classified as a class 1 carcinogen which is four times as poisonous as mercury affecting the Skin, Liver, Nervous system, Cardiovascular and Endocrine system. The toxicity of different arsenic compounds are depicted in table. Inorganic arsenic is carcinogenic to humans. Acute, high dose, oral exposure to arsenic typically leads to Black foot disease, extreme thirst, gastro intestinal irritation, hypotension, cancer in liver, lung, skin, bladder and kidney.

Drinking water limit for arsenic by various guidelines are

Arsenic can occur in several oxidative states. In natural waters, arsenic is found in inorganic form as oxyanions of trivalent arsenite or pentavalent Arsenate.

The various available technologies for removal of Arsenic from water are

1. Preoxidation: Oxidants used world wide are chlorine, ozone, Fenton's reagent, potassium permanganate, UV light.
2. Coagulation-Filteration: Coprecipitation with alum or ferric salts.
3. Membrane Seperation: reverse osmosis (RO) and electro dialysis reversal (EDR).
4. Ion exchange: Anion exchange resins.
5. Adsorption: Different metal based arsenic adsorbents.

The advantages and disadvantages of different methods are

Comparison of main arsenic removal technologies

Technology	Advantages	Disadvantages
Oxidation and sedimentation: air oxidation, Chemical oxidation	Relatively simple, low initial and running cost, oxidizes other impurities and kills microbes	Slow process, partial removal even at low input arsenic concentration
Coagulation and filtration: alum coagulation, iron coagulation	Relatively low capital cost Simple in operation Common available chemicals are used	Produces toxic sludge Inefficient removal of arsenic (III) Pre-oxidation is required
Membrane techniques: nanofiltration, reverse osmosis	Well-defined and high removal efficiency No toxic solid waste produced Capable of removal of other contaminants	High capital and running cost High-tech operation and maintenance Arsenic-rich rejected water is produced
Ion exchange	High removal efficiency Other anionic contaminants also removed	High capital and running cost High TDS (presence of other anions) water reduces efficiency Replacement or regeneration is required
Sorption techniques	Relatively well known and commercially available Well defined technique Many possibilities and scope for development	Arsenic-rich solid waste Constant running Cost

The key challenges in fluoride remediation is

1. Selective removal of fluoride in presence of other ions in water.
2. Efficiency and cost effectivness of remediation methodologies.
3. Disposal issues of fluoride rich solid or liquid waste.

The key challenges in arsenic remediation is

1. Inefficient removal of arsenic (iii)
2. Inconsistent performance of arsenic removal techniques.
3. High capacity and running cost for effective removal technologies.
4. Disposal issues of arsenic rich solid or liquid waste.

ENVIRONMENTAL FACTORS INFLUENCING FOETAL ORIGIN OF CHILDHOOD AND ADULT DISEASES: A CHALLENGE FOR TRANSLATIONAL RESEARCH

Dr B.G.Ranganath

Genetic causes as an explanation for disease susceptibility is well accepted. This is demonstrated by different ethnic groups that live in the same geographic areas and share similar environmental risks have differences in disease prevalence and disease markers.

Other explanations for ethnic difference and inter-individual differences in disease risk are being considered. For ex, high incidence of metabolic disease are found in ethnic groups in whom the average birth weight is low or the rates of gestational diabetes and maternal obesity are high (Barker's hypothesis). The theory of interest the environmental influences, especially nutrition and stress during mammalian development leads to permanent changes in the epigenome, which in turn increase the risk of chronic metabolic and cardiovascular diseases in later stages of life has compelling evidence (foetal origins of adult disease). Developing organisms have a wide window of susceptibility to the epigenetic changes. The periconceptional period of development is sensitive to suboptimal nutrition. Also, nutritional constraints and over nutrition in pregnancy cause metabolic dysfunction later in life.

The importance of the periconception period in later metabolic dysfunction can be observed in retrospective studies in the population exposed prenatally to the Dutch hunger winter. Individuals who were exposed in the later gestational stages had low birth weight and later developed metabolic dysfunction including diabetes and hypertension. Whereas, those exposed to the famine periconceptionally and in the first trimester of pregnancy did not have low birth weight than unexposed individuals, but as adults they experienced a high prevalence of obesity and coronary heart disease.

India currently has the highest rates of neonatal mortality (0.94 million deaths or 34/1000 live births) and the highest rates of low birth weight (30% of births). A similar situation existed in Britain in the early 20th century and paradoxically the epidemic of coronary heart disease coincided with the improved living standards and nutrition in the later part of 20th century. This postulation offers a good explanation for the increasing prevalence of diabetes and coronary heart disease in India.

The strategy of government of India is to provide supplementary feeds to pregnant mothers in the urban slums and rural areas and iron and folic acid supplementation universally to all pregnant mothers to improve the outcome of pregnancy. But the implementation of this strategy through integrated child development services (ICDS) and reproductive and child health programme (RCH) is not uniform.

Also, studies on pregnant rats show that nutritional and endocrinal intervention result in

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phenotypic changes that persist for at least two generations. The mean age at marriage for rural women is 20.2% and the total fertility rate among illiterate rural mothers is 4.1 compared to 2.4 in literate urban mothers.

Hence there is a need to consider the complex social circumstances which may favour foetal under nutrition like, age at marriage, spacing between births, child bearing age and social customs and traditions detrimental to the well being of the pregnant mother and the new born child. The environmental stress of under nutrition continues to the early childhood which may impair the growth and development. Significant proportion of the new born children do not receive breast feeding within 30 minutes of birth, the weaning is delayed and the supplementary feeds given to the growing child have poor nutritional value or are contaminated.

The multidisciplinary field of the developmental origins of health and disease (DOHaD) examines how the environmental factors during foetal development changes the capacity of the organism to cope with its environment in later life. The recognition that the environment interacts with the gene has great implications for public health strategies. Significant strides have been made in understanding the role of pre and post natal environment in influencing health throughout life which are being recognized as the DOHaD. This includes increased risk of chronic diseases like cardiovascular diseases, type-2 diabetes, hypertension, metabolic syndrome, schizophrenia, osteoporosis, obesity and asthma. This understanding of environment interacting with genes has implications for public health actions especially in the prevention of type-2 diabetes and cardiovascular diseases. Also, the associations related to the developmental origins of health and disease could be explored.

This may require firstly, a significant understanding is required on the health and nutritional status of the community in form of comprehensive surveys and longitudinal studies aiming at exploring associations between the putative exposure and the developmental origins of disease. These epidemiological studies backed by genetic laboratory methods will provide scientifically valid information on environmental health problems. Second, initiating programmes to prevent and control diabetes, cardiovascular diseases, asthma based on the light of evidence. These programmes should incorporate education component on safe and responsible use of insecticides, importance of ground water recharging to prevent the hazards of consuming deep underground water and tobacco use. Lastly, awareness on DOHaD needs to be undertaken among health care personnel and students of health sciences in the form of courses on environmental health. Hence it becomes important to launch a comprehensive environmental health education and research programme involving stake holders from the health sector, biomedical and genetics, sociologists, education sector, the local government and the media.

Some putative exposures that could be considered in the developmental origins of disease are discussed.

1. Kolar in India is possibly the world's oldest and longest running gold mine, where gold mining and gold processing operations have

been documented from 4,000 years to recent times. Mining at the Kolar Gold Fields (KGF) site within Kolar District in Karnataka state was operational for more than a century and was discontinued since the year 2000. KGF employed cyanidation to extract gold from the ore, and openly discharged more than 32 million metric tonnes of tailings containing cyanide and acid at several locations. Gold mining and processing (purification procedures) are sources of releases of a number of well-studied environmental toxicants, including heavy metals that are often co-mined with the gold (mercury, arsenic) or used in processing gold ore (mercury, cyanide) and may be released to the environment via acid drainage from mining operations and leaching from mine tailings to soil and water in these areas. Acute and chronic exposures to metals and cyanide used for gold extraction in the Kolar gold field mining area and potential health outcomes needs to be studied.

2. The Kolar area also uses significant amounts of pesticides in floriculture, horticulture and sericulture. Reportedly, local hospitals commonly treat patients with acute toxicity due to accidental and intentional consumption of pesticides. Chronic pesticide exposures and potential health outcomes including endocrine disruption, reproductive and developmental effects and cancers have not been studied.

3. Other major industries in Kolar include quarrying, stone crushing and brick and roof tile manufacturing. Potentially each of these may have significant impacts on the environment, and on health, depending on the specific types of materials and practices that are involved including environmental releases.

4. Kolar district (lat. 13° 2′ 13° 20′ N and long. 77° 56′ 78° 17′ E) is a semiarid and a drought prone district. Less than 14% of the land is under forest cover. More than 90% of the drinking water requirements are met by groundwater resources, which are threatened by declining water table levels and poor water quality. Kolar has the highest number of dried up wells in Karnataka. Deep tube wells exceeding 1000 ft are commonly used for drinking water supply. Around 16% of the villages in Kolar district are affected by excess fluoride concentration in groundwater ranging from 1.5 to 4.05 mg/, leading to endemic fluorosis in Kolar. The exposure to high levels of fluoride on the developmental origins of disease needs to be considered.

5. Kolar has lower socio-economic status larger by virtue of geographic disadvantages. Pediatricians in Kolar have reported a high prevalence of congenital malformations (dysmorphia and neurodevelopmental anomalies), but no formal monitoring program has been put in place. They also have reported that the prevalence of serious neurodevelopmental impairments among children in Kolar may be higher than other regions. While no formal surveillance system is in place, there is some limited evidence, based on clinical evaluations of children in the region and only very limited laboratory investigations that have involved clinicians in the region, consultation with outside institutions and use of local Institutions genomic laboratory, to complete a modest diagnostic workup.

It is most likely that most of these cases represent the normal background of birth defects and childhood diseases in this population, however, in the absence of adequate surveillance data local physicians are concerned that they are observing

an unusual pattern of disease prevalence, and that such prevalence is associated not only with potential environmental exposures (arsenic and heavy metals) but also with the fact that this population has low mobility and relatively high consanguinity among parents. This population is therefore an opportunity to study gene-environment associations in relation to birth defects and other adverse child health outcomes, in a population that is very high risk on the basis of both environmental and genetic factors.

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GENETIC ABERRATION IN BONE DISORDERS

Dr. Kalyani. R

Introduction

Human beings normally has 22 pairs of autosomal chromosomes with one pair of sex chromosomes. Human genome consists of 7 billion base pairs. Till today nearly 30,000 genes has been detected.

Chromosomal Disorders can be classified as:

A. Numerical.
   1. Autosomes.
   2. Sex chromosomes.

B. Structural.
   1. Single gene disorder.
   2. Single gene disorder with non-classic inheritance.

C. Multifactorial Inheritance.

Numerical Autosomes, examples are:

1. Down Syndrome (Trisomy 21)
2. Trisomy 18.
3. Trisomy 13.
4. Trisomy 8.
5. Trisomy 5.

Numerical Sex Chromosomes, examples are:

1. Klinefelter Syndrome. (47, XXY)
2. Trisomy X. (47, XXX)
3. Turner Syndrome. (45, X)

Structural Single gene disorder

1.Autosomal Dominant. Eg: Marfan syndrome. Ehlers-Danlos syndrome. Osteogenesis imperfecta. Achondroplasia. Multiple hereditary exostosis.

2. Autosomal Recessive. Eg: Ehlers-Danlos syndrome. Alkaptonuria. Idiopathic hyperphosphatasia.

3. X Linked. Eg: Hypophosphatemic rickets (Vitamin D resistant). Rett syndrome.

Structural Single gene disorder: Disorders associated with defects in

1. Structural proteins. Eg: Osteogenesis imperfecta. Ehlers-Danlos syndrome. Marfan Syndrome. Multiple hereditary exostosis.
2. Receptor protein. Eg: Vitamin D resistant rickets.
3. Enzymes. Eg: Lysosomal storage diseases. Alkaptonuria.
4. Protein that regulate cell growth. Eg: Neurofibromatosis Type 1 and 2.

Structural Single gene disorder with nonclassic inheritance

1. Triplet repeat mutations. Eg: Fragile X syndrome.
2. Mutations in mitochondrial genes
3. Genomic imprinting. Eg: Prader Willi Syndrome. Angelman Syndrome.
4. Gonadal mosaicism

Multigene / Multifactorial Inheritance, examples are,

1. Osteoarthritis.
2. Osteoporosis.
3. Rheumatoid arthritis.
4. Scoliosis.
5. Neural tube defects.
6. Congenital talipes equinovarus.
7. Developmental dislocation of hip.

Clinical presentation in bone disorders are,

A. Congenital.

B. Developmental.

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Classification of congenital disorders

1) Congenital disorders of trunk and upper extremity
   - Congenital torticollis
   - Congenital elevation of scapula
   - Congenital pseudoarthrosis of the clavicle
   - Congenital radioulnar synostosis

2) Congenital disorders of hip and pelvis
   - Congenital dislocation of hip
   - Coxa vara

3) Congenital disorders of the lower limb
   - Congenital dislocation of the knee
   - Congenital pseudoarthrosis of the tibia
   - Congenital talipes equinovarus

4) Congenital absence of part or all of long bones- Radius, Tibia, Fibula

Table 1: Classification of developmental disorders

Cartilaginous	**Bony**	**Miscellaneous**
Disturbed chondroid formation	Disturbed osteoid formation	• Cleidocranial dysostosis
Heterotrophic chondroblastic proliferation	**Deficient osteoid formation**	• Nail patella syndrome
• Multiple exostosis	• Osteogenesis imperfecta	• Marfans syndrome
• Enchondromatosis		• Chromosomal abnormalities producing skeletal dysplasias
Abnormal chondroblast maturation	**Increased osteoid formation**	
• Achondroplasia	• osteopetrosis	
• Metaphyseal dysostosis	• osteopoikilosis	
Abnormal ephiphyseal centre	**Abnormal osteoid production**	
• Multiple ephiphyseal dysplasia	• Fibrous dysplasia	
• Spondyloepiphyseal dysplasia	• Neurofibromatosis	
	• Pseudoarthrosis	
Abnormal mucopolysaccharide metabolism		
• Hurlers disease		
• Morquios disease		

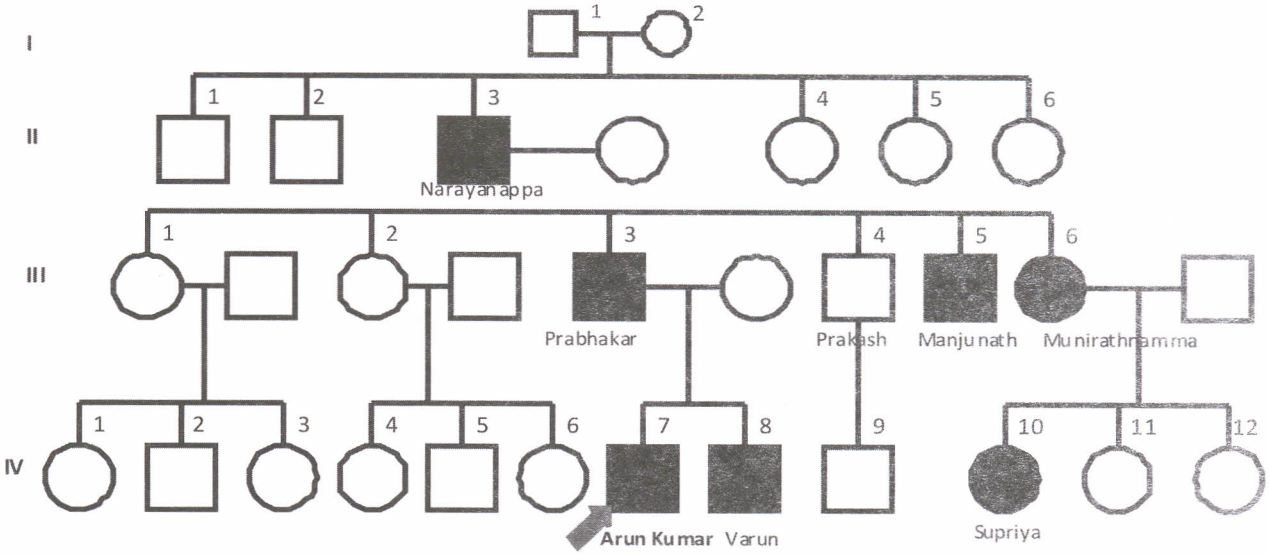
Two cases are presented.

First case: Multiple Hereditary Exostosis in a Family for Three Generations.

The index case was a 10 year male presented with multiple swelling in hand, forearm, leg, right knee and chest since 4 years. Past history was not significant. Family history revealed similar complaints in grandfather, father, paternal uncle, paternal aunt & her daughter and younger brother. **Examination of index case showed**

multiple painless swelling seen over clavicle, chest, scapula, forearm, around knee joint and ankle. CVS, RS, CNS & Per-abdomen examination was within normal limits.

Fig 1: Family pedigree of 3 generations with HME



Karyotype : 46, XY **Proband – Index case 10 yrs**

Table 2: Clinical presentation in family members of 3 generations

Clinical Features	II -3 Grandfather	III – 4 Father	III – 5 P Uncle	III – 6 P Aunt	IV – 7 Index case	IV -8 Younger brother	IV-10 Aunt's daughter
Age in yrs	65	40	35	30	10	06	08
Exostosis	Multiple; around knee joints, ankle joint, elbow joint.	Multiple; knee jts, elbow jts, forearm and left scapula	Multiple; knee jts, elbow jts, ankle jts and left scapula	Multiple; around knee jt.	Multiple; knee jts, elbow jts, ankle jts, both scapula, clavicle and ribs	Small around knee jt	Small around knee jt
Deformity	Knee jt	Knee and elbow jt	Knee elbow and ankle jt	Knee jt	Knee, elbow and ankle jt	Knee jt	Knee jt
Surgical excision	No	Done	No	No	Done	No	No
Malignant degeneration	No	No	No	No	No	No	No
Karyotyping	Normal	---	----	-----	Normal	Normal	---

The synonyms are, Hereditary Multiple Exostoses, Hereditary Multiple, Osteochondromata, Multiple Cartilaginous Exostoses, etc. It is a rare autosomal dominant genetic disorder characterized by multiple bony growths or tumors (exostoses). These are often seen on the growing end (metaphysis) of the long bones of the legs, arms, and digits. Bony growths are covered by cartilage and usually continue to grow until shortly after puberty. The prevalence of the disease is 1 in 30,000 in general population. Usually presents as painless mass around joints at the age of 4-5 years which slowly progresses with age till puberty. Boys and girls can both be affected. Male:Female ratio is 1.5:1 with incomplete penetrance in females. Older literature claimed that boys were more severely troubled by MHE, but bigger series of patients studied recently do not support this theory. Patients present with exostoses or bony bumps, on their bones which can vary in size, location and number depending on the individual. Although any bone can be affected the common site affected is distal femur, proximal tibia, fibula, humerus, pelvis and shoulder blades. Face and skull are generally unaffected. of exostosis varies significantly between and within families. Exostosis can be sessile or pedunculated.

Genetically: HME are of three types.

a. Type I is caused by mutation in the gene encoding exostosin-1 (8q23-q24).

b. Type II is caused by mutation in the gene encoding exostosin-2 (11p11-p12).

c. Type III has been mapped to a locus on chromosome 19.

d. There is some evidence for an additional multiple exostoses locus.

e. Penetrance appeared to be 100%.

Severe phenotype was shown to be significantly associated with EXT1 mutations. Moderate phenotype was associated with EXT2 mutations. EXT1 mutation were found to have more exostosis, more limb mal-alignment with shorter limb segments and height, and more pelvic and flatbone involvement. The ccomplications are bony deformity, skeletal abnormalities, short stature, compression of soft tissue (nerve compression), reduced range of motion and malignant transformation (0.5-3% in MHE). The mean age of onset malignant_degeneration was 31 years; it seldom occurred before the tenth or after the fiftieth year.

Second case: Secondary chondrosarcoma in a case of multiple exostosis.

First admission: A twenty two year old male presented with multiple swellings since childhood, increase in the size of swelling on right low back since three months, weakness and inability to walk using right lower limb since One week, pain on right side of low back since one week. None of other family members had similar swellings. Examination showed right Knee joint three exostosis, right shoulder joint two, left knee joint six, both wrist joints two, right hip joint two, lumbar region - one large. Local examination showed diffuse swelling on right low back extending from T12 to L5. Neurological examination showed motor power of 3/5 in right lower limb and negative deep tendon reflexes. Provisional diagnosis was Multiple Exostoses with lower motor neuron type of weakness in right lower limb secondary to mass in right paraspinal region. X ray of dorsolumbar spine showed bony growth with mature cortical bone and marrow with erosion of

cortex. Area of erosion showed mottled calcification. Radio imaging of the mass showed exostoses arising from vertebrae with calcification and compression of right kidney. FNAC and biopsy showed features of osteochondroma.

Second Admission: After six months: Patient complained of increase in size of the same swelling in right lower back with pain, numbness and paresis of right lower limb. Provisional diagnosis: Secondary Chondrosarcoma L3 L4 L5. FNAC confirmed diagnosis of Chondrosarcoma. Surgical debriment was made as complete exicion was not possible because of local infiltration.

Clinical signs of malignant transformation are sudden increase in the size of tumor with pain after puberty.An increase in thickness of cartilaginous cap of more than 1 centimeter on x-ray should raise a suspicion of Chondrosarcoma. Histologically most patients have grade II Chondrosarcoma.

Genetic aberration in bone are classified as (Table 3, 4 & 5)

1) Non-Neoplastic Lesions.
2) Benign Tumours.
3) Malignant Tumours

Table 4: Genetic Aberration In Bone Non-Neoplastic Lesions

Non-Neoplastic lesions	Chromosome	Protein
Achondrogenesis	5q31-q34	Diastrophic dysplasia sulfate transporter
Achondroplasia	4p16.3	Fibroblast growth factor receptor 3
Ehlers- Danlos syndrome typeII	9q34.2-q34.3	Collagen V, α1 polypeptide
Ehlers- Danlos syndrome type III	2q31	Collagen III, α1 polypeptide
Ehlers-Danlos syndrome type VII A1	17q21.31-q22.05	Collagen , α1 polypeptide
Ehlers-Danlos syndrome type VII A2	7q22.1	Collagen I , α2 polypeptide
Hypophosphatemia, hereditary	Xp22.2-p22.1	Hypophosphatemia
Marfan syndrome	15q21.1	Fibrillin 1
Osteogenesis imperfecta	17q21.31-q22.05	Collagen I, α1 polypeptide
Pseudohypoparathyrodism	20q13.2	Guanine nucleotide binding protein, α- stimulating activity polypeptide 1

Table 4: Genetic Aberration In Bone Benign Tumours

Benign tumour	Chromosome	Gene
osteochondroma	8q22-24	EXT1 and EXT2
Enchondroma	Chr 6 and 12	
Enchondromatosis		RB1, CDKN2A and TP53 overexpression, mutation in PTHR1gene
Chondroblastoma	Chr 5 and 8, 8q21	TP53 mutation
Chondromyxoid fibroma	6q13, 6q25	
Synovial chondromatosis	1p13-p22	
Osteoid osteoma	22q13	
osteoblastoma	Hypodiploid to hyperdiploid	MDM2 amplification, TP53 deletion, MET protooncogene
Desmoplastic fibroma	Trsomies 8 and 20	

Table 5 : Genetic Aberration In Bone Malignant Tumours

Malignant tumour	Chromosome	Gene
Chondrosarcoma	Trisomy 22	CDKN2A tumor supressor gene
Dedifferentiated chondrosarcoma	Chr 1 and 9	TP53 mutation
Mesenchymal chondrosarcoma	t (13;21)	
Osteosarcoma	1p11-13,1q11-12, 1q21-22, 11p14-15, 14p11-13	Overexpression MET and FOS
Adult Fibrosarcoma	2q21, partial tri-tetrasomy	
Infantile fibrosarcoma	t (12;15)	NTRK3 receptor tyrosine kinase gene
Ewing sarcoma	t (11;22)	EWS gene

Investigations in such cases are clinical examination, family history, genetic analysis, and prenatal diagnosis which will be helpful in genetic counseling and gene therapy.

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REVIEW OF INFANT AND CHILDHOOD CONGENITAL ANOMALIES

Dr. J.Krishnappa

At present working as Associate professor, SDUMC, kolar. Previously worked as a senior pediatrician at district hospital, life member of central IAP and ex-president IAP Kolar branch, published 4 research scientific articles in both national and international scientific journals.

Kolar has low socio-economic status largely by the virtue of its geographic disadvantages. The academic and practicing pediatricians of kolar district, Karnataka, India, reported a high prevalence of congenital malformations including cardiac anomalies, neurodevelopmental disorders, chromosomal and genetic abnormalities and isolated and multiple birth defects. But the epidemiology of these abnormalities was not assessed. There is a need for clinical, biochemical and genetic analysis of these conditions. The reasons may be due to untreated underground drinking water, chronic pesticide exposure, high prevalence of consanguinity and low socioeconomic status.

The following were the abnormal cases encountered at rural based hospital like R L Jalappa Hospital and Research Center, Kolar, Karnataka.

DOWNS SYNDROIVIE-trisomy21

- Mongolian face
- Low set ears, epicanthic folds.
- Hyper-telorism
- Protruded tongue
- Cleno-dactyly
- Simian crease
- Saddle gap
- 47 xx 21

TURNER SYNDROME-presented with primary amenorrhoea

- Hypertelorism
- Short fernale
- Low set ears
- Short webbed neck
- Shield chest with wide spacing of nipples
- Corg. Lymphedema
- 45XO

EDWARD SYNDROME (TRISOMY 18)

- Low set ears
- WideAF
- Wasting
- Open metamorphic features
- Long philtrum
- Hypertelorism
- Fisting or campylodactyly
- 2^{nd}, 3^{rd}, 4^{th} overlapping over 5^{th}.
- Rockerbottom foot
- Wide spacing between first and second toe
- Overlapping of 3^{rd} over 4^{th} toe

PROGERIA like syndromesenile like appearance inappropriate to age.Atrophy of subcutaneous fat, alopaciaStretched shiny skin with under nutrition. Scleroderma over extremities.Bird like facies, pinched nose, small mouth with peaked nose, crowding of teeth.

Dr. J. Krishnappa is Associate Professor of Pediatrics, Sri DevarajUrs Medical College, Kolar, Karnataka

COSTELLO SYNDROME

- Elongated facies, lowset and posterior placed ears, hyper-telorism ante-verted nostrils depressed nasal bridge.
- Masroitephaly.
- Short neck short and small digits in upper and lower limbs,
- Delayed milestones, widely placed teeth high arched palate

PRADER WILLI SYNDROIVIE

- Severe MR
- Obesity----D M
- Low hairline
- Flat facies
- Hypertelorism
- Depressed nasal bridge
- Shielding of chest
- Short neck
- Low set and out placed ears
- Hypotonia

FANCONI ANAEMIA

- Absent radius
- Biiateral absence of thumb
- Short statu re with secondary malnutrition
- Fanconi anaeimia

KLEIPPEL TRINANI SYN DROME

- Newborn with hypertelorism, depressed nasal bridge
- Macrodactyly - disproportionate growth of digits.
- Asymmetric limb hypertrophy.
- Hemihypertrophy of the right pectoralis.
- Vericosities (testicular)

HYDROCEPHALUS

- Macrocephaly
- Sun setting sign
- Depressed nasal bridge
- Widening of cranial sutures

CONGENITAL MICROCEPHALY

- MICROCEPHALY
- CRANIOSYNOSTOSIS
- SCISSORING OF LOWER LIMBS

GIANT NEVUS

- Infant with giant hyper pigmented path on ventral and dorsal aspect of the trunk
- Giant hyper pigmented hairy nevus

MENINGOMYELOCELE

- Soft none-pulsatile, non-transluscent mass seen in the midline of ilio sacral region.
- Primary neural tube defect.

ARTHROGYRPOSIS MULTIPLEXA CONGENITA

- Newborn with microceplialy with depresssed nasal bridge, hypertelorism, long philtrum.
- Contractures and stiffness in all the muscles.
- Multiple curved joints of both upper and lower limbs.

FANCONI SYNDROMEHUNTER SYNDROME

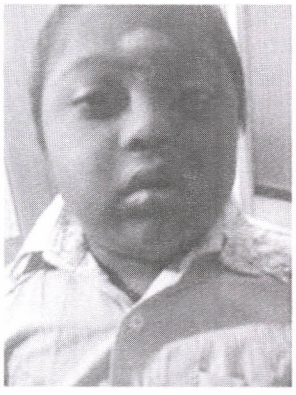
- Coarse facies
- Depressed nasal bridge
- Long philtrum
- Low hairline
- Flared nostrils
- Hypertelorism
- Hypertrophied alveolar bridge

CONGENITAL HYPOTHYROIDISM

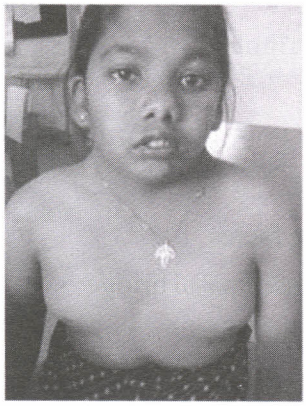
- INCREASED HAIR
- DEPRESSED NASAL BRIDGE
- DULLLOOKING FACE
- ANTEVERTED NOSTRILS

SACRAL AGENESIS

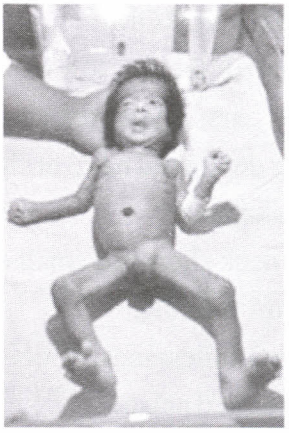
- Infant of diabetic mother
- Macrosomia
- Short neck
- Shortlimbs
- Absence of creases or skin folds in iliosacral region and hip joint.
- Sacral agenesis.



DOWNS SYNDROIVIE-trisomy21

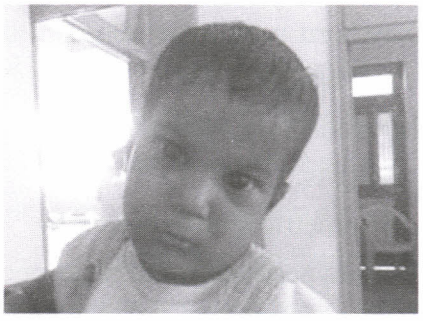


TURNER SYNDROME-presented with primary amenorrhoea



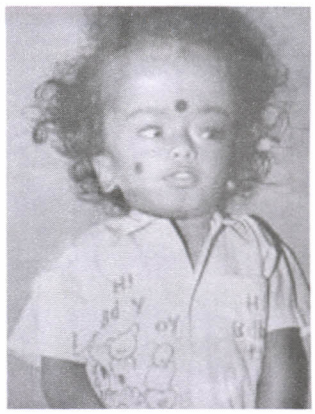
EDWARD SYNDROME (TRISOMY 18)

PROGERIA like syndrome



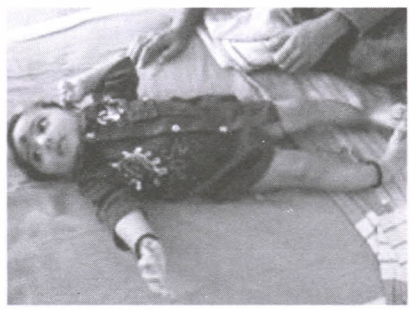
COSTELLO SYNDROME

PRADER WILLI SYNDROIVIE

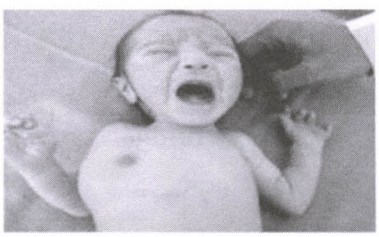


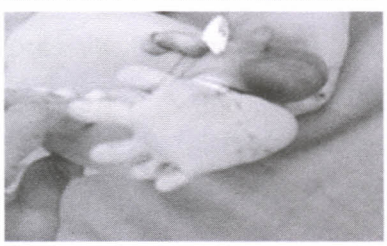
HYDROCEPHALUS

FANCONI ANAEMIA

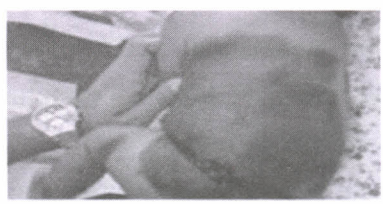


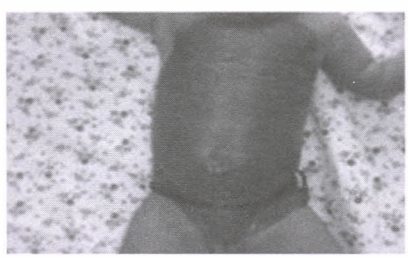
CONGENITAL MICROCEPHALY





KLEIPPEL TRINANI SYN DROME





GIANT NEVUS

- Mal aligned teeth
- Enlarged tongue
- J shaped sella tursica
- Claw hand
- Kyphoscoliosis
- Widening of medial border of clavicle
- Umbilical hernia

ALBINISM

- Both the siblings fair in color completion
- Grey hairs
- Blue eyes(iris)

CONGENITALAMNIOTIC BAND

- Asymmetry of digits in left upper limb
- Presence of thumb and 5^{th} digit in female patient
- 2^{nd}, 3^{rd} and 4^{th} fingers are absent

COLLODION BABY

- New born with parchement like membranous skin (cellotape wrapped),
- Ectropion--eversion of eye lids
- Fish like mouth (ectrobium)

CEREBRAL PALSY

- Under nourished male patient with quadriplegia(UNM) contractures and severe mental retardation
- Muscle wasting scissoring and cortical thumb.

CYTIC HYGROMA

- New born with swelling in the right side of the neck, soft in consistency, transillumination test positive.

DANDY WALKER SYNDROME

- Infant with hydrocephaly with flat occiput and lowset ears
- High arched palate long phlitrium hypertelorism long external ear pinna
- CT showed-cystic expansion of the -4^{th} ventrical

GASTROSCHISIS

- New born with abdominal contents out of the rnalunited and abdominal wall.
- Abdominal contents are not covered by peritonial sac.

PIEBALDISM

- Tuft of hypo pigmented hair and scalp in the frontal region.
- Hypo pigmented patch on the right knee joint and extending from lower half of thigh to upper half of leg.
- Hypo pigmented patch on right chest.
- Father has similar features.

HEMANGIOMAS

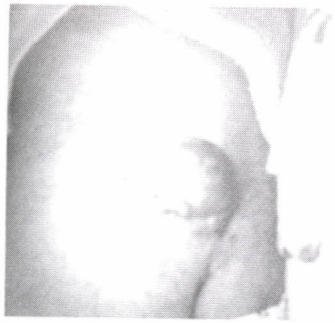
- Mulptile venous hemangiomas

CRANIOFRONTO NASAL DYSPLASIA WITH CLEFT LIP AND CLEFT PALATE WITH CONGENITAL CATARACT

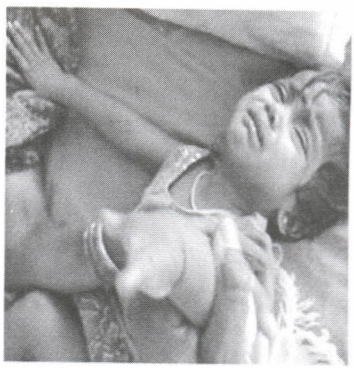
- Frontal bossing
- Depressed nasal bridge
- Congenital cataract
- Hyperteloris
- Cleft lip with cleft palate with perinatal teeth.
- Complete cleft lip with cleft palate with VSD

EHLER DANLO5 SYNDROME

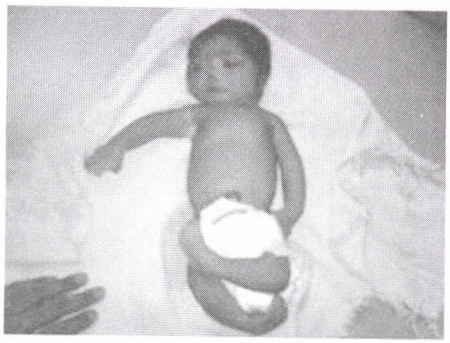
- INCREASED ELASTICITY OF SKIN AND HYPERFLEXION OF JOINTS



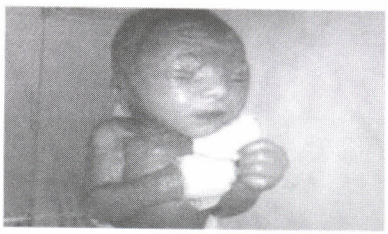
MENINGOMYELOCELE

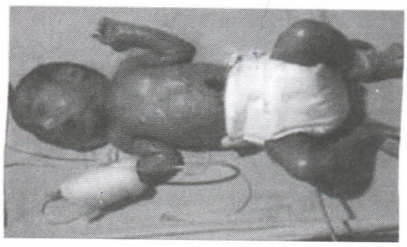


CONGENITALAMNIOTIC BAND

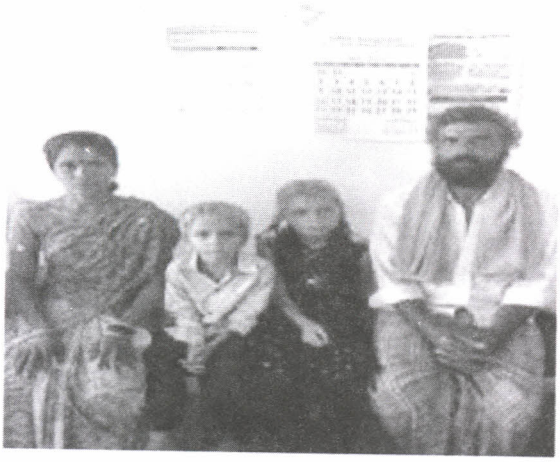


ARTHROGYRPOSIS MULTIPLEXA CONGENITA

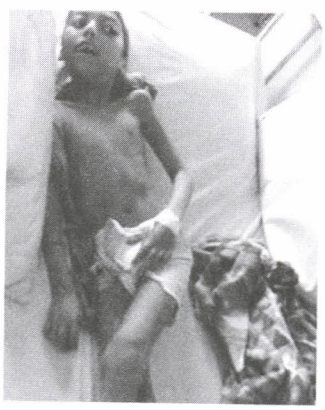




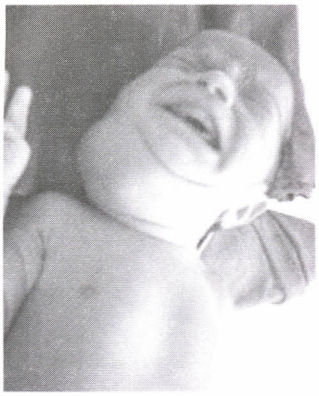
COLLODION BABY



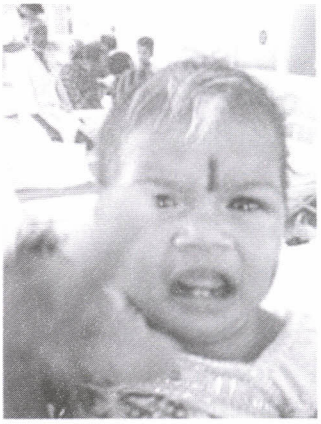
ALBINISM



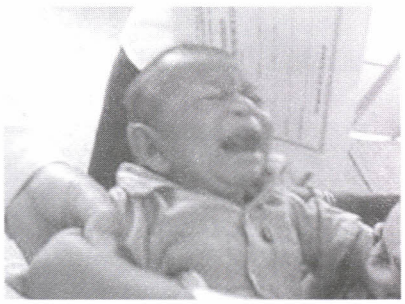
CEREBRAL PALSY



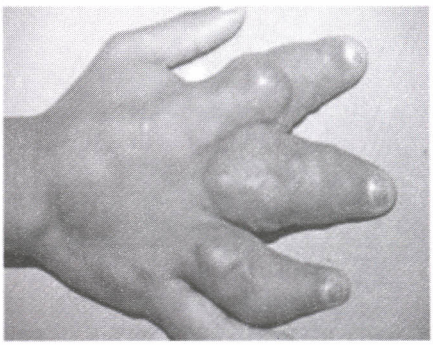
CYTIC HYGROMA



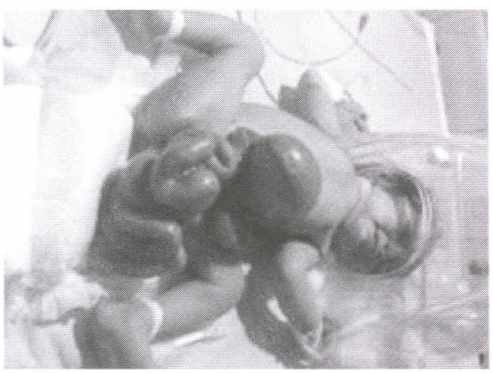
PIEBALDISM



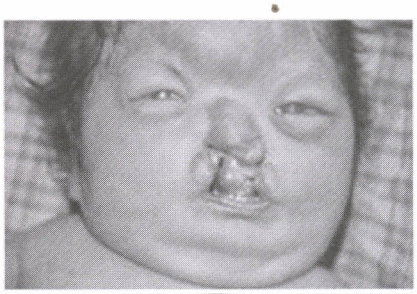
DANDY WALKER SYNDROME

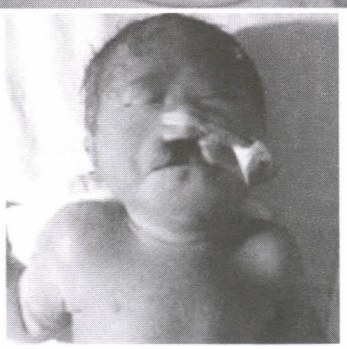


HEMANGIOMAS

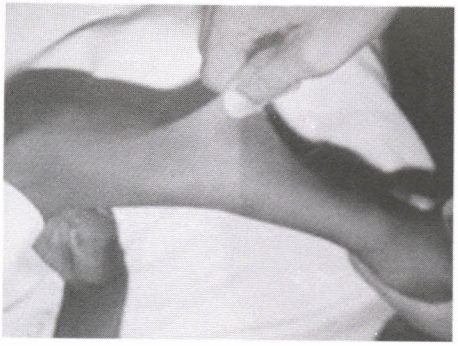


GASTROSCHISIS

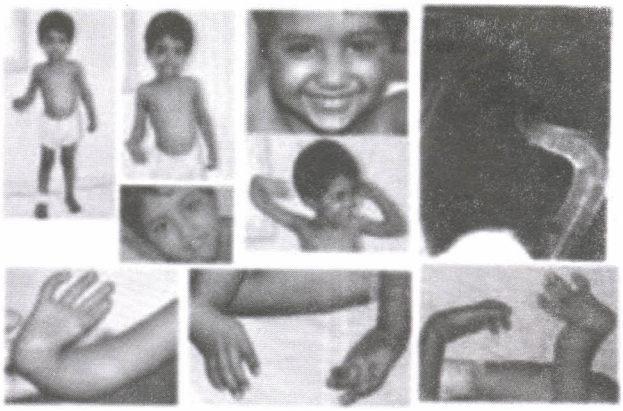




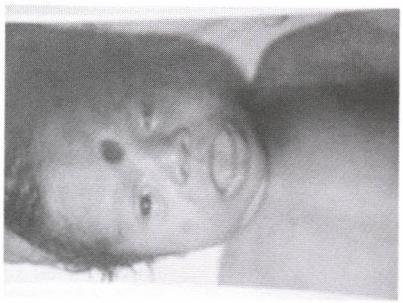
CRANIOFRONTO NASAL DYSPLASIA WITH CLEFT LIP AND CLEFT PALATE WITH CONGENITAL CATARACT



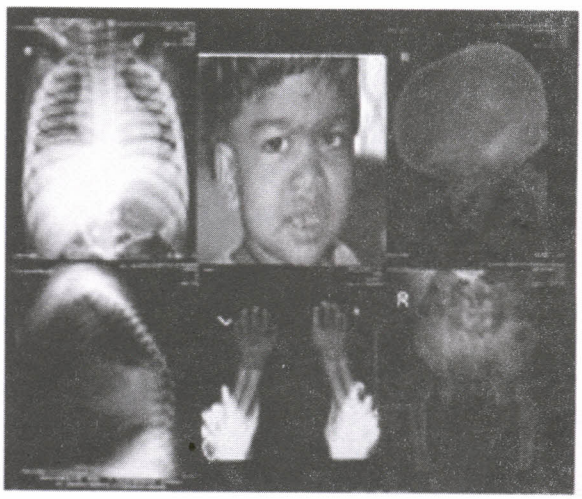
EHLER DANLO5 SYNDROME



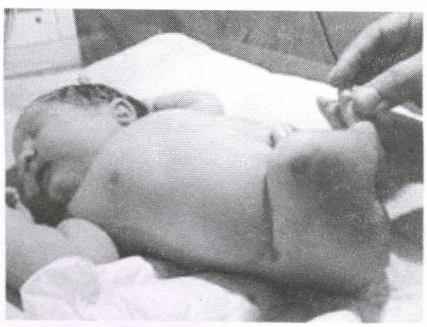
FANCONI SYNDROME



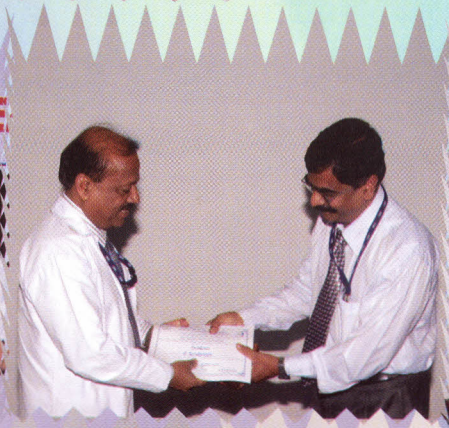
CONGENITAL HYPOTHYROIDISM



HUNTER SYNDROME



SACRAL AGENESIS




FETAL ORIGIN OF CHILDH
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HIGHER EDUCATION AND RESEARCH
-563 101
EARCH WORKSHOP
SDUAHER
FETAL ORIGIN OF CHILDHOOD AND ADULT DISEASES:
GENETIC, EPIGENETIC AND ENVIRONMENTAL PERSPECTIVE