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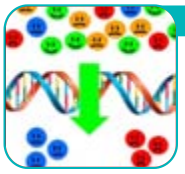
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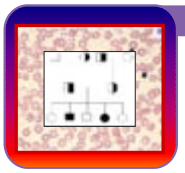
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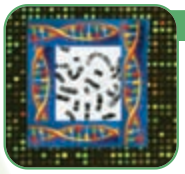
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Editorial

A National Program on Beta Thalassemia Prevention : Are we ready?

'Prevention is better than cure' holds true for all diseases but is especially true for most genetic disorders where prevention is still the only option. Even for the disorders for which there is a definitive cure, it is often expensive, cumbersome and associated with major risks. Preventive strategies play an important role if the disorder is common in a population. For such disorders nationwide screening and prevention program is cost effective. Beta thalassemia fulfills all these criteria in India for consideration for including in a nationwide screening program. Availability of reliable and sensitive screening tests is one important advantage for beta thalassemia.

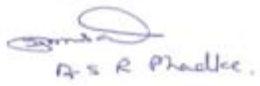
Beta thalassemia can manifest with variable severity. Thalassemia major is the most serious form associated with lethality unless optimally treated; but most of the other homozygous cases with the thalassemia intermedia phenotype also have serious problems and disease associated complications and require continued long term treatment. The only curative treatment is bone marrow transplantation available to only lucky ones with HLA matched sib and financial resources. Hypertransfusion therapy and iron chelation, if done on a regular basis and properly, have a good outcome. Availability of oral iron chelators has increased the ease of iron chelation therapy and improved compliance to it. But, given a choice, most families would opt for birth of a child unaffected with thalassemia and this is possible by way of prenatal diagnosis. Most of the families with one child with thalassemia major opt for prenatal diagnosis in the subsequent pregnancies. Prenatal diagnosis for beta thalassemia is available in many parts of India and now it's the time for primary prevention of beta thalassemia.

The carrier frequency for beta thalassemia varies from 3% to 17%. Hence, this is a good candidate for a nationwide prevention program. Mediterranean countries have eradicated thalassemia about two decades ago. Geneticists in India started pilot projects on screening of pregnant women for beta thalassemia carrier state and screening of spouses of carrier females. Genetic counseling and prenatal diagnosis was offered to the couple if both of them were found to be carriers of beta thalassemia. These projects were sponsored by the Indian Council of Medical Research. These projects in addition to providing experience to the laboratories were effective in many ways by creating awareness amongst obstetricians and common public. There were many other activities for screening population groups and creating awareness amongst laypersons by agencies like ICMR and the Thalassemics Society of India. This has led to increased awareness amongst the general population in major cities and areas with high prevalence of beta thalassemia.

In this issue Dr Rao and his colleagues have shared their experience about the level of awareness about thalassemia in the general population. In spite of some limitations of carrying out such studies, the results are very encouraging. As per the authors, about 40% people from various socioeconomic backgrounds in the study group had heard about 'Thalassemia' and majority of the respondents no longer seemed to view genetic disorders and birth defects as a result of parents' sins. The level of awareness may vary from region to region but in many cities couples are undergoing or being offered beta thalassemia carrier screening during early pregnancy. Early pregnancy or pre-pregnancy period is the best period for carrier screening as the family is more receptive to the issues related to the health of the pregnancy. Screening of college-going students may be easier (our studies as well as those of many others working in the field have shown good take-up of screening tests by college students), but they are less likely to remember the results when necessary and those who test positive for carrier status may also not disclose the fact later to the spouse for fear of stigmatization. Similarly, screening of children and minors for carrier status of any genetic disorder is not ethically justified if the results are not useful medically to the child. In our experience, spreading the message for carrier screening through the family of a child with

thalassemia major is quite effective and cascade screening of extended family members of an affected child is another successful approach.

However, screening of extended family members or high risk ethnic groups will be only partially effective in preventing the disease in the general population. The efforts of many aware obstetricians in screening pregnant women are commendable. But the screening program will be successful only if it is a centralized program with a nation-wide network, quality control, follow up and regular audit of the tests. If we want to eradicate beta thalassemia a population- based government supported program is the only way. The will of some state governments for such screening programs is obvious and government support to genetic disorders in general is improving. It appears possible that India is likely to come out of its 'third-world developing country status' sometime in the near future and with continued decrease of infant mortality rates (reaching less than 30 in some states) and better control of infectious diseases, the time seems right to initiate measures for control of genetic disorders and birth defects. Thalassemia prevention can be the first venture in this direction. Success of pilot projects and increasing public awareness has shown that this is feasible. This experience will help in planning of a feasible system for a nationwide screening program. Active media participation and support from doctors will play an important role in the success of the program. Of course a lot of work needs to be done towards creating quality labs, an improved system of sample transport and better counseling facilities in addition to increasing awareness. India being a very big country, the challenge is enormous but we have to take it. Thalassemia prevention is the need of the hour. Let us work towards it!



Shubha Phadke
1st October, 2011

Neurofibromatosis Type 1

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ABSTRACT

Neurofibromatosis type 1 (NF1) is an autosomal dominant disorder with variable and age dependent clinical features. Here two children with clinical diagnosis of NF1 are reported and issues related to NF1 diagnostic criteria in the pediatric age group are discussed.

INTRODUCTION

Early diagnosis of certain genetic disorders in children not only helps in correct management and avoids unnecessary testing, but also in research activities and genetic counseling. One such disorder is neurofibromatosis type1 (NF 1), inherited as an autosomal dominant disorder with variable clinical features and complete age dependent penetrance.[1] NF1 needs to be differentiated from other simulating and overlapping genetic disorders, as prognosis and management options differ. Reported here are two cases of neurofibromatosis type 1 diagnosed at a young age. Discussed in brief are the early manifestations of NF1, advantages of early diagnosis, management, differential diagnosis, DNA diagnosis and therapeutic options.

CLINICAL REPORTS

Subject 1: First born 11-months-old male child presented with history of unilateral anterior bowing of the leg on left side with patchy pigmentary skin lesions. There was no family history of similar complaints and/or history suggestive of neurofibromatosis type 1. Developmental milestones were appropriate for the age. Hearing and vision were apparently normal. Father was 34 years and mother was 20 years old and they were non-consanguineous. Baby was born at full term by normal vaginal delivery with birth weight of 2.75 kg. At age 11 months his head circumference was 45.2 cm (40-50th Centile). On examination he had multiple café-au-lait macules (CALM) measuring more than 5 mm in diameter and more than 10 in number. In addition, he had one hypopigmented macule of size 2.5 x 3 cm on back. Left leg showed anterolateral bowing. There was no evidence of polydactyly in any of the limbs examined. His systemic examination was otherwise unremarkable.

His chromosomes were normal. Skeletal survey revealed left sided anterolateral tibial bowing with narrowing of the shaft and maximum point of curve at the junction of middle third and distal third of shaft (Fig 1A). Fibula was normal. Tibia and fibula were normal on right side. Examination of parents did not reveal any features of NF1.

Subject 2: Second born 1-year-6-months-old male child presented with history of facial swelling. Although noticed to have fullness in the newborn period, parents observed left side facial swelling at age 8 months and since then noticed very slow progression in size. There was history of atopic dermatitis since the age of 5 months. Hearing and vision were apparently normal. He was born by normal vaginal delivery at term with birth weight of 2.5 kg. His elder brother of age 9 years was normal and had no health issues. There was no family history suggestive of neurofibromatosis type 1. His anthropometric measurements at age 1 year 6 months: head circumference 46.5 cm (40th Centile) and length 77 cm (10th Centile). He had facial swelling involving left cheek and overlying skin pigmentary changes. In addition, he had hypertelorism, down slanting eyes, depressed nasal bridge, micrognathia and low set ears (Fig 1B). Skin

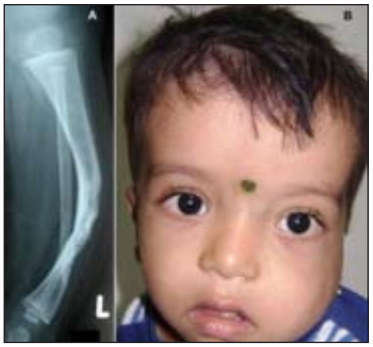

Fig 1. A: Left sided anterolateral tibial bowing with narrowing of the shaft and maximum point of curve at the junction of middle third and distal third of shaft.

B: Left side facial swelling with overlying skin pigmentary changes and facial dysmorphism.

examination revealed multiple CALM all over the body (Fig 2A). Other findings were short neck and bilateral flat feet. His developmental milestones were appropriate for the age.

Chromosome analysis revealed a normal male karyotype. MRI of brain revealed unidentified bright objects (UOBs) (Fig 2B). Eye examination was unremarkable.

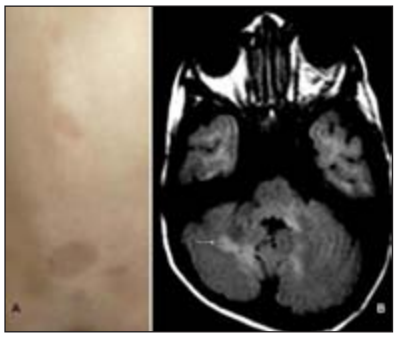

Fig 2. A: Skin showing multiple café-au-lait spots. **B:** Axial T2-Weighted MR imagesshow high signal intensity lesions in cerebellum (arrow)

DISCUSSION

Both the cases did not have peripheral subcutaneous neurofibromas but fulfill diagnostic criteria of NF1. NF1 is an autosomal dominant disorder with prevalence ranging from 1 in 2000 to 1 in 4560.[1,2] As per the diagnostic criteria for NF1, two or more of the following features must be present for clinical diagnosis: six or more café-au-lait macules (› 5 mm in children / › 1.5 cm in adults), two or more cutaneous or subcutaneous neurofibromas or one plexiform neurofibroma, axillary or groin freckling, optic pathway glioma, two or more Lisch nodules (iris hamartomas seen on slit lamp examination), bony dysplasia (sphenoid wing dysplasia, bowing of long bone +/- pseudoarthrosis), and a first degree relative with NF1.[1] As many of the clinical features of NF1 are age dependent, diagnosis especially using these diagnostic criteria in children can be at times difficult.[1] Careful examination, investigations and follow up can exclude all simulating conditions especially in children. NF1 gene is localized to chromosome 17q11.2.[3] The NF1 gene protein product which is called neurofibromin, plays an important role in cell proliferation and differentiation.[3]

Molecular (DNA based) mutation analysis of NF1 gene is more useful in early diagnosis especially in children not fulfilling the diagnostic criteria and without a family history.

NF1 like conditions with underlying NF1 gene mutations include localized (spinal, intestinal forms etc) NF1, mosaic NF1, Watson syndrome and Neurofibromatosis-Noonan syndrome (NFNS or Legius syndrome). These disorders represent the spectrum of manifestations of the NF1 gene defect. NF1 like features with no underlying NF1 gene defect include autosomal dominant CALM (some might be having NF1 gene mutations), NF1-like syndrome caused by SPRED 1 mutation) and AD neurofibromas etc.[3]

Children with NF1 can present with multiple CALM, hypopigmented macules, plexiform neurofibroma (PN), macrocephaly, height at the lower end of normal range (10-25th centile), axillary freckling, bony abnormalities, learning disabilities and/or unidentified bright objects (UBOs) in neuroimaging.

CALM commonly seen in NF1 (in majority appear in infancy), are not pathognomonic, as they can be found in the general population (3-36%) and multiple CALM are seen in 1% of general population.[3] Presence of isolated CALM in children needs careful follow-up in the absence of a family history to rule out NF1 and other related disorders. In both cases reported here, multiple significant CALM were seen, an important clue for the diagnosis with associated features. In addition, subject 1 had a hypopigmented macule, also a feature of NF1.

Axillary freckling is one of the common age dependent skin manifestations seen in NF1 but it is not unique to NF1. Axillary freckles are commonly seen in the axilla and groin region in the form of hyperpigmented tiny macules (1-3 mm size). They are present in 90% of the adults with NF1.[3]

Subject 2 presented with facial plexiform neurofibroma. In presence of other clinical features suggestive of NF1, routine histopathology of skin lesions is not necessary. Neurofibromas in NF1 could

be plexiform neurofibromas (PNs) and cutaneous/subcutaneous neurofibromas. Certain behavior and presentation of PNs are characteristic – most of them are congenital, 50% of them occur in the head and neck region, they originate from peripheral nerve sheath, 4-13% of them have a lifetime risk of malignant transformation and rates of progression of these tumors in childhood (< 10 years) are high.[4,5,6] As all PNs are not clinically evident, imaging might be necessary to pick up additional PNs in some cases. In a retrospective study, Jeffrey et al studied 39 patients of head and neck PNs (age range 1-24.5 years) and found high rate of progression in childhood and high rates of recurrence after surgery.[4] Conservative management and monitoring is a better option for management in cases of NF1 with head and neck region PNs. For those requiring treatment in certain situations, surgery still remains a better option of treatment as chemotherapy trials do not show satisfactory results. Surgery is beneficial in situations where PNs are small (can be totally removed), for large lesions manifesting with malignant signs, compressive signs and symptoms (e.g., airway or surrounding peripheral nerves and other vital structures) and for cosmetic reasons.[4]

Cutaneous and subcutaneous neurofibromas are benign. Cutaneous neurofibromas are usually of cosmetic concern for the patients. Subcutaneous neurofibromas can cause pain and neurological deficit but rarely show tendency for malignancy. They usually appear in and around the adolescent period. Clinically it is difficult to predict the number and size of cutaneous neurofibromas even among affected members of the same family.[3,7]

Other skin manifestations are hypopigmented macules, glomus tumors, melanoma, juvenile xanthogranuloma, blue red macules, localized background hyperpigmentation in segmental NF1 and generalized hyperpigmentation.[3]

Skeletal features seen in NF1 are short stature, relative macrocephaly (29-45%), scoliosis (10%-30%), congenital anterior bowing of tibia, bony lacunae, sphenoid wing dysplasia (1-7%) and osteoporosis.[3,8] Long bone dysplasia can be seen in both limbs (14%), with lower limb long bones involvement being common and is associated with relatively poor prognosis. Tibial dysplastic changes may lead to fracture, followed by pseudoarthrosis. Congenital anterolateral bowing of tibia with CALM should indicate diagnosis of NF1, as present in subject 1.[3] Careful follow-up and/or additional investigations (skeletal survey, neuroimaging, NF1 gene mutation screening) of such cases will fulfill the clinical diagnostic criteria leading to correct diagnosis, prevent unnecessary testing and aid in accurate genetic counseling. Eye manifestations including Lisch nodules and optic glioma are seen in 15-20% of children.[3]

Cognitive dysfunctions in NF1 can be behavior problems, learning difficulties and mental retardation.[3,8] Fifty percent of NF1 cases are found to have learning difficulties. Mental retardation is unusual in cases of NF1.[3] UBOs are one of the peculiar clinical features of NF1. UBOs are defined as high signal intensity on T2-weighted MRI brain, as seen in subject 2. They are commonly seen in the cerebellum, pons, medulla, basal ganglia, midbrain and internal capsules. The cause for UBOs is not clear and they may be a feature of abnormal myelination or gliosis. UBOs are reported to be associated with low cognitive functions but not in all studies.[3] Routine screening for UBOs by MRI in children is not recommended. Nonetheless, UBOs might be helpful for clinical diagnosis in atypical pediatric cases of NF1. They are commonly seen in children < 10 years age (79%) with no mass effect except for some basal ganglia UBOs. They are often seen to resolve with age, as they are usually not seen in adults.[3,8,9]

Facial dysmorphism has been noted in some of the children with NF1, especially with those carrying large deletions of NF1 gene.[10] In our subject 2, some of the facial dysmorphic features were noted.

However, facial phenotype is not always predictive of large deletions and vice versa. Currently there is no good correlation of genotype-phenotype, except for deletions (whole gene deletion & small deletion in exon 17 of NF1 gene). Large deletions involving the whole gene are associated with a severe phenotype and mental retardation.[3,10]

In summary, children with possible NF1 might fail to fulfill the diagnostic criteria, as some of the clinical features are age dependent. Such cases need careful follow up and/or additional investigations to get a final diagnosis.

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Polymorphisms in drug response and predisposition to common disease

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Introduction:

The genetic variations (alleles or sequence variants) present among humans, the rarest of which cannot be maintained by mutation alone and which have a frequency of › 1% in a population, are called genetic polymorphisms.[1,2] Table 1 shows the types of genetic polymorphism in humans.

Table 1. Types of genetic polymorphism in the human genome

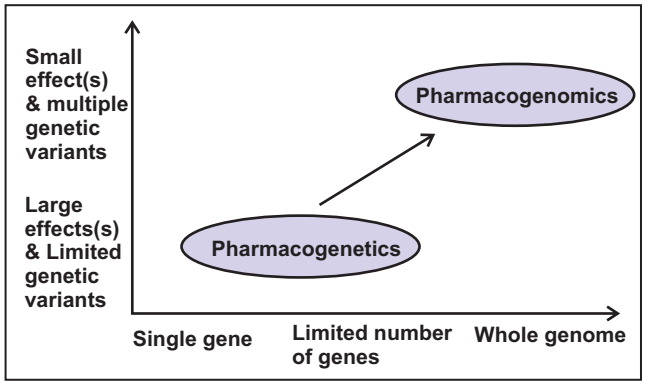
Genetic Variation	Abbrev - iation	Nature	Frequency in human genome
Single nucleotide polymorphism	SNP	Single nucleotide variation at a locus (generally biallelic but rarely triallelic)	12,000,000
Deletions/ Insertions	InDel	Deletion or insertion in the range of 1 to 1000 nucleotides, 1 or 3 base pair deletions more frequent	› 1,000,000
Variable number of tandem repeats	VNTR	Minisatellites (10-100 bp) and microsatellite (2-6 bp)	› 500,000
Copy number variation	CNV	Larger than 1 Kb deletions or duplications of DNA segment which are inheritable	› 1500 loci covering 12% of the genome

(Modified from Brockmöller and Tzvetkov, 2008[3])

Genetic variation affects predisposition to common diseases (e.g. type I diabetes) as well as infectious diseases (e.g. malaria).[4,5] The conventional method to study such pathologic genetic variation in humans has been the candidate gene approach. However, because of its fractional success due to labor, expense, need of prior information about the pathophysiology of disease and its limitation to the coding portion of the gene, the candidate gene approach is giving way to genome wide approaches which give unbiased yet comprehensive information about genetic variation across the genome that confers increased susceptibility to common diseases.[4] Advancements in single nucleotide polymorphism (SNP) typing using DNA microarray technology and the International HapMap project have enabled genome wide association studies to probe for underlying genetic variation as well as to find novel gene loci for various diseases. This newly acquired knowledge about the cause and metabolism of common diseases would help in developing and optimizing novel therapeutic strategies.[5,6,7]

Genetic polymorphism also contributes to the variation in drug response, having an entire spectrum of effects ranging from absence of response to adverse drug reactions (ADRs). Variable drug responses (VDRs) are mainly caused by differences in the pharmacokinetics (i.e. process of drug delivery and removal from target site) and pharmacodynamics (i.e. drug interaction at the target site to produce a response) of the drug in different patients, both of which can be influenced by genetic factors.[2]

The first recognition of pharmacogenetic (study of individual gene variants and variable drug response) variation was in World War II, when African-American soldiers were found to have a greater risk of developing hemolytic anemia than their Caucasian counterparts in response to oxidant drugs (primaquine) given to them. It was later found that Glucose-6-phosphate dehydrogenase (G6PD) gene mutations were the cause for this difference in susceptibility and the African-American population had a higher frequency of G6PD mutations.[8] With advances in technology, pharmacogenetics is moving towards the pharmacogenomics (study of



(Redrawn with modification from Roden et al, 2006.[2])
Fig 1. The transition from pharmacogenetics to pharmacogenomics.

variable drug response at the genomic level) approach (Fig 1).[2]

Different approaches based on the study of genetic polymorphisms are being undertaken for realization of the dream of personalized medicine and for discovery of genetic variations that lead to increased susceptibility to diseases.[4,9] Table 2 summarizes the different candidate gene and genome wide approaches and Fig 2. shows the association between them.

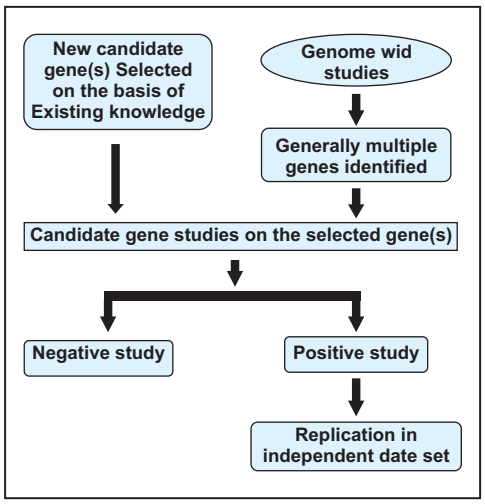
The aim of both the candidate gene and genome wide approaches is to find the underlying genetic

Table 2. Overview of candidate gene and genome wide approaches utilizing genetic polymorphism data

Parameters	Candidate gene approach	Genome wide studies
Nature	Candidate gene approach utilizes the existing genetic knowledge of drug targets, pathway of metabolism and variation which causes increased risk to diseases, to identify candidate genes for further studies	Analysis of entire genome allows identification of genes (and/or novel genes) affecting one or several disease states or increased predisposition to various diseases or variation in drug response
Risk of false discovery	Low due to limited number of genes	High due to large number of analysesperformed by whole genome wide search
Cost of study	Comparatively lower if candidate gene is found to be relevant	Comparatively higher due to greater number of positive results but recent advances in technology (e.g. microarray) are making it more affordable
Sample size	Moderate	Large

(Based on information from Davies, 2006; Hirschhorn and Daly, 2005.[4,9])

Fig 2. Association between candidate gene and genome wide approaches.



(Adapted from Davies et al, 2006.[9])

variation causing VDRs and risk of increased susceptibility to diseases. The candidate gene approach has been in use for some time but with advancement in technology and reduced cost, the genome wide approach is now feasible and is favored as it scans the entire genome for variation rather than a single gene.

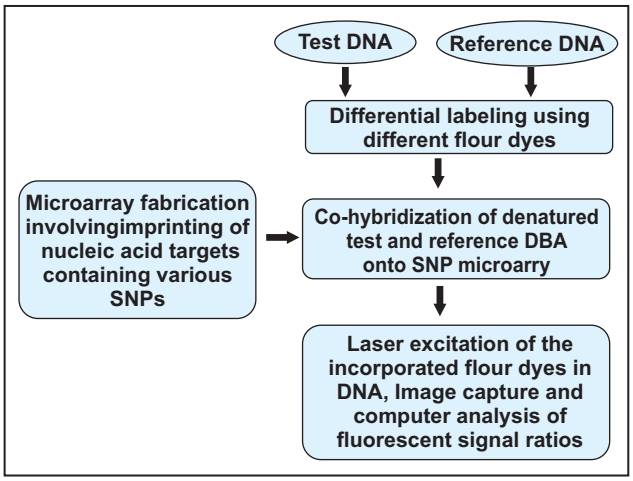
New candidate genes are discovered by genome wide studies which are individually studied emphasizing the complementary nature of both these approaches.

DNA Microarray technology:

With the advent of DNA microarray technology, genome wide studies have become more feasible enabling identification of genetic variation underlying VDRs and influencing the risk of common diseases.[7,10] The microarray itself is the size of a microscopic slide onto which nucleic acid targets are imprinted whose location and sequence is recorded by computer analytic software. The hybridization of test and reference DNA, which are differentially labeled, to nucleic acid targets

containing various SNPs occurs on the microarray chip.

The microarray fabrication involves various methodology; PCR amplification, purification and robotic imprinting forms an integral part of it. Once the probes are spotted on the solid surface or chip, differentially labeled test and reference DNA are



(Modified from Turnpenny & Ellard, 2007.[1])

Fig 3. Strategy of detection in DNA microarray technology.

added followed by competitive co hybridization (of denatured DNA) and washing. Detection is by using laser activation, computer analysis of the signals obtained and calculation of ratio of intensity of various signals obtained from each spot.

The best studied examples of genetic polymorphisms causing VDRs are in genes responsible for drug metabolizing enzymes. CYP2C19 (the gene encoding the 2C19 cytochrome P450 isoform), CYP2D6 (the gene encoding the 2D6 cytochrome P450 isoform) and CYP2C9 (the gene encoding the 2C9 cytochrome P450 isoform) are involved in metabolism of 20%-30% of clinically used drugs.[11] Fig 4 shows the system of

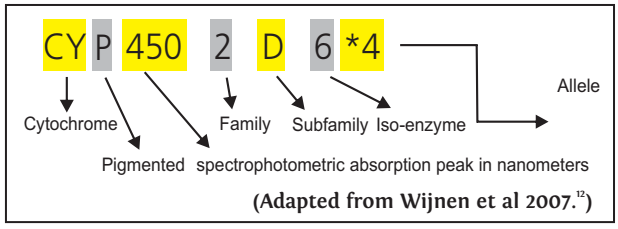


Fig 4. The system of nomenclature of CYP450 enzymes.

nomenclature of CYP450 enzymes & Table 3 shows the examples of drugs and substrate where CYP2C19 and CYP2D6 polymorphism may be relevant. Amplichip™ CYP450 genotyping test (Roche) is a commercially available, microarray- based test which can be used for studying the genetic polymorphism associated with CYP2C19 and CYP2D6. The study of variation in such genes encoding drug metabolizing enzymes would help in understanding VDRs and help in preventing ADRs by genotype-phenotype correlation and appropriate drug dosage adjustment.[3,10]

Families have 40% amino acid sequence identity and Subfamilies have 55% amino acid sequence identity.

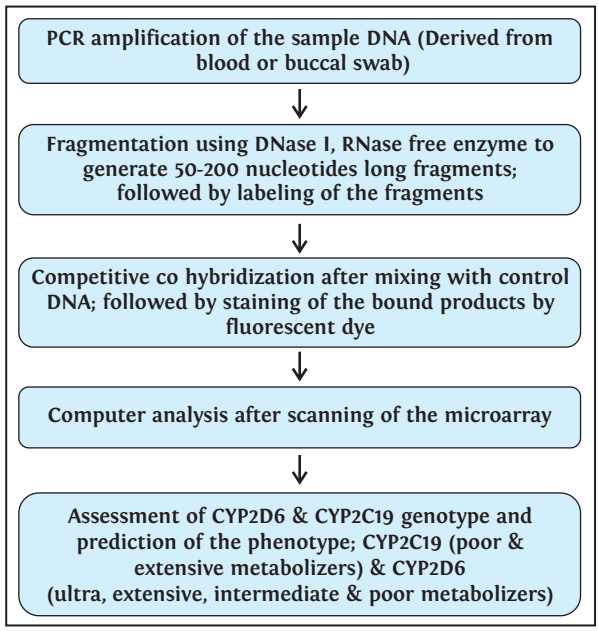
Table 3. Examples of drugs and substrates where polymorphism in Cytochrome P450 2D6 & Cytochrome P450 2C19 may be relevant.

Protein	Abbreviation	Selected substrates, ligands or drugs for which the polymorphism may be relevant
Cytochrome P450 2D6	CYP2D6	Amitriptyline, clomipramine, desipramine, doxepin, duloxetin, imipramine, nortriptyline, trimipramine, paroxetin, venlafaxin; haloperidol, perphenazine; chlorpromazine, perazine, promethazine, thioridazine, zyclopenthixol; aripiprazole, olanzapine; amphetamine, atomoxetin; carvedilol, metoprolol, nebivolol, propranolol, timolol; perhexiline; encainide, flecainide, mexilletine; ondansetron, tropisetron; codeine, tramadol; tamoxifen
Cytochrome P450 2C19	CYP2C19	Omeprazole, esomeprazole, lansoprazole,pantoprazole, rabeprazole; voriconazole; diazepam, alprazolam; amitriptyline,imipramine, doxepin; moclobemide; citalopram; S-mephenytoin, phenytoin, primidone; clopidogrel; proguanil;cyclophosphamide, teniposide

(Modified from Brockmöller & Tzvetkov, 2008.[3])

These genetic polymorphisms should be considered during drug development and treatment to prevent ADRs as well as adjust drug dosage during treatment.

Genotyping can help predict how each individual would respond to specific drugs. For instance, the Amplichip™ CYP450 genotyping test of individuals for 3 alleles of CYP2C19, classifies them into poor & extensive metabolizers and test for 27 alleles & 7 duplications of CYP2D6, classifies them into ultra, extensive, intermediate & poor metabolizers.[10] Fig 5.



(Adapted from de Leon et al, 2006.[10])

Fig 5. Strategy of DNA microarray based genotyping for predicting drug response (illustrative example: Amplichip™ CYP450 genotyping test)

shows the strategy of microarray based predictive testing for drug response using the example of the Amplichip™ CYP450 genotyping test.

Clinical implications of such genotyping tests which predict drug responses are that, on the basis of the predicted phenotype, appropriate dosage adjustments of the drugs can be done by the clinician. Poor or intermediate metabolizers have increased risk of ADRs due to overmedication. Ultra rapid metabolizers metabolize the drug rapidly and do not attain the required therapeutic concentration with standard drug medication and show inadequate response to regular drug doses; therefore, they may require higher drug dosages to achieve the desired therapeutic effect. Extensive metabolizers metabolize the drug at normal optimal levels and show appropriate therapeutic response to standard drug treatment.[10,11] Antidepressants, antipsychotics, anti-epileptics, proton pump inhibitors and beta blockers are some examples of classes of drugs which have a narrow therapeutic window and where drug response predicting genotyping tests might help the clinician in prescription & dosage adjustment.[3] This would help in saving lives of patients as well as in conserving financial resources of the health setup by minimizing hospitalization due to ADRs.[12]

Pharmacogenomics/ Pharmacogenetic (Pgx) Testing: Phases and difficulties en route to clinical practice

The development of any PGx testing is divided into 3 phases namely basic background biomedical research, clinical research and implementation of the test.[13] Fig 6 illustrates the various stages and

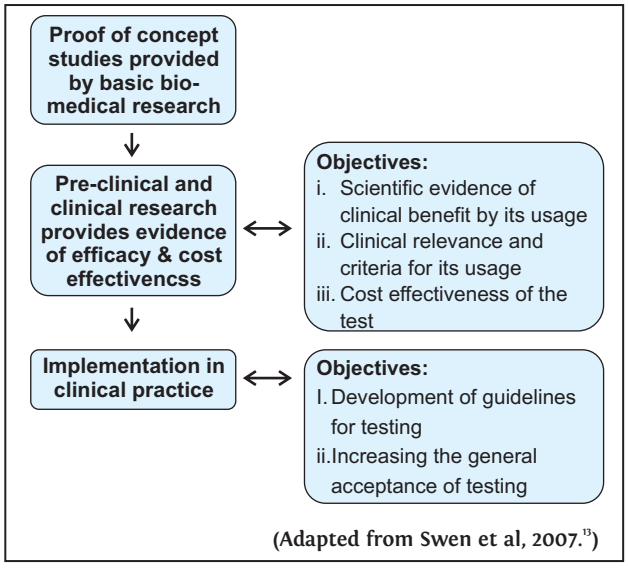


Fig 6. Various stages of evolution of PGx testing and the associated objectives of each stage.

difficulties in each phase of PGx testing.

The clinical relevance and usefulness of the test should be kept in mind while designing a PGx test. The presence of a cheaper alternative or rarity of the condition tested for would not only hamper the prospects of the test being included in clinical practice but would also waste technical & financial

resources. The sensitivity, specificity and predictive value of a PGx test should also be provided in order to make the test acceptable from a clinical perspective as the genotype only partially contributes towards VDRs and there are usually several other non genetic contributory factors involved. Studies involving large target populations, standard & clear genotype-phenotype correlation, study of clinical complications associated with the observed phenotype and success of the test in reducing ADRs are some parameters which would establish the clinical relevance of a PGx test and increase its acceptance into clinical practice. Even from the drug manufacturers' perspective it would not be unappealing if a PGx test increases the candidate population for a drug and allows faster adaptation to the drug & gives them a chance of premium pricing of the drug. [13]

Questions about regulation of PGx testing including issues such as incorporation of pharmacogenomic/ pharmacogenetic information on drug labels, extent of use of pharmacogenomic/ pharmacogenetic information in drug development, social alienation of people having certain genetic variations and economic implications such as discrimination in getting insurance or charging of different rates by insurance companies on the basis of results of PGx testing, still remain to be answered.[2]

The way forward:

To make PGx testing cost effective, acceptable and useful various hurdles are still to be overcome. Ascertaining the inheritability of genetic polymorphism causing VDRs as well as determining the contribution of genotype towards VDRs are some of the most important factors which would contribute to the success of any PGx testing. Family & twin studies are some of the approaches undertaken to study these aspects of VDRs in individuals. The successful identification of candidate genes through various approaches, including genome wide studies, would be the next vital step. The design of a system for universal definition of VDRs (using valid biomarkers or other approaches) as well as of the phenotypes associated with various genotypes would also greatly enhance the pace of different studies in this field. All the present & future scientific studies in the field of PGx testing should be reproducible and incorporate a large sample size to accommodate inter-individual differences due to ethnicity or geographic distribution. To enable this, advancements in technology (e.g. microarray and next generation sequencing techniques) to study numerous polymorphisms in large populations in a rapid, reliable and cost effective manner, would be needed. Moreover these studies should be backed by efficient data management tools & good statistical analysis. There is also a need for easily accessible public domain databases listing the association between genotype-phenotype correlations and VDRs and other related factors (e.g. www.PharmGKB.org). Lastly in order to incorporate PGx testing in the clinical set-up, it would be essential to create awareness among health care staff, patients and the general public.[2]

The majority of the studies published so far consider VDRs in a simplistic manner, focusing on single genes and their variations causing VDRs due to inter-individual differences in pharmacokinetics and pharmacodynamics. This simplistic approach also holds true for identification of genetic variation leading to increased susceptibility to diseases. With the advent of new technologies & novel strategies, the demand for genome wide studies to probe for polygenic variation causing VDRs and increased risk of predisposition to diseases is required.[4,11]

At present the applicability of PGx testing in the clinical setting is limited. Thus, till now we have come as far as establishing the "uniqueness" of an individual but whether that "uniqueness is special or not" still remains to be completely answered.

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MESSAGES

- Every human being is different. The cause of external differences and differences in response to disease causing etiologies and different responses to drugs are due to genetic variations present in DNA.
- The DNA sequence variations which do not cause any disease directly are known as polymorphisms. These polymorphisms are present in normal individuals.
- The polymorphisms in drug metabolizing genes and genes of target molecules are responsible for the differences in drug action.
- G6PD deficiency is a well known example of pharmacogenetics.
- DNA based studies have identified mitochondrial DNA mutation as a cause of deafness induced by aminoglycosides.
- Identification of CYP2C9 and VKORC1 alleles in a patient receiving coumarin group of anticoagulants helps in adjusting the drug dose and reducing the risk of adverse effects by 20%.
- New methods of genome wide studies are being used to identify polymorphisms responsible for adverse effects of drugs and helping to transform pharmacogenetics to pharmacogenomics.
- A lot of ongoing research in the field is expected to change drug treatments for the better by way of identifying individuals with risk of adverse effects and making the drugs safer.
- Pharmacogenomics is an important step towards 'PERSONALIZED MEDICINE'.

Announcement

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at

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Duration : One year

Contact : genetics.clinic@manipal.edu

Knowledge, Attitude and Practice Study of Beta-Thalassemia in Rural Bengal

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INTRODUCTION

Awareness and education are prerequisites of any screening program for genetic diseases, so that people have informed choice whether to be screened and to weigh benefits and risks of screening.[1] Beta-thalassemia carrier screening programs can be mandatory or voluntary. They can also be based on timing of screening i.e. pre-marital (school children) or pre-pregnancy (couples before or immediately after marriage). In all types of screening programs, international guidelines recommend that genetic screening should be voluntary, informed choice be given to the participants and should invariably be followed by counseling. However a review of international practice on carrier screening for beta-thalassemia found that, there is no universally accepted model to deliver genetic screening programs.[2]

Efficacy of targeted genetic screening programs largely depends upon correct knowledge and attitude of people towards screening and reflects on the socio-cultural milieu.[3] A multi-centric study was conducted by the ICMR (Indian Council of Medical Research) task force on Beta –thalassemia carrier screening program in three metropolitan cities i.e. Delhi, Mumbai, and Kolkata, in which 12,000 school children were screened.[4] A validation study after 20 years of this carrier screening program among school children in Mumbai found that the education and awareness was ineffective.[5] Social stigma and negative attitude were largely understood to be the reasons.

However, a report from Mumbai based on a 5-7 year follow up of targeted high risk community screening revealed that carriers had positive attitude and were well aware of all aspects of disease including prenatal diagnosis and prevention.[6] Similar positive results were obtained in another study where 99% of 204 married carrier couples (detected before marriage in a prospective extended family cohort) opted for prenatal diagnosis.[7]

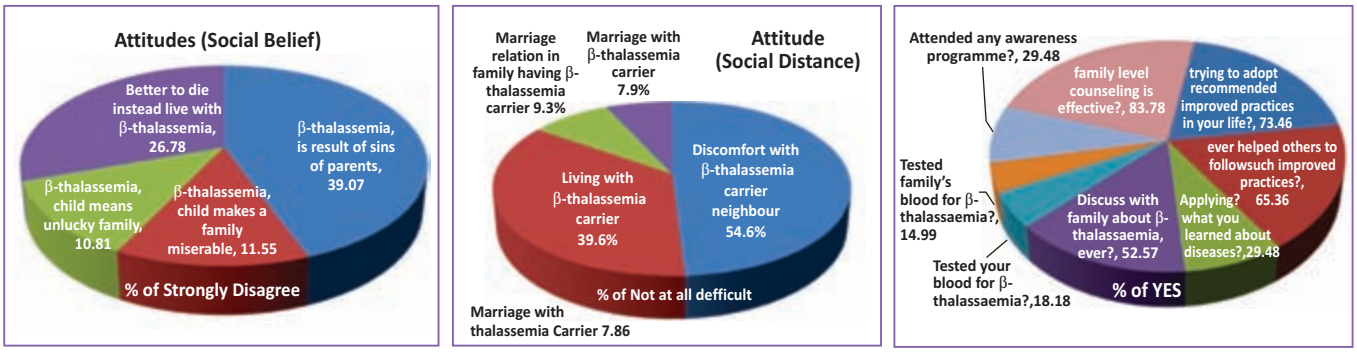
During 2000 -2005, the ICMR task force conducted another beta-thalassemia screening, extending the program to 6 zones in the states of West Bengal, Assam, Gujarat, Maharashtra, Punjab and Karnataka. Two target groups were included for screening at each zone that included 5000 women attending antenatal clinic and 5000 college students, a total of 59892 were covered.[8] Both programs included education and counseling as inbuilt components of the screening. Assessment of the impact of this carrier screening program is yet to be undertaken.

The results point out two very pertinent facts: beta-thalassemia carrier screening among school children was ineffective, where as targeted high risk community based and extended family screening had a positive effect. In India, traditional family ties still play an important role and ancestry based targeted screening could be the model that works.

In the present study we have undertaken a Knowledge, Attitude and Practice Study (KAPs), before implementing a targeted carrier screening program, in the South 24 paraganas district in West Bengal, considered to be a high risk zone for Beta-thalassaemia.[9] The program envisages carrier screening coupled with cascade screening of affected families and family counseling. We believe that KAPs study will help to understand social and cultural imperatives, if any, for success or failure of a screening program, so that there will be some perspective/base line data in evaluating the screening program.

Table 1. Knowledge about Beta- thalassemia

Statements	Response (N=407)	
	Yes (%)	No (%)
Do you know the symptoms of beta-thalassemia?	85 (20.88)	322 (79.12)
Is it a blood disorder?	214 (52.58)	193 (47.42)
Is it an inherited disease?	189 (46.44)	218 (53.56)
How many types of thalassemia are there?	50 (12.29)	357 (87.71)
If yes, do you know the names?	15(3.69)	392 (96.31)
Do you know about beta-thalassemia carrier status?	88 (21.62)	319(78.37)
Can a normal person be a beta-thalassemia carrier?	41(10.07)	366 (89.93)
Beta-thalassemia is treatable but not curable	163 (40.05)	244 (59.95)



The attitude of the people in terms of social belief, social distance and application are depicted in pie charts (Figure 1).

MATERIALS AND METHODS

The results of the present study are part of the targeted beta- thalassemia carrier screening program among high risk communities at family level, in South 24 Paraganas district of West Bengal. One thousand seventy three individuals from rural villages, above the age of 13 years were included in this study. The instruments consisted of a structured Beta- thalassemia awareness schedule comprising of Knowledge, Attitude and Practice and a general introductory schedule for the respondent's demographic details. Only 407 (37.93%), who responded positively to the question 'whether you have heard the word beta-thalassemia?' were considered for further evaluation and were administered the KAP schedule. Approval for the study was obtained from the Institutional Ethics Committee of the Anthropological Survey of India.

RESULTS

According to the information provided by the participants, the study population was drawn from a medium to low socioeconomic and educational background. Majority of the respondents replied that cold, cough, diarrhea, fever, gastritis, asthma and arthritis were the general diseases that people suffer in the area and more specifically children suffer from cold, cough, diarrhea, fever, jaundice, pox and acidity. About 54.52% did not know about genetic diseases. However, majority of people (62.16%) said that they had seen/ knew at least one child to whom blood is being given frequently but, only 22.27% knew that it was due to a genetic disease.

Of the 1073 participants, 407 (37.93 %) who had heard the word beta- thalassemia were considered as respondents for further evaluation. The replies to

the knowledge related questions about beta-thalassemia (Table 1) revealed that most of the respondents had no correct knowledge about beta-thalassemia. The response that beta-thalassemia carrier cannot be a normal person was a very important observation, which the education and counseling programs need to consider seriously.

DISCUSSION

The study revealed a positive attitude in the public perception of thalassemia, as majority of the respondents believed that the birth of an affected child was not due to sins committed by the parents. Social distance was not a criterion against beta-thalassemia sufferers but majority of the study participants were unwilling to have marriage relations with the family having a sufferer and to marry a carrier. This may be due to lack of knowledge, since about 90% responded 'No' to the question "Can a normal person be Beta-thalassemia carrier?" That the disease is highly prevalent in the locality was evident, as about 62% of the villagers had seen children to whom blood was given frequently and they were aware of the seriousness of the problem. Majority responded 'not at all difficult' to the question "Feeling discomfort with a neighbor, who is suffering from beta-thalassemia", which showed that 'social stigma' was not an issue.

Feelings of stigmatization or discrimination as undesirable side effects of genetic screening were reported earlier.[9] However, other studies were emphatic that such feelings were not important.[10,11,12] In the Indian context, the study by Colah et al (2007) assessing the impact of screening and counseling high school children after 20 years, reflected inadequacy of the onetime carrier screening program.[5] This was construed as negative impact of carrier screening programs in India, since a similar twenty year outcome of beta-thalassemia carrier screening program among high school children in Montreal was found to be successful.[13] However, Yagnik et al (1997), in a follow up of 5-6 years in an ancestry based target high risk community beta-thalassemia carrier screening, found the strategy to be highly successful. Similarly, other studies reported positive impact of beta- thalassemia screening.[7,14] In India, there are communities with Beta-thalassemia carrier frequency as high as 17%, and ancestry based target carrier screening program with awareness and community participation could be a viable model.[15] The present study, which is a quantitative, descriptive evaluation of the KAP (knowledge, attitude and practice) before screening, among high risk communities in rural West Bengal, found that the perceptions of the people were positive. Proper education and awareness program with local community participation will be effective for generations, since people witness the seriousness of the disease in their day to day life.

Acknowledgements:

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Teratogenesis and more...

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When you reap what you have not sown[1]

Genetic abnormalities are more often blamed for congenital defects than teratogens. Classical villains like thalidomide, rubella and syphilis have unmistakable signs. However many of the others operate on the sly because "We don't know what we don't study". Parisi et al make a strong case for research on medication effects in pregnancy. In fact, the entire August Issue of Seminars in Medical Genetics is a march to highlight current issues in teratogenesis. Obican et al recall that identification of classical teratogens is attributed to astute clinical observations. The saga on thalidomide, captopril, phenytoin, carbamazepine, penicillamine, diethylstilbestrol, methimazole, and many more began with either singular case reports or small case series. However, in the next article, Jan Friedman states that case series are anathema to epidemiologists. He says that solving these whodunits requires analysis of all available data, their consistency and biological plausibility. Adam et al reviewed the safety during pregnancy of 640 drugs approved by the US Food and Drug Administration (FDA) over the past 30 years. Only five percent drugs changed a full risk category. A mean time of 27 years was spent in travelling through darkness of indeterminacy to finally reach light of precision. In the next article, Jones and Carey stress the role of dysmorphology in clinical recognition of teratogenic syndromes. Wlodarczyk et al complete the circle by saying that some genes like MTHFR, TGFA, ADH1C, PAX3 etc. could still share some of the blame.

MEDdling with leiomyomas[2]

Exome sequencing has revolutionised genetic research. Mapping genes for rare monogenic disorders, even when only a single affected case is available, has become possible. Cancers have long been recognised as genetic disease. Transcriptome analysis of cancer tissues has usually been employed to know the incriminating cancer genes. Virolainen et al have successfully employed exome sequencing to discover the genetic changes in uterine leiomyomas. They examined 18 uterine leiomyomas derived from 17 different patients by exome sequencing, and identified tumor-specific mutations in the mediator complex subunit 12 (MED12) gene in 10. They further analysed 207 tumors and determined that MED12 is altered in 70% (159/225) of tumors from a total of 80 patients. The MED12 gene codes for a 26-subunit transcriptional regulator that connects DNA regulatory sequences to the RNA polymerase II initiation complex. Interestingly, all mutations were restricted to exon 2 incriminating this region in causation of tumorigenesis. Surely, meddling with cancer exomes in addition to cancer transcriptomes would catch the geneticists' fancy.

DeMYSTifying Noonan syndrome and the likes[3]

Noonan syndrome (NS) is characterized by postnatal reduced growth, cardiac defects, distinctive facial dysmorphism, and variable cognitive deficits. The mechanism behind NS is hyperactivation of the RAS/MAPK transduction pathway, with a heterozygous germline mutation in *PTPN11, SOS1, KRAS, RAF1, SHOC2, NRAS, BRAF,* or *MEK1* being detected in approximately 70% of NS/NS-like disorders. Kraft et al found MYST4 gene disruption due to balanced translocation, in a child with NS-like features. Haploinsufficiency of MYST4 led to decreased acetylation of histones (H3) causing hyperactivation of MAPK signaling. Histone acetylation at promoters of genes implies an active transcriptional state. The authors suggest that epigenetic mechanisms should be demystified when mutations in known genes are ruled out for classical monogenic disorders.

Magnum Metabolome Opus starring 37 leads[4]

Genetics underlie the metabolic individuality in humans and there is a great potential to tap this

knowledge for determining susceptibility to complex diseases and tailor pharmaceutical therapy. These so called Genetically Determined Metabotypes (GDMs) were derived from a landmark study published by Suhre et al. They analyzed more than 250 metabolites from 60 biochemical pathways in serum samples from 2820 individuals from two large population based European cohorts. They identified 37 loci that showed exceptionally large effect size (10 – 60% per allele copy in 25 loci) as compared to previous genome wide association studies (GWAS). They have uploaded this data onto a web-server to aid further studies based on these 37 'leads'. This effective exposure of the genetic-metabolic nexus would inspire other groups to produce local adaptations of the study.

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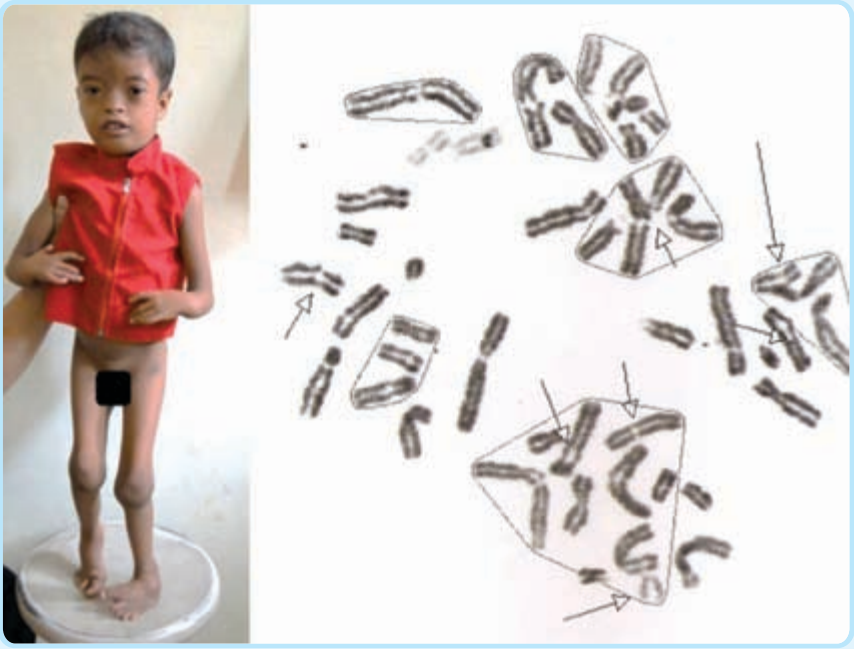
14

Contributed by: Shubha R Phadke, shubharaophadke@gmail.com

The seven-year- old boy with limb defects has short stature with the characteristic cytogenetic findings shown in the figure. Identify this monogenic condition.



Answer to the PhotoQuiz 13 of the previous issue

Coffin- Siris syndrome (OMIM 135900)

Coffin-Siris syndrome (also known as the 'Fifth digit syndrome') is a syndrome of mental retardation associated with multiple congenital anomalies. A major diagnostic clue to this disorder is hypoplasia or absence of the fifth finger and toe nails with radiological evidence of hypoplasia/aplasia of the terminal phalanx of the fifth finger/ toe. Other frequent findings include microcephaly, ``coarse'' facial appearance, feeding difficulties, frequent infections, growth deficiency and short stature. There is evidence to suggest that the disease locus is likely to be 7q32-q34; however, the exact pattern of inheritance has not been established and the gene associated with this disorder has not been identified yet.

Correct responses to PhotoQuiz No. 13 were given by

1. Amaresh Patil K, Davangere
2. Sheetal Sharda, Chandigarh
3. Siddharth Bankda, Manchester

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