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Editorial

Practice of Genomic Medicine: Challenges Ahead

The twenty-first century saw a paradigm shift from genetics to genomics. Exome/whole genome sequencing and microarray based genome analysis are tools available not only for research but also for routine patient care. Now, there is better understanding of the genetic bases of not only classical genetic disorders like monogenic and chromosomal disorders but all disorders including psychiatric disorders, autoimmune disorders, infectious diseases and all types of cancers. Thus, genomic medicine has transgressed all branches of medicine. The various institutes practicing genomic medicine work predominantly in the area of cancers, pharmacogenomics and exome sequencing to identify the etiology of unknown disorders. Cancer genetics includes cancer signatures for diagnosis and prognostication and also, developing personalized treatment based on genetic differences observed between genomes of cancer cells and non-cancer cells of the same individual. Another important area of cancer genomics is mutation detection in cancer susceptibility genes helping not only the patients but also the at-risk family members. Though hereditary cancers account for just 10% of all cancers, identification of mutation carriers who are at risk of developing cancers can be of great help in reducing their risks of developing cancers. Some studies have shown that uptake of cancer gene susceptibility tests is far less than expected and surveillance and preventive measures are not popular. On the other hand, acceptance of double mastectomy by Angelina Jolie after detection of her carrier status for a cancer breast-related gene mutation indicates that such radical preventive measures are acceptable, especially if they reduce the risk of cancer greatly.

Though a lot of these things are being done in clinical settings, there are still many issues to be sorted for genomic medicine to be incorporated into clinical practice. The first of them is lack of enough and convincing evidence for the utility of many of these genomic medicine tests or practices in the clinical settings. For example, though there is evidence about genetic polymorphisms controlling the optimum dose of anticoagulants, there is little evidence that dose calculations of the coumarin group of drugs based on the gene polymorphism profile reduce the risk of bleeding episodes or under-anticoagulation. This suggests that many other genetic and non-genetic factors controlling optimum drug dosage are still unknown. More research is needed to generate evidence for the clinical utility of genomic medicine interventions and to develop guidelines based on the available evidence. The other important challenge in the practice of genomic medicine is the tremendous amount of data generated by the genome based techniques, the enormous task of interpretation and the dilemmas posed by variations of unknown significance. Hopefully, more data from whole genome sequencing and copy number variations in normal and diseased individuals will solve some of the complexities of analysis and counseling issues.

Costs of the investigations, infrastructure and attitudes of clinicians are other important challenges felt by developed countries as well. Cost and insurance coverage are the most important issues for the clinician ordering the test as well as the patient. Development of guidelines will help to sort out some of the issues related to insurance coverage. For genomic medicine to be incorporated into clinical practice, clinicians, heads of institutes, policy makers and the population at large need to be informed and convinced about the utility of interventions based on the results of genome based tests. The resistance to accept this new form of medicine is obvious from the fact that even pedigree drawing, which is the simplest form of

genomic medicine of proven utility, is not yet widely practised by clinicians. Education through various ways like symposia, webinars, guidelines, etc. will play a great role in breaking this barrier towards implementing genomic medicine.

As genomic medicine is evolving, the most important step is DNA banking. Storage of DNA of patients for current research or for testing at a later date is very important. Similarly, DNA banking is important from the patient's as well as the family's perspective, especially if current diagnostic tests have not been able to give an etiological diagnosis. Whatever the purpose may be, detailed record of clinical findings and correct phenotyping is a must for the banked DNA to be useful in the future. India, with its large population and consanguinity, is a good source of clinical material. All the latest technologies have now become available in India. To make the best use of the situation, DNA banking is the first step! If your centre has not started DNA banking, better start immediately. Needless to say, following ethics committee guidelines, taking permission from the Ethics Committee of the institute and obtaining informed consent from the patient/ guardian is mandatory for banking genetic material.

Shubha Phadke
1st July, 2013

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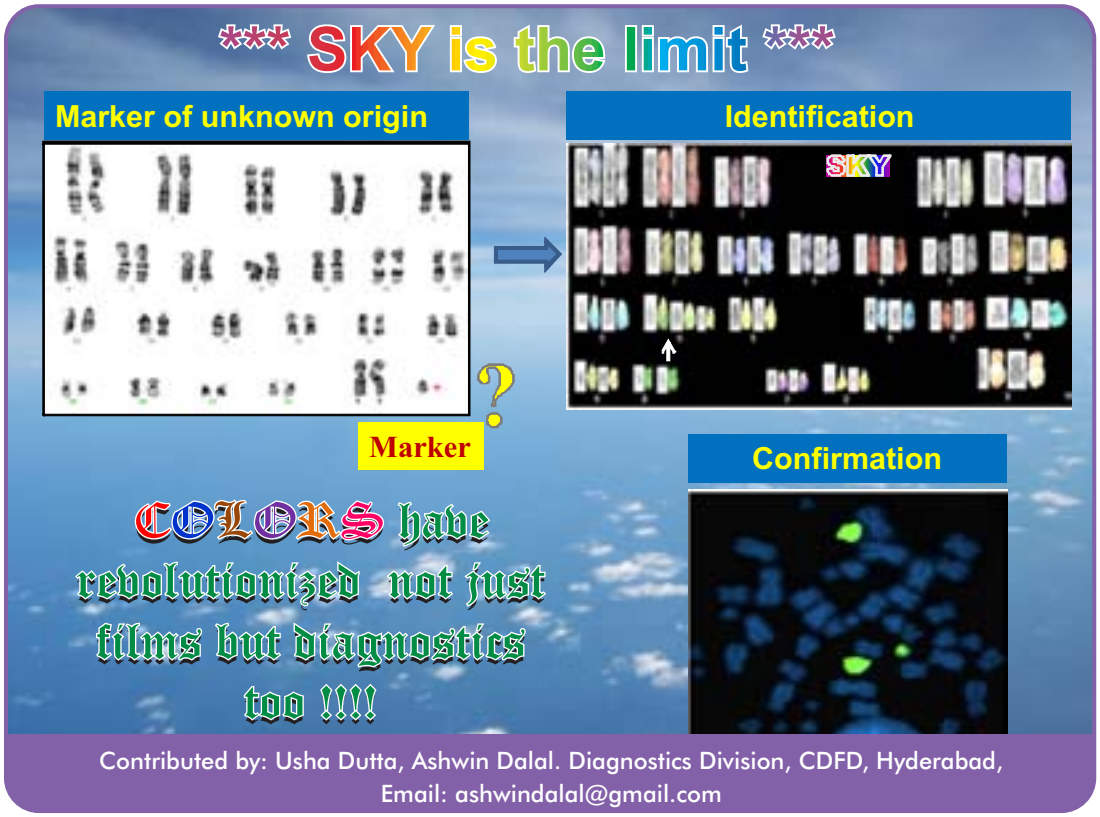

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Laurin-Sandrow syndrome: a case report

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Abstract

Laurin-Sandrow Syndrome (LSS) is a rare polydactyly syndrome of probable autosomal dominant inheritance. Clinical manifestations of LSS include mirror image polysyndactyly of hands and feet, mesomelic bone abnormalities and nasal defects. Here we report a 2 years 7 months old male child with clinical features of LSS.

Introduction

Laurin-Sandrow Syndrome (LSS) is a rare polydactyly syndrome, named after the authors who described this syndrome in the year 1964 and 1970 (Laurin et al. and Sandrow et al. respectively).[1] So far, around 14 cases have been reported worldwide with variable clinical features. Clinical manifestations of LSS include mirror image polysyndactyly of hands and feet, mesomelic bone abnormalities and nasal defects. Nasal defects are important to diagnose LSS. Familial occurrences have been documented in some of the reported cases.[1] So far the underlying gene has not been identified. Here we report a 2 years 7 months old male child with clinical features of LSS.

Clinical Report

The patient, a 2 years 7 months old second born male child, presented with deformities of all four limbs noted at birth. He was born to non-consanguineous parents through a normal vaginal delivery with a birth weight of 2.5 kgs.

On examination his head circumference at presentation was 48.5 cm (50thcentile). Facial features included a short columella with groove, underdeveloped alae nasi, bulbous nasal tip and wide nasal bridge (Fig 1A). Both hands showed symmetrical polysyndactyly of fingers and apparently absent thumbs (Fig 1B & 1C), although the lateral side fingers looked like thumbs due to some surgical procedures he had undergone. Cutaneous syndactyly extended to the tips of fingers (giving an appearance of cup shaped hands) with fused nails. Palmar creases were absent on both sides. Surgical scars could be seen in both hands (Fig 1B & 1C). Lower limb examination revealed hypoplastic legs, bilateral asymmetrical mirror image polydactyly and severe congenital talipoequinovarus. Twelve digits on the right foot and 10 digits on the left foot were seen. In the right foot, both T1 were fused, in the lateral set of toes there was T2-T6 cutaneous syndactyly with independent nails and in the medial set of toes syndactyly of T2-T5 with complete syndactyly of T4-T5 and small T3 were noted. In addition, a digitiform appendix with a nail was attached to the medial aspect of the right foot (Fig 1D). In the left foot, in the medial set of toes, syndactyly of T1-T4, complete syndactyly of T4-T3, small T2, and T5 with bifid nails, and in the lateral set of toes syndactyly of T1-T4, and small T5 attached to T4 were noted (Fig 1E). In

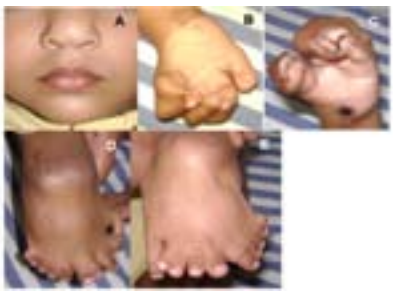

Fig 1. Photograph of the proband.
A – Nasal defects.
B,C – Polysyndactyly of hands.
D,E – Mirror image polysyndactyly of feet.

addition, the child had a right undescended testis. Cardiac evaluation was normal and karyotype was normal 46,XY by routine GTG banding.

Radiological examination revealed normal pelvic bones and femur (Fig 2A). Leg bones showed symmetrical appearance, both showing fibular morphology (Fig 2B). Frontal radiograph of feet

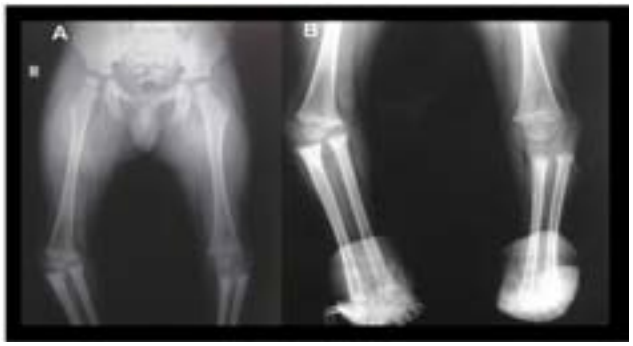

Fig 2.A - Radiograph of the normal pelvic girdle and femora. B - Symmetrical appearance of leg bones, both showing fibular morphology

showed mirror image polydactyly, with the right foot showing 9 metatarsal bones with 12 toes and the left foot showing 9 metatarsal bones with 10 toes. Peripheral toes essentially consisted of auricles without bony components. There was symmetrical arrangement of the distal row of tarsal bones. In addition, finger like preaxial appendix was present on the right side. Great toes were present in the middle with soft tissue- fusion. Severe talipes equinovarus deformity was evident bilaterally (Fig 3A).

Radiograph of hands revealed absence of thumbs and bilateral polysyndactyly. Distal part of the 6th metacarpal bone showed attempted bifurcation (Fig 3B and 3C). Carpal bone development appeared normal.

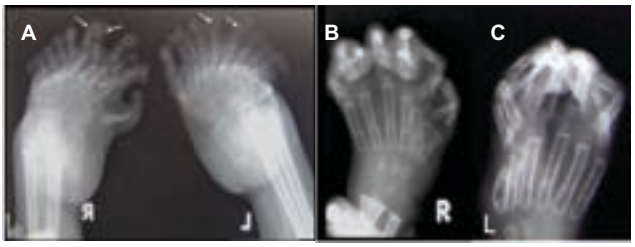

Fig 3. A - Frontal radiograph of foot showing extensive polydactyly and severe bilateral talipes equinovarus deformity. 3. B, C - Radiograph of hands shows absence of thumbs and bilateral polysyndactyly

Discussion

LSS is a rare type of polydactyly syndrome. Most of the reported patients are sporadic. Familial transmission from father to daughter and father to son has been reported. Therefore, it most likely has an autosomal dominant pattern of inheritance.[1,2] In a population based study of the prevalence of polydactyly, Castilla et al classified mirror image polydactyly (high degree of duplication) as one of the 9 rare polydactylies.[3] The birth prevalence of rare polydactylies ranges from 5.4 – 5.7 per 100,000 births in South America and Spain respectively. Rare polydactylies can be an isolated finding or occur in association with other multiple congenital anomalies.[3]

Mirror image polydactyly is reported as a segmental involvement (single limb involvement), an isolated finding (hands and/or feet polysyndactyly) and as a part of a syndrome (LSS).[1,4,5] Al-Qattan et al classified mirror image hand/feet into 8 subtypes depending upon the extent of involvement of mesomelic bones, hands and feet. LSS type mirror image polydactyly is described as subtype 4.[6] Along with mirror image polydactyly and mesomelic bone malformations, nasal defects are the important clinical features to diagnose LSS.[1,5] Various reported nasal defects include short grooved columella, underdeveloped alae nasi, wide nasal bridge, bulbous nasal tip, redundant nasal tissue, cleft nares and philtrum abnormalities.[1,5] Our case had typical features of LSS including nasal defects. Nasal defects seen in our case were short grooved columella, underdeveloped

alae nasi, wide nasal bridge and bulbous nasal tip. Both the hands showed polysyndactyly. Complete syndactyly (syndactyly till tip of fingers with fused nails) gives the hands a cup-shaped appearance. Cup-shaped hands have also been described in other syndromes like Apert syndrome or can occur as an isolated finding.[1] Mesomelic bones and carpal bones were normal in the upper limbs in our case. Lower limbs showed bilateral hypoplastic fibular dimelia, bilateral severe CTEV and mirror image polysyndactyly in our case, all of which have been reported in previous case reports.[1] Mesomelic bone abnormalities are a part of LSS. In LSS both upper and lower limb mesomelic bones can be involved, but the most frequently involved are the mesomelic bones of the lower limbs. Typically, rhizomelic bones are spared. Our case showed bilateral asymmetrical (number of toes and size) mirror-image polydactyly. Both symmetrical and asymmetrical types of mirror image polydactyly are seen in LSS. In addition the child had right undescended testis which has been reported previously.[1] Neurological findings reported include agenesis of corpus callosum, hydrocephalus, developmental delay and neuronal migration defects.[1,5]

The gene defect under lying LSS in not known. Embryonic disruption of limb bud development between fifth and sixth weeks of gestational age have been thought to result in the malformations seen in LSS.[1] Few of the genes studied in patients with LSS include MIPOL1, RAR-B and RAR-G, with no conclusive results.[1,4] A recent paper by Klopocki et al described two fetuses with mirror image polydactyly involving only lower limb, who had large heterozygous deletions (on array CGH) of the region 5q31.[7] This region contains the gene PTX1, the mutations in which have been reported in a family with club foot, tibial hemimelia and duplicated great toes.[8] Based on this family report and its function in the hind limb development, PITX1 gene is considered as one of the causes for the spectrum of limb malformations including mirror image polydactyly. In addition, Klopocki et al studied eight unrelated individuals with mirror image polydactyly and/or tibial hemimelia for PTX1 gene mutations. Out of eight individuals only one showed heterozygous deletion in the coding region of PTX1 gene, suggesting its role in the causation of limb malformations and also genetic heterogeneity. The cause for LSS may or may not be the PTX1 gene. Many genes involved in limb bud development and patterning are known and seem to be good candidates to study in these cases.

References

1. Mariño-Enríquez A, et al. Am J Med Genet A 2008; 146A: 2557-65.
2. Kjaer KW, et al. Am J Med Genet A. 2005 ;137A:148-52.
3. Castilla EE, et al. Am J Med Genet 1996;65:295-303.
4. Innis JW and Hedera P. Am J Med Genet A 2004; 131: 77-81.
5. Kantaputra PN. Am J Med Genet A 2001;98A:210-5.
6. Al-Qattan MM, et al. J Hand Surg B 1998;23: 534-6.
7. Klopocki E, et al. Eur J Hum Genet 2012;20:705-8.
8. Gurnett CA, et al. Am J Hum Genet 2008;83:616-22.

Approach to a child with macrocephaly: the dysmorphologist's view

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Definition

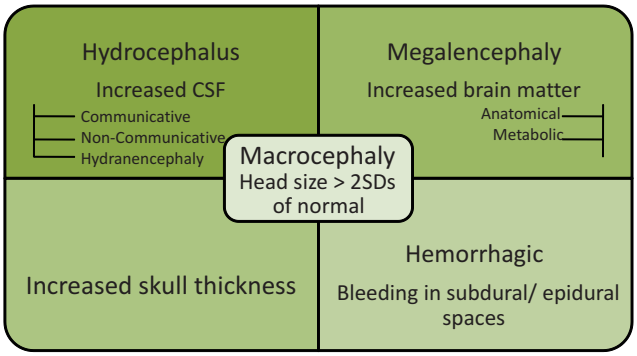
Macrocephaly if present is often the reason for referral to a clinical geneticist. It is an integral part of pediatric examination and an important diagnostic clue for a dysmorphologist. Macrocephaly is defined as head circumference (occipital-frontal circumference -OFC) more than 2SDs or greater than 97th centile above the mean for age and sex matched normal standards. This is an objective definition. Subjectively it is an apparently increased size of the cranium. Many clinicians diagnose macrocephaly based on an initial impression and confirm it by actual measurements and plotting them on charts.

Measurement

Head circumference is measured from just above the glabella (the most prominent point of the frontal bone above the root of the nose) to the most posterior prominent point of the occipital bone using a tape measure. Some standard charts are organized by centiles and others are by standard deviation. Macrocephaly is an absolute term. Ethnic background and overall stature are important and one needs to be familiar with the term relative macrocephaly. The term relative macrocephaly can be used when the head size is at the 75th centile with height at the 5th centile for age and sex or when OFC is 2SDs of the mean but is disproportionately above that for the stature. Parental OFC measurements are important for interpretation and approach.

Types and classification

The etiological types of macrocephaly depend on the factors that determine head size namely brain size (including malformations and space occupying lesions), cerebrospinal fluid (CSF), blood volume, presence of subdural fluid, and overlying tissue



Box 1: Causes of macrocephaly

scalp [Box 1]. Another classification particularly used by clinical geneticists is syndromic, non-syndromic and non-genetic. [Table 1]

Enlargement of the cranial vault leads to secondary changes in the craniofacial profile leading to a high forehead or dolichocephaly, whereas cranial base enlargement can cause mild hypertelorism, down slanting palpebral fissures or a triangular face.

History

The value of history cannot be ignored and in depth analysis of prenatal, perinatal, postnatal and family history is required. Macrocephaly may be detected by prenatal ultrasonography and lethal conditions such as Achondrogenesis type 1 and 2 can be detected but this article focuses on approach to macrocephaly detected after birth.

Table 1: List of causes of macrocephaly

Hydrocephalus	Macrocephaly (Syndromic)	Increased skull thickness	Megalencephaly (Non-Syndromic) - Metabolic
• Aqueductal stenosis • Achondroplasia • Basilar invaginatiion • Benign enlargement • Choroid plexus papilloma • Meningeal malignancy • Dandy walker malformation • Walker - Warburg syndrome • X linked hydrocephalus • Porencephaly • Hydranencephaly • Holoprosencephaly	• Achondroplasia • Neurocutaneous disorders like Epidermal nevus syndrome, Neurofibromatosis 1, Tuberous Sclerosis, Incontinentia pigmenti, Hypomelanosis of Ito, Hemimegalencephaly • Neuro-cardio-facial syndromes like Noonan, Costello, LEOPARD, Cardiofacio cutaneous syndrome • Overgrowth syndromes like Sotos, Weaver, MCMTC, Simpson-Golabi-Behmel, Beckwith-Wiedemann syndrome • With mental retardation FRAXA	• Anemia/ Haemoglobinopathies • Cleidocranial • Craniometaphyseal dysplasia • Epiphyseal dysplasia • Hyperphosphatasia • Orofaciodigital syndrome • Osteopetrosis • Pyknodysostosis • Rickets • Russell Silver syndrome • Gorlin syndrome • Kenny Caffey syndrome • Marshall syndrome • MPS I, II, VI, VII • Proteus syndrome • Lenz-Majewski syndrome • Ruvalcaba-Bannayan syndrome	• Alexander • Canavan • Organic aciduria (Glutaric acid type 1, D2-hydroxy-glutaric aciduria) • Galactosemia • GM2 Gangliosidosis • Globoid leukodystrophy • MSUD • MLC • MLD
MSUD- Maple syrup urine disease MLC-Megalencephalic leukoencephalopathy with subcortical cysts MLD- Metachromatic leukodystrophy MPS -Mucopolysaccharidosis			
Non –Genetic causes Hydrocephalus – Hemorrhage, infections & other causes Subdural effusions Post traumatic Arachnoid cysts			

Onset

OFC at birth if available should be recorded. Birth weight is important; a high birth weight more than 97th centile will indicate an overgrowth syndrome. Macrocephaly is present at birth in syndromes such as Sotos syndrome (SS), Neurofibromatosis type 1 (NF1) and Megalencephalic Leukoencephalopathy

with subcortical cysts (MLC). It appears later in autism, Canavan and Alexander diseases. Among the metabolic disorders Glutaric aciduria type I (GA1), is an exception because unlike other metabolic disorders where macrocephaly manifests during infancy or later, in GA1 macrocephaly may present in the neonatal period.

Growth

Serial values of OFC as well as height and weight should be obtained and plotted on gender and age-dependent graphs. Accelerated growth of the OFC is commonly seen in patients with hydrocephalus which may be identified as part of a syndrome such as Achondroplasia or Walker-Warburg syndrome.

Development

It is important to know whether there is gain of developmental milestones or there is regression. Features of lethargy, vomiting, poor feeding, irritability, and poor weight gain indicate increased intracranial pressure or a metabolic disease.

Family history

Benign familial macrocephaly (BFM), as the name suggests, runs in families. This is an autosomal dominant disorder as are Nf1 and Tuberous sclerosis (TS). Mendelian inheritance patterns may be recognized from the family history such as two male siblings with hydrocephalus may indicate an X-linked hydrocephalus. Additional familial features, seizures, ataxia, tremors or dementia can point to FRAXA syndrome, Autism - macrocephaly-epilepsy, FG syndrome (Opitz and Kaveggia), etc. About 15-35% of autistic children have macrocephaly.

Physical examination

Examination from head to toe is essential. Short stature with limb shortening points to achondroplasia and features for other skeletal dysplasias should also be looked for carefully. Macrosomia denotes overgrowth syndromes, more commonly Sotos syndrome, Macrocephaly-cutis marmorata-telengiectacia congenita (MCMTC), Simpson-Golabi-Behmel syndrome (SGBs) and Weaver syndrome. The shape of the head may give additional clues such as dolichocephaly in Sotos syndrome or frontal bossing in achondroplasia. Increased intracranial pressure which accompanies hydrocephalus is indicated by tense or bulging anterior fontanel or widely separated sutures. Cranial bruits are diagnostic for a vein of Galen malformation. Coarse facies indicate storage disorders such as mucopolysaccharidoses and macroglossia is suggestive of Beckwith-Wiedemann syndrome (BWS). However, microcephaly instead of macrocephaly could be present in some cases of BWS.

Skin examination

Examination of the skin can point to a specific diagnosis such as presence of cafe au lait spots, depigmented skin lesions or linear pigmentation point to NF1, TS or Linear sebaceous nevus syndrome respectively. Lentigines may be present in LEOPARD syndrome or the other RAS/ MAPK related disorders i.e. Noonan, cardio-facio-cutaneous or Costello syndrome.

Eye examination

Eye examination could reveal corneal clouding in the mucopolysaccharidoses (MPS types IH or IH/S, VI, VII), cherry red spot in Tay Sachs disease, Lisch nodules in NF1, or retinal hamartomas in TS.

Additional findings of cardiac murmurs, joint contractures, hepatomegaly, and splenomegaly are features that suggest storage disorders.

Dysmorphology evaluation

Dysmorphism is an important clue for syndrome identification e.g. mandibular abnormalities in pyknodysostosis, prognathism in FRAXA, pointed chin in Sotos syndrome (Figure 1 a&b), wide mouth in SGB, adducted thumbs in X-linked hydrocephalus and hemihypertrophy in Klippel-Trenaunay-Weber syndrome (KTW).

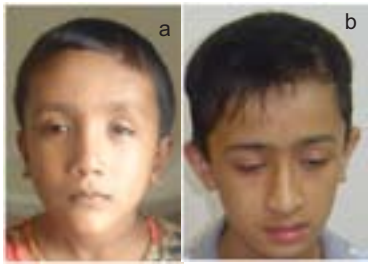


Figure 1. Patients with
[a] Sotos syndrome
[b] Fragile X syndrome

Differential diagnosis and work up

Careful history and physical examination as stated above will result in a diagnosis in many cases. Box 1 lists the causes of macrocephaly. History of developmental regression indicates a leukodystrophy. In Alexander disease the regression is rapid typically presenting before 2 years of age, while in Canavan disease it begins between 2-4 months of age with cognitive delay followed by optic atrophy without retinopathy. MLC (Figure 3) is characterized by infantile macrocephaly but the rapid course of neurodeterioration seen in Alexander or Canavan disease is lacking. Skin lesions are helpful to diagnose neurocutaneous syndromes but minor manifestations such as ash leaf spots in TS may require Wood's lamp examination. Overgrowth syndromes present with macrosomia at birth as in SS, BWS, Weaver or SGBs. Mental retardation if present in a boy with prominent jaw, long ears or long face may point to Fragile X syndrome. Organomegaly indicates possible storage disorder and the investigation can be planned to reach a specific diagnosis. Accelerated growth of the cranium points to hydrocephalus and eye and fundus examination then help further to rule out raised intracranial tension.

Figure 2. Cystic Megalencephalic leucoencephalopathy

Investigations

Clinical diagnosis may be obvious in the first setting as in case of achondroplasia. Characteristic radiological changes confirm the diagnosis. Targeted mutation detection in the FGFR3 gene will help in providing the option of prenatal diagnosis to the family for future pregnancies as shortening of limbs is not prenatally obvious before the third trimester. Additional investigations may be planned directed to manage complications such as brain imaging to rule out hydrocephalus. However in other situations the diagnosis may not be obvious and investigations should be done after performing sufficient literature search in dysmorphology databases (LMD, POSSUM) or websites (OMIM) on macrocephaly. Additional key words are entered in the search criteria for syndrome identification. The current list of syndromes with macrocephaly in OMIM is 277 and will probably keep increasing in the near future. This article discusses the more common genetic conditions that one encounters in a clinic.

The etiological cause of macrocephaly is often determined by brain imaging. In a neonate a neurosonogram may be used for screening as it is a simple bedside technique. However confirmation by MRI brain or CT scan at a later date is often required.

MRI of the brain is the most useful test which can help in reaching a diagnosis. MRI brain in an individual with benign familial macrocephaly (BFM) shows relative enlargement of the subarachnoid compartments and the interhemispheric fissure width over the frontal regions so that frontal lobes appear atrophic. Subcortical cysts in MLC and basal ganglia involvement in glutaric acidura are some other specific findings. Brain scans may demonstrate nonspecific frontal and temporal lobe atrophy. Classical tubers in brain MRI confirms the diagnosis of tuberous sclerosis. Hemimegalencephaly is observed in TS, KTW, Proteus and Epidermal nevus syndromes. CT Scan may be done during assessment of hydrocephalus and follow up after surgery. Subdural haemorrhages specifically can occur in Haemophilia (X linked condition) and glutaric aciduria. Chronic subdural haematomas could be due to coagulopathies (genetic) or infections.

When dysmorphic features are present especially with cognitive deficit but no known syndrome is recognized, chromosomal studies are a must. Now, with MLPA (multiplex ligation-dependent probe amplification) and array CGH, a higher yield of detection of chromosomal anomalies is likely.

Possibilities like rickets, hemoglobinopathies and lead poisoning must be kept in mind while examining and ordering investigations. Skull radiographs may provide clues for diagnosis of craniosynostosis and craniofacial disorders. Finally, exome and whole genome sequencing in unidentified or new entities can be applied.

Description of common syndromic conditions with macrocephaly

Benign Familial Macrocephaly (BFM)

Characteristically they have a high normal head circumference at birth which then increases to more than 2SDs by one year, absence of evidence of syndrome, normal radiographic study of brain and a parent or sibling with macrocephaly or macrocephaly which can be traced through several generations. The criteria for diagnosis have been set forth by DeMyer.

Neurofibromatosis type 1 (NF1)

Around 20-30% of NF1 cases have macrocephaly. NF1 occurs due to mutations in the NF1 gene and diagnosis is easy if café au lait spots, neurofibromas, or family history of NF1 is present. Lisch nodules are characteristically present in the eyes and focal dysplasia of bone in tibia and ethmoid bone. Relative macrocephaly is more common in Nf1. Usually, diagnosis is possible based on clinical findings.

Tuberous Sclerosis (TS)

TS has various skin lesions such as ash leaf spots, facial angiofibromas (Figure 3), Shagreen patches or ungual fibromas with brain (cortical tubers, seizures) and kidney (angiolipomas, cysts) involvement. TSC1 and TSC2 gene mutations are found in about 31% and 69% of cases respectively.

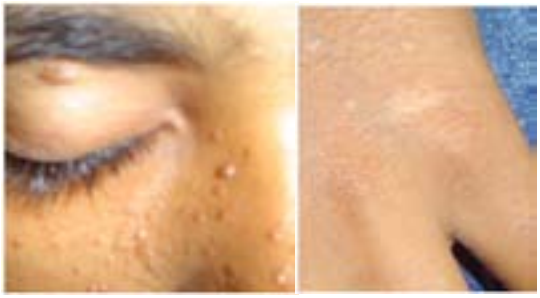


Figure 3. Adenoma sebaceum and ash leaf spot seen in patients with Tuberous sclerosis

PTEN Hamartoma-Tumour syndrome(PHTS)

This spectrum includes Cowden syndrome (CS), Bannayan-Riley-Ruvalcaba syndrome (BRRS), PTEN-related Proteus syndrome (PS), and Proteus-like syndrome. All of these macrocephaly-associated conditions are due to heterozygous mutations in the PTEN (Phosphatase and tensin homolog deleted on chromosome TEN) gene (10q23.31), hence the term PTEN hamartoma- tumor syndromes. All can be inherited as autosomal dominant disorders with extremely variable expression.

Macrocephaly-cutis marmorata-telengiectacia congenita syndrome (MCMCT)

This is characterised by macrocephaly, limb asymmetry, and vascular stains. Birth weight is increased. There is cutis marmorata and a characteristic haemangioma of the philtrum of the upper lip. Cutaneous features may fade with age. There is macrocephaly with ventricular dilatation and polymicrogyria may be present. PIK3CA gene mutations are associated.

Sotos syndrome (SS)

SS is the prototype of overgrowth syndromes and occurs due to mutations in the NSD1 gene. It is an autosomal dominant condition and is characterized by overgrowth, macrocephaly and distinctive facial features. It is usually associated with developmental delay.

Conclusion

This write up focuses on macrocephaly as a major dysmorphic feature which when recognized can point to a syndromic diagnosis and help in the management plan of the individual and genetic counseling for the family.

References

1. Garg BP, et al. Indian J Pediatr 2001; 68:867-71.
2. Williams CA, et al Am J Med Genet A ;2008: 146A 2023-37.
3. Genereviews http://www.ncbi.nlm.nih.gov/sites/GeneTests/review
4. Fenichel GM. Disorders of Cranial volume and shape. In: Clinical Pediatric Neurology : A signs and symptoms approach.Sixth edition. Saunders 2009; pp369-84.

Banking of Genetic Material: A Key to the Future

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Introduction

Single gene disorders are rare by themselves but collectively they are an important cause of morbidity and mortality. The Online Mendelian Inheritance in Man (OMIM) database lists more than 5000 disorders as single gene disorders, inherited in a Mendelian fashion. Of these, the exact genetic basis of only about 3000 single gene disorders has been identified and a large number of genetic disorders remain to be characterized. Today, little can be done to treat let alone cure, most of these diseases. However, knowledge about the disease-causing gene helps in genetic testing for confirmation of the disease and in carrier testing and prenatal diagnosis. Identification of the disease-causing gene also enables characterization of the molecular alterations and genotype-phenotype correlations, which provide insights into the molecular pathogenesis of the disease and help in development of targeted therapeutic interventions.

The data obtained from the Human Genome Project led to the identification of a large number of disease-causing genes through various gene mapping techniques like chromosomal break point mapping, linkage analysis, homozygosity mapping etc. The advent of newer technologies such as microarray and next generation sequencing has further revolutionized molecular genetic analysis, leading to the discovery of many more genes associated with single-gene disorders as well as multi-factorial disorders. Further technological advancements in the coming years are expected to yield further insights into genetic factors as well as epigenetic and epistatic influences underlying diseases. To enable all such research, it is of paramount importance to have good quality and informative clinical data and adequate amounts of appropriate genetic material.

Two techniques of genetic material banking namely DNA banking and lymphocyte immortalization are discussed in detail here.

DNA Banking

DNA banking is the secure, long term storage of an individual's DNA. DNA banking may be appropriate when currently available DNA testing methods have failed to identify the genetic cause of a condition in an affected individual or if genetic testing is not immediately feasible, either because of lack of time due to the affected person being terminally ill or lack of resources. Wherever possible, DNA banking of not only the proband but also of the parents and of affected as well as unaffected sibs and other family members must be done, as these are useful for studying the Mendelian segregation patterns in the family, for carrier testing, for familial association studies etc. DNA banking enables researchers as well as patients and their families to store DNA over long periods of time and then use the material at a later date either for diagnostic testing or for research using newer methods of analysis and molecular genetic tests. DNA can be extracted from peripheral blood (anticoagulated with EDTA) or from any tissue, using standard extraction techniques/ kits. The isolated DNA has to be stored at temperatures below -20°C; temperature of -80°C is ideal for long term storage.[1]

Disadvantages with DNA Banking:

The main disadvantage with DNA banking is that only DNA is available for future molecular testing

but no testing requiring live cells can be done in these specimens. Any research pertaining to identification and characterization of new genes is likely to involve experiments with live cells like gene expression analysis etc. The amount of DNA will be limited to what has been obtained at the time of DNA banking; hence any further requirement for DNA in the future cannot be fulfilled.

EBV mediated transformation of B- lymphocytes

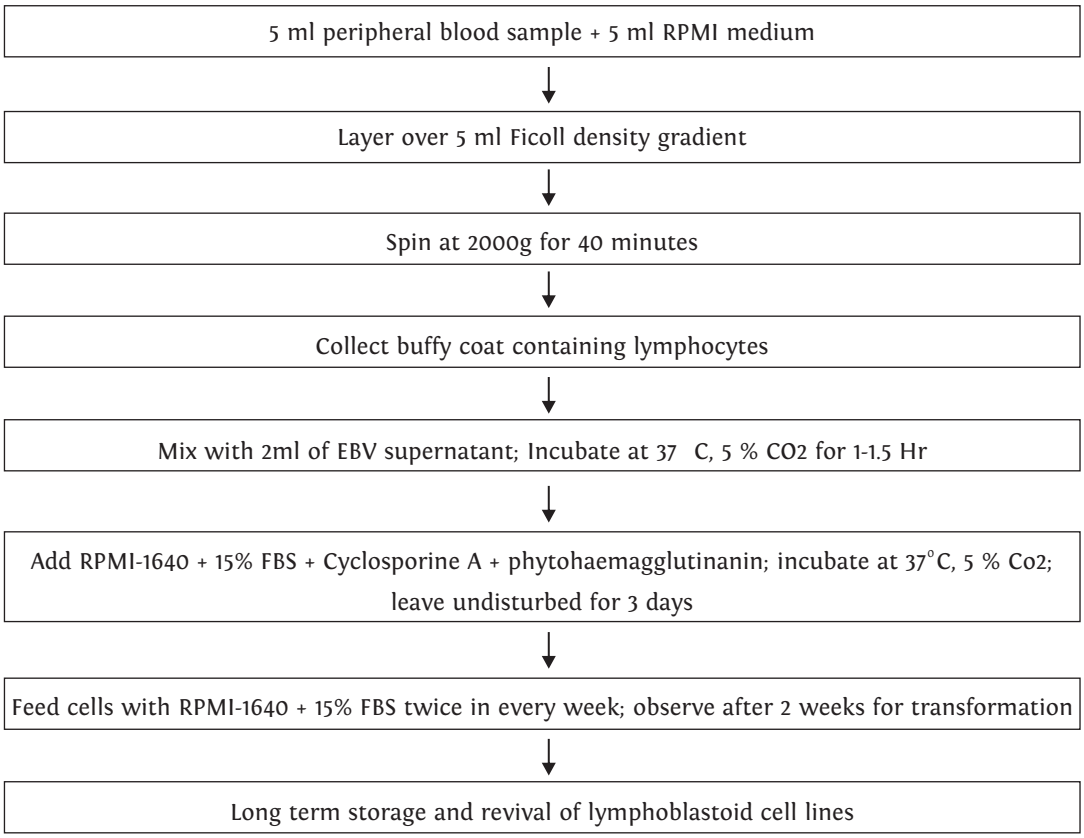
Epstein Barr Virus (EBV) transformation has been developed as a routine method to establish human B- lymphoblastoid cell lines. This is the method of choice for an unlimited source of stable, genomic DNA and for viable cells for biochemical and molecular studies. Lymphocytes can be used either to immediately create lymphoblastoid cell lines or frozen for later immortalization. Steps involved in the establishment of lymphoblastoid cell lines are shown in the flow diagram (Figure 1).[2]

Establishing lymphoblastoid cell lines requires that mononuclear cells be separated from whole blood with a density gradient and then exposed to EBV to induce cell immortalization. Cells are grown in a cell culture medium such as RPMI-1640 (Roswell Park Memorial Institute medium), supplemented with 15% Fetal Bovine Serum (FBS), L-glutamine and antibiotics (penicillin and streptomycin). The roles of individual components used in the process are as follows.

Fetal Bovine Serum:

FBS is commonly used as a supplement to cell culture media. It supports cell growth by providing a broad spectrum of macromolecules, attachment factors, nutrients, hormones and growth factors.

Figure 1: Steps in EBV mediated immortalization of lymphocytes



L-glutamine:

L-glutamine is an unstable essential amino acid required in cell culture media formulations. Glutamine supports the growth of cells that have high energy demands and synthesize large amounts of proteins and nucleic acids. It is an alternative energy source for rapidly dividing cells and cells that use glucose inefficiently.

Ficoll-Paque Density gradient solution:

Ficoll-Paque is normally placed at the bottom of a conical tube, and blood is then slowly layered above Ficoll-Paque. After being centrifuged, the following layers will be visible in the conical tube, from top to bottom: plasma and other constituents, a layer of peripheral blood mono-nuclear cells (PBMCs) called the buffy coat, Ficoll-Paque, and erythrocytes & granulocytes which should be present in pellet form. This separation allows easy harvest of PBMCs.

Phytohaemagglutinin:

Growth of cellular clumps is a typical manifestation of EBV induced transformation. For growth to follow immortalization, the culture must be depleted of T-lymphocytes which can interfere with growth of B- lymphocytes. A method commonly used to prevent T- lymphocyte overgrowth is a combined treatment of Phytohaemagglutinin (PHA) to stimulate lymphocyte growth and cyclosporine A (from fungus Hypocladiuminflatum gams) to kill the growing T-lymphocytes.

Phytohemagglutinin (PHA) was originally employed for its erythrocyte agglutinating ability in obtaining leukocytes from whole blood. PHA has the ability to initiate mitosis among leukocytes, apparently by stimulating the attention of monocytes and large lymphocytes to a state wherein they are capable of division. The fact that PHA initiates mitosis in cultures of whole blood rules out the possibility that its mitogenic action is an indirect one resulting from the removal of red cells through agglutination. PHA agglutinates erythrocytes by linkage of euglobulin portion of mucoprotein PHA molecule with a polysaccharide on the red cell surface. It alters the cell membrane to permit entrance of some substance from the culture medium which in turn, initiates the mitotic process.

Cyclosporine A:

Cyclosporine A (CsA)is believed to inhibit T cell activation by blocking the transcription of cytokine genes, including IL-2 and IL-4. Upon entering T cells, CsA binds with high affinity to cyclophilins and the cyclophilin-CsA complex associates with another cytosolic protein calcineurin. CsA- mediates immune-suppression through inhibition of the calcineurin/ NFAT (Nuclear Factor of Activated T-cells) pathway.[3]

Dimethyl sulfoxide:

After the cell lines are established, they are frozen in liquid nitrogen. For long term storage, the cells are frozen down in RPMI medium containing 20 % FBS and 10% Dimethyl sulfoxide (DMSO), a cryoprotectant. As the suspension of cells freezes, ice crystals form. These ice crystals can puncture the plasma membrane, leading to cell death. DMSO protects the cells by 1) partially solublizing the membrane so that it is less prone to puncture, and 2) interrupting the lattice of the ice, so that fewer crystals form. FBS is added so that the thawing cells get adequate nutrients. Because DMSO is toxic to the cells in an extended duration of exposure, it is important to centrifuge the cells into a pellet and remove as much DMSO containing medium as possible and replace it with fresh growth medium when culturing.

The cells are generally frozen in a programmable freezer (-20°C for 2 hrs, -70°C for overnight and finally to -196°C liquid nitrogen) in order to avoid cellular injury due to direct exposure to -196°C. The cell lines can be revived when required by thawing them at 37°C. Cell clumps can be observed on the 4th day and generally it takes 50-60 days (from the day of reculturing the frozen cells) to get an

adequate number of clumps.

EBV immortalized B cells are described as lymphoblastoid in appearance. Relative to the quiescent precursor B lymphocytes, the immortalized cells have an enlarged appearance due to the increased cytoplasmic volume required to support high rates of RNA and protein synthesis (Figure2). They are typically ovoid or slightly

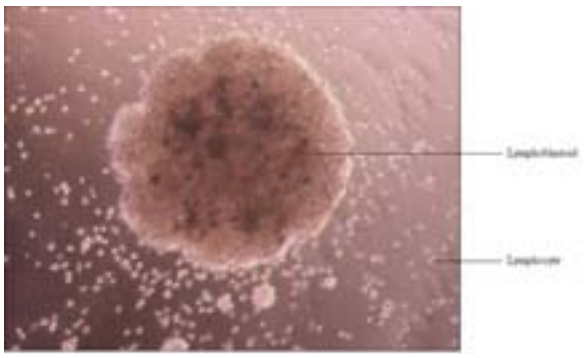


Figure 2: Appearance of an EBV-transformed B lymphocyte at 100X magnification in a light microscope

elongated and frequently have a cluster of short villipodia projecting from the surface. Generation time is between 20-30 hours and undisturbed cultures grow into macroscopic clumps of cells that adhere to each other but not the substratum of plastic culture bottles.

Applications:

EBV immortalized lymphoblastoid B cells are a very good long term source of high molecular weight DNA. Each of the established lymphoblastoid cell lines represents one unique genetic information carrier and can yield unlimited quantities of DNA for downstream applications and research. [2]

- EBV cell lines have been used widely in molecular and genetic research including applications such as transcriptomics and exposure sensitivity studies.
- A major application of EBV immortalization has been to establish cell line resources, which can potentially provide limitless amounts of genomic DNA.
- Consequently banking of EBV cell lines has been of great importance in providing reference material for rare genetic diseases, and the management of large amounts of DNA for the genetic analysis of complex conditions in population and family based disease collections.

Maintenance of records:

Meticulous record keeping and secure storage of the data are very essential as the samples are stored for a long period of time.[1] Complete clinical details relating to each sample must be recorded in a standard format and should preferably be maintained manually as well as in computerized records. Proper labeling of samples should be ensured at every stage of handling. Every sample should be given a unique banking number and the exact DNA box or cryo vial number and location in the refrigerator or liquid nitrogen can, should be noted.

Ethical, legal and social issues:

Written informed consent must be obtained before collecting any genetic material for banking from the patient himself/ herself, or in the case of an under-age minor or intellectually incompetent person, from a parent or guardian. It is important to maintain confidentiality of the patient details as well as of the genetic information relating to each sample. International standardization of ethical requirements and policies with regard to banking of genetic material has been recommended. Such standardization would facilitate protection of individuals' rights as well as future international cooperation in biomedical research[1,4,5]

References:

1. European Society of Human Genetics' PPPC. Eur J Hum Genet 2003; 11: 906-8.
2. Sie L, et al. J Neurosci Res 2009; 87: 1953-9.
3. Kronke M, et al. Proc Natl Acad Sci USA 1984; 81: 5214-8.
4. Godard B, et al. Eur J Hum Genet 2003; 11S2: 88 – 122.
5. McQueen MJ. Clin Chem Lab Med 1998; 36: 545-9.

Announcement

SIAMG-Genzyme Fellowship in Clinical Genetics

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Venue:

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Batch 1 : July 1, 2013 – September 30, 2013

Batch 2 : October 1, 2013 – December 31, 2013

Batch 3 : February 1, 2014 – April 30, 2014

For details, please visit: http://www.iamg.in/ or write to Dr. Ashwin Dalal at info@iamg.in

Announcement

International Conference on Human Genetics and 39th Annual Meeting of Indian Society of Human Genetics [ISHG-2014]

Dates and Venue: 21st to 25th January, 2014
Ahmedabad Management Association [AMA], IIM Road,
Ahmedabad, Gujarat, INDIA

Secretariat Contact: Dr. Jayesh Sheth/Dr. Alok Dhawan/Dr. Frenny Sheth
FRIGE's Institute of human Genetics
FRIGE House, Jodhpur Gam Road, Satellite, Ahmedabad - 380 015.
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Email: secretariat@ishg2014.org, ishg2014@gmail.com

For more details visit conference website: www.ishg2014.org

Microarray in Prenatal Diagnosis

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Introduction

The main aim of prenatal diagnosis is to inform couples about the risk of a birth defect or genetic disorder in their pregnancy and to help couples in making decisions on how to manage the risk. It is usually performed in response to parental request, increased risk of fetal chromosomal abnormality associated with advanced maternal age, high-risk screening test and/or the presence of a congenital malformation identified by ultrasonography.

Prenatal testing is done in samples obtained by either invasive or non-invasive methods. For invasive screening, methods like amniocentesis, chorionic villus sampling (CVS), cordocentesis and for non-invasive testing, methods like maternal alpha-fetoprotein, ultrasonography are used.

Molecular techniques used for prenatal testing include Quantitative fluorescent PCR (QF-PCR), Multiplex Ligation Dependent Probe Amplification (MLPA), Fluorescent In situ Hybridization (FISH) and Microarray analysis. Amniocentesis and CVS provide fetal cells for karyotyping and DNA analysis.

Chromosomal Microarray

Microarray is a rapidly evolving technique for prenatal testing and is now offered in some of the laboratories in India as well. More and more research and experience with microarray technique in prenatal setting is paving the way for this technique to be the first tier technique in prenatal testing. Solinas-Toldo *et al.* published the first article on array based comparative genome hybridization (array CGH) in 1997; since then this technique has become one of the fastest growing due to its ability to screen a sample for thousands to millions of different loci at once.[1]

A DNA microarray chip is a collection of microscopic DNA spots attached to a solid surface and is used to measure the expression levels of large numbers of genes simultaneously or to genotype multiple regions of a genome. Each DNA spot contains picomoles (10^{-12} moles) of a specific DNA sequence, known as probes (or reporters or oligos). These can be a short section of a gene or other DNA elements that are used to hybridize a cDNA or cRNA (also called anti-sense RNA) to the sample (called the target) under high-stringency conditions. Probe-target hybridization is usually detected and quantified by detection of fluorophore, silver, or chemiluminescence-labeled targets to determine relative abundance of nucleic acid sequences in the target. Comparative genomic hybridization is a method for detecting deletions or duplications in the genome by directly comparing an unknown DNA sample to a normal sample and identifying those areas of the genome that are either under or over represented in the study sample.

Microarray analysis is able to detect deletions or duplications that are 100 times smaller (in the kilobase range) than those identified on karyotype. It is established that deletions and duplications too small to be detected by karyotype (submicroscopic) are easily identified by microarray analysis and can cause significant anomalies. Many of these smaller changes occur frequently enough to have well characterized microdeletion or duplication syndromes (e.g. DiGeorge syndrome), whereas others may occur only sporadically but have equally significant phenotypic consequences.

There are many companies providing microarray platforms like Affymetrix, Agilent technologies, Roche and Illumina. There are a broad range of

array designs available, and recent research has focused on the appropriate design for prenatal testing. Affymetrix platforms are most commonly used. For prenatal diagnosis CytoScan 750K and CytoScan HD provide broad coverage for detecting human chromosomal aberrations.

Advantages of chromosomal microarray

The primary advantage of microarray analysis over conventional karyotyping is the ability of an array to interrogate the genome at a higher resolution. Another important advantage is the turnaround time from prenatal sample collection to result availability. Currently, the turnaround time for karyotype is 2–3 weeks, as cells must be cultured and chromosomes analyzed. For microarray analysis, cell cultures are not necessary because there is sufficient DNA in most chorionic villus and amniotic fluid samples. Thus, turnaround times are much shorter.

Armengol *et al* have simultaneously evaluated three screening methodologies in prenatal testing.[2] Techniques like Karyotyping, QF PCR, Chromosomal Microarray Analysis (CMA) and MLPA were compared for their technical performance, sensitivity, specificity and turnaround time. The findings reported were as follows: technical performance was excellent for karyotype, QF-PCR, and CMA (1% failure rate), but relatively poor for MLPA (10% failure). Mean turn-around time (TAT) was 7 days for CMA or MLPA, 25 days for karyotype, and two days for QF-PCR. A total of 57 clinically significant chromosomal aberrations were found (6.3%), with CMA yielding the highest detection rate (32% above other methods). The identification of variants of uncertain clinical significance by CMA (1.9%) tripled that of karyotype and MLPA, but most alterations could be classified as likely benign after proving they all were inherited. In spite of the higher cost for microarray analysis, high acceptability, significantly higher detection rate and lower turnaround time could justify the higher cost of CMA and favor targeted CMA as the best method for detection of chromosomal abnormalities in at-risk pregnancies after invasive prenatal sampling.

Sensitivity was significantly higher for CMA (98.2%) than for other technologies. Youden's index also revealed that CMA combines the highest true to false positive ratio. CMA yielded a superior detection rate in fetuses with abnormal ultrasound (13.3%), but it was also significant in pregnancies with a priori low risk (1.7 and 4.0%, anxiety and advanced maternal age with normal screening, respectively), far above the risk of pregnancy loss by invasive sampling. Overall, CMA detected 32% more alterations than QF-PCR.

Shaffer *et al* concluded that microarray analysis identified clinically significant genomic alterations in 6.5% of cases with one or more abnormal ultrasound findings, the majority of which were below the resolution of karyotyping.[3] Other studies also have shown similar findings.[4,5]

Limitations of chromosomal microarray

Microarray analysis does not detect truly balanced translocations. Standard microarray analysis will also not identify polyploidies because the relative gene content is balanced. Preliminary work suggests that use of SNP data, if present on an array, will identify these. Although microarray is more sensitive than karyotype for detecting small gains or losses, it does not always determine the exact size and location of the added or lost segments. Microarray also does not detect low-level mosaicism.

Another limitation of microarray analysis is the results of unknown clinical significance. As additional experience with microarray analysis is obtained, these should become much less frequent as many of these findings will be able to be reclassified as either benign or pathogenic. However, as with all prenatal testing, some findings are always difficult to interpret. At present, a worldwide consortium (International Standards for Cytogenomic Arrays; ISCA) is collecting array findings and associated phenotypes and organizing them into a database within the National Institute of Health (NIH).

Patient counseling is often difficult due to Variations of Unknown Significance and uncertain phenotype associated with some array findings. Counseling is also difficult in cases of diseases that show variable expressivity, have reduced penetrance, or have less severity or later onset.

Array designs for prenatal testing must minimize findings of uncertain clinical significance because the phenotype is incomplete or unpredictable in these cases and uncertain results can make counseling and parental decisions about pregnancy termination difficult. Whole genome arrays have a potential benefit as opposed to the targeted regions as they may change as new syndromes are identified, and would require constant editing of the array design, whereas use of a whole genome array would reduce this need.

Conclusion

Microarray technique is the most reliable technology with respect to diagnostic capacity, turnaround time, sensitivity and specificity. Its significance in prenatal testing is continuously evolving. Microarray technique in prenatal settings requires a team approach between laboratories performing arrays, clinical geneticists and counselors skilled in communicating the results to the patients. With more and more research and clinical inputs, chromosomal microarray is likely to come into routine use in prenatal testing in the near future.

References

1. Solinas-Toldo S, et al. Genes Chromosomes Cancer 1997; 20: 399-407.
2. Armengol L, et al. Hum Genet 2012; 131:513-23.
3. Shaffer LG, et al. Prenat Diagn 2012; 32:976-85.
4. Breman A, et al. Prenat Diagn 2012; 32:351-61.
5. Fiorentino F, et al. Preanat Diagn 2011;31:1270-82

genetic CLINICS

Papa telling lies - Fair or Unfair

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Life is full of situations in which it is difficult to differentiate between what is correct or wrong. Circumstances and emotions can at times compel sensible people to act in an irrational manner.

This anecdote is about a family affected with Huntington disease. Huntington disease is an adult onset, neurodegenerative, triplet repeat disorder inherited in an autosomal dominant fashion. The disease is caused by expansion of CAG nucleotide repeats in the HTT gene and the risk of transmission of the disease to each offspring of an affected individual is 50%. The proband was a 45 year old female patient who presented with involuntary movements and cognitive decline. She was diagnosed to have Huntington disease based on the molecular genetic test which showed 48 CAG repeats as against the normal of less than 26 repeats in the HTT gene. We counselled the lady and her husband regarding the progressive nature of the disease, non-availability of curative therapy and 50% probability of each of their daughters being affected. Further, we also informed them that although both the daughters were symptom free at present, pre-symptomatic diagnosis could be done to detect whether they were likely to develop the disease in the future. In the absence of a definite treatment to alleviate the symptoms of disease, the main reason for presymptomatic diagnosis in adult onset disorders is to use this information for prenatal diagnosis to prevent transmission of the disease to subsequent generations of the family. Presymptomatic diagnosis for late onset disorders without an effective intervention/ treatment is always done in adults (not in children below 18 years), after a detailed pre-test counseling especially regarding the psychosocial implications of the test.

The patient's husband brought both their daughters aged 26 years (Ms A) and 28 years (Ms B) for presymptomatic testing. The molecular test done after detailed pre-test counseling revealed that Ms A had normal number of repeats whereas Ms B had inherited the increased CAG repeats from her mother and hence was likely to develop the disease at a later age. The father came to enquire for the report and was very upset (especially since he had witnessed his wife's suffering) that one of his daughters had tested positive and was therefore likely to suffer the same gruesome fate of an untreatable degenerative disease as her mother. The father took the reports from us since the daughters were staying in a distant place. He said he would give the reports to his daughters and convey to them the facts about the disease and the option of prenatal diagnosis.

Two years later Ms B presented to us as a primigravida at 8 weeks of pregnancy for prenatal diagnosis. She told us that her father had informed her that her report was normal but it was better to undergo prenatal diagnosis just to rule out the possibility of disease in the fetus. She showed us her report (from our laboratory) which showed repeats in the normal range. We were perplexed as to why her father had told her to undergo prenatal diagnosis inspite of her report being normal. However, when we reviewed our records we found that as per our lab records Ms B had an expanded allele and re-inspection of the report she had shown us revealed the shocking truth that the report available with her was actually a tampered colour photocopy of the original report in which the number of repeats had been, with the help of digital technology, changed to normal. Thanks to digital technology, it was perfect! It is then that it dawned on us that the father in his overprotectiveness had manipulated the report to avoid mental anguish in his daughter before her symptoms started. However, without wanting to reveal the truth, he still wanted to ensure that she would undergo prenatal diagnosis as suggested by us during counseling. We explained the whole situation to Ms B and her husband. They both understood the facts regarding the disease including the likelihood of transmission to the fetus and absence of any known preventive treatment. They both expressed sympathy towards the father and appreciated his efforts to get prenatal diagnosis done inspite of his efforts to hide the truth from his daughter. Prenatal diagnosis revealed that the fetus had inherited the expanded allele from Ms B and the couple opted for termination of the pregnancy

This anecdote raises certain important ethical questions. Was the father right in hiding the truth from his daughter? Was the father's overprotectiveness or affection for his daughter impinging on the daughter's right to know her mutation status? Who was right and who was wrong? These questions do not have a definite "Yes" or "No" answer but such experiences enrich our experience as geneticists to look at the emotional and psychosocial aspects of genetic diseases.

Y-linked hearing loss, craniosynostosis, epilepsy and non-invasive prenatal diagnosis

Contributed by: Girisha KM, girish.katta@manipal.edu

Mystery of genetic basis of Y-linked hearing impairment solved [1]

It is known that the Y chromosome carries genes related to sex determination and spermatogenesis. Male sex determination and hairy ears are not Y-linked traits. The only contender for Y-linked Mendelian trait was a type of hearing impairment reported by Wang et al in 2004 in a large Chinese family. Wang et al have now shown that the apparent Y-linked inheritance in this family is actually due to a complex chromosomal rearrangement including several noncontiguous segments of the Y chromosome and insertion of a 160 kb of DNA from chromosome 1 in the pericentric region of Yp. Interestingly, the segment of chromosome 1 is derived entirely from within a known hearing impairment locus DFNA49. This means we still do not have any inheritable Y-chromosome disorder!

Bye to chorionic villus sampling and amniocentesis? [2]

Non-invasive prenatal diagnosis has been successfully used for prenatal diagnosis of aneuploidy at some centers. Srinivasan et al demonstrate that the next generation sequencing technology can be used for any type of structural chromosomal abnormality. This technology looks promising in finding a way for non-invasive prenatal diagnosis of any chromosomal abnormality. If successfully implemented, this strategy will eliminate the risk of invasive testing and make some skills outdated.

Recent advances in molecular genetics of epilepsy[3]

Epilepsy is often a challenge for the clinician, in terms of diagnosis as well as treatment. Genetic studies in recent years have tried to look into the etiology of these disorders. It is now clear that some forms of epilepsy are monogenic, many are multifactorial and a few are acquired. In a recent review, Hildebrand et al discuss the genes, copy number variations and selected genetic variants that are likely to play important roles in the causation of epilepsy. A good effort worth a look if you are interested in epilepsy genetics!

Two more genes for craniosynostosis [4,5]

Andrew Wilkie and his group have used exome sequencing to identify TCF12 in coronal craniosynostosis and ERF in complex craniosynostosis. These genes have now joined FGFR2, FGFR3, TWIST1, EFNB1, FAM20C and LMX1B to account for approximately 27.1% of the cohort with craniosynostosis. It looks certain that we will have some more genes for craniosynostosis soon!

Cellular interference explains severe X linked phenotype in mosaic males[6]

Loss of function mutations in EFNB1 causes severe phenotype in females presenting as craniofrontonasal dysplasia and males have hypertelorism as the only mild phenotypic manifestation. Functional mosaicism in females due to X inactivation is thought to be the explanation for this phenomenon with the interaction between mosaic cells being referred to as 'cellular interference'. This hypothesis is further supported by Twigg et al, proving that mosaic males really have a severe phenotype. A way not known to interfere with the normal life of cells!

References

1. Wang Q, et al. Am J Hum Genet 2013; 92: 301-6.
2. Srinivasan A, et al. Am J Hum Genet 2013; 92, 167-76.
3. Hildebrand MS et al. J Med Genet 2013; 50:271-9.
4. Twigg SR, et al. Nat Genet 2013; 45: 308-13.
5. Sharma VP, et al. Nat Genet 2013; 45: 304-7.
6. Twigg SR, et al. Hum Mol Genet 2013; 22: 1654-62.

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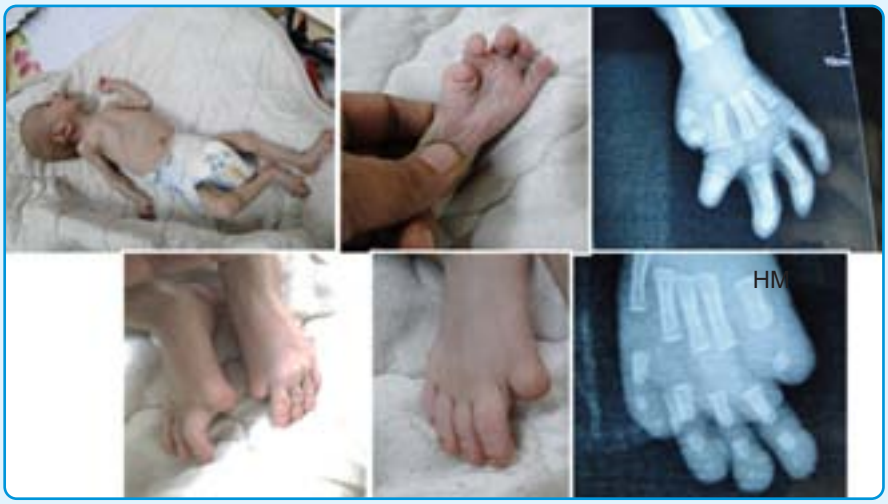
21

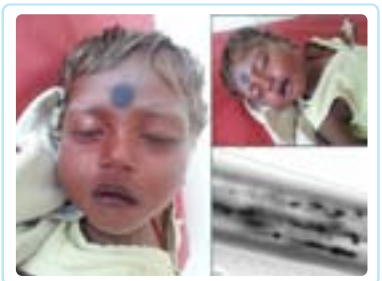
Contributed by: Dr Shubha R Phadke, Email-shubharaophadke@gmail.com

Identify this condition characterized by dysmorphic craniofacial features and typical skeletal and limb anomalies. The child also had hypoplastic clavicles. Please send your responses to editor@iamg.in



Answer to PhotoQuiz 20 of the previous issue

Griscelli syndrome



Griscelli syndrome (GS) is a rare autosomal recessive disorder characterized by hypopigmentation of the skin and hair, presence of large clumps of pigment in hair shafts and an accumulation of melanosomes in melanocytes. GS type1 (OMIM # 214450), wherein hypomelanosis is associated with primary neurologic impairment, is caused by mutations in the MYO5A gene on 15q21.2. GS type 2 (OMIM # 607624), wherein immunologic impairment and hemophagocytosis are associated with the hypomelanosis, is caused by mutations in the RAB27A gene on 15q21.3. GS type 3 (OMIM # 609227), characterized by hypomelanosis with no immunologic or neurologic manifestations, can be caused by mutations in the melanophilin MLPH (on 2q37.3) or MYO5A genes.

Correct responses were given by:

- Dr B Adhisivam, Pondicherry
- Dr G Aruna, Bangalore
- Lt Col Sameer Bhatia, Ludhiana
- Dr Niby J Elackatt, Bangalore
- Dr Krati Shah, Gujarat
- Dr Divya P, Vellore
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