Genetic CLINICS

Official Publication of Society for Indian Academy of Medical Genetics

Volume 8 | Issue 2 | April - June 2015

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Big leaps in diagnostics, small steps in therapeutics

Editorial

Correct diagnosis is the first step to treatment. Treatment is aimed at cure. DNA based diagnosis of monogenic disorders has been established and more diagnostic tests are being added rapidly. Gone is the era of linkage based diagnosis; today, in most centers DNA-based diagnosis is done by mutation detection only. This is due to improvement in the technology of sequencing as well as the development of newer techniques like multiplex ligation probe amplification (MLPA), etc. The problem with large sized genes and genetically heterogeneous disorders has been tackled by the high throughput technique of next generation sequencing (NGS). NGS technology has also been instrumental in identifying causative genes of many rare disorders. Though the pace of diagnostics is far more as compared to that of development of novel treatments and cures, research in the area of treatments is showing great promise. Duchenne muscular dystrophy (DMD) is one such example of a genetic disorder for which many novel therapeutic strategies are being explored and the review article in this issue discusses many of the conventional as well as newer diagnostic and therapeutic aspects related to Duchenne muscular dystrophy. One of the latest treatment strategies showing promise is use of an internal ribosome entry site (IRES), the article about which is mentioned in the Genexpress of this issue. The treatment strategies being innovated and tried are novel and varied. But all of them depend on better understanding of the pathophysiology based on the function of the causative gene and the different degrees of effects of various mutations on protein expression and function.

The story of most of the monogenic disorders e.g. cystic fibrosis, Marfan syndrome, etc., is the same. Identification of the causative gene and understanding of its function and of the pathophysiology of the disease has lead to development of novel treatment strategies for each of them, gene therapy being the common final goal for all. It is becoming obvious that though gene therapy may take longer than was expected, other strategies to manipulate other genes, proteins or pathways may be equally successful in ameliorating symptoms of diseases as has been seen for some diseases like Marfan syndrome, RASopathies, etc. Early diagnosis becomes important for timely intervention for such treatable disorders. For early diagnosis of these disorders newborn screening by sequencing the exome or whole genome of a neonate is an option and technically feasible today. It is being done in clinical settings in patients with difficult clinical diagnoses. One of the articles mentioned in Genexpress of this issue has reported the diagnostic yield of whole exome sequencing to be 25%. Of course analysis of sequence data and interpretation regarding the pathogenic nature of sequence variations are demons arising from the huge NGS data and the bioinformatician's fight against these demons has begun. The tools used in this war are discussed in the article on 'Prediction of Pathogenicity of Sequence Variations' in this issue. Not only laboratory personnel and geneticists but clinicians will need to be well conversant with these tools as they now need to participate in the analysis and interpretation of result data and not limit themselves to only communication of the results to the patients / families. The size of the demon of "variations of uncertain significance" will continue to shrink and whole genome / exome sequencing will become a technique for presymptomatic diagnosis; genetic medicine may then take the form of preventive medicine in the real sense. And, embryonic diagnosis and nonsurgical treatment of congenital malformations such as duodenal atresia or transposition of great vessels or cure for major defects such as holoprosencephaly may not remain just a science fiction.

Dr. Shubha R Phadke
1st April, 2015

Inherited 13q deletion in a first trimester fetus with prenatally detected parietal cephalocele

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Abstract

Parietal cephalocele communicating with the third ventricle is a rare variety of cephalocele. In this case first trimester ultrasonographic evaluation showed a parietal cephalocele which was associated with deletion of the distal part of the q arm of chromosome 13. Karyotyping of the couple revealed a balanced translocation between chromosomes 9 and 13 in the husband. This is the third reported case of parietal cephalocele with 13q deletion indicating that parietal cephalocele may be characteristic for chromosome 13q deletion. This case is also the first report of first trimester detection of parietal cephalocele associated with 13q deletion.

Case report

A 22 year old woman in her fourth pregnancy was referred to our center in view of her bad reproductive history. The first pregnancy was terminated at 19 weeks of gestation due to bilateral pleural effusion in the fetus and there were two spontaneous first trimester miscarriages after that. The present pregnancy was of eight weeks gestation and ultrasonography confirmed a viable gestation of 8 weeks. In view of her past obstetric history, karyotyping of the couple was done, which revealed a balanced translocation between chromosomes 9 and 13 [46,XY,t(9;13)(p24;q22)] in the husband (Fig 1a). The wife's karyotype was normal. Follow up ultrasonography at 12 weeks gestation revealed a parietal cephalocele measuring 4mm×4mm over the midline sagittal suture. The cavity of the cephalocele was in continuity with the third ventricle (Fig 1b). At 14 weeks gestation, follow up ultrasonography confirmed an enlarging cephalocele. Nuchal translucency was within normal limits and rest of the brain was normal in appearance. After genetic counseling, amniocentesis was performed. Fetal karyotype from amniotic fluid cell culture revealed a terminal deletion in the long arm of chromosome 13 from q22 to qter [46,XY,del(13)(q22-qter)] (Fig 1c). The couple opted for termination of the pregnancy in view of the poor prognosis. Fetal autopsy confirmed the parietal cephalocele to be an extension of the third ventricle in between two parietal cerebral hemispheres. The cerebellum, vermis and other brain structures were normal. Associated anomalies noted were hypertelorism, retrognathia, low set ears (Fig 1d), imperforate anus and penoscrotal inversion (Fig 1f), hypoplastic thumbs (Fig 1g & 1h) and talipes equinovarus deformity of the left foot (Fig 1i). No malformations of internal organs were noted.

Reported cases with inherited deletion of 13q are due to balanced rearrangement involving chromosome 13 in parents. The translocation (9;13)(p24;q22) has been reported in a family and was associated with spontaneous miscarriages and partial trisomy 13 in the offspring.[1] The deletion of a large segment of chromosome 13 in this fetus was inherited from the father who had a balanced translocation between chromosomes 9 and 13. The previous miscarriages and fetal hydrops in this couple were most likely due to chromosomal imbalances in the conceptuses.

13q deletion syndrome is an uncommon but well delineated syndrome with neural tube defects, anal atresia, genital abnormalities and hypoplastic thumb as characteristic features.[2,3] Holoproencephaly, Dandy Walker malformation, gut and genital abnormalities have also been reported in 13q deletion and many of these are prenatally detected.[4–7] Neural tube defects of all types including

cephaloceles have often been reported and the critical region is thought to be 13q33-34, which was deleted in the reported fetus.[2,8,9] Absent/hypoplastic thumb and anal atresia present in the fetus are often seen in cases with deletion 13q syndrome.

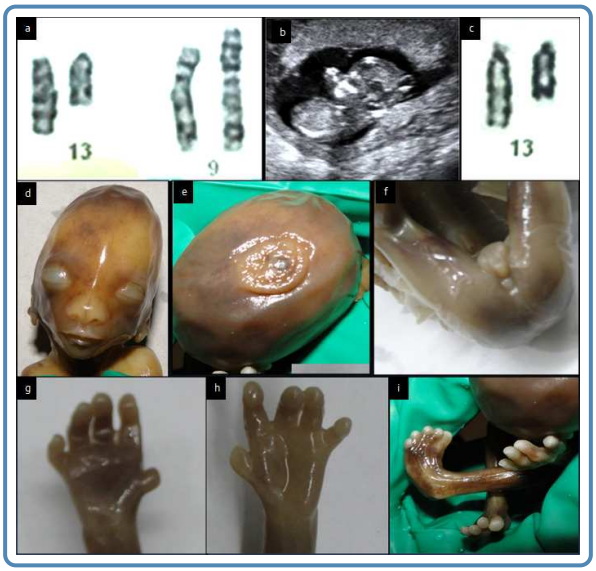

Figure 1 a) Partial karyotype of husband; b) Prenatal ultrasonography showing parietal cephalocele in connection with third ventricle; c) Partial karyotype of fetus; d) External examination of fetus showing hypertelorism, retrognathia, low set ears; e) Parietal cephalocele; f) Penoscrotal inversion and imperforate anus; g) Hypoplastic thumb in right hand; h) Hypoplastic thumb in left hand; i) Left foot showing talipes equinovarus deformity.

The present case came to our attention because of prenatal detection of parietal cephalocele at 12 weeks of gestation. There are limited reports of first trimester detection of parietal cephalocele in literature.[10] Prenatal detection of 13q deletion syndrome is reported and two of them had small parietal encephalocele detected at 16 weeks and 14 weeks of gestation respectively.[7,11] Parietal cephalocele is a rare type of cephalocele and accounts for less than 13% of cephaloceles. This is the third report of prenatal detection of this rare variety of cephalocele in association with the 13q deletion syndrome. Prenatal detection of parietal cephalocele should lead one to suspect the possibility of 13q deletion syndrome and conventional karyotyping or cytogenetic microarray is the preferred method to detect these imbalances.

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GeNeViSTA

Duchenne Muscular Dystrophy

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Abstract

With an incidence of 1 in 3500 affected males, Duchenne Muscular Dystrophy (DMD) is the most common muscular dystrophy and is inherited in an X-linked recessive pattern. Though this disorder has been known for two centuries, delays in its diagnosis and non-uniform practice of care exist even to date. Decades of research has led to better understanding of the pathophysiology of DMD which in turn has resulted in newer therapeutic advances. While these treatment strategies are yet to reach the clinic, they surely have placed an added responsibility on the treating physician to ensure early diagnosis and appropriate management so that maximum benefit can be ensured when these therapies are available. This review aims at bridging diagnostic gaps by describing various signs and symptoms of DMD followed by the currently utilized diagnostic approaches. Also discussed is the staged use of glucocorticoids, the need of multidisciplinary management and the current arenas which are being explored for the cure of this devastating disease.

Introduction

Duchenne muscular dystrophy is recognized as the most common muscular dystrophy with an incidence of 1 in 3500-5000 males.[1] Though still incurable, light seems to be emerging at the end of this long dark tunnel with many new therapies in the making.[2] While these treatment strategies are yet to reach the clinic, they have placed an added responsibility on the treating physician to ensure early diagnosis and appropriate management so that maximum benefit can be ensured when these therapies are available. Delayed diagnosis and inconsistent care in DMD has been reported and this review attempts to bridge these gaps by

a) Describing various clinical presentations – recognizing this will prevent delays in the diagnosis.

b) Discussing the available diagnostic methodologies and importantly their differences, limitations and utility.

c) Providing a brief outline on the management –which will enable uniform care to all thus helping improve quality of life.

d) Outlining the genetic basis, recurrence risks and prenatal diagnosis.

e) Providing an overview of the current novel approaches for treatment.

Clinical Phenotypes

A boy less than 5 years of age presenting with proximal muscle weakness and bilateral calf hypertrophy and 'who is one amongst many affected males' on the maternal side embodies the textbook description of DMD. This typical scenario is encountered only in 70% of the cases. A high index of clinical suspicion is necessary to diagnose the other atypical though not uncommon presentations which include:

a) Sporadic cases of DMD that account for one third of the patients affected with DMD and are usually recognized after onset of clinical symptoms.

b) Asymptomatic elevation of CPK that could be i) noted when investigations are performed for an unrelated reason ii) when an asymptomatic child is investigated due to a positive family history or iii) as a part of newborn screening.

c) Unexplained elevation of serum transaminases – consideration of muscular dystrophy is important as this could prevent unnecessary evaluation for liver dysfunction.

d) Impaired cognition or seizures in patients with symptoms suggestive of DMD- a particular behavior spectrum abnormality ranging from low IQ to an autism spectrum disorder and attention deficit can occur in 20% of Duchenne patients. Epilepsy is observed in 10% of cases.

e) Manifesting carrier females. While females are typically unaffected, mild proximal muscle weakness has been reported in 8-10% of cases.

Inclusive of the above scenarios, the term *dystrophinopathy* also includes the allelic milder disorder Becker Muscular Dystrophy as well as dystrophin-related cardiomyopathy.

When to suspect the disorder?

Proximal muscle weakness brings the disorder to parental notice. This is commonly evident as

- Difficulty in getting up from sitting or squatting position (patient gets up by supporting himself on his legs and thighs, a maneuver named as Gower's sign)
- Difficulty in climbing stairs
- Development of a waddling gait
- An inability to jump or hop or
- Tripping while running
- Other observations include a delay in walking, tip-toe walking, presence of muscle stiffness or cramps or prominent calf muscles.

Disease course

After its onset at 3-5 years of age, the disease follows a relentlessly progressive course culminating in wheelchair dependency by the age of 13 years. While the skeletal muscles bear the major brunt of the disorder, cardiac, respiratory and smooth muscles are also affected albeit at a later stage. Death occurs in the early teens due to respiratory failure which is accentuated by development of scoliosis.[3] The inclusion of glucocorticoids along with multidisciplinary care has gone a long way in changing this natural history and prolonging the life expectancy. Along with increments in life span, available evidence concludes that usage of steroids leads to better quality of life due to delay in wheelchair dependency, scoliosis and hypertrophic cardiomyopathy and better preservation of pulmonary function.[4]

Becker muscular dystrophy is a milder allelic version of DMD with a slower rate of progression, preserved ambulation till the second decade and survival into the fourth decade.

Investigations and diagnosis

1) *Creatine kinase levels (CK):* The first test to be performed on clinical suspicion of DMD is a serum CK level. An elevated serum CK (up to 100-200 times normal) is commonly observed in patients with DMD. Elevation is maximum in the initial stages of DMD followed by a decline later due to fibrous replacement of muscle. Also an increased CK is only present in approximately 30% of carrier females thus making it an unsuitable test for carrier detection.[5]

2) *Molecular diagnosis:* Molecular testing for confirmation is often provided as a two or three tier testing.

   a) **Detection of deletions and duplications:** Deletions in the dystrophin gene are responsible for 65-70% of the cases while duplications are seen in 5-10%. Evaluation for these is possible via multiplex-probe-dependent-amplification (MLPA). However this requires expertise to judge results and is currently available only in limited centers in India. Many laboratories offer multiplex PCR assay. This can be used for studying deletions in only the 'hot spot' regions (within exons 1-20 and 45-50) and cannot detect duplications. Hence MLPA is recommended as the second step if multiplex- PCR results are normal.

   b) **DMD Gene sequencing:** As elucidated above, MLPA results would be normal in 30% of affected individuals, thus necessitating further gene sequencing for detecting point mutations and small genomic rearrangements.[6]

3) *Muscle biopsy:* With current advances in genetic testing the need for muscle biopsy is decreasing. It has been an important practice parameter till recently and is still availed to in atypical situations and hence is briefly discussed here. It is important that the biopsy be performed at centers where facilities are available for immunohistochemistry (IHC) or immunoblotting (IB). If not, then a part of the muscle biopsy sample should be preserved in liquid nitro-

gen and shipped on dry ice. While routine histopathology will demonstrate dystrophy, it is only through IHC/IB that the type of dystrophy can be ascertained. Thus IHC/ IB are central to the diagnosis and need to be performed on every patient undergoing biopsy.[7] Being invasive, it is now reserved for only those cases that do not show a mutation in dystrophin or the pathogenicity of a mutation is not proven. This test helps in differentiating several other dystrophies.

The need for molecular diagnosis is often questioned. The utility is multifold including i) definitive management - allows confident discussion for use of corticosteroids in the treatment ii) carrier detection iii) prenatal testing iv) genotype-phenotype correlation v) application of newer treatment strategies like exon skipping and nonsense mutation read-through.

Management

There are two intertwined branches of dystrophinopathy management: Pharmacological management and Multidisciplinary care.

- Pharmacological management: Corticosteroids are currently the only class of drugs with proven clinical benefit. Both prednisolone/prednisone and deflazacort have been used with equal success, the former being used in nations where deflazacort is not available. While the adverse effect profiles are essentially similar, weight gain is more common with prednisolone/prednisone while cataracts occur more frequently with deflazacort. Both medications have been tested at different doses and the recommended initiating dose for ambulatory patients is 0.75 mg/kg for prednisone and 0.9 mg/kg for deflazacort.

The next pertinent issue is when to start pharmacological management – to make decision making easier, motor performance has been divided into three stages: making progress, plateau and decline. The commonest practice is to wait for the 'plateau phase' usually around 4-6 years of age when the child has stopped making any progress but not yet started to fall downhill. Parental observations, routine follow up visits and timed performance tests help recognition of this stage which in some cases is very short lasting. With loss of ambulation the dose of steroids needs to be scaled down. If however a patient presents for the first time in the non-ambulatory stage, it is still worthwhile considering glucorticoids so as to reduce the need of scoliosis surgery and preserving pulmonary function. An updated immunization schedule with special emphasis on varicella vaccine is essential before starting steroids. Principles for investigating adverse events are similar to other diseases where long term steroids are utilized.[8]

- Multidisciplinary care: Though disease progression is curtailed with steroids, it cannot be avoided and a careful modus operandi to discern involvement of other organ systems should be in place. The cardiac and respiratory muscles are the two major systems whose involvement is fatal and screening via ECG, echocardiography and pulmonary function tests should begin after 6-8 years of age. Similarly wheel chair dependency accelerates development of scoliosis which increases pulmonary burden and careful management for the same is important. A close association with experts in above fields will allow for comprehensive patient care. In addition to physical limitations, the disorder has a major psychosocial impact on the patient as well as the entire family – constant encouragement, discussing options to keep the child as independent as possible (includes rearrangements at home, wheelchair options, splints and orthosis, home tutoring, use of electronic devices) and interaction with other parents faced with the similar challenge play a major role in coping with the disorder.[9]

Genetic Counseling

The entire dystrophinopathy spectrum is an X linked recessive condition. This translates into males being preferentially affected, with females being carriers in a majority. In 1/3 cases there is no family history and the mother tests negative for the mutation detected in her affected son-implying that either it is due to a de novo mutation or germline mosaicism. The risk of recurrence varies according to the maternal carrier status. In obligate cases the risk of recurrence is 50% if the fetus is male. In sporadic cases even in the absence of a detectable genetic mutation in the mother, there exists a 8-20% recurrence risk due to germline mosaicism. Daughters have a 50% risk of being a carrier and the most sensitive method of detection is investigation for the mutation present in her family.

Prenatal diagnosis is possible in families where

mutations are known through chorionic villous sampling from 10 weeks onwards. In cases where genetic testing in the proband is pending, one can resort to linkage analysis provided the pedigree is informative and at least two affected members are available, Linkage involves detection of the high risk chromosome using Short Tandem Repeat markers and carries with itself a 5% risk of recombination which may lead to diagnostic errors.[10]

Newer drugs and emerging therapies

Research targeting various aspects of Duchenne muscular dystrophy is being explored and many compounds are currently in phase I or II clinical trials.[11] Current therapies in the pipeline include:

a) Inducing dystrophin expression – either through exon skipping using anti sense oligonucleotides, read through of stop codons, introducing a functional dystrophin molecule employing viral vectors or by upregulating utrophin expression.

b) Muscle regeneration and replacement- myoblast transfer, mesangioblast transfer and stem cell transfer are all probable modalities to achieve this. Besides this, pharmacological agents which can help muscle regeneration are also being explored and include myostatin inhibitors and IGF-1.

c) Modulation of signaling pathways- Modification of nitric oxide signaling pathways to augment its effect has shown to be beneficial in improving cardiac and skeletal performance in mouse models.

d) Inhibiting fibrosis- Fibrosed muscle signifies irreversible destruction and prevents transfer of effective therapy- hence inhibiting the same is an attractive and advantageous idea and is plausible through myostatin and transforming growth factor inhibition.[12]

Conclusion

Utilizing the above outlined diagnostic methodologies it is possible to detect cases of DMD earlier, thus making it possible to institute early management, detect carrier females and provide prenatal diagnosis. Ensuring uniform care with optimum usage of steroids and multidisciplinary care will contribute to improving the quality of life in affected patients.

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Prediction of Pathogenicity of Sequence Variations

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Introduction

The Human Genome Project revealed full landscape of the human genome at nucleotide level. This enabled us to identify differences between normal human genome (reference genome) and nucleotide sequence of patients. Sequence variations exist at defined positions within genomes and are responsible for individual phenotypic characteristics, including a person's propensity toward a disorder.

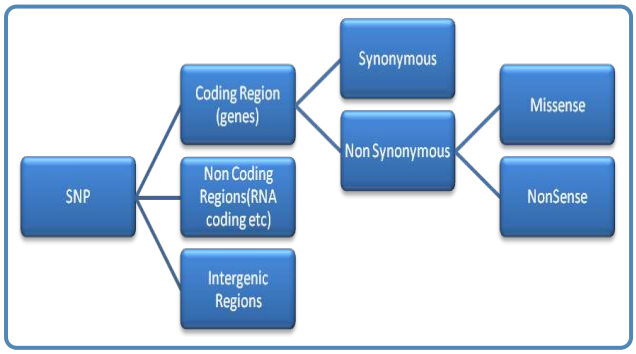

Figure 1 Classification of SNPs.

A Single Nucleotide Polymorphism (SNP) is a variation at a single position in a DNA sequence among individuals. If more than 1% of a population carries the same nucleotide at a specific position in the DNA sequence, this variation can be classified as an SNP. If an SNP occurs within a gene, the gene is described as having more than one allele. SNPs may lead to variations in the amino acid sequence. SNPs, however, are not always present in genes; they can also occur in non-coding regions of DNA. Types of SNPs are listed in figure 1. The synonymous SNPs are probably responsible for inter-individual phenotypic variation. On the other hand, non-synonymous variants are most likely to cause human disease and constitute about 90% of the mutations known to be involved in human inherited diseases.

Human genetic diseases can be caused due to de novo or inherited mutations in genes. The mutations can be detected as variants differing from the sequence in the human reference genome. However many variants may not be disease causing. It is very important to confirm whether a variation is a disease causing mutation or simply a polymorphism since many decisions like treatment, genetic counseling and prenatal diagnosis are dependent on this information.

Pathogenic changes in the nucleotide sequence usually lead to changes in the protein sequence. Protein sequences are subject to mutations in natural evolution as well as in somatic development. The direct effect of a mutation on a protein can be an effect on protein function by a number of different mechanisms. These include

- Changes in protein stability (e.g. destabilization leading to higher degradation rates, and, in the steady state, altered protein concentration).
- Change in the interaction of the protein with other biomolecules, such as other proteins, DNA, RNA or lipids, or change in the interaction with ligands, such as enzyme substrates.
- Changes in the molecular function of a protein can affect the phenotype of cells, tissues and the organism.

The importance of amino acid variation and mutations as genetic factors of human diseases has been known for many years. If the identified variation is a known disease- causing mutation then the prediction is straight forward as it is already classified as a disease- causing variant. On the other hand, if the variation is novel, then it needs to be classified as a disease-causing variant or a polymorphic SNP.

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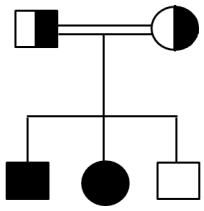
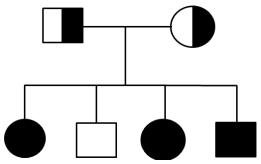
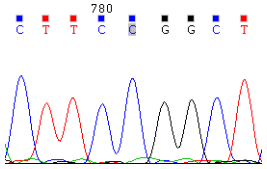
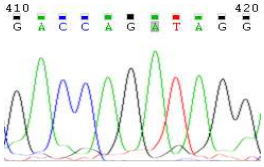
<tr><td>Pedigree</td><td>  </td><td>  </td></tr>
<tr><td>Sequence Chromatogram</td><td>  </td><td>  </td></tr>
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<tr><td>SIFT</td><td>Score (0) Damaging</td></tr>
<tr><td>PolyPhen</td><td>Score (1) Probably disease causing</td></tr>
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<table>
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<tr><td>MutationTaster</td><td>Damaging</td></tr>
<tr><td>Human Splicing Finder</td><td>Broken Wild type donor. Most Probably affecting the splicing.</td></tr>
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<tr><td>Interpretation</td><td>Variant is likely to be disease causing mutation based on these evidences:
1. Variant is homozygous in patient
2. Variant is heterozygous in both parents and normal sibling
3. Variant is not present in SNP databases and in 100 normal individuals
4. Variant is predicted to be disease causing by prediction software</td><td>Variant is likely to be disease causing mutation based on these evidences:
1. Variant is homozygous in patient
2. Variant is heterozygous in both parents and normal sibling
3. Variant is not present in SNP databases and in 100 normal individuals
4. Variant is predicted to be disease causing by prediction software</td></tr>
<tr><td>Disorder</td><td>Niemann-Pick Disease</td><td>Metachromatic Leukodystrophy</td></tr>
<tr><td>Gene</td><td>SMPD1
• Located on Chromosome 11p15
• Has 6 exons
• So far 116 mutations have been identified</td><td>ARSA
• Located on Chromosome 22q13
• Has 8 exons
• So far 189 mutations have been identified</td></tr>
</table>

Table 1 Illustration of the utility of various methods for assessing the pathogenic potential of a genetic variant.

The gold standard for classifying a variant as disease-causing or a polymorphism, is to conduct functional analysis. This is done by recreating the mutation in vitro and studying its effect on the function of that particular protein. Functional analysis can be done by employing cells in culture or in transgenic animals modified with specific variant genes or sequence polymorphisms of interest. The major drawback of these methods is that these procedures are laborious, expensive and time consuming and hence not feasible in routine clinical diagnostics.

In the absence of readily available functional validation methods, different approaches are used to gather evidence "for or against" the likelihood that the particular variant is disease- causing or not. In order to classify the obtained variation as mutation or polymorphism, the following strategies can be followed:

- The identified variation is screened against

Tool	Input	Output	Interpretation
MutationTaster http://www.mutationtaster.org/	Sequence with specific mutation or mutation position and mutated nucleotide	Effect of Mutation	–
SIFT http://sift.jcvi.org/	Ensemble Protein ID and Mutation position and Mutated amino acid	Score (0-1)	Score <0.05 damaging Score > or = 0.05 tolerated
PolyPhen-2 http://genetics.bwh.harvard.edu/pph2/	Amino acid sequence, Wild type amino acid and mutated amino acid along with position of mutation	HumVar score (0-1) HumDiv score (0-1)	Higher the score higher the probability to cause disease
HANSA www.cdfd.org.in/HANSA/	Amino acid sequence, Mutation position, wild type and mutated amino acids	Difference of wild type and mutated amino acid	Higher the difference higher the disease causing ability

Table 2 List of Mutation prediction software and their score interpretation.

SNP databases like dbSNP, 1000 Genome Project, Exome Variant Server etc. dbSNP is a database of known polymorphisms seen in humans. Hence if a variant is found to be described in dbSNP as a polymorphism then the variant can be classified as a non-disease causing polymorphism. Further the variant also needs to be looked for in 100 normal individuals from same ethnic population to identify whether it is a polymorphism or not.

- The identified variation is screened in mutation databases like the Human Genome Mutation Database (HGMD) etc. These databases contain a list of known mutations in a particular gene and if the variant is found in any of these databases then it can be classified as a disease-causing mutation. It is always important to refer to the original paper to see if functional analysis was done for the mutation, specifically in cases of rarely described mutations.
- Segregation analysis: Single gene diseases follow typical patterns of inheritance of variants in families. In case of an autosomal recessive disease, the parents and unaffected siblings of the affected child will be heterozygous for the mutation. This information regarding status of the same variant in family members can help us to postulate regarding disease- causing mutations. In the same way, a severe phenotype of an autosomal dominant condition is more likely to be due to a de novo mutation (i.e. present in the proband but not the parents).
- Pathogenicity potential prediction software: Several pathogenicity prediction software have been developed to predict the likelihood of a particular variant to be disease-causing or not, i.e. MutationTaster, SIFT, PolyPhen, Human Splicing Finder (in case of splice site variations) and HANSA.[1–5] However it is important to note that the results of these software are only predictions and have to be interpreted in association with other information regarding the variant. The obtained variation is analyzed with prediction software. An illustration of the use of these methods is shown in Table 1.

Computer based approaches play an important role in providing reliable results in a shorter time and are very easy to handle. Several methods (Table 2 & 3) for assessing the effects of mutation on protein function and abnormal mRNA splicing patterns (resulting in exon skipping, cryptic splice site use, high levels of intron inclusion) have been developed over the years. To assess a mutational effect, such methods typically use the physicochemical properties of amino acids, as well as information about the role of amino acid side chains in protein structure. These methods

Tool	Input	Output	Interpretation
NNSplice	Single/multiple sequences	Score (0-1)	Higher score implies greater potential for splice site
GENSCAN	Single sequence ≤1 million bp	Probability score (0-1)	Higher score implies a higher probability of correct exon
MaxEntScan	Single/multiple sequences (5': 9 bp (−3 to +6); 3': 23 bp (−20 to +3))	Maximum entropy score (log odds ratio)	Higher score implies a higher probability of the sequence being a true splice site
Human Splicing Finder	Single sequence ≤5,000 bp	S & S score (0-100)	Higher score implies greater potential for splice site
SROOGLE	Target exon along with two flanking introns	Different scores with their percentile scores (0-1)	Higher percentile score implies a higher ranking of the splice site within pre calculated distributions

Table 3 Summary of input, output, and interpretation of prediction scores for selected currently available *in silico* tools for 5' and 3' splice site prediction with user-friendly web interface.

combine all essential properties of both the original and substituted residues (e.g. size, polarity), structural information (e.g. surface accessibility, hydrogen bonding) and evolutionary conservation, and then are trained to distinguish between known functionally deleterious variants and presumably neutral variants. These methods assess effect of a mutation by a score computed based on a particular theoretical model. Most of these computational approaches are validated on variants with pronounced phenotypic effects, e.g. functionally deleterious and disease related variants. Such variants usually involve loss of function of a mutated gene.

Functional studies are the most accurate and reliable method for characterizing the effect of an SNP on the structure and function of the protein. However the limitation lies in the laborious, costly and time consuming procedures needed for these studies. Computer-assisted (*in silico*) technologies are considered to be efficient alternatives to in vitro experiments and are thought to have the potential to speed up the interpretation of pathogenic potential of variants pending functional validation.

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Exome sequencing reaches the clinic

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Exome sequencing as a diagnostic test in the clinic[1,2]

Since its first use nearly five years ago, next generation sequencing has made significant inroads into research as well as diagnostic laboratories. Now here comes the proof for applying whole exome sequencing in the clinic for the diagnosis of genetic disorders.[1,2] Studies have shown that the diagnostic yield of exome sequencing is likely to be around 25%. Compared with other traditional diagnostic tests, this yield is of immense value for physicians. The cost of the test having come down significantly in the last two years, this is proving to be an important tool in the hands of clinicians. If give it to an expert clinician along with a well-defined phenotype, the success is bound to be far higher.

Another attempt to treat Duchenne muscular dystrophy[3]

Duchenne muscular dystrophy is a common genetic disorder and still lacks a specific curative therapy. Several efforts in the past have failed and ongoing trials are yet to yield a tangible result that can be translated to clinical practice. Wein and colleagues have demonstrated a truncated isoform generated by exon skipping that protects muscle from contraction-induced injury and corrects muscle force to the same level as that observed in control mice.[3] To begin with, they have demonstrated that this particular isoform results from usage of an internal ribosome entry site (IRES) within exon 5 in muscle from individuals with minimal symptoms despite the presence of truncating mutations. There's a long way to go, but several such efforts are probably necessary before we have a permanent solution, that continues to elude both clinicians and families.

Consanguinity affects the fetal outcome[4]

We all know consanguinity increases the incidence of birth defects, but how does it affect the fetal outcome? A study by Becker and colleagues has thrown light on this important aspect that we all face in our clinics routinely.[4] After adjusting for several factors, the incidence of congenital anomalies was found to be 2% for non-consanguineous couples versus 5.9% for consanguineous couples (6.1% in first cousin progeny and 1.9% beyond first cousin) i.e. an excess of 3.9%. The authors have concluded that prevalence of major fetal anomalies associated with consanguinity is higher than in evaluations based only on postnatal life, a message to take home for all those who marry a relative!

Genetic basis for febrile seizures[5,6]

We knew it, right? Many children with febrile seizures including MMR vaccine related febrile seizures have similar history in a sib or a parent. Some preempt the attack by medications when MMR vaccine is given. Two papers now provide the basis for these observations. Schubert and colleagues have shown that mutations in the *STX1B* gene explain autosomal dominant fever associated epilepsy whereas Feenstra and colleagues have identified common genetic variants associated with general and MMR vaccine–related febrile seizures.[5,6]

Susceptibility to enteric fever[7]

Susceptibility to infections is multifactorial and extremely rarely Mendelian. Dunstan and colleagues have identified HLA-DRB1 as a genetic locus that has a role in susceptibility to enteric fever.[7] Their

study conducted in subjects from Vietnam and Nepal, though lacking matched controls, implicates HLA-DRB1 as a major contributor to resistance against enteric fever, presumably through antigen presentation.

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GeNeEvent – PediGen2015

PediGen2015, the second national Pediatric-Genetics conference was held at Deenanath Mangeshkar Hospital & Research Center, Pune from February 13-15, 2015. It was inaugurated at the hands of Prof Wayne Grody, UCLA, USA, and a former president of American College of Medical Genetics on February 13th 2015, following three concurrent symposia on IEM, Molecular Diagnostics and Clinical Genetics. The following one and a half days were busy with didactic lectures, case presentations and debates and special events such as Genzyme postgraduate genetics quiz. The delegates actively participated in the quiz as well as in cross-word completion. E-poster and Essay competition also received a good response from the PG students.

The conference was a great success due to participation of esteemed national and international faculty from USA, Canada, UK, Germany, Australia and Netherlands. ICMR, Genzyme, Lal Pathlabs, Centogene, MedGenome, Strandlife, GenepathDx, Neogen Labs, Serum institute and Biorad supported this unique event which was also granted four credit hours by the Maharashtra Medical council. PediGen2015 was a joint venture of Deenanath Mangeshkar Hospital and Pune chapter of Indian Academy of Pediatrics.

Winners of various competitions held during PediGen2015 are as follows:

PediGen2015 Winners

Event	Name of Delegate	Institute	Title
Essay Competition Winner	Dr. Dhanyalakshmi	Sanjay Gandhi Institute, Lucknow	*The evolved Homo sapien: My vision.*
Quiz Winners	Dr. Ami Shah Dr. Vineeti Dalal	Nanavati Institute, Mumbai	
Quiz Runnersup	Dr. Surabhi Goverdhan Dr. Sonal Mirani	Datta Meghe Institute of Medical Sciences, Sawangi, Wardha	
E-posters 1st Prize	Dr. Umesh Kalane	Deenanath Mangeshkar Hospital, Pune	*Peters plus syndrome: A sporadic case with a novel locus on chromosome 21*
E-posters 2st Prize	Dr. Nisha Chandra Babu	Bharati Hospital, Pune	*Treatable causes of Inborn errors of Metabolism*
E-posters 3st Prize	Dr. Karen Moras	Bangalore Medical College, Bangalore	*Organic acidemia presenting as shock in an infant*

PhotoQuiz - 28

Contributed by: Anju Shukla

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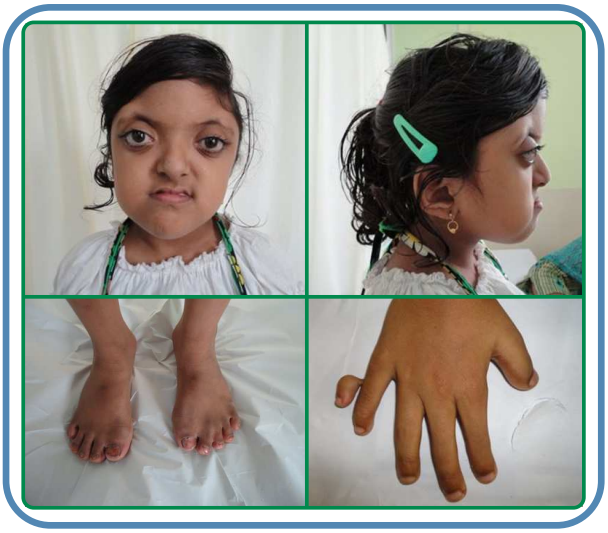
An 8-years-old girl had clinical features of cloverleaf skull, telecanthus, midface retrusion, brachydactyly, cutaneous syndactyly, postaxial polydactyly, broad thumbs and great toes with normal intelligence and presence of brachymesophalangy on radiography. Identify the condition.

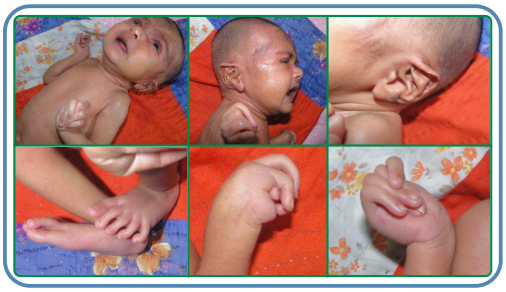
Please send your responses to editor@iamg.in
Or go to http://iamg.in/genetic_clinics/photoquiz_answers.php
to submit your answer.



Answer to PhotoQuiz 27

Congenital Contractural Arachnodactyly

Congenital Contractural Arachnodactyly (Beals syndrome; OMIM #121050) is a rare autosomal dominant connective tissue disorder caused by mutation in *FBN2* (Fibrillin-2) gene. It is characterized by a Marfanoid habitus, contractures, arachnodactyly, scoliosis, crumpled ears and rarely aortic dilatation.



Correct responses were given by:

1. Girish Subramaniam, Nagpur
2. Prashanth Verma, Saudi Arabia
3. Rekha Goyal, Jaipur
4. Niby J Elackatt, Bengaluru
5. Beena S, Chennai
6. Ravi Goyal, Kota, Rajasthan

Gaucher Disease

A Treatable Lysosomal Storage Disorder

YOU can make the difference!

- Chronic progressive disease with multi-systematic pathology
- Inherited enzyme insufficiency
- May cause disability, negatively impact quality of life and shorten life span
- Causing hepatosplenomegaly, anemia, thrombocytopenia and bone involvement
- Increases the risk of hematological malignancies, in particular multiple myeloma (up to 50x)
- Majority of children with Gaucher disease will see a pediatrician in their pursuit of a diagnosis!

- A simple Dried Blood Spot (DBS) test can be used to definitely establish the diagnosis

Early recognition of Gaucher disease is important because safe and effective treatment is available with Cerezyme (imiglucerase for injection).

Cerezyme™
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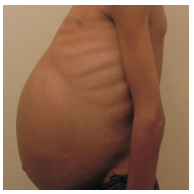
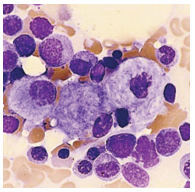
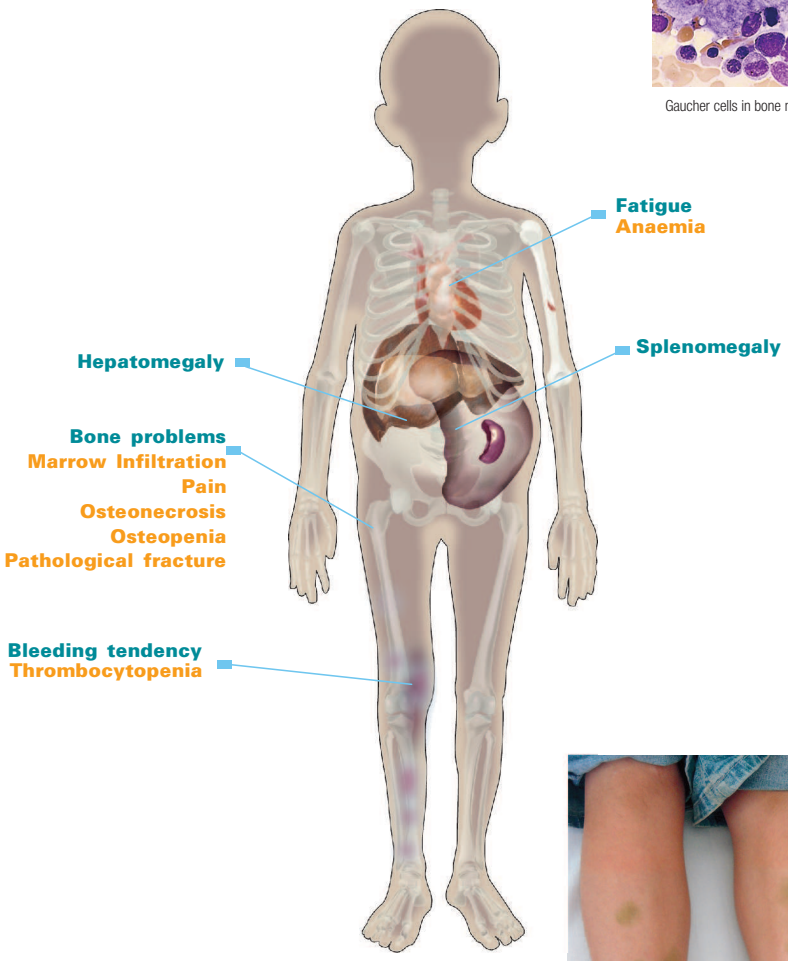


Enlarged Liver and Spleen



Gaucher cells in bone marrow



Hematoma[6]

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