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Editorial

Genetics for Clinicians: Lessons to be Learnt from Cancer Genetics

Clinical and medical genetics have gained popularity in India due to prenatal diagnosis. Prenatal diagnosis is the ray of hope to the families at risk of genetic disorders, most of which still continue to remain severe, life-threatening, handicapping and untreatable. The idea of helping families with genetic disorders using modern genetic techniques is exciting for clinicians and more and more clinicians are taking interest in genetic disorders and the applications of modern genetic diagnostic tests in clinical practice. The field of medical genetics, fetal medicine specialists and genetic laboratories are getting well established in India.

The second wave of genetics which is carrying clinicians towards modern molecular medicine is cancer genetics. Cancer is a common disease as compared to traditional genetic disorders like chromosomal disorders and monogenic disorders. And cancer is a genetic disease. Mutation in a gene in one cell of an organ of a body is the beginning of non stop growth of cells which progresses to the cancerous process. Better understanding of genetic defects has led to better understanding of the pathogenesis development of diagnostics and therapeutic strategies. The article in this issue by Dr Kaur and Dr Prakash has provided a good overview of the subject. Most of the diagnostic criteria especially for hematological malignancies include molecular defects consistently associated with the specific type of malignancy. Most of the commonly used genetic tests for cancer diagnosis and prognostication are available in India. Techniques mainly used for research in cancers like Genome Wide Association Studies, RNA expression profiling, copy number variations studies and exome sequencing are being used in clinical practice for developing personalized treatments for cancers. In this era, surgeons, oncologists, hematologists, internists, ophthalmologists, skin specialists, bone specialists or pediatricians cannot deal with cancers without the knowledge of medical genetics.

The other role of genetics in cancers is in the field of hereditary cancers. It is well said about inherited cancers that while all cancers have a genetic basis, 'some cancers are more genetic'. Five to ten percent of cancers are inherited in the family due to a germline mutation in the cancer predisposing gene. There is high risk of this mutation getting transmitted to the next generation and hence, the family members are at increased risk of cancers. This calls for providing genetic counseling to the affected member and his or her at-risk relatives. Mutation detection by molecular techniques plays an important role in identifying at-risk individuals and providing them surveillance and preventive measures. Identification of patients with familial cancer syndromes from the common types of sporadic cancers is the responsibility of the treating physician or surgeon.

Thus an oncologist of the twenty first century needs to have a thorough knowledge of cancer genetics and stay abreast with recent developments. Principles of cancer genetics are no different from those of medical genetics. One needs the knowledge of basic genetics, molecular techniques and genetic counseling. At present, the undergraduate and postgraduate medical courses do not give required stress on these aspects. How do we bridge this gap between current medical training and rapidly advancing knowledge of cancer genetics or medical genetics in general? Recently, we conducted one experiment in this direction. An eight lecture course on cancer genetics was organized in the Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow for postgraduate students. In addition to the introductory lectures there were lectures on various hereditary cancers; each talk illustrated some principle of cancer genetics like Knudson hypothesis, use of mutation testing, genetic

counseling, issues of screening family members, microsatellite instability, etc. Some prototypes of common cancers like cancer of the urinary bladder and malignant melanoma were also discussed to highlight use of genetic tests in follow up and development of treatments based on understanding of the molecular pathogenesis. Each of the participants was given one recently published article related to cancer genetics, to discuss and present. The pre-course test results showed an average marking of 8 out of 25, while just after 8 lectures the post course evaluation showed a much better performance, with the participants securing around 16 out of 25 marks on an average. This indicates that updating knowledge in this area is not a difficult task, if the clinician wants to learn. More important than the post course performance were the successful presentations of cancer genetics-related articles highlighting latest molecular techniques, which depicted not only gain of knowledge, but also helped students lose their inhibitions to learn so called 'difficult DNA- based articles'.

The success and positive feedback of this course proves the utility of such short courses and stresses the need to conduct them in all medical institutes. This is essential to equip the next generation clinicians with the knowledge of basic genetics and molecular genetics. These trained clinicians and medical teachers of tomorrow will spread the light of genetics and will prove to be of great importance not only in patient management but also pave ways in the direction of cancer research.

Shubha Phadke
1st April, 2013

Announcement

Second International conference of the Indian Society for Inborn Errors of Metabolism (ISIEM) (IEMCON-2013)

Conference : 5th – 7th April, 2013
Workshop : 4th & 8th April, 2013
Organized by : Center of Medical Genetics, Sir Ganga Ram Hospital, New Delhi and ISIEM
Highlights : Approach, Diagnosis & Management of IEMs. Newborn screening & Newer advances in metabolic disorders. Renowned faculty from across the world would be participating in the unique meeting designed for all Geneticists, Pediatricians & Neonatologists.

For further information, please contact Dr IC Verma, Dr Ratna Puri or Dr Sunita Bijarnia-Mahay.
Email: iemcon2013@gmail.com or visit: http://iemcon2013.com/

Molecular diagnosis of Spondylothoracic Dysostosis – novel mutations: Case report and literature review

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Abstract

Spondylothoracic dysostosis (STD) is an autosomal recessive disorder characterized by abnormal vertebral segmentation throughout the spine, with complete bilateral fusion of the ribs at the costovertebral junction producing a "crab-like" configuration of the thorax. The condition is prevalent in the Puerto Rican population, although it is a pan-ethnic disorder. We report a fetus with STD which was diagnosed on the basis of characteristic radiographs (AP and lateral). The diagnosis was then confirmed by gene testing. We discuss the diagnostic criteria of STD and spondylocostal dysostosis (SCD) and review the molecular genetics of vertebral segmentation defects.

Introduction

Spondylothoracic dysostosis, aka dysplasia, (STD, MIM#277300) is an autosomal recessive disorder with high prevalence in the Puerto Rican population. The shortened spine and trunk can severely affect respiratory function thus causing early lethality in about 44% of cases.[1] A form of generalized vertebral segmentation defects with mal-aligned ribs was first described by Jarcho and Levin in 1938, a phenotype which is slightly different to STD and better designated as SCD.[2,3] Over time the nomenclature for widely varying forms of abnormal vertebral segmentation has become somewhat confusing, with the terms spondylocostal dysplasia, costovertebral dysplasia, Jarcho-Levin syndrome, SCD and STD all being used interchangeably. A more logical and simplified nomenclature has recently been proposed.[4]

Case Report

A 30 year old second gravida was referred to our department at 20 weeks of pregnancy. Her level II ultrasound showed multiple hemivertebrae/ butterfly vertebrae in the region of dorsal and lumbar spine (Fig 1). The spine was short. There were no other malformations detected on ultrasound. The marriage was non-consanguineous. There was no history of bleeding, fever, rash or drug exposure during the pregnancy. Her TORCH IgM serology was negative. She had previously suffered one early miscarriage.

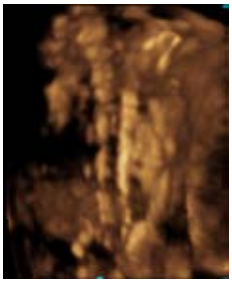
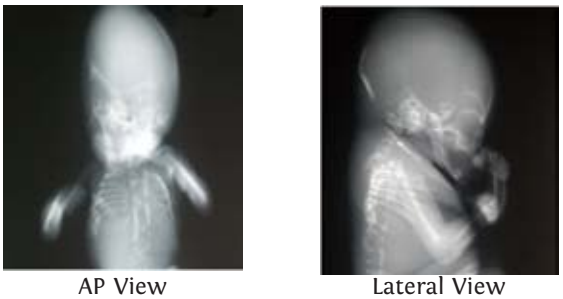


Fig 1: 3D USG of fetus

According to the ultrasound findings she was counseled that the baby would have a poor chance of survival due to respiratory insufficiency. Following this, the couple opted for termination of pregnancy and the fetus was sent for autopsy. The weight of the fetus was 354 g, corresponding to 21

weeks gestation. Crown rump length was 13 cm, corresponding to 17-18 weeks of pregnancy. Foot length was 3.6 cm, corresponding to 21 weeks of gestation.

The fetus had a large mouth, depressed nasal bridge, almost absent neck and short trunk. The limbs were of normal length and the arms extended beyond the knee joint. The abdomen was distended. The extremities were normal with no polydactyly. X-ray of the fetus showed hemivertebrae throughout the spine with the ribs showing a 'crab-



AP View Lateral View

Fig 2. X-ray of Fetus

like' appearance (Fig 2). The X-rays were sent for expert opinion prior to mutation analysis. It was noted that the vertebral pedicles were prominent, giving rise to the so-called 'tram-line' appearance of the spine. The ribs fanned out from the posterior costo-vertebral origins with no points of fusion along their length. These signs were considered to be consistent with a MESP2-associated form of segmentation disorder rather than the more commonly encountered DLL3-associated SCD, in which the pedicles are not ossified in early life and the appearance has been designated 'pebble-beach' sign.

Molecular analysis

Genomic DNA was extracted from the fetus and both parents. Mutation analysis of MESP2 gene was performed according to the protocol previously described.[5] Mutation analysis identified compound heterozygosity for two MESP2 mutations – c.178_188delins7 and c.348_351dupCCAG, thus confirming the diagnosis of autosomal recessive STD. Sequencing analysis showed that mother was heterozygous for the frameshift mutation c.178_188del ins7 in exon 1 of the MESP2 gene. This mutation is a deletion of eleven nucleotides (AGCTCCCGAGC) and an insertion of seven nucleotides (GGCTCGG) at nucleotide 178 (c.178_188delins7) which results in a premature termination codon at 118 (p.Ser60fs). Mother was therefore confirmed to be a carrier of STD. Sequencing analysis of father's DNA showed heterozygosity for the frameshift mutation c.348_351dupCCAG in exon 1 of the MESP2 gene. This mutation is a duplication of four nucleotides (c.348_351dupCCAG) and results in a premature termination codon at 367 (p.Ser118fs). Father was therefore confirmed to be a carrier of STD. The risk that any subsequent pregnancy will be affected with STD is 25%.

Discussion

The term "Jarcho-Levin syndrome" is used frequently for all radiological phenotypes that include multiple segmentation defects of the vertebrae (M-SDV) and abnormal rib alignment. The diverse radiologic phenotypes with M-SDV that make up the 'spondylocostal dysostoses' has highlighted the need to rationalize nomenclature for these poorly understood disorders. The International Consortium for Vertebral Anomalies and Scoliosis (ICVAS) has proposed algorithms for classification of these disorders. The Clinical Algorithm, used for routine reporting of SDV, identifies seven broad categories or groups based on the involvement of single or multiple vertebrae, regional or generalized involvement and whether or not a syndrome is defined or undefined.[4]

The classification is premised on the following.

1. As the system deals with segmentation and malformations of the vertebrae occurring early in morphogenesis, all dysplasias are essentially excluded;
2. The eponymous term Jarcho–Levin should be

discontinued because it is used too broadly and is confusing. Klippel–Feil anomaly has more specific usage and should be retained;

3. SCD/STD should be referred to as dysostoses, not dysplasias; furthermore, SCD and STD should be used to describe very specific phenotypes;

4. Apart from established syndromes that include SDV, and the monogenic forms of SCD, the ICVAS classification relies on a systematic description of the radiological features; it will inevitably evolve a more genotype–phenotype correlations are discovered. Spondylocostal dysostosis (SCD) refers to a radiological phenotype that includes generalized segmentation defects of the vertebrae (G-SDV) with abnormalities of the ribs, e.g. rib fusion, malalignment, and/or abnormal rib number (usually reduced).

The radiological phenotype is characterized in general by:[3]

1. Abnormal segmentation of virtually all vertebrae, with at least ten contiguous segments affected;
2. Pebbled beach appearance of vertebrae in fetal life and early childhood;
3. A mild degree of scoliosis, which is usually non-progressive;
4. Malalignment of at least some ribs with a variable number of intercostal rib fusions, and sometimes a reduction in rib number;
5. Overall, a general symmetry to the shape of the thorax (at least, no major asymmetry).

Based on differences in radiological findings, it may be possible to identify the various sub-types of SCD. The majority of these cases show autosomal recessive inheritance and the Notch signaling pathway genes DLL3 (SCD1), MESP2 (SCD2), LFNG (SCD3) and HES7 (SCD4) have been identified.

SCD Type1 is due to mutation in the DLL3 gene. In early childhood the vertebral bodies are ovoid and vary in size and shape giving rise to the 'Pebble beach' sign. The ribs may show fusion distal to the costovertebral articulation.

SCD Type 2 is due to mutation in the MESP2 gene. There is generalized SDV with more angular features in the spine than in type 1. The lumbar vertebrae are relatively mildly affected.

SCD Type 3 is due to the LFNG gene mutation. All vertebrae show major segmental abnormalities and the spine is severely shortened (though to date only one family has been reported).

SCD Type 4 is due to mutation in the HES7 gene. Segmentation anomalies of the vertebrae are more severe and resemble those seen in STD.

Spondylothoracic dysostosis is characterized by: [6]

1. Abnormal segmentation of all vertebral segments with characteristic "sickle cell shaped vertebrae" when seen in cross-section on CT scan;
2. Severe shortening of the spine, especially the thoracic spine;
3. Rib fusions typically occurring posteriorly at the costovertebral origins, where the spinal shortening is most severe. The ribs usually appear straight and neatly aligned without points of fusion along their length. On antero-posterior x-ray the ribs characteristically 'fan out' from their costovertebral origins in a 'crab-like' fashion;
4. The early radiographic prominence of the vertebral pedicles gives rise to a distinctive radiographic appearance that has been called the 'tramline sign' (which is not seen in SCD type 1); the vertebral bodies have no regular form or layout.3
5. Overall, a general symmetry to the shape of the thorax.

STD occurs most frequently in Puerto Ricans of Spanish descent. The prevalence of STD has been estimated at one in 12,000 live births in the Puerto Rican population. No exact prevalence data exist for the rest of the world.

Our case was diagnosed as STD based on the distinguishing radiological features – more severe shortening of spine, posterior fusion of ribs, which were neatly aligned and straight without any point of fusion along their lengths, fanning of the ribs and 'tram line' sign of early radiographic prominence of vertebral pedicles. There was no apparent intrinsic rib abnormality. STD is caused by mutation in the MESP2 gene. Mutation analysis in our case showed it to be a compound heterozygote for two MESP2 mutations c.178_188delins7 and c.348_351 in exon 1, confirming autosomal recessive STD. The MESP2 mutation c.178_188delins7 removes 11 nucleotides (AGCTCCCGAGC) and inserts 7 nucleotides (GGCTCGG) at nucleotide 178, resulting in a premature termination codon at 118 (p.Ser60fs). The MESP2 mutation c.348_351dupCCAG in exon 1 is a duplication of 4 nucleotides (CCAG), which results in a premature termination codon at 367 (p.Ser118fs). Both parents were carriers of one mutation. These mutations produce a more severe phenotype compared to the MESP2 mutations that cause SCD type 2.

The MESP2 gene, a member of the basic helix-loop-helix (bHLH) family of transcriptional regulatory proteins essential to a vast array of developmental processes, is critical for normal somitogenesis. The gene is involved in the Notch signaling pathway. Whittock et al. identified the human MESP2 gene in the chromosome 15q26.1 region of the human genome in a consanguineous family of Lebanese Arab origin with 2 offspring affected with SCD.[5] MESP2 gene has 2 exons and spans approximately 2 kb. The MESP2 gene is predicted to produce a transcript of 1,191 bp encoding a protein of 397 amino acids. The MESP2 protein contains an N-terminal bHLH region and a unique CPXCP motif immediately C-terminal to it.

To date, most individuals reported with STD have had nonsense mutations in exon 1 of MESP2, which are predicted to result in nonsense-mediated decay; however, several affected individuals are heterozygous for a nonsense mutation and a missense mutation.[7]

In 12 Puerto Rican probands with STD, Cornier et al. identified 3 different biallelic mutations in the MESP2 gene. The most common allele was E103X consistent with a founder effect in this population. In a 12-year-old girl of Puerto Rican origin with a severe form of STD they identified a homozygous 307G-T transversion in exon 1 of MESP2, resulting in a glu103-to-ter (E103X) substitution in the bHLH domain.[7] The mutation was predicted to produce a nonfunctional protein and be susceptible to nonsense-mediated RNA decay. The patient also had scoliosis, a tethered spinal cord, and malrotation of the right kidney. In 10 of 12 additional Puerto Rican probands with the disorder, the authors identified homozygosity for the E103X mutation. One patient was compound heterozygous for the E103X and 373C-G transversion resulting in a leu125-to-val (L125V) substitution. Another 2 sibs, who were third cousins of an E103X homozygous proband, were compound heterozygous for the E103X and a 688G-T transversion in exon 1 resulting in a glu230-to-ter (E230X) substitution. Heterozygous carriers were unaffected. In general, E103X homozygotes were more severely affected than compound heterozygotes. The findings were consistent with a founder effect in the Puerto Rican population. Our case is a compound heterozygote for the MESP2 mutations c.178_188delins7 and c.348_351 in exon 1. These are previously unreported mutations. The recurrence risk is 25% in each pregnancy. Prenatal diagnosis is possible by mutation analysis on chorionic villus tissue at 10-12 weeks of pregnancy. Prenatal diagnosis can also be made by ultrasound of the fetal spine, sometimes as early as 14 weeks gestation. If the couple opt to continue an affected pregnancy, the delivery should be arranged in a tertiary center where intensive neonatal care is available, due to the high risk of respiratory

insufficiency. The genetic testing strategy in a proband with M-SDV or G-SDV is determined primarily by the radiographic appearance of spine, as follows:[8]

1. DLL3 is usually sequenced first as it is the most commonly implicated gene; if normal, MESP2 gene is sequenced next;
2. If the radiological features are closer to the STD phenotype, MESP2 gene is sequenced initially; if normal, HES7 may then be tested, followed by DLL3;.
3. If severe truncal shortening is observed LFNG may be sequenced first, followed by DLL3.

In summary, we describe a case of autosomal recessive Spondylothoracic Dysostosis, conceived to a non-consanguineous Indian couple, due to previously unreported protein truncating mutations in MESP2, and we have reviewed the literature and nomenclature on monogenic forms of abnormal vertebral segmentation in humans.

References

1. Cornier AS, et al. Am J Med Genet 2004; 128: 120–6.
2. Jarcho S, Levin PM. Bull Johns Hopkins Hosp 1938; 62: 216-26.
3. Turnpenny PD, et al. Dev Dynamics 2007; 236: 1456–74.
4. Offiah A, et al. Am J Med Genet Part A 2010; 152A: 1357–71.
5. Whittock NV, et al. Am J Hum Genet 2004; 74: 1249–54.
6. Corneir AS. Spondylothoracic Dysostosis. In: GeneReviews at GeneTests. http://www.genetests.org. Accessed on 5 Feb 2013.
7. Cornier AS, et al. Am J Hum Genet 2008; 82: 1334–4.
8. Turnpenny PD, Young E, (International Consortium for Vertebral Anomalies and Scoliosis) ICVAS. Spondylocostal Dysostosis, Autosomal Recessive. In: GeneReviews at GeneTests. http:// www.genetests.org. Accessed on 6 Feb 2013.

Contributed by: Dr Prajnya Ranganath

If you have a patient with muscle weakness or seizure,
Send him to a neurologist and a geneticist for good measure.

If you have a patient with an enlarged liver or spleen,
He could be having an acquired problem or a defect in some gene.

If you have a patient with a defect in the heart,
It may be isolated or of some syndrome it could be a part.

If you have a patient who is not thriving well or short,
He may have skeletal dysplasia or a syndrome of some sort.

Keep a genetic etiology always in mind,
Especially if a case confounds you, it is very likely to be of a genetic kind.

Cancer Genetics: From Bench to Bedside

Dr Anupriya Kaur, Dr Gaurav Prakash

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The incidence and prevalence of cancers is rapidly increasing in our country. In the years to come, malignant disorders will become one of the most common and challenging diseases for health care providers. The development of newer genetic techniques and a better understanding of cancer related cellular pathways have established a close relationship between the fields of genetics and oncology. In fact, a Familial Cancer Clinic has now become an integral part of successful Oncology Practice.[1] Today geneticists are providing oncologists with not only diagnostic tests but also the key knowledge of cancer genetics which helps them to assess the prognosis of a patient suffering from cancer, selection of the most appropriate anticancer therapy and also monitoring of the response to treatment. Therefore it is prudent for anyone involved either in dealing with cancer patients directly or indirectly in cancer research to have a basic understanding of cancer genetics and its clinical applications.

Cancer is a genetic disease. The process of oncogenesis begins with a mutation in a single gene in a single cell and then progresses to cause genomic instability leading to accumulation of mutations in that cell. These mutations affect the functioning of 3 major classes of cancer genes: Proto-oncogenes, Tumor suppressor genes, and DNA mismatch repair genes. The normal functions of the gene get disturbed causing the affected cell to undergo uncontrolled growth and proliferation, which ends in the form of cancer. All cancers are due to genetic alterations; however, a positive family history of cancer is often absent. This is due to the fact that in most cases the cancer causing mutations are acquired during the life of an individual. But in certain cancers a mutated gene involved in oncogenesis is inherited from a parent and further mutation(s) are thereafter acquired in the life of the individual which ultimately results in malignancy. Such cancers are labeled as the Hereditary Cancer Syndromes (Table 1). The likelihood of getting a positive family history in

Table 1: Important Hereditary Cancer Syndromes

Hereditary cancer syndromes	Common cancers
Hereditary Retinoblastoma	Retinoblastoma, Pinealoma
Familial Adenomatosus Polyposis Coli	Colon cancer, Gastric cancer, Fibroma
Hereditary Non-Polyposis Colon Cancer	Colon cancer, Brain cancer, Uterine cancer, Gastric cancer
Hereditary breast and ovarian cancer syndrome	Breast cancer, Ovarian cancer, Uterine cancer, Colon cancer, Pancreatic cancer, Melanoma, Gastric cancer
Neurofibromatosis 1 and 2	Neurofibromas
Tuberous sclerosis	Benign Tubers
Ataxia telangiectasia	Acute leukemia
Von Hippel-Lindau syndrome	Renal cell cancer, Hepatoblastoma, Brain cancer
Fanconi anemia	Acute leukemia
Li-Fraumeni sysndrome	Breast cancer, Sarcoma, Brain cancer, Adrenal cancer

these cases is higher than in the other cases. Also, like for other multi-factorial disorders, now researchers have identified several susceptibility loci for various cancers, which predispose individuals in some families to a higher tendency for a malignancy compared to the rest of the population. Besides this, there are certain Mendelian disorders with predisposition to cancers. A good family history and pedigree analysis is invaluable in all such cases.

This review focuses on two facets of cancer genetics –one which has already come in routine clinical practice of oncology and the other which deals with improving our understanding of tumor biology ultimately aiming at personalized anticancer therapy. Table 2 lists the application of some genetic tests in commonly seen cancers. Chronic

Table 2: The clinical applications of genetic testing in some common cancers

Disease	Genetic Aberration	Currently used genetic technique for testing	Clinical implication	Biological Sample
Chronic Myeloid leukemia	t(9,22) Philadelphia chromosome	Karyotyping, FISH	1-Diagnosis 2-Assessesment of response to therapy	Blood or Bone marrow
	bcr: abl transcripts	Real time PCR	Monitoring response to therapy	Blood or Bone marrow
Acute promyelocytic leukemia	PML-RARA transcripts	RT-PCR	For diagnosis and monitoring of response to therapy	Peripheral blood or Bone marrow
Acute Myeloid leukemia	Several chromosomal aberrations	Karyotyping	Prognostication and Selection of optimal therapy	Blood or Bone marrow
	FLT3 internal tandem duplication, NPM gene mutation, CEBPA mutation	PCR	Prognostication and Selection of optimal therapy	Blood or Bone marrow
Breast Cancer	ERBB2 amplification	FISH	Selection of Therapy (Trastuzumab)	Tumor tissue
	Multiple gene mutations	Mammaprint: 70 gene Tissue microarray	Assessment of need of adjuvant chemotherapy after surgery	Tumor tissue
		OncotypeDX: 21 gene expression by RT PCR	Assessment of need of adding chemotherapy with hormonal therapy in early stage cancer	Tumor tissue
Colon cancer	K–RAS mutation	PCR	Selection of Therapy (only patients with wild type K-RAS respond to anti EGFR therapy)	Tumor tissue

Myeloid Leukemia (CML) is a prime example of how the discovery of the presence of a chromosomal aberration i.e Philadelphia chromosome t(9,22) lead us to understand the whole molecular mechanism of this malignancy. Oncologists are now regularly using the Tyrosine Kinase Inhibitor (Imatinib) specifically designed to target the fusion gene product of the translocation (bcr-abl fusion gene) and FISH (Fluorescence insitu hybridization) and Real-Time PCR (polymerase chain reaction) are being routinely used for measuring reduction in the probe signals and bcr-abl transcripts respectively, to monitor the cytogenetic and molecular response to treatment. While conventional karyotyping, FISH and PCR based tests have become standard techniques in cancer management, the present technological advancements (Microarray and Next Generation Sequencing-NGS) are expected to become routine tests in the future.

A review of the current literature on cancer genetics shows that this area of genetics is also evolving into cancer genomics. Two major biotechnological advancements which are expected to play a centre-stage role in the near future in the field of cancer research are Microarray and NGS.The major applications of microarray in oncology have been for Gene Expression Profiling (GEP), array CGH (comparative genomic hybridization) and the use of SNP (single nucleotide polymorphisms) platforms for genome wide association studies(GWAS). In GEP the genomic signature of a tumor is assessed by studying RNA expression. Microarray provides information about which genes are over-expressed or under-expressed in the cancerous tissue as compared to normal tissue. GEP has been found to correlate with patient prognosis in certain hematological as well as solid malignancies. For example, GEP has enabled classification of Diffuse Large B cell Lymphoma (DLBCL) into 2 different subtypes viz activated B cell type and germinal center type, of which the activated B cell type is known to have a worse outcome. Currently scientists are trying to develop special chemotherapy protocols for this subgroup of DLBCL patients. In the times to come it is anticipated that this knowledge would be available at the time of diagnosis itself and thereby, could be used in optimizing therapy of such patients. Similarly tissue microarray for breast cancer, Mammaprint is a study of 70 key genes. Based on the information on expression of these genes in a given patient, an oncologist can decide whether a patient requires chemotherapy or not. Going by the possible short term and long term side effects of anticancer drugs, this information is highly valuable for certain breast cancer patients who have early stage disease and are likely to have only borderline benefit with chemotherapy.

NGS is expected to play an important role in oncology in the future. An International research consortium tested the RObustness of Next generation sequencing (IRON study) on 3 genes (TET2, CBL, KRAS) which are important for myeloproliferative neoplasms. This study showed that together with the output of high-quality long reads and fast run time of such sequencing systems, there was also utility of deep sequencing in clinical applications.[2] Also, large scale cancer genome studies, such as The Cancer Genome Atlas (TCGA) and the International Cancer Genome Consortium (ICGC) are applying next-generation sequencing technologies to tumors from 50 different cancer types to generate more than 25,000 genomes at the genomic, transcriptomic, and epigenomic level, and will provide the foundation for a complete catalog of oncogenic mutations.[3]

Recently there has been a shift from population based GWAS studies to microarray based studies focusing on personalization of cancer care.[4] Personalization of anticancer therapy traditionally has meant identifying a person's inherited (germline) allele status for certain important gene(s) affecting the pharmacokinetics or dynamics of a particular anticancer drug to be used for their treatment. Studies have been initiated to use NGS for personalizing oncology practice. Integrative high

throughput sequencing techniques are now aiming to identify the somatic alterations in a patient's cancerous tissue and thereafter using this information for tailoring the anticancer therapy accordingly. In simplified terms, for example, such sequencing techniques may provide hundreds of mutations in a given biopsy specimen. Pathway specific expertise is then utilized and the most likely pathway(s) in a given mutational landscape is/ are chosen, blocking which is expected to control the tumor process. Such a strategy has been shown to work out practically in a time relevant frame period of 4 weeks.[5] However, in the times of direct-to-consumer testing, a word of caution needs to be given that before any test is offered to a patient proper pre- and post-test counseling sessions need to be conducted, as through them we ensure that the patient is prepared for the various possible implications of testing andis also made aware of our current limitations of differentiating significant from non-significant genomic changes found after testing.

There are now a fair number of examples of use of the cancer genetics-related information in the selection of targeted anti cancer agents. Table 3 illustrates few such examples. CML is again exemplary to highlight role of genetic information in modification of a patient's treatment. It is now understood that one of the common causes of treatment failure with imatinib for CML is acquisition of mutations in the tyrosine kinase domain. Kinase domain mutation studies are employed to identify specific mutations causing drug resistance and then the most appropriate second line drug is selected which is active against the given mutation.

To conclude, our knowledge about cancer genetics has expanded tremendously. We are now aiming to identify the molecular genomic signature of a particular cancer and use this information for targeted anticancer therapy. Providing personalized medicine in cancers on a routine basis shall be the most rewarding outcome of cancer genetic research. Indeed, the knowledge and application of cancer genetics can become our most powerful weapon to conquer Cancer "The emperor of all maladies".[6]

References

1. Duncan PR, Lin JT. J OncolPract 2011; 7: 39 – 42..
2. Kohlmann A, et al. Leukemia2011;25: 1840-8.
3. Hudson TJ, et al. Nature 2010; 464:993–8.
4. Shao L, et al. PLoS One 2012;7:e29534.
5. Roychowdhury S, et al. SciTransl Med 2011;3: 111-121.
6. Mukherjee S. The Emperor of all Maladies. London: HarperCollins Publishers;2011.

Table 3: Important examples of targeted anticancer therapy determined by genetic alterations

Disease	Genetic alteration	Targeted anticancer drug/s
Chronic Myeloid Leukemia	bcr-abl Tyrosine kinase present	Tyrosine Kinase Inhibitor- Imatinib, Dasatinib, Nilotinib
Non Small Cell Lung Carcinoma	EGFR mutation present	Anti EGFR drugs -Erlotinib, Gefitinib
Melanoma	BRAF mutation present	Vermurafenib
Colorectal cancer	K -RAS allele wild type	Anti EGFR monoclonal antibodies- Cetuximab, Panitumumab
Breast cancer	ERBB2 over expression	Anti HER2 Monoclonal antibody Trastuzumab
Acute Promyelocytic Leukemia	PML-RARA transcript present	All Trans Retinoic Acid(ATRA)

The Spinocerebellar Ataxias

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Cerebellar ataxias are a group of disorders with progressive incoordination of gait and are often associated with poor coordination of hands, speech, and eye movements. They can be classified into sporadic and inherited ataxias. The inherited ataxias are further divided into autosomal dominant, autosomal recessive, X-linked and mitochondrial ataxias.

The spinocerebellar ataxias (SCAs) are a group of autosomal dominant hereditary ataxias characterized by both clinical as well as genetic heterogeneity. They are much less common than the sporadic and acquired causes of ataxia but nonetheless represent a unique type of mutation i.e. triplet repeat expansions. These disorders usually manifest in adulthood but sometimes can have a more severe and early onset due to expansion of these triplet repeats during transmission from the parent to the offspring. This phenomenon of earlier and more severe onset in successive generations of an affected family is called anticipation and trinucleotide repeat mutations, which tend to expand during transmission from one generation to the next, are called "dynamic mutations".[1] SCAs also exhibit extreme genetic heterogeneity with at least 30 genes or genetic loci known till date. These two characteristics pose certain special issues in genetic diagnosis and counselling. Hence, SCAs are being discussed here as a prototype of triplet repeat disorders with special emphasis on issues related to diagnostic testing and genetic counselling.

Clinical features

The prevalence of SCAs is 1 to 3 per 100,000 worldwide. Till date, 31 types of SCAs have been identified. Although SCA9 has been reserved, no clinical or genetic information regarding this type has been published. Polyglutamine expansion SCAs i.e those involving expansion in the CAG repeat units, are more frequent than the other forms of SCA. SCA3 is the most frequent subtype in the world but rare in India (3%). SCA 2 is the most frequent type of spinocerebellar ataxia in India.[2]

The mean age at onset of most SCAs is generally in the third or fourth decade of life, and is mainly, but not only, determined by the number of CAG repeats in the corresponding gene.[3] Gait disorders are the initial symptom in two-thirds of all patients with SCA. Classic findings of cerebellar involvement including wide based gait, rebound phenomenon, dysmetria, past pointing etc. are present. The unique clinical features and genes for some of the common types of SCA are summarized in Table I.

Pathogenesis

As for the other neurodegenerative disorders, protein misfolding has been classically hypothesized to play a role in the pathogenesis as shown by the presence of intracellular inclusions in these disorders.[4] Lately, small oligomers rather than large inclusions have been proved to be "toxic" with large polymerized structures being the "protective" forms. The abnormal aggregates have also been shown to interfere with normal homeostasis in the neurons leading to large scale protein misfolding. In certain animal models, resistance to autophagy i.e. failure to degrade the abnormal protein has been demonstrated, contributing to the pathogenesis of the disease. Dysregulation of gene expression due to localization of the mutant protein in the nucleus with inhibition of acetone deacetylases and repression of transcription has been shown with consequent use of deacetylase inhibitors as therapeutic targets in some animal models.[5] A few

Table I: Unique clinical features, associated genes and type of nucleotide repeat in common SCAs

Common types of SCA	Unique clinical features	Genes involved	Type of trinucleotide repeat
SCA1	Pyramidal signs (67%) Brainstem oculomotor signs (74%)	ATXN1	CAG
SCA2	Peripheral nerve involvement (68%)	ATXN2	CAG
SCA3	Dystonia(24%)	ATXN3	CAG
SCA6	Pure cerebellar ataxia Purkinje cells degeneration with no other specific symptoms	CACNA1A	CAG
SCA7	Retinal degeneration Decrease in visual (83%) and auditory (24%) acuity	ATXN7	CAG
SCA10	Epilepsy	ATXN10	ATTCT
SCA17	Epilepsy	TBP	CAA/CAG

recent trials on mouse models of SCA have used small interfering RNAs to decrease the amount of mutant proteins in the cell: a potentially attractive therapeutic strategy for the future.

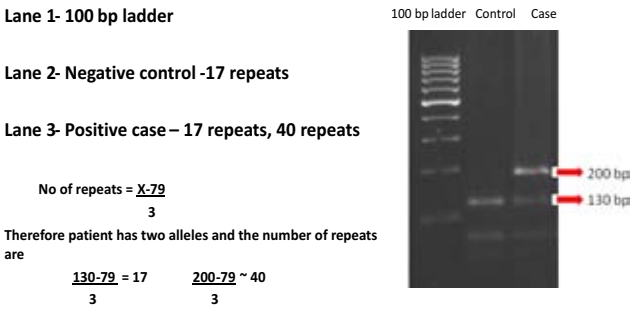
Diagnosis

To approach a case of hereditary ataxia, detailed three generation family history should be elicited. Complete neurologic examination of clinical symptoms and signs along with neuroimaging should be carried out. On finding a positive family history of ataxia or recognizing a clinical phenotype frequently with evidence of cerebellar atrophy in imaging, the next step would be to identify an ataxia-causing mutation. In sporadic cases, exclusion of non-genetic causes of ataxia like alcoholism, vitamin deficiencies, multiple sclerosis, vascular disease, primary or metastatic tumors, or paraneoplastic diseases associated with occult carcinoma of the ovary, breast, or lung should be done before proceeding to genetic testing.

Genetic Testing

Since the SCAs are caused due to repeat expansions, primers are designed complementary to flanking regions of the repeats in respective genes for each type of SCA and PCR products are run on agarose gel or capillary electrophoresis to identify the size of the products. The repeat number is deduced from the size of PCR product as shown in Figure 1.

PCR products analysed on 2% agarose gel



Because of the broad clinical overlap, most laboratories that test for the hereditary ataxias have a battery of tests including testing for SCA1, SCA2, SCA3, SCA6, SCA7, SCA10, SCA12, SCA14, and SCA17. Many laboratories offer them as two groups in a stepwise fashion based on the population frequency, testing first for the more common ataxias e.g SCA1, SCA2, SCA3, SCA6, and SCA7 in one panel. This panel is available in the Centre for DNA Fingerprinting and Diagnostics as well as in the Centre for Cellular and Molecular Biology, Hyderabad and in a few other private laboratories in India. If a strong clinical indication of a specific diagnosis exists based on the affected individual's examination or if the

family history is positive for a known type, testing can be performed for a single disease. The interpretation of test results can be complex because the exact range for the abnormal CAG repeat expansion has not been fully established for many of these disorders

Issues in Genetic Counselling

These disorders are inherited in an autosomal dominant manner and hence, most affected individuals will have an affected parent. However,family history may appear to be negative because of

1. early death of a parent,
2. failure to recognize autosomal dominant ataxia in family members,
3. late onset in a parent,
4. reduced penetrance of the mutant allele in an asymptomatic parent, or
5. a de novo mutation.

The risk to sibs depends on the genetic status of the proband's parents. If one of the proband's parents has a mutant allele, the risk to the sibs of inheriting the mutant allele is 50%. Individuals with autosomal dominant ataxia have a 50% chance of transmitting the mutant allele to each offspring.[6]

Testing of at-risk asymptomatic adult relatives and children

Testing of adult relatives of individuals with autosomal dominant cerebellar ataxia is possible after molecular genetic testing has identified the specific disorder and mutation in the family. Such testing should be performed after proper genetic counselling. It should be emphasized that testing is not useful in predicting age of onset, severity, type of symptoms, or rate of progression in asymptomatic individuals.Molecular genetic testing of asymptomatic individuals younger than age 18 years who are at risk for adult-onset disorders for which no treatment exists is not considered appropriate, primarily because it negates the autonomy of the child with no compelling benefit.

Anticipation

Anticipation is observed in the autosomal dominant ataxias in which CAG trinucleotide repeats occur. It refers to earlier onset and increasing severity of disease in subsequent generations of a family. In the trinucleotide repeat diseases, anticipation results from expansion in the number of CAG repeats that occurs with transmission of the gene to subsequent generations. ATN1 (DRPLA) and ATXN7 (SCA7) have particularly unstable CAG repeats. In SCA7, anticipation may be so extreme that children with early-onset, severe disease die of disease complications long before the affected parent or grandparent is symptomatic.

Prenatal Testing

Prenatal diagnosis for the hereditary ataxias is possible by analyzing fetal DNA by chorionic villus sampling at about ten to 12 weeks gestation or amniocentesis at about 15 to 18 weeks gestation in case the type of SCA is known in the particular family.

Treatment of Manifestations

Management of ataxias is usually directed at providing assistance for coordination problems through established methods of rehabilitation medicine and occupational and physical therapy.

- Canes, walkers, and wheelchairs are useful for gait ataxia.
- Special devices are available to assist with handwriting, buttoning, and use of eating utensils.
- Speech therapy may benefit persons with dysarthria. Computer devices are available to assist persons with severe speech deficits.

Newer treatment strategies

Many therapies are being tried in animal models of hereditary ataxia with partial success in controlling the various manifestations of the disease. Lithium has been shown to improve the motor incoordination, behaviour as well memory in mice model of SCA1 while thiamine has been found to be effective in SCA 2. Novel peptides which bind the mutant proteins in SCA 3 and reduce their aggregation lead to decreased motor manifestations. Also, recently Sodium valproate has been found to decrease apoptosis and alleviate signs of neurodegeneration. Single oligonucleotides have been designed to target almost all the SCAs to reduce mutant protein RNA and decrease the symptoms.

References:

1. Schols L. Lancet Neurol 2004; 3:291–304.
2. Saleem Q. Hum Genet 2000 ;106:179-87.
3. Schmitz-Hubsch T. Neurology 2008; 71:982–9.
4. Riley BE. Genes & development 2006; 20:2183–92.
5. Butler R. Nat Rev Neurosci2006 ;7:784–96.
6. Bird T. Hereditary ataxia overview. GeneReviews at GeneTests. At http;// www.genetests.org. Accessed on February 6, 2013

Announcement

Hajdu-Cheney syndrome (HJCS:OMIM 102500) is a rare genetic disorder characterised by generalised (osteoporosis) and focal (acro-osteolysis) bone loss, variable congenital abnormalities, faltering growth and dysmorphic features. Autosomal dominantly transmitted mutations in the gene encoding Notch2 cause HJCS.Current medical management is through the use of the bone anti-resorptive agents, bisphosphonates, such as disodium pamidronate, to maintain bone mass. The treatment may not always be effective, nor does it prevent or improve the acro-osteolysis, which necessitates the development of new treatment options. Dr Melita Irving and her team at Guy's Hospital and King's College, London, UK are seeking to determine the incidence of Hajdu Cheney Syndrome worldwide to help inform a drug development programme. They are keen to hear about any known patients and affected relatives to help gauge the number of individuals with the condition.

Please mail to Melita.Irving@gstt.nhs.uk

Announcement

Twelfth ICMR Course on Medical Genetics and Genetic Counseling

29th July to 10th August, 2013

Organized by:

Dr Shubha Phadke
Department of Medical Genetics
Sanjay Gandhi Postgraduate Institute of Medical Sciences, Raebareli Road, Lucknow 226 014
E-mail: shubharaophadke@gmail.com

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For details and application form visit www.iamg.in

Non-invasive prenatal diagnosis and more...

Contributed by : : Dr Parag Mohan Tamhankar. Email: paragtmd@gmail.com

Massively parallel sequencing promises a more "gentle approach" to the fetus [1]

The classical method of diagnosing fetal chromosomal abnormalities has been karyotyping or fluorescent in situ hybridization following amniocentesis or chorionic villus sampling. The invention of "Non-invasive Prenatal Diagnostics" (NIPD) i.e. using cell free fetal DNA in the maternal plasma to diagnose fetal genetic abnormalities has revolutionized fetal diagnosis. The conquests of NIPD have been fetal sex assessment, fetal RhD determination, fetal trisomy 21 and diagnosis of fetal single gene disorders such as achondroplasia, myotonic dystrophy, and Huntington disease (dominant diseases that are de novo or paternally inherited). Now, to add to the list, diagnosis of fetal trisomy 18, trisomy 13, and monosomy X is possible using massively parallel sequencing (MPS). Srinivasan et al published a series of seven cases wherein fetal microdeletions, duplications, translocations, and trisomy 20 were detected by MPS, including a microdeletion as small as 300 kb. Slowly but surely technology promises a shift that is more gentle on the mother and the fetus.

Using a Thorn to Draw Another [2]

Enzyme replacement therapy (ERT) using Myozyme (alglucosidase alfa) has revolutionized management of Pompe disease. However, as expected, antibodies develop to the foreign protein in a significant group of patients. Patients who have sustained high antibody responses enter a prolonged phase of clinical decline resulting in death despite ERT. Banugaria et al targeted the plasma cell source of high sustained antibody titres; a regimen based on bortezomib (Velcade) was used in combination with rituximab, methotrexate, and intravenous immunoglobulin. Three patients were treated and a massive reduction in antibody response was observed. Thus, using immunomodulatory "antibodies" targeting the plasma cell repertoire was found to be safe and effective.

Refining molecular basis for autism [3]

Copy number variants (CNVs) of genomic regions/ genes have been implicated in autism and intellectual disability. However, these CNVs may occur in the normal population. Girirajan and colleagues explored the genomic hotspots with repeat architecture. They targeted segmental duplication mediated hotspots and deletion hotspots that are flanked by repeat sequences. They found that gene disruptive events were enriched in autistic individuals and genes such as DPP10, PLCB1, TRPM1, NRXN1, FHIT, and HYDIN were affected. Interestingly, autism severity increased directly as the size of duplications increased; however, it was neither affected by size of deletions nor gene disruptive events. They concluded that autism was a net effect of imbalance of multiple genes rather than disruption of single genes.

References

1. Srinivasan, et al Am J Hum Genet 2013; 92:167-76.

2. Banugaria, et al. Genet Med 2013; 15:123-31.

3. Girirajan, et al. Am J Hum Genet 2013; 92: 221-37.

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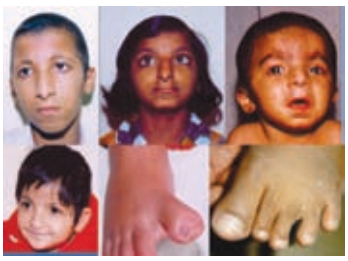
Contributed by: Dr Prajnya Ranganath, Email-prajnyaranganath@gmail.com

This 10 months old male child was brought for evaluation of recurrent infections, hepatosplenomegaly, developmental delay and hypopigmented hair. Hair microscopy (HM) showed typical findings. Identify the condition.

Answer to PhotoQuiz 19 of the previous issue

Rubinstein Taybi syndrome (OMIM # 180849 & 613684)



Rubinstein Taybi syndrome is a multiple malformation disorder with typical craniofacial features (downslanting palpebral fissures, columella extending below the nares, grimacing smile and talon cusps), broad thumbs and great toes, short stature and moderate to severe intellectual disability. Other associated features that may be present include ocular anomalies such as coloboma and cataract, congenital heart defects, renal abnormalities and cryptorchidism. It is an autosomal dominant disorder that almost always occurs due to a de novo mutation. CREBBP and EP300 are the only two genes currently known to be associated with Rubinstein Taybi syndrome and together account for up to 50 – 60% of the cases.

Correct responses were given by:

- Anupriya Kaur, Chandigarh
- Akanchha Kesari, via email
- Prashant Kumar Verma, via email
- Nupur Sarkar, Bhopal
- Beena Suresh, Chennai
- Krati Shah, Bristol
- Niby J Elackatt, Bangalore
- Kalpana Gowrishankar, Chennai
- Prochi Madon, Mumbai
- A M Kulkarni, Davangere
- Ranjith Kumar, New Delhi
- Mohandas Nair, Kozhikode
- Sheetal Sharda, Chandigarh

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