NEUROLOGY

GENETIC TESTING IN NEUROLOGICAL DISORDERS - RADIOGENOMICS

*Sheffali Gulati
**Sonali Singh
**Rahul Sinha

Abstract: *"Radiogenomics" is a new emerging field which correlate imaging features with genotype of disease. It has potential to play an important role in medicine, particularly neuro-oncology, metabolic and neurodegenerative disorders. Genetic testing confirms the diagnosis, helps in prognostication, predicts the risk of recurrence. But selecting the right genetic test is of prime concern and is difficult. Radiological finding or radio phenotype is useful in selecting the genetic tests needed. Role of radiogenomics is bidirectional. It predicts genotype on the basis of radiological phenotype and vice versa. So it plays an important role in bridging the gap between phenotype and genotype. It will further help the clinician to go for targeted sequencing of the gene which will be cost effective and time saving. For example the pediatric tumours which are studied on the basis of radiogenomics are medulloblastoma and glioblastoma. New generation genomic sequencing is very useful in sequencing the DNA at faster rate and lower cost. Whole genome sequencing, whole exome sequencing, clinical exome sequencing and target gene panel sequencing are various tests which are available. Radiogenomics is an important tool to indicate the likely genotype and directs towards the right genetic investigation.*

Keywords: *Whole genome sequencing, Whole exome sequencing, Gene panel sequencing, Radio genomics.*

"Radiogenomics" is a new emerging field which correlates the imaging characteristics of a disease (radio phenotype), with its genetic or molecular features (genomics or genetic phenotypes). It has potential to play an important role in medicine. The 1990s marked the beginning of genetic evolution In field of neurology too, genetics has an important role to play. Radio genomics still has a long way ahead before it becomes usable in daily clinical practice. First, it requires standardisation of imaging protocols, including image acquisition and post-processing, as well as robust segmentation algorithms that require minimal operator input.[1,2] Most of the studies mentioned in the literature involve novel imaging techniques that specically interrogate aspects of underlying tumor biology and biochemical pathways which have great potential in neuro-oncology. Radiomics applies to advanced computational methods to automatically extract and analyze hundreds or thousands of quantitative imaging descriptors (radiomic features) from a tissue-of-interest on medical imaging data, thus earning the -omics suffix to describe the field.[3] In the current era of precision medicine, identification of genetic variation is of prime importance. Genetic testing confirms the diagnosis, helps in prognostication, predicts the risk of recurrence in patient and their relatives. Importance of genetics is well known, however selecting the right genetic test is of prime concern (Table I). In resource limited setting as ours, each investigation is planned carefully. Here comes the role of radiology. Radio genomics aims to predict genotype on the basis of radiological phenotype and vice versa. Thus it plays an important role in bridging the gap between phenotype and genotype.

Clinico-radiological phenotyping helps to answer the question "Which genetic test should I ask for?"

Field of radio genomics can be extended to metabolic neurodegenerative disorders which have good homogeneity in clinical, radiological and genetic domain.[4] It will further help the clinician to go for targeted sequencing of the gene which will be cost effective and time saving. For example the pediatric tumours which are studied on the basis of radio genomics are medulloblastoma and glioblastoma. The genetic traits underlying were IDH mutations and 1p/19q co deletion.[5] IDH protein or Isocitrate dehydrogenase (IDH) 1 and 2 are metabolic enzymes that are mutated in a wide range of blood and solid tumor cancers.

* Chief ,
Child Neurology Division,
Center of Excellence and Advanced Research on Childhood Neurodevelopmental Disorders

** Senior Resident, D.M Pediatric Neurology,
Department of Pediatrics, AIIMS,
New Delhi.

email : sheffaligulati@gmail.com

Table I. Comparison of features of different genetic tests

	Karyotype	FISH	Microarray	DNA sequencing
Detects large deletions or duplications	Yes	Yes	Yes	-
Detects deletions or duplications in part of a chromosome	-	Yes	Yes	-
Detects small deletions or duplications	-	-	Yes	-
Detects translocations	Yes	-		-
Detects sequence changes and single gene mutations	-	-	-	Yes

We should be clear that growing popularity of next generation sequencing cannot undermine the importance of karyotyping and microarrays etc. genetic tests should be ordered only after narrowing down the differentials clinically.

Next generation genetic sequencing is a technological advance which enable sequencing of million base pairs of DNA at a faster rate and lower cost. Depending on the extent of genes covered, next generation sequencing offers following tests (Table II).

- Whole genome sequencing (WGS) : WGS covers all introns (non coding regions) and exones (coding regions) i.e. determines the complete DNA sequence of an organism's genome at a single time.
- Whole exome sequencing (WES): It is a genomic technique for sequencing all of the protein-coding regions of genes in a genome (Exons) - about 20,000 genes, in one single test.
- Clinical exome sequencing : This is a test for identifying disease-causing DNA variants within the 1% of the genome which codes for proteins (exons) or flanks the regions which code for proteins (splice junctions) about 5,000-6,000 genes.
- Target gene panel sequencing: These are useful tools for analyzing specific mutations in a given sample. E.g. Inborn errors of metabolism, immune deficiency, epilepsy.

Table II. Comparison of features of next generation sequencing tests

Next generation sequencing (NGS)			
	Clinical exome sequencing	**Whole exome sequencing**	**Whole Genome sequencing**
Coverage	5000-6000 clinically relevant genes	20,000 genes	Covers coding and non-coding regions of genome
Depth of coverage	High 100X	High 100X	Low 30X
Limitations	Restricted number of genes Cannot detect novel genes	Cannot detect variants outside the exonic regions	Complicated interpretation of data Very costly
Clinical Use	Clinical	Clinical	Currently mostly used in research setting
Ability to detect copy number variants (CNV)	Cannot detect	Cannot detect	May be analyzed to detect CNV

Note: Good phenotyping remains the corner stone of deciding the appropriate genetic test and increasing likelihood of a positive yield.

Facts to be remembered while requesting NGS

1. Is this the right test for the disorder? - e.g. Triplet repeats cannot be detected by NGS, southern blot is the technique used.
2. Is the required gene included? - If we do not know what we are looking for, NGS may not provide the answer
3. Depth and coverage of the gene - e.g. SMN gene has poor coverage by NGS
4. Yield and cost
5. Turnaround time
6. Interpretation - Interpretation becomes complicated when a number of variants are detected with inaccurate phenotype.

In pediatric neurology, imaging has emerged as an important aid in bridging the gap between phenotype and genotype. Imaging findings in certain disorders are specific for a particular genetic variation. There are others where imaging findings narrows down the differentials, but multiple genes are implicated. In the former, targeted gene sequencing can be done, however the latter requires gene panel testing. Role of radiogenomics is bidirectional. Phenotype can predict the genotype, as well as genotype helps to predict likely phenotype. A simplified way of classifying these disorders are[6]

a) Disorders with clinical, imaging and genetic homogeneity

b) Disorders resulting from different gene mutation but having similar clinical and imaging findings.

c) Disorders with different clinical and imaging findings, inspite of involvement of a same gene

Common neurological diseases in pediatric population with diagnostic imaging findings are discussed briefly.

1. Pantothenate kinase 2 (PANK2) associated neurodegeneration with brain iron accumulation (NBIA)

Gene involved is PANK2, which provides instructions for making an enzyme called pantothenate kinase 2

MRI brain reveals the presence of "Eye of tiger sign" which indicates PANK2 mutation (Fig.1). There is hypointensity in globus pallidi on T2 weighted images resulting from iron deposition. There is foci of T2 hyperintensity (superomedial aspect) of variable size present within the area of T2 hypointensity representing pallidal destruction.[7]

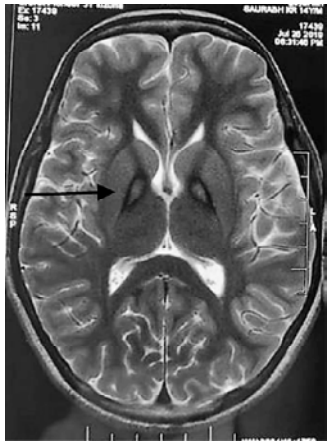


Fig.1. T2 weighted image - Eye of tiger sign. Hypointensity in globus pallidi with foci of hyperintensity medially (black arrow).

Differential diagnosis: Other disorders of neurodegeneration with brain iron accumulation (NBIAs) (non-PANK2) may also have globus pallidus T2 hypointensity but lack T2 hyperintense foci. All cases with PANK2 mutation have eye of tiger sign during course of illness but not all eye of tiger sign cases showed PANK2 mutation.

2. Infantile neuroaxonal degeneration

The gene is PLA2G6, which provides instructions for making an enzyme called A2 phospholipase.

MRI brain shows progressive cerebellar atrophy, vertically oriented splenium and claval hypertrophy. Some patients may show thinning of optic nerves and chiasma, and T2 hypointensity in the globipallidi (corresponding to iron deposition) (Fig.2).

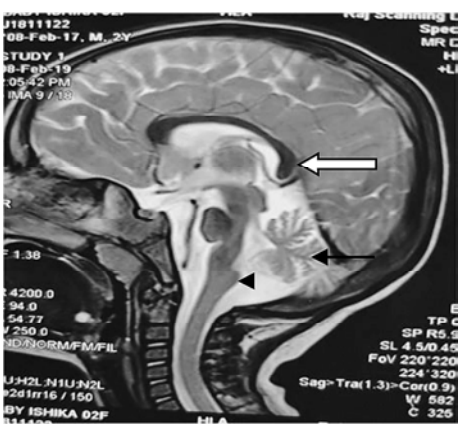


Fig.2. T2 weighted image shows cerebellar atrophy (black arrow), claval hypertrophy (black arrow head), vertically placed splenium (white arrow)

Differential diagnosis: Other NBIAs do not show cerebellar atrophy.

3. Megalencephalic leukoencephalopathy with subcortical cyst (MLC1)

The gene is MLC1 which encodes a membrane protein known as MLC1 which is found primarily in the brain. MRI shows swollen subcortical white matter. Subcortical cysts characteristically begin from anterior temporal lobes. It eventually involves frontal and parietal lobes too (Fig.3 a&b).

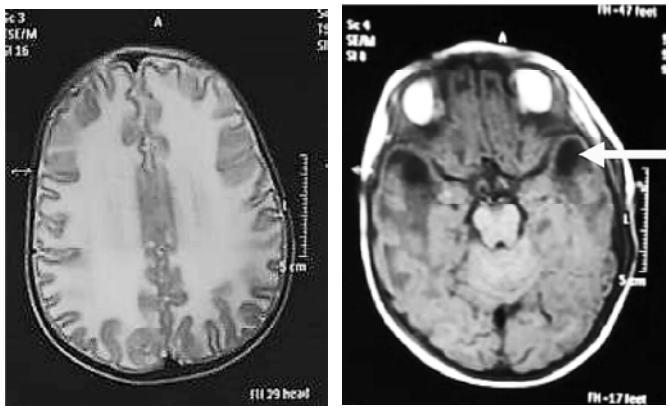


Fig.3. T2-weighted a&b MRI images of the brain show bilateral symmetrical diffuse white matter hyperintensity along with bilateral temporal cysts on FLAIR image.

4. Cerebral Adrenoleukodystrophy

The gene is ABCD1 (ATP binding cassette transporter) which provides instructions for producing the adrenoleukodystrophy protein (ALDP), which is located in the membranes of peroxisomes.

MRI findings: Most common pattern is predominant posterior white matter involvement. Usually first area to be affected is middle of corpus callosal splenium, extending into parieto-occipital areas. MRI shows T1 hypointensity and T2/FLAIR hyperintensity of affected white matter. There is characteristic contrast enhancement of inflammatory leading edge of demyelination (Fig.4).[8]

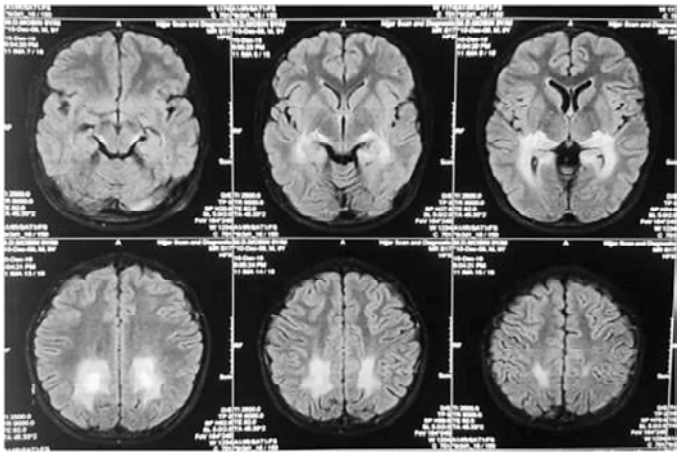


Fig.4. FLAIR images show Posterior predominant white matter involvement. Lowe score for this patient- 9-10.

5. Krabbe

The gene is GALC which encodes for an enzyme called galactocerebroside beta galactosidase (GALC)

CT scan shows high density in bilateral thalami, caudate nuclei, corona radiata, cerebellar dentate nuclei. MRI shows abnormally bright thalami on T1 weighted images. On T2 weighted images cerebellar nuclei, corticospinal tracts are abnormally hyperintense. Some also show enlarged optic nerves (Fig.5 a&b).[9]

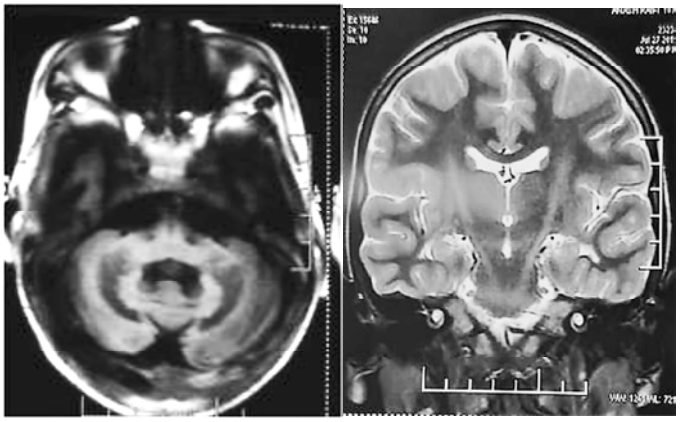


Fig.5 a&b. MRI image shows signal changes in bilateral dentate nuclei, Cortico-spinal tract involvement

6. Vanishing white matter

The gene is EIF2B, which encodes one of five subunits of eukaryotic translation initiation factor 2B.

MRI shows diffuse white matter signal changes. Abnormal hypointensity on T1, hyperintensity on T2.

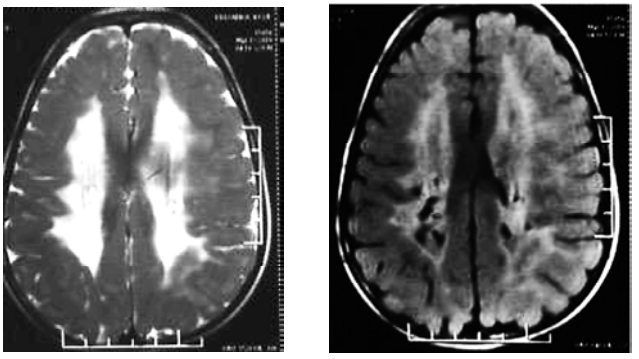


Fig.6. a&b. T2, FLAIR images show cavitating white matter involvement with sparing of subcortical white matter.

White matter eventually cavitates. On diffusion weighted images, diffusivity is reduced in areas which are in process of cavitation. Subcortical fibers may be spared early in the disease. Temporal lobes may be relatively spared (Fig.6 a&b).[10]

7. Alexander disease

The gene is GFAP, which encodes a protein called glial fibrillary acidic protein of mature astrocytes.

MRI changes: Frontal predominant cerebral white matter signal changes, periventricular rim with T1 hyperintensity and T2 hypointensity, abnormal signal changes in basal ganglia, thalami, brainstem is seen in MRI. Contrast enhancement is noted in periventricular region and lower brainstem regions (Fig.7).

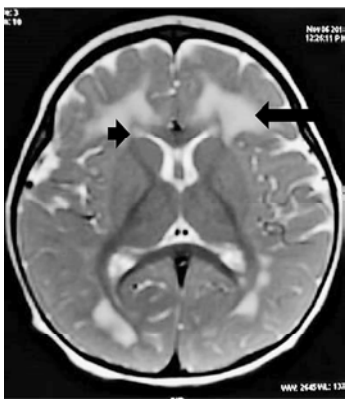


Fig.7. T2 weighted MRI image shows bilateral cerebral white matter hyperintensity predominantly in frontal lobes (long black arrow). In addition, there are focal ring-like lesions in frontal white matter at the tip of frontal horns (short black arrow).

8. Glutaric Aciduria I

The gene is GCDH, which provides instructions for making the enzyme glutaryl-CoA dehydrogenase.

MRI findings: Open sylvian fissures due to hypoplastic frontal and temporal opercula and T2/FLAIR hyperintensity in basal ganglia, delayed myelination is seen in MRI brain. During acute decompensation, cerebellum nuclei are involved. Chronic subdural hematoma is noted in few of the cases (Fig.8).[11]

9. Canavan disease

The gene is ASPA, which encodes an enzyme that catalyzes the conversion of N acetyl L aspartic acid (NAA) to aspartate and acetate.

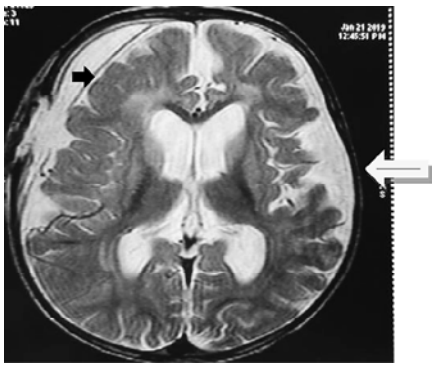


Fig.8. T2 weighted MRI image shows delayed myelination, abnormal signal change in bilateral basal ganglia, open sylvian fissures(white arrow) and subdural collection (black arrow).

MRI findings: MRI reveals T1 hypointensity and T2/ FLAIR hyperintensity in the cerebral white matter. The subcortical white matter is affected early in the disease and appears swollen. Abnormal signal changes seen in corpus striatum, cerebellar dentate nuclei, dorsal pons, portions of cerebellar peduncles. Contrast enhancement is not reported. MR spectroscopy reveals elevated NAA peak (Fig.9).[12]

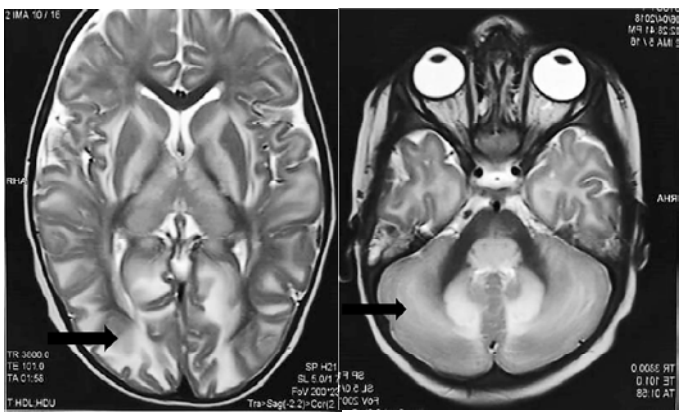


Fig.9. T2 weighted MRI images show hyperintensity in cerebral white matter, cerebellar dentate nuclei (Black arrows).

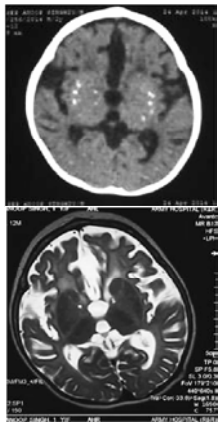
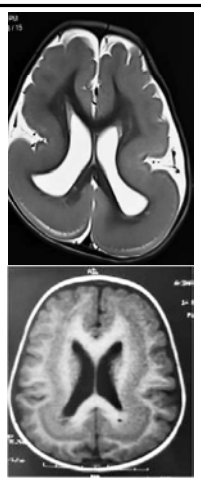
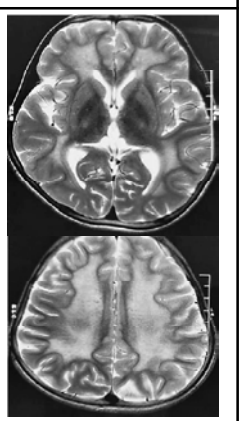
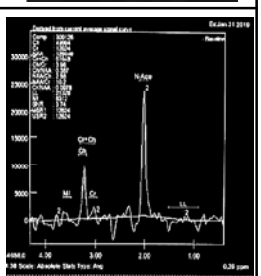
Others disorders which have characteristic imaging phenotype and reliably predicts the genotype are

1. L2 Hydroxy glutaric aciduria

The gene is L2HGDH, which encodes an enzyme called L-2-hydroxyglutarate dehydrogenase

MRI findings: Bilaterally symmetrical involvement of subcortical U fibres. Deep periventricular white matter, corpus callosum and internal capsule remain spared even

Table III. MRI features of few prototype conditions

Conditions	Genes	Radiology	Images
Aicardi-Goutieres Syndrome	TREX1, RNASEH2, RNASEH2C, RNASEH2A, SAMHD1, ADAR1	Punctate calcification especially in basal ganglia, white matter and/or dentate nuclei. White matter abnormalities, cerebral atrophy.	
Lissencephaly and subcortical band heterotropias	ARX, DCX, LIS1, RELN, TUBA1A, VLDLR	Brain surface appears smooth with absence (agyria) or abnormally wide gyri (pachygyria). Gyri typically ≥3 cm wide, cortex 10-15mm. Posterior to anterior or anterior to posterior gradient.	
Metachromatic leukodystrophy	ASA, PSAP	Abnormal signal changes in deep and periventricular white matter. Subcortical white matter is spared late in the course of disease. High resolution images show stripes of normal and affected myelin giving a tigroid pattern or leopard skin sign or stripe sign	
Creatine deficiency	GAMT, AGAT, SLC6A8	MRI is essentially normal in AGAT deficiency, GAMT deficiency shows bilateral T2 hyperintensity with reduced diffusivity in globi pallidi MR spectroscopy shows reduced or absent creatinine.	

in advanced cases. An anterior to posterior gradient can be observed. Basal ganglia is involved. Dentate nuclei is affected but cerebellar white matter and brainstem are spared. Vermis is significantly atrophied.[13]

Differential diagnosis: Canavan disease: Brainstem is involved and putamen and caudate nuclei are spared. Elevated NAA on MR spectroscopy.

2. Menke's disease

The gene is ATP7A, which provides instructions for making a protein that is important for regulating copper levels in the body.

MRI findings: Progressive brain atrophy, bilateral subdural hematoma, elongated and tortuous cerebral arteries. In absence of asphyxia or physical trauma these MRI findings are highly suggestive of Menkes.[14]

3. ACTA2 mutation

The gene is ACTA2, which encodes protein , smooth muscle actin that is involved in vascular contractility and blood pressure homeostasis.

MRI findings: Arterial ischemic stroke, MRA shows dilated proximal internal carotid artery (ICA), stenosis of terminal ICA, twig like abnormally straight intracranial arteries and absence of collaterals.[15]

4. Leukoencephalopathy with brainstem and spinal cord involvement and lactate elevation (LBSL)

The gene is DARS2, which encodes an enzyme called mitochondrial aspartyl-tRNA synthetase, which is important in the synthesis of proteins in mitochondria.

MRI findings: Signal abnormalities in cerebral white matter (subcortical white matter relatively spared), dorsal column and lateral cortico-spinal tracts in cervical spinal cord, medial medullary pyramids. Besides these signal changes may be seen in splenium of corpus callosum, internal capsule, superior and inferior cerebellar peduncles, trigeminal nerves and cerebellar white matter. On MR spectroscopy lactate can be elevated.[16]

Gene panel sequencing: There are number of other genetic neurological diseases where neuroimaging helps to narrow down the differentials. Some of these abnormalities can be caused by mutation in multiple genes. In these cases multiple gene panel where they are sequenced concomitantly at one step, is required rather than single gene targeted sequencing done one after another.[17]

Few of prototype disorders and their neuroimaging findings are mentioned in Table III.

In above mentioned conditions genetic diagnosis becomes important for therapeutic implication too. For example, all patients with creatine deficiency may not benefit with creatine supplement. Those with GAMT, AGAT deficiency show response however those with creatine transporter protein defect usually do not benefit from creatine supplement. Radiogenomics also helps to validate novel mutations as pathogenic by phenotypic categorization.[17] Thus it has a bidirectional role.

Conclusion

Radiogenomics is emerging as an important tool in the field of pediatric neurogenetics. It points out the likely genotype and directs towards the right genetic investigation. The radiogenomics has great potential to accelerate precision medicine, but it is still early in its evolution especially in pediatric neurological conditions apart from tumours. Optimum protocols for image acquisition and reconstruction must be identified and standardized, and robust protocol should be developed which should have excellent precision. Furthermore, databases need to be generated to incorporate imaging features with clinical and genetic data.

Points to Remember

- ***"Radiogenomics" is a new emerging field which correlates the imaging characteristics of a disease (radiophenotype), with its (genotype) genetic or molecular features.***
- ***Next generation genetic sequencing is a technological advance which enable sequencing of million base pairs of DNA at a faster rate and lower cost.***
- ***Whole genome sequencing, whole exome sequencing, clinical exome sequencing and target gene panel sequencing are various tests which are available.***
- ***Knowledge about the various features of these tests is essential to decide the specific test needed.***
- ***Good clinical phenotyping remains the corner stone of deciding the appropriate genetic test and increasing likelihood of a positive yield. But radiophenotype helps bridging the gap between clinical phenotype and genotype, by helping to select the type of genetic test is needed.***

References

1. Balmaceda C, Critchell D, Mao X, Cheung K, Pannullo S, DeLaPaz RL, et al. Multisection 1H magnetic resonance

spectroscopic imaging assessment of glioma response to chemotherapy. J Neurooncol 2006; 76(2):185-191.

2. Yates LR, Gerstung M, Knappskog S, Desmedt C, Gundem G, Van Loo P, Aas T, et al. Subclonal diversification of primary breast cancer revealed by multiregion sequencing. Nat Med 2015; 21(7):751e9.
3. Lambin P, Rios-Velazquez E, Leijenaar R, Carvalho S, van Stiphout RG, Granton P, et al. Radiomics: extracting more information from medical images using advanced feature analysis. Eur J Cancer 2012; 48(04):441-446
4. Shashi V, McConkie-Rosell A, Rosell B, Schoch K, Vellore K, McDonald M, et al. The utility of the traditional medical genetics diagnostic evaluation in the context of next-generation sequencing for undiagnosed genetic disorders. Genet Med 2014; 16:176-182.
5. Venkatesh HS, Chaumeil MM, Ward CS, Haas-Kogan DA, James CD, Ronen SM. Reduced phosphocholine and hyperpolarized lactate provide magnetic resonance biomarkers of PI3K/Akt/mTOR inhibition in glioblastoma. Neuro Oncol 2012; 14(3):315-325.
6. Sudhakar SV, Muthusamy K, Arunachal G, Shroff M. Genomics and radiogenomics in inherited neurometabolic disorders – a practical primer for pediatricians. Indian J Pediatr 2019.
7. Kruer MC, Boddaert N. Neurodegeneration with brain iron accumulation: a diagnostic algorithm. SeminPediatr Neurol 2012; 19:67-74.
8. Kim JH, Kim HJ. Childhood X-linked adrenoleukodystrophy: clinical-pathologic overview and MR imaging manifestations at initial evaluation and follow-up. Radiographics 2005; 25:619-631.
9. Provenzale JM, Peddi S, Kurtzberg J, Poe MD, Mukundan S, Escolar M. Correlation of neurodevelopmental features and MRI findings in infantile Krabbe'sdisease. Am J Roentgenol 2009; 192:59-65.
10. Van der Knaap MS, Valk J. Magnetic Resonance of Myelination and Myelin Disorders. Berlin:Springer 2005.
11. Nunes J, Loureiro S, Carvalho S, Pais RP, Alfaiate C, Faria A, et al. Brain MRI findings as an important diagnostic clue in glutaric aciduria type1.Neuroradiol J 2013; 26:155-161.
12. Vasquez ML, RenaultIZ. Understanding genetics in neuro imaging. Neuroimag Clin N Am 2015; 25:1-16.
13. Steenweg ME, Salomons GS, Yapici Z, Uziel G, Scalais E, Zafeiriou DI, et al. L-2-hydroxyglutaric aciduria: pattern of MR imaging abnormalities in 56 patients. Radiology 2009; 251:856-865.
14. Menkes, J. H. Kinky hair disease. Pediatrics 1972; 50: 181-182.
15. D'Arco F, Alves CA, Raybaud C, Chong WKK, Ishak GE, Ramji S, et al. Expanding the distinctive neuroimaging phenotype of ACTA2 mutations. Am J Neuroradiol 2018; 39:2126-2123.
16. Van der Knaap MS, Salomons GS. Leukoencephalopathy with Brain Stem and Spinal Cord Involvement and Lactate Elevation. 2010May25[Updated2015Feb12]. In:AdamMP, Ardinger HH, Pagon RA, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2018.
17. Biesecker LG, Green RC. Diagnostic clinical genome and exome sequencing. N Engl J Med 2014; 370:2418-2425.

NEWS & NOTES