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The Genome Revisited

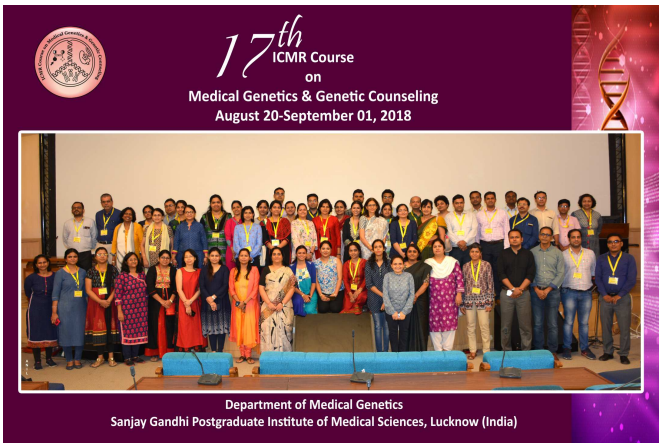
Editorial

The field of Medical Genetics has been growing by leaps and bounds. We have come a really long way since the initial description of heritable traits by Gregor Mendel in the 1860s. The elucidation of the human genome sequence, which would have seemed like science fiction until even half a century back, has become a reality and now we are actually looking at ways of modifying it through genome editing techniques. The explosion of information about the human genome has been paralleled by the tremendous advances in genetic testing technologies. The genetic diagnostic armamentarium has been rapidly expanding, beginning with the development of the karyotyping technique for chromosomal analysis in the 1950s to the evolution of the Next generation sequencing technology in the 21st century. Each technique when introduced, opens up a world of diagnostic possibilities, only to be overshadowed by a more advanced technology with an even more fascinating scope and a higher diagnostic yield.

The chromosomal microarray (CMA) technology, which began to be used extensively in clinical practice from the late 2000s onwards, was one such development. CMA made genome-wide screening of copy number variations possible at a much higher resolution than conventional karyotyping, so much so that it became the recommended first-line genetic test for the evaluation of unexplained intellectual disability and uncharacterised multiple malformation syndromes. It has retained its pre-eminent position as the molecular cytogenetic test-of-choice for almost a decade now. But it seems as though it is going to be gradually replaced by the newest entrant in the arena – Whole genome sequencing (WGS). The GenExpress in this issue summarizes three recently published studies by Halgren et al., Zhou et al. and Van Opstal et al., which have demonstrated the superiority of WGS over CMA in the elucidation of molecular cytogenetic aberrations in the prenatal as well as postnatal scenario. WGS can detect CNVs at a far higher resolution than CMA and can help to exactly map chromosomal breakpoints down to the last nucleotide.

As technology continues to evolve, we are going to keep revisiting the genome, delving further into its intricacies and unearthing more and more of its secrets. Therefore, it is essential for all of us, working in the field of Medical genetics, doctors and scientists alike, to stay abreast of these rapid developments. Genetic Clinics endeavours to play a small but significant role as a travel guide for this journey through the human genome.

Dr. Prajnya Ranganath
Associate Editor, Genetic Clinics
1st October, 2018



ICMR Course on Medical Genetics & Genetic Counseling completed its Seventeenth annual event to popularise genetics and make clinicians aware about the applications of latest developments in genetics in patient care. This two week long course was attended by 50 doctors from all over India. More than 500 doctors have been provided training during these annually held courses and have been introduced to the fascinating world of medical genetics.

- Basic, clinical, molecular genetics simplified.
- Genetic counseling and prenatal diagnosis demystified.
- Exposure to common and rare genetic clinical scenarios.

Megaconial Muscular Dystrophy with a Novel Mutation in the *CHKB* Gene

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Abstract

Congenital muscular dystrophy is a genetically heterogenous disorder. We report two sisters with megaconial myopathy who presented with developmental delay, congenital muscular dystrophy and acanthosis with ichthyosis. About 36 cases have been described in world literature. Ours is the first case report from India. Whole exome sequencing identified a novel homozygous nonsense mutation in the *CHKB* gene.

Introduction

Congenital muscular dystrophies (CMD) are clinically and genetically heterogeneous group of inherited muscle disorders in which weakness is first apparent at birth or in infancy. The prevalence and incidence of CMD in different regions of the world are poorly known. Few studies are limited to epidemiologic figures of prevalence (Norwood et al., 2009) and a recent review estimated the overall prevalence of CMD to be 0.99/100,000 (Mah et al., 2016) which may have been an underestimate because of limited availability of diagnostic means. The relative frequency of CMD subtypes also varies in different populations.

Clinical presentation of motor delay, proximal weakness, hypotonia and learning difficulties raise the possibility of CMD. Serum creatine phosphokinase (CPK) is usually the first investigation to detect CMD. Neurophysiological studies in CMD cases may suggest myopathic pattern and muscle biopsy shows features of dystrophic changes. Further classification is based on immunohistochemistry with different antibodies e.g. merosin or alpha, beta, gamma and delta dystroglycan. With the advent of next generation sequencing (NGS)-based testing, the causative gene can be identified. CMD-causative pathogenic variants can be identified in 25%-50% of cases, underscoring the need for ongoing investigation into the genetic causes of CMD (Peat et al., 2008).

Choline kinase beta gene (*CHKB;* MIM *612395) mutations are associated with a rare recessive congenital muscular dystrophy with mental retardation called congenital megaconial type muscular dystrophy (MIM#602541). This gene codes for the choline kinase beta enzyme (EC 2.7.1.32) which catalyzes the first step of phosphatidylcholine (PC) biosynthesis. Here, we describe a novel nonsense mutation in exon 2 of the *CHKB* gene in 2 sisters with congenital megaconial muscular dystrophy.

Case Report

Two sisters (8 years and 5 years old) with intellectual disability, born to third degree consanguineous parents, were referred for evaluation.

The elder sibling (Patient 1) was born through vaginal delivery at 34 weeks gestation. There was history of delayed cry at birth. She had neonatal hyperbilirubinemia for which phototherapy was given and she was kept in the neonatal intensive care unit (NICU) for 10 days. She developed head control at 6 months of age and was able to sit by 8 months of age. However, after this she stopped acquiring new skills and developed autistic features. She never achieved standing or walking. There was no speech and no eye contact. She was not able to interact or follow any commands. There was no history of seizures and there was no cranial nerve involvement. There was a history of severe itchy skin lesions present since birth on the neck, back, axilla, antecubital regions and the abdomen.

On examination, the head circumference was

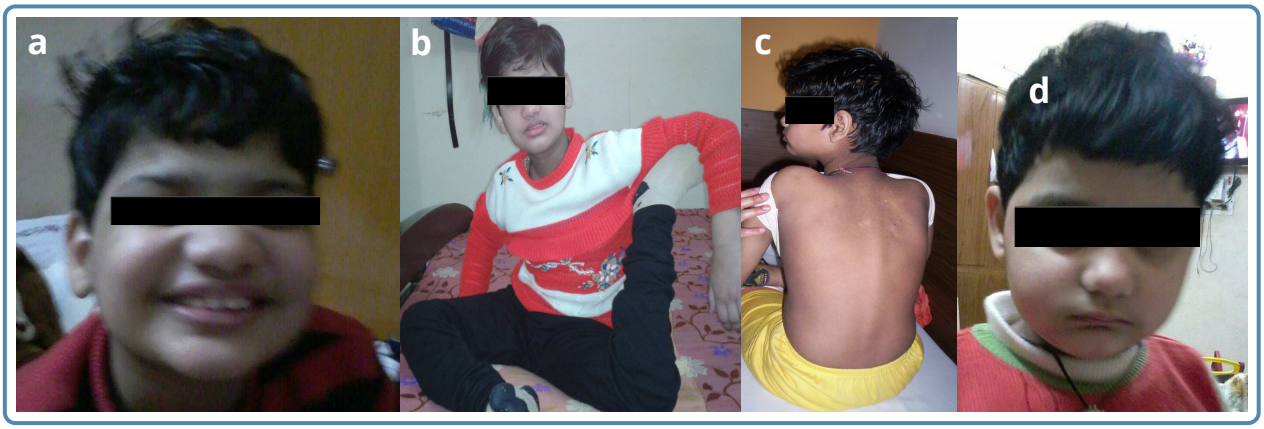

Figure 1 a) Clinical photograph of Patient 1. b) Joint laxity seen in Patient 1. c) Ichthyosis-like skin lesions in Patient 1. d) Clinical photograph of Patient 2.

45 cm (< 3rd centile for age) (Figure 1a). There was severe generalized hypotonia, hyporeflexia and joint laxity (Figure 1b). The muscle bulk was normal. Acanthosis nigricans-like lesions were present in the axilla, neck and abdomen with marked pruritus-related scarring (Figure 1c).

The younger sibling (patient 2) was born at 32 weeks gestation and had pruritic skin lesions since birth. The severity of lesions was less when compared to the elder sib (Figure 1d). She also had developmental delay along with joint laxity. Like her sister she had attained head holding at 6 months and achieved sitting at 8 months and no further milestones thereafter.

Age of death of the elder sibling was 10 years and for the younger one was 8 years. The cause of death was attributed to renal failure in both of them. Before death the elder sibling had developed aggressive behaviour whereas the younger one had become quiet for 3 months.

We investigated Patient 1 (the elder sibling) as both sibs had similar presentations but she was more severely affected of the two. Her serum CPK was 1109 IU/L. Electrophysiological study was suggestive of a myopathic pattern. Her MRI Brain showed multiple punctate perivascular and subcortical hypodensities. Mass tandem spectrometry, organic acid analysis, fundus examination, hearing evaluation and karyotype were normal. Evaluation for congenital disorders of glycosylation through transferrin isoelectric focusing was normal. Her muscle biopsy showed features suggestive of muscular dystrophy. Immunohistochemistry for dystrophin 1, 2, and 3, alpha, beta, delta and gamma sarcoglycans, merosin 80 and 300, and alfa dystroglycans was normal. Skin biopsy from the axilla showed hyperkeratosis and parakeratosis with acanthosis. Collagen VI staining of the skin done to exclude Ullrich muscular dystrophy was normal. In view of ichthyosis, steroid sulphatase gene deletion analysis was done and this was normal.

Whole exome sequencing was then done through the Illumina platform and a sequence variant was detected in exon 2 of the *CHKB* gene: c.331 C>T; p.Gln111Ter. This is a novel sequence variant which results in a stop codon. It was present in a homozygous state in both the affected sisters whereas both parents were heterozygous carriers for the same. The sequence variant is not present in the 1000 Genome, ExAC, gnomAD and also the combined VarSome Databases and it is predicted to be likely pathogenic.

Muscle biopsy was reanalysed in view of the above mutation results and cytochrome oxidase (COX) and succinate dehydrogenase (SDH) staining was done. Typical features of megaconial myopathy were seen with mega mitochondria at the periphery of the fibre, seen with COX and oxidative enzyme stains, and depletion of it at the centre of the fibre (Figures 2 & 3). This feature is specific of this disease and not seen in other mitochondrial conditions.

Discussion

Megaconial myopathy was first described in 1998 in 4 Japanese patients from three unrelated families, with mitochondrial structural changes. It is characterized by neonatal hypotonia, developmental delay and its pathological signature i.e. enlarged

mitochondria displaced to the periphery of the fibres in muscle biopsy, leaving the centre devoid of organelles (Nishino et al., 1998). The characteristic enlarged mitochondria at the periphery of the muscle fibres have given rise to the name 'megaconial myopathy'.

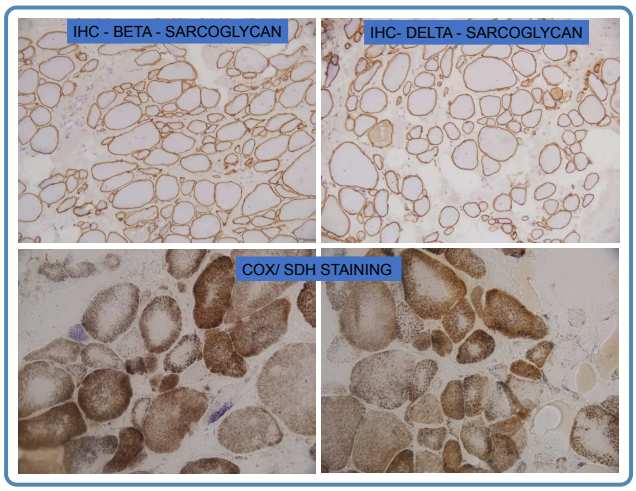

Figure 2 Images of muscle biopsy and immunohistochemical staining for beta sarcoglycan and delta sarcoglycan and COX/SDH staining in Patient 1.

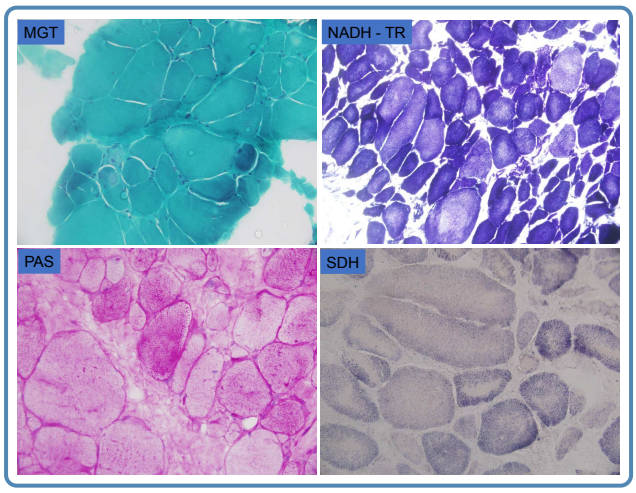

Figure 3 Modified trichrome Gomori (MGT), nicotinamide adenine dinucleotide tetrazolium reductase (NADH-TR), Periodic acid Schiff (PAS) and succinate dehydrogenase (SDH) staining of muscle biopsy showing prominent mitochondria at the periphery of the muscle fibres.

About 36 patients (4 Japanese, 10 Turkish, 3 British, 1 French, 1 African-American, 16 Spanish and 1 Italian) with mutations in the *CHKB* gene have been characterized and published since then (Mitsuhashi et al., 2011; Gutierrez Rios et al., 2012; Quinlivan et al., 2013; Mitsuhashi et al., 2013; Castro-Gago et al., 2014; Cabrera-Serrano et al., 2015; Haliloglu et al., 2015).

The age of presentation varies from 0-28 years. Both our patients presented with skin lesions since birth. Both the sisters had severe developmental delay and they were never able to walk. All patients described in literature had developmental delay. The Spanish patient described by Castro- Gago et al. (2014) had mainly motor delay. The female patient reported from Australia had intellectual disability. Two of the 15 patients described by Mitsuhashi et al. (2011) never walked. All patients in the study described by Haliloglu et al. (2015) had global developmental delay predominantly involving gross motor (n=14), and language domains (n=15). There was a delay in the age at independent walking (n=14), with one child still not able to walk at the age of 6 years 1 month. Intellectual disability was mild (n=7), moderate (n=2), or severe (n=4) in this group of Spanish patients (Haliloglu et al., 2015).

Skin changes include ichthyosis-like changes. Both the sisters in the current study had acanthosis-ichthyosis like changes which were present since birth. Skin lesions were seen mainly in the neck, axilla and abdomen. The severity was lesser in the younger sib. There was significant pruritus in both of them, more so in the elder sib. The severity was so marked that at times she had to be sedated to avoid the itching. Pruritus was lesser in the younger girl. Skin biopsy showed parakeratosis and hyperkeratosis in the elder sib. Five out of the 15 patients described by Mitsuhashi et al. (2011) had skin changes. Skin changes were not seen in the patients described from Australia and Africa (Castro-Gago et al., 2014; Cabrera-Serrano et al., 2015). In the study by Haliloglu et al. (2015), skin changes were present in the form of diffuse ichthyosis-like changes (n=11), exfoliative and desquamative lesions mainly involving the neck, trunk, and face (n=7), hirsutism (n=1), and atopic dermatitis (n=1). The mean age at recognition of skin findings was 3 years 8 months (birth-17 yrs).

Marked hypotonia and joint laxity were present in both sisters. Hypotonia was noted in 9 out of the 15 patients described by Mitsuhashi et al. (2011). Hypotonia was also seen in patients of

Australian and African origin (Castro-Gago et al., 2014; Cabrera-Serrano et al., 2015).

Both our patients had raised serum CPK (Patient 1 - 1109 IU/ L, Patient 2- 405 IU/ L). Serum CPK was raised in all patients described by Mitsuhashi et al. (2011) and in 12 out of 15 of the Spanish patients described by Haliloglu et al (2015).

Our patient showed changes typical of muscular dystrophy which include variation in muscle fibre size. IHC for dystrophin 1, 2 and 3 and alpha, beta, gamma and delta sarcoglycans was normal. COX and SDH staining were suggestive of mitochondrial abnormalities. All affected individuals in the Mitsuhashi series exhibited nonspecific dystrophic features; Gomori trichrome, NADH-TR, SDH, and COX staining showed prominent mitochondria at the periphery as well as central areas devoid of mitochondria. The typical muscle dystrophic changes and typical mitochondrial structural changes were present in all muscle biopsy cases of Haliloglu et al. (2015); enlarged mitochondria were easily identified by Gomori trichrome, SDH, and COX stains.

The *CHKB* gene consists of 11 exons. Mutations reported so far include nonsense, frame shift, missense, nonframeshift deletions, and splice-site mutations, in exons 1, 4, 5, 6, 7, 8, 9, and 11 (Mitsuhashi et al., 2011; Gutierrez Rios et al., 2012; Quinlivan et al., 2013; Mitsuhashi et al., 2013; Castro-Gago et al., 2014; Cabrera-Serrano et al., 2015; Haliloglu et al., 2015). Eight out of 36 patients have been reported to have mutation in exon 5, 8/36 in exon 8 and 6/36 in exon 9. The c.677+1 G>A is the commonest mutation reported and has been found in 6/36 patients. The homozygous nonsense pathogenic variant in exon 2 found in our patients is a novel variant. We have not done functional studies to confirm the pathogenicity of this variant but the segregation pattern in the family and clinical and immunohistological phenotype in the patient are supportive of the diagnosis.

Conclusions

We hereby report the first case of megaconial myopathy in two sisters from India with a novel homozygous mutation in the *CHKB* gene. The skin manifestations such as ichthyosis and acanthosis nigricans along with joint laxity in a case of congenital muscular dystrophy are major clues towards this diagnosis.

Acknowledgments

We would like to acknowledge Prof Carsten Bonneman for helping with the Whole exome sequencing for these patients.

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Fatty Acid Hydroxylase- Associated Neurodegeneration - A Rare Case of Neurodegeneration with Brain Iron Accumulation (NBIA)

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Abstract

Fatty acid hydroxylase associated neurodegeneration is a rare disorder which belongs to the group of disorders of neurodegeneration with brain iron accumulation (NBIA). We present a case of a 9-year-old girl who presented with gradually progressive stiffness of limbs with speech delay and neuroimaging findings of T2 hypointensities in the globus pallidus and substantia nigra suggestive of brain iron accumulation. Targeted exome sequencing by next generation sequencing (NGS) revealed a novel homozygous splice site likely pathogenic variant in intron 6 of the *FA2H* at position c.1039+2T>G, confirming the diagnosis of Fatty acid hydroxylase-associated neurodegeneration (FAHN), a subtype of NBIA. FAHN has never been reported from the Indian subcontinent before. This report further emphasizes the use of good clinical evaluation and NGS in diagnosing rare disorders which otherwise are difficult to recognise.

Introduction

Iron is vital to life but it may generate neurotoxic reactive oxygen species if inappropriately handled. Neurodegeneration with brain iron accumulation (NBIA) is a group of inherited neurologic disorders in which iron accumulates in the basal ganglia (most often in the globus pallidus and/or substantia nigra) resulting in variable degree of progressive dystonia, spasticity, parkinsonism, neuropsychiatric abnormalities, with or without optic atrophy or retinal degeneration [Gregory et al., 2018]. This disorder has 10 subtypes based on involvement of different genes. It is a slowly progressive disorder with marked genetic and clinical heterogeneity. We present a patient with progressive motor regression with spasticity and mild optic atrophy due to novel biallelic variant in the *FA2H* gene (Fatty acid hydroxylase associated Neurodegeneration, FAHN), affecting GT donor splice site in intron 6. The role of thorough clinical examination, high level of suspicion and the use of next generation sequencing (NGS) is emphasized in this report.

Case report

The proband was a 9-year-old girl, the second child of non-consanguineous parents from Bangladesh, born by Caesarean section at term gestation, with a birth weight of 2.8 kg. She required no special care at birth or in the perinatal period. She achieved developmental milestones appropriately till 2 years of age, after which the parents noted some difficulties in mobility. Initially she used to run on toes but then her condition worsened and the difficulty became evident in walking as well. She was able to walk independently till 4 years of age, which at the time of examination regressed to walking a few steps with great difficulty, using support.

There was speech delay, although she started speaking monosyllables at 2 years, no further progress in speech was noted and at 9 years, she had unclear speech with only few words in her vocabulary. Hearing evaluation showed normal result. There was no history of any seizure or acute illness associated with lethargy or coma at any time. No history of visual impairment or abnormal movement of eyes (nystagmus) was given. Her intellect appeared normal, although it was not formally assessed.

On examination, she was alert and cooperative and her anthropometric measurements were corresponding to 25th centile for the age (as per WHO standards). On general examination, no

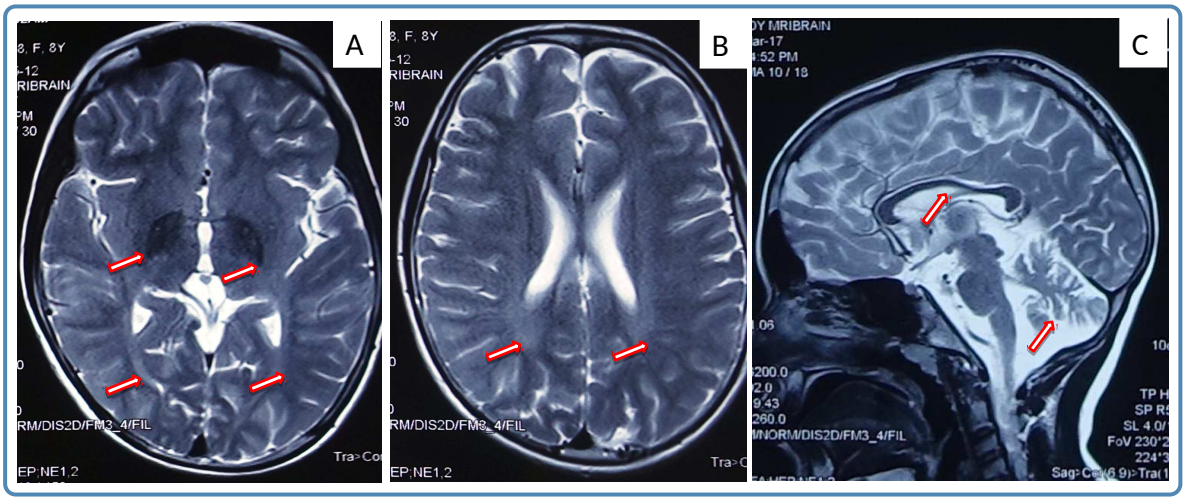

Figure 1 MRI of the brain (T2 images) showing hypointensity in bilateral globus pallidus (Fig 1A), white matter hyperintensities (Fig 1A & 1B), thin corpus callosum and cerebellar hypoplasia (Fig 1C). Figures A & B axial view, figure C) sagittal view.

dysmorphic features were present. The respiratory and cardiovascular system and abdominal examination was normal. On central nervous system (CNS) examination, generalised spasticity was noted involving distal joints of lower limbs more than upper limbs. Power was normal, and deep tendon reflexes were brisk in both the upper and lower limbs. On eye evaluation, mild optic atrophy was noted. There was no nystagmus or KF ring.

Parents had sought medical consultation 6-months prior at which time she had been evaluated. Plasma lactate and ammonia were 23 mg/dl (ref. 4.5–20 mg/dl) and 54 µmol/L (ref 9–35 µmol/L) respectively. Thyroid profile, renal function tests and liver function tests were normal. Metabolic investigations including Tandem Mass Spectrometry (TMS) and urine Gas Chromatography Mass Spectrometry (GC-MS) showed no specific etiology. Eye evaluation showed mild disc pallor suggestive of optic atrophy. Visual evoked potential test revealed mild delay in amplitude and latency in both the eyes further confirming the diagnosis of optic atrophy. Brain MRI showed mild prominence of cerebellar foliae, ponto-cerebellar hypoplasia and white matter hyperintensities on bilateral parieto-occipital regions. Globus pallidus and substantia nigra hypointensities were appreciated in T2 weighted images (Figure 1).

Based on clinical profile of an early-onset neurodegenerative disorder and characteristic MRI findings, the possibility of neurodegeneration with brain iron accumulation (NBIA) was considered. To confirm the diagnosis, next generation sequencing (NGS)-based targeted clinical exome analysis was performed. The patient was detected to have a novel homozygous splice donor site likely-pathogenic variant at position c.1039+2T>G in the *FA2H* gene (NM_024306.4) confirming the diagnosis of Fatty acid hydroxylase-associated neurodegeneration (FAHN). Another phenotype associated with *FA2H* is Spastic paraplegia type 35. However, this phenotype is now considered as a part of the spectrum of FAHN. The family was counselled regarding the genetic basis of the disorder, lack of specific treatment, risk of recurrence in their future pregnancies and availability of prenatal diagnosis.

The variant, c.1039+2T>G affects GT donor splice site of exon 6 of the *FA2H* gene (NM_024306.4). It has not been reported in both the 1000 genomes and ExAC databases. The *in silico* prediction of the variant was damaging or deleterious by various online predictive tools. The reference region was conserved across species. Analysis of this variant with the help of "Human splicing finder" revealed broken GT donor splice site due to this change.

Discussion

The *FA2H* gene codes for the enzyme, fatty acid 2-hydroxylase enzyme which catalyses hydroxylation at position 2 of N-acyl chain of ceramide moiety which is an important component of the myelin sheath (Gregory et al,2018; Schneider et al., 2013). Pathogenic variants in this gene have been found to be associated with demyelinating leukodystro-

phy (HSP35) and the NBIA - FAHN. The exact cause of disturbed iron metabolism and abnormal accumulation in the basal ganglia in FAHN is still not clear. A reasonable hypothesis developed is that the pallidum being a high metabolic demand structure, is vulnerable to subacute oxidative stress from mitochondrial dysfunction caused by intrinsic or extrinsic factors [Hayflick et al; 2014], and that the iron accumulation is triggered by the apoptotic cascade or cellular damage [Kruer et al., 2017]. The iron discarded from demyelination is not able to be re-used and hence gets accumulated in these structures because of specific predilection (Schneider et al., 2013).

Fatty acid hydroxylase-associated neurodegeneration (FAHN) is a subtype of NBIA (Gregory et al., 2018). It is a rare, autosomal recessive disorder, and till date a total of just over 50 individuals of diverse ethnicity with FAHN have been described (Kruer et al., 2017; Mari et al., 2018). The disorder can be recognised by the characteristic features of childhood-onset progressive spastic paraplegia that progresses to tetraparesis, or focal dystonia, ataxia, dysarthria, intellectual decline, and optic atrophy, accompanied by iron deposition in the brain. MRI studies demonstrate bilateral globus pallidus T2 hypointensity, consistent with iron deposition, prominent pontocerebellar atrophy, mild cortical atrophy, white matter lesions and corpus callosum thinning with variability among patients. Spastic paraplegia 35 is now considered as a part of the same disorder. Disease progression in FAHN is intermittent and there may be a period of clinical stability. Although premature death often occurs in the third or fourth decade secondary to a combination of nutrition-related immunodeficiency and respiratory compromise, life span is variable and genotype-phenotype correlation is difficult to make.

NBIA is an important group of neurodegenerative disorders to be identified in childhood, because of the recognisable clinical and radiological features. The common features in the group include a history of regression of milestones after a variable period of normal development, spasticity or extrapyramidal symptoms, and a variable neuroradiological picture. Differential diagnosis in this clinical setting include white matter disorders such as Metachromatic leukodystrophy, Krabbe disease, Pelizaeus- Merzbacher disease, Adrenoleukodystrophy, Juvenile Huntington disease, Friedreich's ataxia and Hereditary Spastic Paraplegias.

MRI of the brain typically shows hypo-intensities in the globus pallidus, substantia nigra and other basal ganglia structures. Cerebellar atrophy and visual impairment secondary to optic atrophy is not present in Pantothenate kinase-associated neurodegeneration (PKAN). The typical MRI features are: 'eye of the tiger' sign in PKAN; cerebellar atrophy in PLA2G6-associated neurodegeneration (PLAN) and Aceruloplasminemia; T2 -weighted signal hyperintensity with a central band of hypointensity in the substantia nigra in Beta-propeller Protein-Associated Neurodegeneration (BPAN); and cerebral, cerebellar and brain stem atrophy in Kufor-Rakeb syndrome (Gregory et al., 2018). Differential diagnosis for basal ganglia hypointensities include multiple sclerosis, Juvenile Huntington disease and Fahr disease. The age of onset of these disorders is however much later than for NBIAs.

The diagnostic yield of single gene disorders, including NBIA has increased greatly over the years owing to NGS technology. We previously described one case of *PLA2G6* -related neuronal degeneration using this technology (Goyal et al., 2015). Another study reported mutations in 15 families with PLAN using Sanger sequencing method from India (Kapoor et al., 2016). Whole genome sequencing technology offers even better diagnostic yield as it covers not only the coding regions but the entire genome including any copy number variation. Our case of FAHN adds to the spectrum of NBIA disorders that are seen in the Indian subcontinent.

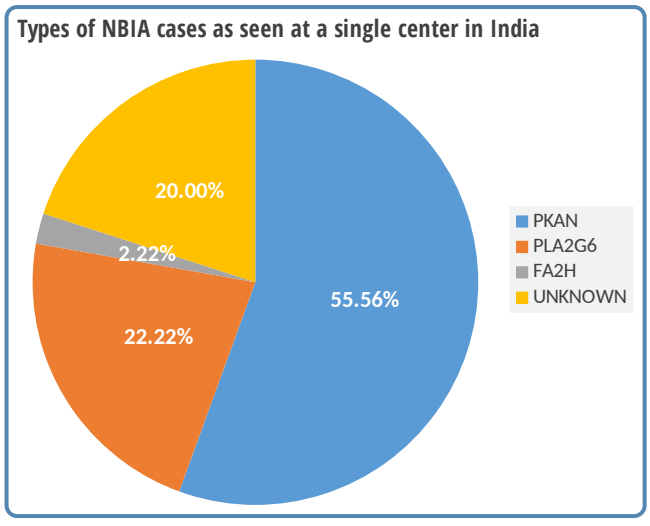

Figure 2 The distribution of cases of NBIA types seen over last 15 years at Institute of Genetics and Genomics Sir Ganga Ram Hospital New Delhi

In our own experience from the genetic clinic at Sir Ganga Ram Hospital of the last 15 years or so (Figure 2), from approximately 45 cases diagnosed with NBIA using clinical, neuroradiological and molecular methods, PKAN remains the most frequent followed by PLAN, with FAHN now adding a new type. Our findings are in agreement with those of others from various parts of the world. Our case emphasizes the importance of thorough clinical examination and the use of advanced molecular techniques like next generation sequencing in the accurate diagnosis of these rare disorders.

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Upcoming Gene Events (October 2018 – March 2019)

October 2018

- XXVI World Congress of Psychiatric Genetics 2018
  11 – 15, October 2018. Glasgow, Scotland.
- American Society of Human Genetics 68th Annual meeting, 2018
  16 – 20, October 2018. San Diego, CA, USA.

December 2018

- Fourth International Birth Defects Conference and Fifth Annual Conference of Society for Indian Academy of Medical Genetics
  13 – 15, December 2018. Christian Medical College, Vellore, India.

January 2019

- Fifth National Conference of the Indian Society for Inborn Errors of Metabolism (ISIEM)
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GeNeViSTA

Genetics of Parkinson Disease

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Abstract

Parkinson disease is a common neurodegenerative disorder, characterized by bradykinesia, tremor, rigidity and difficulty in initiating movement. It is caused due to loss of dopaminergic neurons in the substantia nigra in the midbrain. Sporadic forms account for 90% of cases and manifest by 60 years of age. Both environmental and genetic factors have been implicated in sporadic forms. Various genes with autosomal dominant, recessive and X linked inheritance have been identified for monogenic forms of Parkinsonism. This brief review is about the latest advances in the understanding of the genetics of Parkinsonism and some of the novel therapeutic approaches that are being tried.

Introduction

Parkinsonism refers to a constellation of symptoms like bradykinesia, rigidity, resting tremor and postural instability. Parkinsonism can occur as a side effect to drugs like neuroleptics, as a complication of stroke or as a manifestation of other autosomal dominant genetic disorders like Huntington disease, Spinocerebellar ataxias and Frontotemporal dementias with Parkinsonism. Parkinson disease is the second most common neurodegenerative disease, preceded by Alzheimer disease and is the most common primary cause for Parkinsonism (Pankratz et al., 2004). The most important pathology that defines Parkinson disease is the loss of dopaminergic neurons in the substantia nigra in the midbrain with Lewy body inclusions (Pankratz et al.,2004). In the population above 60 years of age, the estimated worldwide prevalence is 1% and in the population above 80 years, the prevalence is as high as 4% (de Lau et al., 2006). Even though the median age of onset is 70 years, around 4% of individuals manifest symptoms before 50 years of age (Lin et al., 2014)

Classification based on the age of onset of symptoms

Based on the age of onset, Parkinson disease can be classified as:

1. Juvenile: Onset before 20 years
2. Early onset: Onset before 50 years
3. Late onset: Onset after 50 years

Causes of Parkinson disease

Environmental, genetic and epigenetic mechanisms have been implicated in Parkinson disease.

- Non-genetic/ environmental causes which have been shown to be associated with Parkinson disease include:

1. Occupational exposure to herbicides, pesticides, heavy metals
2. Head trauma
3. Smoking, which has an inverse association with Parkinson disease (de Lau et al., 2006)
4. Coffee and alcohol consumption
5. Dietary factors and physical activity

- Heritable or genetic factors: Monogenic forms account for 5-10% of cases of Parkinson disease. Following the identification of a missense variant in the *SNCA* gene in an Italian family with autosomal dominant Parkinson disease, several other genes and loci have been implicated in Mendelian forms of Parkinson disease. The loci were named as PARK followed by a number, in the order of their discovery. These genes are thought to be involved in various cellular processes like synaptic transmission, lysosome mediated autophagy and mitochondrial quality control (Trinh et al., 2013). Mendelian forms of Parkinson disease can be inherited in autosomal dominant, autosomal recessive or X linked manner. Table 1 summarizes the genes implicated in Parkinson disease.

Table 1 Genes associated with Mendelian forms of Parkinson disease.

Loci	Gene	Mode of inheritance	Clinical phenotype
PARK1 (4q21-22)	*SNCA*	AD	EOPD
PARK2 (6q25.2–q27)	*PRKN*	AR	Juvenile onset Parkinson disease
PARK3 (2p13)	Unknown	AD	Classical PD
PARK4 (4q21-22)	*SNCA*	AD	EOPD due to heterozygous triplication in *SNCA* gene.
PARK5 (4p13)	*UCHL1*	AD	Single family with late onset PD
PARK6 (1p36.12)	*PINK1*	AR	EOPD
PARK7 (1p36.23)	*DJ1*	AR	EOPD
PARK8 (12q12)	*LRRK2*	AD	Classical PD
PARK9 (1p36)	*ATP13A2*	AR	Juvenile onset atypical Parkinson disease (Kufor-Rakeb syndrome)
PARK10 (1p32)	*Unknown*	AD	Classical PD
PARK11 (2q37.1)	*?GIGYF2*	AD	Late onset PD; unconfirmed
PARK12 (Xq21-25)	*Unknown*	X linked	Classical PD
PARK13 (2q13.1)	*HTRA2*	AD	Classical PD; unconfirmed
PARK14 (22q13.1)	*PLA2G6*	AR	Adult onset dystonia-Parkinsonism
PARK15 (22q12.3)	*FBX07*	AR	Early onset Parkinsonian pyramidal syndrome
PARK16 (1q32)	*Unknown*	Not known	Susceptibility to Classical PD
PARK17 (16q11.2)	*VPS35*	AD	Classical PD
PARK18 (3q27.1)	*EIF4G1*	AD	Classical PD
PARK19 (1p31.3)	*DNAJC6*	AR	Early onset and juvenile PD
PARK20 (21q22.11)	*SYNJ1*	AR	EOPD
PARK21 (3q22)	*Unclear*	AD	Classical PD
PARK22 (7p11.2)	*CHCHD2*	AD	Classical PD
PARK23 (15q22.2)	*VPS13C*	AR	EOPD

AD: Autosomal dominant
AR: Autosomal Recessive
PD: Parkinson disease
EOPD: Early onset Parkinson Disease

Autosomal dominant PD: Heterozygous variants in the *SNCA, LRRK2, VPS35, CHCHD2* and *EIF4G1* genes cause autosomal dominant forms of Parkinson disease. Generally autosomal dominant forms tend to manifest at a later age compared to autosomal recessive forms. *SNCA* is the first gene in which a mutation was identified in Parkinson disease and this gene codes for alpha- synuclein, which is the primary protein found in Lewy bodies. Disease causing variants in *SNCA* could be single nucleotide variants or gene duplications and triplications. The p.Gly2019Ser variant in *LRRK2* accounts for 5-7% of autosomal dominant forms (Nichols et al.,2005).

Autosomal recessive PD: Autosomal recessive forms have an earlier onset of disease, mild non-motor symptoms and a slow progression. They are caused due to biallelic variations in *PRKN* which codes for Parkin, *PINK1, ATP13A2, DNAJC6, SYNJ1* etc.

X-linked PD: PARK 12 is the only locus that has been shown to demonstrate X-linked transmission. *ATP6AP2* is the gene that has been implicated in X-linked Parkinsonism (Korvatska et al., 2013).

Pathogenesis of Parkinson disease

The genes identified as causing idiopathic Parkinsonism are shown to affect four different processes: synaptic transmission, endosomal trafficking, lysosomal autophagy and energy metabolism.

- Synaptic transmission: Alpha- synuclein, which is found in presynaptic terminals in the central and autonomous nervous system, is involved in exocytosis and synaptic release of neurotransmitters and it is the main component of Lewy bodies. Triplications of the *SCNA* gene which codes for alpha synuclein lead to earlier onset of symptoms compared to duplications, implicating a dosage effect in the pathogenesis. The exact mechanism by which alpha synuclein leads to neuronal death and spreads throughout the CNS still remains unexplained. There are various theories regarding the spread of alpha synuclein pathology in the brain, like 'selective vulnerability hypothesis' and 'pathogenic spread hypothesis' (Lill, 2016).

LRRK2 codes for a protein kinase, which regulates glutamate transmission and striatal signal transduction (Lin et al., 2014). *DNAJ6,* a biallelic mutation of which causes autosomal recessive forms of Parkinsonism, encodes a protein auxilin, which aids in clathrin mediated synaptic vesicle recycling. Synaptojamin, a protein coded by *SYNJ1,* forms complexes with auxilin and has been implicated in autosomal recessive Parkinsonism.

- Endosomal trafficking: This is a complex process by which the receptors or vesicles are internalized and then recycled in the Golgi complex or degraded in the lysosomes. *VPS35* and *DNAJC13* are implicated in endosomal trafficking causing Parkinsonism.

- Lysosomal autophagy: Alpha synuclein, which gets accumulated in cells in Parkinsonism, is not degraded by lysosomes and it is not clear whether this is the cause or effect of dysfunction in that pathway. Accumulation of intracellular alpha synuclein is found in many disorders like neuronal ceroid lipofuscinosis, Gaucher disease and Neimann-Pick type C. Heterozygous carriers of mutations in *GBA*, which in the homozygous state cause Gaucher disease, have an increased prevalence of Parkinsonism and Lewy body-associated dementia. It is postulated that accumulation of glucosylceramide due to decreased GBA enzyme activity, results in decreased lysosomal degradation of alpha synuclein (Mazzulli et al.,2011). *ATP6AP2* which is implicated in X-linked Parkinsonism and *ATP13A2* code for lysosomal proteins and when mutated cause impairment in lysosomal autophagy.

- Energy metabolism in mitochondria: Mitochondrial dysfunction has been implicated in the pathogenesis of Parkinsonism and several mutations in genes in the common pathway in mitophagy in mitochondria cause Parkinsonism. The most important among them are *PARK2, PINK1, FBXO7* and *DJ1*.

Genetic testing for Parkinsonism

Three-generation pedigree, detailed family history and evaluation need to be done in every family to determine whether the cases are simplex or familial. Age of presentation of affected individuals and their relevant medical records should be collected and noted in detail. No formal guidelines have been formulated to regulate genetic testing in Parkinson disease (Klein et al., 2012).

- Whom to test?

1. Early onset PD with atypical features and/ or family history
2. Any patient with juvenile onset of Parkinson disease irrespective of family history
3. Late onset disease with a strong family history

- Which genes to test? Testing strategy can be stepwise single gene testing or multigene panel testing. In families with autosomal dominant inheritance, the European Federation of Neurological Sciences recommends screening for mutations in *LRRK2*. In specific populations with familial and sporadic PD, the same federation recommends screening for the *LRRK2* mutation - p.G2019S. Testing for Parkin, *PINK1* and *DJ1* is indicated in families with autosomal recessive PD.

- Ethical concerns regarding molecular testing: Without appropriate pretest counseling by a trained Medical Geneticist and/ or genetic counselor, molecular testing for Parkinson disease should not be attempted. Direct- to- consumer testing is available and is being used by patients and healthy at-risk individuals. With many susceptible loci being identified without ample evidence to prove causality, genetic counseling is crucial before molecular testing is ordered. Susceptibility testing should be strongly discouraged, especially in healthy individuals. Providing prenatal diagnostic testing for an adult onset disease is still debatable.

Genetic counseling

A family with an affected individual should be counseled regarding the environmental, epigenetic and genetic factors, which can cause Parkinson disease. Since Parkinson disease is a common neurodegenerative condition, the lifetime risk of developing this condition is 1-2% (Elbaz et al., 2002). The empiric risk of recurrence in a family with a sporadic case of late onset classical PD is 3-7%. (Elbaz et al., 2002). In monogenic forms, depending on the pattern of inheritance, the risk of recurrence will vary.

Therapeutic strategies

The treatment strategies being tried for Parkinson disease include pharmacologic therapy, therapies based on molecular mechanisms of disease, cell-based therapy and gene therapy.

a. Pharmacological therapy: The main intention of pharmacological methods is to achieve symptom control by normalizing dopamine levels. This includes monotherapy with dopaminergic drugs like levodopa, combination of levodopa-carbidopa, monoamine oxidase B inhibitors (MAO B inhibitors) and Catechol-o-methyl transferase inhibitors, which increase the levels of dopamine. Dopamine agonists like pramipexole and ropinirole can also be tried in early stages of disease. Non-dopaminergic drugs, which are useful, include anticholinergic compounds, antiviral drugs like amantadine, norepinephrine and serotonergic receptor and muscarinic related compounds. The main drawbacks of these pharmacological agents are that they cannot alleviate non-motor symptoms like dementia and mood disorders and they do not correct the abnormalities in cholinergic and serotonergic pathways.

b. Therapeutic strategies based on molecular mechanism of disease:

1. Small molecular therapy: Table 2 shows the various small molecules that have been tried in Parkinson disease.

2. Cell based therapy: Attempts to improve dopamine levels in the brain by transplanting fetal midbrain tissues and adrenal medullary tissues to mice models started as early as 1980s (Parmer, 2018). These transplants were later shown to induce transplant related side effects (Barker et al., 2015). Moreover due to limited supply of human fetal ventral mesencephalic tissue and related ethical concerns, grafting to human brain had limited clinical utility. With the discovery of human embryonic stem (hESC) cells in 1998, attempts to use them for producing dopaminergic neurons began. Several stem cell sources for grafting were considered and these were pluripotent embryonic stem cells, induced pluripotent stem cells (iPSCs), mesenchymal stem cells and induced neurons obtained by reprograming somatic cells (Barker et al., 2015). Many research groups have completed preclinical trials with GMP (Good Manufacturing Practice) level cell manufacturing and now clinical trials are on their way (Barker et al., 2017).

3. Gene therapy-based approach: Viral vector mediated approach using lenti virus, adeno virus and recombinant adeno virus has been tried in animal models. (Maiti et al., 2017). Triple gene therapy by delivering the genes required for the three enzymes to produce dopamine from L Dopa has also been tried. RNA interference to silence *SNCA*, Parkin and *PINK* is another approach. CRISPR-cas9 mediated genome editing has been used to develop a stable cell line, which expresses *SNCA*. More studies in animal and cellular models are required before gene-based therapy can be tried on human beings.

c. Surgical therapy: The two surgical approaches that are used for Parkinsonism include deep brain stimulation and pallidotomy or thalamotomy.

Conclusion

Parkinson disease is a common neurodegenerative condition, which occurs due to interplay between environmental, epigenetic and genetic factors. Only 5-10% of Parkinson disease is due to monogenic causes. Genetic testing for Parkinson disease should be attempted with utmost care only after appropriate pretest counseling. Newer modalities of treatment for Parkinsonism like cell-based therapy are on the horizon with clinical trials being conducted now.

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Table 2 Small molecule therapy being tried for Parkinson disease.

Small molecule	Targeted protein	Model	Effect	Reference
BIOD303	SCNA *(Synuclein)*	Neuronal cell culture	Synuclein accumulation decreased	Moore et al., 2015
ELN484228 and ELN484217	SCNA *(Synuclein)*	Cortical neuron from embryonic rat	Rescue of synuclein-induced disruption of vesicle trafficking and dopaminergic neuronal loss and neurite retraction	Toth et al., 2014
Flavonoid epigallocatechin gallate (EGCG)	SCNA *(Synuclein)*	OLN-93 oligodendrocyte cell line	Neuroprotective effect by decreasing cytotoxicity	Lorenzen et al., 2014
Oligomer modulator anle138b	SCNA *(Synuclein)*	PD mouse model	Improved survival 50 weeks after onset of symptoms	Levin et al., 2014
PREP inhibitor, KYP-2047.	SCNA *(Synuclein)*	Homozygous A03P mice	Increases clearance of protein by increasing autophagy	Savolainen et al., 2014
NOS inhibitor, NG -nitro-L-arginine methyl ester (L-NAME)	Parkin	Mice model	Protection against dopamine neurotoxicity	Singh et al., 2013
STI 571	Parkin	Cell model (SH-SY5Y)	Neuroprotective	Ko et al., 2010
K 560	LRRK2	Cellular and mice models	Prevented neuronal death by inhibiting HDAC1 and HDAC2 (Histone deacetylase)	Choong et al., 2016
Nurr1 agonists (Amodiaquine and chloroquine)	Nurr1	Mice	Neuroprotective	Kim et al., 2015

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GeNeXpreSS

Whole Genome Sequencing: The Way Forward for Molecular Cytogenetics

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Utility of NGS in Prenatally Detected Balanced Chromosomal Rearrangements (Halgren et al., 2018)

Prenatally detected de novo balanced chromosomal rearrangements have been reported to be associated with a 6-9% risk of adverse outcome, but the postnatal long-term morbidity in these antenatally detected cases has not been adequately studied. In this study, Halgren et al. have obtained long term follow-up data, for a mean period of around 17 years, from an existing national registry as well as through clinical follow-up, for 41 individuals who had prenatally detected de novo apparently balanced chromosomal rearrangements (BCRs) but no antenatal ultrasound anomalies (where the pregnancy was continued), and found that as many as 27% of them developed neuropsychiatric or neurodevelopmental disorders. Samples could be obtained from 32 out of these 41 individuals. Chromosomal microarray (CMA) in all these 32 cases was normal. Next generation sequencing (NGS)-based mate-pair sequencing could be done in 29 cases, out of which 21 had intragenic or non-genic disruptions. The findings included disruption of genes (*ARID1B, NPAS3, CELF4*) or regulatory domains of known developmental genes (*ZEB2, HOXC*), or complex BCRs which correlated with the adverse outcomes in these patients. This study demonstrates that NGS outperforms CMA in the characterization of prenatally detected de novo BCRs and can help in more accurate prognostication of these cases. NGS-based mate pair sequencing may soon replace CMA in the evaluation of de novo BCRs, specifically in cases with structurally normal fetuses, where the diagnostic yield of CMA is very low.

Utility of WGS in Copy Number Variation Analysis (Zhou et al., 2018)

Conventionally, sequencing technologies have been stated to be unsuitable for identification of copy number variations (CNVs), and molecular cytogenetic techniques especially chromosomal microarray (CMA) have been recommended for CNV analysis. In their study, Zhou et al. have studied the ability of three Whole Genome Sequencing (WGS) strategies - short insert, 3 kb insert mate pair and 5 kb insert mate pair (each at 1X, 3X and 5X coverages) to detect CNVs. They have investigated how these strategies perform relative to each other and also compared their yield to 17 currently used high-density oligonucleotide arrays. A set of gold standard CNVs generated for the 1000 Genomes Project (CEU subject NA12878) was used as the benchmark. WGS strategies with even low coverage were found to be able to detect significantly more CNVs and gold standard CNVs with validation, when compared to the high-density oligonucleotide array platforms. Thus, WGS appears to be having a higher sensitivity than even the best performing arrays, in detecting CNVs, with a lower percentage of unvalidated CNV calls.

WGS–based NIPT for Prenatal Cytogenetic Analysis (Van Opstal et al., 2018)

A new study from Netherlands by Van Opstal et al. has reported the yield of Whole Genome Sequencing (WGS)- based non-invasive prenatal testing (NIPT) for the detection of chromosomal aberrations other than common chromosomal aneuploidies, among participants of the TRIDENT study (Trial by Dutch laboratories for Evaluation of

Non-invasive prenatal Testing). Out of the 2527 cases where whole-genome shallow massively parallel shotgun sequencing was performed in the cell-free DNA derived from the maternal blood sample, in 78, one of the common trisomies was reported (trisomy 21/13/18) and in 41 (1.6%), some other chromosomal aberration was found. Further cytogenetic evaluation of the chorionic villi/ amniotic fluid/ fetal blood/ fetal skin/ placental samples revealed that out of these 41 chromosomal aberrations detected through NIPT, 10 were fetal, 22 were placental and 1 was a maternal CNV; in 7 cases, the origin of the aberration remained unresolved and in one case, cytogenetic follow-up could not be done. Nine of the 10 fetal chromosomal aberrations were associated with an abnormal clinical phenotype and 13 of the 22 placental aberrations were associated with fetal congenital anomalies and/or poor fetal growth. This study demonstrates the utility of WGS-based NIPT in the detection of chromosomal aberrations other than common trisomies and also in the identification of confined placental chromosomal aberrations that may affect the pregnancy outcome.

WGS as the First–tier Genetic Test for Pediatric Patients (Lionel et al., 2018)

The utility of Whole-genome sequencing (WGS) as a comprehensive testing platform in suspected pediatric genetic disorders was explored in a study by Lionel et al. One hundred and three patients with a clinical phenotype suggestive of an underlying genetic disorder were recruited from pediatric non-genetic subspecialty clinics. The diagnostic yield was compared with that of conventional step-wise genetic testing. Diagnostic variants were identified in 41% of cases. WGS was able to detect all the molecular aberrations detected by conventional genetic testing methods as well as an additional 18 variants, including structural and non-exonic sequence variants. WGS was thus shown to provide a higher diagnostic yield as a primary test, when compared to conventional genetic tests, in a clinically heterogeneous cohort.

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PhotoQuiz - 42

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This seven months-old girl child, born to non- consanguineous parents, was evaluated for severe global developmental delay. She was operated for cleft lip and cleft palate. Her karyotype was normal. Identify the intracranial anomaly seen in the MRI Brain images and the likely genetic etiology.

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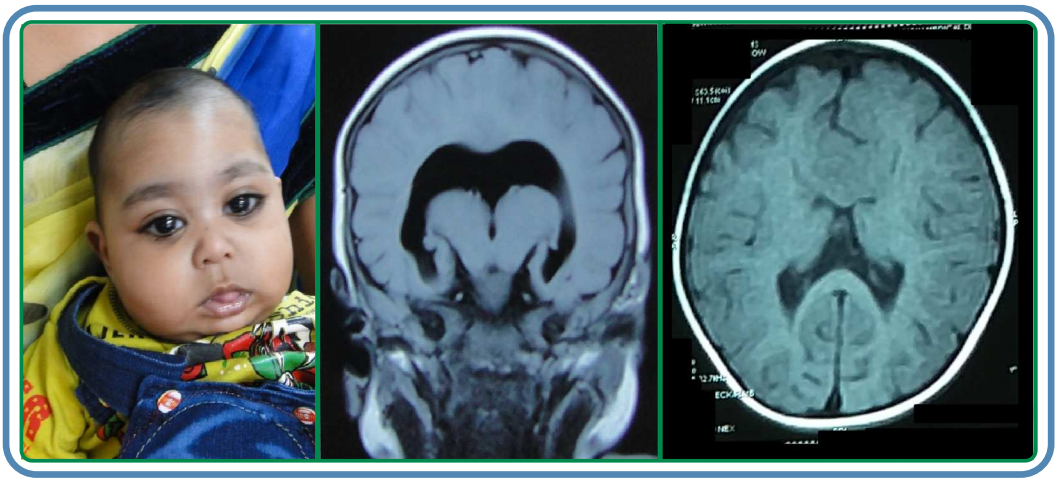

Answer to PhotoQuiz 41

Williams-Beuren syndrome (OMIM # 194050)

Williams Syndrome (WS), also known as Williams-Beuren syndrome is characterized by cardiovascular disease (peripheral pulmonary stenosis, supravalvular aortic stenosis, hypertension), distinctive facies, connective tissue abnormalities and intellectual disability (usually mild). They have a friendly personality and attention deficit disorder. Other findings include growth retardation, endocrine abnormalities like hypercalcemia, hypothyroidism and early puberty. Williams Syndrome (WS) is a microdeletion syndrome caused by a heterozygous deletion of 1.5-1.8-Mb Williams-Beuren syndrome critical region (WBSCR) that encompasses the elastin gene (*ELN*) at chromosome 7q11.23. It is inherited in an autosomal dominant manner. However, most of the cases are *de novo*.

Correct Responses Were Given By:

1. Sarah Bailur, Hyderabad
2. Veronica Arora, New Delhi
3. Suvarna Magar, Aurangabad
4. Dr Jayarekha Raja, Chennai
5. Kanika Singh, New Delhi
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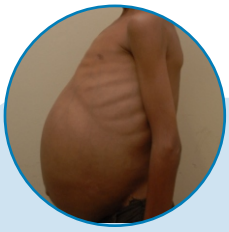

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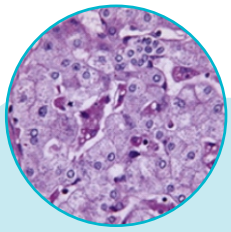

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