In this special issue of Genetic Clinics, we are publishing five selected abstracts from the ones that were submitted for the 4th International Conference on Birth Defects (ICBD) and the 5th Annual National Conference of the Society for Indian Academy of Medical Genetics (SIAMCON 2018), held in Christian Medical College, Vellore, Tamil Nadu, on 13th – 15th December 2018. Of these, 2 won the prizes for the best oral paper presentation (first and second), 2 for the best poster presentation (first and second) and 1 was awarded a special prize.

ABSTRACT 01

I. Paper awarded the first prize for oral presentation:

Mitochondriopathies: Further delineation of clinical, radiological and genotypic spectrum

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Aim: To define clinical, radiological and genotypic spectrum of mitochondriopathies.

Objectives:

1. Clinical, radiological and molecular characterization of mitochondriopathies in the Indian population
2. To identify novel phenotypes of mitochondriopathies and their underlying genetic mechanism

Methods: This case series is a part of a larger cohort of individuals with neurodevelopmental disorders. Clinical and radiological evaluation was performed for all families by a medical geneticist. This was followed by exome sequencing for index patients in families without definite clinical diagnosis and Sanger sequencing in families with definite clinical diagnosis. Validation of the identified pathogenic variant and bi-allelic segregation analysis was performed by Sanger sequencing.

Results: In the above-mentioned cohort eighteen families were diagnosed with mitochondriopathies. Six truncating and nine missense variants were identified in nuclear genes *NDUFAF6, NDUFV2, NDUFV1, SURF1, SDHB, MGME1, TYMP, PNPLA8, AUH, ACO2, CLPP,* and *GCDH*. Ten of these variants are novel. Among these families, disorders of the respiratory chain complexes (n=12), disorders of mtDNA maintenance (n=3), and disorders of phospholipid metabolism (n=1) were noted. Additionally, two novel disorders of the respiratory chain complexes were identified along with the causative genes, *ISCA1* and *NAXD*. Final diagnosis in these families with underlying genetic variants is given in table 1.

Discussion and Conclusion: Mitochondriopathies are a group of clinically and radiologically heterogenous conditions and have a complex underlying pathophysiology involving genetic variants in either the nuclear or mitochondrial genome. This implies that the inheritance patterns observed with these disorders are diverse too, including Mendelian and mitochondrial inheritance, the former being more commonly observed. We report 18 families with mitochondriopathies (Table 1) and their clinical, radiological and genotypic spectrum. Developmental delay, neuroregression, seizures, cardiac and eye abnormalities were noted to be common clinical features. Characteristic radiological findings were noted in majority of the families. Application of exome sequencing in this heterogenous cohort helped in identification of molecular cause in known mitochondriopathies and elucidation of novel phenotypes.

ABSTRACT 02

II. Paper awarded the second prize for oral presentation:

Genetic and Phenotypic Heterogeneity in Waardenburg Syndrome

Mr. Somashekar PH[1], Dr Sheela Nampoothiri[2], Dr Kalpana Gowrishankar[3], Dr Radha Rama Devi[4], Dr Neerja Gupta[5], Dr Dhanya Lakshmi Narayanan[6], Dr Anupriya Kaur[7], Dr Shruti Bajaj[8], Dr Sujatha Jagadeesh[9], Dr Leslie Lewis[10], Dr S Shailaja[11], Dr Girisha KM[1], Dr Anju Shukla[1]

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Aim and Objective: Analysis of phenotypic and genetic diversity in Waardenburg syndrome (WS).

Materials and Methods: We investigated a cohort of 15 families (17 subjects). Fourteen of these families were clinically diagnosed with WS and one family with isolated non-syndromic hearing loss (NSHL). Genetics testing was done by Sanger sequencing or Whole Exome Sequencing (WES).

Results: We identified thirteen single nucleotide variants (SNV) and one copy number variation (CNV) in genes known to cause WS. Intra familial phenotypic variability and non-penetrance were observed in families diagnosed with WS1, WS2 and WS4 with pathogenic variants in *PAX3*, *MITF* and *EDNRB* respectively. We observed gonosomal mosaicism for a variant, c.256A>T in *PAX3* in an asymptomatic father of two affected siblings. Biallelic novel missense variant, c.1021C>G in *MITF* was identified in a patient with WS2. A variant, c.673G>A in *EDNRB* in homozygous state was identified in a patient diagnosed as WS2. Two pathogenic variants, c.166C>T in PAX3 and c.1047delC in *EDNRB* in heterozygous state were identified in subject diagnosed as WS1. Extended exome analysis for CNVs revealed 0.17 Mb heterozygous deletion encompassing *SOX10* in a patient diagnosed with WS 4. A homozygous known stop-gain variant, c.71G>A in *GJB2*, known to cause Deafness, autosomal recessive 1A was identified in a subject diagnosed as WS1. A novel stop-gain variant, c.1608C>G in *ADGRV1* and a known missense variant, c.575C>A in *TYR* known to cause Usher syndrome 2C and albinism respectively were identified in a subject diagnosed as WS2.

Discussion and Conclusion: Our cohort demonstrates intra and inter familial phenotypic variability and non-penetrance in families with WS. We report gonosomal mosaicism in WS1 and biallelic variants in *MITF* and *EDNRB* causing WS2. Blended phenotype of non-syndromic hearing loss and albinism mimicked WS. A phenocopy of WS1 was observed in a subject with a reported pathogenic variant in GJB2, known to cause isolated NSHL. These novel and infrequently reported observations exemplify the genetic heterogeneity and phenotypic diversity of WS.

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ABSTRACT 03

III. Paper awarded the first prize for poster presentation:

Genetic analysis of clinically diagnosed Neurodegeneration with brain iron accumulation (NBIA cases) - Identification of common and rare subtypes

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Introduction: Neurodegeneration with brain iron accumulation is a heterogenous group of disorders characterised by the accumulation of iron in the basal ganglia that results in dystonia, spasticity, intellectual and motor decline, neuropsychiatric disabilities, and optic atrophy or retinal degeneration. Current diagnosis is facilitated by Brain MRI findings of "eye of tiger" sign in the typical form of NBIA. Genetic studies helps in confirming the diagnosis, delineating the subtype and genetic counselling. In India data on genetically de[FB01?]ned NBIA cases are limited and underdiagnosed. Hence we aim to identify the spectrum of pathogenic variants in patients diagnosed with NBIA.

Patients and methods: Nineteen patients with clinically diagnosed NBIA (2014-2018) were included in this analysis. These patients were referred from the department of Neurology and presented with any one or more of clinical features such as regression in milestones, dystonia, deterioration of vision and hearing, and spastic quadriparesis. Neuroimaging revealed iron deposition in the basal ganglia con[FB01?]rming NBIA. We have performed NGS based screening in 5 patients and targeted single gene analysis in 14 patients to identify the genetic variations causing NBIA.

Results: The mean age of patients was 8.4±4.4yrs and male to female ratio was 5:4.5. We have identified homozygous/compound heterozygous variants in 16 patients in which 10 were classified as novel variants. One patient was identified to have heterozygous variant and another patient was found to be negative for any mutations. Interestingly, one patient who was initially suspected to be NBIA based on MRI brain was found to be compound heterozygous for variants in *GLB1* gene confirming GM1 gangliosidosis on Clinical exome sequencing.

Molecular analysis helped us to stratify NBIA cases into 5 different subtypes. Seven patients were categorised into PLA2G6-Associated Neurodegeneration (PLAN) confirmed by genetic mutations. Six patients were subtyped into (PKAN) Pantothenate Kinase-Associated Neurodegeneration due to *PANK2* gene mutation. One patient was identified to have a rare form of FA2H; Fatty Acid Hydroxylase-Associated Neurodegeneration (FAHN) and the other patient with C19ORF12; Mitochondrial Membrane Protein-Associated Neurodegeneration (MPAN) mutation. One patient was genetically proven rare case of BPAN with *WDR45* gene mutation (Figure 1).

Conclusion: In conclusion, the proportion of patients with PLAN subtype was higher than reported in literature. Mutations in *PLA2G6* gene in exons 16 & 7 in four patients indicates that those exons can serve as hotspots for genetic testing. The hot spots in *PANK2* gene were found to be exons 1 and 2. Establishing molecular analysis (*PLAN* and *PKAN* genes) assisted us to genetically confirm the diagnosis of NBIA in 68% of cases and we could offer prenatal diagnosis for two families. The advancement in Next generation sequencing and Clinical exome analysis furnished better understanding of genotype-phenotype correlation including differential diagnosis of NBIA as GM1 gangliosidosis.

ABSTRACT 04

IV. Paper awarded the second prize for poster presentation:

Spondyloepiphyseal dysplasia congenita caused by biallelic c.3190C>T in *COL2A1*

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Aim and Objective: The aim of the study was to identify the genetic cause in a family with clinical features of spondyloepiphyseal dysplasia.

Patients/ Material and methods: We ascertained a consanguineous family with two affected siblings, a 7 years old male (proband 1) and 10 years old female (affected sibling) who presented with gait abnormalities. In proband 1 clinical features of exaggerated lumbar lordosis, scoliosis, lower limb length discrepancy and mild joint laxity were noted. Only exaggerated lumbar lordosis was noted for affected sibling. Both had radiographic features suggestive of mild platyspondyly with irregular end plates, epiphyseal dysplasia of femora with delayed carpal ossification. The parents were clinically unaffected.

Whole exome sequencing was done for the proband 1 followed by segregation analysis and validation of the variant in proband 1, his parents and proband 2 was done by Sanger sequencing.

Results: Analysis of exome data revealed a novel missense variant c.3190C>T p.(Arg1064Cys) in exon 47 in homozygous state in *COL2A1* gene in the proband 1. The variant was detected in homozygous state in proband 1 and affected sibling. The parents were heterozygotes for the variant.

Discussion: Heterozygous pathogenic variants in *COL2A1* are known to be implicated in the pathogenesis of several types of skeletal dysplasia collectively known as type II collagenopathies. In the recent literature, two families have been reported with homozygous pathogenic variants in COL2A1. The affected individuals reported had short stature, kyphoscoliosis, barrel-shaped chest, short neck, flat face, waddling gait, brachydactyly and myopia. The clinically unaffected parents were heterozygotes for the condition. However, our probands had milder phenotype in comparison to the previously reported individuals. Here we report an additional family with spondyloepiphyseal dysplasia congenita which further validates the pathogenicity of homozygous missense variants in COL2A1, leading to spondyloepiphyseal dysplasia congenita.

ABSTRACT 05

Analysis of Homozygosity Stretches Around Homozygous Pathogenic Variations for Autosomal Recessive Disorders in Indian Patients from Consanguineous and Non-Consanguineous Families

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We compared stretches of homozygosity around homozygous pathogenic / likely pathogenic sequence variations causing autosomal recessive disorders in consanguineous and non-consanguineous families. The exome data of the cases in whom the homozygous pathogenic / likely pathogenic sequence variations were identified was analysed. All 24 cases with AR disorders from consanguineous families were homozygous for the disease causing variations (12 out of 24 being novel variations) and had large (Average – 77.2 Mb,Range - 5 Mb to 271 Mb) stretches of homozygosity around the disease causing pathogenic or likely pathogenic variations. For AR disorders from non-consanguineous families, the disease causing variations were in homozygous form in 13 (9 being novel) out of 19 cases and 6 were compound heterozygous. In the cases with homozygous pathogenic variations from non-consanguineous families; there were stretches of homozygosity around the causative sequence variations (Average - 27.9 Mb, 0.6 Mb to 188 Mb).

We also reviewed our data of SNP microarray of cases from 50 consanguineous and 50 non-consanguineous families. In cases born to consanguineous parents the sizes of Regions of Homozygosity (ROH) regions were 28 Mb to 770 Mb. The average number of ROH more than 5 Mb were 11.59 (1 to 25). Amongst 50 cases from non-consanguineous families, 26 had at least 1 ROH more than 5 Mb (Average 2.33 of 26 cases). The sizes of runs of homozygosity regions varied from 3 Mb to 49 Mb (0.10 % to 1.7% of total genome; average 0.74%).

In India the custom of marriages amongst caste groups has been followed for ages. We have seen that for many rare autosomal recessive (AR) disorders, the affected individuals are homozygous for rare disease causing pathogenic variations, suggesting effects of inbreeding. Long stretches of homozygosity around homozygous rare pathogenic variants supports the notion that the system of marriages between closed groups (castes) has many founder mutations and using the strategy of homozygosity by descent even in non-consanguineous families can be fruitful in identifying novel pathogenic variations and novel genes.

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