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ABSTRACT BOOK

Poster #10

Clinical, Biochemical & Molecular Characteristics of GM1 & GM2 Gangliosidosis - an Experience from Northern India

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Background: Gangliosidosis, a lysosomal storage disorder, caused by accumulation of GM1 /GM2 gangliosides. These are a group of inherited metabolic diseases of autosomal recessive inheritance arising due to defects in HEXA, HEXB, GLB1 genes encoding lysosomal proteins.

Objectives: To study the disease spectrum of gangliosidosis in northern India particularly Punjab state and to analyze the clinical, biochemical and molecular characterization of these neuronopathic lysosomal storage diseases.

Methods: Over 12 years (2010-2022), 11 patients were diagnosed with sphingolipidosis in our institute. The majority of the patients presented mainly with CNS manifestations. Specific enzyme assays in serum, leucocytes and other relevant investigations, including neuroimaging studies, were conducted.

Results: The age of presentation varied from 9 months to 4 years. M:F ratio was 1.8:1. Parental consanguinity was present in one case only. In all the patients, biochemical assays depicted the relevant enzyme deficiencies. Out of all the cases, 4 cases were of GM1 gangliosidosis and 7 cases were of GM2 gangliosidosis (Tay Sachs disease). In 5 patients, molecular tests were available for HEXA, HEXB & GLB1 genes involving various exons. Out of these, 4 patients harbor novel variants of gene mutations.

Conclusion: There is paucity of literature about gangliosidosis prevalence & molecular characterization in Northern India. Our study on these 11 patients put new insight into the diagnosis, of novel mutations including genetic complexity and management in developing countries and lay a cornerstone for further research in this area for understanding pathogenesis, and helping people in prevention through genetic counseling and prenatal diagnosis.

Poster #11

Kidney Biopsy as Diagnostic Biomarker for Early Fabry Nephropathy

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Background

Kidney biopsy (KB) is an important tool in assessing renal involvement in Fabry disease (FD).

Objectives

We used KB to assess histological changes and the relationship between glomerular structure and renal function in patients with FD who had normoalbuminuria/microalbuminuria.

Methods

KB specimens were obtained from 11 patients, with genetically confirmed FD who had not yet received FD specific therapy. The evaluation of Fabry nephropathy included measurement of serum creatinine and eGFR, measurement of the urine albumin-to-creatinine ratio (UACR), and KB.

Results

The mean age at the time of renal biopsy was 38.8 ± 16.9, range 10-63 years. Six patients had chronic kidney disease (CKD) 1, five patients had CKD 2 and three patients had CKD 3. Eight patients presented normal UACR and three patients showed microalbuminuria.

Histological findings showed segmental sclerosis in 3 patients, one with CKD 1, one with CKD 2, and one with CKD 3. Global sclerosis was present in 2 patients: 1 with CKD 3 and 1 with CKD 2. Interstitial fibrosis was observed in three patients. Podocyte GL3 deposits were present in all patients, and glomerular endothelial cell and tubular epithelium cell GL3 deposits in 10 patients.

Conclusion

Specific FD findings were observed in all patients, and in some cases were accompanied by nonspecific chronic lesions, demonstrating that KB has an essential role for early Fabry nephropathy assessment. This biomarker can help to identify patients at high risk of progression and can potentially be used to guide clinical decisions for FD specific therapy initiation.

Poster #12

Abnormal PRE-mRNA Splicing in Exonic Fabry Disease-Associated Mutations of the GLA Gene

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Background: For the rare X-linked Fabry disease (FD, MIM #301500), a molecular therapy based on the pharmacological chaperone (PC) 1-deoxygalactonojirimycin (DGJ) was internationally approved in 2016. The effectiveness of PC therapy depends largely on the genetics of the patient. The predominant type of mutations that respond to PC therapy are point mutations in protein-coding exonic gene regions, so-called missense mutations. Objective: A GLP-validated and FDA-approved pharmacogenetic cell culture assay (GLP-HEK assay) allowed the determination of amenable α-galactosidase A (gene symbol: GLA, E.C. 3.2.1.22, AGAL) genotypes in vitro. However, in vitro activity measurement has the limitation that only the theoretically synthesised missense AGAL enzyme can be analysed, i.e. it is assumed that exonic point mutations pathomechanistically alter only the amino acid sequence and thus the protein folding behaviour of AGAL, while abnormal splicing activity is excluded. Methods: We examined exon mutations at or near exon-intron junctions using a minigene reporter assay to investigate possible abnormal splicing events. Results: In all cases examined, we found evidence of an unanticipated splicing disorder. Mutations in exon 4 at both the 3` acceptor splice site (c.548GT) and the 5` donor splice site (c.638AG/T) resulted in massive exon skipping. A mutation in exon 1 (c.194GT) and a mutation in exon 2 (c.358CG) that did not directly affect an intron/exon junction also showed altered RNA splicing. Conclusion: Abnormal splicing can lead to a reduction in enzyme activity and alter the eligibility for PC treatment in FD.

Poster #13

MPS Type IIIA - Sanfillipo Syndrome: Patient with Two Homozygous Variants in the SGSH Gene

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Background:

Mucopolysaccharidosis type IIIA (MPSIIIA, Sanfillipo syndrome) is a rare lysosomal storage disorder caused by biallelic loss-of-function variants in the SGSH gene, encoding enzyme involved in the breakdown of glycosaminoglycans (GAGs). Accumulation of GAGs lead to progressive neurolodegeneration and behavior problems, as well as musculoskeletal, auditory, visual, cardiovascular and respiratory problems.

Case report:

We report a 5 yo male with developmental regression and behavior problems evident from 3 years. He also had hearing loss, short stature, hepatosplenomegaly, joint deformations and coarse facial features including thick lips and eyebrows, broad nose, and frontal bossing.

Results:

NGS panel revealed two homozygous variants of uncertain significance (VUS) in the SGSH gene: p.(Asp440_His446dup) and p.(Leu438Ile). Biochemical investigation showed markedly elevated urinary GAGs and decreased levels of N-sulphoglucosamine sulphohydrolase, confirming the diagnosis of MPSIIIA. Both parents were heterozygous for each variant, confirming their cis phase.

Discussion:

P.(Asp440_His446dup) vatiant results in the insertion of 7 amino acids, but preserves the reading frame of the encoded protein. The p.(Leu438Ile) variant affects a highly conserved amino acid within the sulfatase domain of the protein and all in silico tools predict it to be deleterious. Both variants are absent in the GenomAD and neither of them has been reported in the medical literature. Currently there is insufficient evidence to confirm or exclude exactly which of the detected variant is pathogenic.

Conclusion:

Present case emphasizes the importance of biochemical investigations in patients suspected of MPS and illustrates the importance of parental testing for confirmation of cis/trans phase of the detected variants.

Poster #14

Molecular Genetics

Twelve Patients with Enzymatic Study Compatible with Pompe Disease and a Single Variant in the GAA Gene

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Background

Pompe disease (PD) is an autosomal recessive metabolic disorder caused by pathogenic variants in the acid alpha-glucosidase gene (GAA) that produces biochemical defects in the lysosomal acid alpha 1,4-glucosidase. We have analyzed 30 patients with reduced enzymatic activity and in 12 of them we have only found a heterozygous variant in the GAA gene.

Objectives

To analyze the variants of the carriers to assess if there is the possibility of dominant hereditary susceptibility

Methods

Enzymatic activity was detected by fluorometric techniques and the genetic study was carried out using Next-Generation Sequencing covering the exomes and areas close to the splicing.

Results

The 12 patients carrying a heterozygous variant in GAA with reduced activity enzyme study had 8 intronic variants (6 c.-32-13TG; 2 c.1194+5GA) 3 missense (c.1445CT; c .854CG; c.2065GA) and a frameshift (c.1396_19397insG)

Conclusion

Perhaps the possibility exists that variants that do not completely abolish gene expression produce protein products that compete with the healthy enzyme and make these patients susceptible to a defect in overall enzyme activity. It will be interesting to continue with the study of the expression to verify this hypothesis

Poster #15

Clinical Types and Geographical Distribution of Variants in the GBA Gene in Mexican Patients with Gaucher Disease

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Background: Gaucher disease (GD) is one of the 5 most prevalent lysosomal disorders in Mexico. It has three clinical forms as follows: Type 1, non-neuronopathic; type 2, acute neuronopathic; and type 3 subacute neuronopathic based on presence and progression of neurological manifestations. GD is caused by pathogenic variants in the human glucocerebrosidase gene (GBA, locus 1q21) leading to deficit or absent enzymatic activity of B-glucocerebrosidase leading to the accumulation of glucocerebroside in the cells o the macrophage-monocyte system. Here, we present the distribution of clinical phenotypes and genetic variants in Mexican patients affected with GD.
Methods. We report the molecular analysis of the GBA gene, clinical type of GD and geographical distribution in historical clinical records of 95 Mexican belonging to the Instituto Mexicano del Seguro Social.
Results: 25 pathogenic variants were identified in the GBA gene, and c.1226AG (p.N409S) and c.1448TC (p.L483P) had the most prevalent alleles frequencies (0.371 and 0.373). The most prevalent genotype in this population sample was c.1226AG; c.1448TC (0.361) 80% of the patients had GD type 1 and the 20% was conformed by Type 3 (18% and type 2, 2%) with a clear geographical distribution of non-neuronopathic forms in the northern region of the country and neuronophatic foms in the western-central region and minimal presence in the southern region.
Conclusions. We defined the clinical, molecular and geographical profiles in Mexican patients with GD.

Poster #16

Parkinson`s and Alzheimer`s Disease are Manifestations of Substrate accumulation due to Dysfunctional Lysosome and Disregulated Autophagy leading to Neuro Degeneration

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BACKGROUND: In our earlier in-vitro study using SH-SY5Y cell line we have shown that a Phyto compound has protective and regenerative ability against rotenone induced neurotoxicity by improving, Glycolysis, ATP and Mitochondrial health (MMP).

OBJECTIVE: To evaluate the efficacy of the same Phyto compound in Alzheimer's Model of in vivo.

METHODS: Neurodegeneration and Cognitive disability was induced by intraperitoneal administration of Aluminium Chloride in Wistar rats and the Neuro protective effect of the orally administered Phyto compound was evaluated through: Levels of oxidative markers like, LPO, SOD, GSH and GPX; Neuronal markers like, B Amyloid, Gamma Secretase, B Secretase, APP and AChE and the Inflammatory markers like, IL6, TNF-α, NFkB in Hippocampus and Cortex and behavioural and Histopathological studies.

RESULTS: In this 4 weeks' study all the markers were significantly negatively impacted in Alcl3 group and they were all positively reversed close to control group in Phytocompound treated animals. Histopathology: Presence of NFT, Gliosis, Mononuclear cell infiltration and Neuro degeneration in Hippocampus and Cerebral cortex of Al Cl group of animals. Though mild NFT could be observed, Gliosis, Neuro degeneration, Glial nodule or Mononuclear cell infiltration were totally absent in treated animals. Behavioural tests revealed a significant improvement in Recognition, Spatial memory and Learning in treated animals.

CONCLUSION: Though AD and PD are two different manifestations, they have the same pathology of lysosomal dysfunction and dysregulated autophagy. As these could be reversed by improving Glycolysis, ATP and Mitochondrial health, efficacy of this Phyto compound in LSDs especially Gaucher, Pompe and Fabry diseases can be explored.

Poster #17

Involvement of Circadian Clock Mechanisms in Fabry Disease, a Genetic Lysosomal Storage Disorder

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Fabry disease (FD) is a rare genetic lysosomal storage disorder, inherited in an X-linked manner. In FD, the mutations in the GLA gene encoding for a lysosomal enzyme alpha-galactosidase A lead to a deficiency in the enzyme activity. This results in excessive accumulation of the particular type of fat called glycosphingolipid (mainly globotriaosylceramide) inside cells and tissues, including the cells lining the blood vessels of the skin as well as the heart, kidney and nervous system. This progressive disease often starts in early childhood and affects multiple systems, thus negatively influencing patients' quality of life and life expectancy.

In order to better understand the systemic effects of this disorder, we have investigated the hypothesis that circadian clock mechanisms play a role in FD pathogenesis. We show that core clock and clock-controlled genes show altered expression, phase and rhythmicity in synchronized FD patient fibroblasts compared to healthy controls as assessed by the qPCR analyses and real-time-recording of clock::gene reporters. Moreover, the regulation of several antioxidant genes, responsive to lysosomal stress, show dampened circadian expression in FD patient cells. Furthermore, healthy control fibroblasts show a circadian variation in the alpha-galactosidase A enzyme activity, suggesting that altered circadian regulation of this enzyme may play an important role in FD.

Further work will test whether timed supplementation of the recombinant alpha-galactosidase A enzyme to patient cells (used as enzyme replacement therapy in FB) can rescue their altered circadian rhythms. All together, these data suggest a novel involvement of the circadian clock mechanisms in the pathophysiology of lysosomal disorders.

Poster #18

Lysosome and Olfactory Neuroepithelial Dysfunction

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Background: Olfactory neuroepithelium is a specialized structure and sensitive to external biotic and abiotic milieus. This structure is guarded by non-specific vascular elements (i.e., goblet cells, mast cells, rodlet cells, plasma cells and macrophages.) for cell mediated neural protection.

Objectives: The accumulation of lysosome-like structures in the vascular elements within the olfactory neuroepithelium can trigger neural stress in vertebrates?

Methods: Olfactory structure of Lepidocephalicthys guntea fixed in 2.5% glutaraldehyde, 1 hr. at 4ºC and 1% osmium tetraoxide in 0.1 M PBS (pH-7.2) for 1 hr., followed by dehydration, staining and viewed under transmission electron microscope (TEM:TECNAI,G20 FEI at AIIMS-New Delhi).

Results: Olfactory neuroepithelium shows mucus secreting goblet cells with holocrine secretary droplets. Macrophage diversity within olfactory neuroepithelium is frequently observed and closely associated with rodlet cells and mast cells. Rodlet cells and macrophages are packed with phagolysosome-like structures. A few macrophages are heavily packed with cellular debris; primary lysosomes, multivesicular bodies (MVBs) and phagolysosome. It is also evident that macrophages are heavily loaded with lysosome-like bodies within the cytoplasm.

Conclusion: A few vascular cells (i.e., macrophages, mast cells, etc.) are directly involved in cell mediated neural protection but high pathogenic load and frequent accumulation of lysosome-like structure significantly distort the stability of cellular function (?) of the neuroepithelial tissue concern.