Using recombinant glycogen branching enzyme and structural biology techniques to understand therapeutic opportunities for APBD

Or Kakhlon
Hadassah-Hebrew University Medical Center, Jerusalem, Israel
Wyatt Yue
Structural Genomics Consortium, University of Oxford, UK

Glycogen branching enzyme 1 (GBE1) plays an essential role in glycogen biosynthesis, mutations of which lead to the heterogeneous early-onset glycogen storage disorder type IV (GSDIV) or the late-onset adult polyglucosan body disease (APBD). Our group has an ongoing interest in studying the catalytic and disease mechanism underlying the human glycogen biosynthetic machinery, which consists of the priming enzyme glycogenin (GYG1/2), the elongation enzyme glycogen synthase (GYS1/2), and the branching enzyme (GBE1).

My current study is aimed at validating two potential therapeutic avenues for APBD: pharmacological chaperoning for GBE1, and small molecule inhibition of GYS1. My presentation hence will consist of two parts:

- Characterization of the recombinant GBE1 mutant protein p.Y329S linked with APBD, and structure-guided design of a stabilizing peptide
- Effect of the small molecule guaiacol towards activity of recombinant glycogenin-glycogen synthase complex

TGMs on the interaction of WT and Y329S GBE1 with membranes: Effects on GBE1 activity and APBD pathophysiology and therapy.

Pablo Escribá
University of the Balearic Islands, Palma, Spain

Rafael Alvarez[a], Jesús Casas[a], David J. López[a], Maitane Ibarguren[a], Alexander Lossos[b], Xavier Busquets[a], Or Kakhlon[b] and Pablo V. Escribá[a]

Adult Polyglucosan Body Disease (APBD) is triggered by GBE1 Y329 mutation and it is characterized by adult-onset neurogenic bladder, spasticity, weakness, sensory loss and more. Albeit being a soluble enzyme, we observed that protein-membrane interactions regulate GBE1 activity. Because soluble proteins can be in contact with a wide variety of cell membranes, we investigated the interactions of purified wild type and Y329S GBE1 proteins with different types of model membranes (liposomes). Moreover, the investigational drug triheptanoin (TH) and some of the triacylglycerol mimetics we designed and synthesized (TGM0 and TGM5) were able to induce marked and significant increases in GBE1 Y329S activity, which may suffice to achieve enough glycogen branching to reverse APBD symptoms. Our results indicate that the Y329S mutation causes exposure of a hydrophobic amino acid stretch whose interaction with cell membranes can either stabilize and *increase* its activity or alter protein-membrane interactions *reducing* the enzyme's activity. In addition, we observed that GBE1 activity is modulated by Ca^{2+} and phosphatidylserine (PS), which could be associated with a metabolic mechanism to regulate energy consumption and storage. In summary, both TGM0 and TGM5 could have higher activity against APBD than TH, although only TGM5 can be developed as a food supplement. Given the (i) thermal stabilization produced by TGM5, (ii) the increase in GBE1 activity induced, and (iii) its omega-3 oil structure, this molecule has a great therapeutic potential for the treatment of APBD.

Identification of therapeutic targets in an APBD Mouse model

Erin Chown
The Hospital for Sick Children, Toronto, Canada

Erin Chown[1], Peixiang Wang[1], Xiaochu Zhao[1], Bart Pedersen[2], Berge Minassian[1]
[1]The Hospital for Sick Children, Toronto, Canada
[2]Ball State University, Indiana, USA

Adult polyglucosan body disease (APBD) is a neuromuscular disease caused by autosomal recessive mutations in the glycogen branching enzyme gene (glycogen storage disease type IV). APBD is characterized by the build-up of poorly-branched glycogen particles which aggregate to form pathogenic inclusions, called polyglucosan bodies. Polyglucosan bodies localized to neuronal axons are understood to cause the progressive neuromuscular symptoms observed in APBD patients. We hypothesized that knockdown of the enzyme responsible for glycogen chain elongation, glycogen synthase, or of a protein involved in its activation, PTG, could mitigate the glycogen branching enzyme deficiency and prevent the formation of polyglucosan bodies. Using the APBD mouse model, we characterized the effect of glycogen synthase or PTG deficiency on the disease phenotype and observed significant behavioural, histological and biochemical rescue. Identification of glycogen synthase and PTG as effective therapeutic targets represents a step towards the development of a therapy for APBD patients.

Antisense Oligonucleotide Therapy for Genetic Disorders

Tamar Grossman
Department of Antisense Drug Discovery, Ionis Pharmaceuticals, Carlsbad, California.

Advances in deciphering the complex roles RNA plays in normal health and disease have been substantial over the past decade, and RNA is becoming an increasingly important target for therapeutic intervention. Antisense oligonucleotides (ASO) are perhaps the most direct therapeutic strategy to approach RNA, and ASO technology has emerged as a powerful alternative to conventional small molecule approaches or gene replacement strategies for the treatment of genetic disorders. ASO are short, synthetic single-stranded DNA sequences designed to bind to target RNA by well-characterized Watson-Crick base pairing, and once bound to the target RNA, can modulate RNA function through a variety of post binding events. ASO-mediated gene silencing occurs either through degradative mechanisms, where the target RNA is cleaved by endogenous nucleases, or non-degradative mechanisms, where sterically bound ASO block or modulate translation, capping, or splicing. The majority of ASO drugs in development work through the RNaseH1 dependent degradation mechanism.

This presentation will cover antisense technology platform and will include new preclinical data on the characterization of mouse Gys1 ASOs, a potential novel therapy approach for APBD and Lafora disease.

Antisense oligonucleotide therapy for APBD

Berge Minassian
The Hospital for Sick Children, Toronto, Canada

Saija Ahonen[1], Tamar Grossman[2*], Peixiang Wang[1], Xiaochu Zhao[1], Berge Minassian[1]*
[1]The Hospital for Sick Children, Toronto, Canada
[2]Ionis Pharmaceuticals, Carlsbad, USA
*contributed equally

Adult polyglucosan body disease (APBD) is an adult-onset variant of glycogen storage disease type IV that manifests as a neuromuscular disease. APBD is recessively inherited due to mutations in the glycogen branching enzyme gene and is characterized by the presence of poorly-branched, precipitated glycogen aggregates, called polyglucosan bodies. Genetic intervention in the APBD mouse model identified glycogen synthase (Gys1), the main enzyme responsible for glycogen chain synthesis, to be an effective therapeutic target for APBD. Antisense oligonucleotides (ASOs) provide a clinically-translatable approach to induce targeted protein knockdown and we therefore aim to test the therapeutic efficacy of Gys1-targeted ASOs in the APBD mouse model. Mice were administered ASOs at one and two months of age via intracerebroventricular injection, prior to their sacrifice at three months of age. Preliminary results show a significant reduction in Gys1 mRNA and protein, and a trend towards polyglucosan body reduction. Tissue analysis and further *in vivo* experimentation is ongoing and the potential of Gys1-targeted ASOs as a therapy for APBD patients remains promising.

Research update: Generation of the mouse model of *Gbe1* intronic insertion/deletion and approaches to treatments

H. Orhan Akman
Columbia University Medical Center, New York, New York

The synthesis of glycogen is catalyzed by the sequential actions of two enzymes: (i) glycogen synthase, which attaches up to about 10 glucosyl units in alpha-1,4-glucosidic bonds to nascent linear chains of glycogen; and (ii) the branching enzyme, which attaches a short branch of approximately 4 glucosyl units to a linear chain in an alpha-1,6-glucosidic bond. Glycogen storage disease type IV (GSD IV) (OMIM 232500) is an autosomal recessive disorder caused by glycogen branching enzyme (GBE) deficiency and leading to the accumulation of an abnormal polysaccharide (polyglucosan, PG) in multiple tissues, including liver, heart, skeletal muscle, and the central nervous system. A late-onset clinical variant, known as adult PG body disease (APBD), causes a neurodegenerative disorder simulating amyotrophic lateral sclerosis (ALS). Most APBD patients are of Ashkenazi Jewish origin and harbor a c.986A>C change in GBE1. Although carriers do not develop the disease, a high number of manifesting heterozygous develop APBD. Onset and prognosis of APBD in these manifesting heterozygous patients are similar to homozygous patients. They have lower enzyme activity and are homozygous at mRNA level for c.986A>C, strongly suggesting the existence of a second mutation in the other allele. Recently, we have discovered this mutation in intron 15 of *GBE1*. This mutation is a complex deep intronic change, deleting 9 bp "GTGTGGTGG" of DNA sequence and replacing it with 19 bp "TGTTTTTTACATGACAGGT" DNA sequence. This sequence contains a strong splice acceptor site, which affects proper RNA synthesis. Unlike the common c.986A>C mutation, location and property of this intronic mutation allowed us to develop antisense oligonucleotide (ASO) treatment to recover the normal mRNA and enzyme synthesis from the affected allele.

A feel-good moment in the realm of APBD awareness

Nicole Schreiber-Agus
Program for Jewish Genetic Health, Montefiore Medical Center, Albert Einstein College of Medicine, Bronx, New York

Description of MJGH

MyJewishGeneticHealth.com is a free, online educational series that was conceived and launched by the Program for Jewish Genetic Health in May 2013. The series comprises individual "lessons" (14 lessons to date) whose topics have been carefully selected and include specific diseases/medical conditions, genetic technologies, and bioethical issues. Each lesson also addresses the question, "What does this mean for me because I am Jewish?"

Most of the lessons begin with a short public service announcement (PSA) about the topic that includes a real patient story. Viewers interested in learning more are directed to register on the site for free access to the associated full lesson that includes a webinar by an expert in the field and helpful resources/written materials. We've had more than 31,000 visitors to the platform its launch, and have close to 900 fully registered students.

The goal of MyJewishGeneticHealth.com is not only to boost awareness and knowledge, but also to enable individuals to become active participants in ensuring their own health and well-being.

Summary of Duke Pediatric Medical Genetics Glycogen Storage Disease Type IV (GSD IV) Research Program (2016)

Priya Kishnani
Duke University School of Medicine, Durham, North Carolina

1. ***A modified enzymatic method for measurement of glycogen content in GSD IV:*** Standard enzymatic method, used to quantify glycogen content in GSD IV tissues, causes significant loss of the polysaccharides during preparation of tissue lysates. We report a modified method including an extra boiling step to dissolve the insoluble glycogen, ultimately preserving the glycogen content in tissue homogenates from GSD IV mice. This study provides important information for improving disease diagnosis, monitoring disease progression, and evaluating treatment outcomes in both clinical and preclinical clinical settings for GSD IV.
2. ***Starch binding domain-containing protein 1 (stbd1) plays a dominant role in glycogen transport to lysosomes in liver:*** A small portion of cellular glycogen is transported to and degraded in lysosomes by acid alpha-glucosidase (GAA) in mammals, but the function and mechanism of this process remain unknown. Here we generated a GAA/Stbd1 double knockout mouse model and identified that Stbd1 is a major mediator for transporting glycogen to lysosomes in mouse liver. Our finding provides a potential novel therapeutic target for both lysosomal and cytoplasmic GSDs.
3. ***Alglucosidase alfa treatment alleviates liver disease in a mouse model of GSD IV:*** Patients with progressive hepatic form of GSD IV often die of liver failure in early childhood. We tested the feasibility of using recombinant human acid-α glucosidase (rhGAA) for treating GSD IV. Weekly intravenously injection of rhGAA at 40 mg/kg for 4 weeks significantly reduced hepatic glycogen accumulation, lowered liver/body weight ratio, and reduced plasma ALP and ALT activities in GSD IV mice. Our data suggests that rhGAA is a potential therapy for GSD IV.
4. ***Systemic correction of murine GSD IV by an AAV-mediated gene therapy:*** Deficiency of glycogen branching enzyme (GBE) in GSD IV results in deposition of polyglucosan bodies in multiple tissues. In this study we demonstrated that a single injection of an AAV-GBE vector into GSD IV (*Gbe1*$^{ys/ys}$) mice at a young age effectively prevented glycogen accumulation in all muscles and, to a lesser extent, in the liver and brain for up to 9 months of age. In addition, the AAV treatment resulted in an overall decrease in plasma activities of alanine transaminase, aspartate transaminase, and creatine kinase. Our data suggests a long-term benefit of AAV-mediated gene therapy for GSD IV.

.

A double-blind placebo-controlled trial of triheptanoin in adult polyglucosan body disease

Raphael Schiffmann
Baylor Research Institute, Dallas, Texas

Schiffmann R[1], Wallace M[1], Rinaldi D[2],Turner J[1], Hogrel JY[3], Blankenship D[1], Mochel F[2,4,5].
[1]Baylor Research Institute, Dallas, Texas, USA;
[2] INSERM U 1127, CNRS UMR 7225, Sorbonne Universités, UPMC Univ Paris 06 UMR S 1127, Brain and Spine Institute, Paris, France
[3] Institute of Myology, Neuromuscular Physiology and Evaluation Lab, F-75013 Paris, France
[4] Neurometabolic Research Group, University Pierre and Marie Curie, Paris, France
[5] Department of Genetics, AP-HP, Pitié-Salpêtrière University Hospital, Paris, France.

BACKGROUND: Adult polyglucosan body disease (APBD) is a progressive neurogenetic disorder caused by a deficiency of glycogen branching enzyme. Patients develop in the 5th or 6th decade of life neurogenic bladder, progressive spastic paraparesis, sensorimotor peripheral neuropathy and sometimes dementia. There currently is no effective therapy. We hypothesized that decreased glycogen degradation leads to cellular energy deficit and that anaplerotic therapy via triheptanoin may augment energy production thus preventing or reversing cellular damage.
METHODS: This was a two-site randomized crossover study over one year of 23 patients (age 35-73 years; 63% male). Vegetable oil served as placebo. Outcome measures included a 6-minute walk test (primary), balance testing, and SF-36 health survey questionnaire for all subjects; the Spastic Paraplegia Rating Scale, finger tap and dynamometer testing in French site subjects. Safety monitoring included adverse events, blood chemistries, urine organic acids and acyl carnitine profile in blood. The linear mixed model was used to analyze this study.
RESULTS: Nineteen patients were eligible for data analysis. At baseline, patients could walk a mean 389±164 meters (range 95-672). The overall mean difference between subjects on triheptanoin versus placebo was 6 meters; 95% CI: (-11, 22); p=0.49. Motion capture gait analysis, gait quality, stair climbing did not show a consistent direction of change. SF36 questionnaire showed significant improvement in mental and physical quality of life scores on triheptanoin. There was no significant difference in the number of patients experiencing adverse events in the two treatment groups. Patients were followed on the open label study for up to 5 years. Most parameters showed progressive decline over time.
DISCUSSION: Triheptanoin had a good safety profile. However, from this study we cannot conclude that it is effective in the treatment of APBD over a 6-month period. We quantified gait, balance, and quality of life data over time to be used in future clinical trials.

Clinical Trials of Guaiacol in Adult Polyglucosan Body Disease Patients

Alex Lossos and Or Kakhlon
Hadassah-Hebrew University Medical Center, Jerusalem, Israel

The FDA-approved food additive Guaiacol (CAS 90-05-1) was discovered as a drug candidate for Adult Polyglucosan Body Disease (APBD) by high throughput screening (HTS), and was confirmed in a mouse model of the disease (Gbe1$^{ys/ys}$) homozygous for the human mutation in Gbe1, p.Y329S. In these mouse studies, Guaiacol restored the significantly shorter lifespan of the Gbe1$^{ys/ys}$ APBD modeling mice to normal levels, without any adverse effects. On the contrary, Guaiacol even corrected penile prolapse in aged Gbe1$^{ys/ys}$ male mice, a urologic problem which perhaps could be related to other urologic problems found in patients. These results motivate us to initiate a phase 1-2 clinical trial to explore the therapeutic potential of Guaiacol in APBD patients.

Patients. Because of a limited patient population, we plan this trial as an open label prospective study on **17** Israeli APBD patients.

Inclusion/exclusion criteria. Diagnosis of APBD must be biochemically and genetically confirmed. Patients will be cognitively fit for informed consent. The inclusion criteria are: Unremarkable CBC, complete biochemistry, urinalysis, ECG, abdominal US.

Prospective testing. All patients will have a pre- and every 3-months on-trial clinical physical and neurological examination, CBC, complete biochemistry, urinalysis, ECG. All patients will have pre- and every 6-months 6-min walk test, EDSS and Spastic Paraplegia Rating Scale forms, orthostatic hypotension and R_R interval testing. All patients will have a pre- and yearly on-trial peripheral nerve conduction study and head MRI. All tests will be conducted until 3, objectively determined, consecutive deteriorations are recorded. The entire trial is expected to last a few years.

Safety. While No-observed-adverse-effect level (NOAEL) has not been rigorously determined, the lowest level in which adverse effects start to show in mice (N=7) is 175 mg/kg administered once sub-cutonuosly(1). In our hands, this level did not show any adverse effects in mice, administered both orally and subcutaneuously (N=6).

Efficacy. We don't have data, both in mice and in humans, on serum levels correlating with an effective Guaiacol dose. The only efficacy data available to us are that in humans Guaiacol was effective already at 0.03 mg/kg(2) and in mice at 22.5 mg/kg (N=6, equivalent to 1.8 mg/kg in human, our unpublished data), both administered orally.

Pharmacokinetics The only study in which Guaiacol levels in the blood were determined was a pharmacokinetic study, not addressing efficacy(3). In that study 32 mg (0.4 mg/kg, assuming average weight is 80 kg) were administered orally to N=8 men. After 30 min, Guaiacol level in the serum was < 0.04 mg/l. The following pharmacokinetic data were obtained for Guaiacol's metabolites Glucuronide and Guaiacol-Sulfate, but not for non-conjugated Guaiacol:

	C_{max} (mg/l)	$t_{1/2}$ (h)	AUC (h*mg/l)	C_p/F (l/h)	V_{area}/F (l)
Glucuronide	0.91 ± 0.38	2.1 ± 0.6	0.97 ± 0.22	33 ± 8	100 ± 24
Sulfate	0.22 ± 0.09	2.5 ± 0.6	0.30 ± 0.13	108 ± 46	396 ± 170

References

1. Martinez Enriquez ME, Del Villar A, Chauvet D, Lopez Valle A, Susano Pompeyo M, Campos Sepulveda AE.. Acute toxicity of guaiacol administered subcutaneously in the mouse. Proc West Pharmacol Soc. 2009;52:92-3.
2. Heatley RV, Evans BK, Rhodes J, Atkinson M. Guaiacol -- a new compound in the treatment of gastro-oesophageal reflux? Gut. 1982;23(12):1044-7.
3. Ogata N, Matsushima N, Shibata T. Pharmacokinetics of wood creosote: glucuronic acid and sulfate conjugation of phenolic compounds. Pharmacology. 1995;51(3):195-204.

Combining High Throughput Screening with Image-Based Phenotyping to advance APBD drug discovery

Or Kakhlon
Hadassah-Hebrew University Medical Center, Jerusalem, Israel

Leonardo Solmesky[1], Netaly Khazanov[2], Hanoch Senderowitz[2], Eddy Pichinuk[3], Alexander Lossos[4], Miguel Weil[3], Or Kakhlon[4]
[1]Kadimastem, Nes Ziona, Israel
[2]Department of Chemistry, Faculty of Exact Sciences, Bar Ilan University, Ramat Gan, Israel
[3]Laboratory for Neurodegenerative Diseases and Personalized Medicine, Department of Cell Research and Immunology, The George S. Wise Faculty for Life Sciences and the Blavatnik Center for Drug Discovery, Tel Aviv University, Tel Aviv, Israel
[4]Department of Neurology, Hadassah-Hebrew University Medical Center, Ein Kerem, Jerusalem, Israel

Glycogen storage diseases (GSD) are caused by intracellular accumulation of insoluble inclusion bodies, chiefly composed of normal, or malconstructed glycogen. In the prototypical GSD Adult Polyglucosan Body Disease (APBD) the pathogenic glycogen accumulates in the cytosol of nerve cells as an aberrant, poorly branched form called polyglucosan. Polyglucosans are formed as a result of deficient activity of the glycogen branching enzyme (GBE).

We have developed a patient cell-based assay in order to identify small molecule inhibitors of polyglucosan body (PB) accumulation. Using this assay in a high throughput format, we have screened the DIVERSet-CL 10,080 compound library and discovered and validated 11 dose-dependent potential hits. Similarities between these hits and drugs interacting with docking targets of the hits have been found and most hits were similar to drugs interacting with the glycogen synthase (GS) activator protein phosphatase 1 (PP1).

We have also generated a novel, personalized analytical tool for assessing drug efficacy. This tool is image-based phenotyping (IBP): a global phenotypic fingerprint of patients' primary skin fibroblasts, which encompasses multiple cellular and subcellular features. IBP enables us to identify image based features within patient cells which may change in response to tested small molecules. This analysis has generated phenotypic signatures of APBD patients and control subjects. Using computational classification methods, we are able to define control and pathological global phenotypes in skin fibroblasts respectively derived from unaffected subjects and APBD patients. This analysis will be used to compare the effect of our 11 hits on cell status.

In summary, combining high throughput screening (HTS) with clustering analysis and especially with IBP constitutes an important upgrade to conventional HTS. As demonstrated in our work on APBD, obtaining as much information as possible on the cellular effects of tested drug candidates is crucial for their future development and will facilitate dealing with their prospective adverse effects.

RarePurposing – The Cures Within Reach Approach

Bruce Bloom
Cures within Reach, Skokie, Illinois

There are 7000+ unsolved diseases, and the vast majority are rare diseases. Cures Within Reach brings together Patient Advocacy funders and rare disease researchers to successfully RarePurpose generic drugs and nutriceuticals that create effective “new” treatments in rare diseases. This presentation will provide background on the CWR approach, including examples of how RarePurposing progressed from genetic discovery to initial ideation, from mouse modeling to clinical trials, from results to publishing, and from dissemination to clinical use in rare pediatric autoimmune and neurological diseases.

Guaiacol – a novel drug candidate for treating Adult Polyglucosan Body Disease

Or Kakhlon
Hadassah-Hebrew University Medical Center, Jerusalem, Israel

Hasan Orhan Akman[1], Or Kakhlon[2], Igor Ferreira[3], Wyatt Yue[3], Leonardo Solemsky[4], Miguel Weil[5], Netaly Khazanov[6], Hanoch Senderowitz[6], Yoseph Caraco[7], Alexander Lossos[2]
[1]Houston Merritt Neuromuscular Disease Research Center Columbia University Medical Center New York, NY 10032, USA
[2]Department of Neurology, Hadassah-Hebrew University Medical Center, Ein Kerem, Jerusalem, Israel
[3]Structural Genomics Consortium, University of Oxford, Oxford, UK
[4]Kadimastem, Nes Ziona, Israel
[5]Department of Cell Research and Immunology and the Blavatnik Center for Drug Discovery, George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv, Israel
[6]Department of Chemistry, Bar Ilan University, Ramat Gan, Israel
[7]Hadassah Clinical Research Center, Hadassah-Hebrew University Medical Center, Ein Kerem, Jerusalem, Israel

Background: APBD is caused by intracellular accumulation of insoluble inclusion bodies called polyglucosans, formed as a result of glycogen branching enzyme (GBE) deficiency. To identify drug candidates for APBD, we used high throughput screening (HTS) of FDA approved drugs, a promising strategy for drug repurposing. One of the discovered candidates was Guaiacol, a flavorant originally prescribed for relieving cough and reflux.
Methods: We performed HTS in fibroblasts from *Gbe1*$^{ys/ys}$ (APBD modeling) mice and from patients using the LOC 1100 FDA approved compound library. The HTS readout was diastase resistant, periodic-acid Schiff reagent positive, polyglucosans. The effect of Guaiacol on glycogen synthase (GYS1) activity was tested by an established biochemical assay, or by on gel assay of a glycogenin-GYS1 chimera, where the effect of Guaiacol on glycogenin activity was excluded by mass-spectrometry.
Results: Consistent with the Guaiacol-mediated increase in inhibitory GYS1 phosphorylation in mice, the drug inhibited both basal and glucose-6-phosphate stimulated GYS1 activity in both purified enzyme and APBD patients' cell lysates. Furthermore, Guaiacol also increased phosphorylation of the master activator of catabolism, AMP-dependent kinase. Guaiacol also restored the significantly shorter lifespan of *Gbe1*$^{ys/ys}$ mice to normal levels, and caused no adverse effects except slightly reduced glucose tolerance. In addition, Guaiacol reduced liver polyglucosan and glycogen levels, and corrected penile prolapse in the aged *Gbe1*$^{ys/ys}$ male mice.
Conclusions: Restoration of the reduced life span of APBD modeling mice by Guaiacol as well as its additional effects are encouraging. Together with the lack of observable significant side effects, our results form the basis for future clinical trial. Interestingly, despite its curative effect, the only organ in which Guaiacol reduced polyglucosans was the liver, which apparently is not pertinent to neurological damage in APBD.

Using recombinant glycogen synthase and structural biology techniques to understand therapeutic opportunities for APBD

Or Kakhlon
Hadassah-Hebrew University Medical Center, Jerusalem, Israel
Wyatt Yue
Structural Genomics Consortium, University of Oxford, UK

Glycogen branching enzyme 1 (GBE1) plays an essential role in glycogen biosynthesis, mutations of which lead to the heterogeneous early-onset glycogen storage disorder type IV (GSDIV) or the late-onset adult polyglucosan body disease (APBD). Our group has an ongoing interest in studying the catalytic and disease mechanism underlying the human glycogen biosynthetic machinery, which consists of the priming enzyme glycogenin (GYG1/2), the elongation enzyme glycogen synthase (GYS1/2), and the branching enzyme (GBE1).

My current study is aimed at validating two potential therapeutic avenues for APBD: pharmacological chaperoning for GBE1, and small molecule inhibition of GYS1. My presentation hence will consist of two parts:

- Characterization of the recombinant GBE1 mutant protein p.Y329S linked with APBD, and structure-guided design of a stabilizing peptide
- Effect of the small molecule guaiacol towards activity of recombinant glycogenin-glycogen synthase complex

Pharmacological treatment of APBD: Potential for treatment by Ibudilast® and/or guaifenesin

H. Orhan Akman
Columbia University Medical Center, New York, New York

Glycogen storage diseases are caused by the defects in either synthesis or degradation of glycogen molecule and affect 1 to 2 out of 100,000 people worldwide. There are more than 14 enzyme defects that have been described causing this disease and list is increasing each year. Our body synthesizes glycogen to store glucose after meals. This is very important to maintain the glucose homeostasis in between meals because glucose is the fuel for tissues to continue their metabolic activities. There are two pathological components of glycogenosis; one is not being able to use the stored glycogen and the second is excess accumulation of glycogen that impairs the function of the cell, particularly in the muscles and neurons where space is limited and their high metabolic rate demands constant fuel supply. Life threatening and debilitating effects of glycogenosis can be ameliorated if we stop the synthesis of glycogen. In order to study the pharmacological compounds that prevent glycogen accumulation, we generated a mouse embryonic fibroblast cell line that reliably shows the glycogen accumulation. Glycogen accumulation can be monitored in this cell line by fluorescent microscopy, after staining glycogen with periodic Schiff reaction. We tested 1700 compounds on this cell line to determine if one or more of those clinically available compounds decrease glycogen accumulation. Our results have shown that guaifenesin, and Ibudilast® decreased glycogen accumulation by 50%. Although it looks like a modest decrease clinically, this value is very significant. In addition, these medicines are already available. Therefore they must be tried for the treatment of glycogen storage diseases.

Testing pharmacophore-based small molecules for stabilizing GBE-Y329S

Or Kakhlon
Hadassah-Hebrew University Medical Center, Jerusalem, Israel

Or Kakhlon[1], Amit Michaeli[2], Wyatt Yue[3], Amiram Goldblum[4]
[1]Department of Neurology, Hadassah-Hebrew University Medical Center, Ein Kerem, Jerusalem, Israel
[2]Pepticom Ltd., Jerusalem, Israel
[3]Structural Genomics Consortium, University of Oxford, Oxford, UK
[4]Institute for Drug Research, School of pharmacy, the Hebrew University of Jerusalem, Jerusalem, Israel

We have designed a peptide which stabilizes the GBE1 Y329S mutant and partially rescues its function in APBD patient-derived cells (Froese *et al* (2015) Hum Mol Genet 24:5667). While the peptide itself is a drug candidate, developing it would require funds sufficient for FDA approval. Therefore, we aimed at testing whether any FDA-approved drugs can serve as structural analogs of the peptide as a GBE1-Y329S stabilizer. Success in this project will lead to a significant reduction in the time and costs of reaching the patient.

We used the 3D binding pattern of the LTKE peptide model to define a pharmacophore. The molecule database was then screened using the pharmacophore. Passing molecules were docked to GBE-Y329S and analyzed by computational chemistry. 28 successful docked molecules are being screened for their effect on GBE activity.

Essential Groundwork – Patient Registry, Genetic Testing, Natural History Studies, Biobanks

Lori Ann Correia
Founder and President of Allele Consulting, LLC, Boston, Massachusetts

The APBD Research Foundation is encouraging and facilitating many initiatives on many fronts in order to be prepared for clinical trials that are on the horizon.

One effort underway since 2014 is a patient registry called **CAP**, **C**olumbia University **A**PBD **P**atient Registry.

A natural history study, the objective of which is to gather prospective information on the manifestations of a condition over time, is just being launched by the National Organization for Rare Diseases (NORD). The study, called **FAN**, which stands for **F**DA and NORD-**A**PBD **N**atural History Study, will be discussed. Duke University is also planning to conduct a different natural history study. It is hoped that funding will come primarily from the FDA. Duke contemplates applying for a grant from the FDA in 2018.

Genetic testing for GBE1, the gene that contains mutations which lead to ABPD, was begun at Mount Sinai Medical in 2015. Screening through their prestigious Jewish genetic screening panel has already resulted in more refined information about the carrier rate for APBD mutations among people of Ashkenazi Jewish descent. In the near future, carrier screening for APBD will be offered by Counsyl, Inc., a genetic testing laboratory.

The Association for Glycogen Storage Disease (United States), or AGSDUS plans to write the Glycogen Storage Disease Type IV (GSD IV) health care guidelines in 2017.

Last but not least, a biobank for APBD patient skin fibroblasts has begun to collect samples from Israeli and American patients.

Presentations of all these initiatives will be provided.

CAP, Columbia University APBD Patient registry – enrollment update and revised questionnaire

Raphael Schiffmann
Baylor Research Institute, Dallas, Texas

Salvatore DiMauro
Columbia University, New York, NY
Principal Investigator, CAP registry

The APBD Research Foundation and the Mailman School of Public Health at Columbia University, along with a team of international researchers and clinicians, established the first APBD registry as of May 1, 2014. The team at Columbia, experienced in maintaining patient registries, follows Institutional Review Board (IRB) approved protocols to ensure security and privacy of registrant information. The registry collects demographic data, family history, clinical observations, and the results of physical and neurological examinations for each patient. The APBD Registry is open to both APBD patients and their family members.

Patients and their family members are encouraged to participate in the registry to aid researchers studying APBD and the effects of various treatment programs. There are a number of promising research initiatives under development, and the success of potential treatments will need to be tested over a period of time. The registry will enable the researchers to more effectively assess the results of proposed treatment programs and will facilitate human trials in the foreseeable future.

Registrants have the option to enroll anonymously with their physician serving as the point of contact.

How information is protected:

- Patient data is encrypted and stored in secured servers with 24/7 security by Columbia University
- All data and web communications are encrypted
- Access to the data is by authorized Columbia University personnel, using secured passwords
- Only de-identified data is provided to authorized parties who have been approved by the APBD Research Foundation in accordance with federal regulations
- Patients can request to enroll anonymously and/or limit the data they provide
- All direct communication with enrollees with done only through the Columbia team

As of December 2016 there are 90 people enrolled in CAP.

Experience with carrier screening for *GBE1* mutations associated with Adult Polyglucosan Body Disease/Glycogen Storage Disease Type IV in the Ashkenazi Jewish and general populations

Ruth Kornreich
Department of Genetic and Genomic Sciences, Icahn School of Medicine at Mount Sinai, New York, New York

R. Kornreich; A.H. Birch; G. Cai; G. Mendiratta-Vij; L. Shi; X. Cai; O. Birsoy; B. Webb; L. Elkhoury; M. Dillon; S. Van Den Berg; E. Bazinet; M. Delio; G. Diaz; F. Suer; L. Edelmann

The Mount Sinai Genetic Testing Laboratory has been performing carrier screening by next generation sequencing for adult polyglucosan body disease (APBD) for the past year. We tested 2776 individuals who were self-reported to be 100% Ashkenazi Jewish. Fifty eight carriers were detected which included 38 individuals who carried the Ashkenazi Jewish (AJ) *GBE1* founder allele, p.Y329S and 16 carriers of the IVS15+5289_5297delGTGTGGTGG-insTGTTTTTTACATGACAGGT AJ founder allele. Interestingly, we also found two individuals with two other likely pathogenic variants and two individuals with the p.Y329C pathogenic variant which has been previously reported in Ashkenazi individuals. The carrier frequency, therefore, in this group was found to be approximately 1 in 48. Importantly, we found one individual who was homozygous for the p.Y329S variant. For the screenees as a whole, we found the carrier frequency to be approximately 1 in 108 with 19 different pathogenic or likely pathogenic variants detected amongst the 21319 individuals tested. More detailed information about our experience will be presented.

Advances in carrier screening: Making the case for APBD

Alana Cecchi
Counsyl, Inc., San Francisco, California

Counsyl is a DNA testing and genetic counseling service that runs a fully automated lab, providing the capability to screen for many rare genetic diseases at scale. Counsyl's *Family Prep Screen*, an expanded carrier screen panel, was first made available in 2009. While professional societies currently recommend testing for a limited number of single gene conditions based on self-reported ethnicity and family history, more than 80% of children born with rare genetic diseases have no such family history. Using technological advances in molecular genetics, Counsyl has made it both possible and affordable to screen for a large number of conditions independent of ethnicity or family history. Carrier screening recommendations by the ACOG and ACMG even differ in terms of recommended diseases to screen for, highlighting the ongoing challenge of determining what conditions to include in prenatal screening guidelines and expanded carrier screening panels.

Counsyl recently published data in JAMA pointing to limitations in the current screening guidelines. The study was a retrospective modeling analysis of expanded carrier screening results from 346,790 individuals spanning 15 self-reported ethnic categories. Results showed that when analyzed and compared to current carrier screening recommendations by professional organizations (ACOG and ACMG), guideline-based screening misses a significant percentage of pregnancies affected by serious conditions. We have proposed a design model for expanded carrier screening that optimizes the clinical detection of at-risk couples by accounting for disease severity, incidence, and gene-specific sensitivity. By utilizing this approach, we recently reviewed more than 650 genes of consideration for addition to our *Family Prep Screen* and elected to add 100 new conditions, including the *GBE1*-related disorders, glycogen storage disease IV (GSD IV) and adult-onset polyglucosan body disease (APBD).

The Importance of Natural History Studies for Rare Diseases

Suzanne Rossov
National Organization for Rare Disorders (NORD), Danbury, Connecticut

According to the National Institutes of Health (NIH), there are approximately 7000 rare diseases, most of which are poorly understood and only a few hundred of which have approved therapies. Unlike more prevalent diseases, rare disease drug development poses more challenges due to the lack of information about rare diseases and candidates for clinical trials. To address the orphan drug development process, natural history studies have been introduced in research to obtain a better understanding and follow the natural progression of rare diseases. Information acquired from these studies can be used to facilitate product development and the approval process. In support of this cause, the National Organization for Rare Disorders (NORD) registry platform is an application designed to empower rare disease patient organizations to collect and manage research-grade, natural history patient reported outcomes data.

FDA Orphan Products Natural History Grants Program: An opportunity for Adult Polyglucosan Body Disease?

Harrison Jones
Duke University School of Medicine, Durham, North Carolina

An upcoming funding opportunity from the FDA Orphan Products Natural History Grants Program: The Orphan Products Research Project Grant (R01; RFA-FD-16-043) will support research that advances rare disease product development by the characterization of the natural history of rare diseases/conditions; identification of genotypic and phenotypic subpopulations; and development/validation of clinical outcomes, biomarkers, and/or companion diagnostics. Dr. Jones and his collaborators at Duke University seek national/international collaborators in an effort to submit a robust APBD-focused application at the next funding deadline (August 2018).

A Biobank of APBD patient skin fibroblasts

Or Kakhlon
Hadassah-Hebrew University Medical Center, Jerusalem, Israel

Or Kakhlon[1], Eduard Pichinuk[2], Miguel Weil[2]
[1]Department of Neurology, Hadassah-Hebrew University Medical Center, Ein Kerem, Jerusalem, Israel
[2]Laboratory for Neurodegenerative Diseases and Personalized Medicine, Department of Cell Research and Immunology, The George S. Wise Faculty for Life Sciences and the Blavatnik Center for Drug Discovery, Tel Aviv University, Tel Aviv, Israel

We have generated a novel, personalized analytical tool for assessing drug efficacy. This tool is image-based phenotyping (IBP): a global phenotypic fingerprint of patients' primary skin fibroblasts, which encompasses multiple cellular and subcellular features. IBP enables us to identify image based features unique to APBD patient cells, which may change in response to tested small molecules, or any other drug for that matter. This analysis has generated phenotypic signatures of APBD patients and control subjects. The accuracy of these signatures improves as skin fibroblasts from more patients are analyzed. Using computational classification methods, we are able to define control and pathological global phenotypes in skin fibroblasts respectively derived from unaffected subjects and APBD patients. These IBP signatures will enable us to **a)** test any therapeutic candidate for its ability to turn a global APBD phenotype into a global unaffected phenotype; and **b)** predict adverse or side effects of any therapeutic candidate based on its effect on multiple cellular features.