Research Workshop
Understanding the role of Glucocerebrosidase (GBA) in Parkinson’s disease
October 23, 2012, New York City, NY

AGENDA
Introduction & Chair’s Opening Presentation: 8:15 – 9:00
- Introduction of MJFF – Kuldip Dave
- Introduction of Meeting Goals and State of the Field - Workshop Chair: Michael Schlossmacher

Part 1: Presentation of Background and Discussion: (20 minute presentations with 10 min Q&A)
- GBA1 Gene – An overview of Gaucher’s in Mice and Man 9:00 – 9:30
  Presenter: Greg Grabowski
- Glucocerebrosidase and Parkinsonism: Do clinical phenotype and neuropathology provide clues to pathogenesis? 9:30 – 10:00
  Presenter: Ellen Sidransky
- GBA in relation to Parkinsonism and other movement disorders. The physician's perspective 10.15 – 10.45
  Presenter: Nir Giladi
- Comparison of LRRK2, GBA and idiopathic PD cohorts in the 23andMe database 10:45 – 11:15
  Presenter: Emily Drabant
- Analysis of alpha-synuclein turnover and enteric nervous system dysfunction in heterozygous GBA L44P X SNCA A53T overexpressing mice 11:15 – 11:45
  Presenter: Ianai Fishbein
- Deficiency of the β-glucocerebrosidase transporter LIMP-2 cause synucleinopathy in mice 11:45 – 12:15
  Presenter: Michael Schwake

Part 2: Key Discussions 12:45 – 2:15
- Biology (45 minutes)
  - Mutant GBA1 Effects I: Gain versus loss of function, or both?
    - Discussion Leaders: Dimitri Krainc and Julianna Tomlinson
  - Mutant GBA1 Effects II: Possible targets – the dysregulation of proteins or lipids
    - Discussion Leaders: Gregory Petsko and Steffanny Bennett
- Therapeutic Development: (45 minutes)
  - Strategies to target GBA1
    - Discussion Leaders: Pablo Sardi and Brandon Wustman
  - How do we select the right cohort for a trial?
    - Discussion Leaders: Bernard Ravina and Peter Reinhart

Part 3: Final Discussion (Led by the chair Dr. Michael Schlossmacher) 2:30 – 4:00 pm
- Genetic risk factors, causative alleles and gene-environment interaction – how best to model these complex phenomena?
- Identify immediate research topics that need to be addressed in order to improve our understanding of GBA gene function and its implication in Parkinson disease
- Identify critical needs in order to move the research forward
- Summary of outcomes and questions where MJFF resources (funding and non-funding) can make a meaningful impact

PARTICIPANTS

Michael Schlossmacher, MD, Chair
Associate Professor of Medicine (Neurology)
OHRI - Division of Neurosciences
University of Ottawa
Ottawa, ONT
mschlossmacher@ohri.ca
charlotte.mccusker@uottawa.ca

Karlheinz Baumann, PhD
Biology Project Leader, Preclinical CNS Research
F. Hoffmann-La Roche AG
Basel – Switzerland
karlheinz.baumann@roche.com

Steffany A. L. Bennett, PhD
Professor
Department of Biochemistry, Microbiology, and Immunology (BMI)
University of Ottawa
Ottawa – ONT
sbennet@uottawa.ca

Zdenek Berger, PhD
Senior Scientist, Neurodegeneration
Pfizer Neuroscience Research Unit
Groton, CT
zdenek.berger@pfizer.com

Emily Drabant, PhD
Research and Development Manager
23andMe, Inc.
Mountain View, CA
emilydrabant@23andme.com

Nir Giladi, MD
Professor and Chairman
Department of Neurology
Director, Movement Disorders Unit
NPF Center of Excellence
Tel Aviv Medical Center
Sackler School of Medicine
Tel Aviv University
Tel Aviv – Israel
nirg@tlvmc.gov.il
livnatbg@tasmc.health.gov.il

**Gregory A. Grabowski, MD**
Director, Division of Human Genetics
Director, Medical Genetics Training Program
Professor, UC Department of Pediatrics
Cincinnati Children's Hospital Medical Center
Cincinnati, OH
greg.grabowski@cchmc.org
joyce.life-ishmael@cchmc.org

**Jennifer Johnston, PhD**
Vice President, Exploratory Research
Elan Pharmaceuticals
San Francisco, CA
jennifer.johnston@elan.com
maria.garcia@elan.com

**Dimitri Krainc MD PhD**
Associate Professor
Department of Neurology
Massachusetts General Hospital
Charlestown, MA
dkrainc@partners.org
samelanson@partners.org

**Robert Nussbaum, MD**
Chief, Division of Medical Genetics
University of California
San Francisco, CA
russbaumr@humgen.ucsf.edu
woldlin@humgen.ucsf.edu

**Gregory A. Petsko, PhD**
Gyula and Katica Tauber Professor of Biochemistry and Chemistry
Chair, Dept. of Biochemistry
Brandeis University
Waltham, MA
petsko@brandeis.edu
killeen@brandeis.edu

**Bernard M. Ravina, MD, MS**
Medical Director, Translational Neurology
Biogen Idec
Cambridge, MA
bernard.ravina@biogenidec.com
ayana.king@biogenidec.com
Pablo Sardi, PhD
Staff Scientist
Genzyme, a Sanofi Company
Cambridge, MA
pablo.sardi@genzyme.com

Michael Schwake, PhD
Assistant Professor / Group Leader
University of Kiel Institute of Biochemistry
Kiel – Germany
mschwake@biochem.uni-kiel.de

Ellen Sidranksy, MD
Chief, Section on Molecular Neurogenetics
National Human Genome Research Institute
Bethesda, MD
sidranse@mail.nih.gov
marichall@mail.nih.gov

Juliana Tomlinson, PhD
Senior Research Associate
OHRI - Division of Neurosciences
University of Ottawa
Ottawa, ONT
jtomlins@uottawa.ca

Brandon A. Wustman, PhD
Director
Amicus Therapeutics
La Jolla, CA
bwustman@amicustherapeutics.com

Ianai Fishbein, PhD
Postdoctoral Scholar
Department of Medicine
UCSF School of Medicine
San Francisco, CA
ianai.fishbein@ucsf.edu

Grisel Lopez, MD
Senior Staff Clinician
Section of Molecular Neurogenetics
Medical Genetics Branch
National Human Genome Research Institute, NIH
glopez@mail.nih.gov
Outcomes Summary and Recommendations for MJFF Staff

The workshop was entitled: “Understanding the role of glucocerebrosidase (GBA1) in Parkinson’s disease”

Take away points from the meeting: Drafted by Dr. Michael Schlossmacher (Chair)

1) There was a general sense of excitement over the unique research opportunities and potential therapeutic avenues that have opened up in the field of Parkinson disease (PD) and dementia with Lewy bodies (DLB) as a result of the emerging interface between the GBA1 gene and alpha-synuclein (SNCA) processing. Owing to the pioneering work carried out by geneticists, it is currently estimated by Dr. E. Sidransky -based on results from worldwide population studies - that between 4% and 17.9% of all patients with typical, late onset PD carry one (or two) mutation(s) in their GBA1 gene.

2) Among Israeli PD subjects of Ashkenazi Jewish heritage, Dr. N. Giladi reports that approximately 14% carry a mutation in the LRRK2 gene, whereas 18% of patients of the same ethnic background carry a mutation in the GBA1 gene. This rate is slightly lower than the approximately 20% published previously by other Israeli investigators, but still ranks as the highest in the world. Across the Israeli population, it was mentioned that there are approximately 6.5% mutation carriers, thereby equaling 250,000 people with an altered GBA1 genotype. In multicenter studies from various regions of the world, the odds ratio to develop PD is greater than >5.0 for carriers of one (or two) GBA1 mutation(s). Although the actual penetrance rate of carriers of a GBA1 mutation is currently unknown, it is clear from these international studies that mutations in the GBA1 gene now represent the number one known genetic risk factor for Parkinson’s disease. To put this in perspective, it is currently estimated that <2% of all patients with sporadic PD in the Western hemisphere carry one of the six known disease-causing mutations in the LRRK2 gene, an area of major investment in research funding and tool development by the Foundation.
3) Furthermore, a mutation in the GBA1 gene now also represents the best known genetic risk factor to develop the related illness of sporadic dementia with Lewy bodies. According to the recent paper by the Dr. K. Marder group, it is estimated that the odds ratio for DLB is 8.28 in GBA1 mutation carriers with an age at onset that is five years below the mean;

4) However, it is also apparent that only 5% of patients with Gaucher disease have been observed to ultimately develop PD. Some of these subjects may develop a phenotype, which is clinically similar but not necessarily identical to typical PD. Several participants argued that carefully assembled groups of Gaucher disease patients represent an ideal cohort to carry out longitudinal studies to identify those additional risk factors (either genetic or environmental in origin) that contribute to the currently poorly understood conversion from carrying the genetic risk to actually developing the disease. However, other participants argued that the relatively low conversion rate from carrier to diseased subject makes it difficult to finance and thus carry out the necessary longitudinal studies of the natural history of the illness. As pointed out by Dr. B. Ravina, the same argument also refers to clinical trials to be carried out over decades in order to discern whether a primary prevention study could be successfully conducted. This scenario was juxtaposed to the recently announced primary prevention study of early onset Alzheimer disease in carriers of APP and Presenilin mutations, which confer a greater than 95% penetrance rate;

5) Details of a relatively new concept in genetic screening were introduced to the participants, namely through subject initiated participation in research efforts carried out by private enterprises in partnership with academics, such as pursued by “23 and me”, which was introduced by Dr. E. Drabant. Participants raised questions as to what the appropriate control cohorts look like for those subjects that were identified to carry distinct genotypes, and what the reliability/accuracy rates are with respect to patients’ communicating their own diagnoses. However, it was presented that this effort, as initiated outside of conventional academic research labs, has also contributed to the successful identification of two novel loci in PD. Upon their publication in the public domain, these can be further validated now;

6) One emerging and currently poorly understood topic of research is that of the spectrum of accumulating substrates. From currently published papers, such as by the Dr. G. Grabowski group and the Dr. P. Sardi team, it appears that the accumulation of glucosylsphingosine (GS) is more relevant as a potentially toxic metabolic product in vivo than the previously better understood accumulation of glucosylceramide (GC), which represents the traditionally known substrate of the GBA1 gene product, “GCase enzyme”. As pointed out by Dr. S. Bennett there is an enormous variety of GC lipids, and that their complexity is greater than that of PC lipids. In accordance, the mass spectrometry-based accurate identification of GS and GC lipids and their reliable quantification by appropriate means represent unmet needs in the field. It is pointed out by two participants of the meeting with neurolipidomics expertise, Dr. G. Grabowski and Dr. S. Bennett, that this is a potential area in which the Foundation could support the research endeavors of the community.
7) Several participants pointed out the pros and cons of currently available animal models. None are currently deemed to be perfectly faithful models of either Gaucher disease or Parkinson disease in humans. It was pointed out by Dr. G. Grabowski that the metabolism surrounding GBA1 function differs at multiple levels between mice and humans, and that therefore certain approaches to mimic distinct mutations that occur in humans have not resulted in the desired outcomes in rodents. It appears that multiple research teams around the world are currently exploring ways by which mice carrying mutations in the SNCA and GBA1 genes are being crossed to generate bigenic, PD-linked mutant mice. Furthermore, it is suggested that conditional models of mutant gene constructs for GBA1 as well as conditional models for its functional removal after development in the central nervous system are to be pursued. Again, this is an area where the Foundation with its experience in the generation of animal models and their characterization could potentially help the PD research community;

8) With respect to suitable animal models for preclinical development, a lively topic of discussion was on the fact that a variety of already developed compounds and currently available enzyme replacement therapies for Gaucher disease could be explored for their repositioning in alpha-synuclein-linked diseases. The purpose would be reducing its concentration, including of oligomeric and toxic species in the nervous system in currently available, preclinical animal models of SNCA misprocessing, as well as lowering its neuropathological features in the forebrain and brainstem. Such experimental approaches have already begun, foremost in pharmaceutical and biotech industries, and have included the use of small molecule chaperones, as pursued by Dr. B. Wustman’s team at Amicus, for example, as well as enzyme replacement therapy via gene delivery using viral vectors, as presented by Dr. P. Sardi, and potential catheter mediated delivery of the actual enzyme into the central nervous system, which was mentioned as well;

9) Another topic that was the subject of discussions was introduced by the Dr. R. Nussbaum team and focused on the biological half-life of the proteins of interest, namely that of SNCA and GBA1, in both cell culture models as well as in living mammals. This is of particular relevance to those who pursue a gain-of-function theory behind the link, as defended by Dr. J. Tomlinson and Dr. M. Schlossmacher. The authors propose a biochemical relation between mutant GBA1 protein expression and SNCA misprocessing in cells and in vivo, where a mutation in the former negatively affects the turnover/degradation of the latter. From the opposite view, namely that of a loss-of-function theory underlying the observed link between the two gene products, several researchers felt that it was important to discern how much substrate accumulation does / has to occur downstream of heterozygous mutations in the GBA1 gene to allow for a significant rise in SNCA species. It was pointed out by Dr. D. Krainc that many of these studies ask for proper experimentation using primary neuronal cultures, iPS cells, and lysosomal isolates from critical brain areas from suitable models (rather than whole brain homogenates). Furthermore, no consensus has been reached –as per Dr. Krainc- as to how to isolate neuronal lysosomes as well as brain lysosomes in a standardized manner;

10) In discussions of the detection of various mutations in the GBA1 gene, the question arose as to whether there are some less aggressive versus more aggressive genotypes, which could help predict the likelihood of conversion, severity of symptoms, disease progression as well as the
age of onset of PD. There were thoughts on both sides of the spectrum, and Dr. Grabowski reminded other participants that there are currently over 350 mutations known. It was pointed out by geneticists in the room that several patients from different geographic corners of the world have been described who carry one wild type allele and one null allele and have Parkinson disease, which provides an argument against the “gain-of-a-mutant GBA1 function” theory because no mutant protein is expressed (without a second somatic hit affecting the single wt allele);

11) From a cell biological perspective, it was also discussed that the trafficking of the GBA1 protein from its synthesis to its further maturation in various organelles to its final translocation to the lumen of lysosomes (as well as its apparent secretion into the extracellular milieu) deserved further investigation. This topic includes the function of other proteins, such as LIMP2, which according to Dr. M. Schwake et al. plays a critical role in the processing of GBA1 and its successful delivery to lysosomes. Intriguingly, in their studies these authors demonstrated that by increasing LIMP2 expression the level of SNCA could be lowered in lysosomes. These data are similar to those pursued by Dr. P. Sardi and colleagues, who showed that increasing amounts of wild type GBA1 itself promoted a reduction in SNCA pathology in their mouse model (Pubmed ID: 21730160).

12) Some participants felt that the currently available repertoire of antibodies and tools to measure the enzymatic activity of the GBA1 protein/enzyme as well as its detection in rodent brain is currently insufficient, which could be an area where the Michael J Fox Foundation may want to provide support to the research community. The latter is of relevance to the further exploration of a bidirectional interaction between GBA1 protein (dys)function and accumulation of misprocessed SNCA species, as proposed by the Dr. D. Krainc team. Along the same lines, a topic that was introduced recently by Sidransky and co-workers deals with the potential complex formation between activators of GBA1 activity within lysosomes at lower pH (such as Saposin C, PC lipids), GBA1 itself, and unexpectedly, SNCA protein, a finding which begs to be explored further;

13) To this day it remains a mystery and of great scientific interest as to how a hydrolase (GBA1/GCase), which cleaves a sugar off of complex lipid structures (GC; GS), is able to activate - still unknown- proteolytic enzymes that efficiently process SNCA and therefore reduce the risk of its aggregation, accumulation and presumed toxicity, most likely within lysosomes. Therefore, protease activity (‘synucleinase’) downstream of wt GBA1 function is of growing interest to some researchers;

14) Dr. B. Ravina pointed out in his comments that it is of great importance to strategically design longitudinal cohorts that include mutant GBA1 carriers in a way so that they can be jointly analyzed with other cohorts from other regions of the world in future meta-analysis to increase the power of meaningful and significant observations. Several participants also discussed the important topic of pre-motor symptoms and signs in PD, which is already being incorporated into some natural history studies of GBA1-linked PD and DLB;
15) An interesting topic of discussion beyond simple gain of function and loss of function mechanisms behind the observed link was the mechanistic issues raised by Dr. G. Petsko. He digested the existing body of literature and identified four possible events, which could be involved in the pathogenesis. First, he reviewed the literature on ER stress; second, he raised the issue of abnormal lysosomal autophagy, where too much or too little activity can promote disease; third, he discussed interference with the normal secretory pathway, which also could further add to ER stress; and fourth, he mentioned the still poorly understood issue of inflammation that in and by itself could contribute to the disease process;

16) Toward the end of the workshop, the topic of biomarker definition was brought up as is often the case during discussions on and planning of concrete intervention trials for PD (and DLB). Along with it, the question of successful target engagement in biological fluids was raised. In the case of GBA1 protein, its enzymatic GCase activity would be the target of interest, and by inference, the monitoring of its activity or quantifying the concentration of its substrates in readily accessible components of the CNS, such as cerebral spinal fluid, would be desirable. At the current time, it appears that although GBA1 protein levels can be determined in CSF, the quantification of lipid substrates in the same biological fluid compartment has not yet been accomplished;