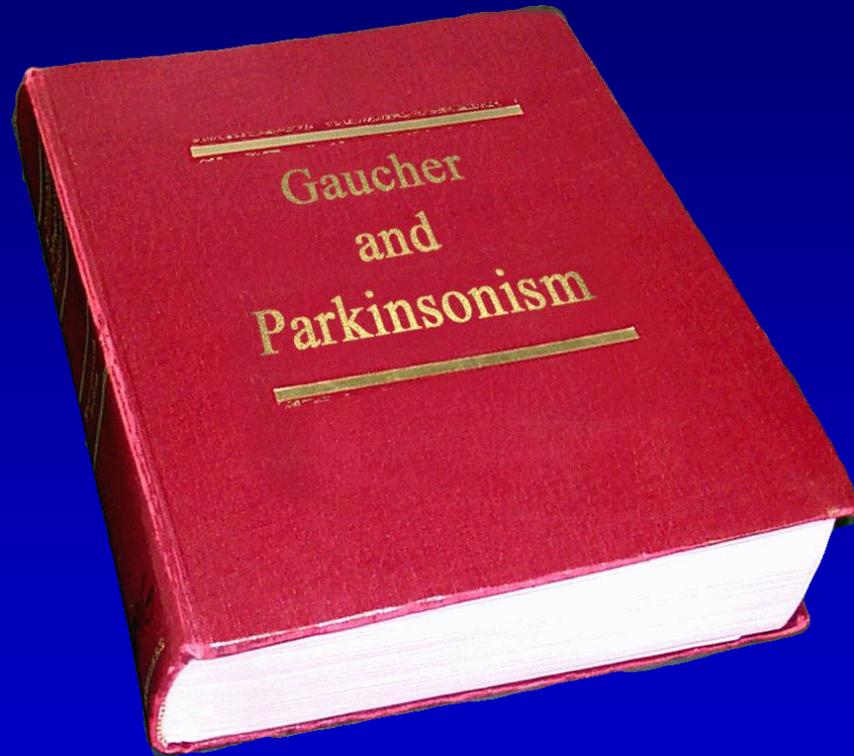


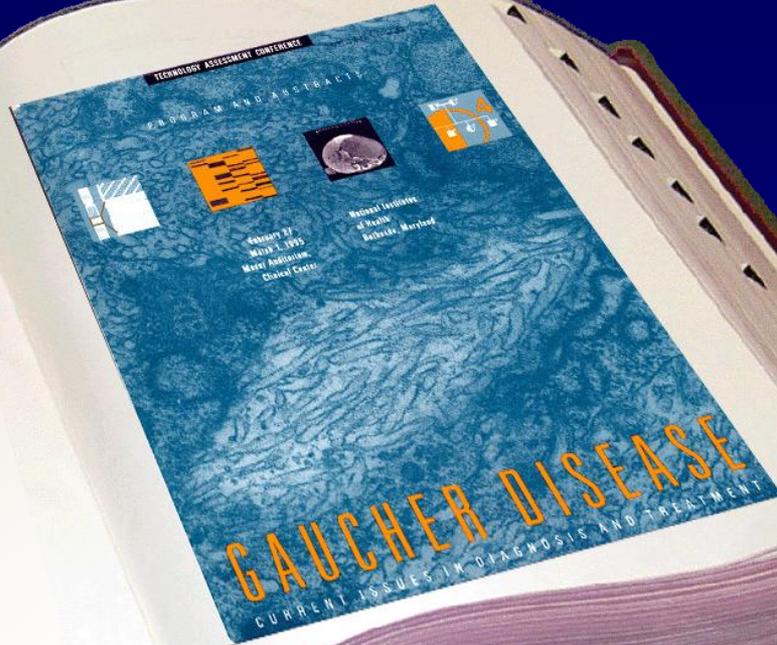
The Genetics of Parkinson Disease: Insights from a Mendelian Disorder



Ellen Sidransky M.D,
Medical Genetics Branch
National Human Genome
Research Institute, NIH



CAUTION!



This story may challenge how we think about the genetics of Mendelian disorders and complex diseases!

Blurred boundaries between simple and complex genetic disorders

**“Simple”
single gene
disorders**

Gaucher, CF, PKU,

**Mendelian
disorders**

**“Complex”
multi-gene
disorders**

PD, Diabetes, Asthma,
ADHD

**Mendelian
disorders**



Single primary gene with large
effect + multiple modifiers

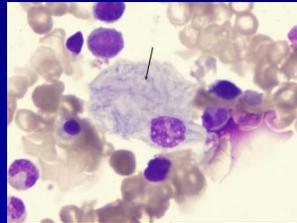
Multiple primary genes with small
effects + some modifiers

Introduction

Gaucher Disease (GD)

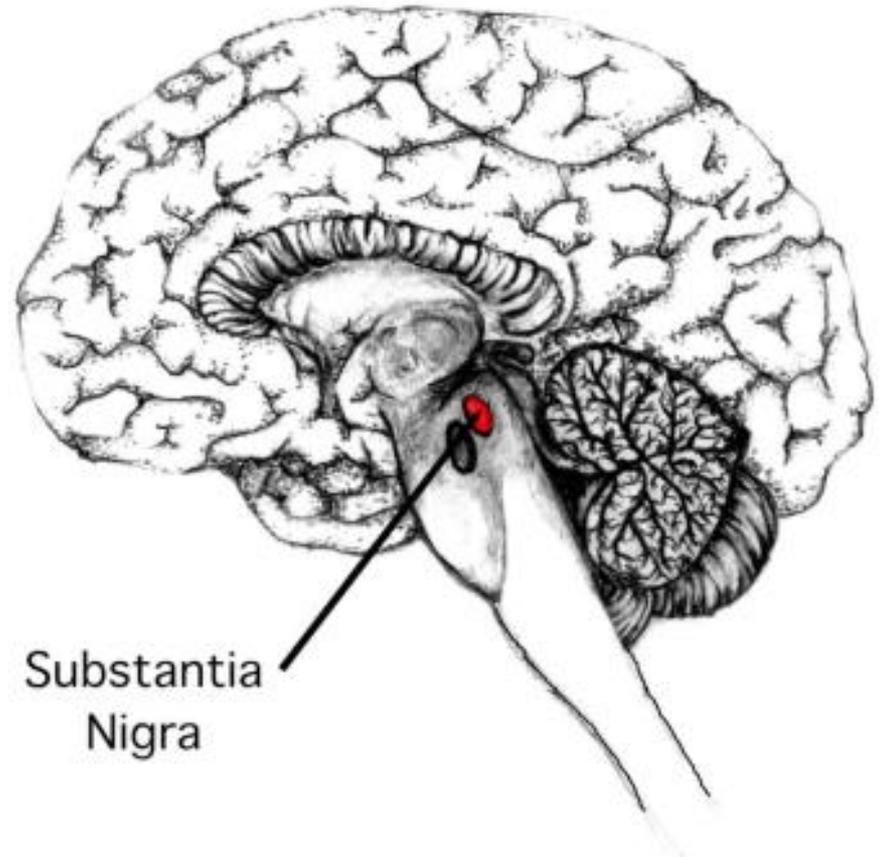


- Deficiency of enzyme, (glucocerebrosidase)
- Variable age of onset
- Rare recessive, single gene disorder
- Symptoms include enlarged spleens and livers, low platelet counts, bone and brain involvement
- Multi-organ involvement

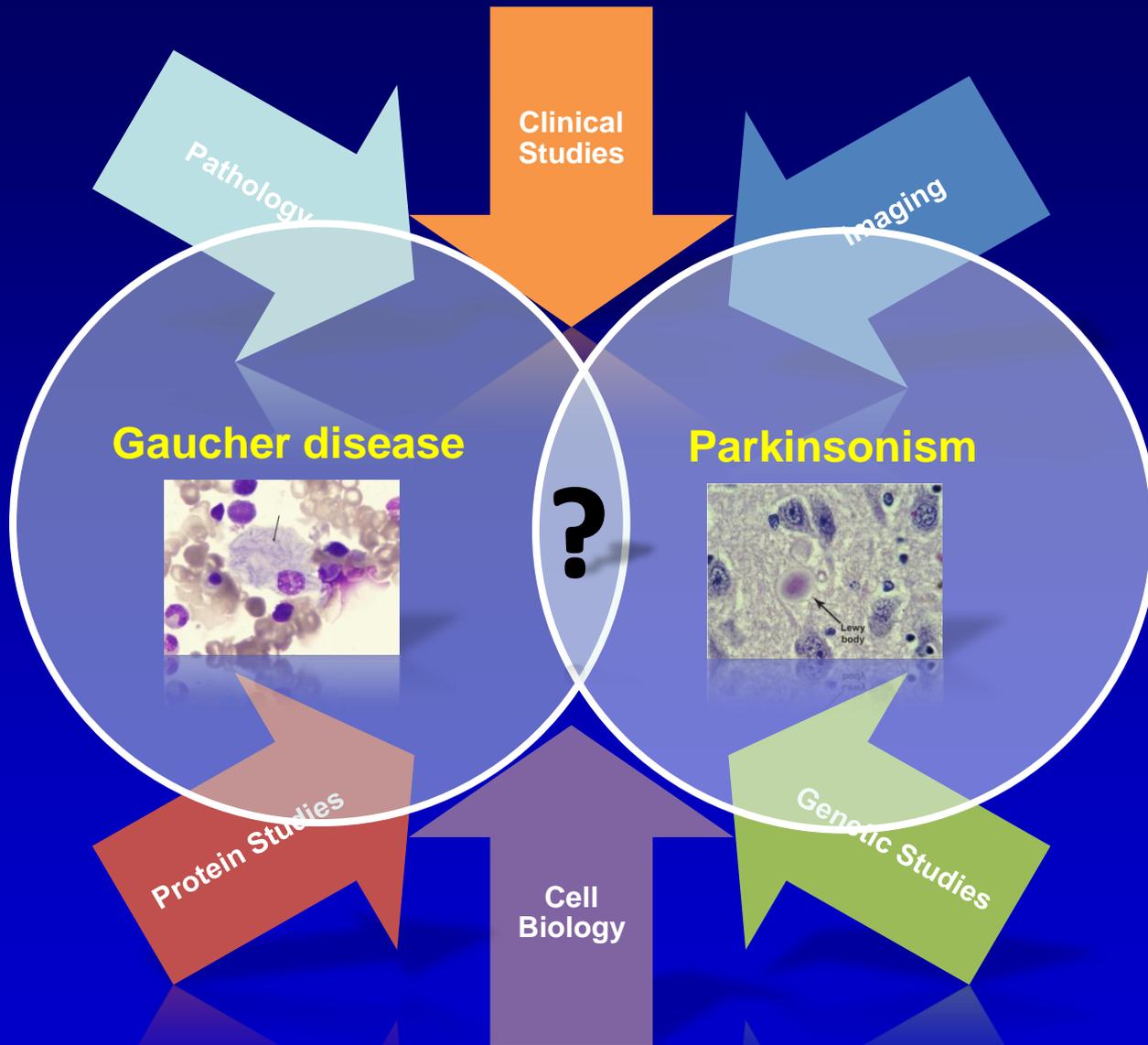


Parkinson Disease (PD)

- Loss of dopaminergic neurons + Lewy bodies (aggregates, α -syn)



Gaucher disease and parkinsonism



Mendelian disorders provide a window into complex disease

Diagnosis of PD

- Parkinsonism - term that describes motor features of Parkinson disease
- Parkinson disease - clinically and pathologically defined process (*UK PD Society Brain Bank Criteria*)

Includes: Bradykinesia (slowness of initiation of voluntary movement & reduction in speed and amplitude of repetitive actions)

And at least one of the following:

Muscular rigidity

4-6 Hz rest tremor

Postural instability not caused by primary visual, vestibular, cerebellar, or proprioceptive dysfunction

Parkinson DiseaseS

- “Classic” idiopathic PD, largely sporadic
- Environmental - MPTP PD (“frozen addicts”)
- Lewy body dementias- DLB and AVLBD
- Parkinson plus
 - Multiple system atrophy (MSA)
 - Progressive supranuclear palsy (PSP)
 - Corticobasal degeneration (CBD)
 - Spinocerebellar degenerations (SCAs)
- Genetic PD: PARK1, PARK2, etc.
- Over 15 PARK genes now described-some, but not all, associated with Lewy body pathology

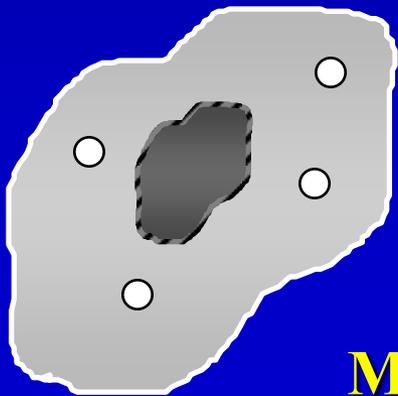
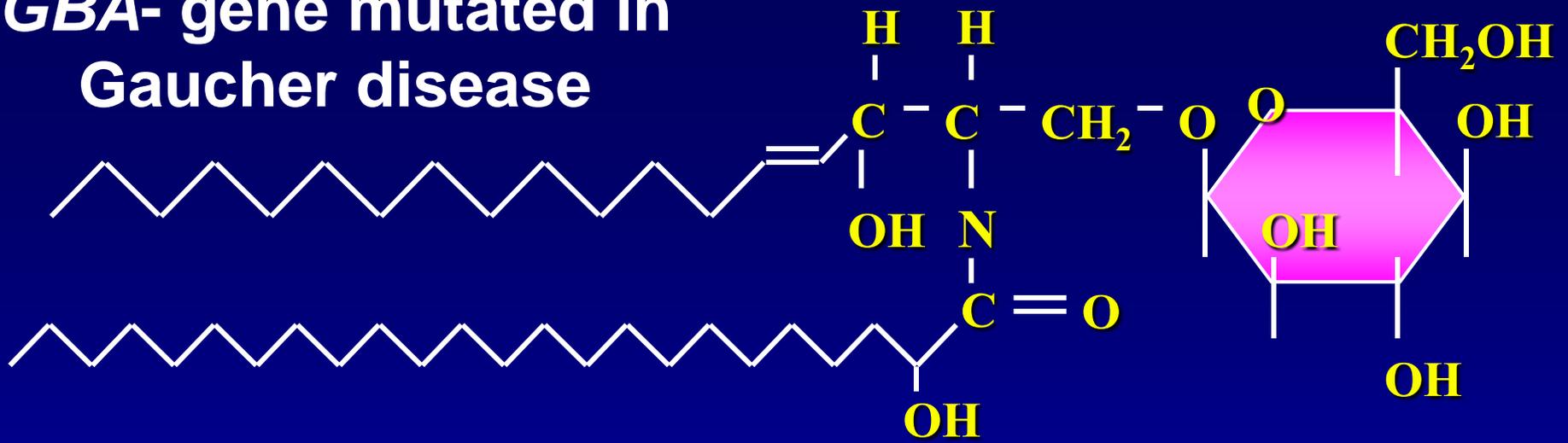
Autosomal Dominant

- **SNCA (PARK1)=Rare cause of hereditary PD, point mutations, multiplications of α -synuclein**
- **LRRK2 (PARK8)=Frequent, incomplete penetrance function unknown, variable pathologic findings**

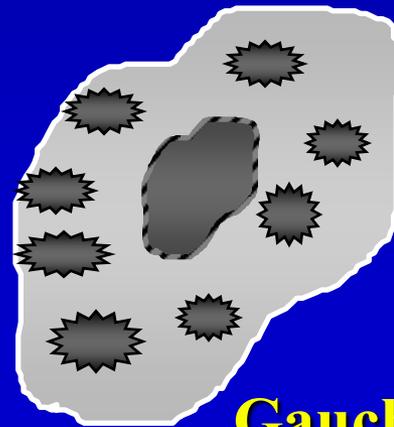
Autosomal Recessive

- **Parkin (PARK2)=Early onset, slow progression, found in 10-20% in patients with early-onset (<45 y)**
- **PINK1 (PARK6)=Rare, onset 3rd and 4th decade, slow progression**
- **Kufor-Rakeb disease (PARK9)=Rare, pyramidal degeneration, cognitive dysfunction**

GBA- gene mutated in Gaucher disease

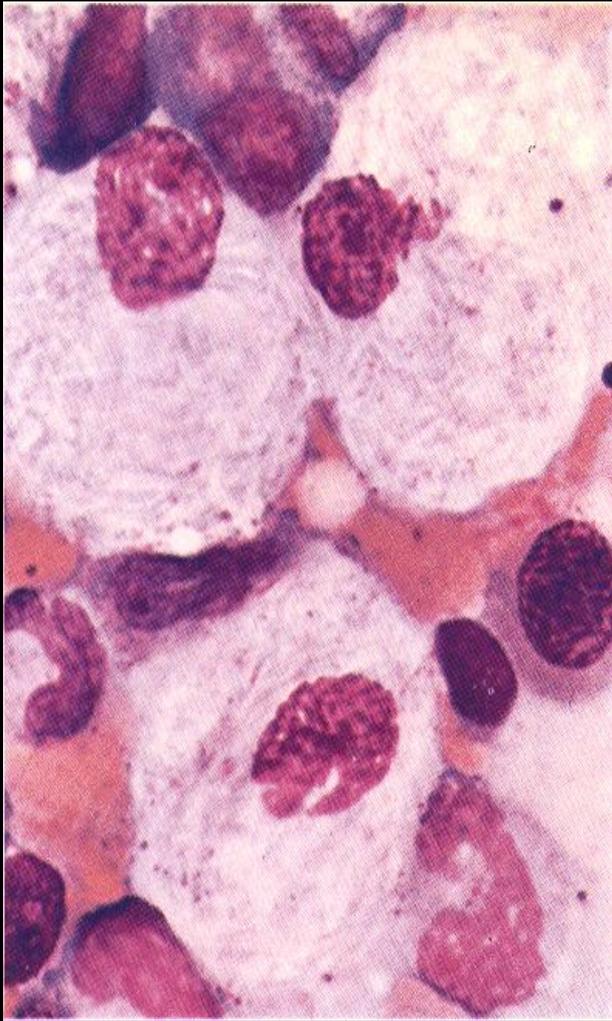


Macrophage

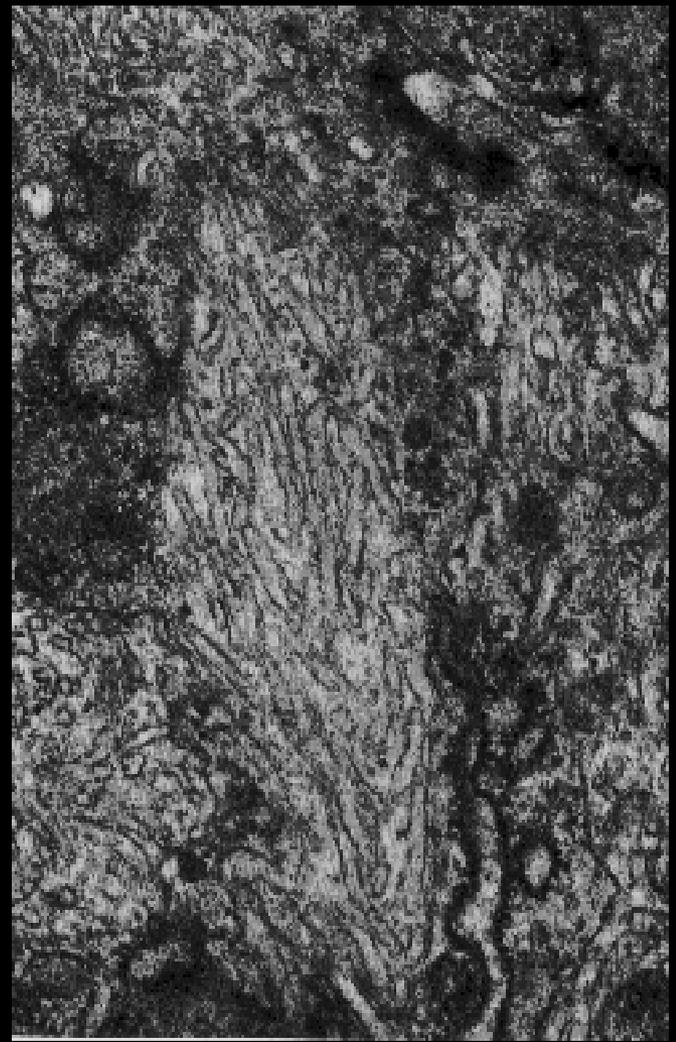


Gaucher Macrophage

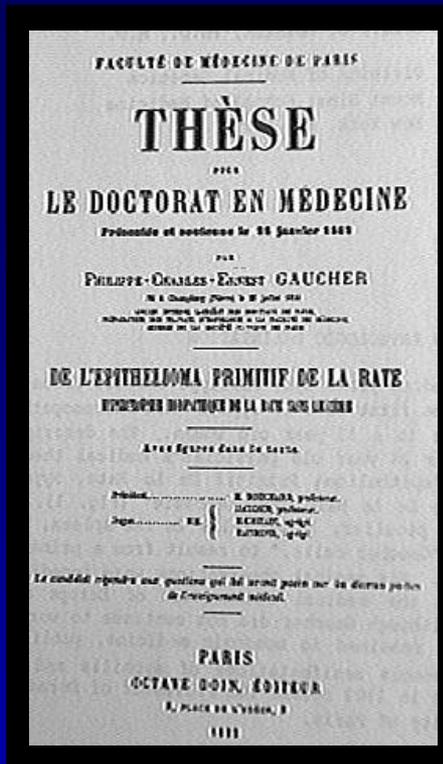
The Gaucher Cell



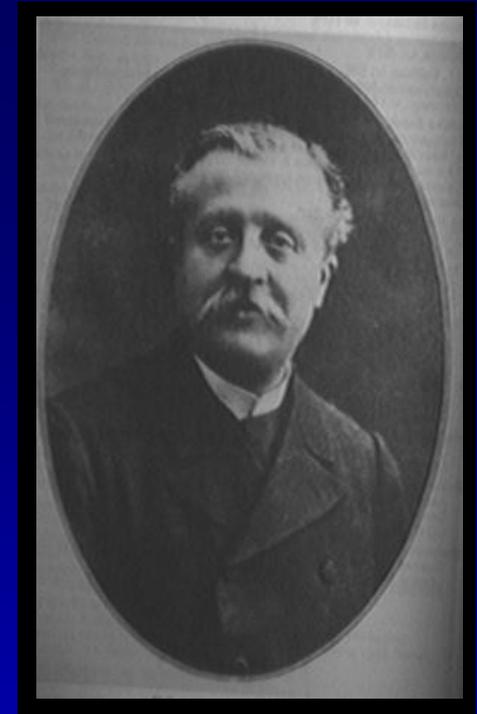
Bone Marrow Smear



**EM of a Gaucher cell showing a
lipid engorged lysosome**



Philippe Gaucher 1854 - 1918

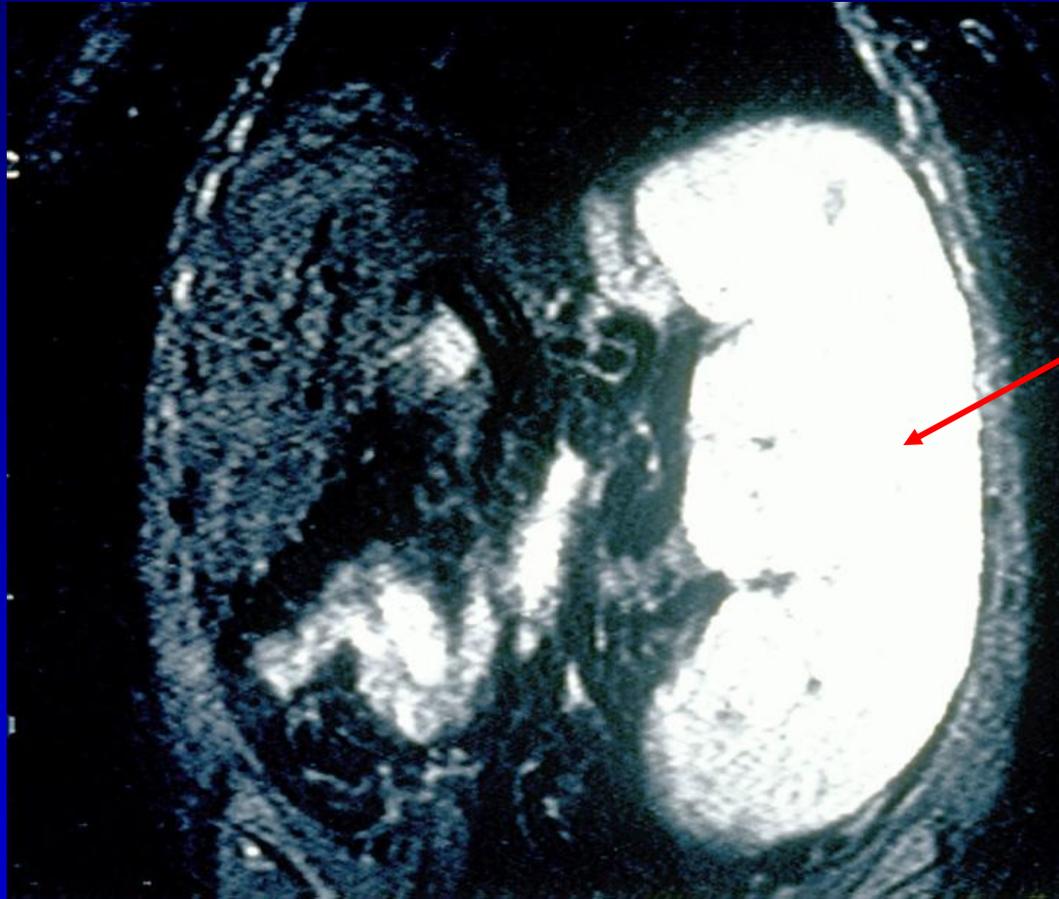


- Recognition of Gaucher disease -- 1882
- Nature of the stored material -- 1935
- Enzymatic defect (Dr. Brady) -- 1965
- Era of molecular biology -- 1981
- Enzyme replacement -- 1991
- Animal model of Gaucher disease 1992
- Association with parkinsonism -2000

Clinical Manifestations of Type 1 Gaucher Disease

- Very heterogeneous in phenotype
- Presents at any age with variable progression
- Common signs and symptoms - hepatosplenomegaly, bone involvement, anemia, thrombocytopenia
- Many asymptomatic individuals
- Autosomal recessive - panethnic
- More frequent among Ashkenazi Jews

Abnormal MRI of a patient with Type 1 Gaucher disease



Enlarged
spleen

Xrays showing Erlenmeyer flask deformity and osteopenia



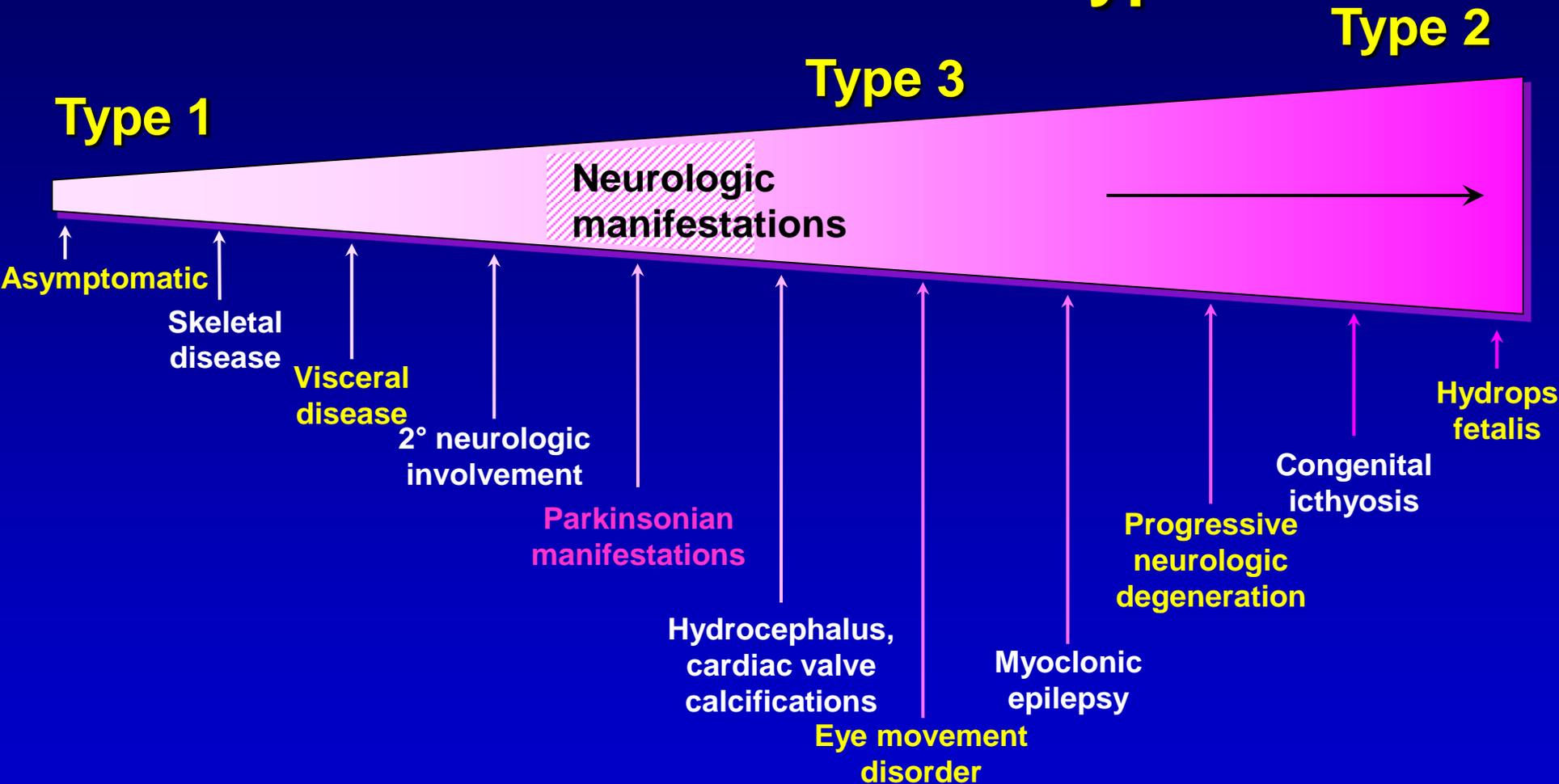
Clinical Manifestations of Type 2 Gaucher Disease

- Stereotypic presentation
- Usually presents at 3 - 6 months, can present in the neonatal period
- Death by age 2 -3 years
- Common signs and symptoms -
hepatosplenomegaly,
seizures, neurologic
deterioration, may have
associated ichthyosis
- Rare form

Clinical Manifestations of Type 3 Gaucher Disease

- Heterogeneous phenotype
- Includes neurologic involvement-
slowing and looping of horizontal
saccades, may include ataxia,
dementia, myoclonic epilepsy
- Visceral and bone involvement
- Panethnic, with a geographic
isolate in Sweden

Gaucher Disease: A Continuum of Phenotypes

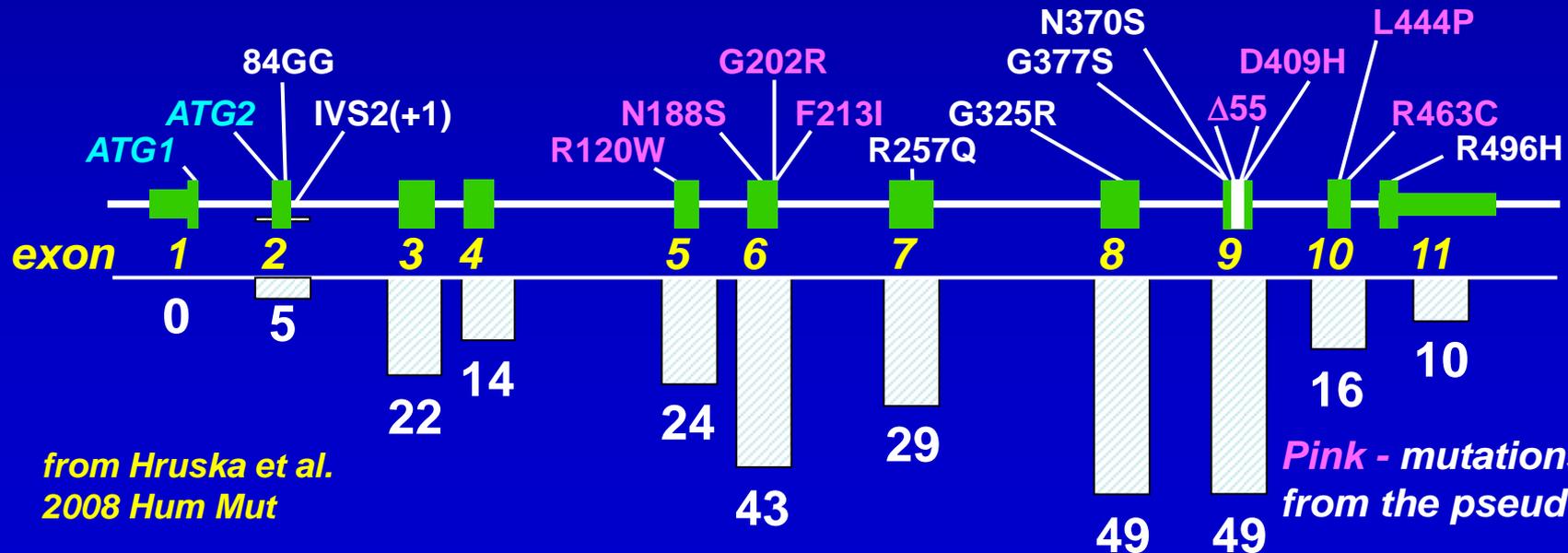


Glucocerebrosidase Gene (*GBA*) Locus on 1q21



- Pseudogene sharing 96% sequence homology 16kb downstream
- Some mutant alleles originate from the pseudogene (rec alleles)
- > 300 mutations, point mutations, frame-shifts, deletions and insertions

GBA # of reported mutations per exon



Neither the amount of lipid stored nor the enzyme activity correlate well with the patient phenotype

Genotype-Phenotype Studies

Some correlations exist, but.....

- **Clinically different patients can have the same genotype**
- **Clinically similar patients can have many different genotypes**
- **Siblings with the identical genotype exhibit different clinical manifestations and responses to therapy**

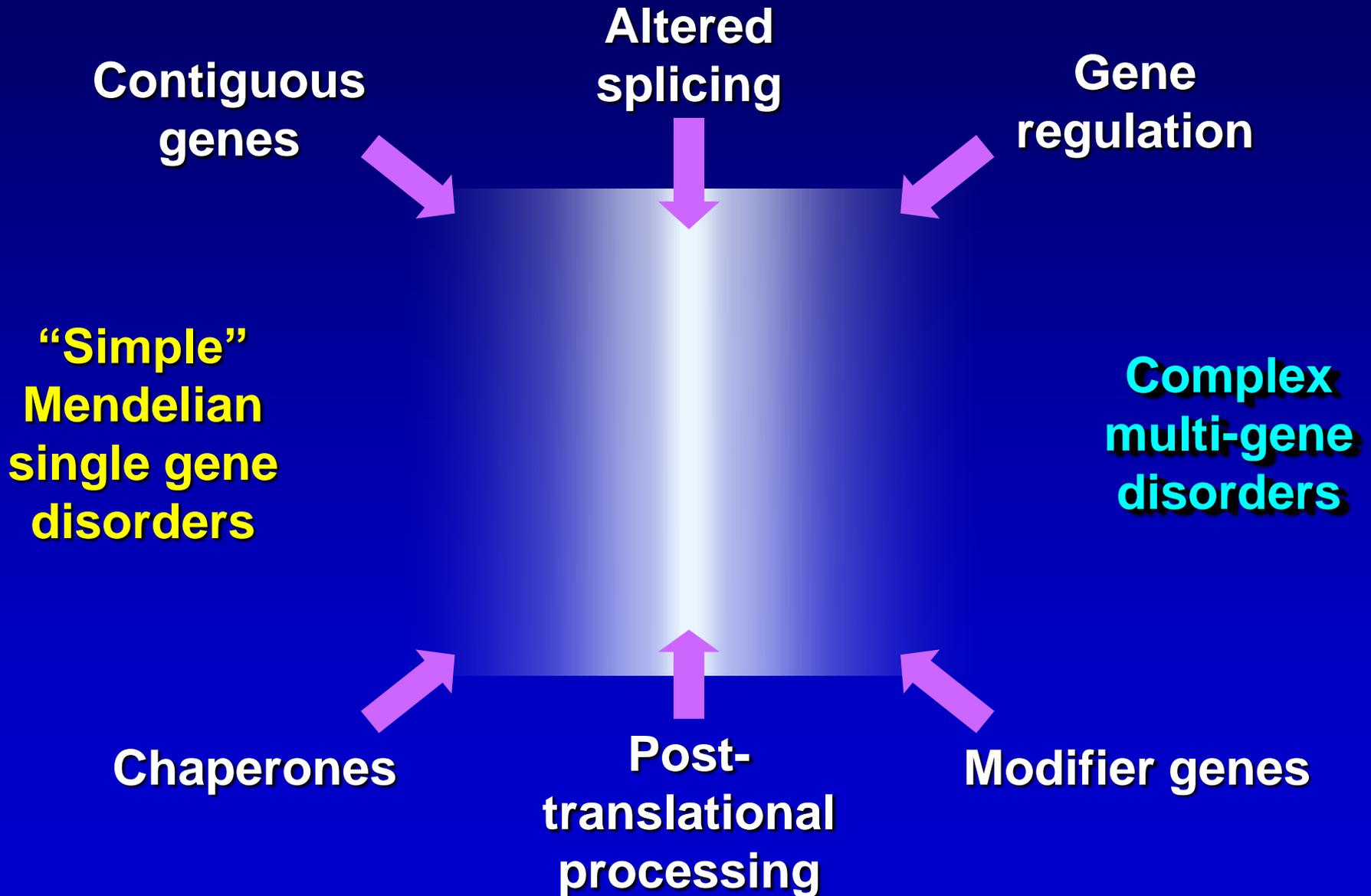
Study of 32 L444P homozygotes

Goker-Alpan et al. J Med Genet 2005

- All have slowed horizontal saccades
- Degree of systemic and neurological manifestations highly variable
- Residual enzyme activity between 1%-13% with no correlation to clinical symptoms
- Outcomes range from death in childhood to autism to successful college students
- Specific neurocognitive deficits noted

Studies of discordant siblings and patients with the same genotype can be used to identify factors modifying phenotype

Multiple factors impact phenotype and blur boundaries between simple and complex genetic disorders



Chapter 1

Probands with both Gaucher disease and parkinsonism

W. B. SAUNDERS COMPANY Philadelphia London Toronto
London Bombay Calcutta

Medical
Dictionary

This story began my clinic in 1996, with a single patient with GD who developed parkinsonism...

- **Mild Gaucher disease- diagnosed at age 19**
- **Tremor began at age 42 with progressive rigidity, masked facies, difficulty initiating movements and rapid deterioration of gait**
- **No improvement with pallidotomy or enzyme replacement therapy**
- **Progressive dementia -death at age 54**

Was this a co-incidence?

- **Other cases found in literature (Neudorfer *et al* 1996) and other clinics**
- **In 2003, we published a series of 17 such subjects**

Patients with Gaucher disease and parkinsonism

Patient	Sex	Genotype	Ethnic Background
1	M	R463C/R120W	Canadian
2	M	N370S/V394L	Hungarian/Jewish
3	M	N370S/IVS2+1	Polish
4	F	L444P/D409H + dup.	American
5	F	G377S/G377S	Italian
6	M	N370S/RecNcil	French
7	M	N370S/c.84insG	American
8	M	N370S/N370S	Ashkenazi
9	M	N370S/c.1263-1317del	French
10	M	N370S/c.500insT	Spanish
11	F	N370S/?	Italian (sib of 12)
12	F	N370S/?	Ashkenazi
13	F	N370S/N370S	Ashkenazi
14	F	N370S/L444P	Italian
15	M	N370S/N370S	Ashkenazi
16	F	N370S/N370S	Ashkenazi
17	M	N370S/N370S	unknown

Clinical data on 17 patients with Gaucher disease and parkinsonism

Primary Gaucher Disease Manifestations

- Most had relatively mild Gaucher disease manifestations

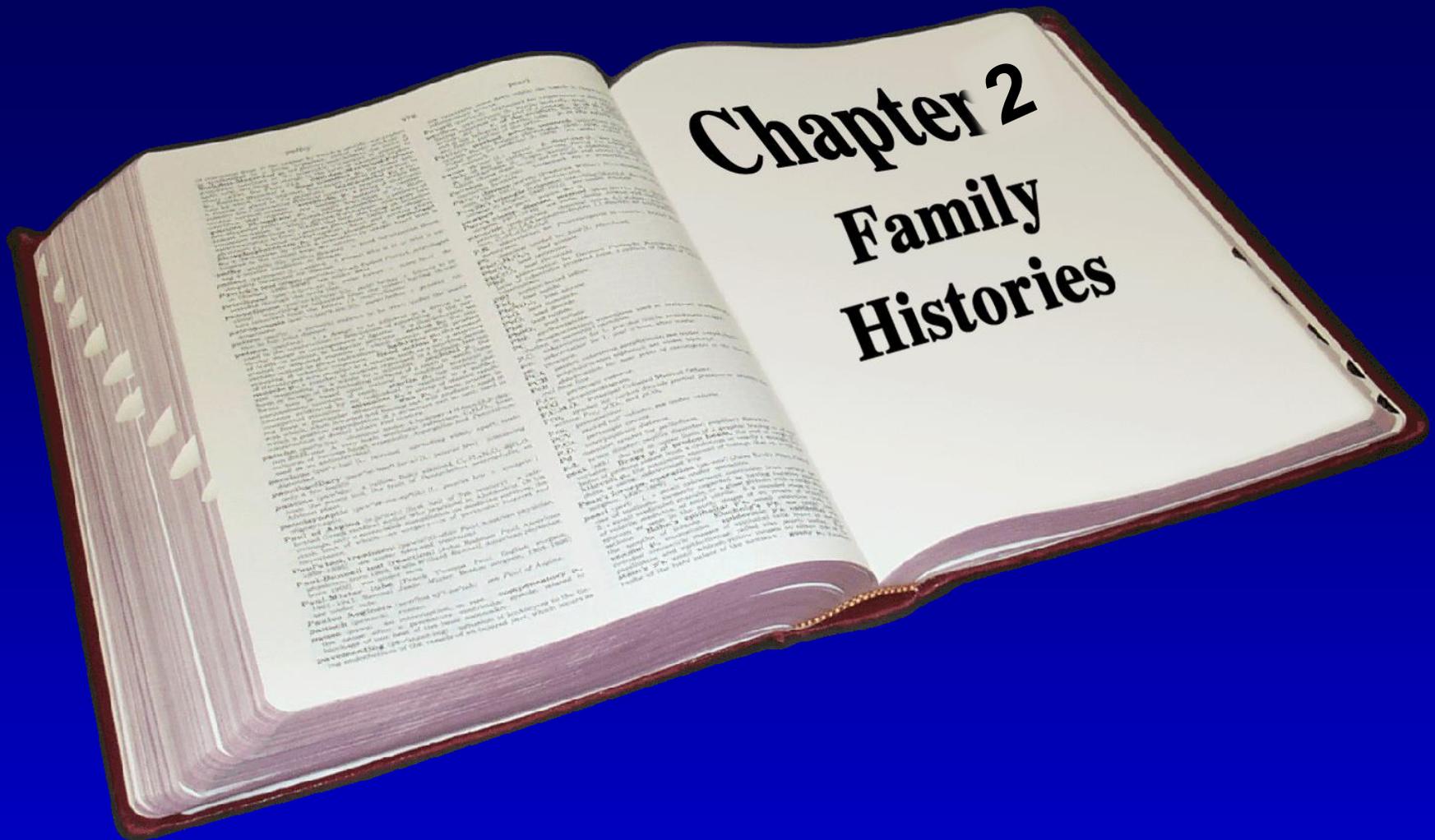
Primary Parkinsonian Manifestations

- Tremor
- Bradykinesia
- Rigidity
- Masked facies
- Several had cognitive decline and/or dementia

	<u>Range</u>	<u>Mean</u>
● Age at diagnosis of Gaucher disease	9-62 yrs	35 yrs
● Age at onset of parkinsonian symptoms	38-63 yrs	48 yrs

Therapy

- L-Dopa response was variable
- No change with Cerezyme



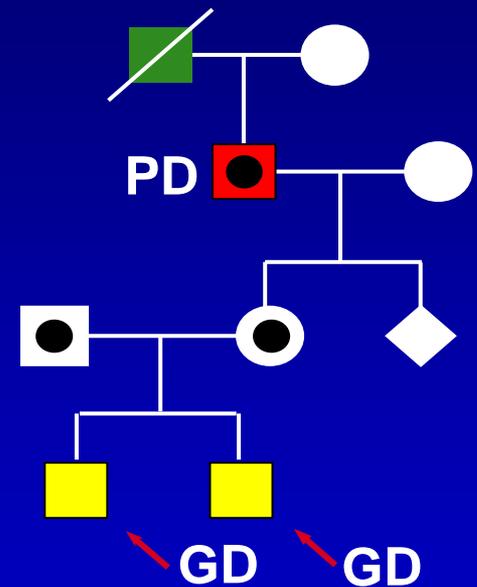
Chapter 2

Family Histories

Are Gaucher carriers more susceptible to parkinsonism?

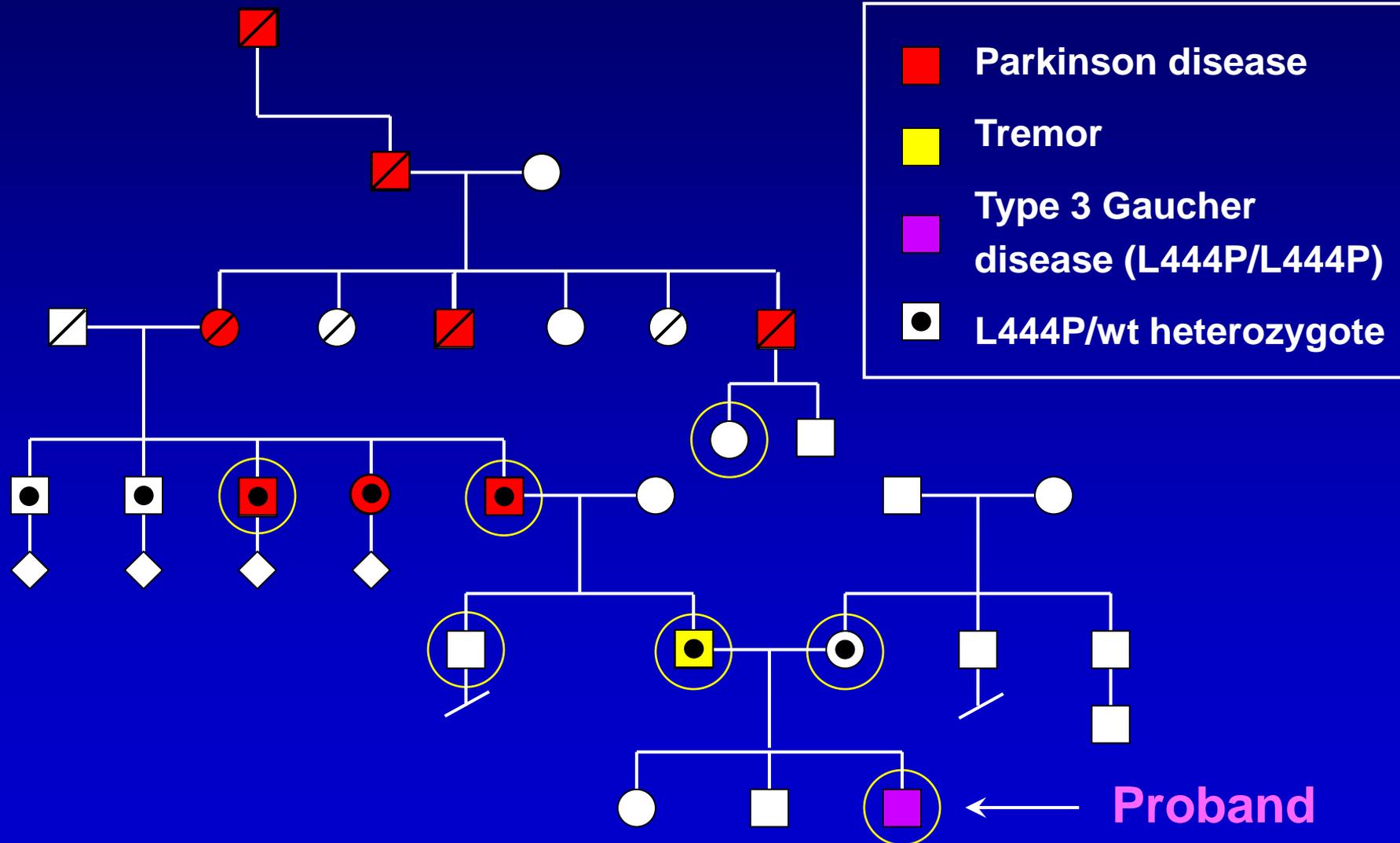
Family histories reveal parkinsonism in heterozygotes

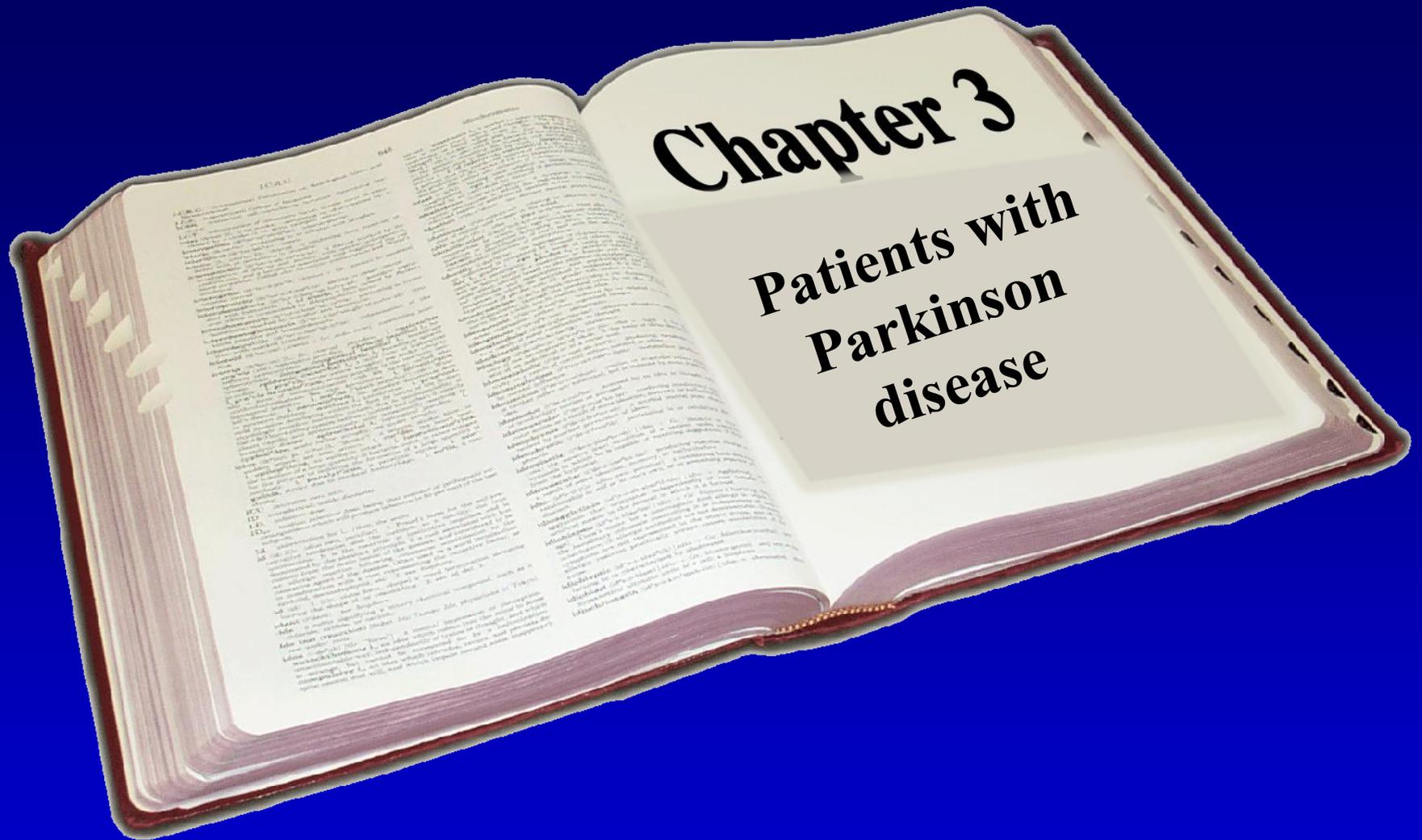
- In a prospective study, **12** of **45** Gaucher probands had relatives with parkinsonism.
- Often, this was a parent or grandparent who was a Gaucher carrier.
- This was subsequently seen in other Gaucher centers including in Jerusalem.



Heterozygotes are at increased risk for parkinsonism

Pedigree of a patient with Gaucher disease





***This time we studied patients with Parkinson disease
(with no known Gaucher disease)***

Identification of *GBA* mutations in PD

- Autopsy sample was donated from a subject with Gaucher disease and parkinsonism
- Tissue requested from 2 age-matched PD subjects
- Both “controls” had GCase activity in the heterozygote range and *mutations in GBA!*
- We sequenced DNA from 57 brain samples with PD- *8 carried GBA mutations, none in 44 controls*

This has propelled the community studying the genetics of PD in a new research direction. Many replication studies published around the world.

However, many of the initial studies were greeted with skepticism due to limitations in power or controls and because GWAS did not identify *GBA*

Studies of *GBA* in Different PD Cohorts

Study	Location	% <i>GBA</i> mutations in PD patients	% <i>GBA</i> mutations in controls
Nichols <i>et al.</i> 2009	Cincinnati	4.1% of 1325	1.1% of 359
Mitsui <i>et al.</i> 2009 *	Japan	9.4% of 534	0.4% of 544
Gan-Or <i>et al.</i> 2008	Tel Aviv, Israel	17.9% of 420	4.2% of 333
Bras <i>et al.</i> 2008 *	Portugal	6.1% of 230	0.7% of 430
Lesage <i>et al.</i> 2010 *	France	6.7% of 1391	1.0% of 391
Neumann <i>et al.</i> 2009 *	UK	4.2% of 720	1.2% of 257
Kalinderi <i>et al.</i> 2009 *	Greece	5.8% of 172	1.5% of 132

*sequenced

Green- subsequent to NEJM paper

This association has persisted!



A database for Parkinson's disease genetic association studies developed by Massachusetts General Hospital/Harvard Medical School, The Michael J. Fox Foundation and the Alzheimer Research Forum



THE MICHAEL J. FOX FOUNDATION FOR PARKINSON'S RESEARCH

The PDGene database is supported by a grant from [The Michael J. Fox Foundation](#) in partnership with the [Alzheimer Research Forum](#).



ALZHEIMER RESEARCH FORUM

Top PDGene Results

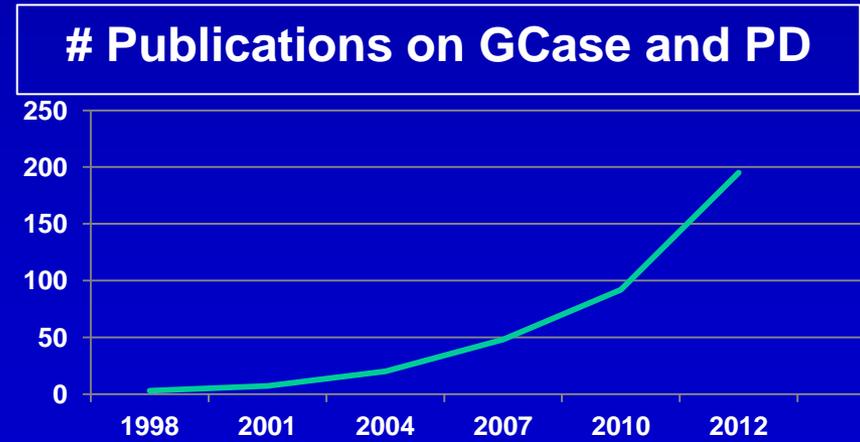
[View Top Results](#)

Methods

1. **GBA**
2. [LRRK2](#)
3. [SNCA](#)
4. [MAPT/STH](#)
5. [PINK1](#)
6. [CYP2D6](#)
7. [APOE](#)
8. [MAOB](#)
9. [ELAVL4](#)
10. [GWA_2q36.3](#) [see more]

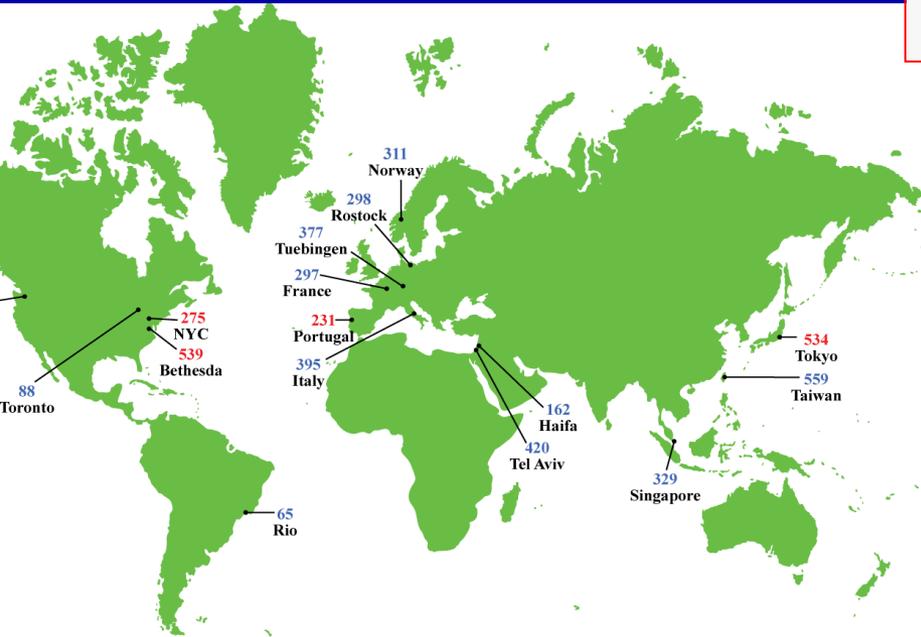
“The GBA example is an illustration of how an important genetic risk factor for a complex disease can evade detection by systematic analysis: it only came onto the radar because of astute clinical observation.”

Rogaeva and Hardy, Neurology - June 10, 2008 Editorial



International multi-center study of *GBA* mutations in PD

16 centers joined,
contributing 5691 *GBA*
genotypes from patients
with PD and >5000 from
controls



The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

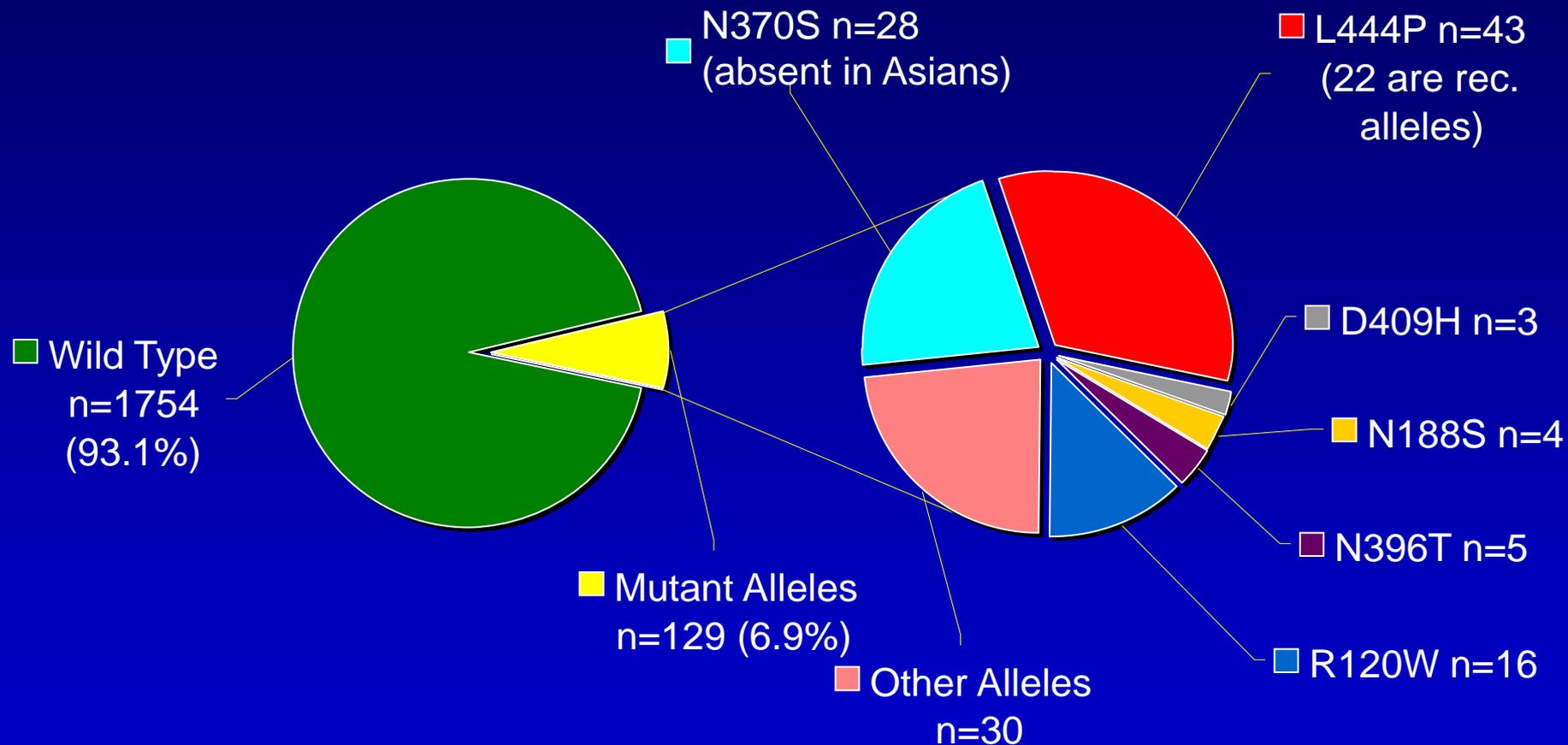
Oct 2009

Multicenter Analysis of Glucocerebrosidase Mutations in Parkinson's Disease

E. Sidransky, M.A. Nalls, J.O. Aasly, J. Aharon-Peretz, G. Annesi, E.R. Barbosa, A. Bar-Shira, D. Berg, J. Bras, A. Brice, C.-M. Chen, L.N. Clark, C. Condroyer, E.V. De Marco, A. Dürr, M.J. Eblan, S. Fahn, M.J. Farrer, H.-C. Fung, Z. Gan-Or, T. Gasser, R. Gershoni-Baruch, N. Giladi, A. Griffith, T. Gurevich, C. Januario, P. Kropp, A.E. Lang, G.-J. Lee-Chen, S. Lesage, K. Marder, I.F. Mata, A. Mirelman, J. Mitsui, I. Mizuta, G. Nicoletti, C. Oliveira, R. Ottman, A. Orr-Urtreger, L.V. Pereira, A. Quattrone, E. Rogaeva, A. Rolfs, H. Rosenbaum, R. Rozenberg, A. Samii, T. Samaddar, C. Schulte, M. Sharma, A. Singleton, M. Spitz, E.-K. Tan, N. Tayebi, T. Toda, A.R. Troiano, S. Tsuji, M. Wittstock, T.G. Wolfsberg, Y.-R. Wu, C.P. Zabetian, Y. Zhao, and S.G. Ziegler

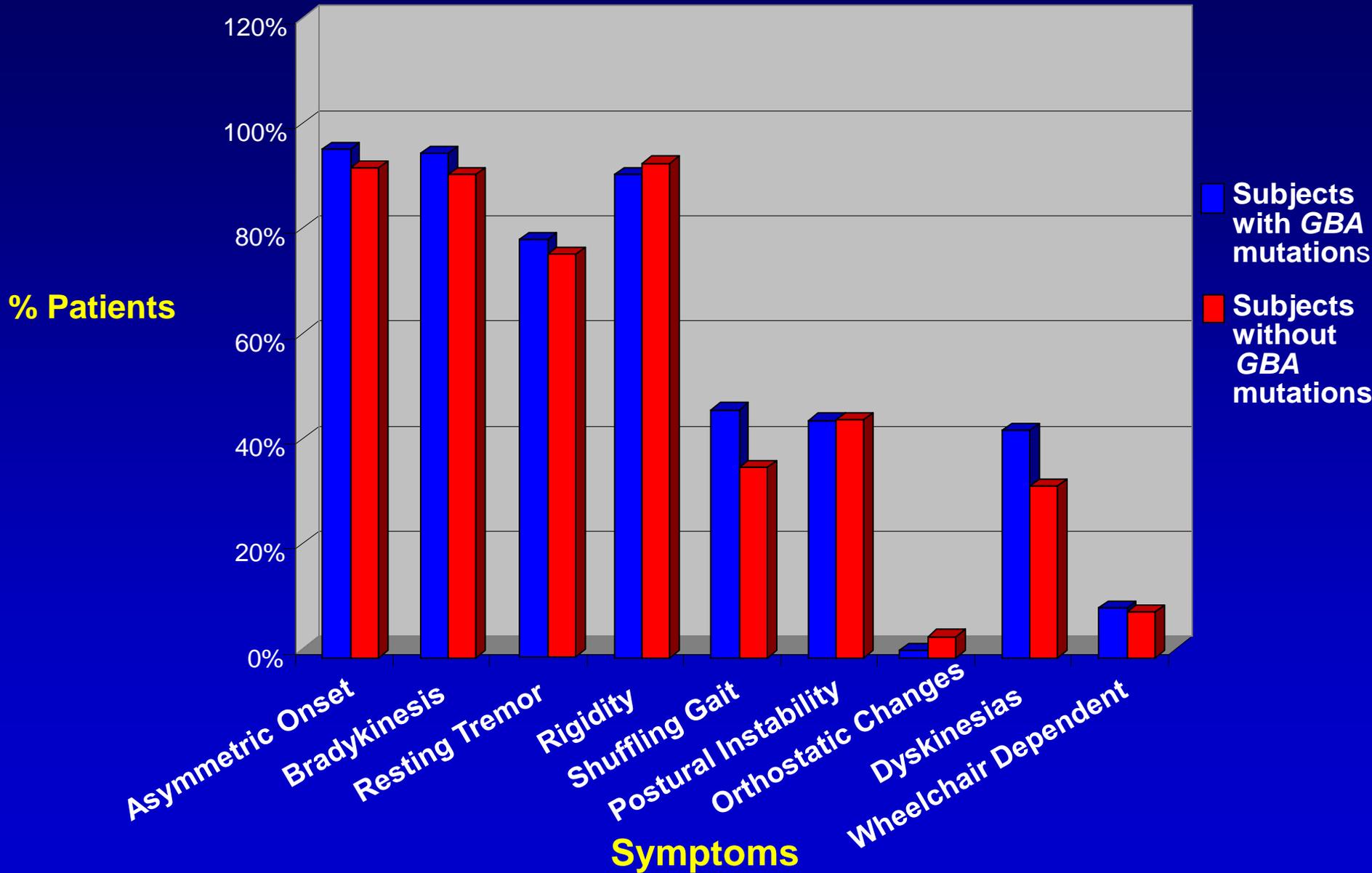
- Subjects with PD are >5 times more likely to have a mutation in *GBA*
(Overall OR=5.43; 95%CI=3.89-7.57)

Non-Ashkenazi Jewish Patients (n=1883) Full sequencing of *GBA*

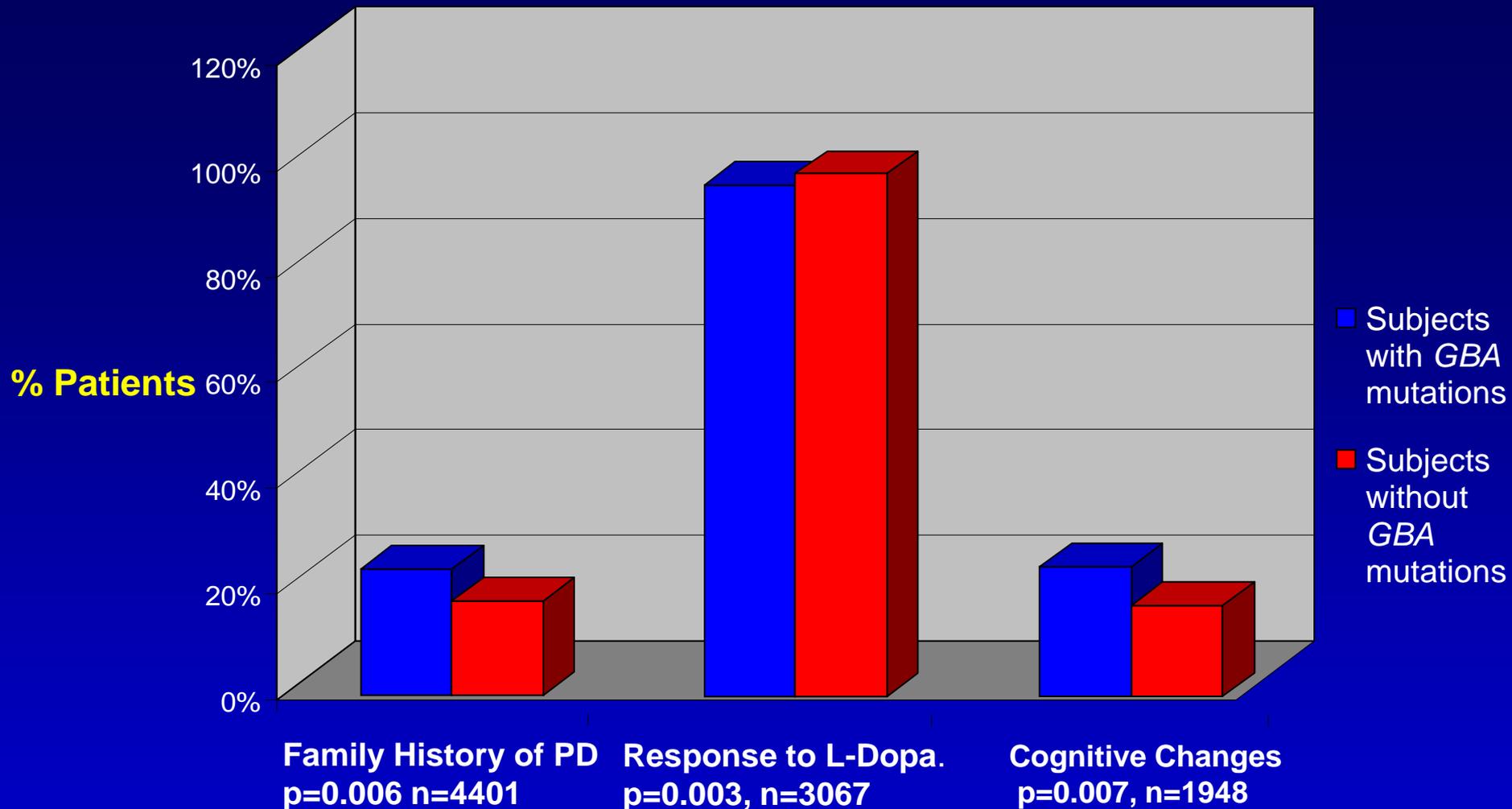


**Screening for 2 mutations would fail to detect
at least 42% of mutant alleles**

Clinical Characteristics of ~3000 PD Patients With and Without *GBA* Mutations



Other Factors

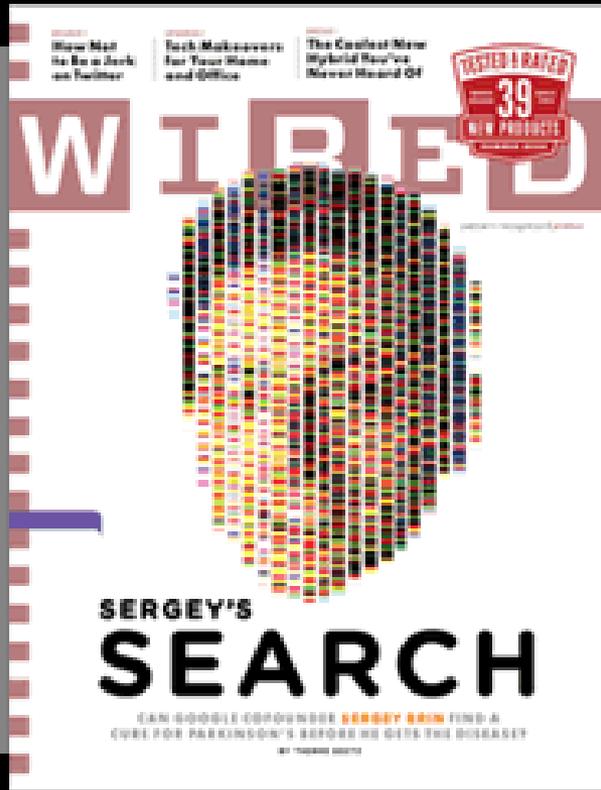


However many subjects with cognitive decline may have been excluded because of diagnostic criteria for PD
Patients with **GBA** mutations have an earlier onset of parkinsonian manifestations (mean 4.3 yr, $p < 0.001$)

Conclusions from study

- Combined analysis of 5691 patients showed that subjects with Parkinson disease are >5 times more likely to have a mutation in glucocerebrosidase
- Screening methods are crucial- in some cohorts >40% of *GBA* mutations are not L444P or N370S
- Age at onset is earlier in patients with Gaucher mutations; Parkinson symptoms vary and do not differ from patients without mutations
- Studies of a rare Mendelian disease can provide insight into a common complex disorder

Mutations in glucocerebrosidase are the most significant genetic risk factor for PD identified



July 2010

Can research based on direct-to-consumer data surpass standard research methods?

23andMe study on the genetics of Parkinson disease

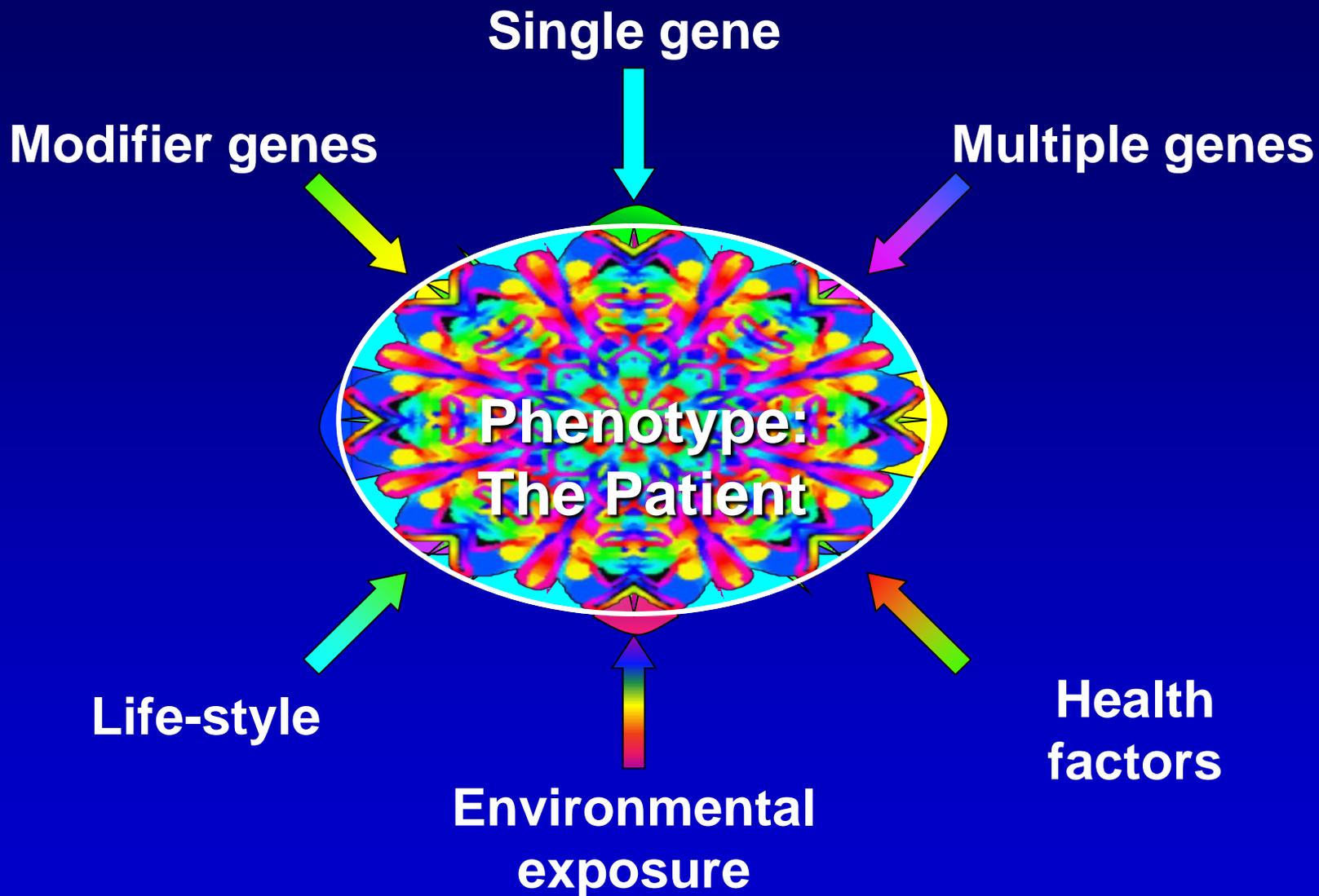
- *“23andMe researchers run a database query based on 3,200 subjects. The results are returned in 20 minutes.”*
- *“The results are that people with GBA are 5 times more likely to have Parkinson’s, which is squarely in line with the NEJM paper.”*

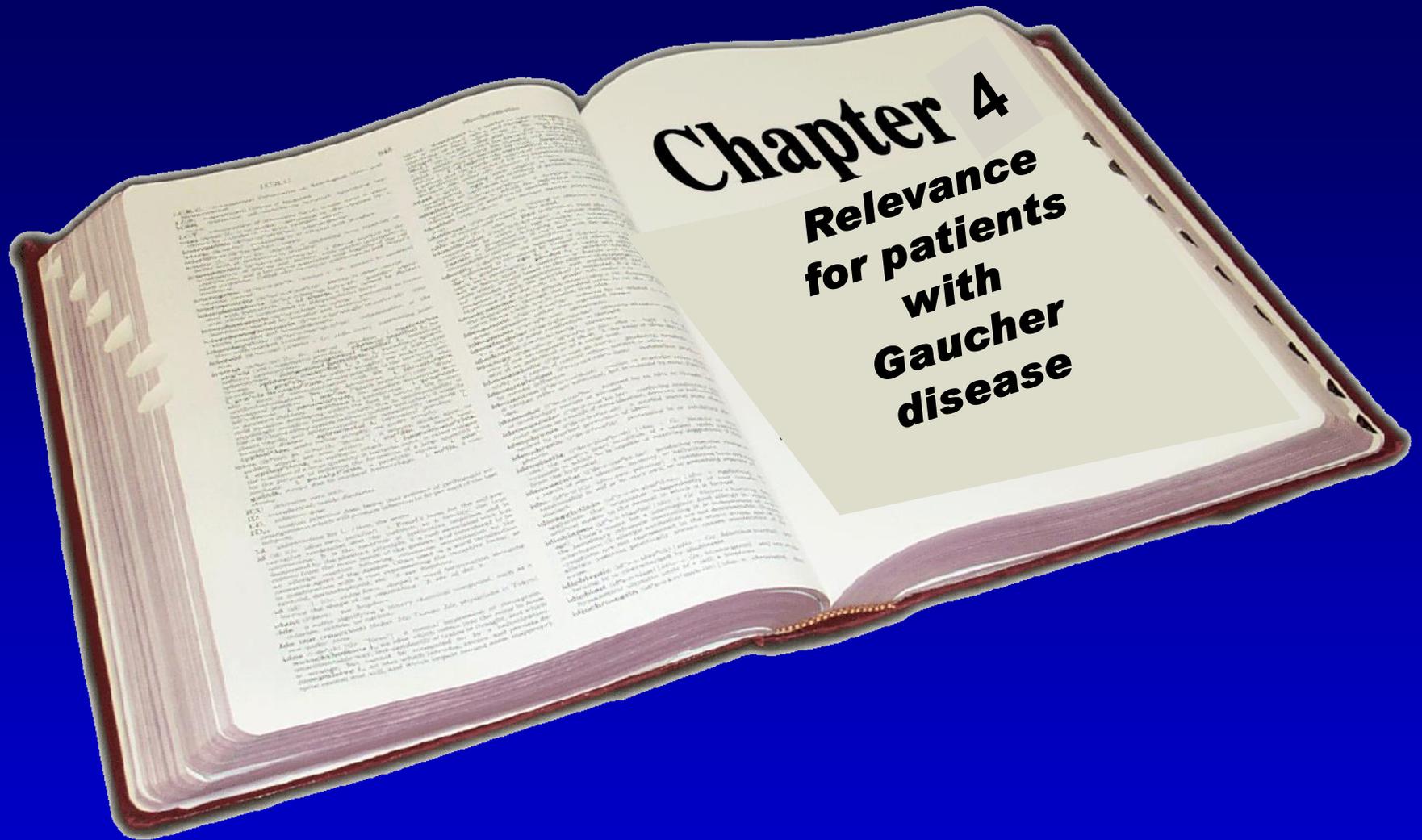
Mutations in GBA are the most significant genetic risk factor for PD identified to date!

However, the vast majority of patients with Gaucher disease and Gaucher carriers do NOT develop parkinsonism!

A GBA mutation is a risk factor for the development of parkinsonism.

Different Risk Factors Contribute to Genetic Disease





What does this finding mean for patients with Gaucher disease and GBA mutation carriers?

Estimates of the frequency of PD in patients with Gaucher disease and Gaucher carriers

International Gaucher registry: PD in 68 of 1130 pt > age 60. Probability of PD is 5-7% by age 70; 9-12% age 80. No predictive GD-profile. (Rosenbloom *et al.* 2011)

Other studies from individual clinics: Probability of PD is 5-13 % by age 60, 15-30% by age 80.

Better and larger studies still needed- Lifetime RR is around 5-8 X higher than general population.

Clinical studies of GD with parkinsonism

- Patients with GD have classic Parkinson disease, a more aggressive early-onset form and/or a familial form.
- Multiple genotypes seen: N370S is common.
- Most respond to Ldopa: No improvement with ERT or SRT.
- Olfactory and cognitive impairment relatively common.
- Among 12 patients at NIH, mean age at PD diagnosis: 49yr; mean UPDRS III Score: 26.3

Prospective patient study: Clinical features and PET imaging in subjects with *GBA* mutations (Collaboration with K.F. Berman, NIMH)

Goals:

1. To better characterize the PD phenotype associated with *GBA*.
2. To study F-dopa uptake and evaluate PET as a surrogate marker in subjects with *GBA* mutations.
3. To establish earliest signs of PD in at-risk subjects.

Included: {
GD/PD
PD-no GD
GD with +FH of PD
GD carriers with +FH of PD

Clinical Studies

- ❖ Physical exam
- ❖ Neurologic exam (UPDRS)
- ❖ Neurocognitive evaluation
- ❖ Olfactory testing
- ❖ Screens for non-motor symptoms

Imaging Studies

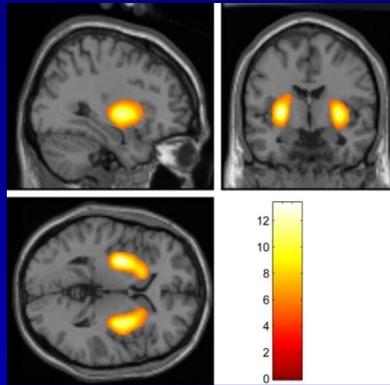
- ❖ MRI (structural abnormalities)
- ❖ F-18 Dopa PET (L-Dopa metabolism)
- ❖ 15-H₂O PET (cerebral blood flow)
- ❖ Trans-cranial sonogram (TCS) (midbrain structures)

PET studies: ^{18}F -fluorodopa and H_2^{15}O

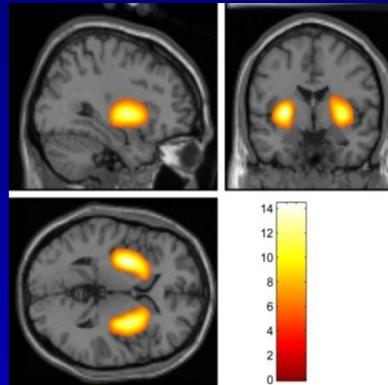
Measure regional brain dopamine synthesis and resting rCBF respectively

Studies of 44 patients completed

Striatal dopamine synthesis (Ki value)



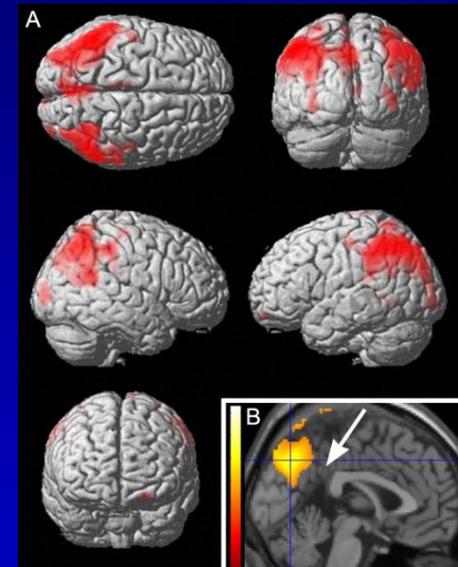
Controls > PD



Controls > GD-PD

rCBF studies: PD-GD vs. controls

Regions with lower rCSF displayed on
A) rendered templates B) midsagittal section



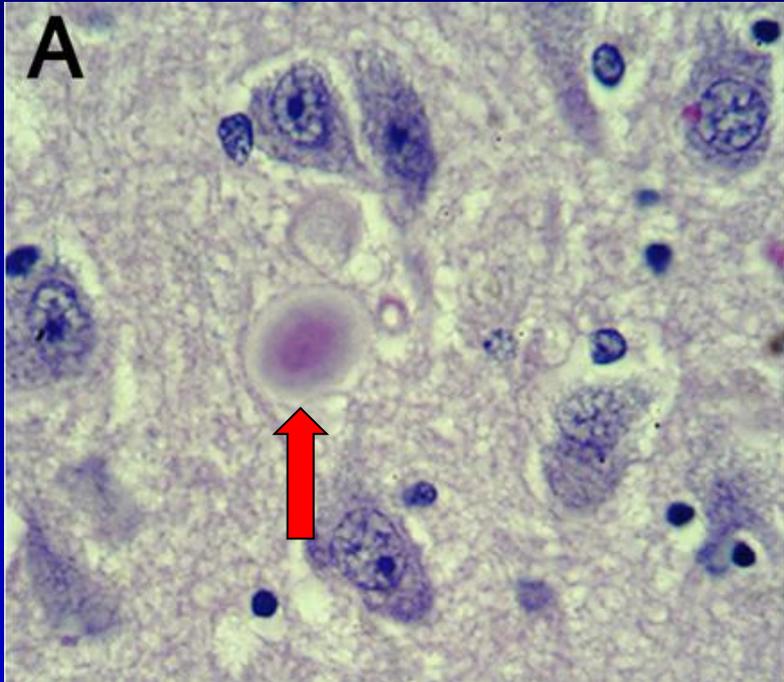
Spares
post.
cingulate
gyrus

^{18}F -fluorodopa: Pattern of dopamine loss in *GBA*-associated PD similar to sporadic PD.

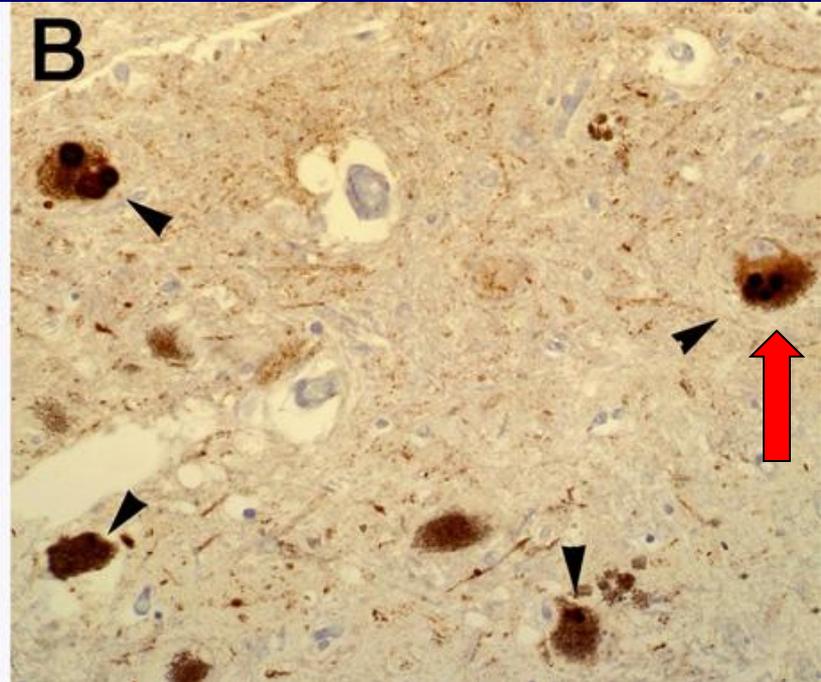
rCBF: Less activity in areas affected in neurodegenerative disorders like AD (inf parietal lobules & precuneus bilat)- may explain cognitive impairment in GD-PD.

Among 14 pt with GD and 7 carriers (no PD, but +FH), only 2 showed evidence of dopamine loss. Longitudinal studies needed. *(Brain 2012)*

Brain samples from patients with GD and parkinsonism show Lewy bodies, inclusions characteristic of PD and related disorders



H&E, 400X

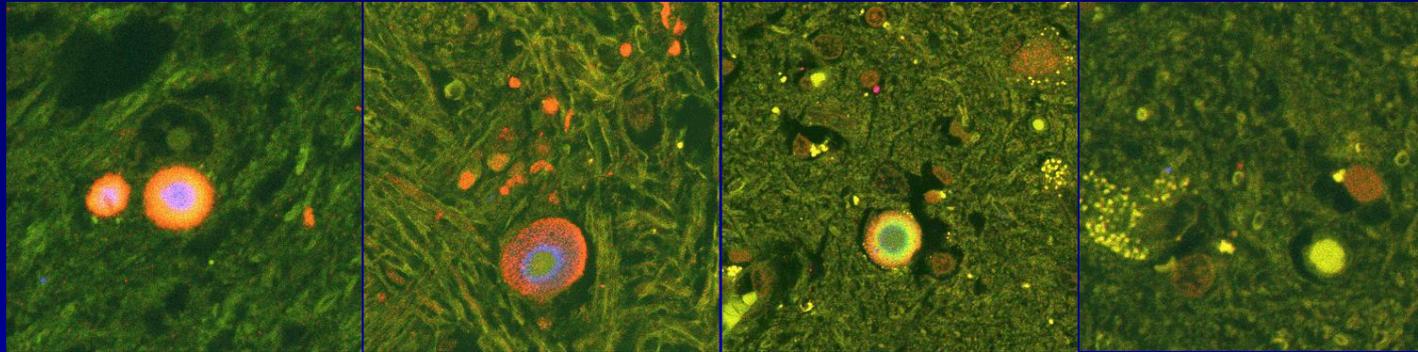


**α -Synuclein antibody,
200X**

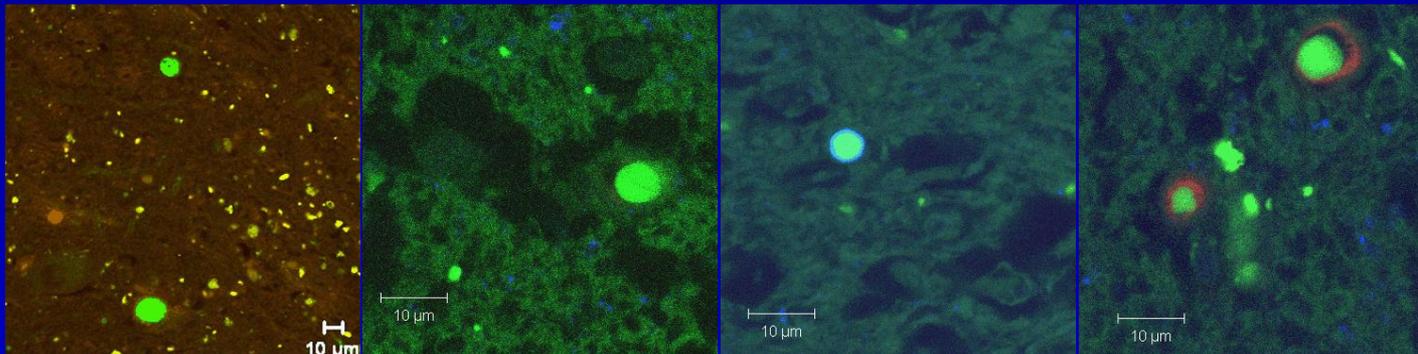
**Patients also have gliosis of hippocampal CA2-4- regions
also affected in Lewy body dementia**

Studies of mechanism: Glucocerebrosidase is found in Lewy bodies in patients with *GBA* mutations

ACTA Neuropath 2010



LBs from subjects with parkinsonism and *GBA* mutations-most + for GCase



LBs from subjects with parkinsonism but no *GBA* mutations-little GCase staining

Brain samples stained with antibody; **GCase**, **α -synuclein** and **ubiquitin**

Multicenter analysis of *GBA* in Dementia with Lewy bodies (DLB) *JAMA Neuro In Press*

GBA genotypes from 11 centers
721 cases with DLB, 1962 controls.

450 cases were autopsied, 80% of cases had full GBA sequencing
Significant association found.

Odds ratio = 8.28 (95%CI = 4.78 – 14.88).

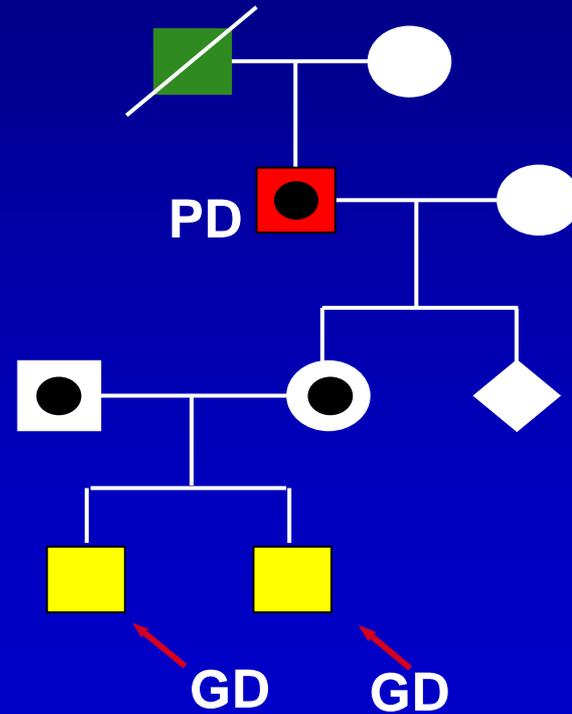
Age at diagnosis ~ 5 years earlier in *GBA* carriers with DLB.

Mutations associated with higher PD scores (H&Y, UDPRS).

GBA mutations play a larger role in DLB than PD!

Pilot studies of *GBA* mutations in MSA and Essential Tremor have *not* shown a similar association.

Why would being a carrier for one genetic disorder put you at risk for another unrelated illness?



Carriers for other Mendelian disorders are at risk for other complex diseases

Methyltetrahydrofolate reductase



Atherothrombotic disease

Factor V & prothrombin



Stroke, recurrent miscarriages

Alpha-1 antitrypsin



Chronic obstructive pulmonary disease

CFTR



Obstructive azospermia, chronic pancreatitis

Glycerol kinase



Diabetes

Glucocerebrosidase



Parkinson disease

TREM 2 (Nasu-Hakola disease)



Alzheimer disease

Protein misfolding

Missense mutation

Conformation change in protein (misfolding)

Unstable protein

Altered transport and/or protein-protein interactions

Stable protein

Organelle dysfunction

Traffic jam

Aggregation

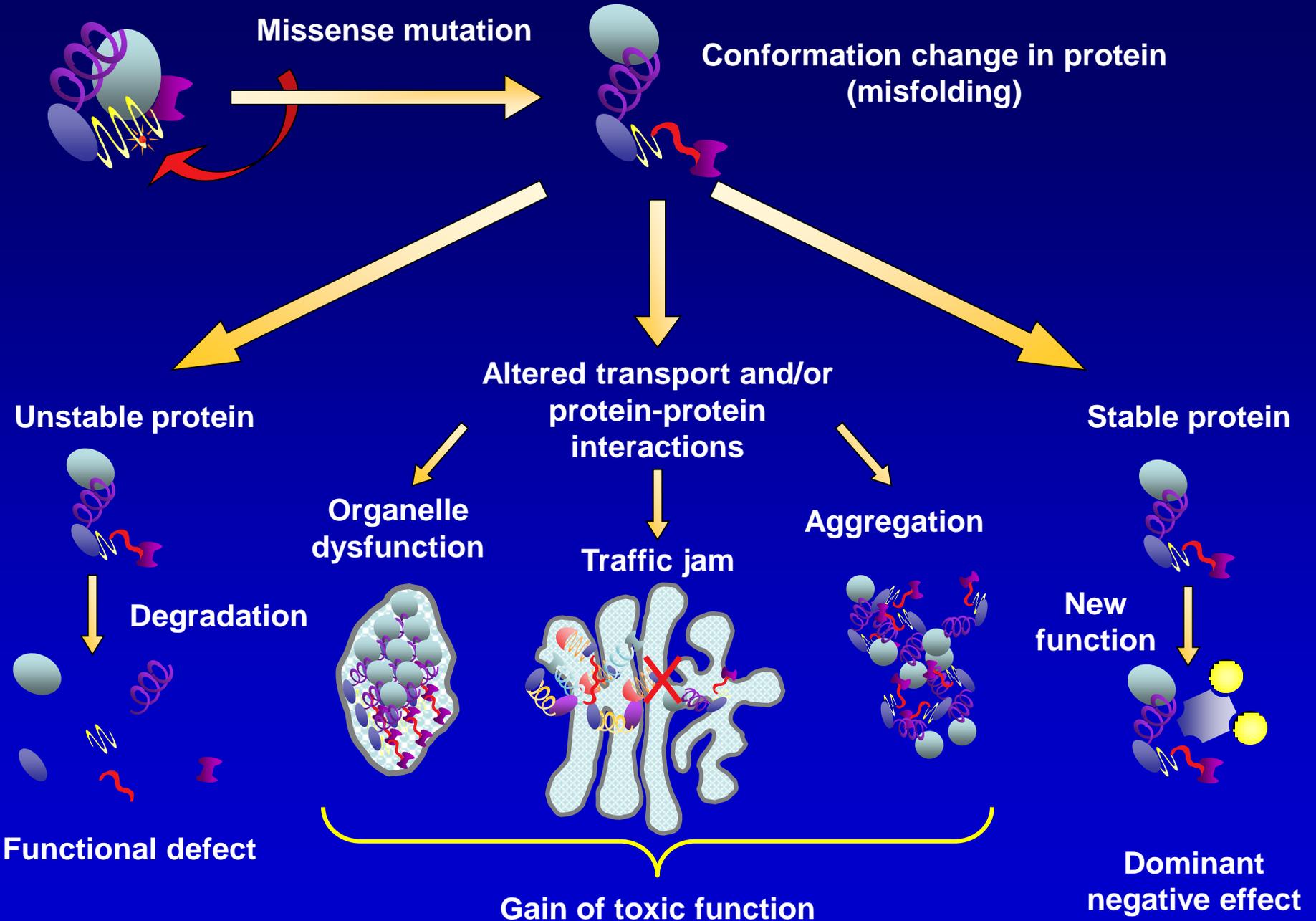
Degradation

New function

Functional defect

Gain of toxic function

Dominant negative effect



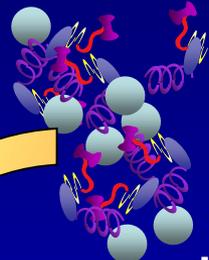
How can mutations in a metabolic enzyme lead to parkinsonism?

Gain-of-function:

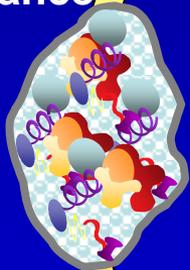
PD in *GBA*
heterozygotes

GCase in LBs

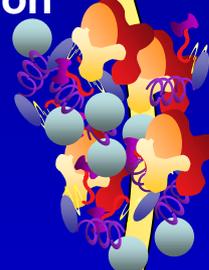
Formation of insoluble α -synuclein aggregates



Organelle dysfunction:
decreased aggregate clearance



Increased aggregate formation



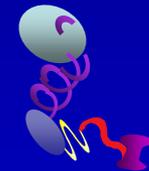
Neuronal cell death

Loss-of-function:

Null alleles
CBE effect

Mutant glucocerebrosidase may lead to...

Unstable or deficient protein



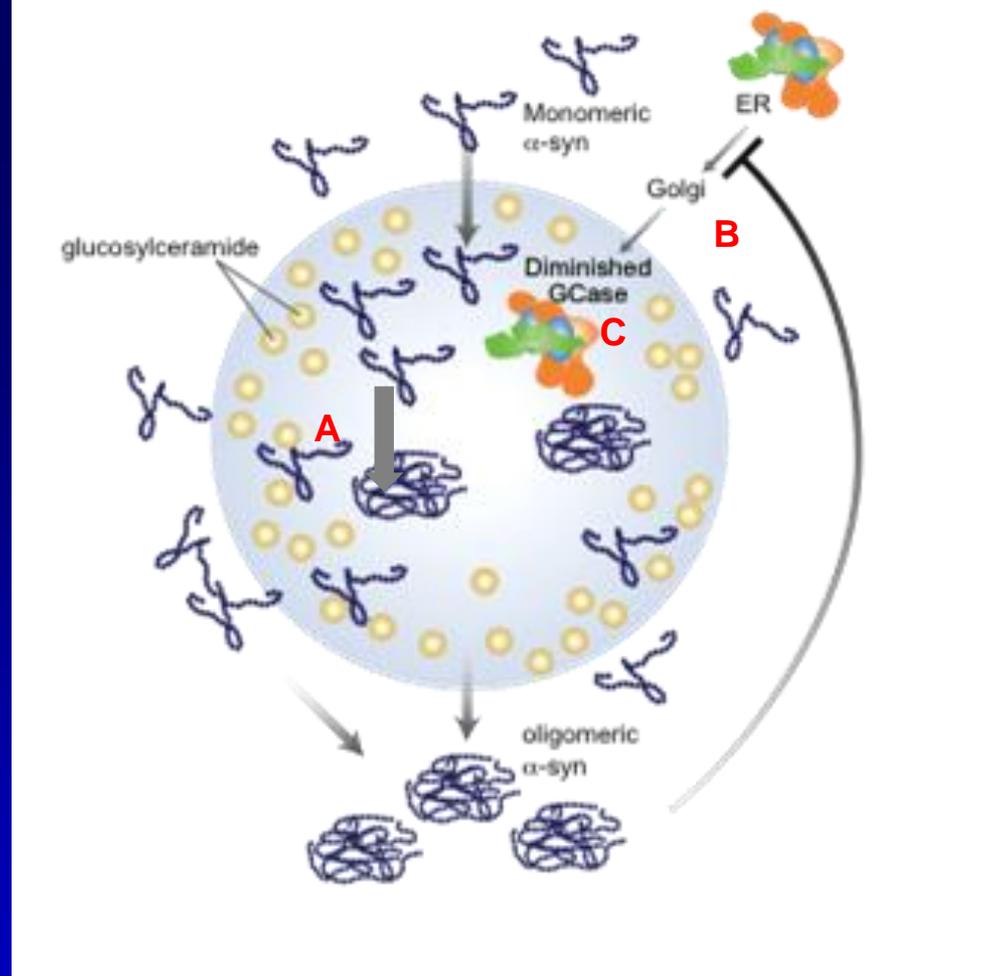
Degradation

Not enough enzyme =
Lipid accumulation

Neuronal cell death

Bidirectional feedback loop theory (Mazzulli et al. Cell, 2011)

Study with shRNA, neuronal cells, iPSc, C. Elegans, human and mouse brain

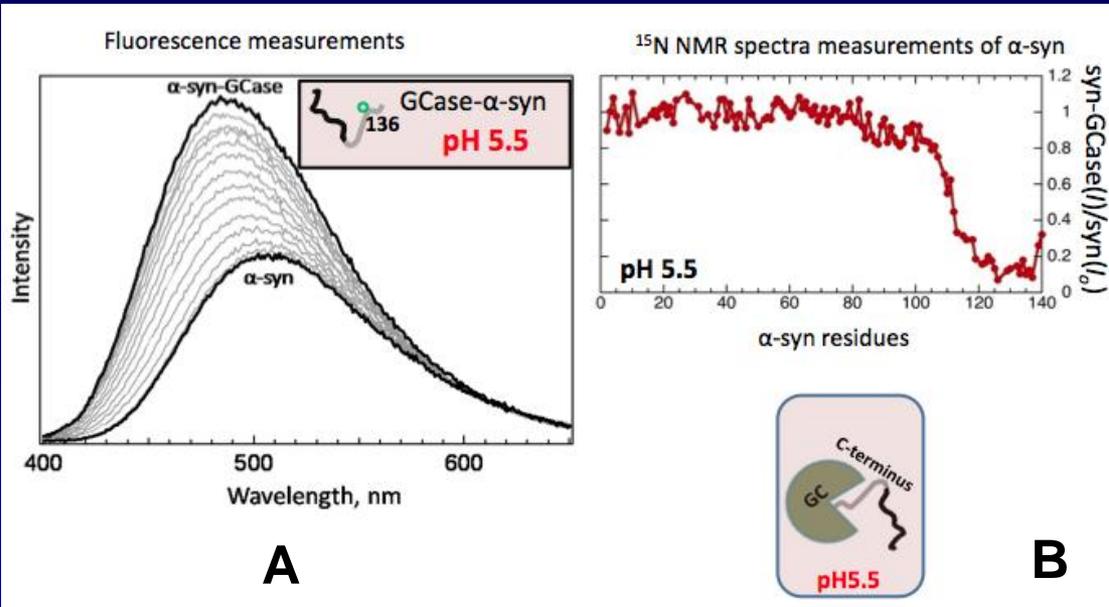


- A) Lysosomal **GluCer** promotes formation of soluble **α-syn** oligomers and fibrils
- B) Increased **α-syn** oligomers block ER-Golgi trafficking of **GCCase**
- C) Decreased lysosomal **GCCase** amplifies increased **GluCer** and stable **α-syn**

However, questions remain since Gaucher carriers do not have **GluCer** accumulation and lysosomal dysfunction. **Story may still be more complicated.**

A molecular link between α -synuclein and glucocerebrosidase

Collaboration with J. Lee, NHLBI (*JBC* 2011)



A) Dns-136- α -syn + GCCase-spectrum shifts indicate physical interaction.

B) NMR shows interaction is at C-terminus of α -synuclein.

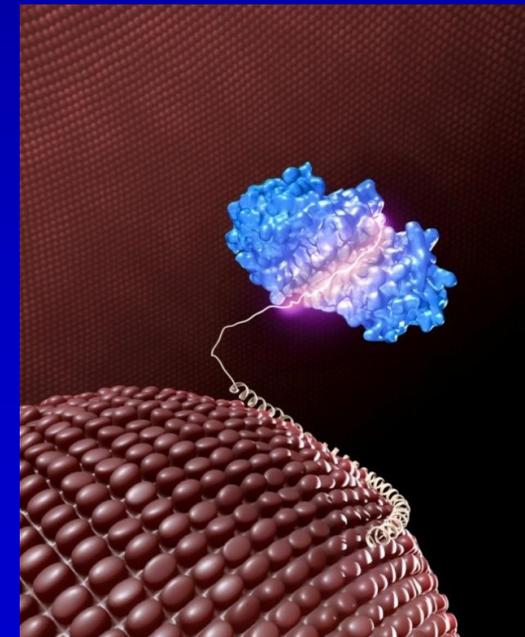
C) In brain extracts, GCCase co-immunoprecipitates with α -syn.

All 3 occur at pH 5.5 (not at pH 7, with N370S GCCase or with α -glu).

Model: C-terminal region of α -syn interacts with GCCase (glowing). N-terminal helix bound to a glycolipid-rich vesicle in lysosomes.

Membrane bound α -syn interacts with GCCase and inhibits activity

Could this binding at lysosomal pH facilitate α -synuclein degradation or prevent aggregation?



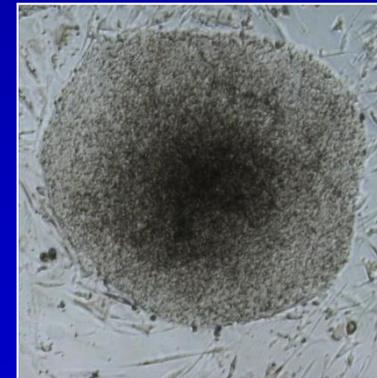
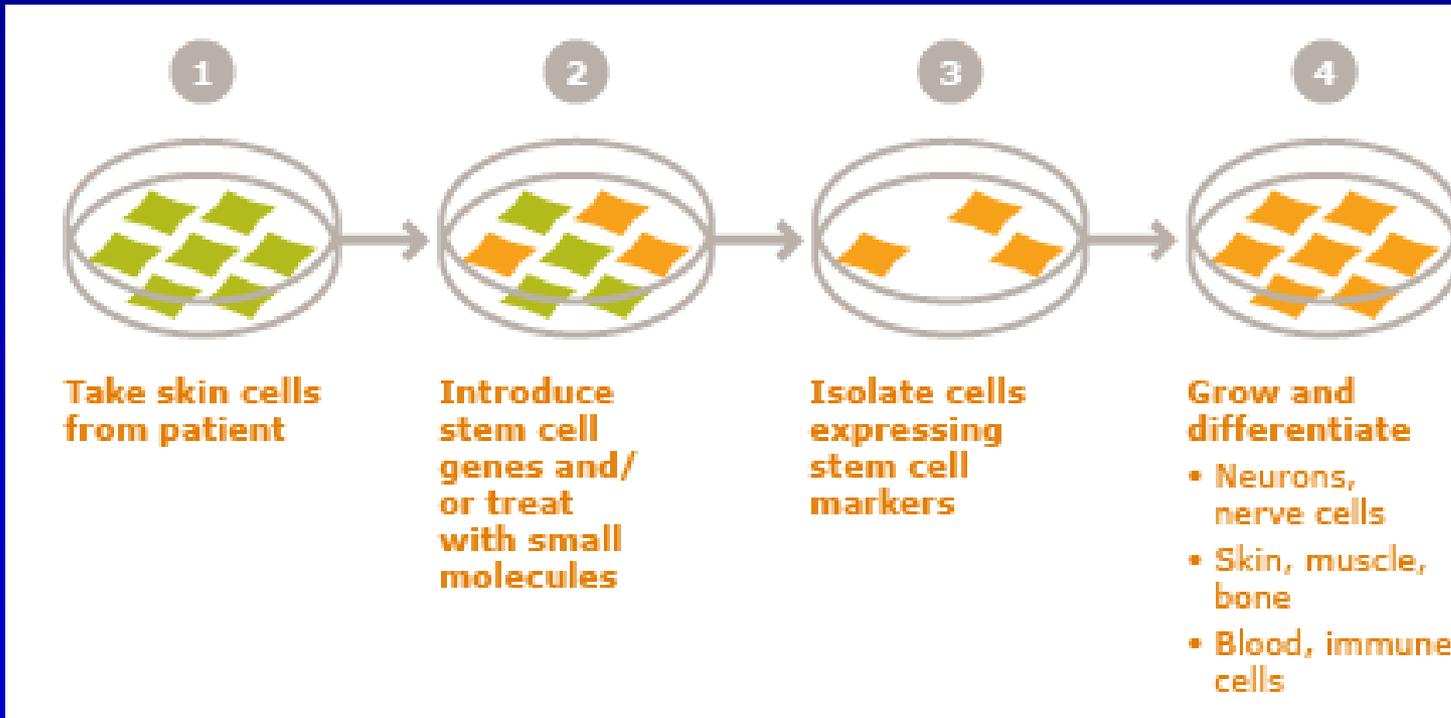
Needed: Better models for studies

Mouse models do not mimic the human phenotype.

Skin fibroblasts, used in some studies don't show lipid storage.

Patient-derived induced Pluripotent Stem cells (iPSCs cells)

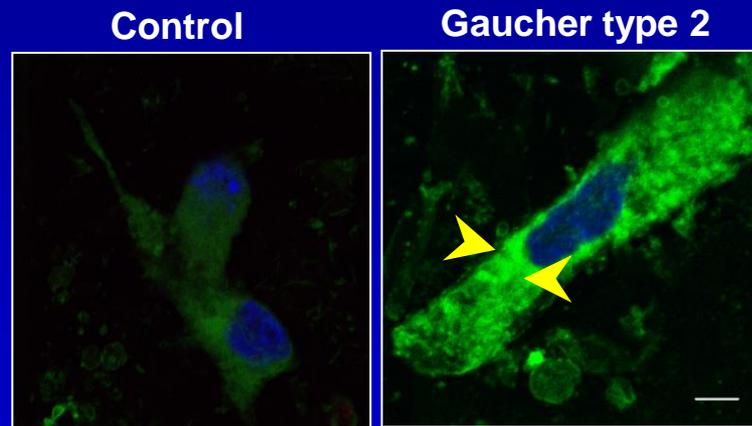
iPSCs are reprogrammed adult fibroblasts that can grow for extensive periods and be used to form any cell type.



iPS cells from type 2 GD

Development of Gaucher iPSC cells

- iPSCs generated from type 2 GD fibroblasts (L444P/IVS2+1).
- Differentiated into Gaucher macrophages- show lipid storage.
- Neurons now generated. Will make iPSC lines from patients with PD and Gaucher disease.



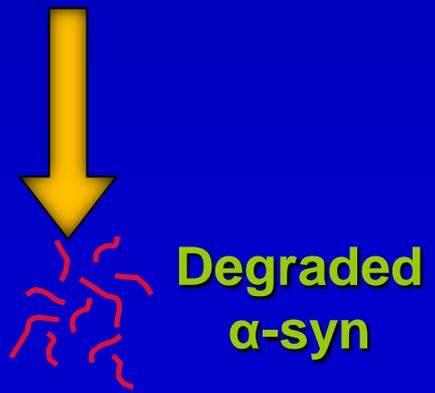
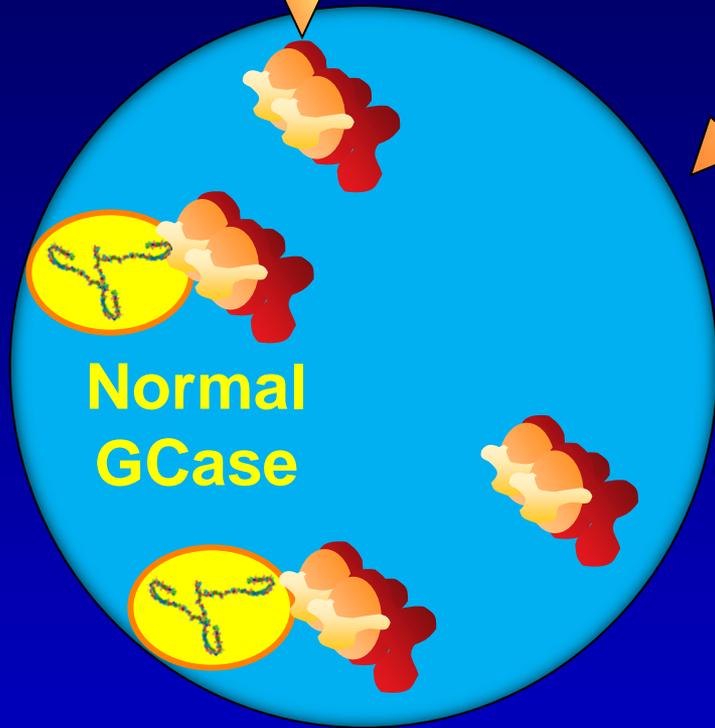
hiPSC –derived macrophages
(fed with labeled ghost cells)

DAPI 
Bodipy-GlcCer 

**Proteosomal
and/or
mitochondrial
pathways**

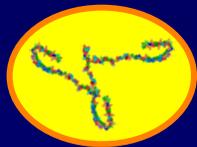


**Normally
functioning
lysosome**

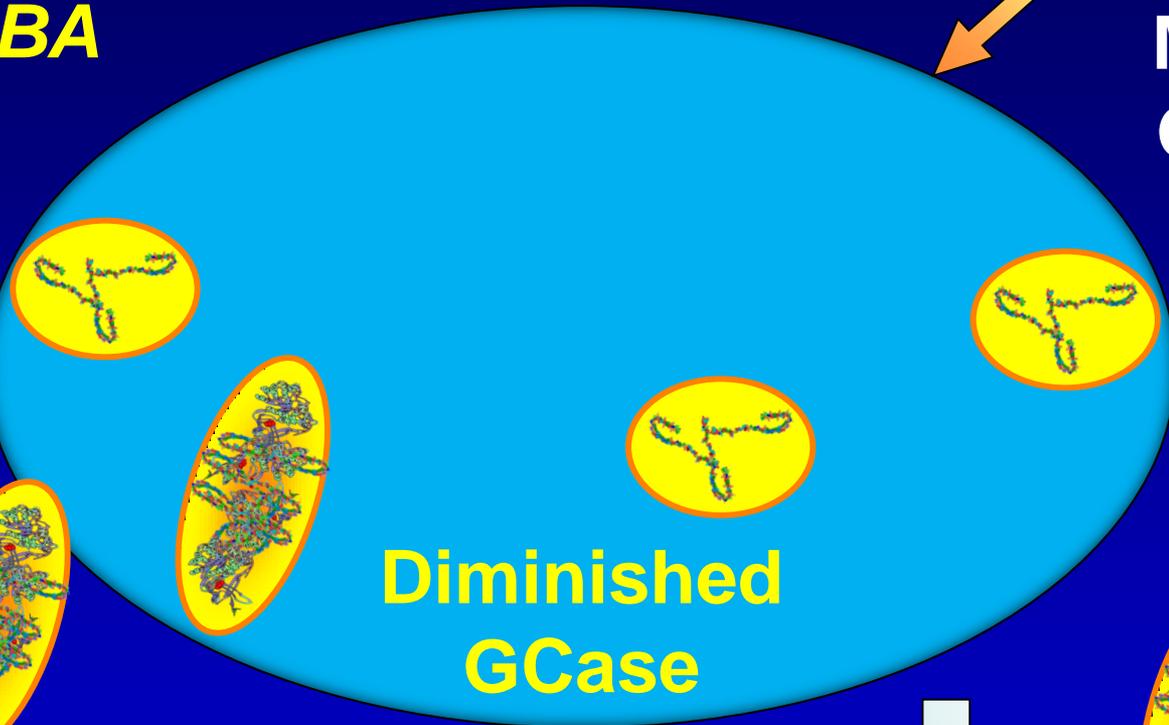
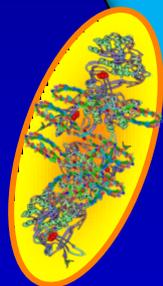
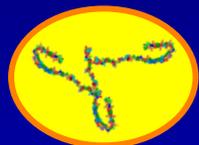
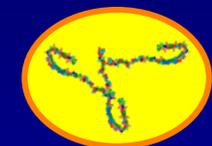


**Lysosome
with mutant
GBA**

α -syn

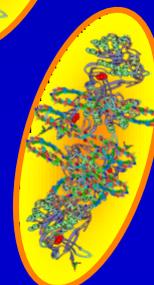
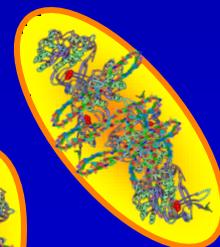
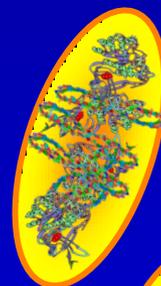


**Mutant
GCase**



**Diminished
GCase**

α -syn
aggregates

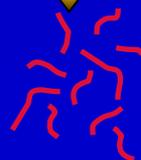


With aging:

↓ # lysosomes

↓ function

↑ α -syn



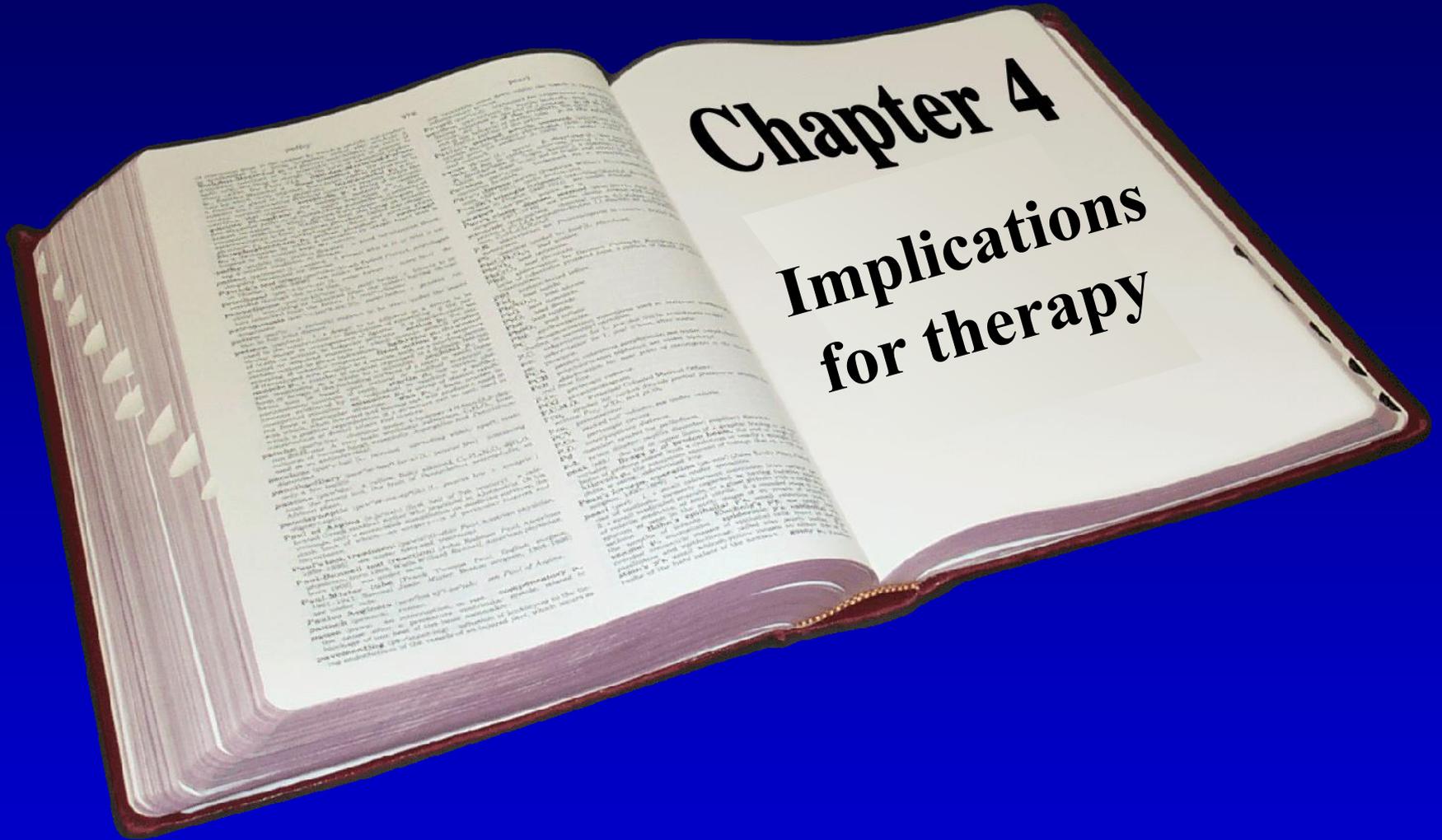
**Degraded
 α -syn**



**Neuronal
cell death**

Chapter 4

Implications for therapy



Enzyme Replacement Therapy

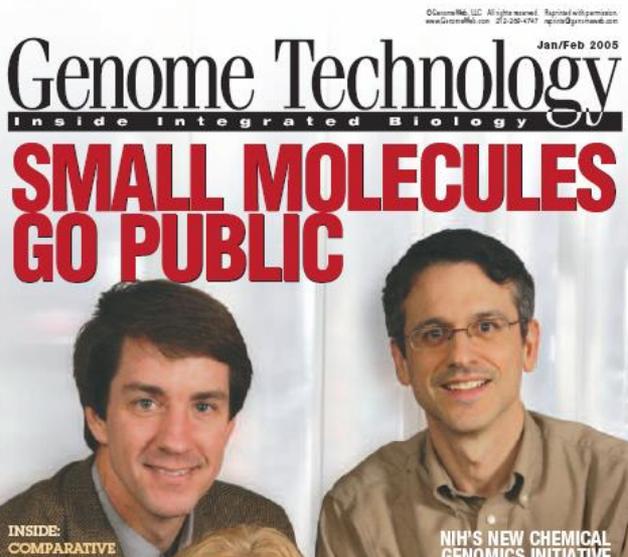
Results:

- ↑ Hemoglobin levels in 4-6 months
- ↑ Platelet counts
- ↓ Splenic and hepatic volume
- ↑ Feeling of well-being & growth velocity

- High cost (\$100,000 - \$400,000/patient/year)
- Response is highly variable; IV preparation
- Does not alter CNS manifestations



We have been working with NCGC to develop small molecule therapies



PNAS

Three classes of glucocerebrosidase inhibitors identified by quantitative high-throughput screening are chaperone leads for Gaucher disease

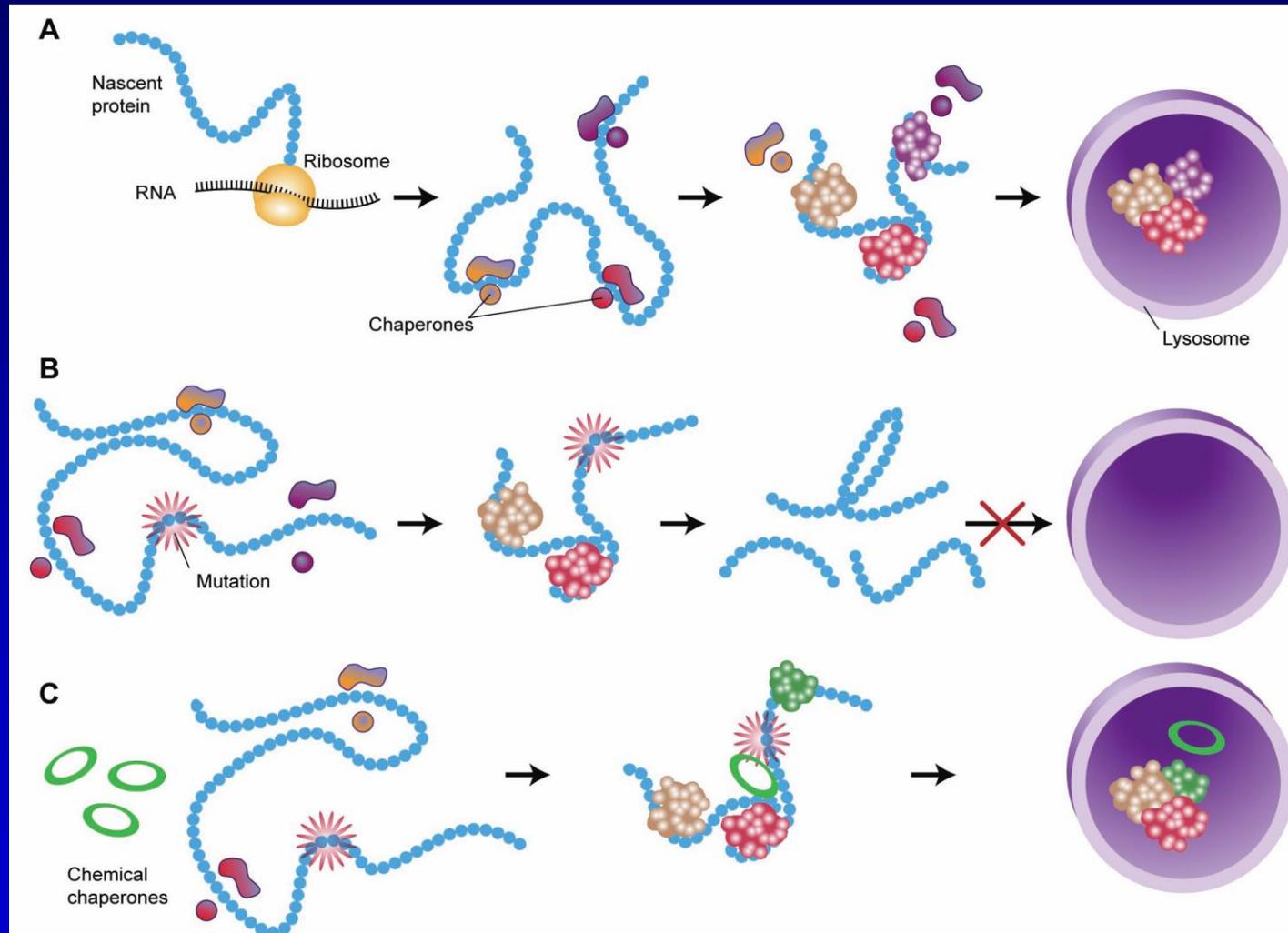
Wei Zheng*, Janak Padia*, Daniel J. Urban[†], Ajit Jadhav*, Ozlem Goker-Alpan[†], Anton Simeonov*, Ehud Goldin[†], Douglas Auld*, Mary E. LaMarca[†], James Inglesse*, Christopher P. Austin^{**}, and Ellen Sidransky^{†‡}

*NIH Chemical Genomics Center, National Human Genome Research Institute, National Institutes of Health, 9800 Medical Center Drive, MSC 3370, Bethesda, MD 20892-3370; and [†]Medical Genetics Branch, National Human Genome Research Institute, National Institutes of Health, Building 35 Rm1A213, 35 Convent Drive, Bethesda, MD 20892-3708

Chemical chaperone therapy for Gaucher disease

A) GCase is synthesized in the ER, glycosylated and folded, attaining functional tertiary structure in the lysosomes

Chaperones:
assist in folding
proteins



Chemical chaperones may partially correct the enzyme deficiency in Gaucher disease

B) However, mutant GCase may be misfolded and degraded.

C) Chemical chaperones may bind to the active site and enhance folding, allowing delivery to the lysosome.

High Throughput Screening (HTS)

Screen Goal:

- To identify activators and inhibitors of glucocerebrosidase
- To find potential pharmacological chaperones

Assay Development:

- Use commercially available enzyme
- Miniaturize enzymatic assay
- Fluorogenic readout
- Homogenous assay in 1536- well plate format



Primary Screening:

HTS with 7-point concentration-response curves for all compounds in library collection (60,000- 500,000)



New GCase chaperones as therapy for GD

New screening approach: (*Goldin et al. PLoS One 2012*)

- **Patient spleen- source of mutant GCase (N370S/N370S).**

- **HT Screen performed with 250K compounds.**

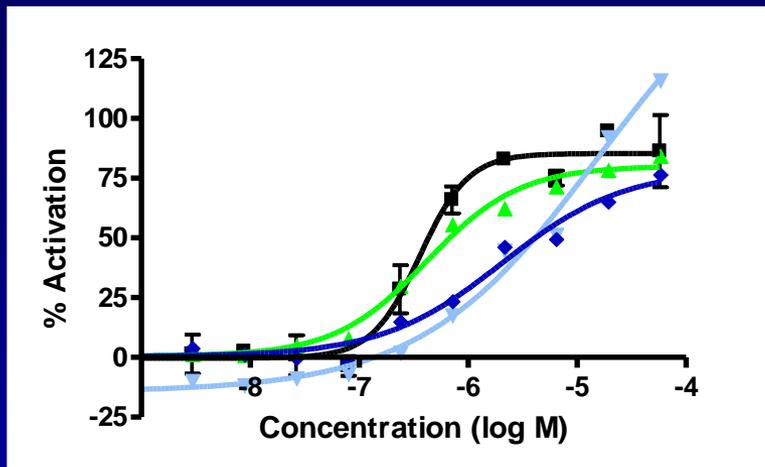
New compounds identified: both activators & inhibitors.

- **Lead compound reverses lipid storage and enhances enzyme activity.**

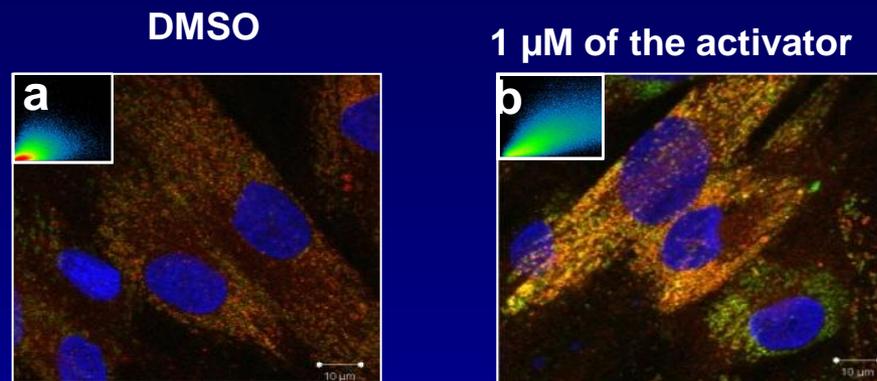
- **Plan to advance development through NIH TRND and/or with partners in industry.**

Small molecule therapy may stabilize and/or activate mutant GCase and be used to treat GD as well as GBA-associated PD

Results with lead compound *J Med Chem* 2012



GCCase activity with lead activator in four different assays

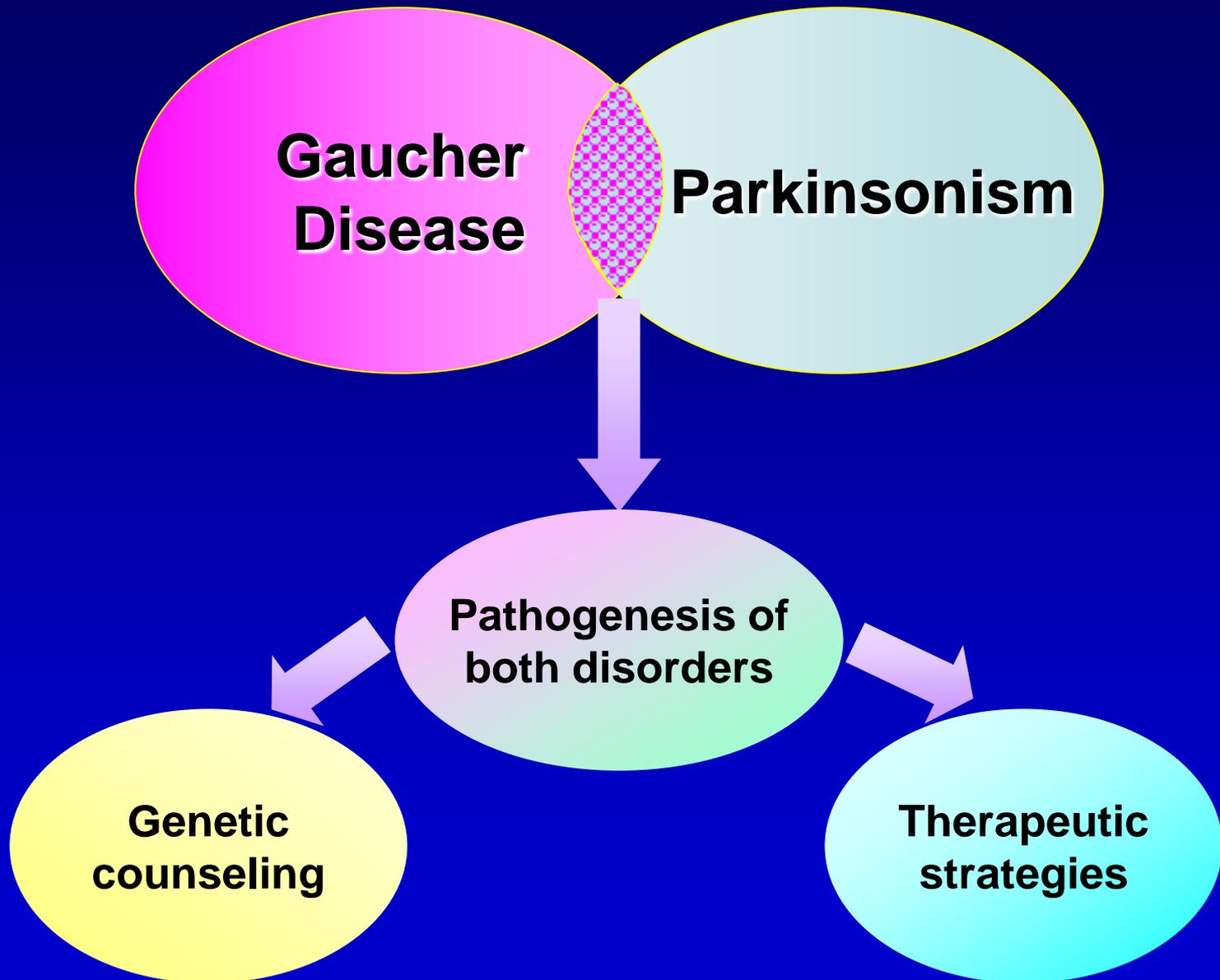


N370S/N370S fibroblasts GCCase; LAMP-2

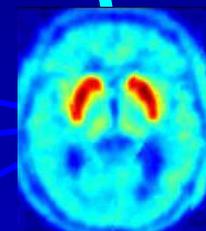
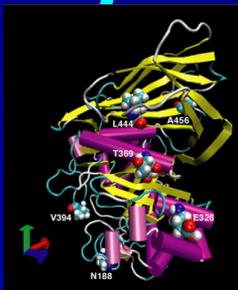
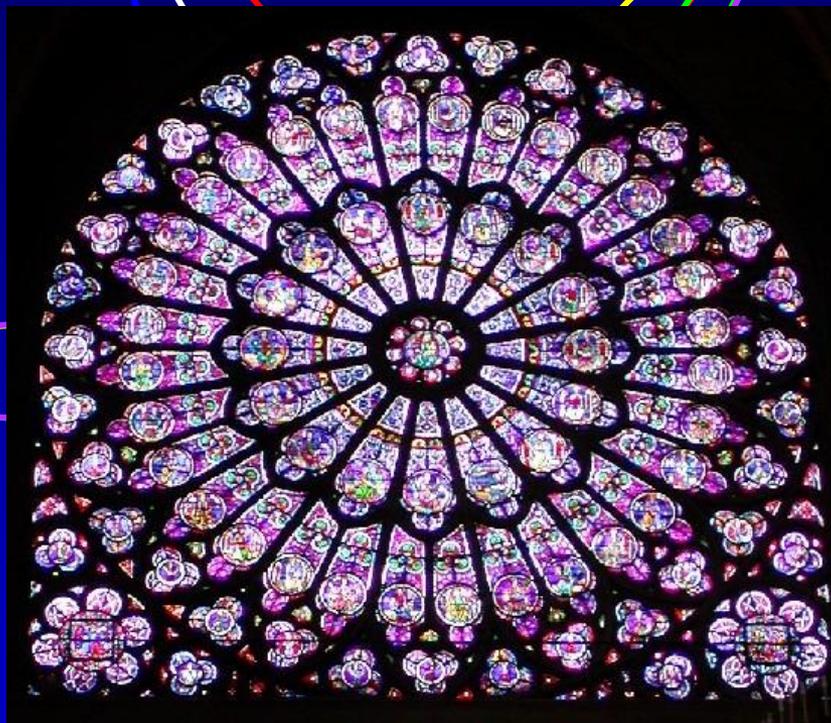
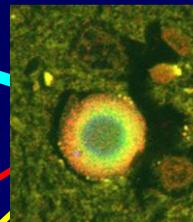
Storage is cleared by compound

Compound is selective for GCCase and did not activate other hydrolases.

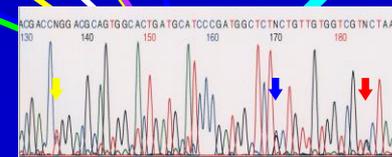
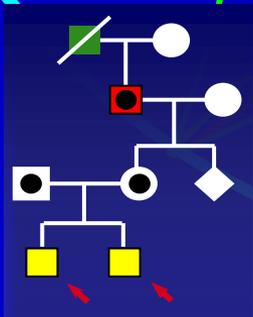
Understanding the links between the two disorders



Complexity in Mendelian disorders



A window into complex disorders



Acknowledgements

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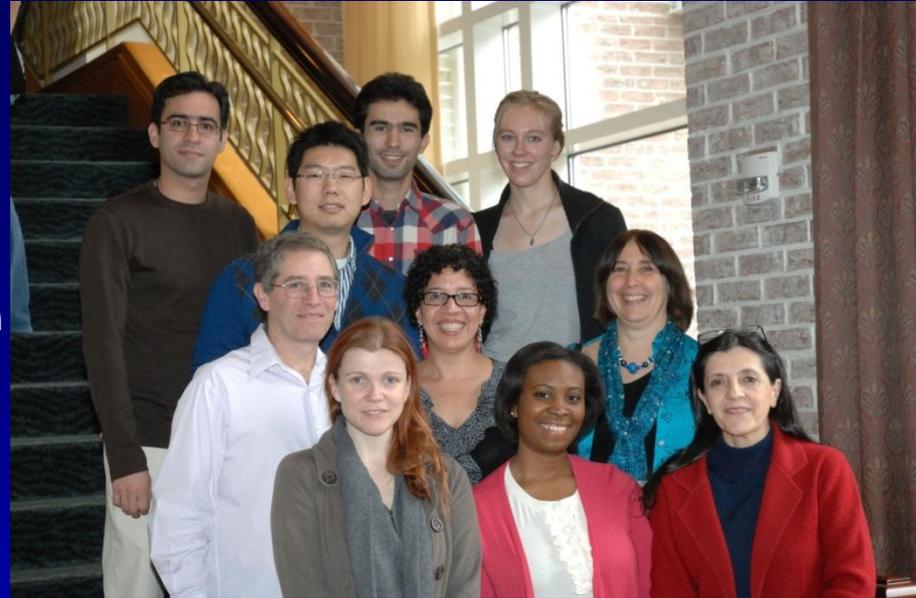
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