Parkinson's disease –

focus on proteotoxicity

Parkinson's Disease (PD)

Chronic, degenerative neurological disorder affecting 1-2% people over age 60

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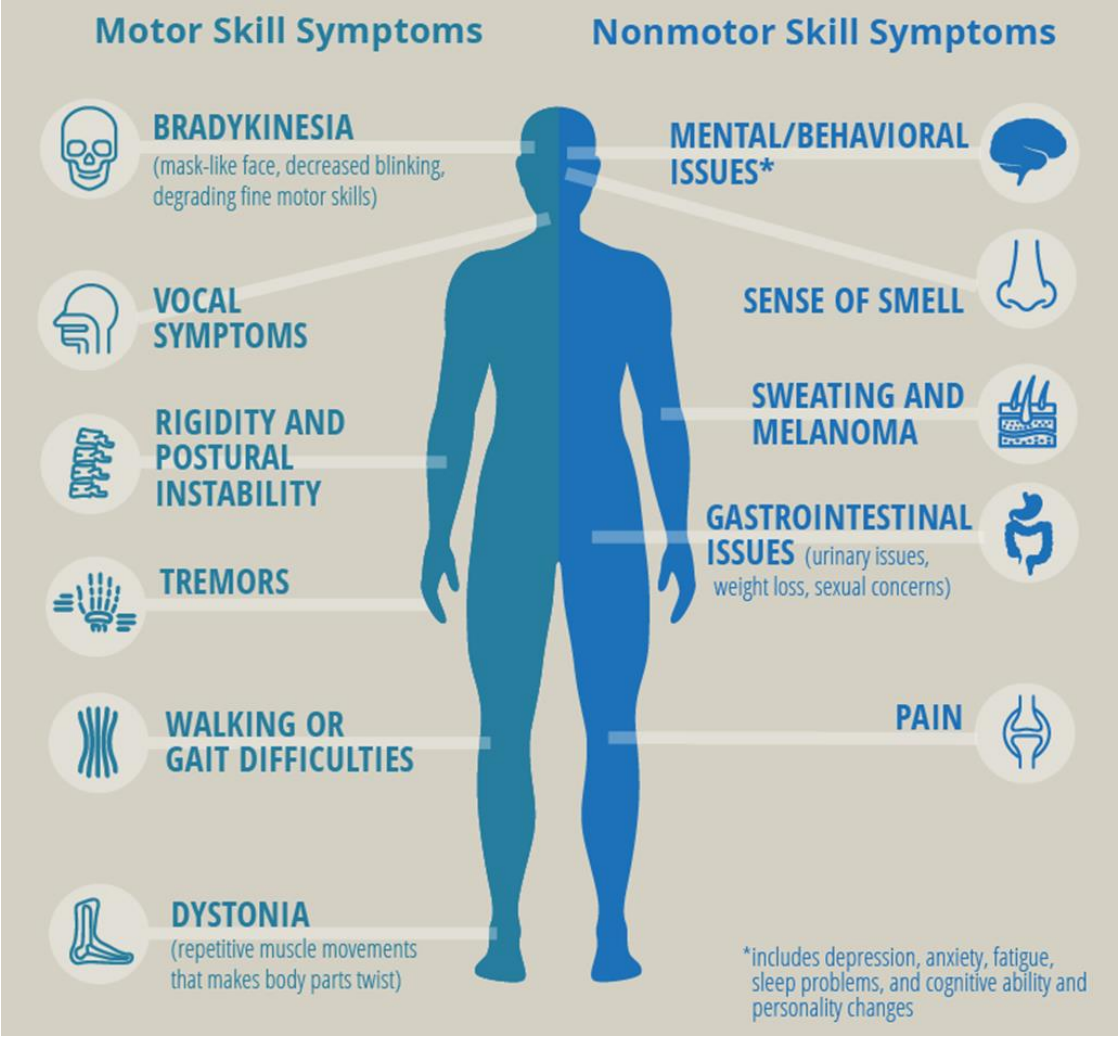
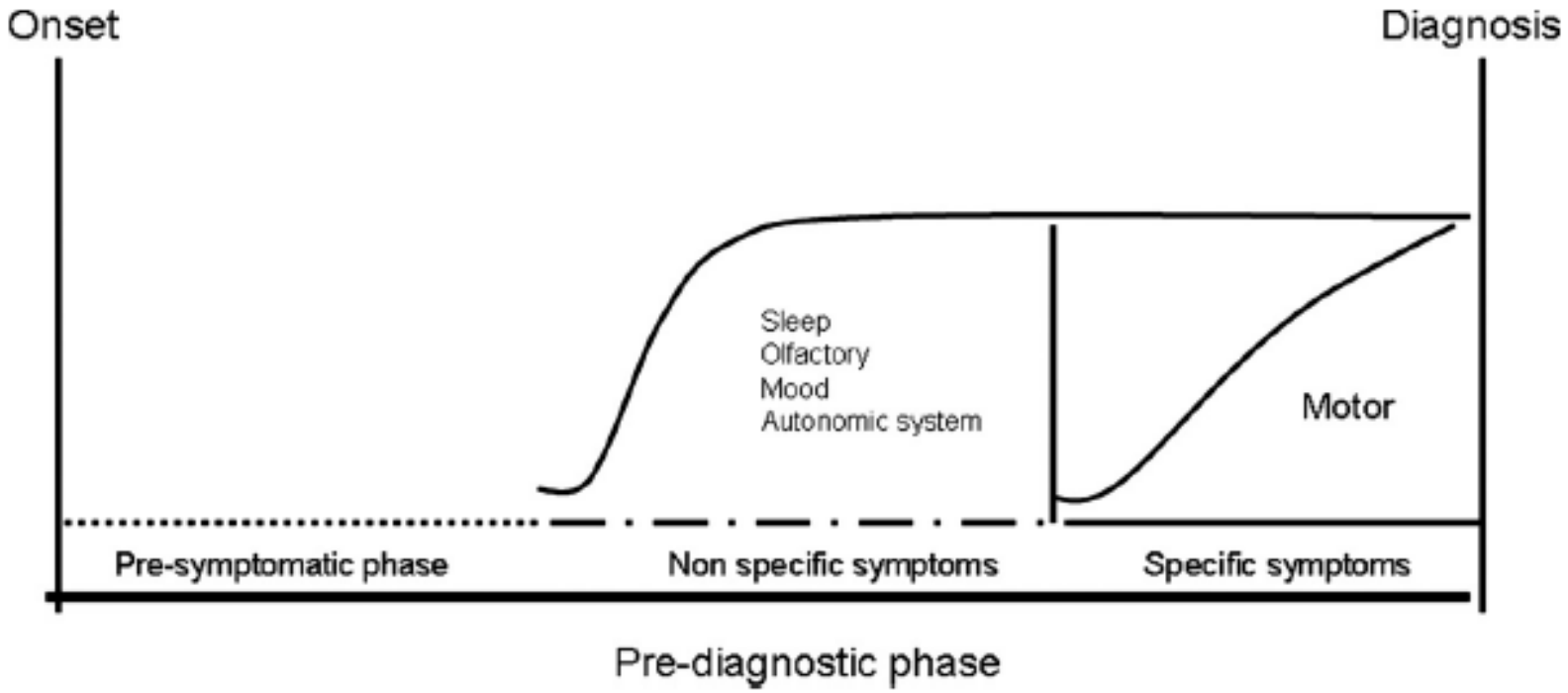
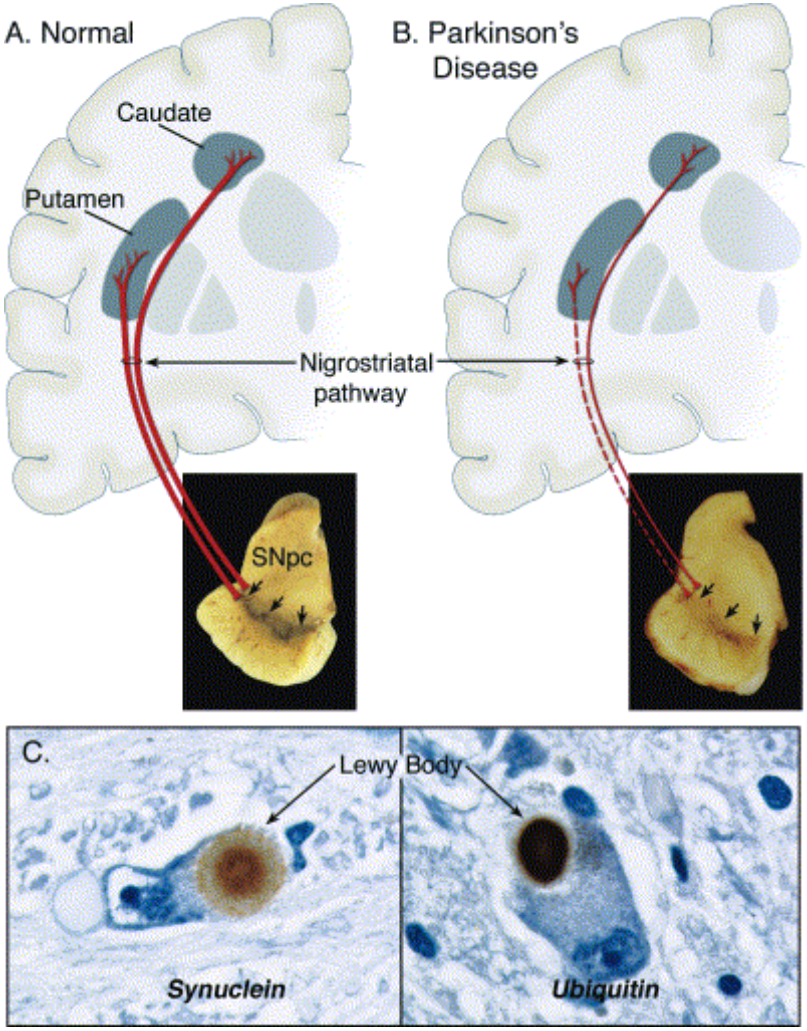


Years from disease onset to diagnosis



PD hallmarks



- Loss of dopamine-producing neurons in the substantia nigra pars compacta (SNpc)

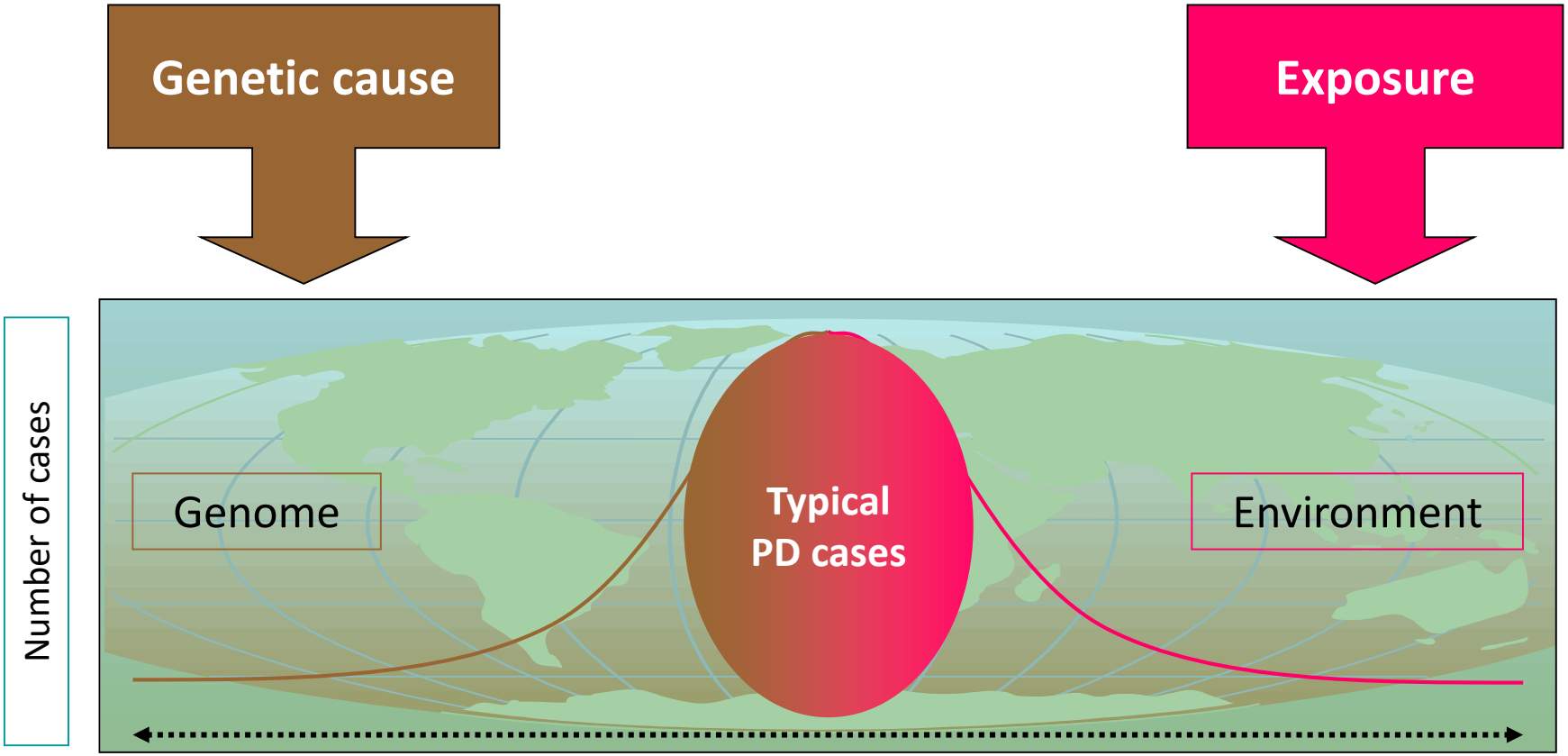
↓ dopamine

PD is a movement disorder

- Alpha-synuclein-enriched inclusions known as Lewy bodies

Dauer W. et al, Neuron, 2003

Causes of PD



- 5-10% of PD patients have a monogenic form of PD with Mendelian inheritance
- ~15% of PD patients have family history

Genetics of PD: monogenic variants associated with PD

Table 1 Selected genetic loci associated with familial Parkinsonism

Locus	Locus map	Inheritance pattern	Gene	Clinical features	Reference
PARK1/4	4q22.1	AD	*SNCA*	Early onset, rigidity, cognitive impairment	Polymeropoulos et al. 1997
PARK2	6q26	AR	*PRKN*	Juvenile onset, dystonia	Kitada et al. 1998
PARK6	1p36.12	AR	*PINK1*	Early onset, dystonia	Valente et al. 2002
PARK7	1p36.23	AR	*PARK7*	Early onset, dystonia	Abou-Sleiman et al. 2003
PARK8	12q12	AD	*LRRK2*	Classic PD	Funayama et al. 2002
PARK9	1p36.13	AR	*ATP13A2*	Early onset, cognitive impairment	Di Fonzo et al. 2007
PARK14	22q13.1	AR	*PLA2G6*	Early onset, cognitive impairment, dystonia	Paisán-Ruíz et al. 2009
PARK15	22q12.3	AR	*FBXO7*	Early onset	Di Fonzo et al. 2009
PARK17	16q11.2	Unknown	*VPS35*	Adult onset, cognitive impairment, dystonia	Zimprich et al. 2011
PARK19a/b	1p31.3	AR	*DNAJC6*	Early onset, cognitive impairment	Edvardson et al. 2012
PARK20	21q22.11	AR	*SYNJ1*	Early onset, seizures	Krebs et al. 2013
PARK21	3q22	AD	*DNAJC13*	Classic PD	Vilariño-Güell et al. 2014
PARK23	15q22.2	AR	*VPS13C*	Early onset, rapid progression, cognitive impairment	Lesage et al. 2016

The inheritance pattern, gene, clinical features, and relevant reference for each locus are provided. Classic PD refers to symptoms that resemble sporadic PD. Abbreviations: AD, autosomal dominant; AR, autosomal recessive; PD, Parkinson's disease.

Annu. Rev. Neurosci. 2021. 44:87–108 https://doi.org/10.1146/annurev-neuro-100720-034518

Genetics of PD: over 90 risk loci identified

Table 4 Genes associated with single nucleotide polymorphisms that modulate Parkinson's disease risk

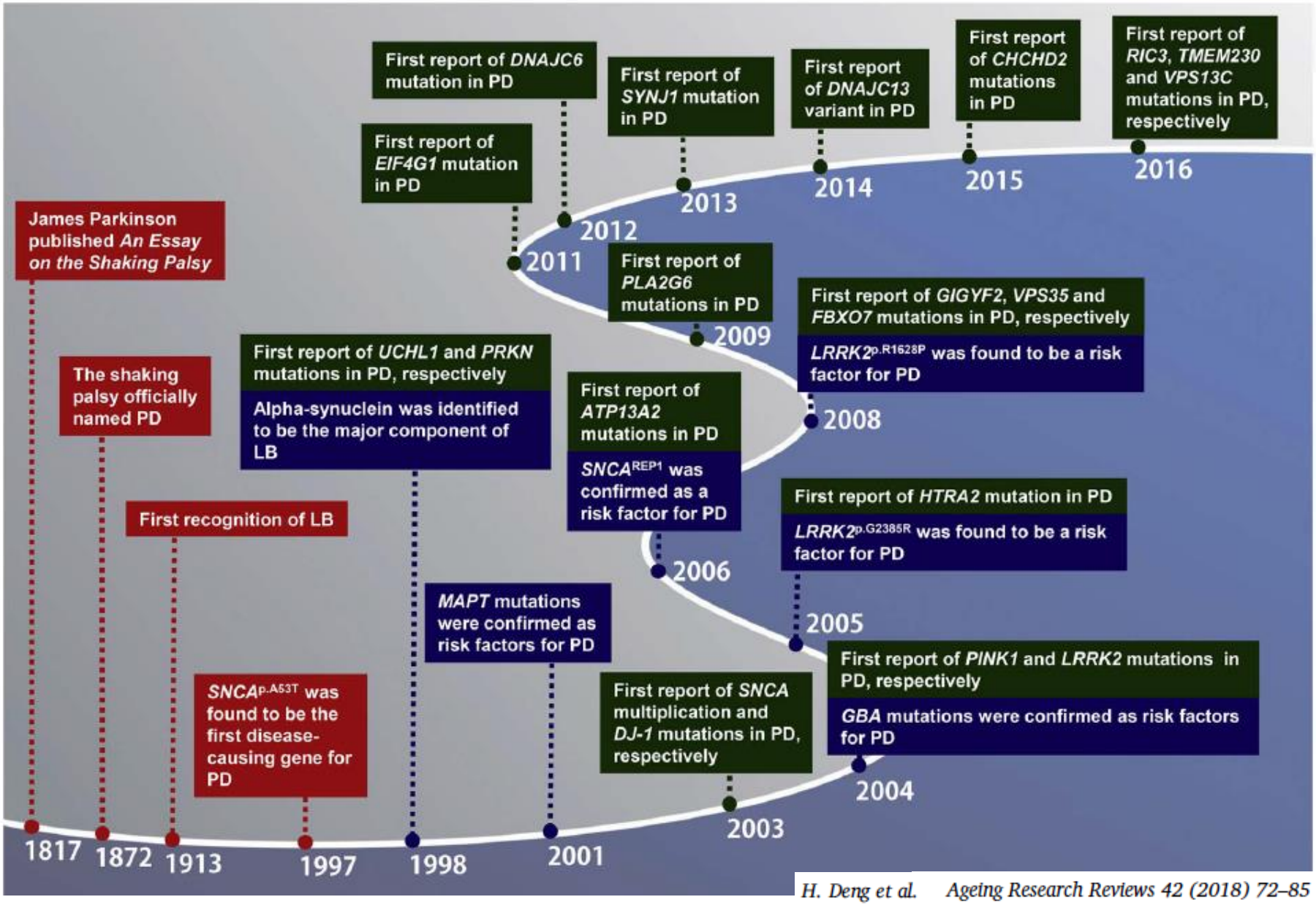
Category	Candidate genes
Cytoskeleton	*CAB39L, TUBG2, MAPT, DNAH17, ANK2, PDLIM2, SORBS3*
Endosomal and vesicular trafficking	*VAMP4, SIPA1L2, SNCA, CHMP2B, LRRK2, BIN3, RIMS1, DDRGK1, SYT4, ATP6V0A1, GBF1, ARHGAP27, SH3GL2*
Immune system	*FCGR2A, IL1R2, HLA-DRB6, HLA-DQA1, FYN, CD19, CD38, NOD2, TRIM40, FAM49B, ITIH3, ITIH4, TLR9, STAB1*
Ion channels, transporters, and neurotransmitter signaling	*KCNS3, KCNIP3, TMEM163, SCN3A, CHRNB1, CLCN3, GCH1, NCKIPSD, CAMK2D*
Lipid metabolism and signaling	*SPTSSB, ELOVL7, DGKQ*
Lysosome and autophagosome	*GBA, CTSB, GALC, KAT8, TMEM175*
Mitochondria	*SLC41A1, COQ7, VPS13C, BAG3, MCCC1, CRLS1, MICU3*
Nucleus and gene regulation	*NUCKS1, CCNT2, SATB1, KPNA1, MED12L, LCORL, MBNL2, MEX3C, MIR4697, TOX3, UBTF, LSM7, BRIP1, ASXL3, RPS6KL1, PSMC3IP, SREBF1, RAI1, KANSL1, RNF141, RPS12, CDC71, PHF7, NUPL2, ZNF184*
Ubiquitin pathway	*UBAP2, BAP1, KLHL7*
Miscellaneous	*ITPKB, LINC00693, DYRK1A, OGFOD2, FAM171A2, ZNF646, FAM47E, FBRSL1, MIPOL1, SCAF11, PAM, TMEM229B, CRHR1, STH, SPPL2C, DLG2, C5orf24, C8orf58, GS1–124K5·11, ALAS1, NISCH, GPNMB, FAM200B, STK39*

All genes are organized into functional categories. The miscellaneous category is reserved for genes with unknown function or with functions that do not fit into the other categories.

Polygenic risk score (PRS) accounts for 16-36% of the genetic contribution to idiopathic non-monogenic PD

Annu. Rev. Neurosci. 2021. 44:87–108 https://doi.org/10.1146/annurev-neuro-100720-034518

Milestone in PD research



H. Deng et al. Ageing Research Reviews 42 (2018) 72–85

Risk factors

age

heredity

sex (↑ risk in man > women)

head injury and pesticide exposure

smoking and caffeine consumption ↓ risk

Drugs and treatments

L-dopa

Dopamine agonists

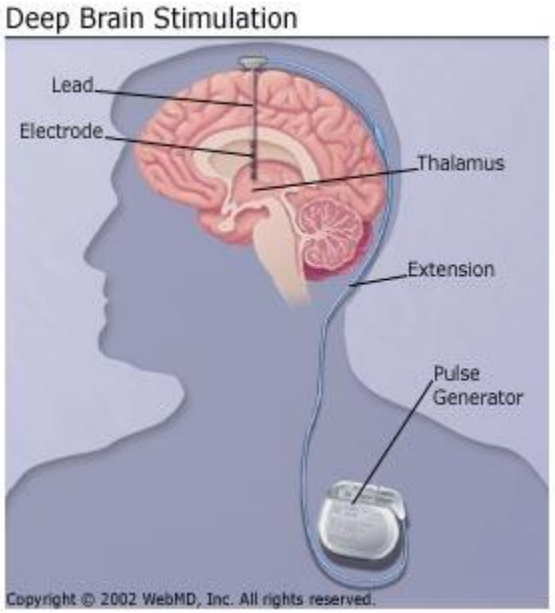
MAO-B inhibitors

COMT inhibitors

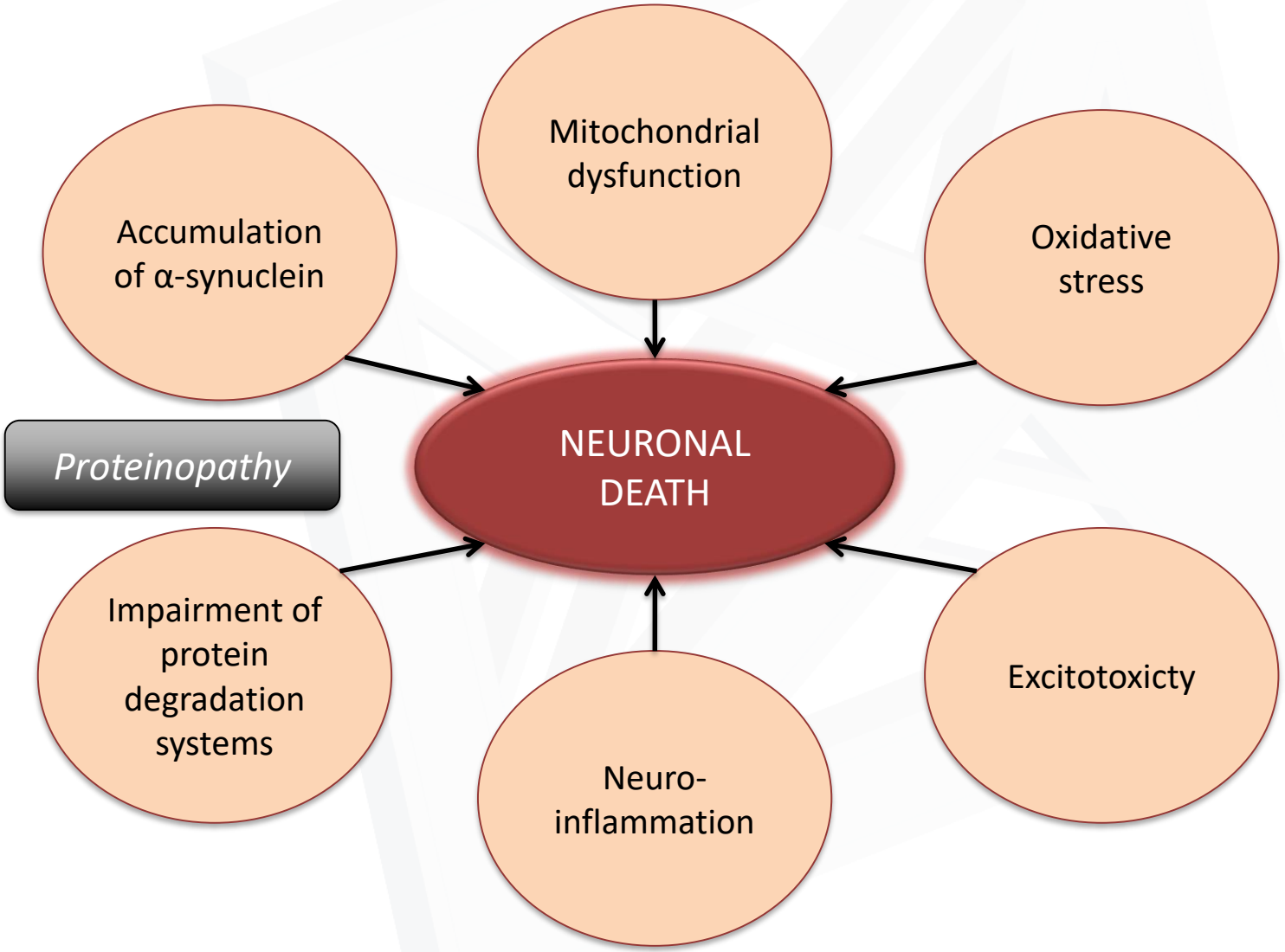
tDCS

DBS (surgical procedure)

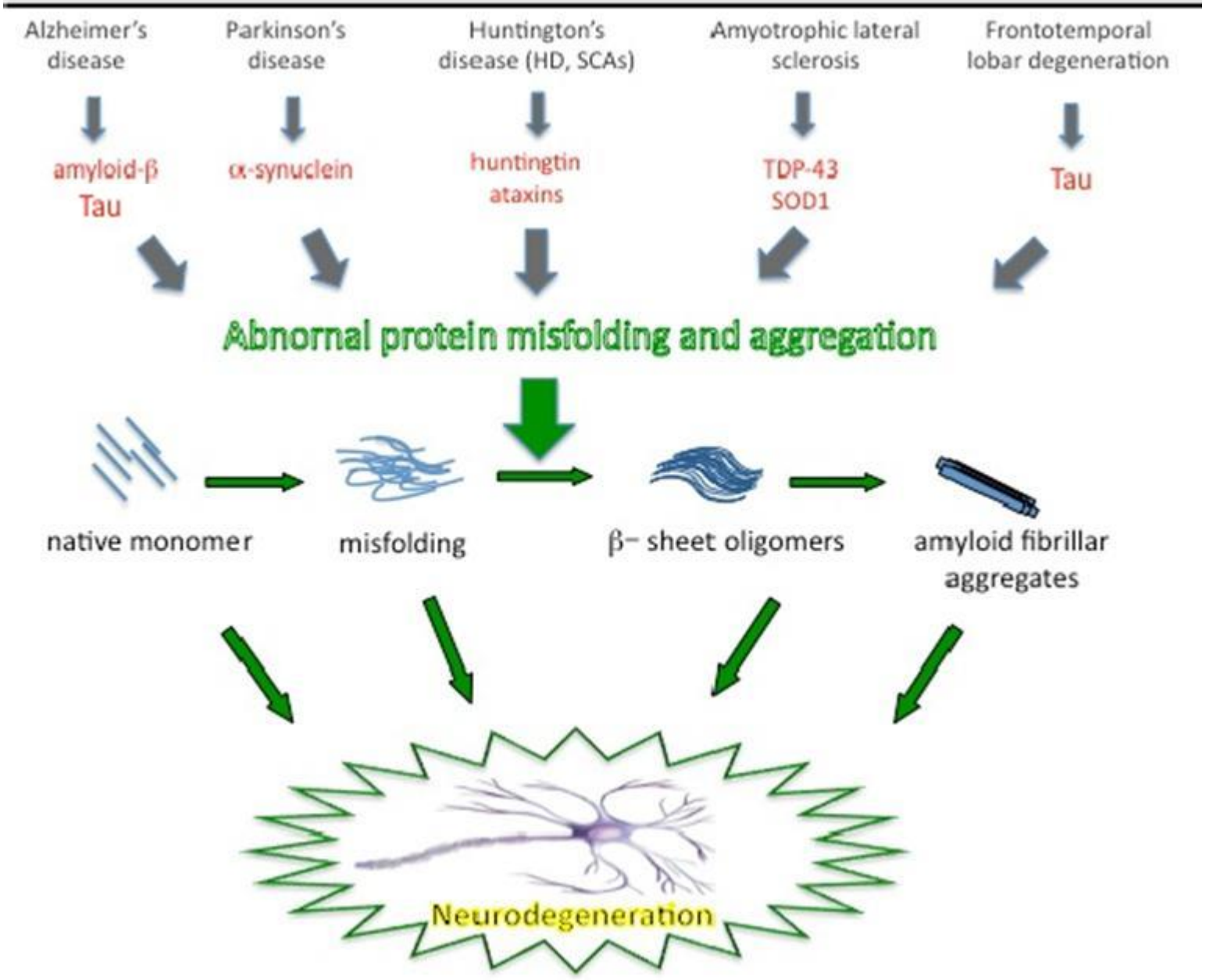
Most common targets:
subthalamic nucleus (STN)
globus pallidus interna (GPi)



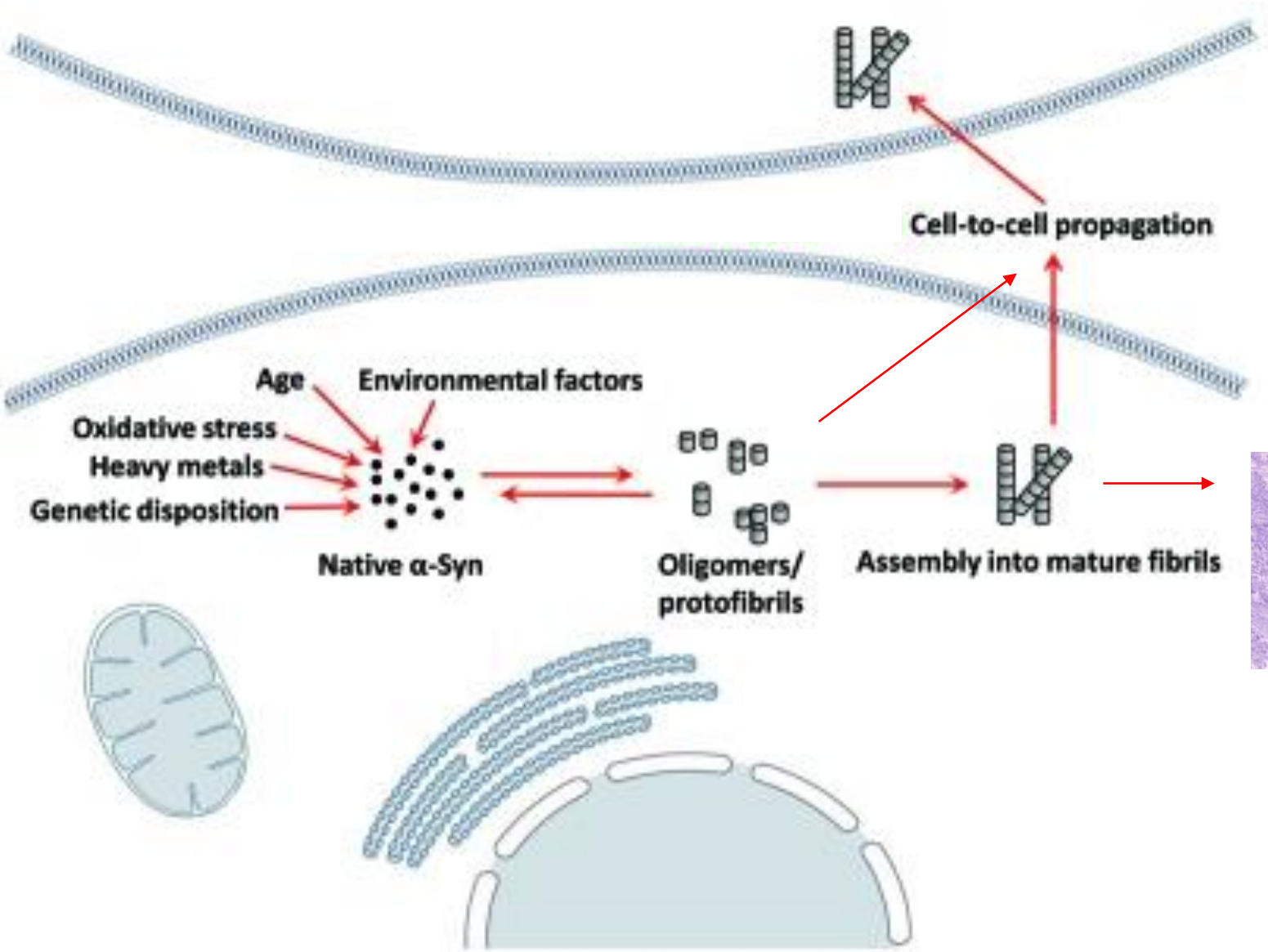
Pathogenic mechanisms

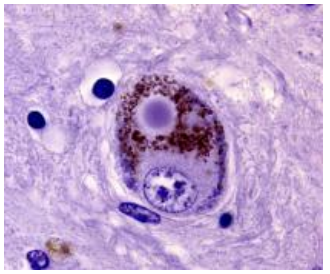


Proteinopathies



Alpha-synuclein toxicity and PD

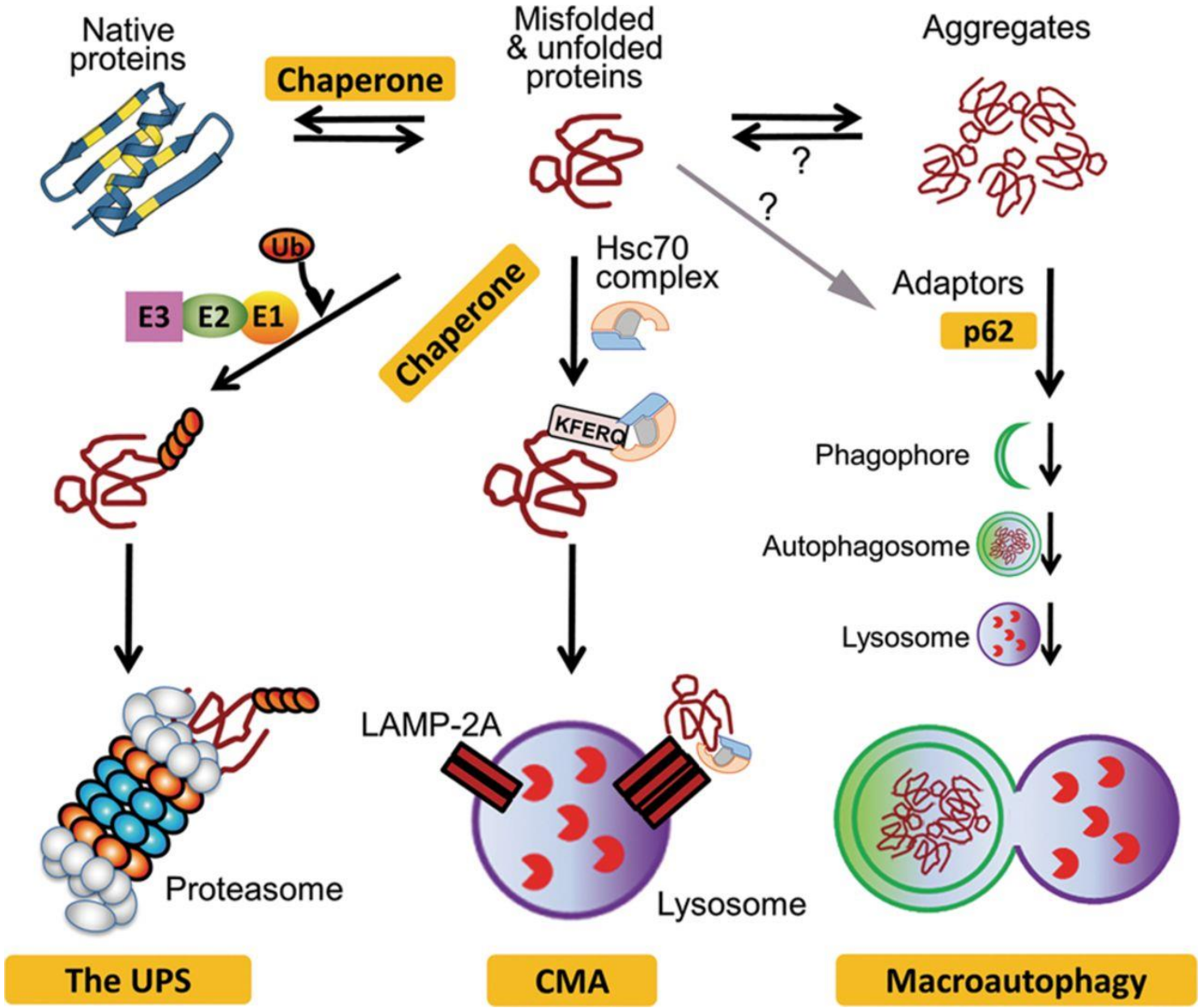




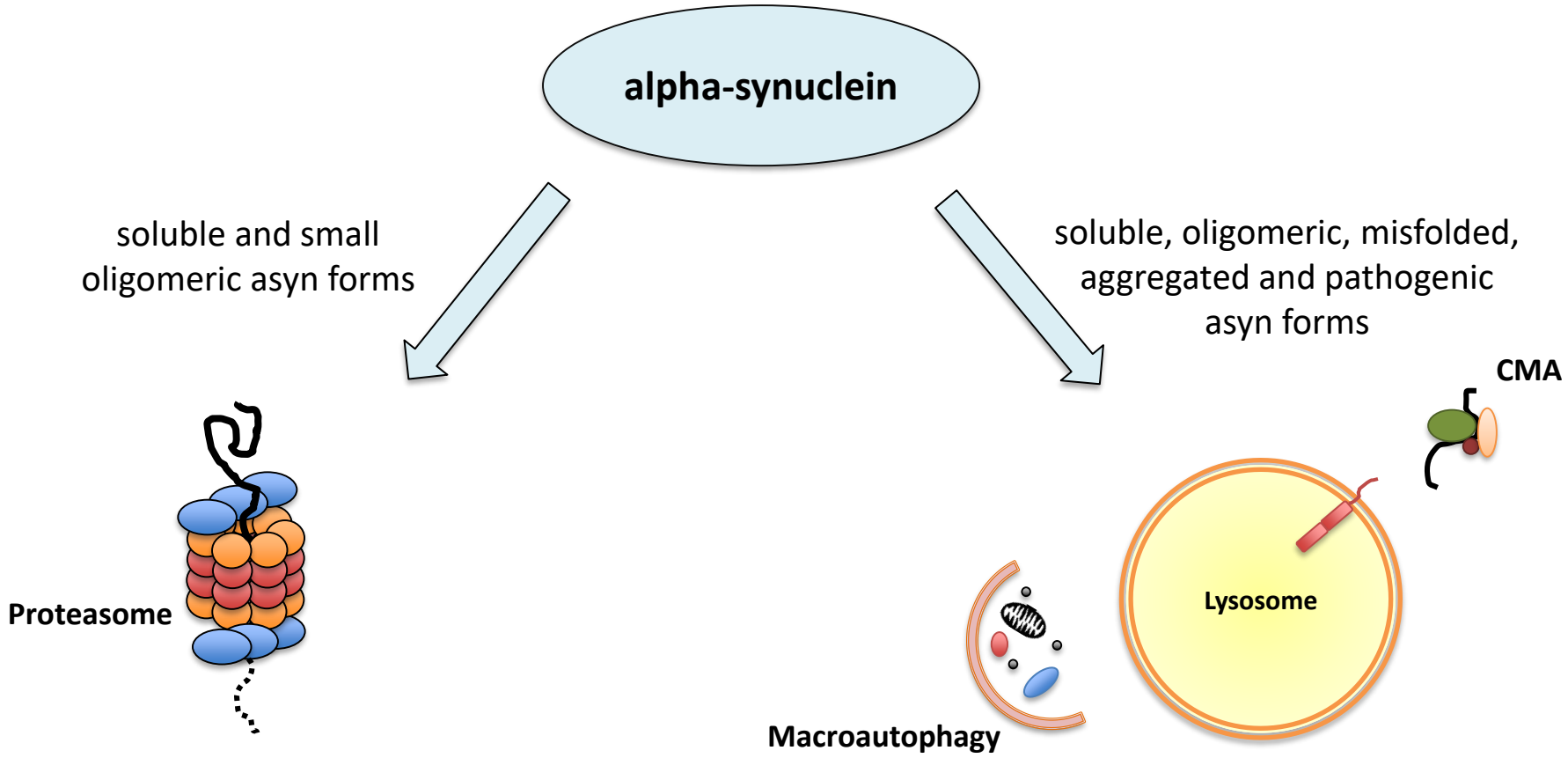
Lewy bodies

modified from: Pemberton and Melki, Commun Integr Biol. 2012 January 1; 5(1): 94–95

Protein quality control systems



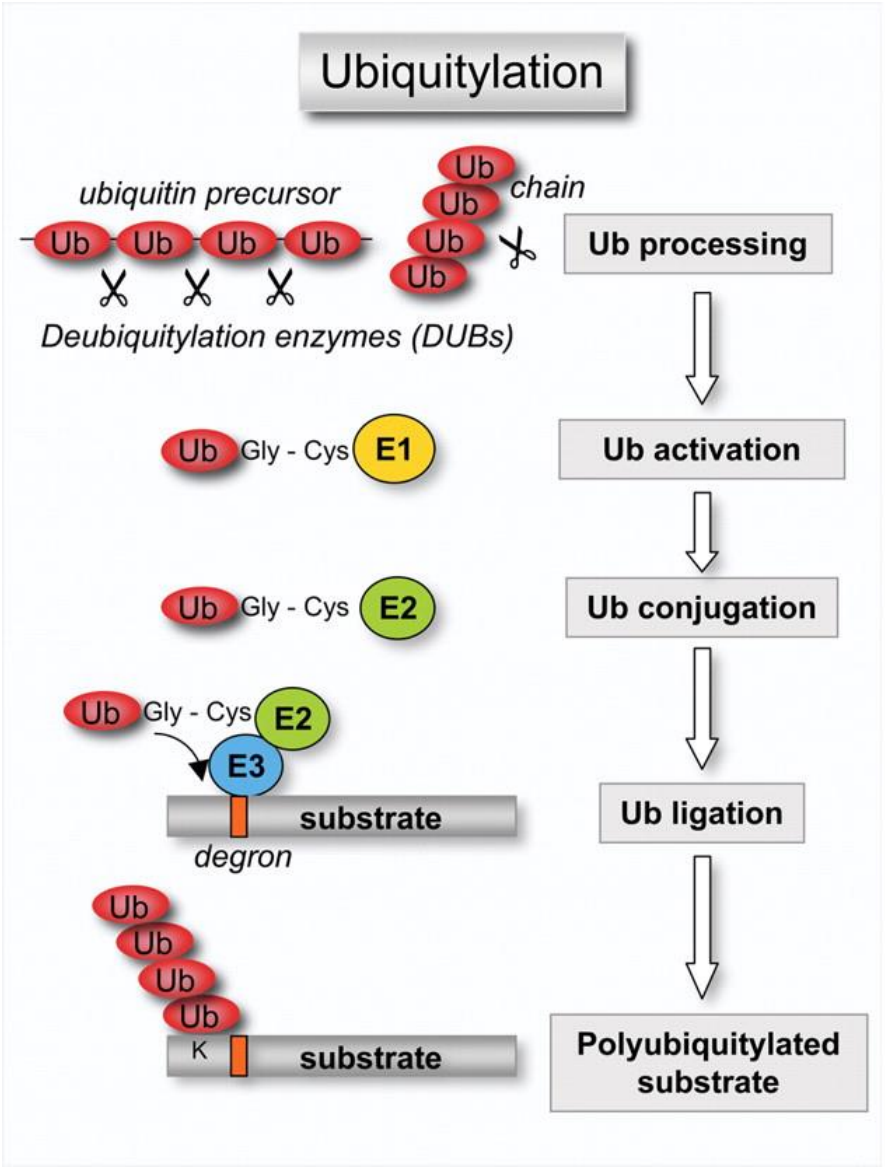
Alpha-synuclein catabolic systems

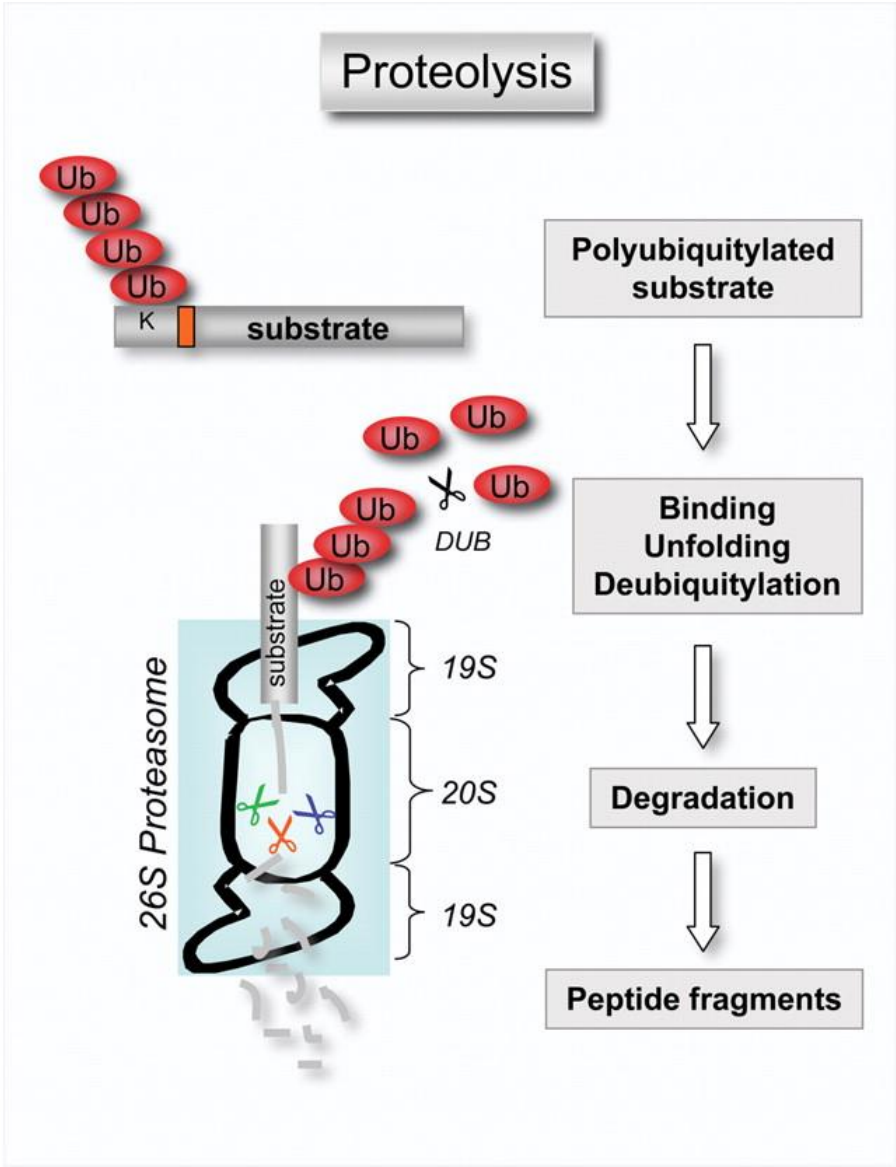


Ubiquitin-Proteasome System (UPS)

Autophagy-Lysosome Pathway (ALP)

Ubiquitin-proteasome system (UPS)





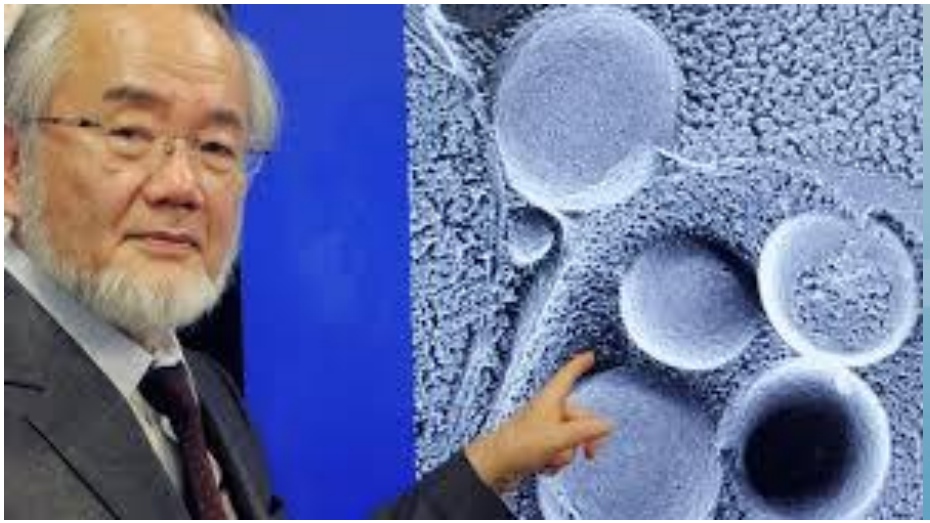
UPS alterations and PD

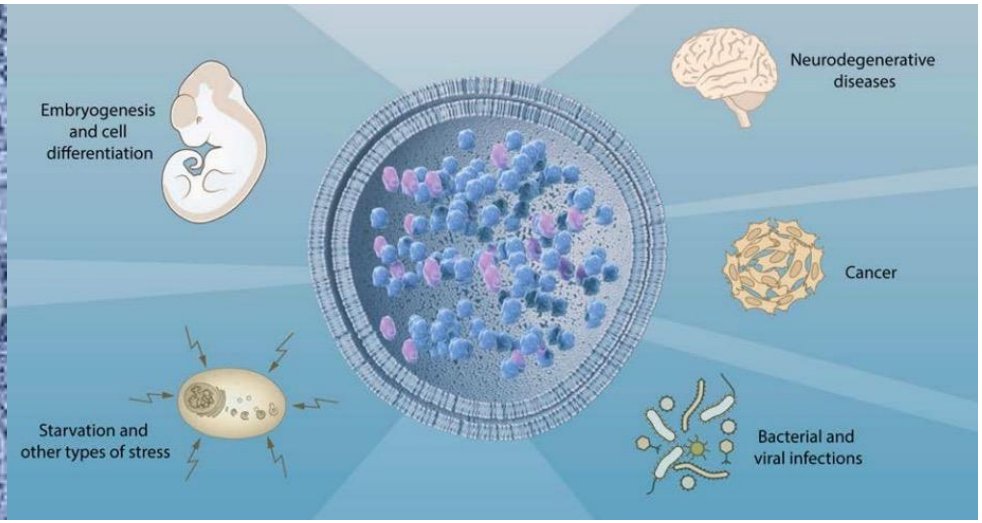
- Lewy bodies are ubiquitin-positive
- Genetics of PD:
    - Parkin (PARK2) is an E3 ubiquitin-ligase
    - UCHL1 (PARK5) is a DUB
- ↓ function/expression of 20S subunit in SNc of PD patients
- UPS inhibitors → animal models of PD

The 2016 Nobel Prize in Medicine

Yoshinori **Ohsumi**,
Tokyo Institute of Technology

Autophagy



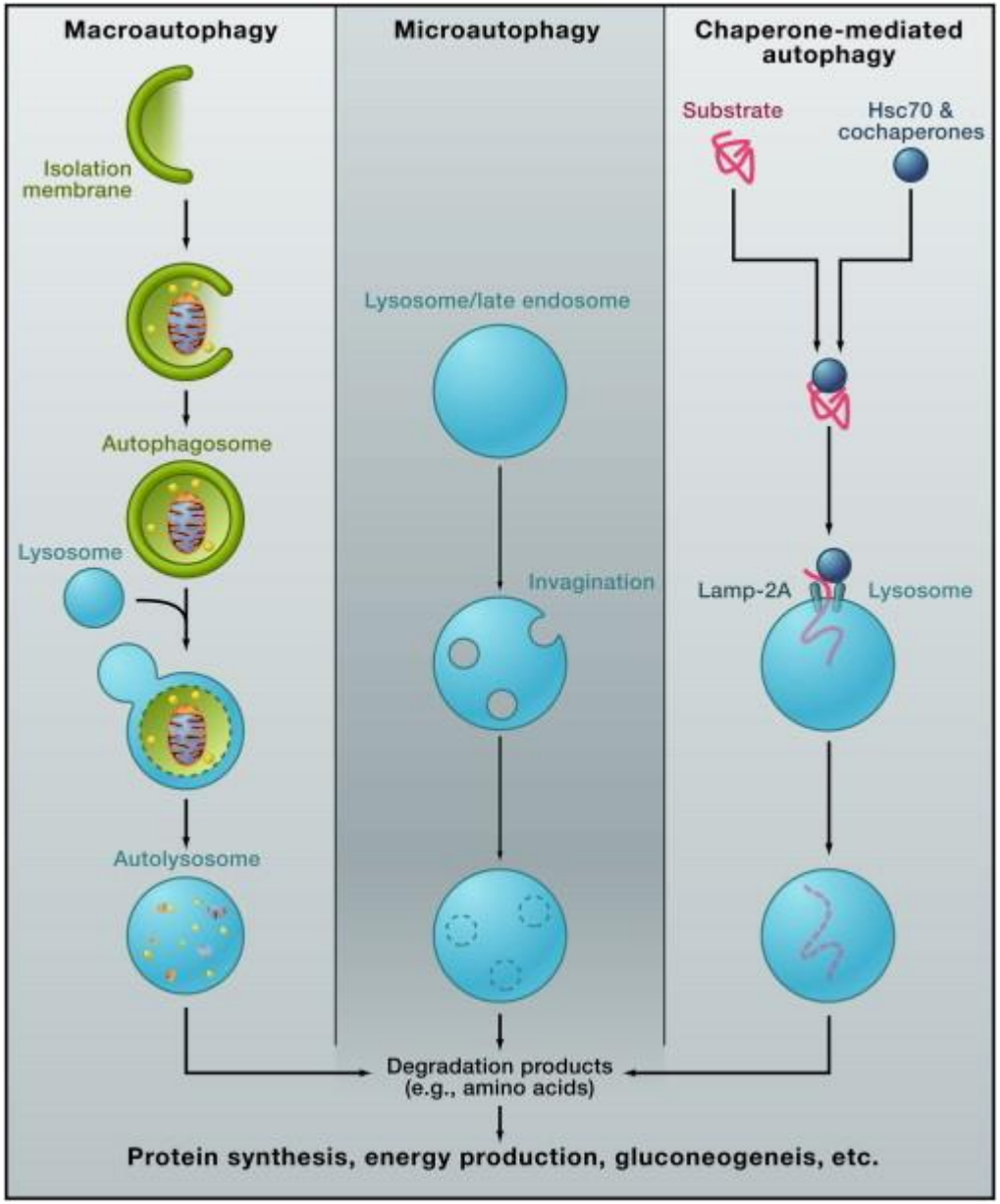


International Award «Lombardia è Ricerca» - 2019

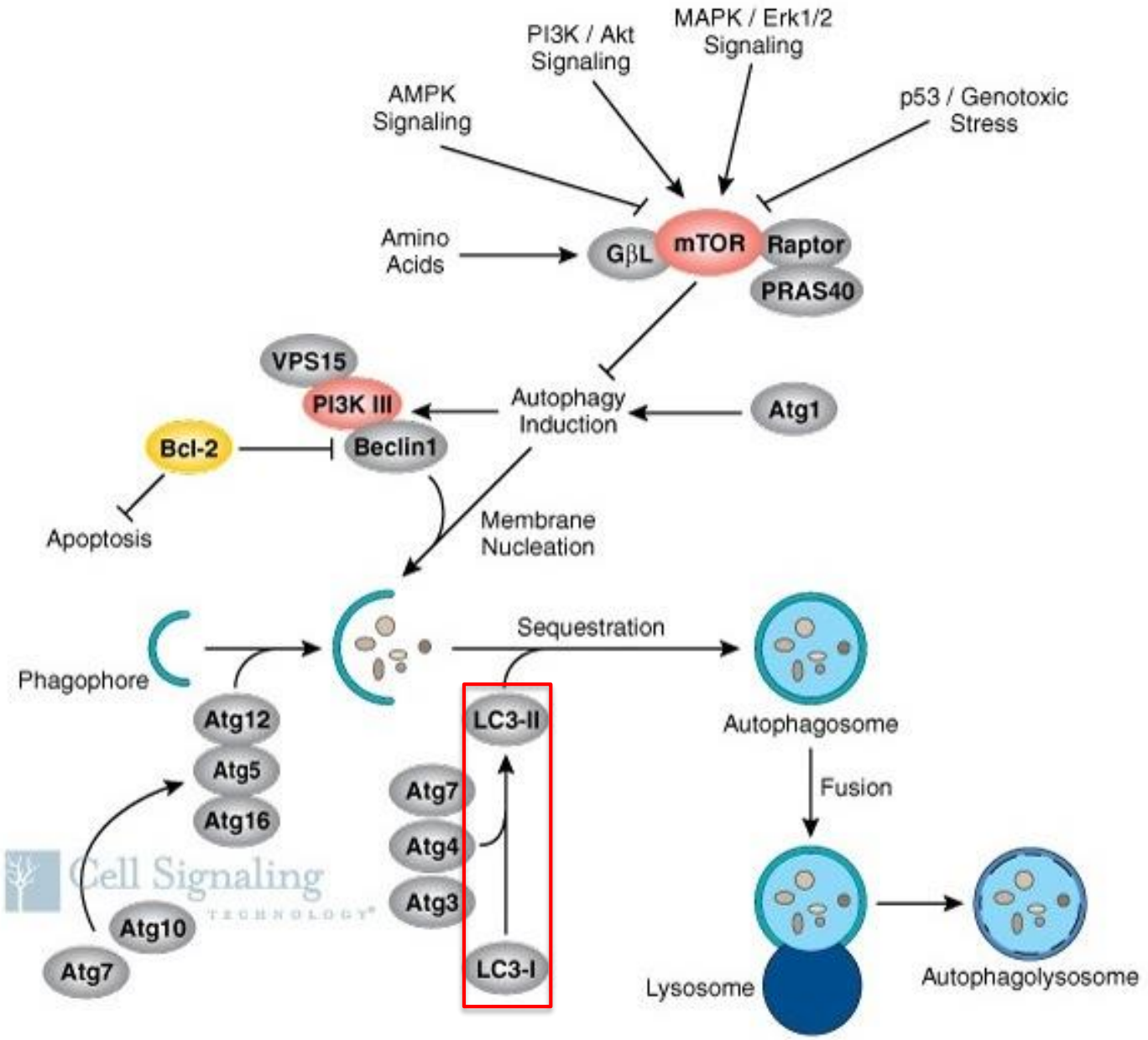
Caloric restriction → ↑ autophagy → ↑ longevity

Guido Kroemer, molecular biologist, University of Paris Descartes

Types of autophagy



(Macro)autophagy



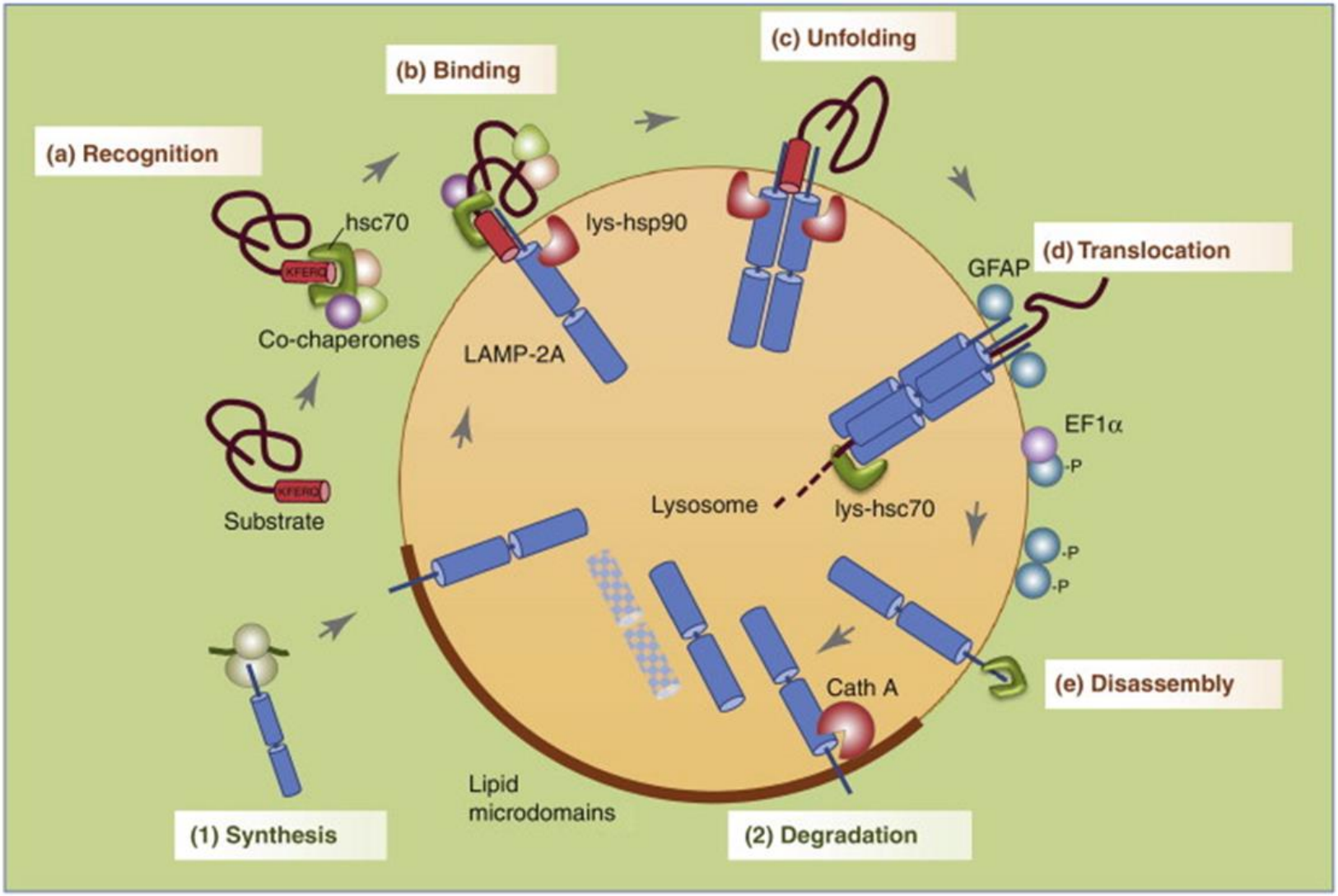
Macroautophagy alterations and PD

- autophagosome accumulation in SNc of PD patients and in animal and cell models of PD
- PINK1, parkin: key role in mitophagy
- LRKK2 …

Table 1
Familial and other genes involved in Parkinson's disease[a] and their roles in the autophagy-lysosomal pathway.

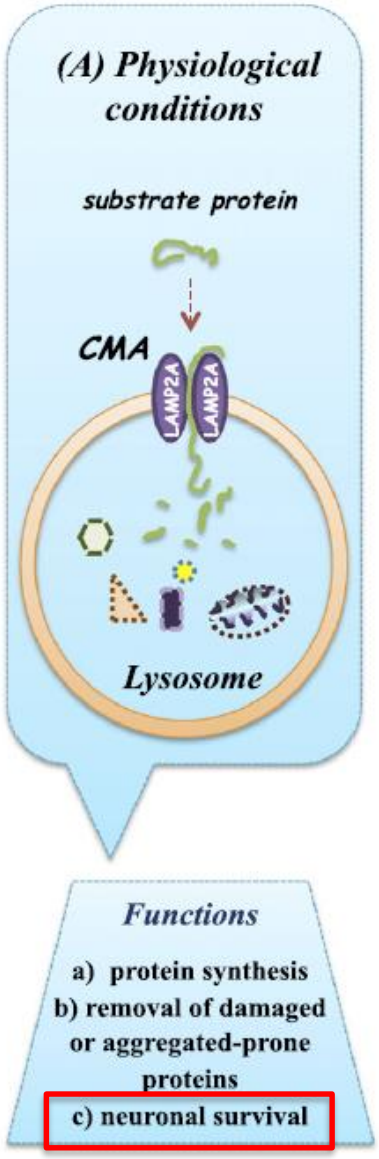
Gene	Genetic and clinical features	Role in ALP
Autosomal dominant PD genes		
SNCA	Rare point mutations and gene multiplication lead to EOPD. Several point mutations and gene duplication associated with LOPD.	Degraded by the ALP; overexpression or point mutations may lead to dysfunction of CMA, microautophagy and macroautophagy.
LRRK2	Several mutations were reported, most common is p.G2019S. Typical phenotype, with lower rates of non-motor symptoms.	Regulates endolysosomal transport, lysosomal function and potentially mitophagy.
VPS35	Very rare, only one mutation (p.D620 N) was reliably confirmed as associated with PD. Typical phenotype, potentially with earlier age at onset.	Involved in endolysosomal transport regulation.
Autosomal recessive PD genes		
PRKN (Parkin)	Bi-allelic mutations are the most common recessive genetic cause of EOPD. Dystonia is common, early onset but slowly progressive.	Regulates mitophagy through interaction with PINK1 and targeting of dysfunctional mitochondria for degradation by the lysosome.
PINK1	The second most common recessive genetic cause of EOPD. Anxiety may be more common	Regulation of mitophagy through the same pathway as *PRKN*
PARK7 (DJ-1)	Rare cause of recessive EOPD. Early onset, more dystonia at presentation	Regulation of mitophagy in a parallel pathway to that of *PRKN/PINK1*
VPS13C	Very rare mutations were reported. Early onset, rapidly progressive PD with rapid cognitive decline.	Activates the *PRKN/PINK1* mitophagy pathway.
Other genes, involved in PD and lysosomal storage disorders		
GBA	More than 100 mutations were reported in PD. Typical PD with earlier onset on average, and wide spectrum of non-motor symptoms. Prominent cognitive decline and neuropsychiatric features	A lysosomal hydrolase involved in degradation of glycosphingolipids. Bi-allelic mutations may cause Gaucher disease.
SMPD1	Rare mutations, mainly in Ashkenazi Jews, were associated with PD in different studies. Typical PD, possibly with earlier onset.	A lysosomal hydrolase involved in degradation of sphingolipids. Bi-allelic mutations may cause Niemann-Pick type A/B disease.
ASAH1	Burden analysis suggested association with PD, yet this association needs to be replicated. No information on clinical presentation is available.	A lysosomal enzyme responsible for the degradation of ceramide. Bi-allelic mutations may cause Farber disease or spinal muscular atrophy with progressive myoclonic epilepsy.
GLA	No genetic evidence for involvement, yet enzymatic activity is reduced in PD patients. No information on clinical presentation is available.	A lysosomal hydrolase involved in glycolipid degradation. Bi-allelic mutations may cause Fabry disease.

Chaperone-mediated autophagy (CMA): a selective form of autophagy



Kaushik and Cuervo, Trends in Cell Biology. 2012

Physiological functions of CMA



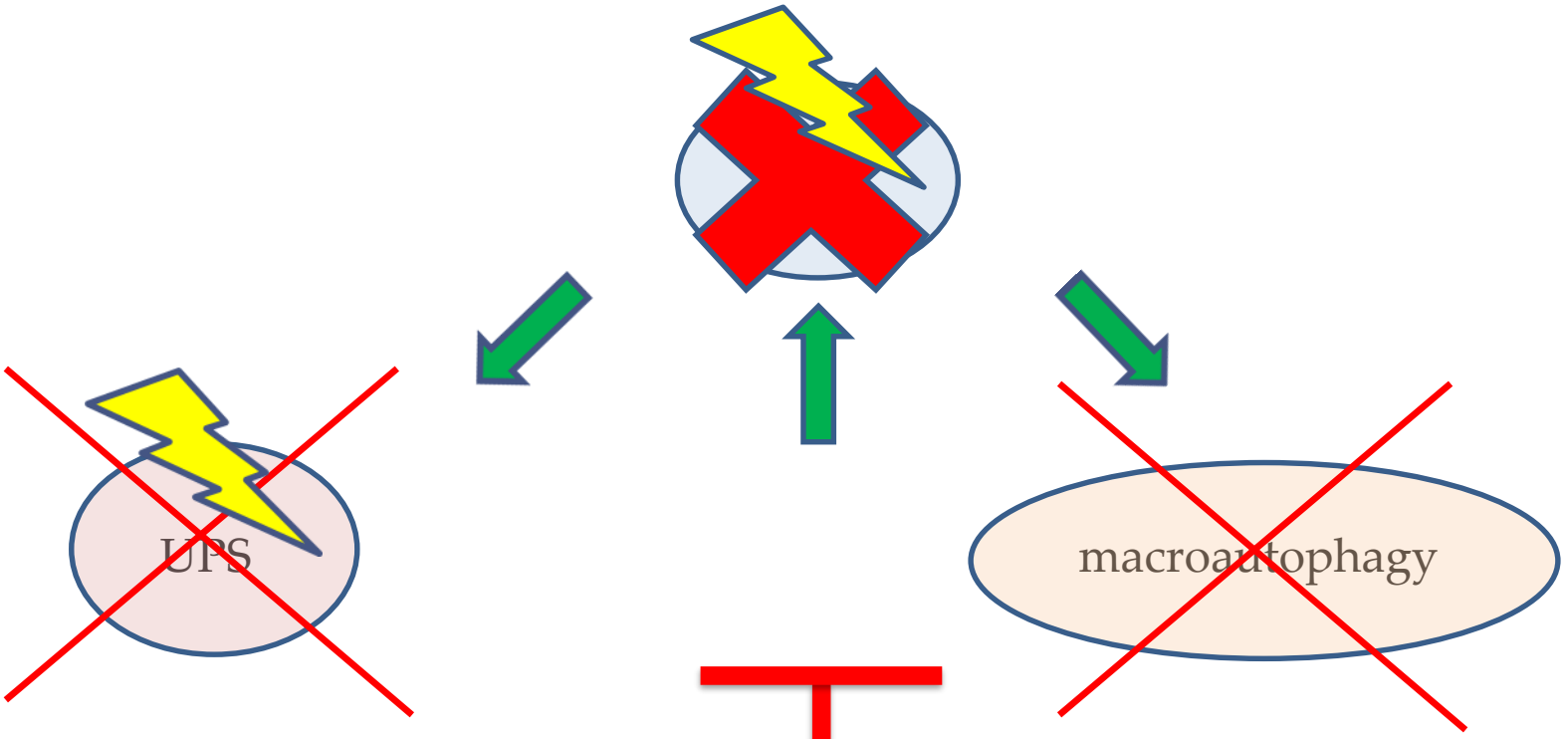
M. Xilouri, L. Stefanis / Molecular and Cellular Neuroscience 66 (2015) 29–36

B

General functions	Consequences of failure
Energy during Starvation Amino acids	• Energetic compromise • Susceptibility to stress
Quality Control Damaged proteins	• Proteotoxicity • Cellular degeneration • Susceptibility to stress
Cell-type specific functions	**Consequences of failure**
Neuronal Survival MEF2D	• Neurodegeneration
Kidney Growth Pax2	• Kidney disorders
Antigen Presentation Cytosolic proteins	• Altered immunity
Transcription regulation FOX, IκBα	• Altered cellular processes

from Cuervo and Wong, Cell Research (2014) 24:92-104

Cross talk between different proteolytic systems



- defects in macroautophagy or UPS → ↑ CMA
- CMA blockage → ↑ macroautophagy
- CMA alterations → UPS perturbation

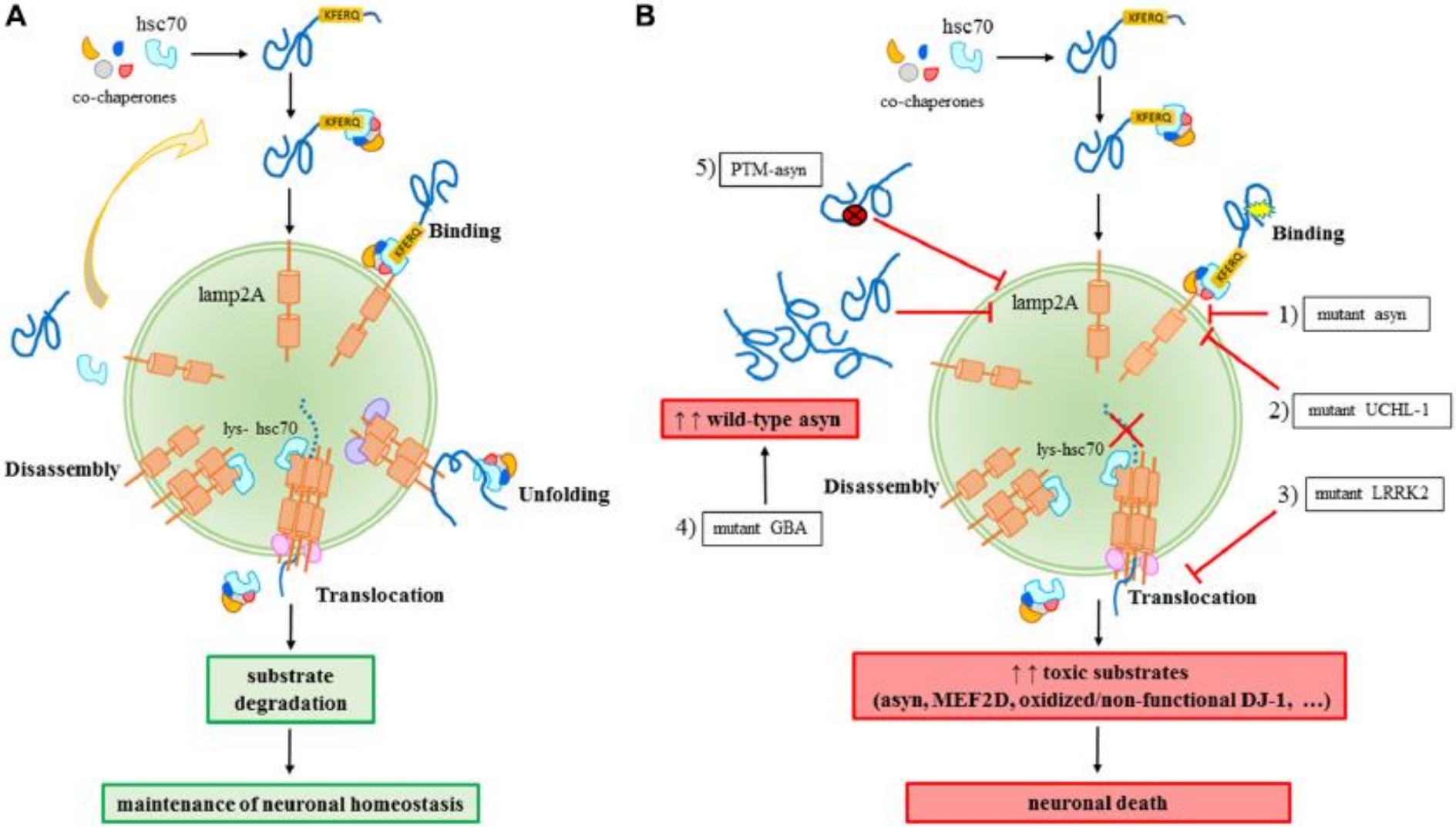
AGING

frontiers in Molecular Neuroscience

MINI REVIEW
published: 23 December 2016
doi: 10.3389/fnmol.2016.00157

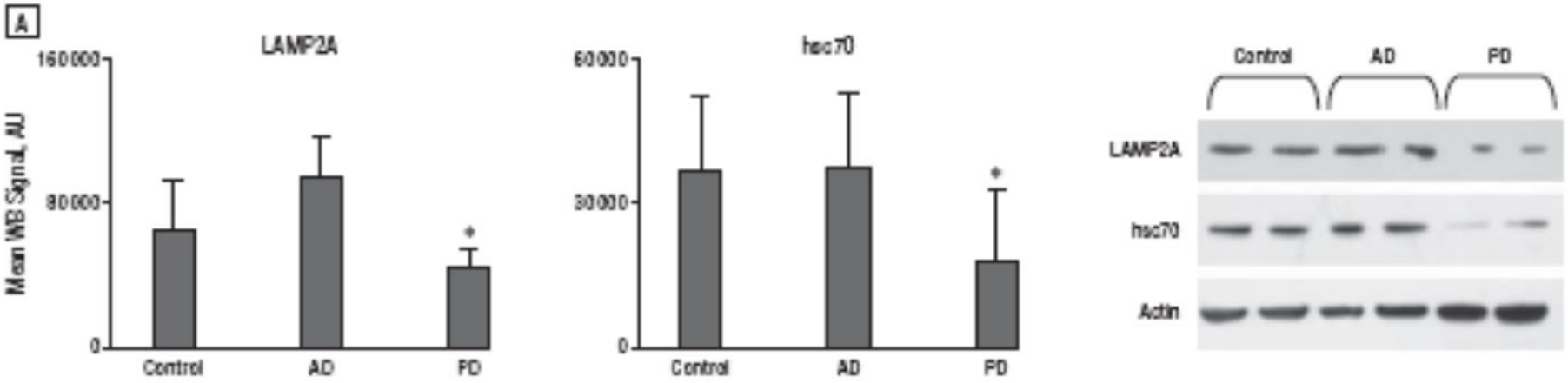
Role of Chaperone-Mediated Autophagy Dysfunctions in the Pathogenesis of Parkinson's Disease

Gessica Sala[1], Daniele Marinig[1,2], Alessandro Arosio[1] and Carlo Ferrarese[1,3]*



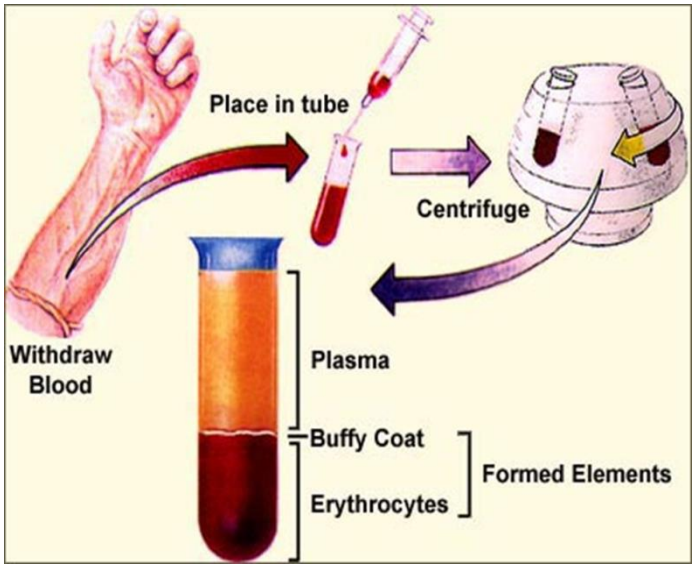
CMA alterations in PD patients

- ↓ **lamp2A and hsc70** levels in **advanced stages** in dopaminergic neurons of PD patients (Alvarez-Erviti et al., Arch Neurol 2010)

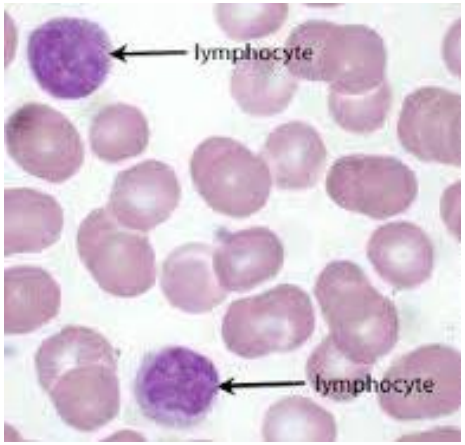


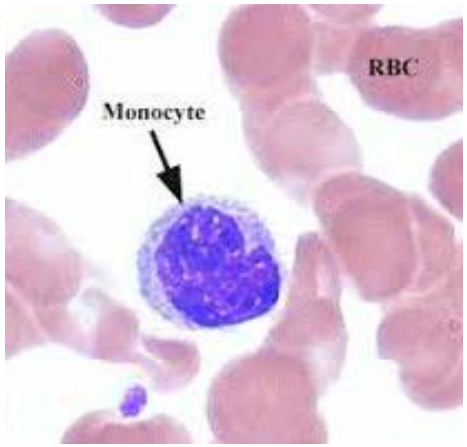
Possible role for deregulation of microRNA (Alvarez-Erviti et al., Cell Death Dis 2013) and sequence variation in lamp2 promoter region (Pang, Neurosci Lett 2012)

Aim 1: to search for autophagy dysfunctions in *ex vivo* cells from PD patients



PBMCs



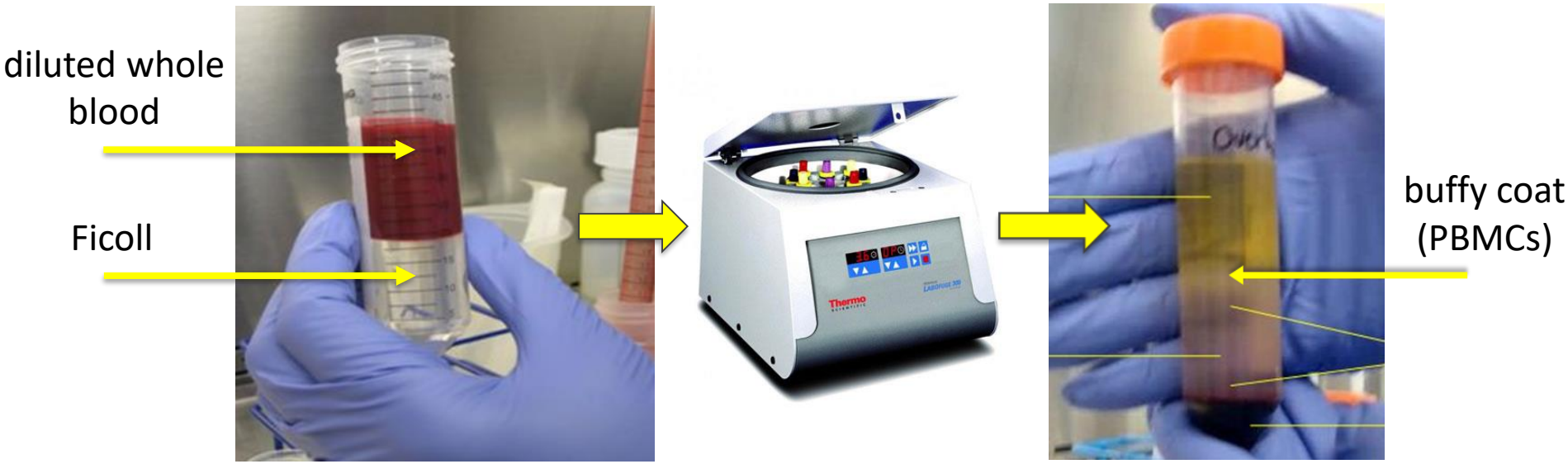


→ Identification of new PD biomarkers

- early diagnosis
- personalized therapy
- monitoring of drug efficacy in clinical trials

Methods

PBMCs isolation through density gradient centrifugation



Protein expression → WB
IF
FRA (insoluble proteins)
Dot blot (soluble proteins)

Gene expression → real time PCR

Neuroscience Letters 477 (2010) 6–10

Contents lists available at ScienceDirect

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journal homepage: www.elsevier.com/locate/neulet

Alpha-synuclein nitration and autophagy response are induced in peripheral blood cells from patients with Parkinson disease

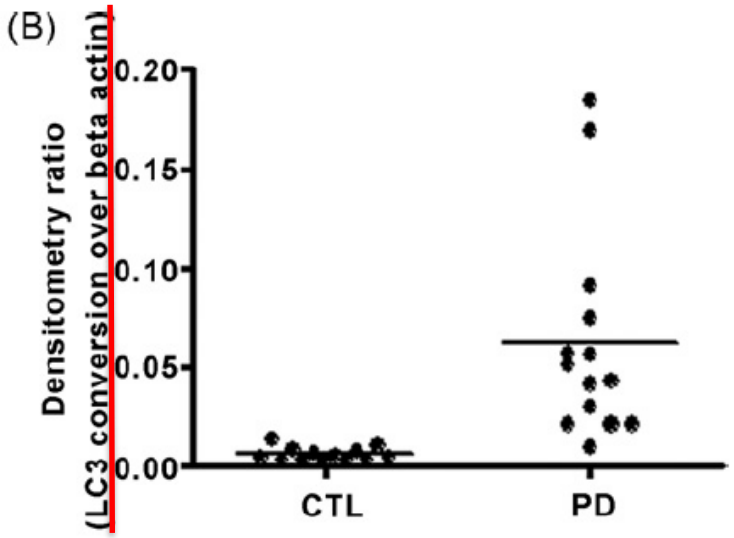
Alessandro Prigione[a,1], Fabrizio Piazza[a], Laura Brighina[a,b], Barbara Begni[a], Alessio Galbussera[b], Jacopo C. DiFrancesco[a,b], Simona Andreoni[a], Roberto Piolti[b], Carlo Ferrarese[a,b,c,*]

[a] *Laboratory of Neurobiology, Department of Neuroscience and Biomedical Technologies, University of Milan-Bicocca, Italy*
[b] *Department of Neurology, University of Milan-Bicocca, San Gerardo Hospital, Monza, Italy*
[c] *Scientific Institute "E. Medea", Bosisio Parini, Italy*

Table 1
Subject demographic.

	PD patients	Controls
Number	25	30
Sex (M/F)	12/13	18/12
Age at study (years)	65 ± 9.9	60.1 ± 13
Age at onset (years)	58 ± 9.8	
UPDRS III score	29.2 ± 12	
Hoehn and Yahr stage	2.4 ± 0.9	
MMSE score	24.4 ± 5.6	
GDS score	5.3 ± 4.1	
Levodopa dosage (mg/die)	460 ± 197	
REP1 259 bp[a]	22	18
REP1 261 bp[a]	22	41
REP1 263 bp[a]	6	1

Values are expressed as mean ± standard deviation.
[a] REP1 allele number (2 per participant); allele frequencies (the number of participants is half the number of alleles indicated).



↑ autophagosomes in PD PBMCs

BRAIN RESEARCH 1546 (2014) 46–52

Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

Brain Research

Research Report

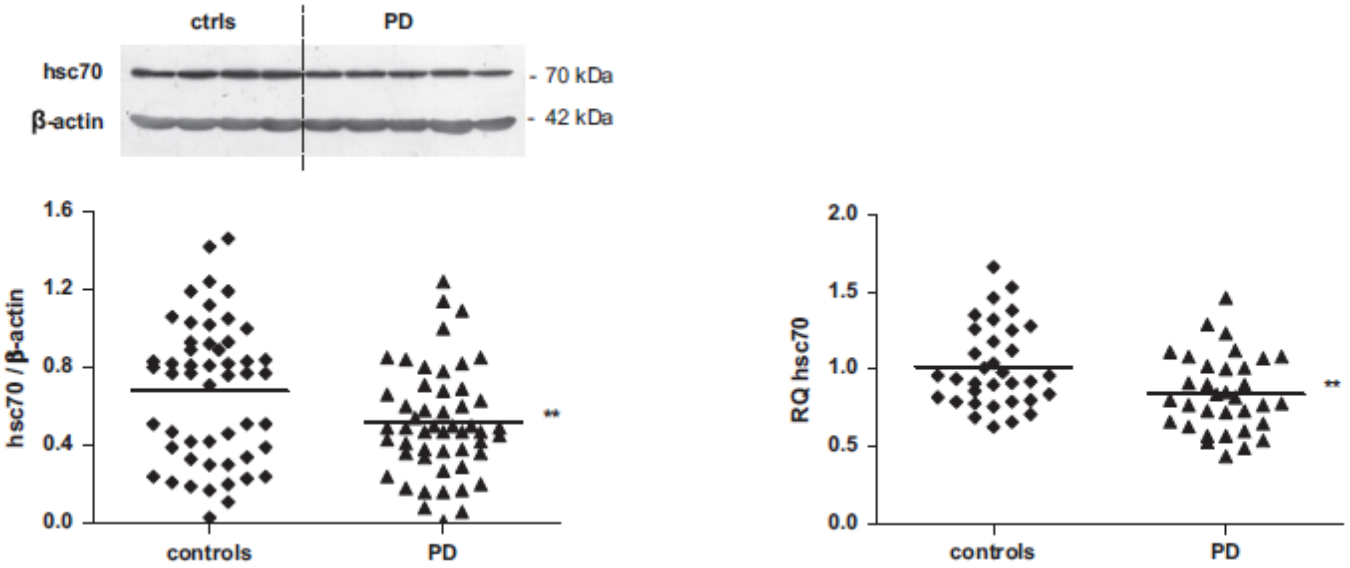
Reduced expression of the chaperone-mediated autophagy carrier hsc70 protein in lymphomonocytes of patients with Parkinson's disease

Gessica Sala[a,*], Giovanni Stefanoni[a,b], Alessandro Arosio[a,c], Chiara Riva[a], Laura Melchionda[a], Enrico Saracchi[a,b], Silvia Fermi[a,b], Laura Brighina[b], Carlo Ferrarese[a,b]

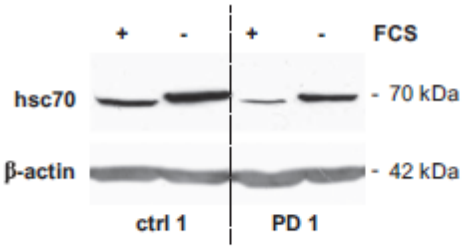
Table 1 – Demographic and clinical characteristics of the enrolled population.

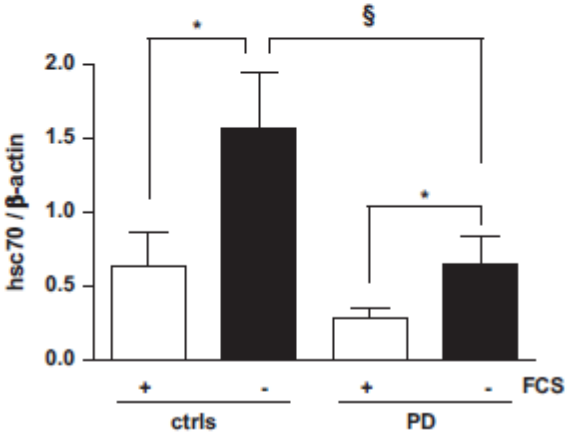
	Controls	PD patients
Number	53	53
Age at study (years)	66.1±7.2	66.9±9.7
Sex (M/F)	34/19	36/17
Age at onset (years)	n.a.	64.6±9.6
Disease duration (years)	n.a.	2.5±2.6
UPDRS III score	n.a.	15.6±10.3
Hoehn and Yahr stage	n.a.	1.7±0.7
MMSE score	29.20±1.3	28.3±2
Treated/untreated	n.a.	39/14
(L-Dopa)		(26)
(Dopamine agonists)		(14)
(Rasagiline)		(5)

n.a.=not applicable.

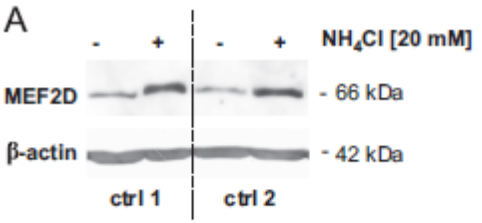


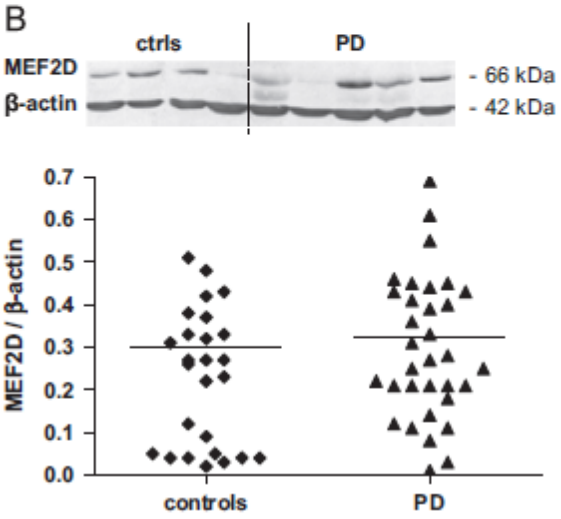
↓ hsc70 mRNA and protein levels in PD PBMCs

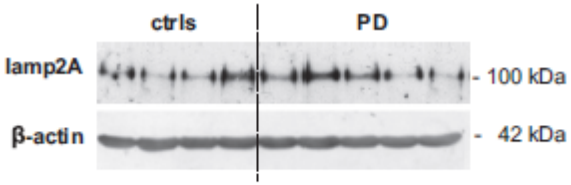


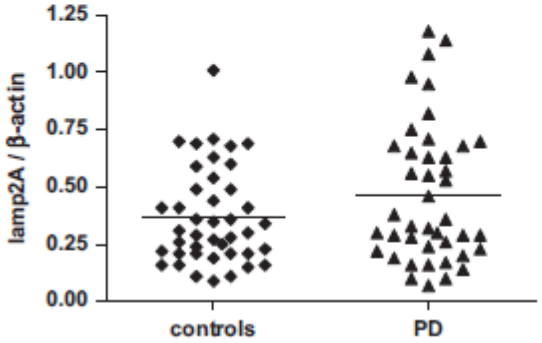


↓ hsc70 in PD PBMCs after starvation









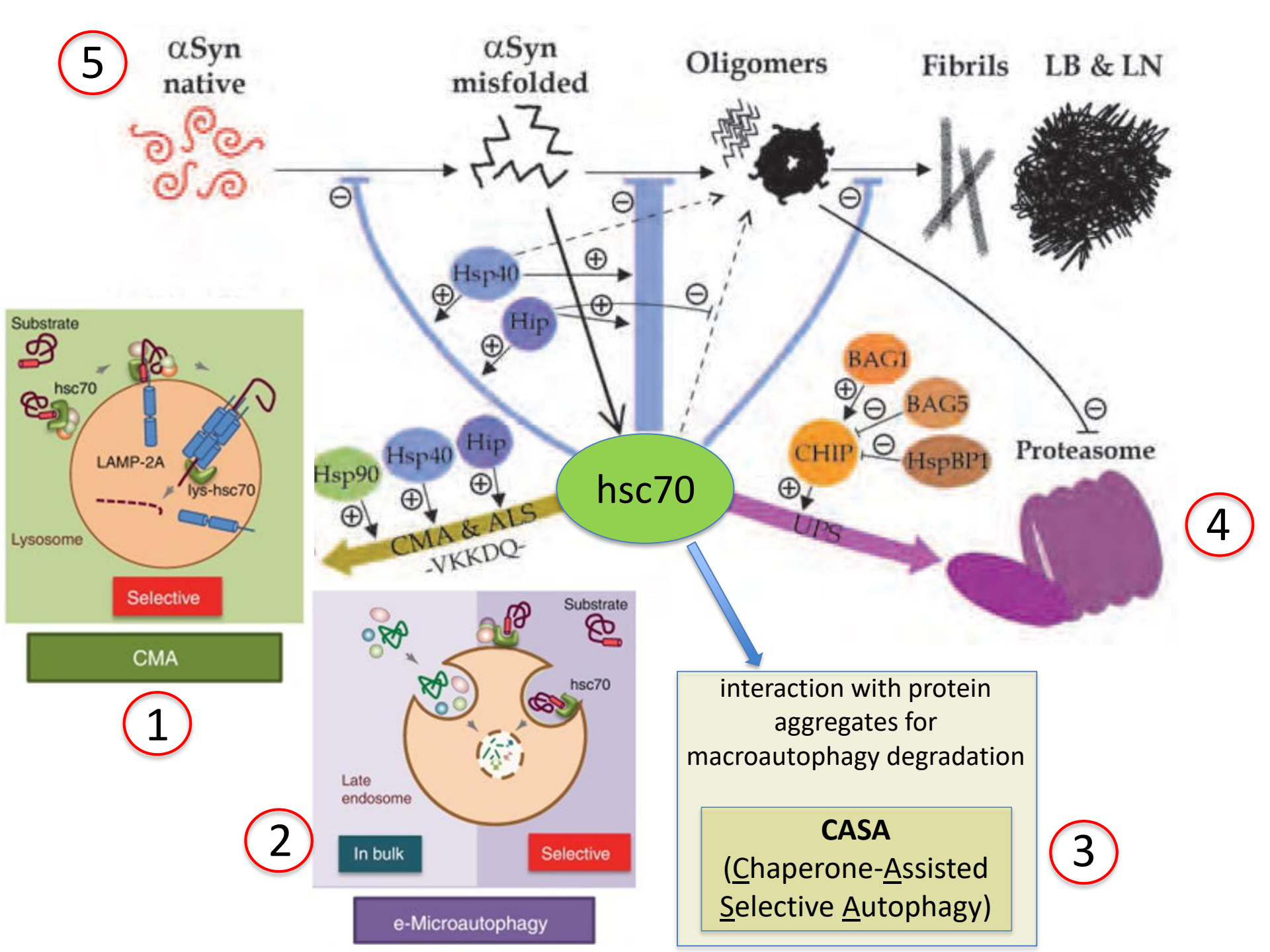
= lamp2A and MEF2D mRNA levels
no correlation hsc70-clinical characteristics

systemic ↓ of hsc70

⇩

possible 'trait' biomarker for PD

Sala et al., Brain Res 2014


5
αSyn native
αSyn misfolded
Oligomers
Fibrils
LB & LN
Hsp40
Hip
BAG1
BAG5
CHIP
HspBP1
Proteasome
Hsp90
Hsp40
Hip
hsc70
CMA & ALS
-VKKDQ-
UPS
4
Substrate
hsc70
LAMP-2A
lys-hsc70
Lysosome
Selective
CMA
1
Substrate
hsc70
Late endosome
In bulk
Selective
e-Microautophagy
2
interaction with protein aggregates for macroautophagy degradation
CASA
(Chaperone-Assisted Selective Autophagy)
3

↓ hsc70

↑ cell susceptibility to stressors
↑ protein accumulation/aggregation

hsc70 up-regulation as
therapeutic approach