Ninewells Hospital and Medical School & MRC Protein Phsophorylation and Ubiquitylation Unit, Dundee

Edinburgh Research Interest Group Meeting

200 years on: Genes, Genetics and Signalling pathways in Parkinson's disease

Esther Sammler MD PhD

Consultant Neurologist and Hon Senior Clinical Lecturer

22 April 2017

MRC Protein Phosphorylation and Ubiquitylation Unit

A few things upfront…

We are hoping to start our own Research Interest Group in Tayside

https://shakyteam.com Fundraising for Parkinson's UK

24%

£14,664

raised of £60,000 target
by 188 supporters

Donate

Share on Facebook

Marc van Grieken

Please support 'Shaky team from Shaky Toun'

I am cycling the 81 mile Etape Caledonia for **Parkinson's UK** because I want to fund Parkinson's research

Team members: The 'shaky team': Donald Coltart, Nick James, Stuart Henderson, Pete Murray, Keith Vance, Ian Findlay and me.

Event: Etape Caledonia 2017, 21 May 2017 Team: Shaky Team from Shaky Toun

PARKINSON'S UK
CHANGE ATTITUDES.
FIND A CURE.
JOIN US.

Parkinson's UK
We offer support and fund research to find a cure for Parkinson's

Overview

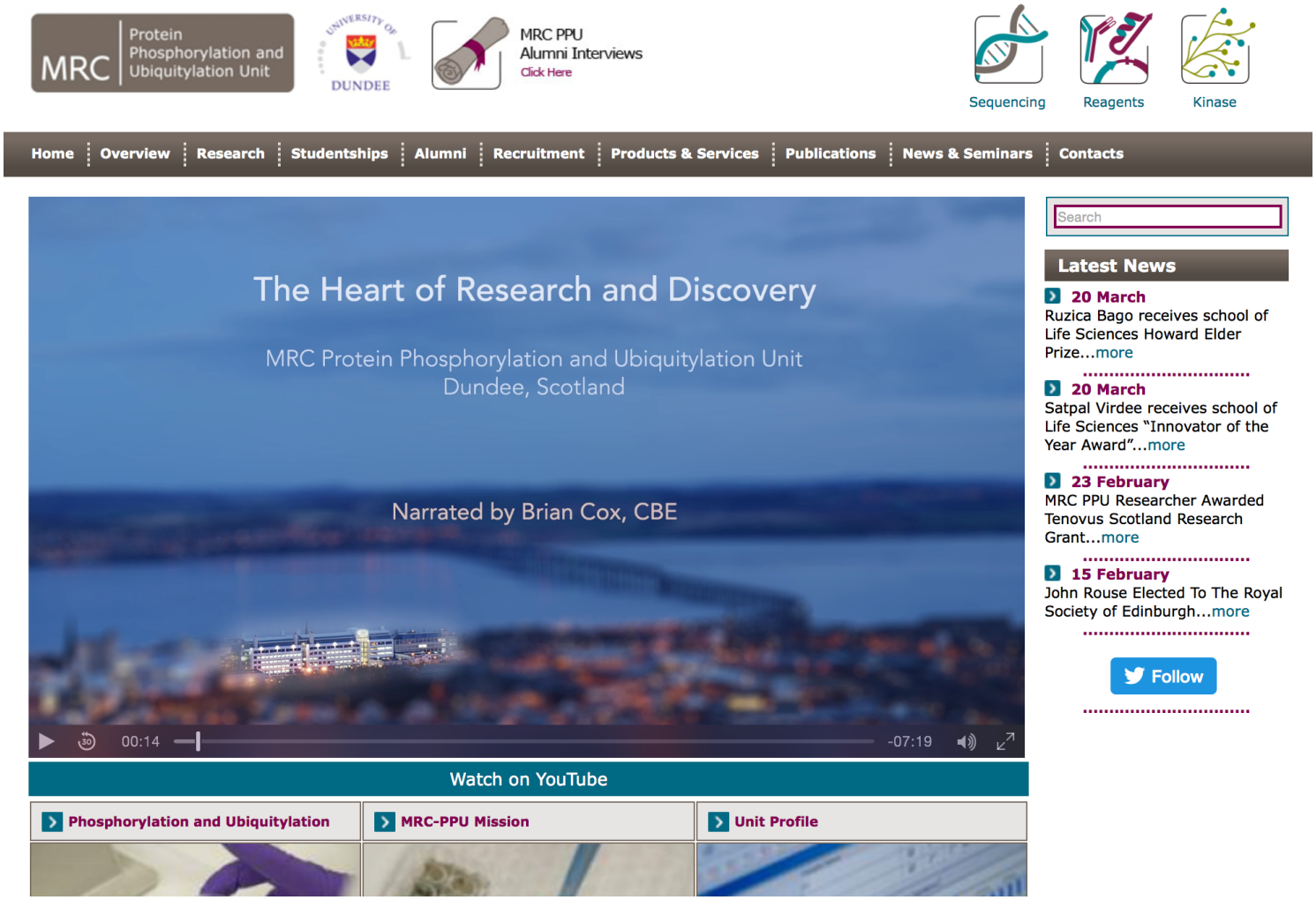
- → Introduction
- → What on earth is “Protein Phosphorylation”?
- → Parkinson’s disease
- → Hereditary Parkinson’s disease: Focus on LRRK2

- → *New developments in biomarker developments in LRRK2 associated PD*

About myself…

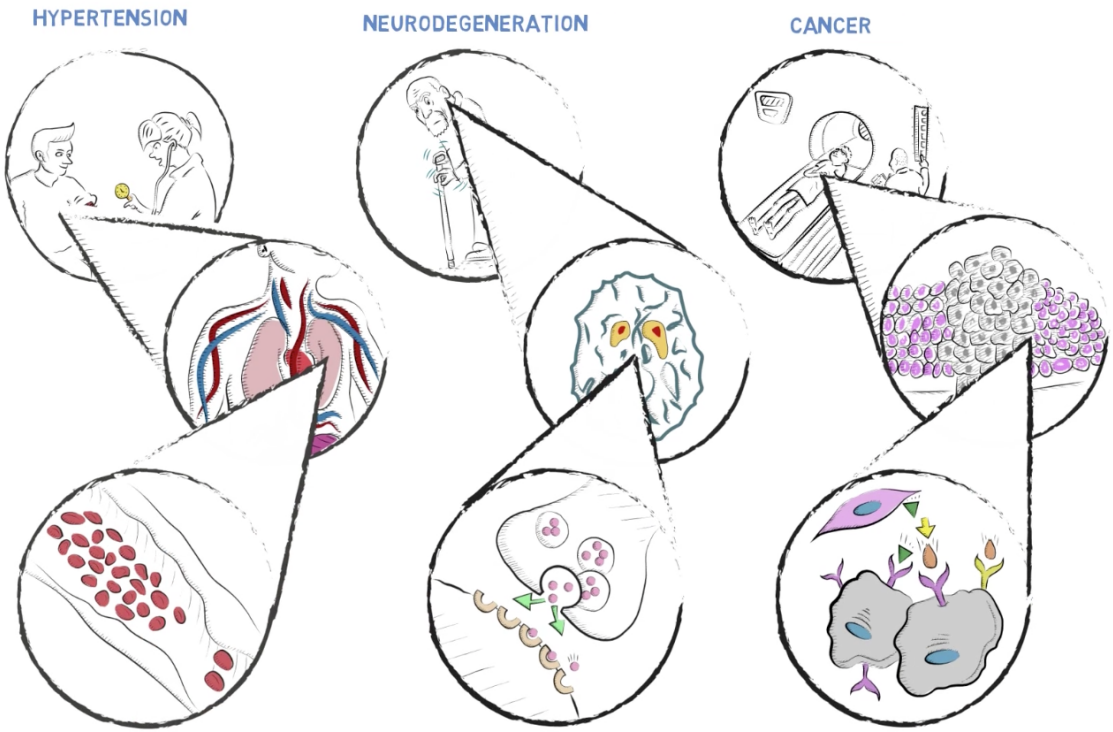
- → Originally from Germany
- → Scottish Neurology Training programme 2008 - 2015
  - → Wellcome Trust Clinical PhD Programme 2010-2014
    Professor Dario Alessi, MRC PPU, Dundee
  - → SCREDS Clinical Lectuer 2014 – 2015 (MRC PPU)
- → Consultant Neurologist in Dundee
  - → Movement disorders / Parkinson's disease
  - → Neurogenetics
- → AHSP Clinical Fellowship
  - → to set up translational link between clinical movement disorder service and MRC-PPU / Dario Alessi

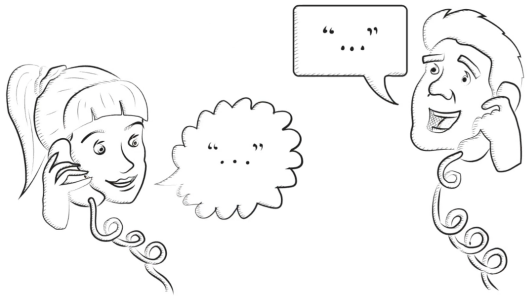
MRC Protein Phosphorylation and Ubiquitylation Unit

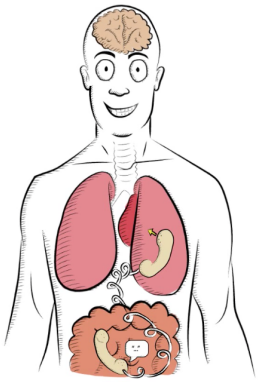


Please visit the MRC PPU website for a link to the movie clip…

CELL SIGNALLING – Protein Phosphorylation and Ubiquitylation

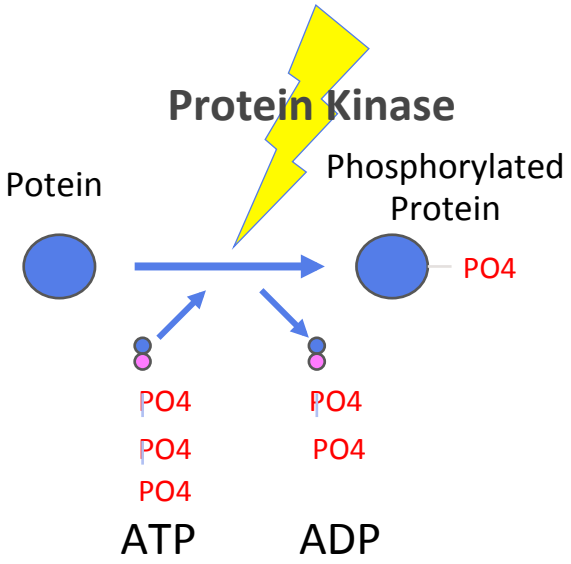






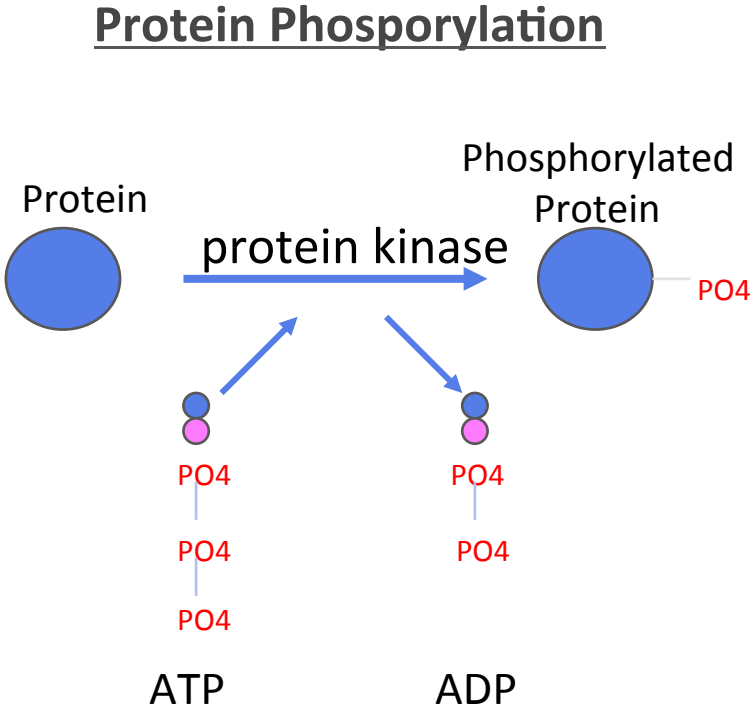
CELL SIGNALLING

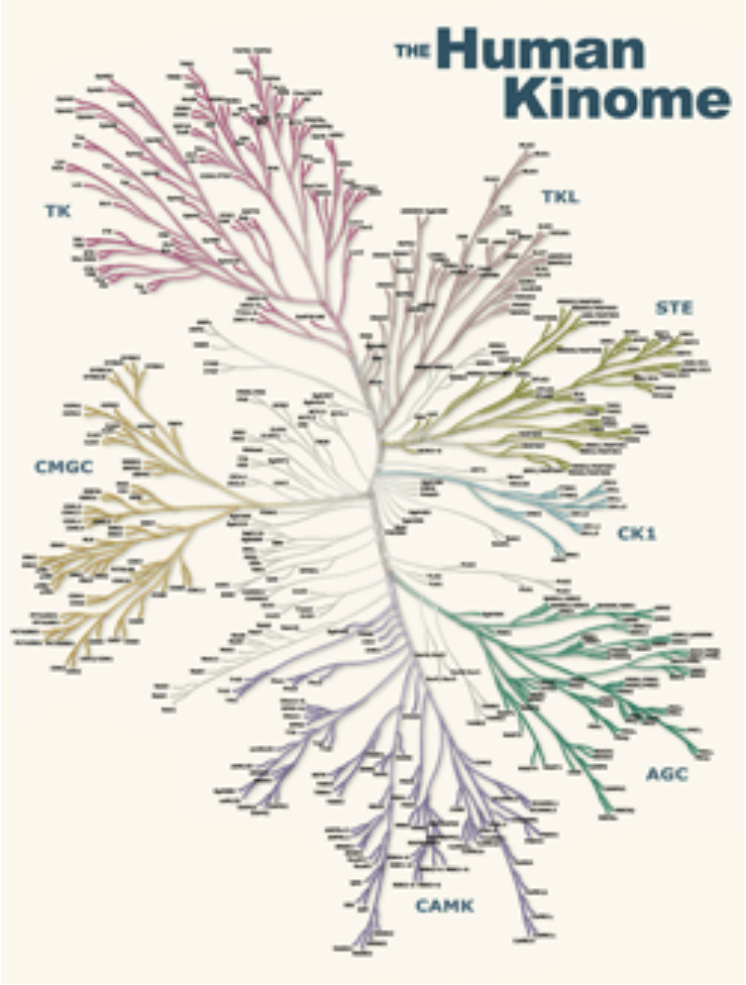
CELL SIGNALLING – Protein Phosphorylation and Ubiquitylation



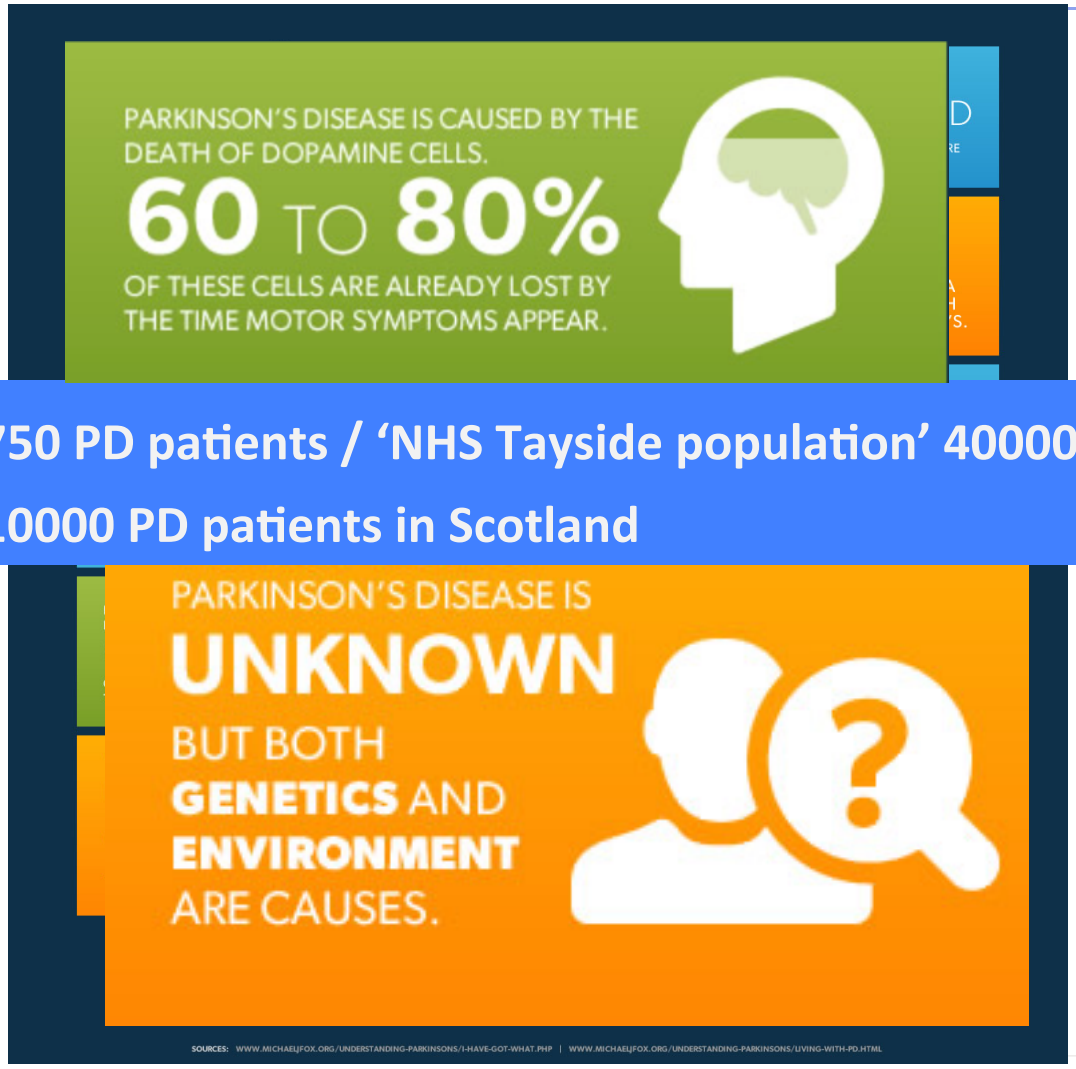
- Phosphorylation is the most general regulatory control mechanism in cells
- Nobel Price for Krebs & Fisher 1992 for discovering protein phosphorylation
- Abnormal phosphorylation identified as cause or consequence of many human diseases
- Many drugs target protein phosphorylation to alter the course of diseases

Comprehensive catalogue of Human Kinases





Parkinson's disease - Overview



750 PD patients / 'NHS Tayside population' 400000

10000 PD patients in Scotland

5% Familial

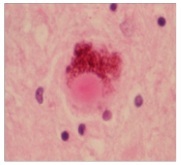
95% Idiopathic

Timeline for Parkinson's disease

Clinical Era

1817 — James Parkinson describes 'shaking palsy'

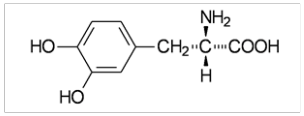
Pathology Era



1912 — Lewy discovers inclusions

1967 — L-Dopa introduced into practice

Treatment Era



1980s — Deep Brain Stimulation surgery

Genetic Era

1997 — Discovery of gene mutations in families with Parkinson's

2004

Biochemical Era

Present day — Understanding function of genes

Parkinson's disease – Clinical features

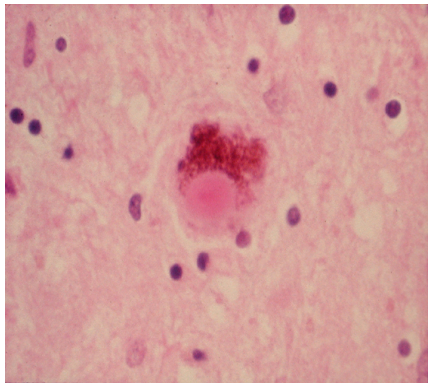
Motor symptoms

- → Tremor
- → Bradykinesia
- → Rigidity
- → Postural instability

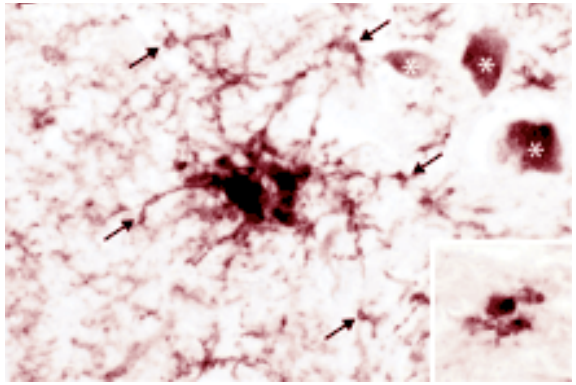
Non-motor symptoms

- → Sleep disorders
- → Hallucinations
- → Gastrointestinal dysfunction
- → Depression
- → Cognitive impairment / dementia
- → Anosmia

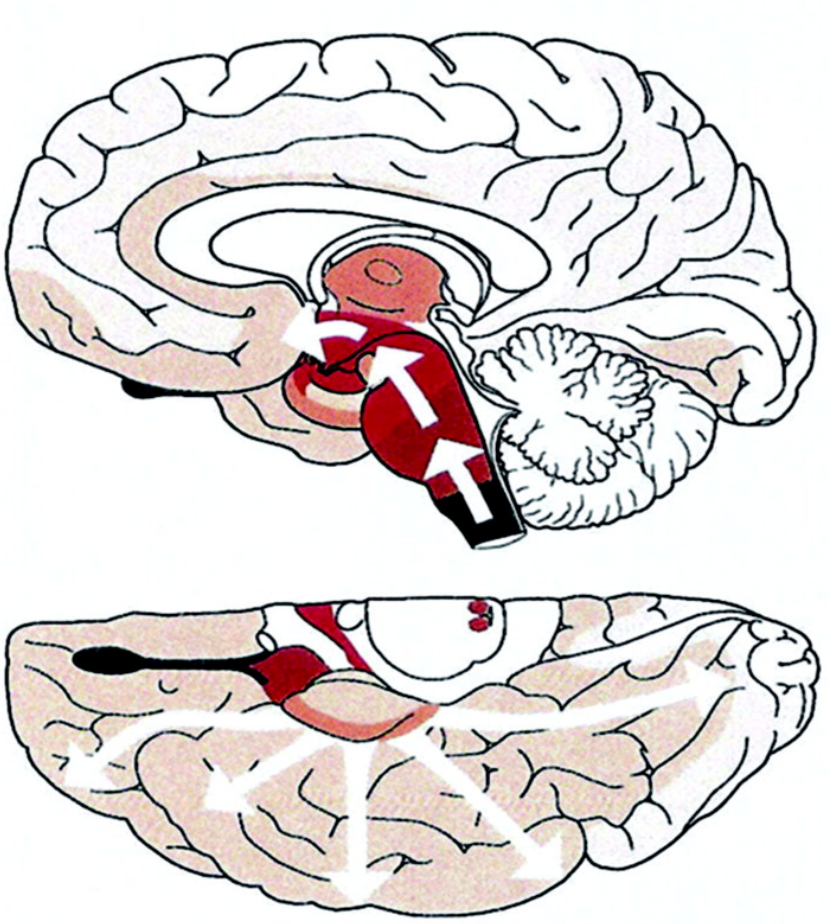
Pathology of Parkinson's disease – Lewy body formation



H.E

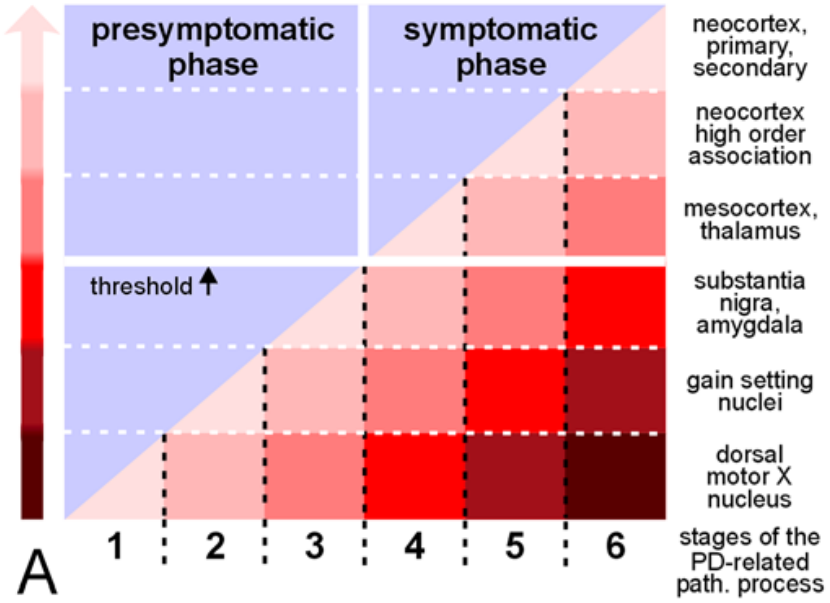


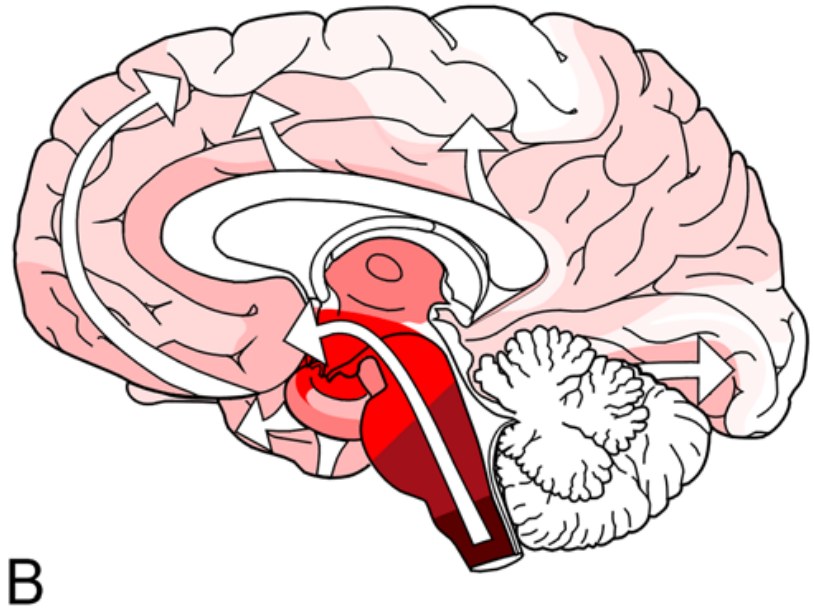
anti-HLA-DP/DQ/DR
Microglia activation



Hawkes C H , Deeb J Pract Neurol 2006;6:272-277

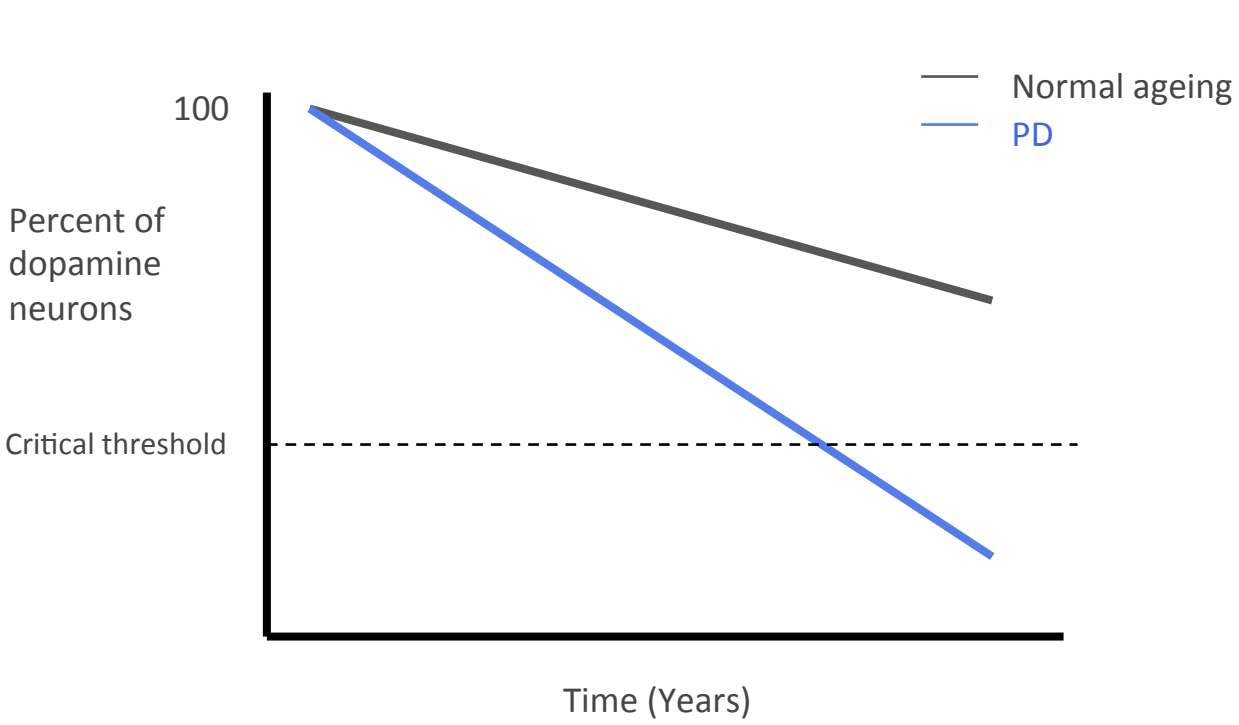
Widespread neuronal loss ≈ non-motor symptoms

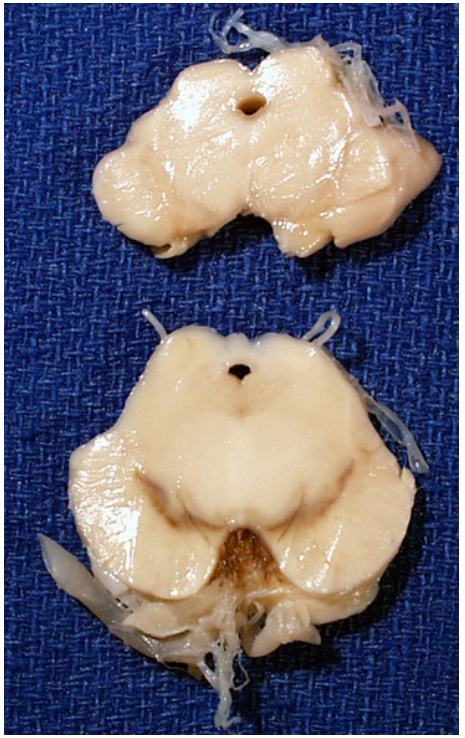




Braak H, Ghebremedhin E, Rub U, Bratzke H, Del Tredici K. Cell Tissue Res. 2004 Oct;318(1):121-34.

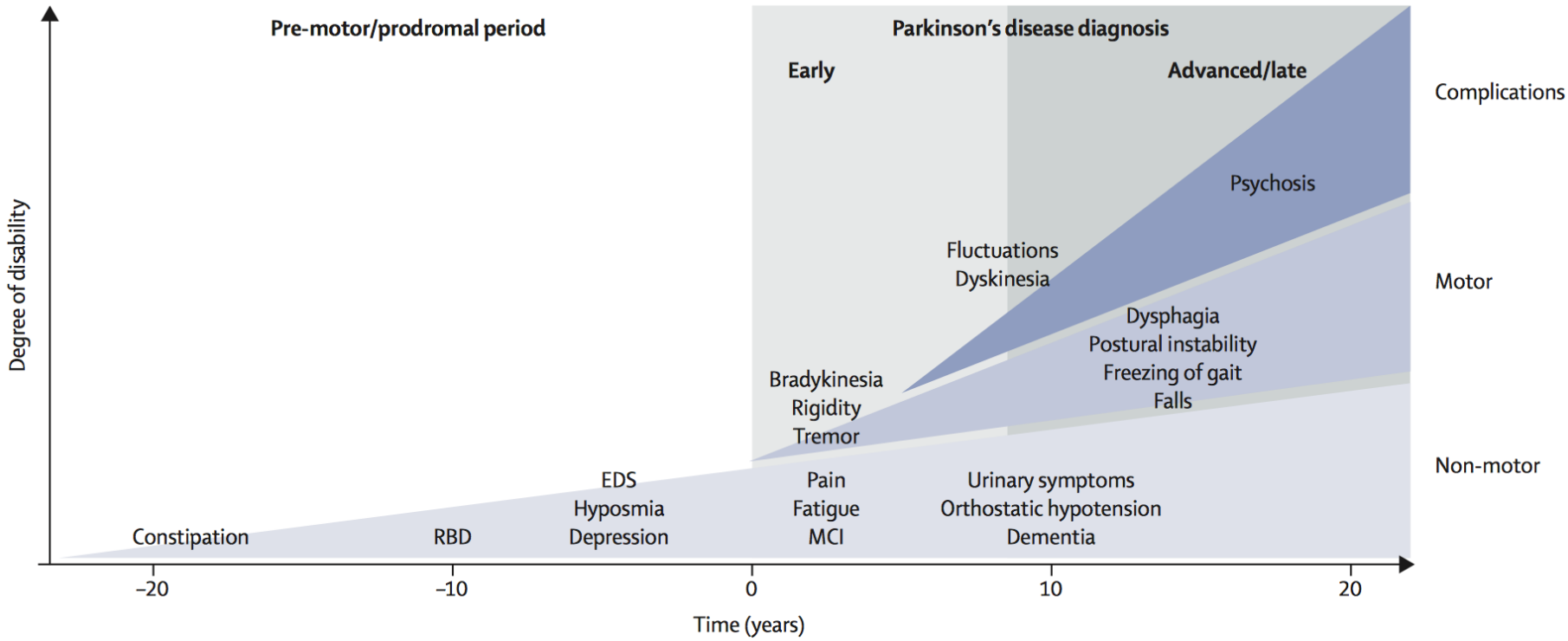
Pathology of Parkinson's disease – neuronal loss





Loss of dopaminergic neurons from the pars compacta region of the substantia nigra - approx 60% loss of neurons (80% depletion in striatal dopamine) gives PD symptoms

PD - Clinical features and time course of progression

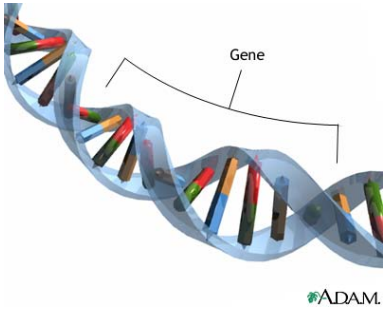


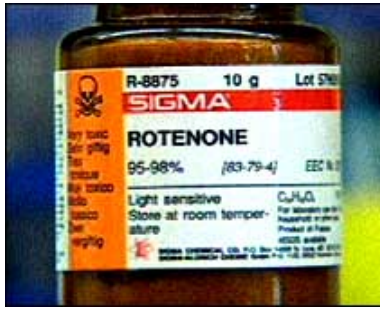
(RBS=REM Sleep behaviour disorder, EDS=excessive daytime sleepiness, MCI=mild cognitive impairment), Fig from thelancet August 2015

Idiopathic Parkinson's disease

COMPLEX TRAIT

Susceptability genes **+/-** **Environmental triggers** **+/-** **Age**





Familial Parkinson's disease

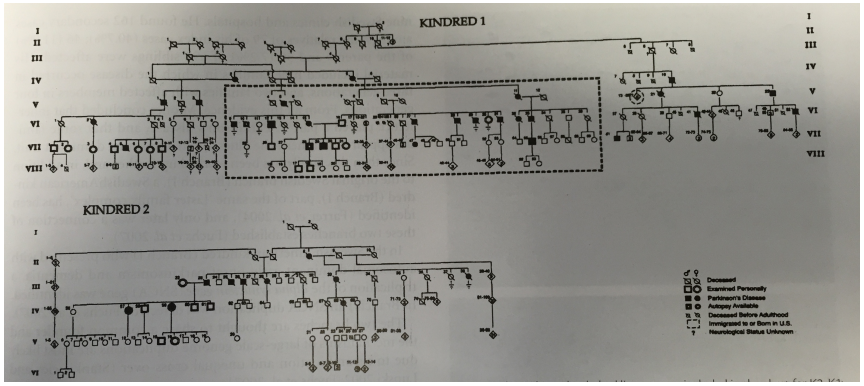
Staff Group 1886

Back row, left to right: **Horsley, Beevor, Cumerbatch, Buzzard, Burdendall Carter, Ormerod, Adams.**
Front row: **Marcus Gunn, Bastian, Jackson, Ramskill, Radcliffe, Gowers, Semon, Ferrier.**

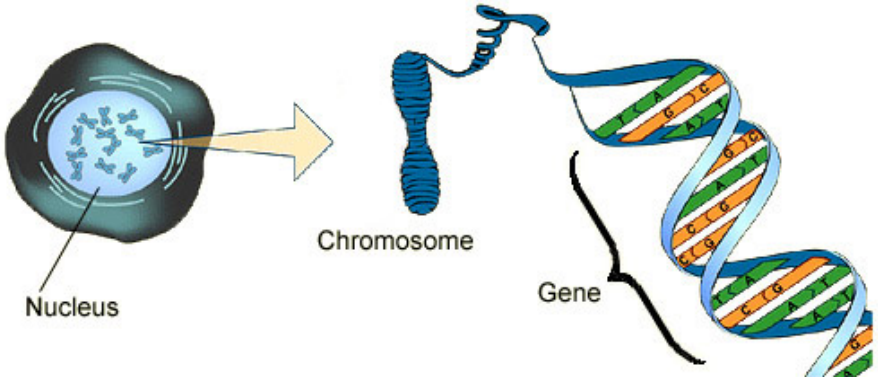
→ William Gowers noted a positive family history in 15% of PD patients in 1902

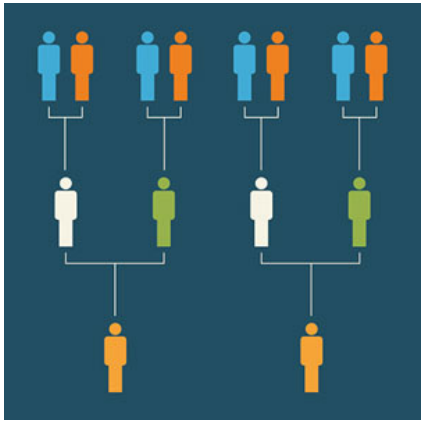
→ Pedigree of autosomal dominant PD (1990)

→ This family was later found to have a point mutation in α-synuclein (1996/7)



Genetics and Parkinson's disease





- → Genes are like recipes to make make proteins
- → Changes / mutations in alpha-Synuclein were first discovered in 1997
- → Since then identification of many more genes implicated in PD
- → Only a few people get Parkinson's as a direct result from a genetic mutation
- → Rare mutations provide a tool to understand the general disease process

The current list of locus symbols for hereditary PD

PARK	Gene	Inheritance	Onset	Function
PARK1	*SNCA*	AD	Classical PD or EO	Protein folding
PARK2	*PARKIN*	AR	EO	Ubiquitinylation
PARK5	UCHL1[preliminary]	AD	Classical PD	Ubiquitinylation
PARK6	*PINK1*	AR	EO	Phosphorylation
PARK7	DJ-1	AR	EO	Protein folding
PARK8	LRRK2	AD	Classical PD	Phosphorylation
PARK9	ATP13A2	AR	Complex PD with EO	Membrane biology
PARK13	HTRA2	AD or risk factor	Classical PD	Protease
PARK14	PLA2G6	AR	EO plus dystonia	Metabolism
PARK15	FBXO7	AR	Complex PD with EO	Ubiquitinylation
PARK16	Rab7L1	Risk	Complex late	Confirmed susceptibility locus
	GAK	RISK	Late PD	Phosphorylation
PARK17*	VPS35	AD	Classical PD	Vesicle trafficking
PARK18*	EIF4G1	AD[unconfirmed]	Classical	Translocation factor
PARK19*	DNAJC6	AR	EO	Vesicle trafficking
PARK20*	SYNJ1	AR	Complex EO or classical	PIP-like domain
PARK21*	DNAJC13	AD	Late onset PD	Unconfirmed
PARK22*	CHCHD2	AD	Late onset PD	Confirmed
PARK23*	VPS13C	AR	EO	Vesicle trafficking
	GBA	Risk factor	Classical PD	Metabolism
	CHCHD2	AD[Asian]	Classical PD	Transcription factor
	PODXL[preliminary]	AR	EO	Membrane biology
	TMEM230	AD	Classical	Vesicle Trafficking
	GCH1	Risk factor	Classical	Metabolism

*Not approved by HGNC

Six genes involved in Phosphorylation and Ubiquitylation

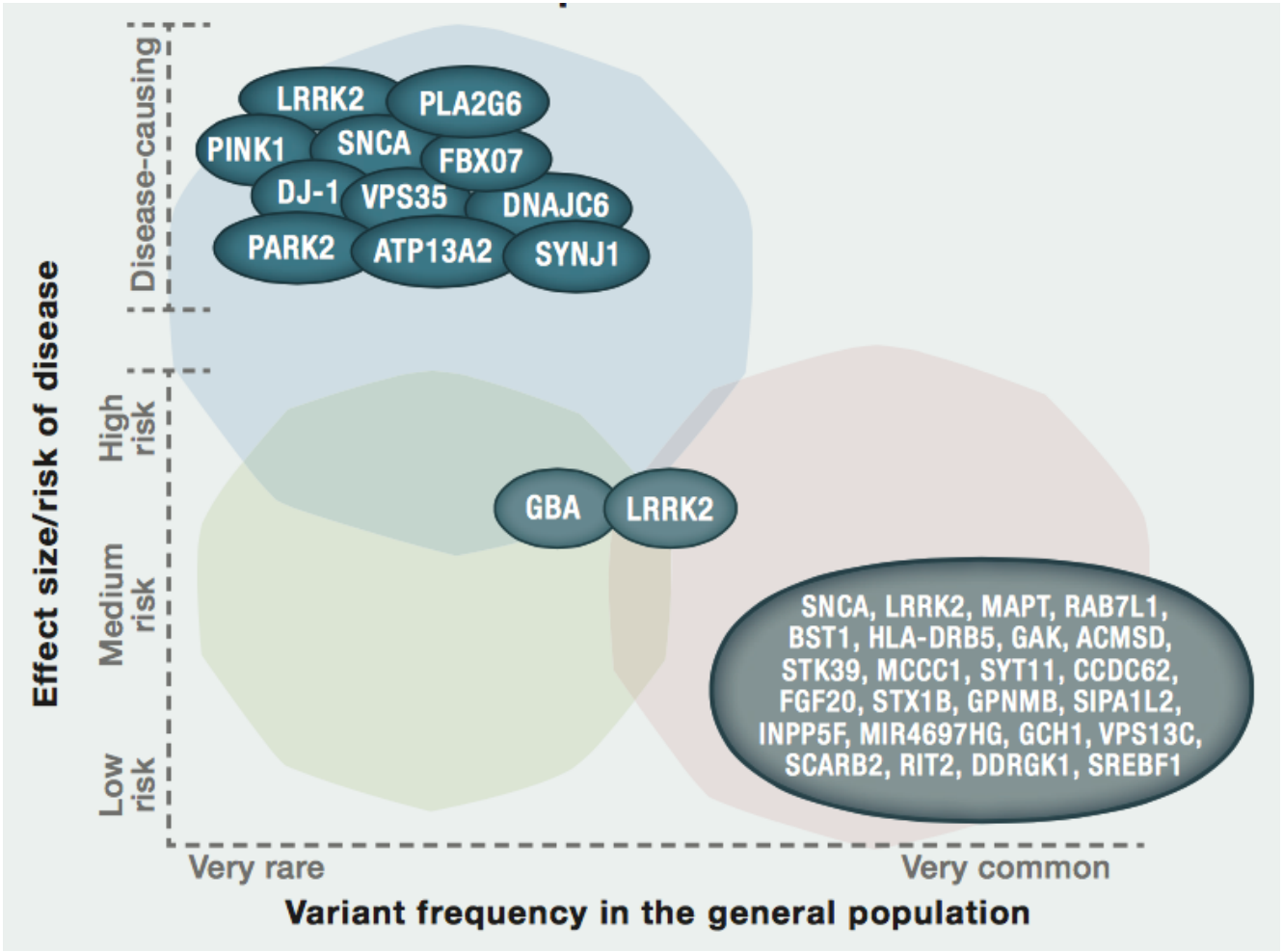
PARK	Gene	Inheritance	Onset	Function
PARK1	*SNCA*	AD	Classical PD or EO	Protein folding
PARK2	***PARKIN***	**AR**	**EO**	**Ubiquitinylation**
PARK5	**UCHL1**[preliminary]	**AD**	**Classical PD**	**Ubiquitinylation**
PARK6	***PINK1***	**AR**	**EO**	**Phosphorylation**
PARK7	**DJ-1**	**AR**	**EO**	Protein folding
PARK8	**LRRK2**	**AD**	**Classical PD**	**Phosphorylation**
PARK9	ATP13A2	AR	Complex PD with EO	Membrane biology
PARK13	HTRA2	AD or risk factor	Classical PD	Protease
PARK14	PLA2G6	AR	EO plus dystonia	Metabolism
PARK15	**FBXO7**	**AR**	**Complex PD with EO**	**Ubiquitinylation**
PARK16	Rab7L1	Risk	Complex late	Confirmed susceptibility locus
	GAK	**RISK**	**Late PD**	**Phosphorylation**
PARK17*	VPS35	AD	Classical PD	Vesicle trafficking
PARK18*	EIF4G1	AD[unconfirmed]	Classical	Translocation factor
PARK19*	DNAJC6	AR	EO	Vesicle trafficking
PARK20*	SYNJ1	AR	Complex EO or classical	PIP-like domain
PARK21*	DNAJC13	AD	Late onset PD	Unconfirmed
PARK22*	CHCHD2	AD	Late onset PD	Confirmed
PARK23*	VPS13C	AR	EO	Vesicle trafficking
	GBA	Risk factor	Classical PD	Metabolism
	CHCHD2	AD[Asian]	Classical PD	Transcription factor
	PODXL[preliminary]	AR	EO	Membrane biology
	TMEM230	AD	Classical	Vesicle Trafficking
	GCH1	Risk factor	Classical	Metabolism

Miratul Muqit

Dario Alessi

MRC Protein Phosphorylation and Ubiquitylation Unit

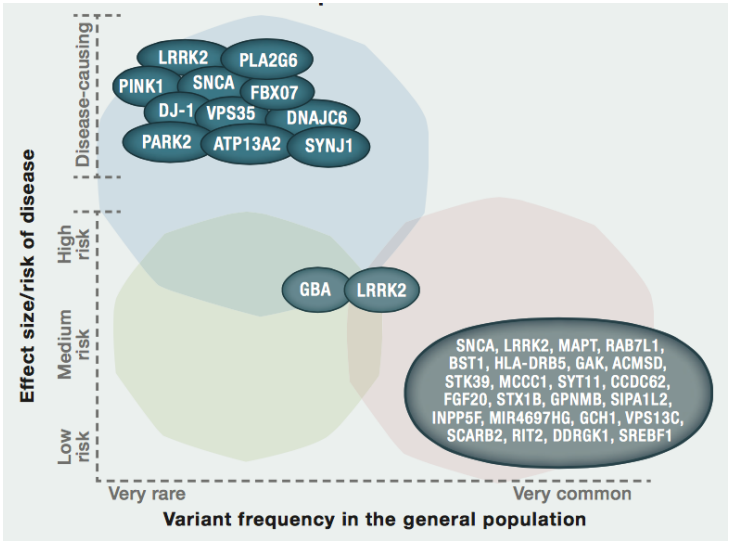
Genetic landscape of Parkinson's disease



DOI http://dx.doi.org/10.1016/j.cell.2015.01.019

Above and beyond Mendelian PD: LRRK2 as Risk

- GWAS link LKKR2 polymorphisms to an increased risk for idiopathic PD
- Clinically there is significant overlap between idiopathic and LRRK2 PD
- Thus, LRRK2 genetically links idiopathic and familial forms of PD



Mutations in LRRK2 cause autosomal dominant Parkinson's

Back to back publication in Neuron by 2 independent groups

Neuron, Vol. 44, 601–607, November 18, 2004, Copyright ©2004 by Cell Press

Mutations in *LRRK2* Cause Autosomal-Dominant Parkinsonism with Pleomorphic Pathology

Alexander Zimprich,[1,2,11] Saskia Biskup,[3,11]
Petra Leitner,[1] Peter Lichtner,[3] Matthew Farrer,[4]
Sarah Lincoln,[4] Jennifer Kachergus,[4] Mary Hulihan,[4]
Ryan J. Uitti,[5] Donald B. Calne,[6] A. Jon Stoessl,[6]
Ronald F. Pfeiffer,[7] Nadja Patenge,[1]
Iria Carballo Carbajal,[1] Peter Vieregge,[8]
Friedrich Asmus,[1] Bertram Müller-Myhsok,[9]
Dennis W. Dickson,[4] Thomas Meitinger,[3,10,*]
Tim M. Strom,[3,10] Zbigniew K. Wszolek,[5,*]
and Thomas Gasser[1,*]

Thomas Gasser (Tübingen)

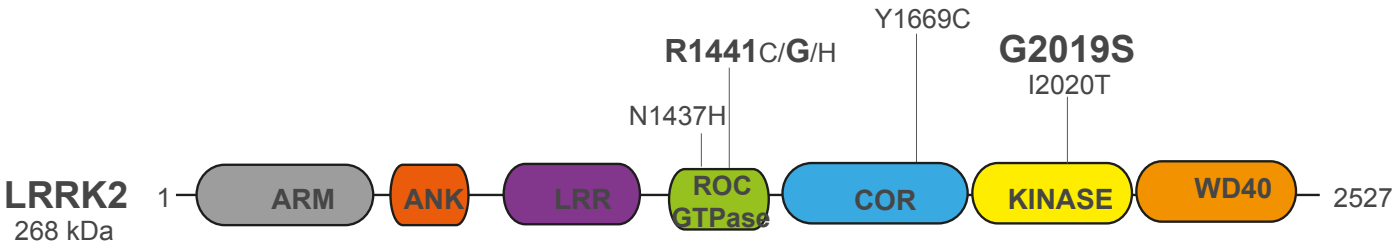
Neuron, Vol. 44, 595–600, November 18, 2004, Copyright ©2004 by Cell Press

Cloning of the Gene Containing Mutations that Cause *PARK8*-Linked Parkinson's Disease

Coro Paisán-Ruiz,[1,11] Shushant Jain,[2,3,11]
E. Whitney Evans,[4] William P. Gilks,[3] Javier Simón,[1]
Marcel van der Brug,[5] Adolfo López de Munain,[6,7]
Silvia Aparicio,[1] Angel Martínez Gil,[8]
Naheed Khan,[3] Janel Johnson,[4]
Javier Ruiz Martinez,[9] David Nicholl,[10]
Itxaso Marti Carrera,[7] Amets Saénz Peña,[6]
Rohan de Silva,[3] Andrew Lees,[3]
José Félix Martí-Massó,[7] Jordi Pérez-Tur,[1,*]
Nick W. Wood,[2,*] and Andrew B. Singleton[4,*]

Andrew Singleton (NIH Washington)

LRRK2 associated Parkinson's



- **LRRK2 genetically links familial and sporadic PD** Mutations in LRRK2 are the most common cause of late-onset autosomal dominant and sporadic Parkinson's (from 1- 2% to up to 40% in different populations)
- **LRRK2** encodes a large multi-domain protein and functions as a ***Protein Kinase***
- All pathogenic LRRK2 mutations reside in the catalytic core of the protein.
- As such LRRK2 is potentially drug-able and several pharmaceutical companies are already undertaking pre-clinical research with promising LRRK2 inhibitors

Autosomal dominant: LRRK2

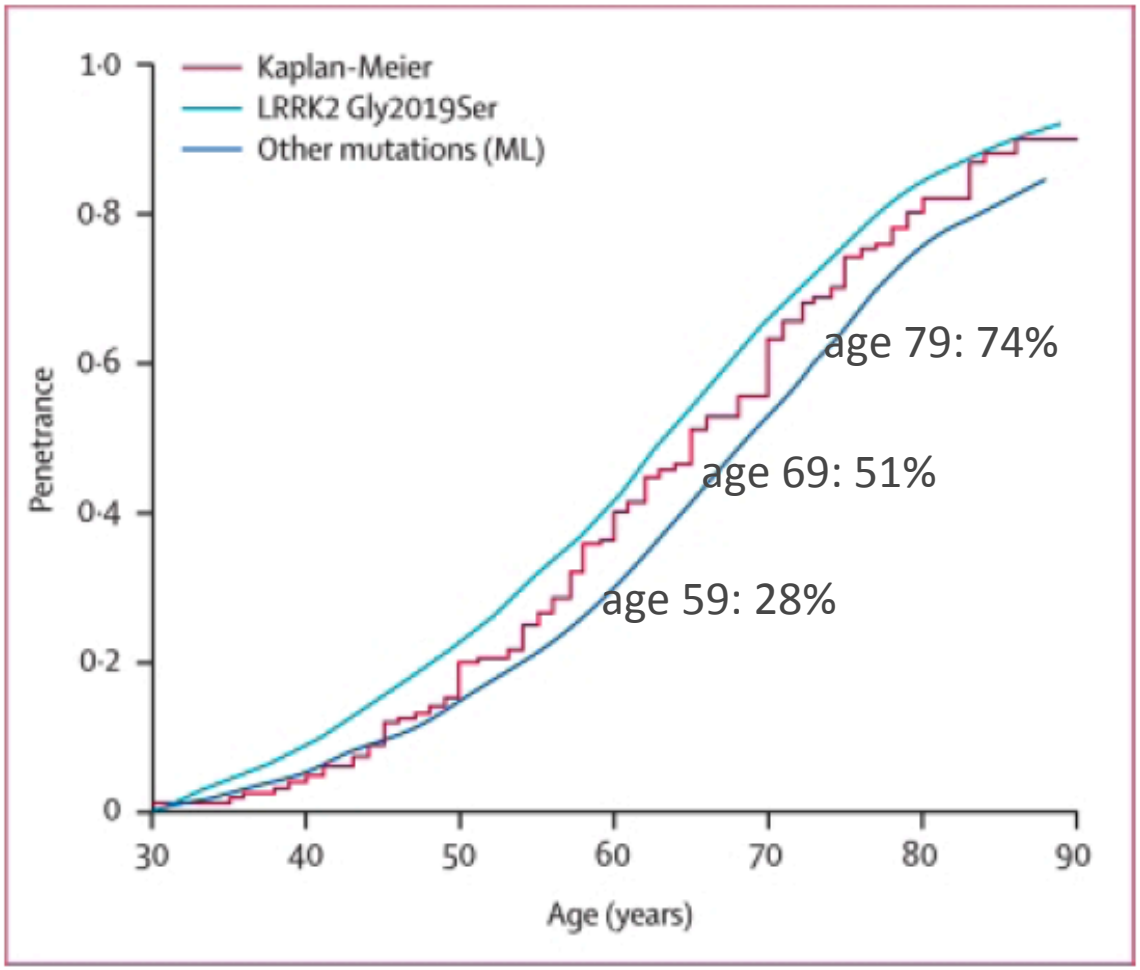
- Changes in the LRRK2 gene are the greatest genetic contributor to Parkinson's
- Six mutations have repeatedly been shown to segregate with disease in an autosomal dominant fashion. These are thought to result in a gain of function
- Frequency of LRRK2 mutations varies dependent on ethnicity:

  British: 1.6% of sporadic PD (Gilks et al., Lancet 2005), Ashkenazi Jews:

  29% of familial and 13% of sporadic cases (Ozelius et al. NEJM),

  North African Arab Berbers: 37% (Lesage et al. NEJM 2006)
- Since 2004, some 1500 papers have been published, but information on mode of action is still vague

Autosomal dominant: LRRK2 G2019S – age dependent penetrance



Healy et al. Lancet Neurol 2008

18,000 patients screened; G2019S mutations in 354
Phenotype similar to IPD

Autosomal dominant: LRRK2 encodes a protein kinase

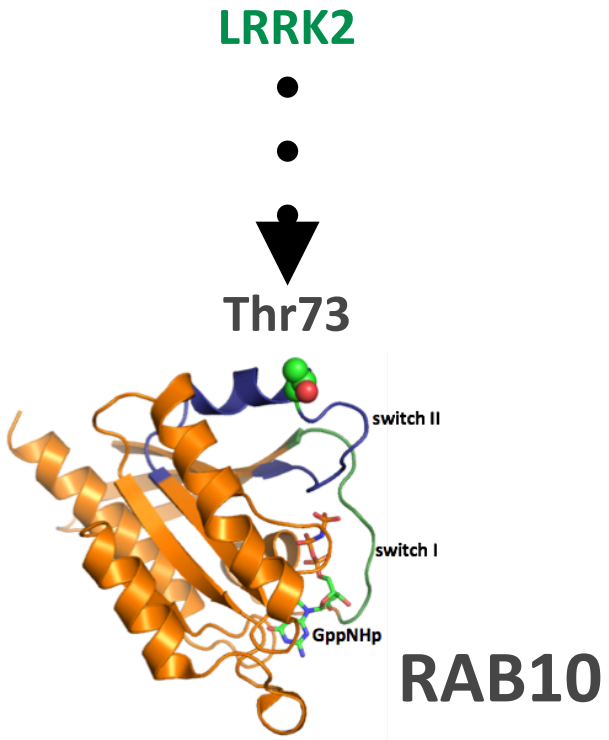
Alessi lab discovers first phosphorylation target of LRRK2

LRRK2 directly phosphorylates a subset of Rab GTPases including Rab10 at Thr73, within their Switch-II effector binding motif

eLIFE
elifesciences.org

Phosphoproteomics reveals that Parkinson's disease kinase LRRK2 regulates a subset of Rab GTPases

Martin Steger[1], Francesca Tonelli[2], Genta Ito[2], Paul Davies[2], Matthias Trost[2], Melanie Vetter[3], Stefanie Wachter[3], Esben Lorentzen[3], Graham Duddy[4†], Stephen Wilson[5], Marco AS Baptista[6], Brian K Fiske[6], Matthew J Fell[7], John A Morrow[8], Alastair D Reith[9], Dario R Alessi[2*], Matthias Mann[1*]



Biomarkers in LRRK2 associated Parkinsonism

LRRK2 mediated phosphorylation of RabGTPases

Esther
Ivonna Fan (postdoctoral researcher in Dario Alessi's lab)
Andy Howden (Postdoc with Prof D Cantrell)
Alessi lab

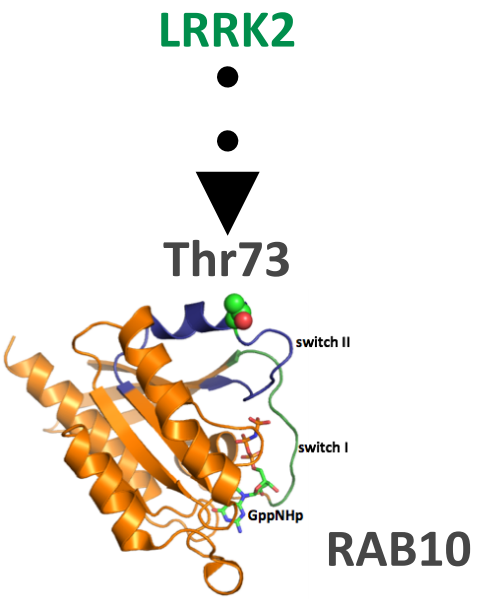
LRRK2 mediated Rab phosphorylation?

Biomarker for LRRK2 associated PD?

Ability to monitor LRRK2 pathway activity in PD patients?

Assess efficacy and target engagement of administered LRRK2 inhibitors?

Suitability for translation into human system?



eLIFE elifesciences.org

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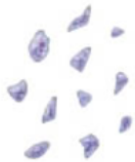
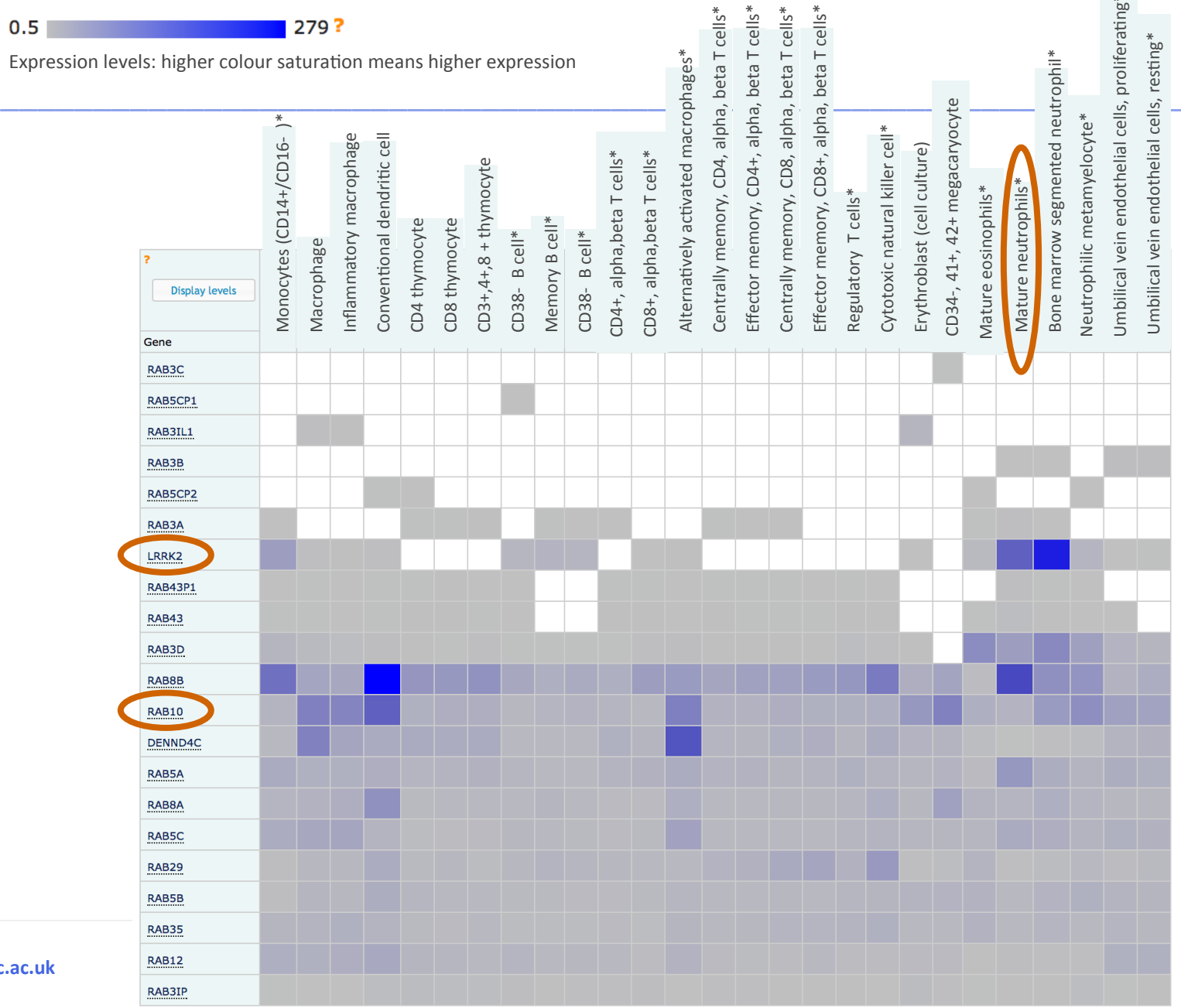
Formed elements of the blood

CELL TYPE	ILLUSTRATION	DESCRIPTION*	CELLS/μL (mm^3) OF BLOOD	DURATION OF DEVELOPMENT (D) AND LIFE SPAN (LS)	FUNCTION
Erythrocytes (red blood cells, RBCs)		Biconcave, anucleate disc; salmon-colored; diameter 7–8 μm	4–6 million	D: about 15 days LS: 100–120 days	Transport oxygen and carbon dioxide
Leukocytes (white blood cells, WBCs)		Spherical, nucleated cells	4800–10,800		
Granulocytes					
▪ Neutrophil		Multilobed nucleus; inconspicuous cytoplasmic granules; diameter 10–12 μm	3000–7000	D: about 14 days LS: 6 hours to a few days	Phagocytize bacteria
▪ Eosinophil		Bilobed nucleus; red cytoplasmic granules; diameter 10–14 μm	100–400	D: about 14 days LS: about 5 days	Kill parasitic worms; complex role in allergy and asthma
▪ Basophil		Bilobed nucleus; large purplish-black cytoplasmic granules; diameter 10–14 μm	20–50	D: 1–7 days LS: a few hours to a few days	Release histamine and other mediators of inflammation; contain heparin, an anticoagulant
Agranulocytes					
▪ Lymphocyte		Spherical or indented nucleus; pale blue cytoplasm; diameter 5–17 μm	1500–3000	D: days to weeks LS: hours to years	Mount immune response by direct cell attack or via antibodies
▪ Monocyte		U- or kidney-shaped nucleus; gray-blue cytoplasm; diameter 14–24 μm	100–700	D: 2–3 days LS: months	Phagocytosis; develop into macrophages in the tissues
Platelets		Discoid cytoplasmic fragments containing granules; stain deep purple; diameter 2–4 μm	150,000–400,000	D: 4–5 days LS: 5–10 days	Seal small tears in blood vessels; instrumental in blood clotting

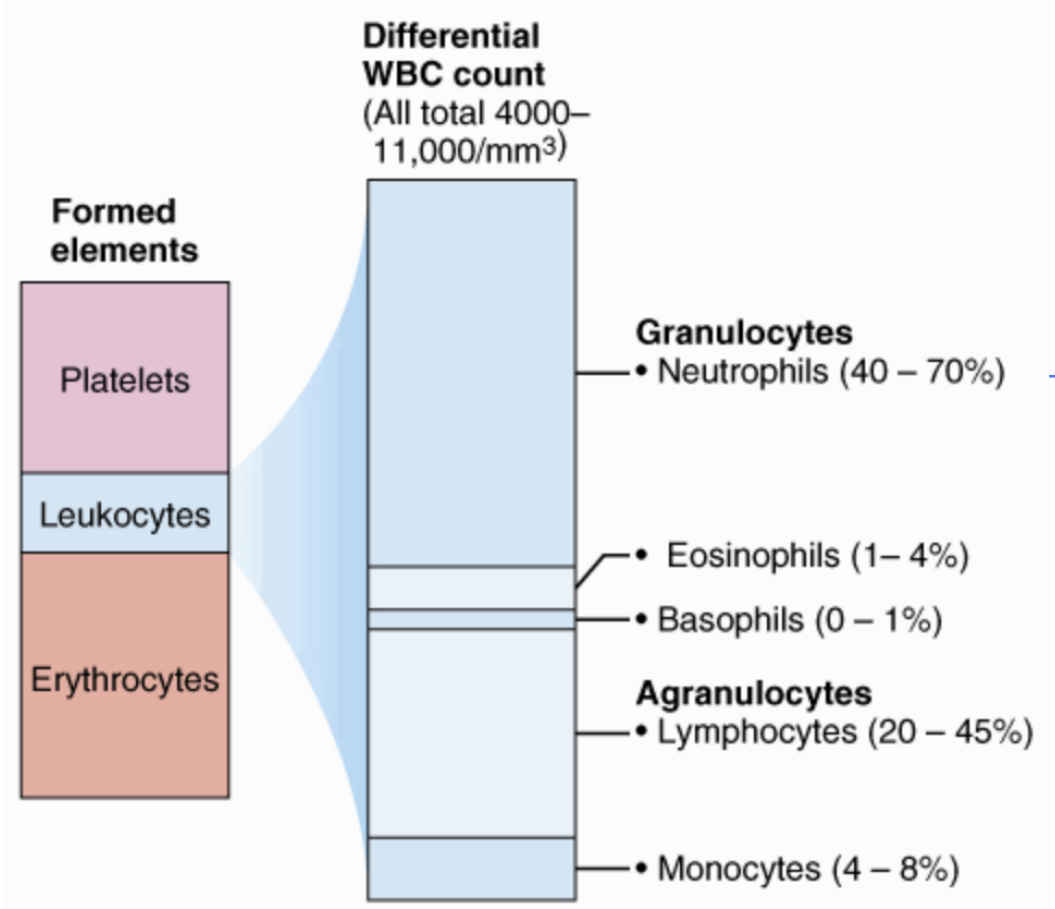
'PBMC'

EMBL-EBI Expression Atlas



Strand-specific RNA-Seq or rRNA-depleted total RNA from primary cells of different haemotopoetic lineages from healthy individuals in the Blueprint epigenome project (*uncultured vs cultured primary cells)

Differential cell count of white blood cells ('leukocytes')



Neutrophils

Peripheral blood mononuclear cells 'PBMC'

Advantages of neutropils

Homogeneous cell population

~60% of white blood cells in blood

Kit isolation takes 15-20 min

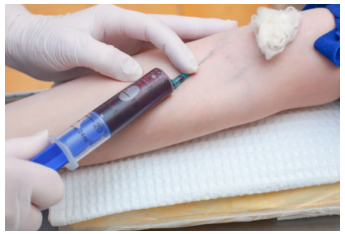
Neutrophils 98-99% pure

0.5-1mg of protein from 20 ml of human blood

Analysis of LRRK2 in Human Neutrophils: Workflow

Venesection (20 ml blood)

5 min

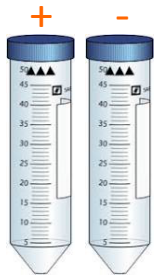


Neutrophil isolation via negative selection

30 min

LRRK2 Inhibitor treatment

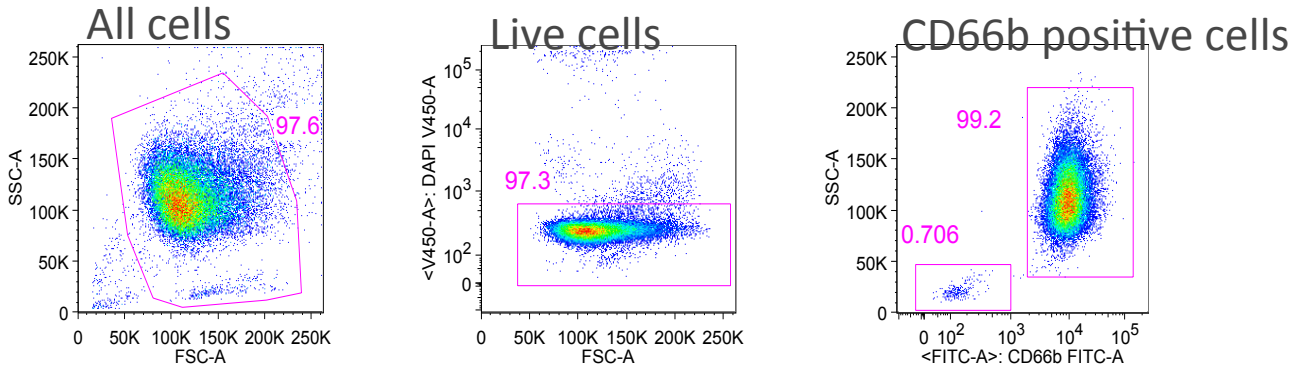
30 min



Cell lysis and storage at -80°C

ANALYSIS

High Purity and Efficacy of Neutrophil Isolation from 20ml of Human Peripheral Blood from healthy donors

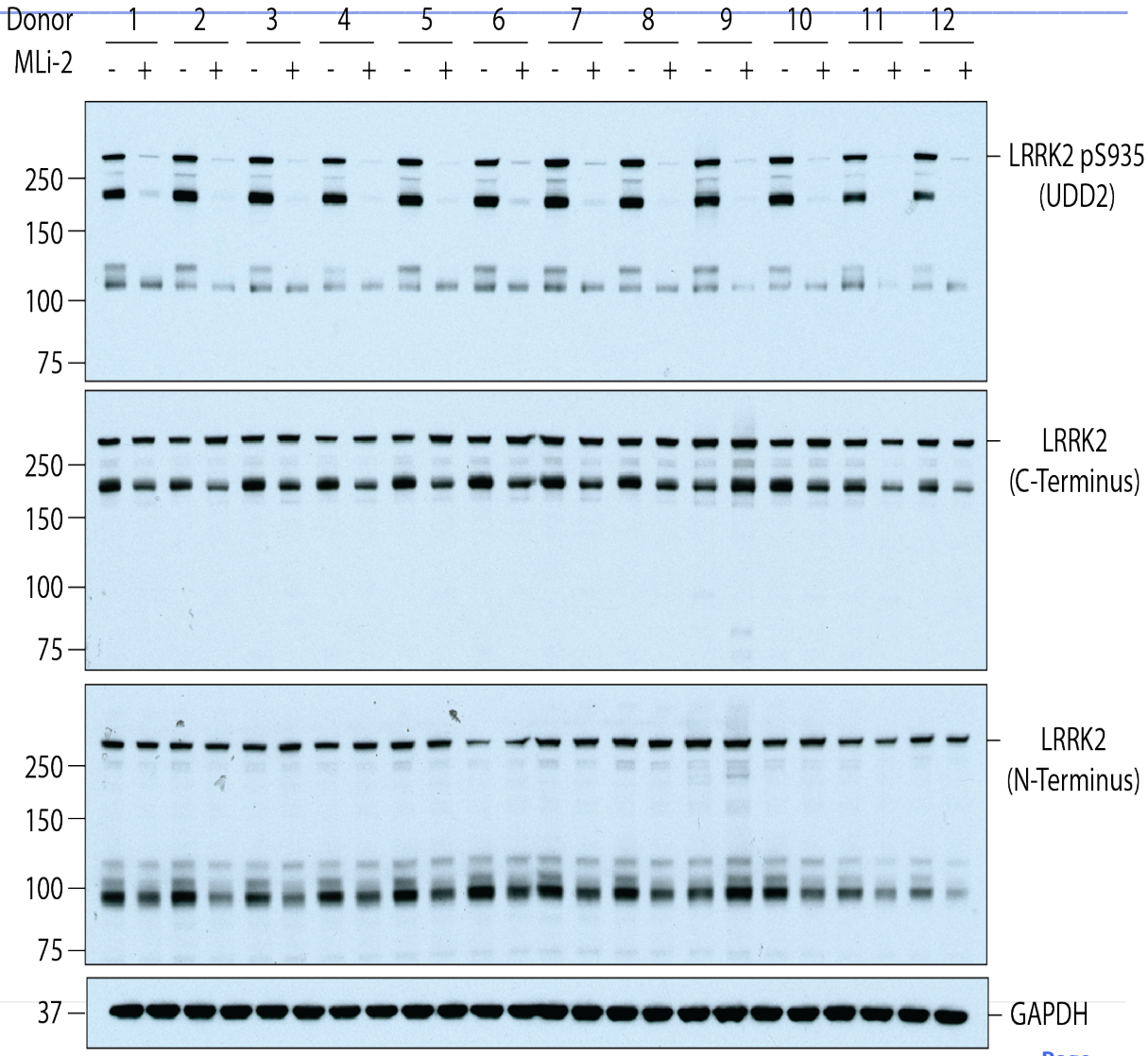


Donor	Gender	Cell counts (million/ ml)	Purity	Lysate protein conc. (µg/µl)	Total protein amount (µg/ condition)
1	Male	2.27	98.20%	4.87	1105
2	Female	1.33	99.40%	2.67	355
3	Male	1.47	97.60%	3.25	477
4	Male	1.2	99.10%	4.95	594
5	Female	1.58	99.20%	4.58	723
6	Female	1.44	99.30%	3.85	554
7	Male	1.63	99.10%	4.34	707
8	Male	1.53	96.90%	4.41	674
9	Female	1.61	99.20%	1.31	210
10	Male	3.7	99.30%	2.13	788
11	Male	2.12	99.00%	1.24	262
12	Female	5	99.80%	2.79	1395

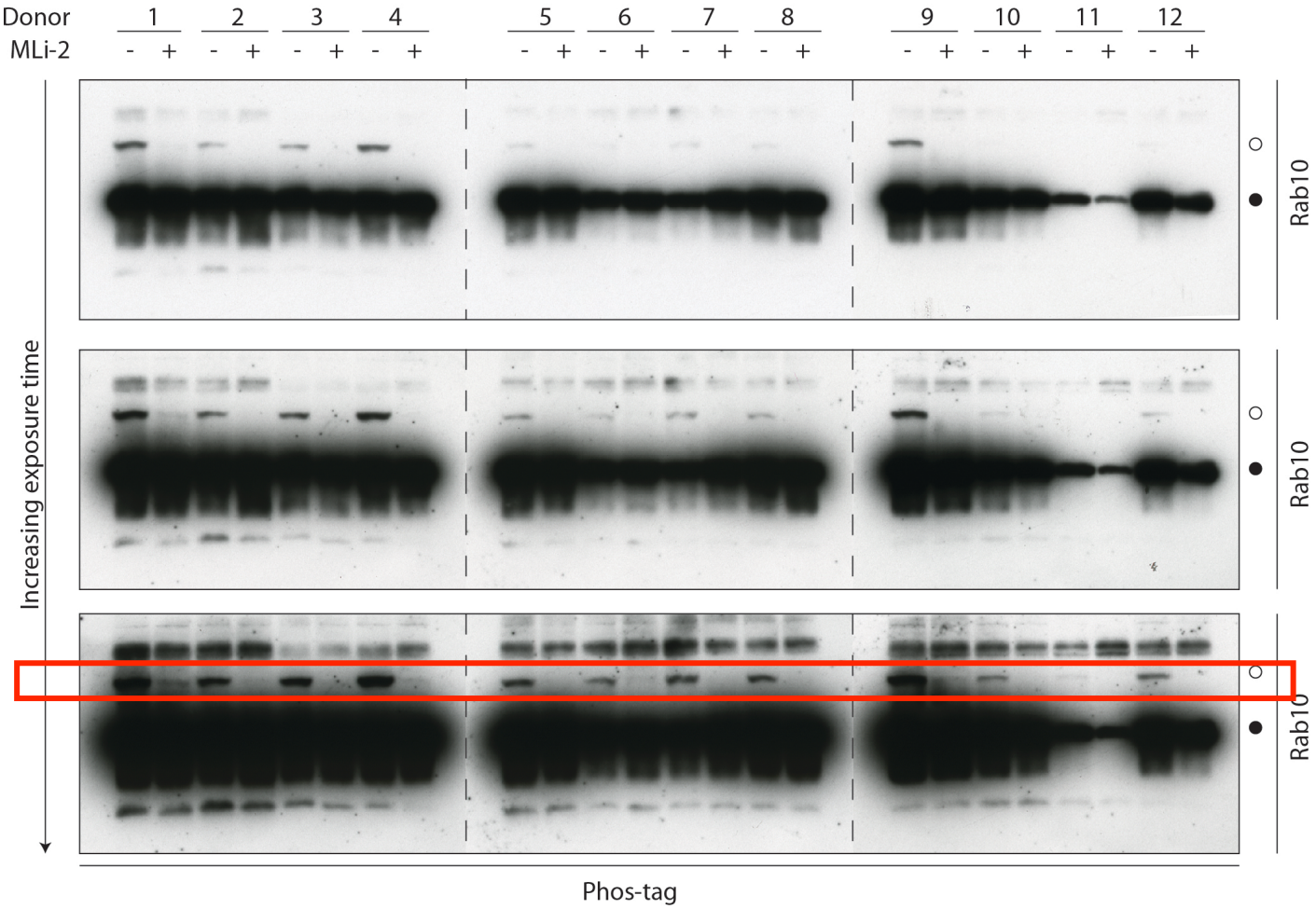
LRRK2-dependent Rab10 Phosphorylation in Human Neutrophils

LRRK2 control blots
MLi-2: specific LRRK2 Inhibitor

- Note that auto-phosphorylation at pS935 LRRK2 is dephosphorylated upon treatment with MLi2

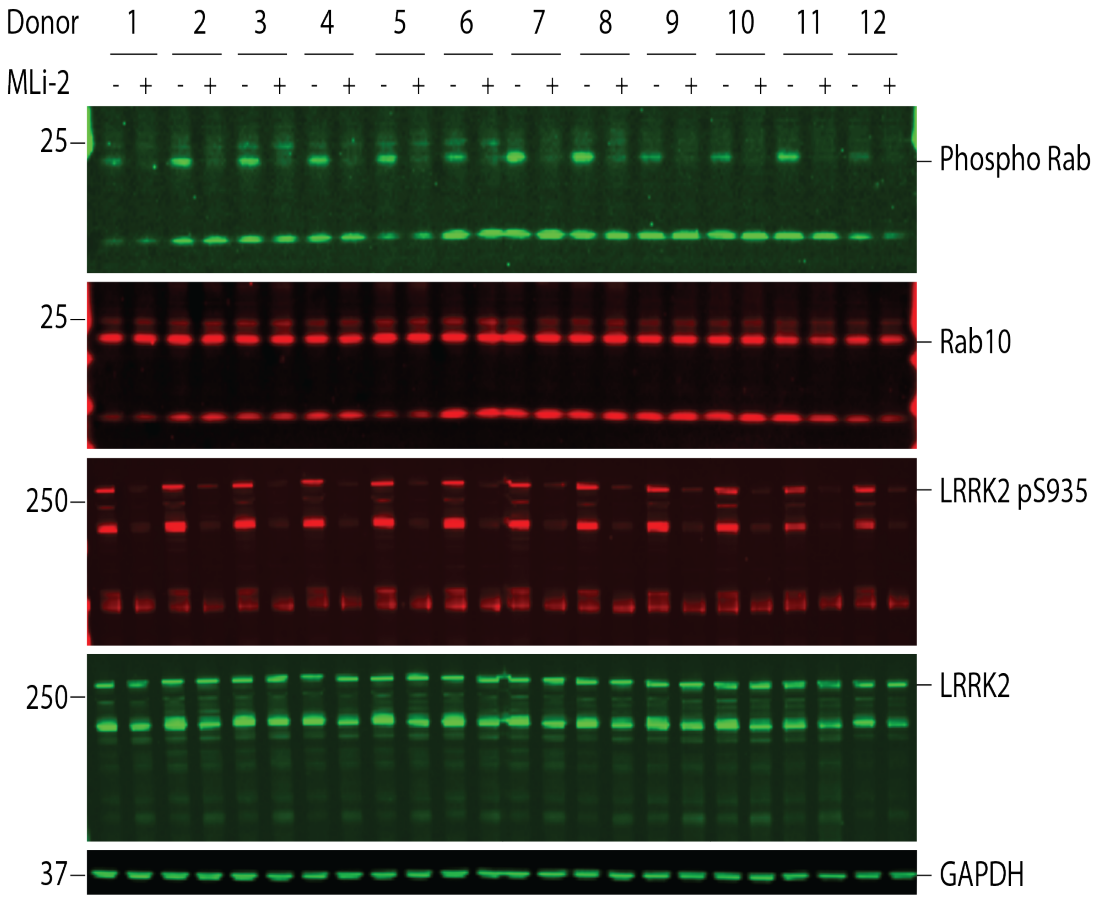


Analysis of LRRK2 in Human Neutrophils- Rab 10 Phos-tag assay



Rab 10 phosphorylation (red box) is detectable in all 12 samples and disappears upon specific inhibition of the protein kinase LRRK2

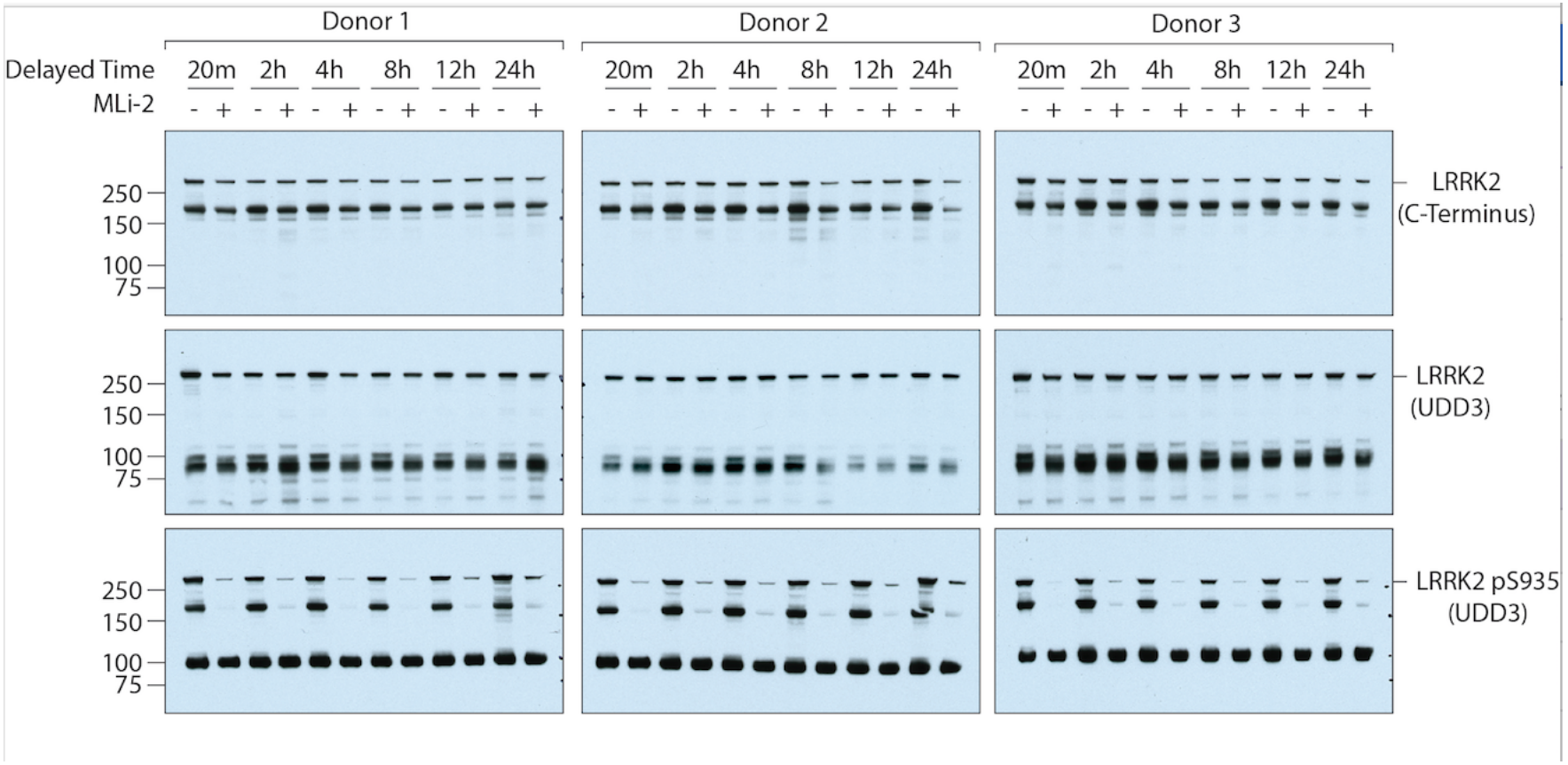
LRRK2-dependent Rab10 Phosphorylation in Human Neutrophils



Phospho-specific Rab antibodies (top panel) have been developed, which now allow quantitative analysis of LRRK2 dependent Rab phosphorylation

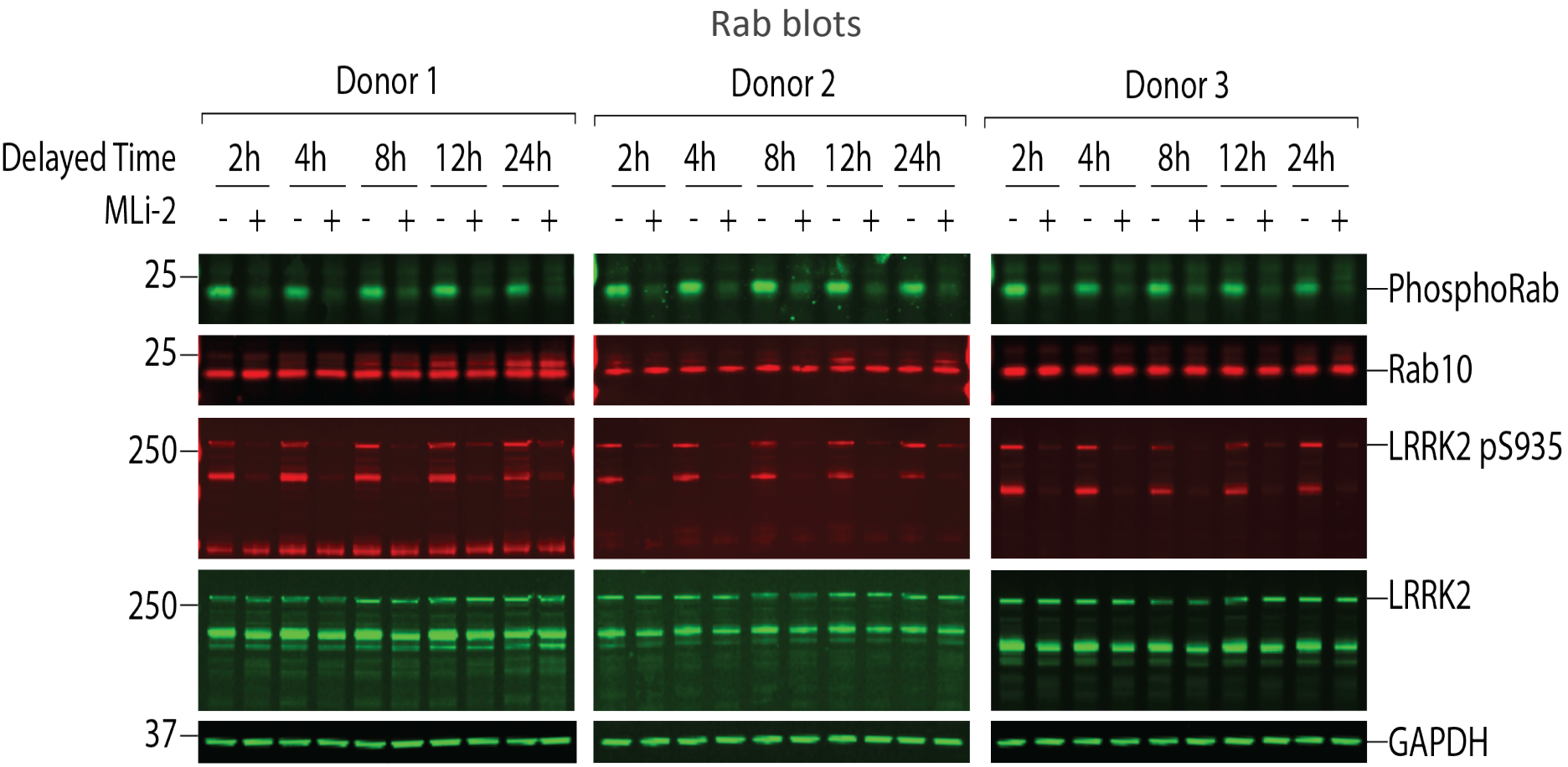
Delayed processing experiments:
Feasibility study for future study in patient samples

LRRK2 control blots



LRRK2 control blots

Delayed processing experiments:
Feasibility study for future study in patient samples



Blood can be stored at room temperature for 24 hours prior to neutrophil isolation without impacting on LRRK2-mediated Rab phosphorylation.

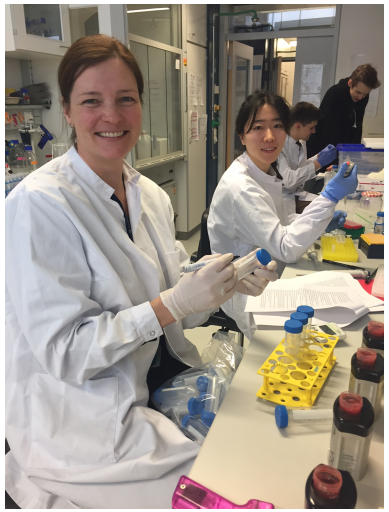
Pilot study in patients now underway

G2019S LRRK2 associated Rab phosphorylation

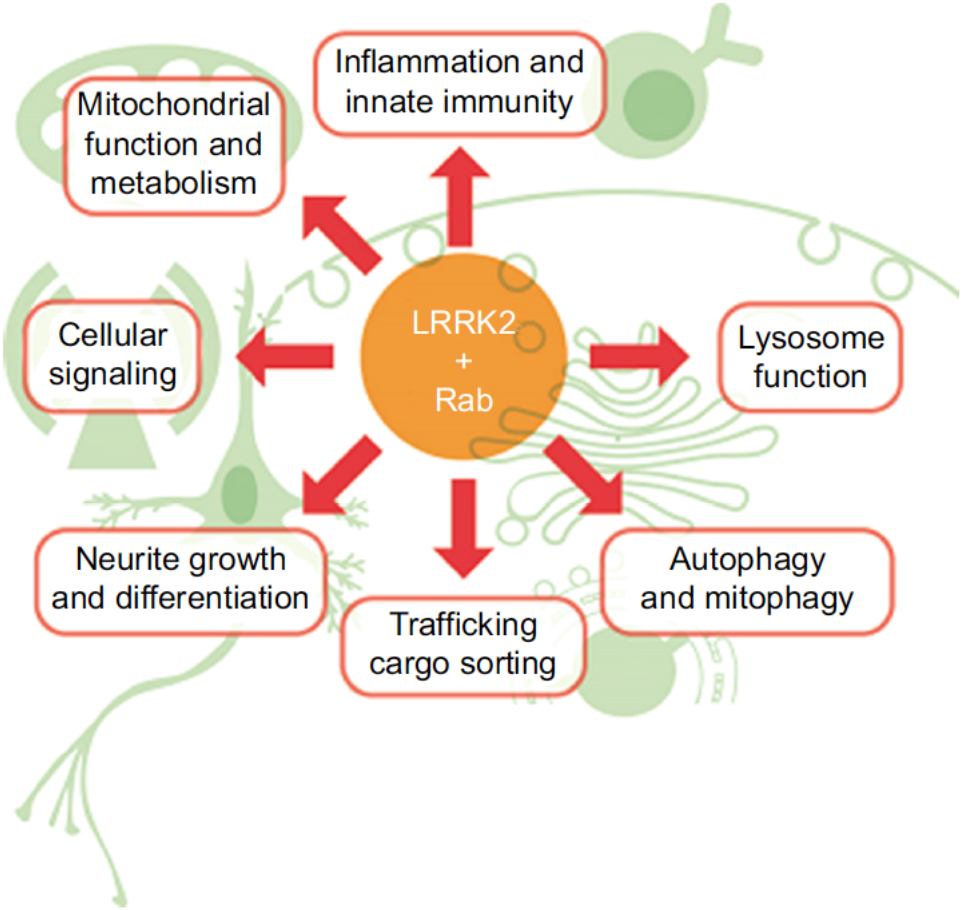
Esther Sammler, Alessi lab
Ivonna Fan, Alessi lab

In collaboration with

Professor Thomas Gasser
Dr. Kathrin Brockmann
University of Tuebingen, Germany



Rab proteins as potential mediators of LRRK2 pathology



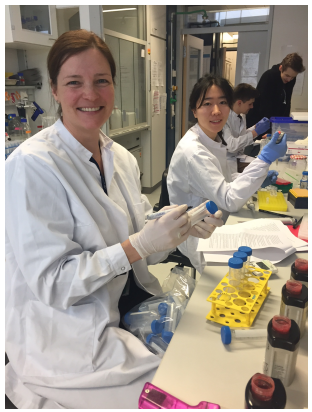
Additional evidence for Rab biology in PD

- PINK1 has also been shown to target Rabs as substrates
- PARK-Rab39B associated with x-linked EO PD +/- intellectual disability
- Rab7L1 has been identified as risk factor

Summary

- Genetic research in Parkinson's provides a tool to study the early molecular mechanisms of the disease
- Important basic research findings are often not efficiently translated into the human system
- Professor Dario Alessi's lab together with international collaborators has recently identified Rab proteins as a substrate of LRRK2 (2016)
- We have now developed a robust assay to quantitatively asses LRRK2 mediated Rab phosphorylation in peripheral blood samples
- The assay provides a tool to
  - monitoring LRRK2 pathway activity in a blood sample
  - assessing efficacy and target engagement of administered LRRK2 inhibitors?
- More research and more samples are needed to explore whether LRRK2 mediated Rab phosphorylation could serve as a biomarker for Parkinson's

Thank you



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