



# FROM BASIC SCIENCE DISCOVERY TO BENCH TO BEDSIDE PARKINSON'S RESEARCH

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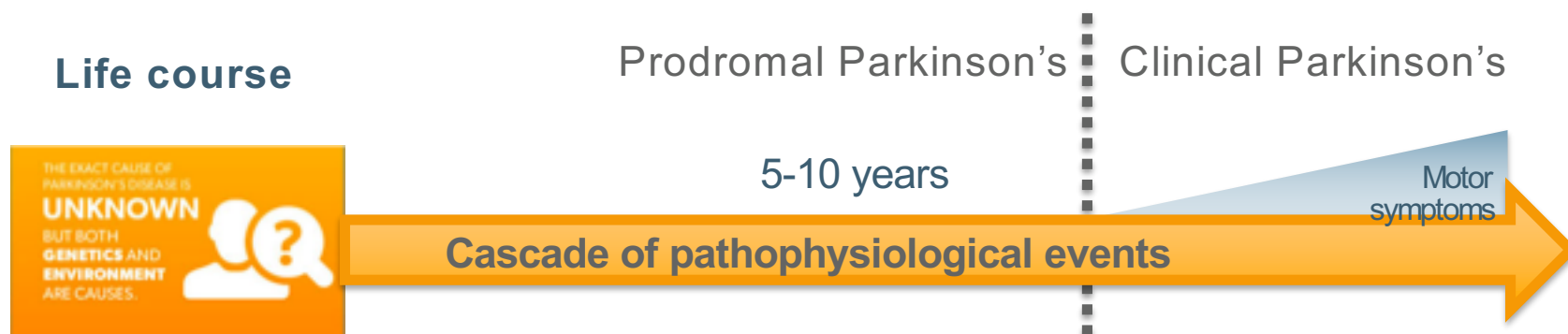
Honorary Clinical Principal Investigator, MRC PPU

# WHAT IS PARKINSON'S DISEASE?

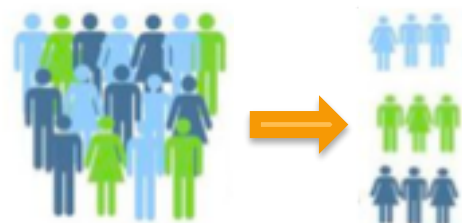


Modified: <https://www.michaeljfox.org/page.html?what-is-parkinsons-infographic>

# WISHLIST OF A CLINICAL NEUROLOGIST



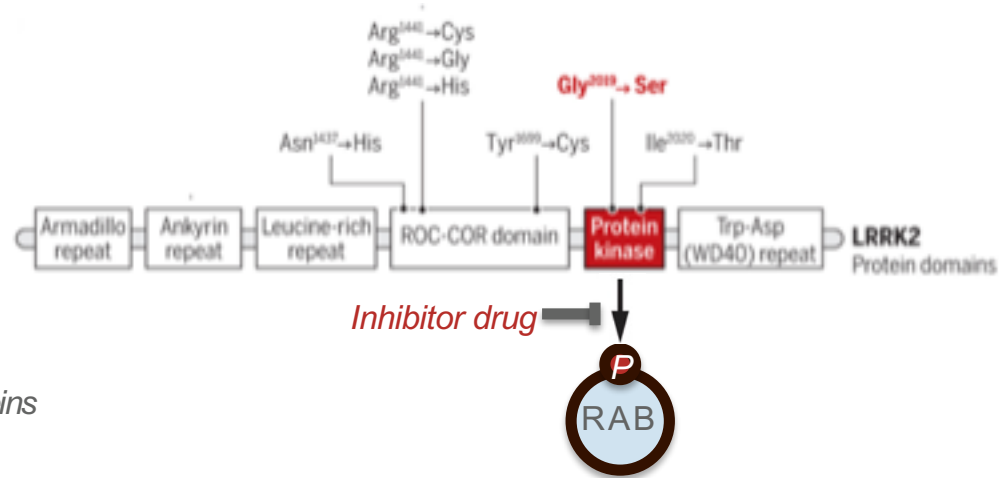
- » Simple test for diagnosis
- » Markers of PD progression
- » Disease modifying / neuroprotective treatments
- » Better symptomatic treatments
- » Better 'patient stratification' methods / precision medicine
- » Biomarkers to predict people at risk for developing PD



# LEUCINE RICH REPEAT KINASE 2 (LRRK2)

2004

LRRK2  
Disease causing mutations



2016

*RAB* proteins  
LRRK2 phosphorylates a  
subgroup of 14 RAB proteins  
at the equivalent site

- » Genetic changes in LRRK2 cause Parkinson's disease
- » LRRK2 functions as a PROTEIN KINASE and all LRRK2 mutations activate kinase activity
- » RAB proteins are phosphorylation substrates of LRRK2
- » **Bench to bedside translation: true in the human setting and clinical utility thereof ?**

# POPULATION SPECIFIC FREQUENCY OF LRRK2 G2019S

- » LRRK2 is the most frequently mutated gene in PD
- » The G2019S mutation represents 4% of familial and 1% of sporadic PD across all populations



<https://doi.org/10.1016/j.parkreldis.2010.11.008>

# BENCH TO BEDSIDE LRRK2 RESEARCH IN PARKINSON'S



Can we measure LRRK2 controlled RAB phosphorylation in human bio samples?








Can we develop a simple and robust test for LRRK2 activity / Rab phosphorylation?

What bio sample is most suitable?

Would this test allow identifying individuals who might benefit from future LRRK2 inhibitor treatment?

Reverse translation? From bedside back to bench?

# FORMED ELEMENTS OF THE BLOOD

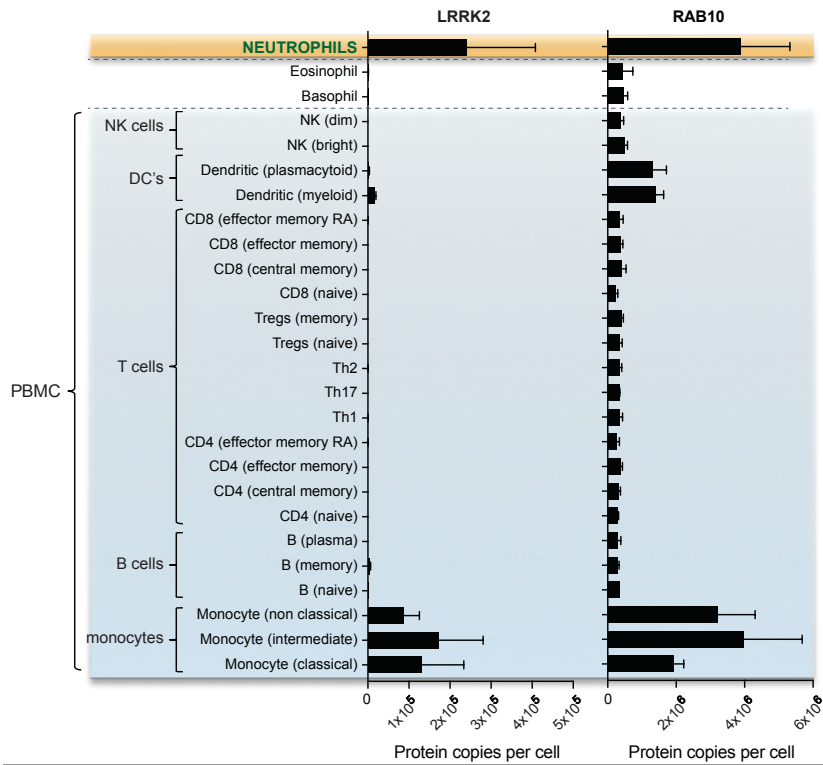
CELL TYPE	ILLUSTRATION	DESCRIPTION*	CELLS/ $\mu\text{L}$ ( $\text{mm}^3$ ) OF BLOOD	DURATION OF DEVELOPMENT (D) AND LIFE SPAN (LS)	FUNCTION
<b>Erythrocytes</b> (red blood cells, RBCs)		Biconcave, anucleate disc; salmon-colored; diameter 7–8 $\mu\text{m}$	4–6 million	D: about 15 days LS: 100–120 days	Transport oxygen and carbon dioxide
<b>Leukocytes</b> (white blood cells, WBCs)		Spherical, nucleated cells	4800–10,800		
<b>Granulocytes</b>					
• Neutrophil		Multilobed nucleus; inconspicuous cytoplasmic granules; diameter 10–12 $\mu\text{m}$	3000–7000	D: about 14 days LS: 6 hours to a few days	Phagocytize bacteria
• Eosinophil		Bilobed nucleus; red cytoplasmic granules; diameter 10–14 $\mu\text{m}$	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 $\mu\text{m}$	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
<b>Agranulocytes</b>					
• Lymphocyte		Spherical or indented nucleus; pale blue cytoplasm; diameter 5–17 $\mu\text{m}$	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 $\mu\text{m}$	100–700	D: 2–3 days LS: months	Phagocytosis; develop into macrophages in the tissues
<b>Platelets</b>		Discoid cytoplasmic fragments containing granules; stain deep purple; diameter 2–4 $\mu\text{m}$	150,000–400,000	D: 4–5 days LS: 5–10 days	Seal small tears in blood vessels; instrumental in blood clotting

**Neutrophils**  
40-70%

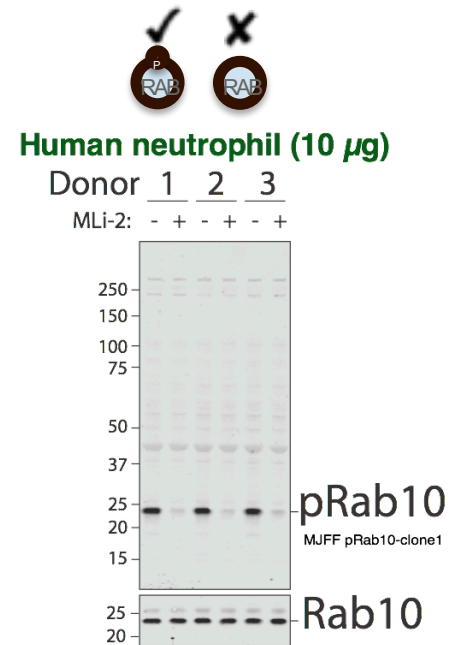
**PBMCs**  
29-69%

# LRRK2 MEDIATED RAB10<sup>THR73</sup> PHOSPHORYLATION IN HUMAN PERIPHERAL BLOOD

- » Human Neutrophils express high levels of LRRK2 and Rab10
- » Availability of highly sensitive and phospho-specific antibodies for pRAB10<sup>Thr73</sup>



## MJFF-phospho-Rab10<sup>Thr73</sup>



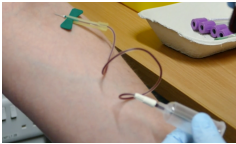
Pavel Lis



# BLOOD TESTS TO LOOK FOR LRRK2 CONTROLLED RAB PHOSPHORYLATION

» Neutrophil isolation via negative immunomagnetic selection is quick and efficient

**Venesection  
(20 ml blood)**



**3 min**

**Neutrophil isolation  
via negative selection**



**40 min**

**+/- LRRK2 Inhibitor  
treatment (MLi-2)**



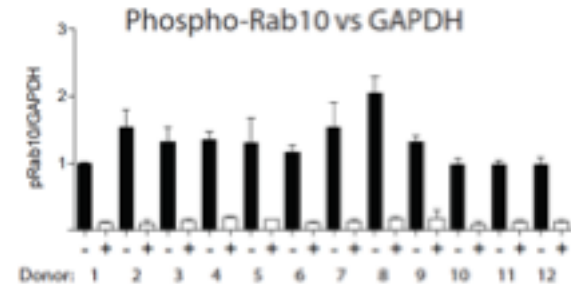
**30 min**

**Cell lysis &  
storage (-80 C)**

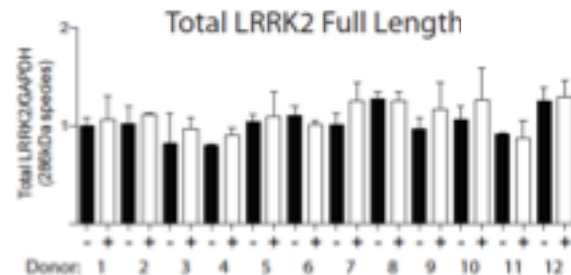
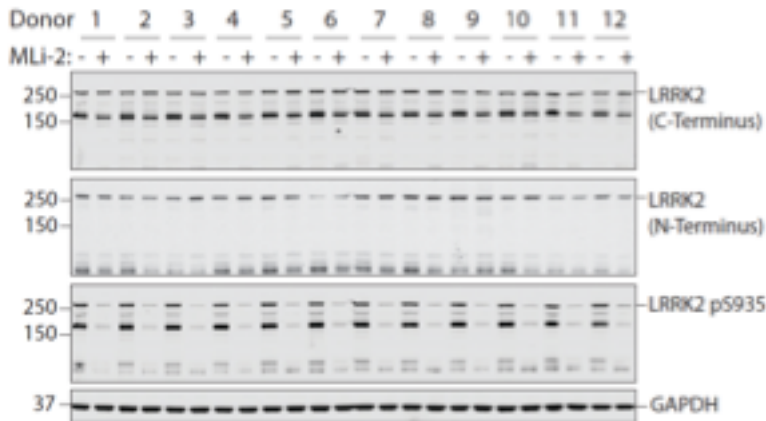
**Analysis by quantitative immunoblotting with MJFF-phosphoRab10 Ab for pRab10 as well as total Rab10, pS935 LRRK2, total LRRK2, GAPDH**

# SIMPLE AND ROBUST 'LRRK2 PHOPHO-RAB10' BLOOD TEST IN NEUTROPHILS FROM HEALTHY VOLUNTEERS

## Rab10 and phospho-RAB10<sup>pThr73</sup>



## LRRK2 and phopho-LRRK2<sup>pS935</sup>



# SIMPLE AND ROBUST 'LRRK2 PHOPHO-RAB10' BLOOD TEST IN NEUTROPHILS FROM HEALTHY VOLUNTEERS

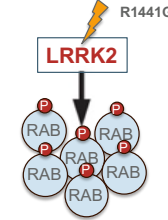
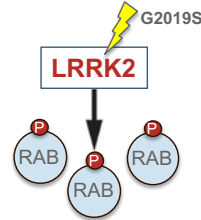
Can we measure LRRK2 controlled RAB phosphorylation in human bio samples? ✓

Can we develop a simple and robust test for LRRK2 phopho-Rab10? ✓

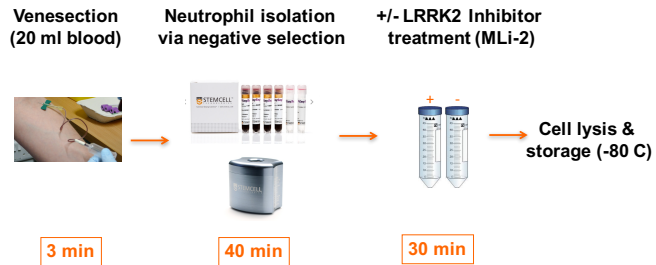
Which biomaterial is suitable? **Human white peripheral blood cells** ✓

# DOES THIS TEST ALLOW IDENTIFICATION OF INDIVIDUALS WITH INCREASED LRRK2 ACTIVITY?

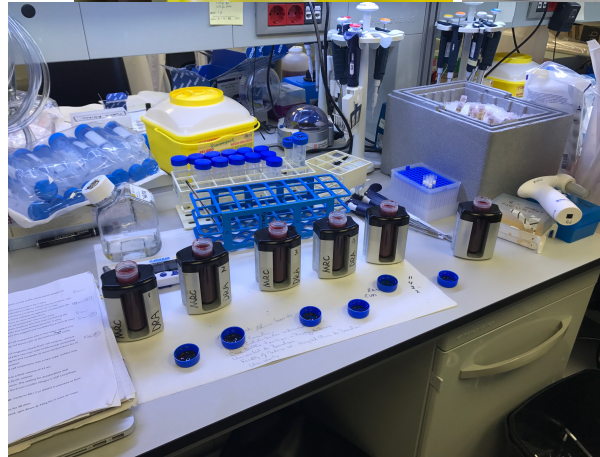
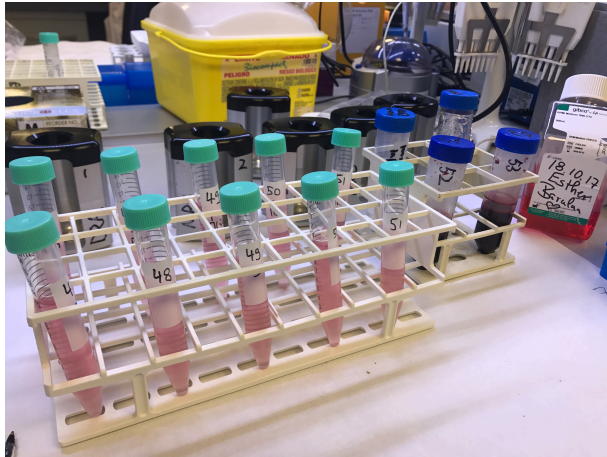
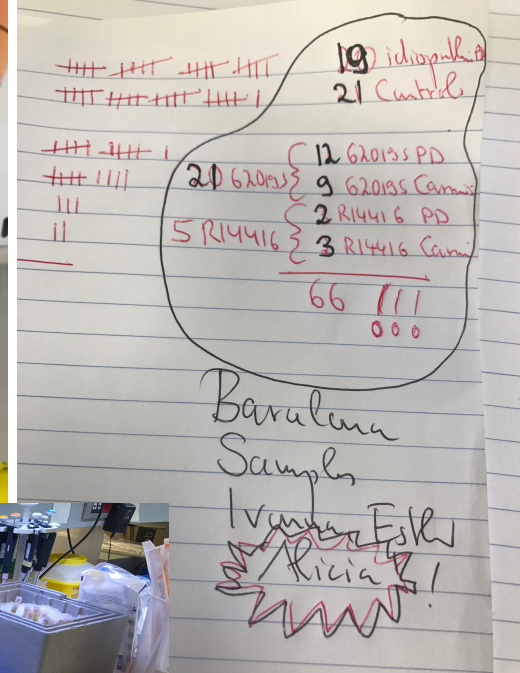
- » Collaboration with Eduardo Tolosa in Barcelona, Spain
- » Blood samples from 66 individuals:
  - 26 with LRRK2 mutations
  - 19 idiopathic Parkinson's
  - 21 healthy controls



# MEASURING LRRK2 KINASE PATHWAY ACTIVITY IN BLOOD CELLS OF PATIENTS WITH FAMILIAL AND NON FAMILIAL PARKINSON'S



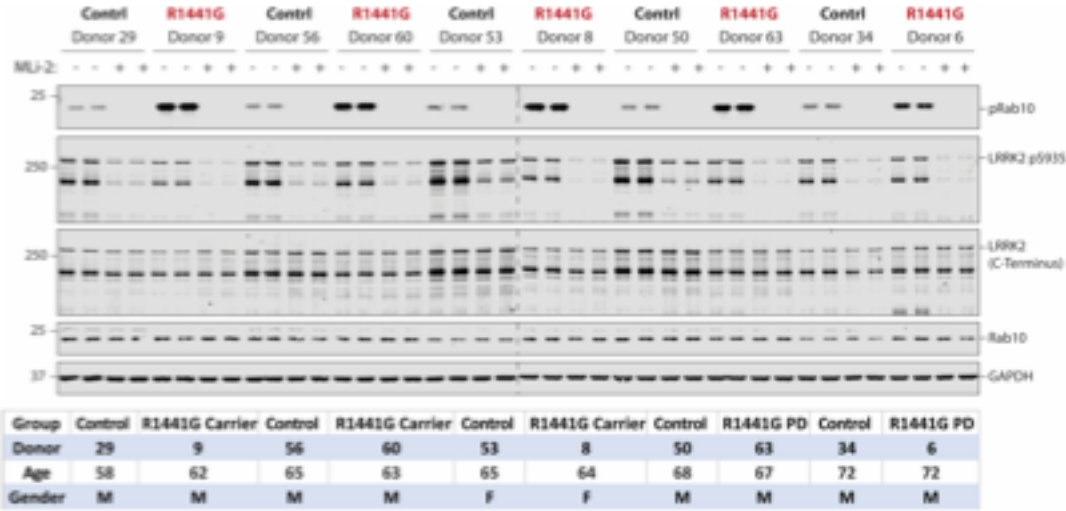
Analysis by quantitative immunoblotting with MJFF-phosphoRab10 Ab for pRab10 as well as total Rab10, pS935 LRRK2, total LRRK2, GAPDH



Sample collection and processing in Barcelona for subsequent analysis in Dundee



# SIGNIFICANT INCREASE IN LRRK2 ACTIVITY IN LRRK2 PD WITH THE BASQUE MUTATION (R1441G)




# SIMPLE AND ROBUST 'LRRK2 PHOPHO-RAB10' BLOOD TEST IN NEUTROPHILS FROM HEALTHY VOLUNTEERS

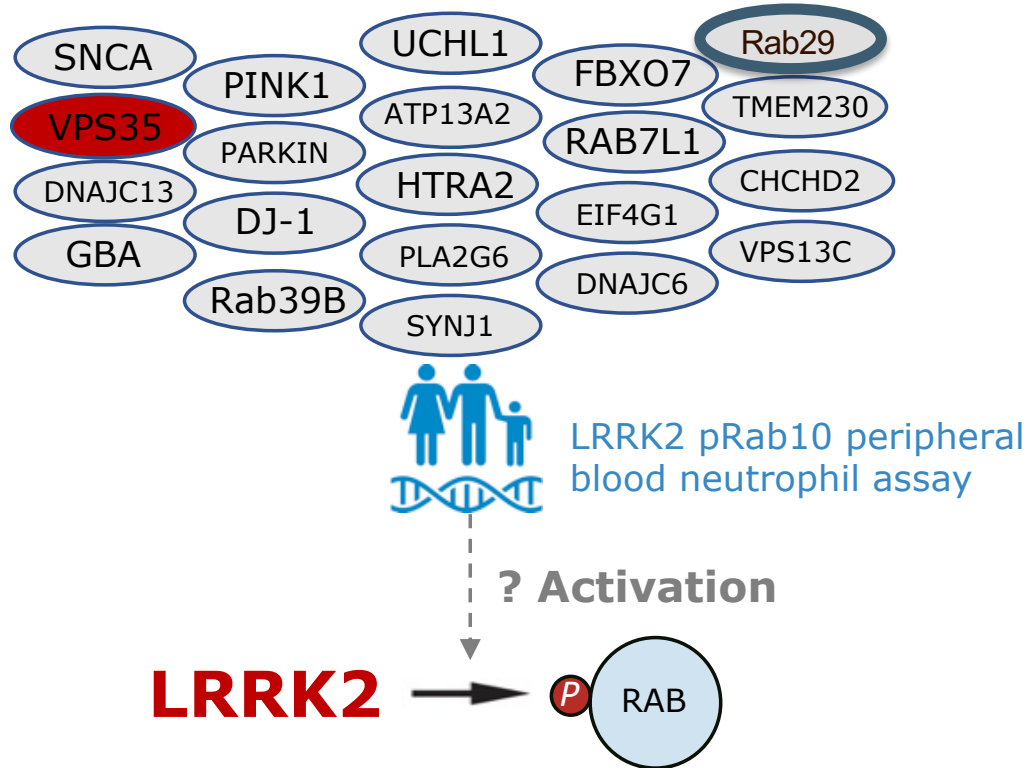
Can we measure LRRK2 controlled RAB phosphorylation in human bio samples? 

Can we develop a simple and robust test for LRRK2 phopho-Rab10? 

Which biomaterial is suitable? **Human white peripheral blood cells**

Can this test identify individuals who might from future LRRK2 inhibitor treatment? 

# IS THERE CROSSTALK BETWEEN LRRK2 AND OTHER PD GENE PRODUCTS





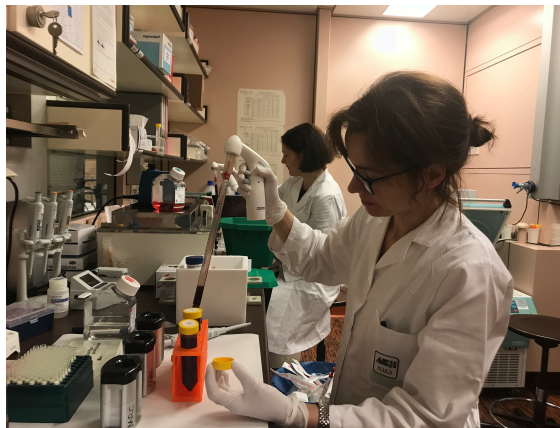
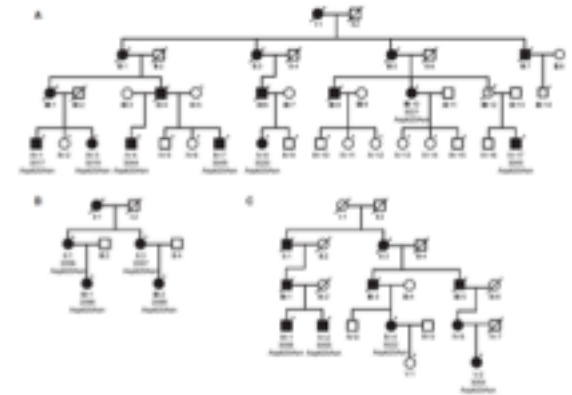
# VALIDATION: VPS35 D620N MUTATION ACTIVATES LRRK2 KINASE ACTIVITY

## REPORT

### A Mutation in *VPS35*, Encoding a Subunit of the Retromer Complex, Causes Late-Onset Parkinson Disease

Alexander Zimprich,<sup>1,14,\*</sup> Anna Benet-Pagès,<sup>2,14</sup> Walter Struhal,<sup>3,14</sup> Elisabeth Graf,<sup>2,14</sup> Sebastian H. Eck,<sup>2</sup> Marc N. Offman,<sup>4</sup> Dietrich Haubenberger,<sup>1</sup> Sabine Spielberger,<sup>5</sup> Eva C. Schulte,<sup>2,6</sup> Peter Lichtner,<sup>2</sup> Shaila C. Rossle,<sup>4</sup> Norman Klopp,<sup>7</sup> Elisabeth Wolf,<sup>5</sup> Klaus Seppi,<sup>5</sup> Walter Pirker,<sup>1</sup> Stefan Presslauer,<sup>8</sup> Brit Mollenhauer,<sup>9</sup> Regina Katzenschlager,<sup>10</sup> Thomas Foki,<sup>1</sup> Christoph Hotzy,<sup>1</sup> Eva Reinthaler,<sup>1</sup> Ashot Harutyunyan,<sup>11</sup> Robert Kralovics,<sup>11</sup> Annette Peters,<sup>7</sup> Fritz Zimprich,<sup>1</sup> Thomas Brücke,<sup>8</sup> Werner Poewe,<sup>5</sup> Eduard Auff,<sup>1</sup> Claudia Trenkwalder,<sup>9,12</sup> Burkhard Rost,<sup>4</sup> Gerhard Ransmayr,<sup>3</sup> Juliane Winkelmann,<sup>2,6,13</sup> Thomas Meitinger,<sup>2,13</sup> and Tim M. Strom<sup>2,13,\*</sup>

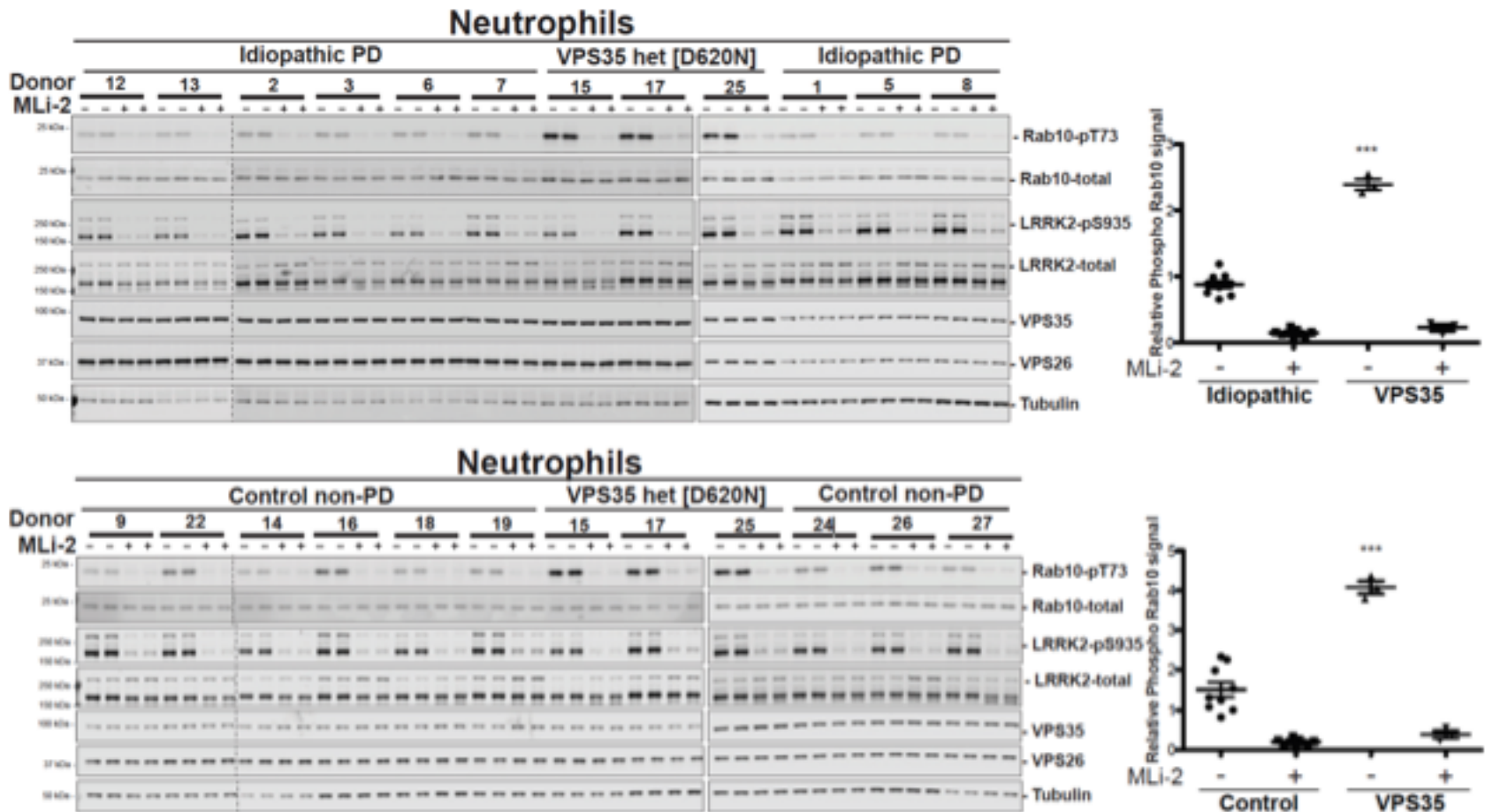
168 The American Journal of Human Genetics 89, 168-175, July 15, 2011



Francesca Tonelli

Theresa Koenig & Alexander Zimprich

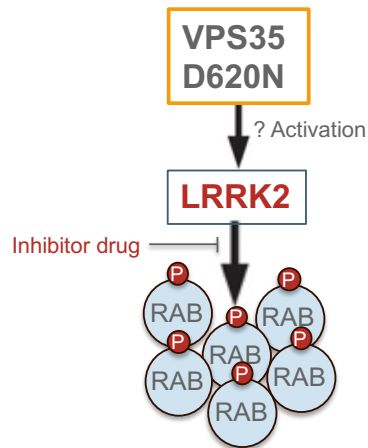
# VALIDATION OF VPS35 D620N ACTIVATION OF LRRK2 KINASE PATHWAY IN PD PATIENTS WITH VPS35 D620N MUTATION



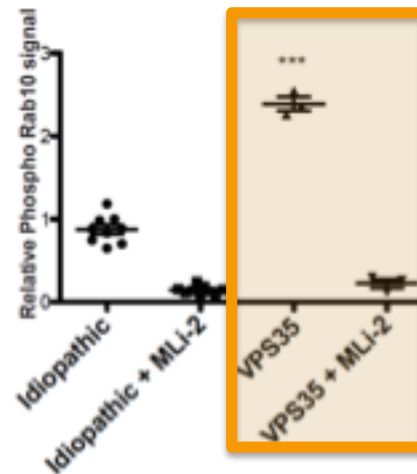
# LRRK2 PHOPHO-RAB10<sup>THR73</sup> ASSAY BE OF USE IN OTHER GENETIC PD CASES

- » VPS35 D620N upregulates LRRK2 kinase activity in cell based assays
- » Validation in human neutrophils of VPS35 D620N patients / Alexander Zimprich (Vienna)
- » Patients with VPS35 D620N mutations might also benefit from LRRK2 kinase inhibitors

In mice



In man



3 PD patients with VPS35 (D620N)  
8 idiopathic PD

# SIMPLE AND ROBUST 'LRRK2 PHOPHO-RAB10' BLOOD TEST IN NEUTROPHILS FROM HEALTHY VOLUNTEERS

Can we measure LRRK2 controlled RAB phosphorylation in human bio samples? 

Can we develop a simple and robust test for LRRK2 phopho-Rab10? 

Which biomaterial is suitable? **Human white peripheral blood cells**

Can this test identify individuals who might from future LRRK2 inhibitor treatment? **Individuals with LRRK2 (R1441G) and VPS35 mutations** 

Reverse translation? **Important cross talk between PD causing molecules** 

# SUMMARY AND OUTLOOK: BENCH TO BEDSIDE

- » Set up a robust & facile assay to interrogate LRRK2 signalling pathway in human blood
- » This test identifies patients with increased LRRK2 activity (LRRK2 R144G and VPS35 D620)
- » Validates VPS35 (D620N) as potent activator of LRRK2 in the human system (*novel!*)
- » Utility in patient stratification & target engagement studies in LRRK2 inhibitor trials?
- » The MJFF (via Fox-Bionet) has implemented our assay, evaluation in Dundee
- » About to start a Parkinson's UK funded research study "Biomarkers in Parkinson's in Scotland"
- » Studying the role of the LRRK2 pathway in other monogenetic PD

# ACKNOWLEDGEMENTS

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» TOLOSA GROUP BARCELONA

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» Members of the Rab Detection Initiative