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Centre for Applied Neurogenetics

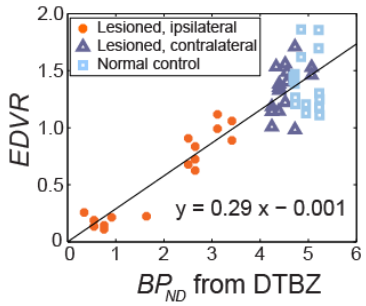
Genetic Discovery to Therapeutic Target

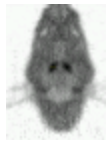
Jordan Follett & Emil Gustavsson, March 12th, 2019

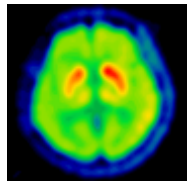
Learning objectives

1) Describe a brief history of the genetics in Parkinson's disease
    I. Genetics as a diagnostic and prognostic tool
    II. Stratifying patients for clinical trials and therapeutic interventions
2) Illustrate how genetic information can be used in physiologic modelling (mice, rats) and recapitulate stages of disease progression
3) Show how 'neuroprotection' (disease modification) may be achieved

Predict and prevent?





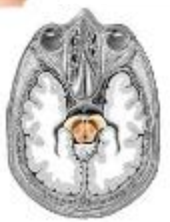


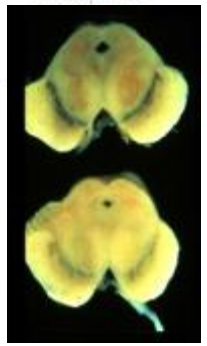
4

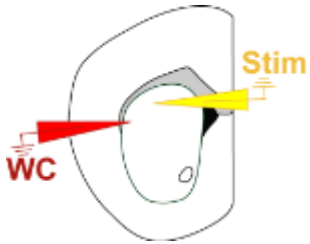
Imaging & biomarkers

5

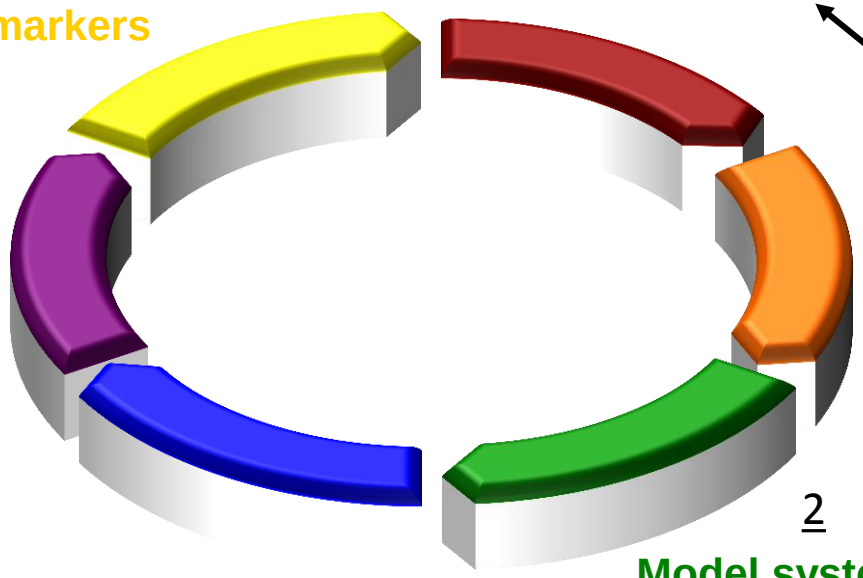
Neurology & neuropathology



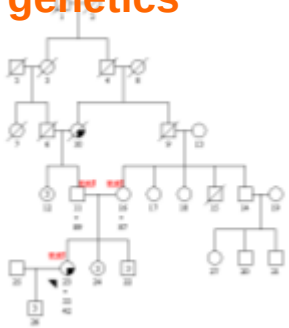




Pathophysiology & pharmacology

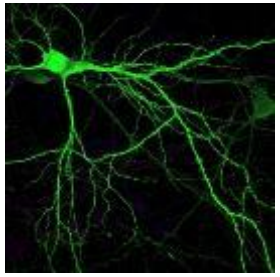


1 **Population and family genetics**



2

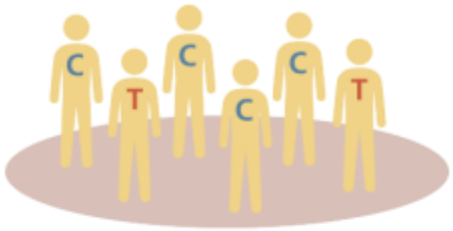
Model system design and characterization

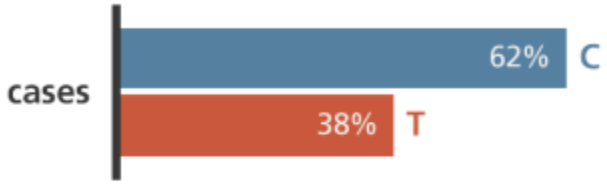


3

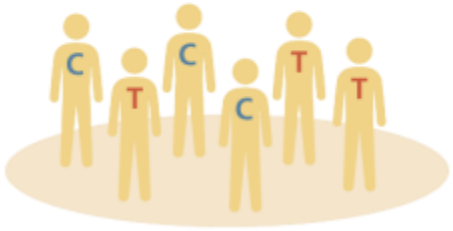
Discovery cell biology & drug screening

Association studies





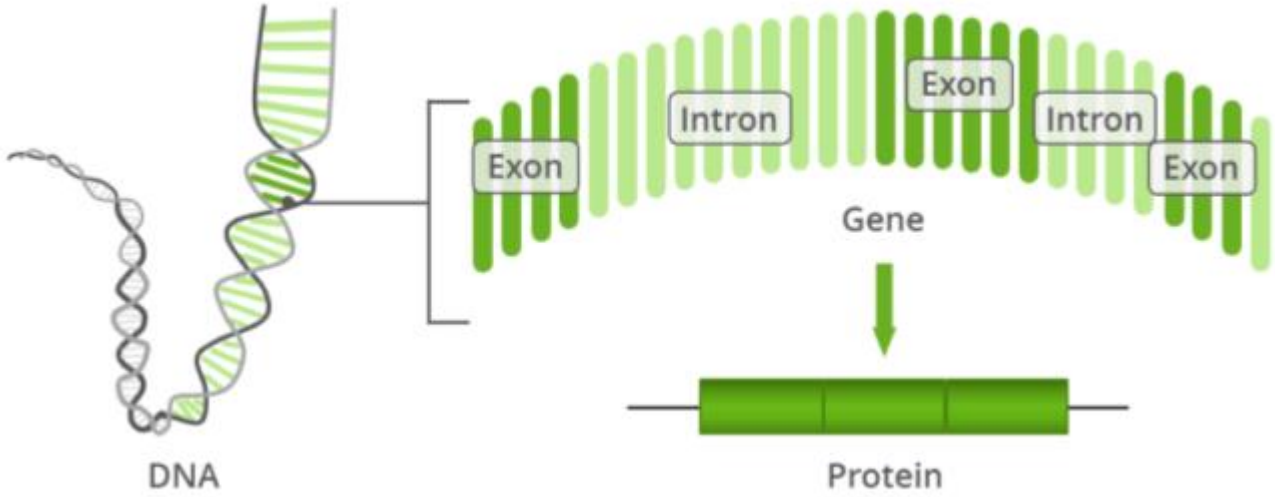
Cases
People with Parkinson's disease



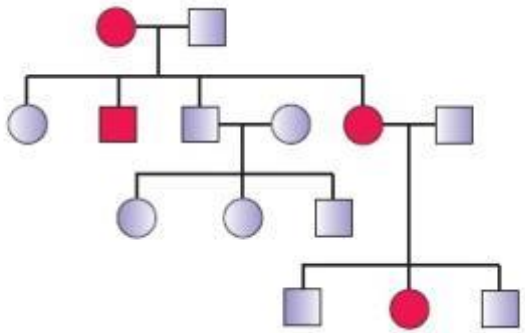
Controls
Neurologically healthy

Familial studies

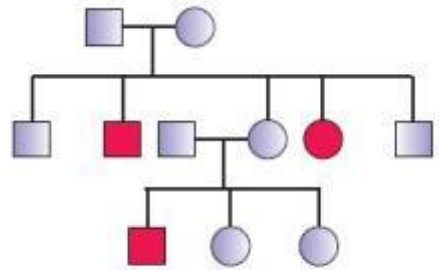
The exome consist of all exons, the 1-2% of the genome that encode the proteins



Autosomal dominant



Autosomal recessive

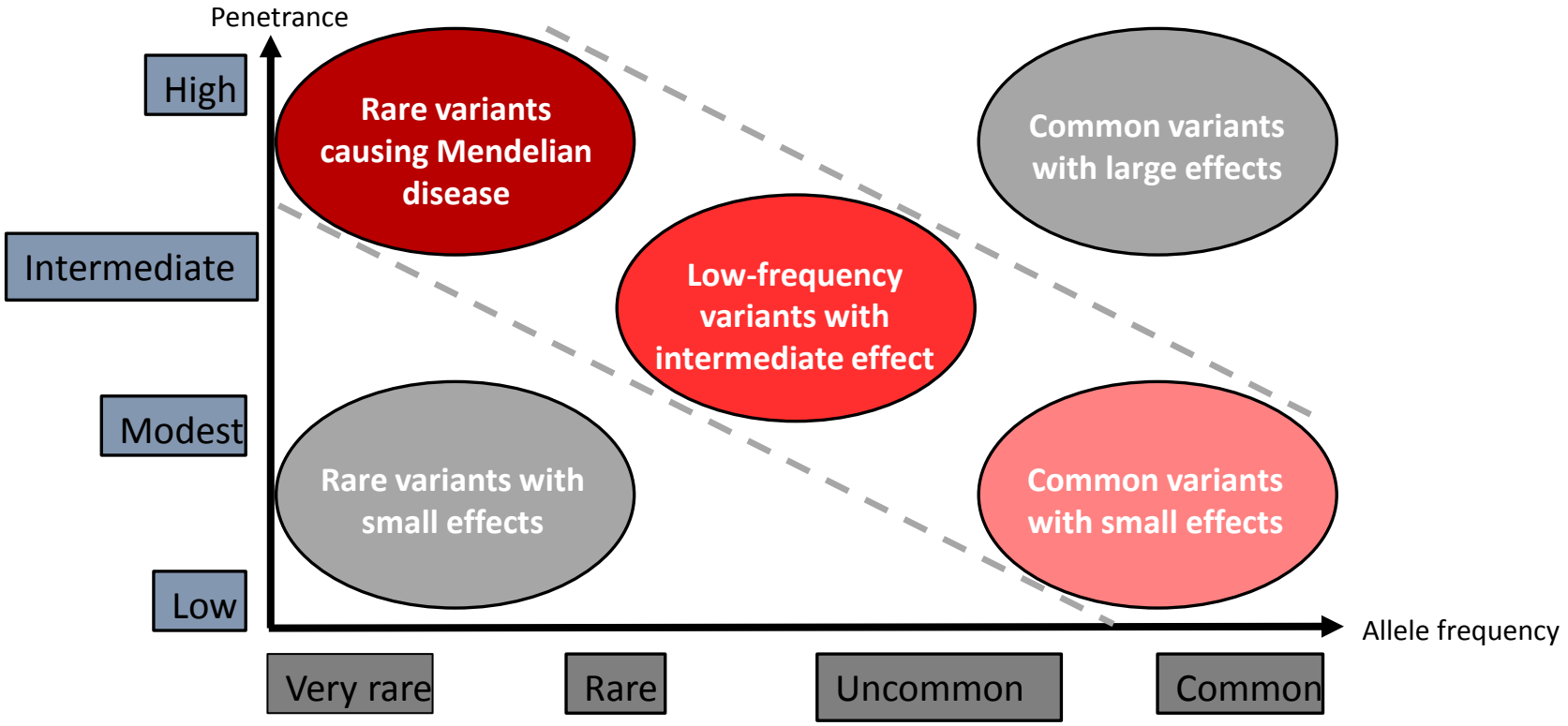


Common variants with low effect

- Increase susceptibility
- Polygenic risk scores
- Penetrance
- Some exceptions e.g. APOE

Rare variants with high effect

- Rare variants are extremely helpful in diagnosis
- Most commonly they cause rare disorders
- Monogenic forms of complex disease



Penetrance is the proportion of people with a particular genetic change (such as a mutation in a specific gene) who exhibit signs and symptoms of a genetic disorder.

Allele frequency is the relative frequency of an allele (variant of a gene) at a particular locus in a population

Science 1996

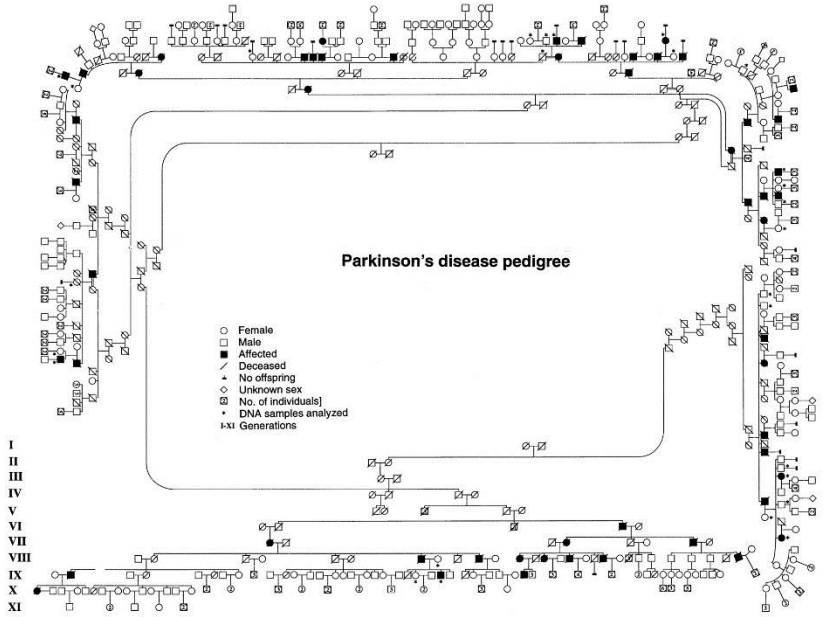
Mapping of a Gene for Parkinson's Disease to Chromosome 4q21–q23

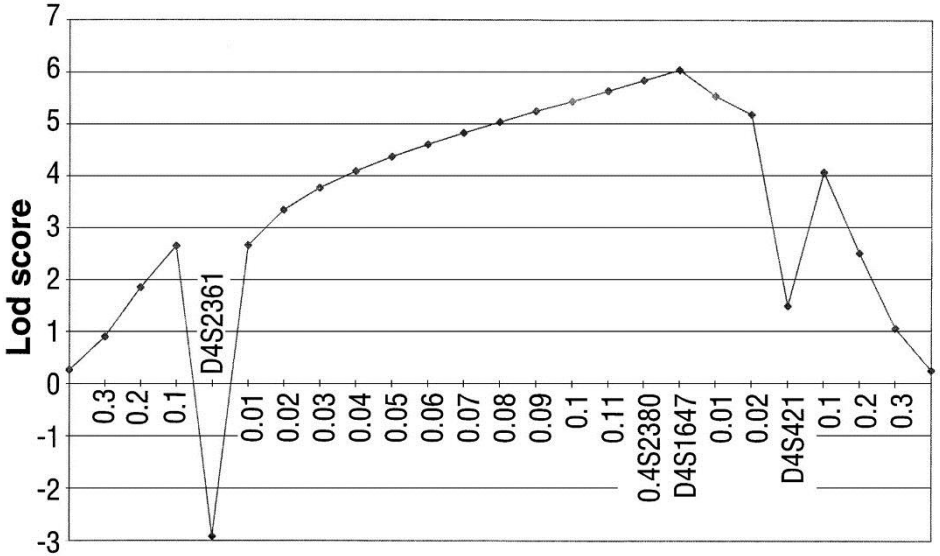
Mihael H. Polymeropoulos,* Joseph J. Higgins, Lawrence I. Golbe, William G. Johnson, Susan E. Ide, Giuseppe Di Iorio, Giuseppe Sanges, Edward S. Stenroos, Lana T. Pho, Alejandro A. Schaffer, Alice M. Lazzarini, Robert L. Nussbaum, Roger C. Duvoisin

Science 1997

Mutation in the α-Synuclein Gene Identified in Families with Parkinson's Disease

Mihael H. Polymeropoulos,* Christian Lavedan†, Elisabeth Leroy†, Susan E. Ide, Anindya Dehejia, Amalia Dutra, Brian Pike, Holly Root, Jeffrey Rubenstein, Rebecca Boyer, Edward S. Stenroos, Settara Chandrasekharappa, Aglaia Athanassiadou, Theodore Papapetropoulos, William G. Johnson, Alice M. Lazzarini, Roger C. Duvoisin, Giuseppe Di Iorio, Lawrence I. Golbe, Robert L. Nussbaum

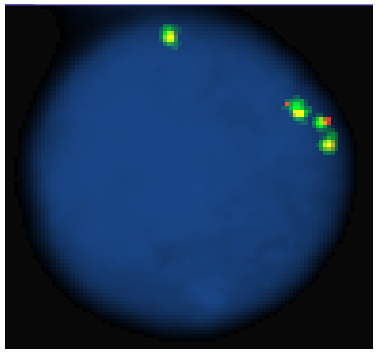


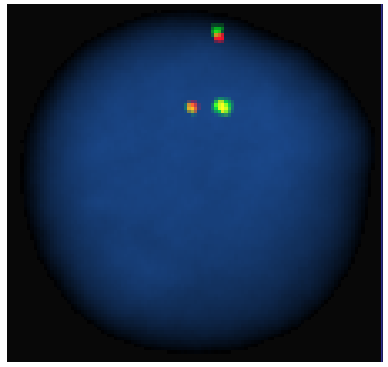


Science 2003

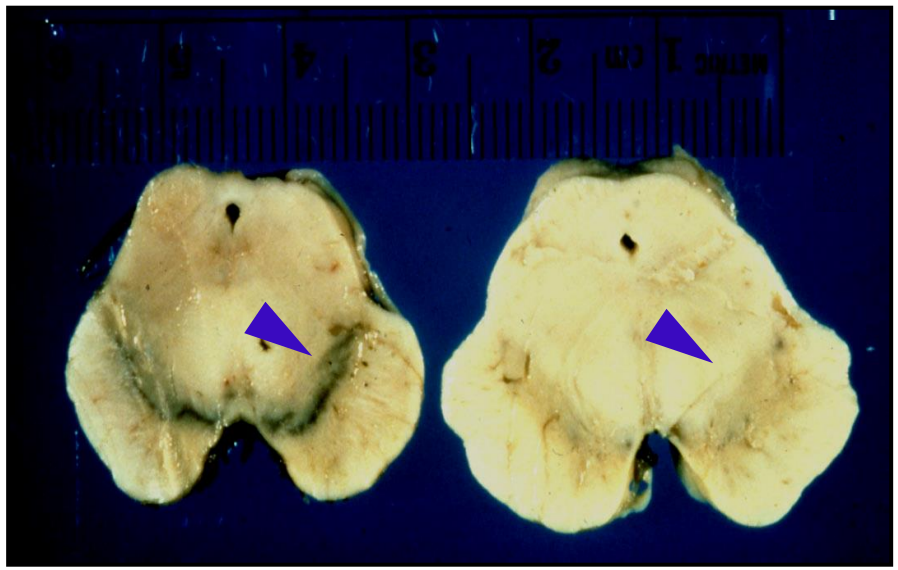
α-Synuclein Locus Triplication Causes Parkinson's Disease

A. B. Singleton,[1*†] M. Farrer,[4†] J. Johnson,[1] A. Singleton,[2] S. Hague,[1] J. Kachergus,[4] M. Hulihan,[4] T. Peuralinna,[1] A. Dutra,[3] R. Nussbaum,[2] S. Lincoln,[4] A. Crawley,[2] M. Hanson,[1] D. Maraganore,[5] C. Adler,[6] M. R. Cookson,[1] M. Muenter,[6] M. Baptista,[1] D. Miller,[1] J. Blancato,[7] J. Hardy,[1] K. Gwinn-Hardy[2]

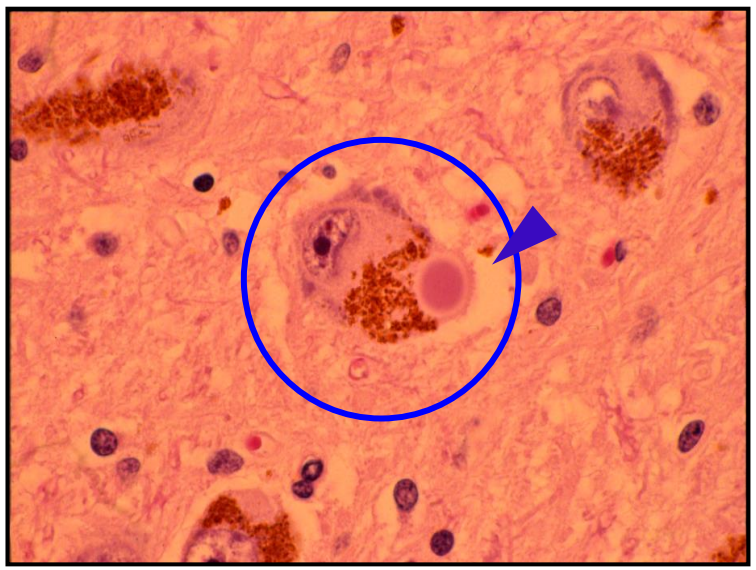




Loss of pigmented neurons of the substantia nigra

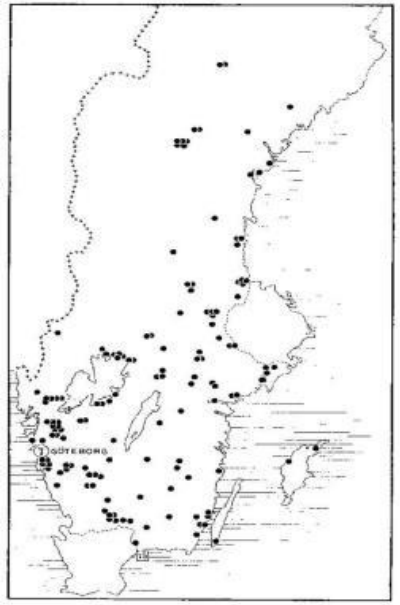


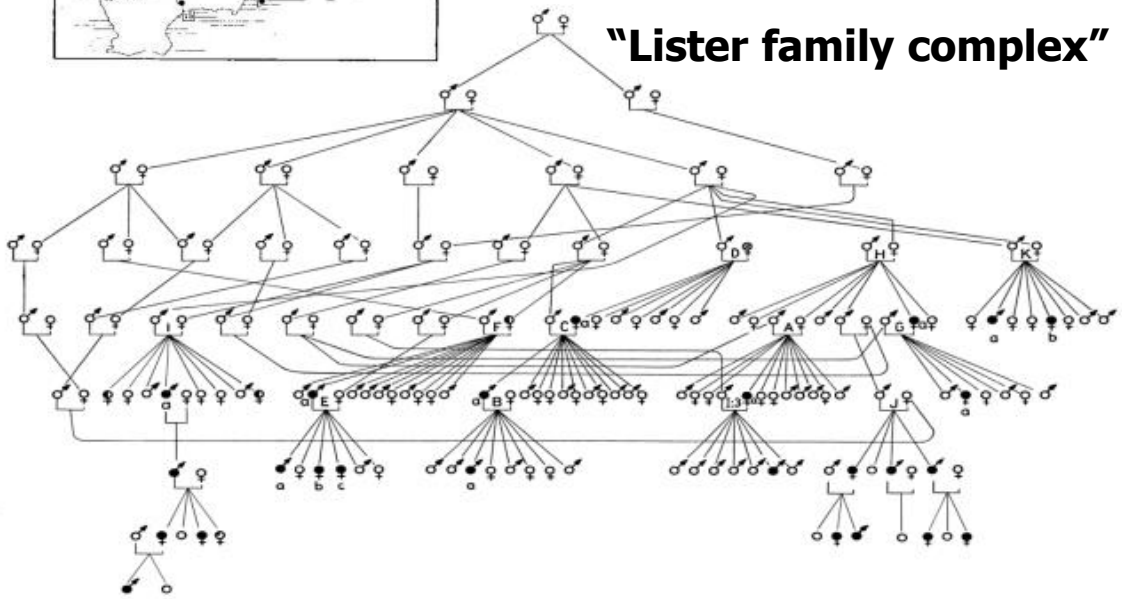
Lewy body inclusions



Phenotypic variation in a large Swedish pedigree due to *SNCA* duplication and triplication

NEUROLOGY
2007





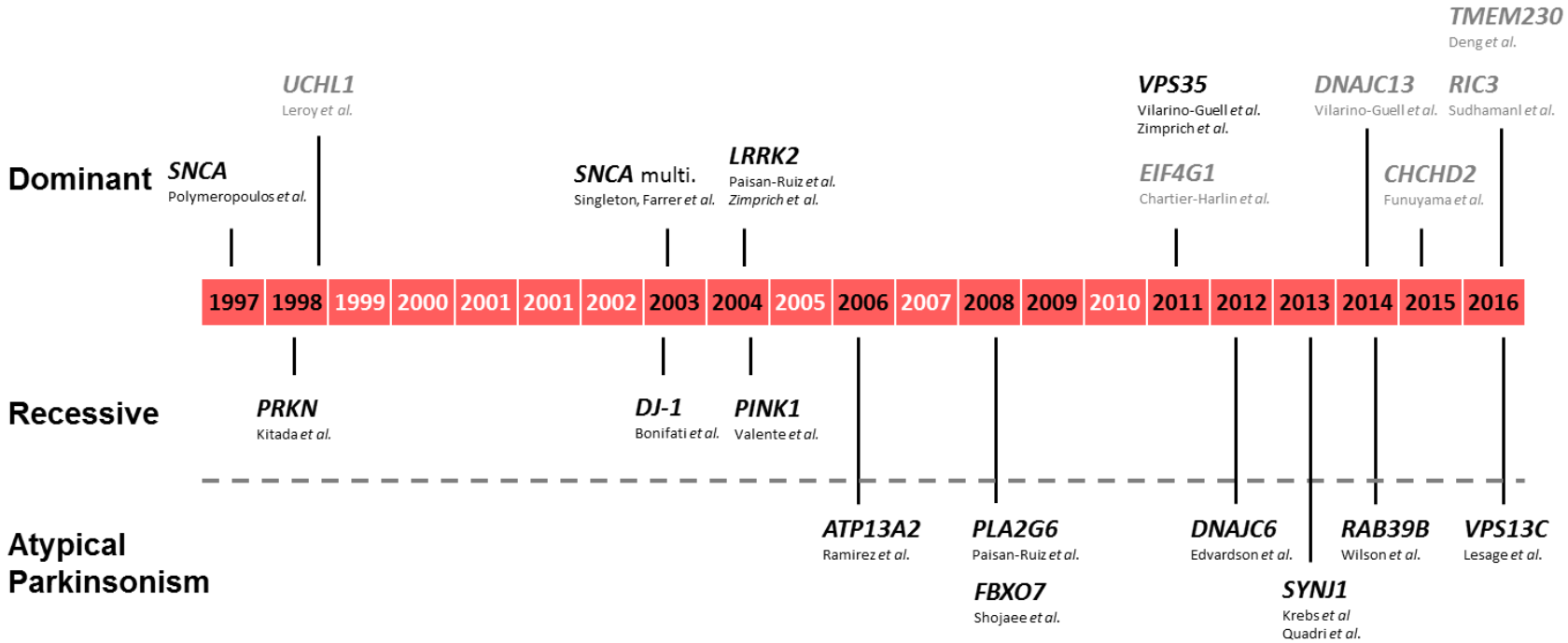
"Lister family complex"

frontiers in Neurology 2018

A Meta-Analysis of α-Synuclein Multiplication in Familial Parkinsonism

Adam Book[††], Ilaria Guella[††], Tara Candido[1], Alexis Brice[2], Nobutaka Hattori[3], Beomseok Jeon[4], Matthew J. Farrer[†] and the SNCA Multiplication Investigators of the GEoPD Consortium[‡]*

SNCA multiplication families (N)	CNV3	CNV4	
Previously published	34	25	9	32 affected and 2 unaffected
New	25	25	-	23 affected and 2 unaffected
Total	**59**	**50**	**9**	**55 affected and 4 unaffected**


TMEM230
Deng et al.
UCHL1
Leroy et al.
VPS35
Vilarino-Guell et al.
Zimprich et al.
DNAJC13
Vilarino-Guell et al.
RIC3
Sudhamanl et al.
Dominant
SNCA
Polymeropoulos et al.
SNCA multi.
Singleton, Farrer et al.
LRRK2
Paisan-Ruiz et al.
Zimprich et al.
EIF4G1
Chartier-Harlin et al.
CHCHD2
Funuyama et al.
1997
1998
1999
2000
2001
2001
2002
2003
2004
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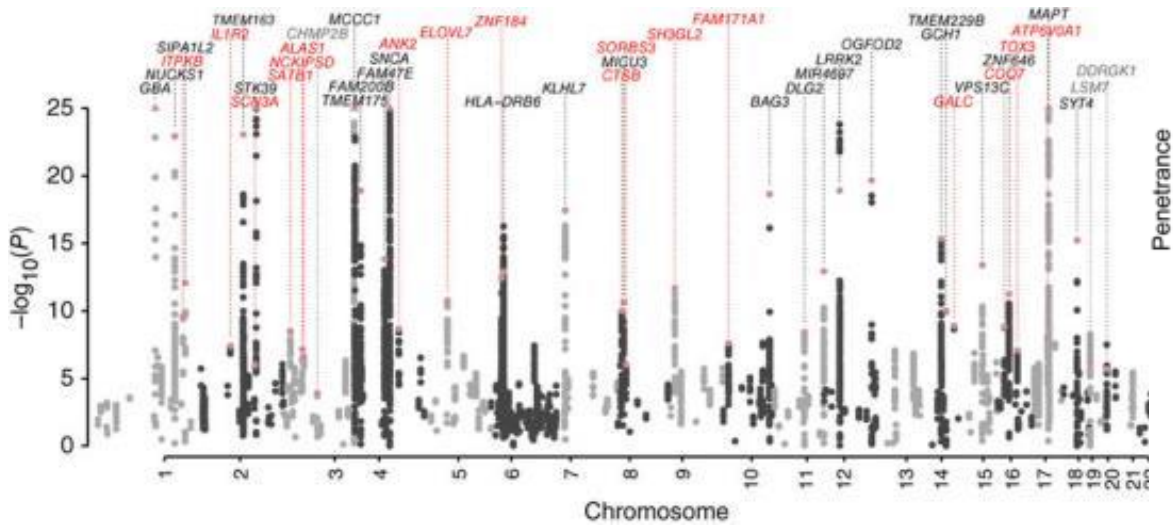
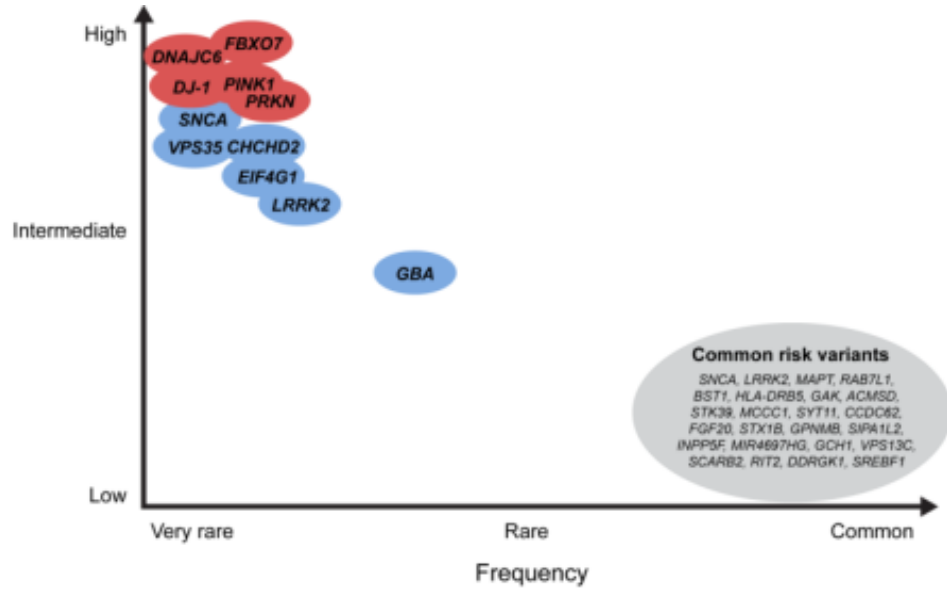
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
2016
Recessive
PRKN
Kitada et al.
DJ-1
Bonifati et al.
PINK1
Valente et al.
Atypical Parkinsonism
ATP13A2
Ramirez et al.
PLA2G6
Paisan-Ruiz et al.
FBXO7
Shojaee et al.
DNAJC6
Edvardson et al.
SYNJ1
Krebs et al
Quadri et al.
RAB39B
Wilson et al.
VPS13C
Lesage et al.

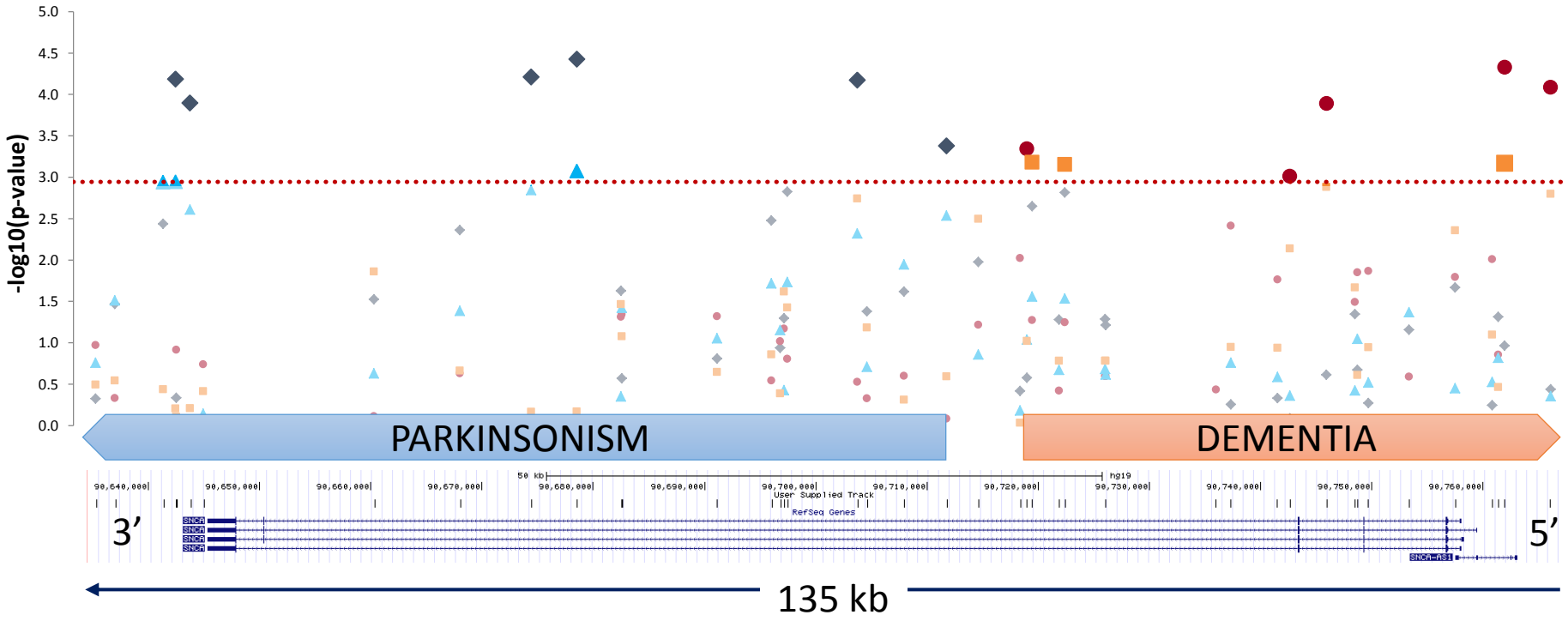

−log10(P)
Chromosome
TMEM163
IL1R2
SIPA1L2
ITPKB
NUCKS1
GBA
STK39
SCN3A
MCCC1
CHMP2B
ALAS1
NCKIPSD
SATB1
FAM200B
TMEM175
ANK2
SNCA
FAM47E
ELOVL7
ZNF184
HLA-DRB6
KLHL7
SORBS3
MIGU3
CTSB
FAM171A1
SH3GL2
BAG3
DLG2
LRRK2
MIR4697
OGFOD2
TMEM229B
GCH1
GALC
VPS13C
MAPT
ATP6V0A1
TOX3
ZNF646
CCC7
SYT4
LSM7
DDRGK1


High
Intermediate
Low
Penetrance
DNAJC6
FBXO7
DJ-1
PINK1
PRKN
SNCA
VPS35
CHCHD2
EIF4G1
LRRK2
GBA
Common risk variants
SNCA, LRRK2, MAPT, RAB7L1, BST1, HLA-DRB5, GAK, ACMSD, STK39, MCCC1, SYT11, CCDC62, FGF20, STX1B, GPNMB, SIPA1L2, INPP5F, MIR4697HG, GCH1, VPS13C, SCARB2, RIT2, DDRGK1, SREBF1
Very rare
Rare
Common
Frequency

A meta-analysis of genome-wide association studies identifies 17 new Parkinson's disease risk loci

CHR:BP[a]	SNP	Candidate gene[b]	Effect allele alternate allele	EAF in 1000 Genomes	$P_{discovery}$	$OR_{discovery}$	P_{NeuroX}	OR_{NeuroX}	P_{joint}	OR_{Joint}	OR_{Joint} (95% CI)
1:226916078	rs4653767	*ITPKB*	C/T	0.315	2.40×10^{-10}	0.92	0.017	0.93	1.63×10^{-11}	0.92	0.90–0.94
2:102413116	rs34043159	*IL1R2*	C/T	0.352	3.83×10^{-8}	1.07	1.91×10^{-4}	1.11	5.48×10^{-11}	1.08	1.06–1.10
2:166133632	rs353116	*SCN3A*	T/C	0.385	9.73×10^{-7}	0.94	8.98×10^{-3}	0.93	2.98×10^{-8}	0.94	0.92–0.96
3:18277488	rs4073221	*SATB1*	G/T	0.132	3.02×10^{-9}	1.11	0.583	1.02	1.57×10^{-8}	1.10	1.06–1.13
3:48748989	rs12497850	*NCKIPSD, CDC71*	G/T	0.347	6.80×10^{-8}	0.93	0.040	0.94	9.16×10^{-9}	0.93	0.91–0.96
3:52816840	rs143918452	*ALAS1, TLR9, DNAH1, BAP1, PHF7, NISCH, STAB1, ITIH3, ITIH4*	G/A	0.996	2.25×10^{-7}	0.68	0.095	0.73	3.20×10^{-8}	0.68	0.60–0.78
4:114360372	rs78738012	*ANK2, CAMK2D*	C/T	0.106	2.11×10^{-9}	1.14	7.5×10^{-3}	1.12	4.78×10^{-11}	1.13	1.09–1.17
5:60273923	rs2694528	*ELOVL7*	C/A	0.115	1.69×10^{-11}	1.15	6.25×10^{-5}	1.19	4.84×10^{-15}	1.15	1.11–1.20
6:27681215	rs9468199	*ZNF184*	A/G	0.172	3.44×10^{-13}	1.12	0.302	1.04	1.46×10^{-12}	1.11	1.08–1.14
8:11707174	rs2740594[c]	*CTSB*	A/G	0.753	9.54×10^{-11}	1.10	7.95×10^{-3}	1.08	5.91×10^{-12}	1.09	1.07–1.12
8:22525980	rs2280104	*SORBS3, PDLIM2, C8orf58, BIN3*	T/C	0.367	9.06×10^{-7}	1.06	7.87×10^{-3}	1.08	2.53×10^{-8}	1.07	1.04–1.09
9:17579690	rs13294100	*SH3GL2*	T/G	0.371	1.99×10^{-12}	0.91	0.037	0.94	4.84×10^{-13}	0.92	0.89–0.94
10:15569598	rs10906923	*FAM171A1*	C/A	0.306	2.37×10^{-8}	0.93	0.133	0.96	1.35×10^{-8}	0.93	0.91–0.96
14:88472612	rs8005172	*GALC*	T/C	0.424	1.20×10^{-9}	1.08	0.022	1.06	8.77×10^{-11}	1.08	1.05–1.10
16:19279464	rs11343	*COQ7*	T/G	0.454	1.46×10^{-9}	1.07	0.019	1.06	9.13×10^{-11}	1.07	1.05–1.10
16:52599188	rs4784227	*TOX3*	T/C	0.265	8.29×10^{-8}	1.08	1.47×10^{-4}	1.12	9.75×10^{-11}	1.09	1.06–1.12
17:40698158	rs601999	*ATP6V0A1, PSMC3IP, TUBG2*	C/T	0.699	8.03×10^{-9}	0.93	NA	NA	NA	NA	NA

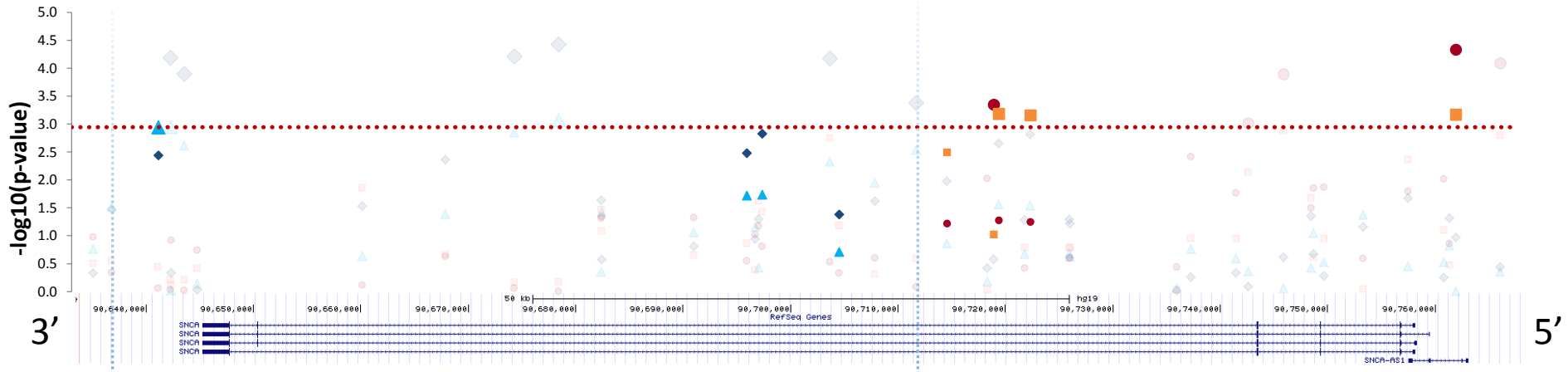
Alpha-synuclein (SNCA) - allelic association



1. PD (n=1443) vs CTR (922)
2. DLB (n=273) vs CTR (922)
3. PD no-CI (620) vs CTR (922)
4. PDD (147) vs CTR (922)

Red line = Bonferroni correction
Analysis adjusted for age and gender

Haplotype-based association



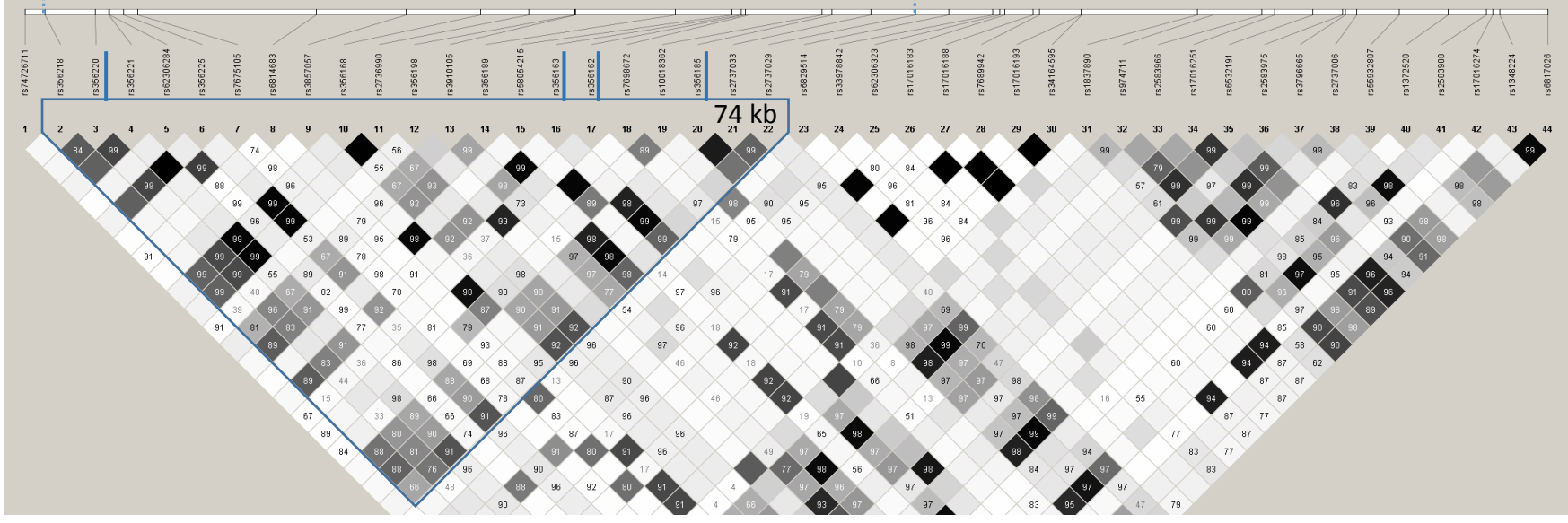
T **AT** **G**

PD: 6.7% vs 2.4%, $p<1.0x10^{-4}$, OR(95%CI)=3.24(2.27-4.63)

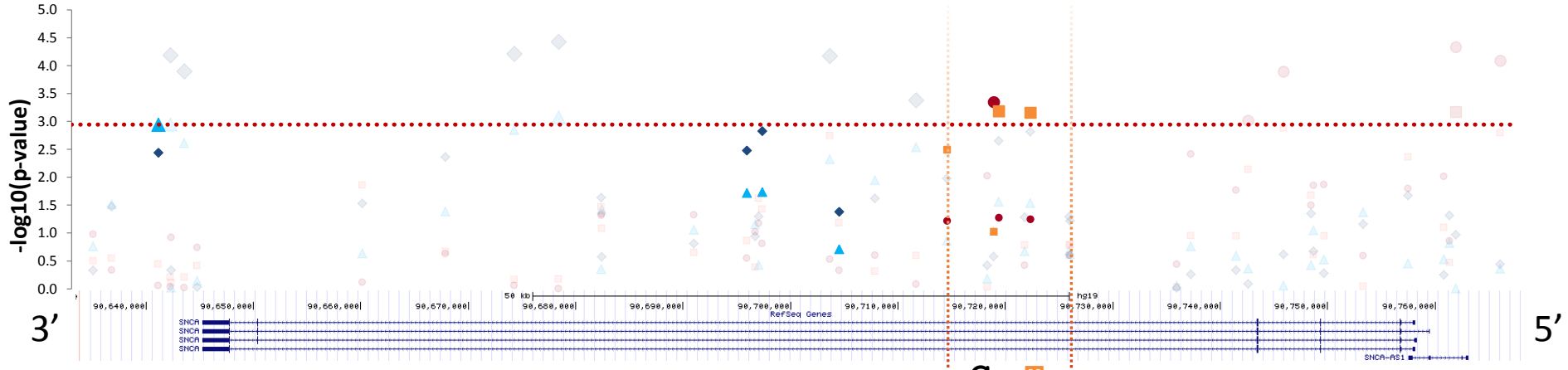
PD no-CI: 8.2% vs 2.4%, $p<1.0x10^{-4}$, OR(95%CI)=3.88(2.63-5.73)

DLB: 0.6% vs 2.4%, $p=9.0x10^{-4}$, OR(95%CI)=0.23(0.11-0.47)

A 74kb haplotype spanning from intron 4 to the 3’ end of the SNCA gene is highly associated with an increased risk of PD.



Haplotype-based association

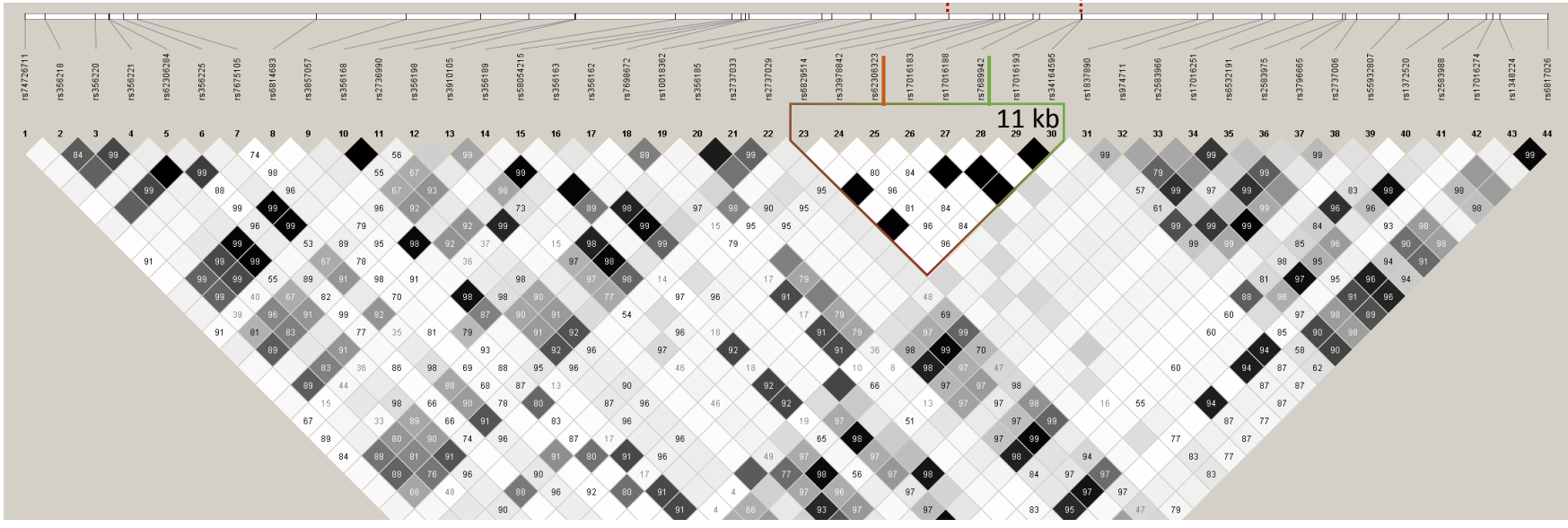


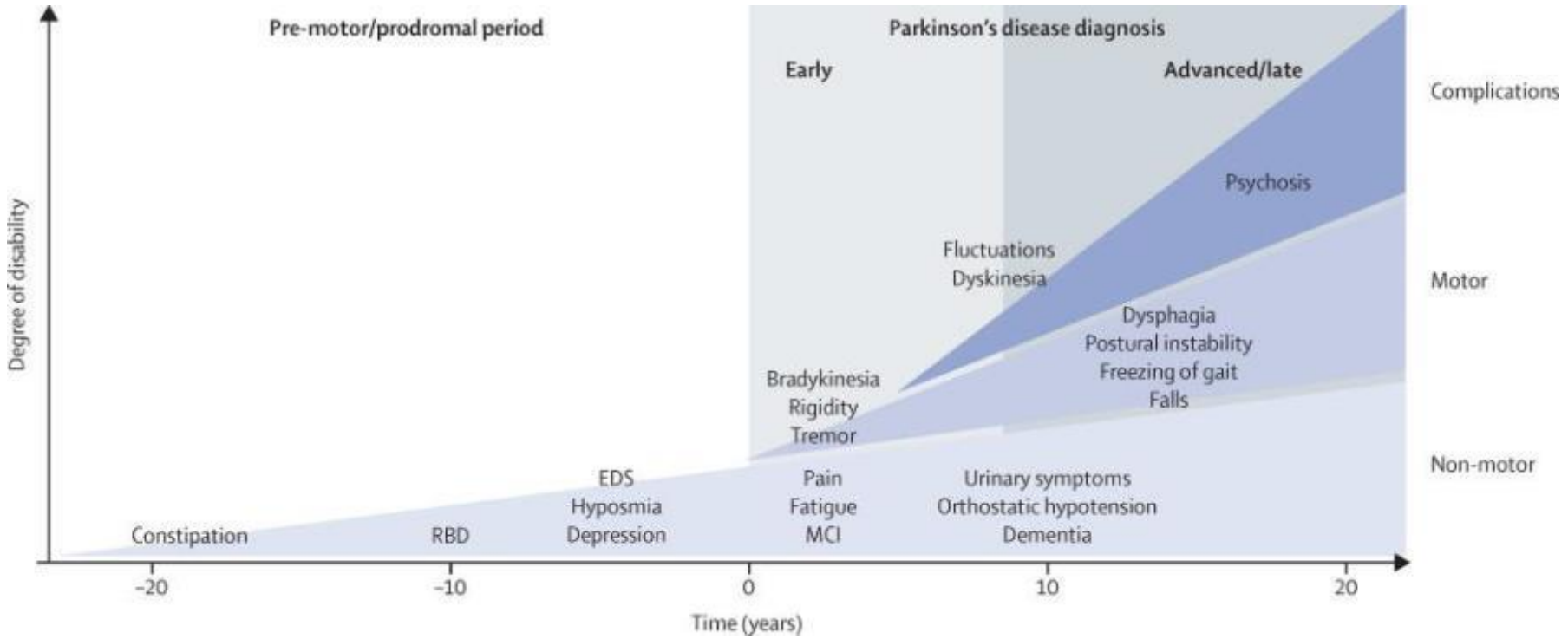
An 11kb haplotype in intron 4 is associated with both DLB and PDD. However, the associated alleles are different in the two diseases.

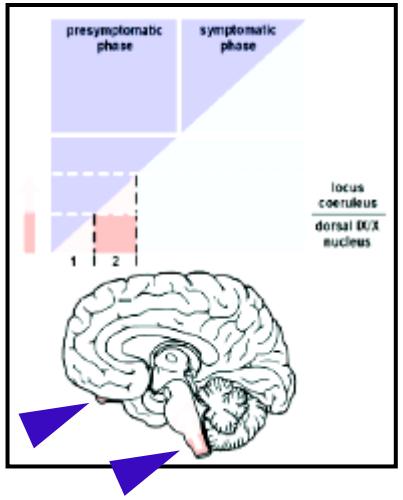
PD: 7.3% vs 4.9%, p=0.01, OR(95%CI)=1.55(1.21-1.99)

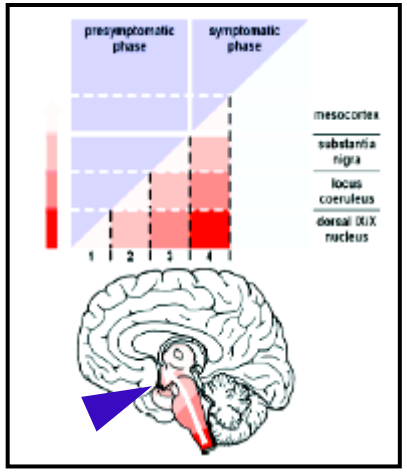
PDD: 9.1% vs 5.0%, p=0.01, OR(95%CI)=2.01(1.33-3.04)

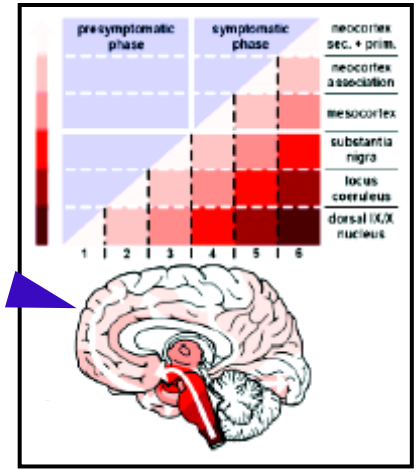
DLB: 15.3% vs 11.6%, p=0.02, OR(95%CI)=1.37(1.13-1.66)



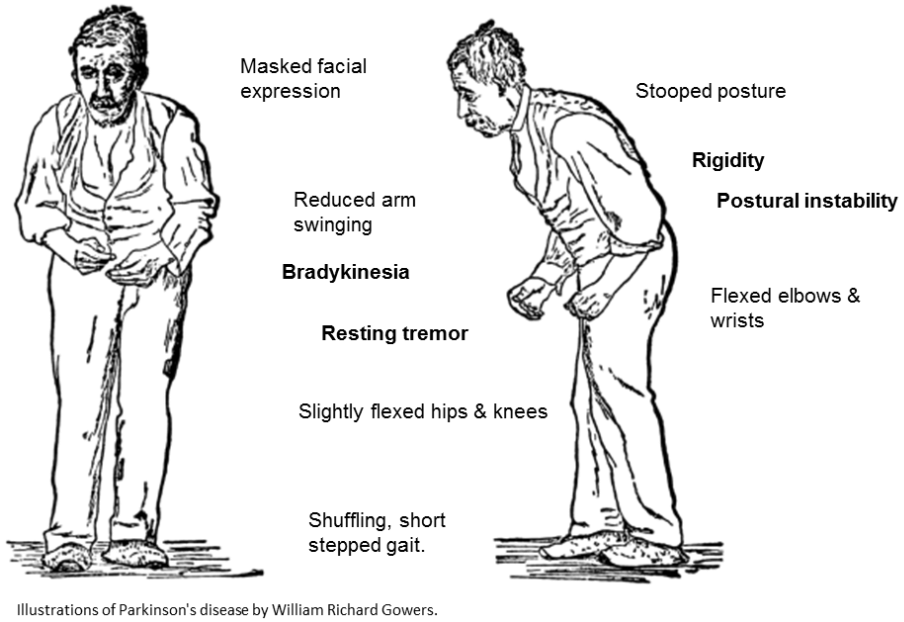

Pre-motor/prodromal period
Parkinson's disease diagnosis
Early
Advanced/late
Complications
Psychosis
Fluctuations
Dyskinesia
Motor
Dysphagia
Postural instability
Freezing of gait
Falls
Bradykinesia
Rigidity
Tremor
Non-motor
Constipation
RBD
EDS
Hyposmia
Depression
Pain
Fatigue
MCI
Urinary symptoms
Orthostatic hypotension
Dementia
Degree of disability
-20
-10
0
10
20
Time (years)


presymptomatic phase
symptomatic phase
locus coeruleus
dorsal IX/X nucleus


presymptomatic phase
symptomatic phase
mesocortex
substantia nigra
locus coeruleus
dorsal IX/X nucleus

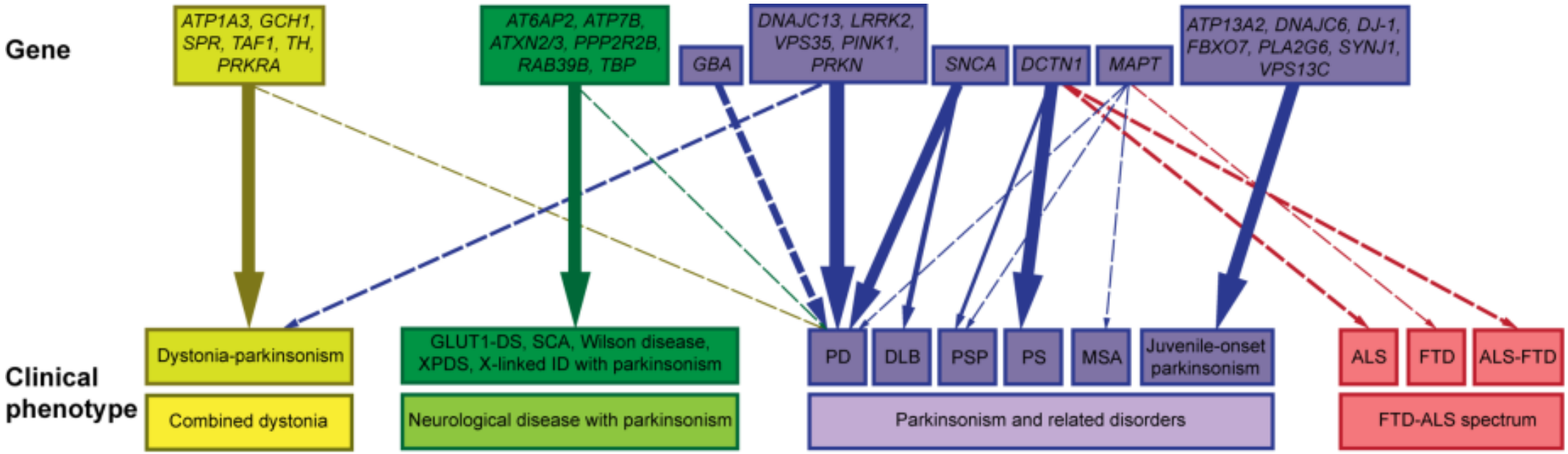

presymptomatic phase
symptomatic phase
neocortex sec. + prim.
neocortex association
mesocortex
substantia nigra
locus coeruleus
dorsal IX/X nucleus

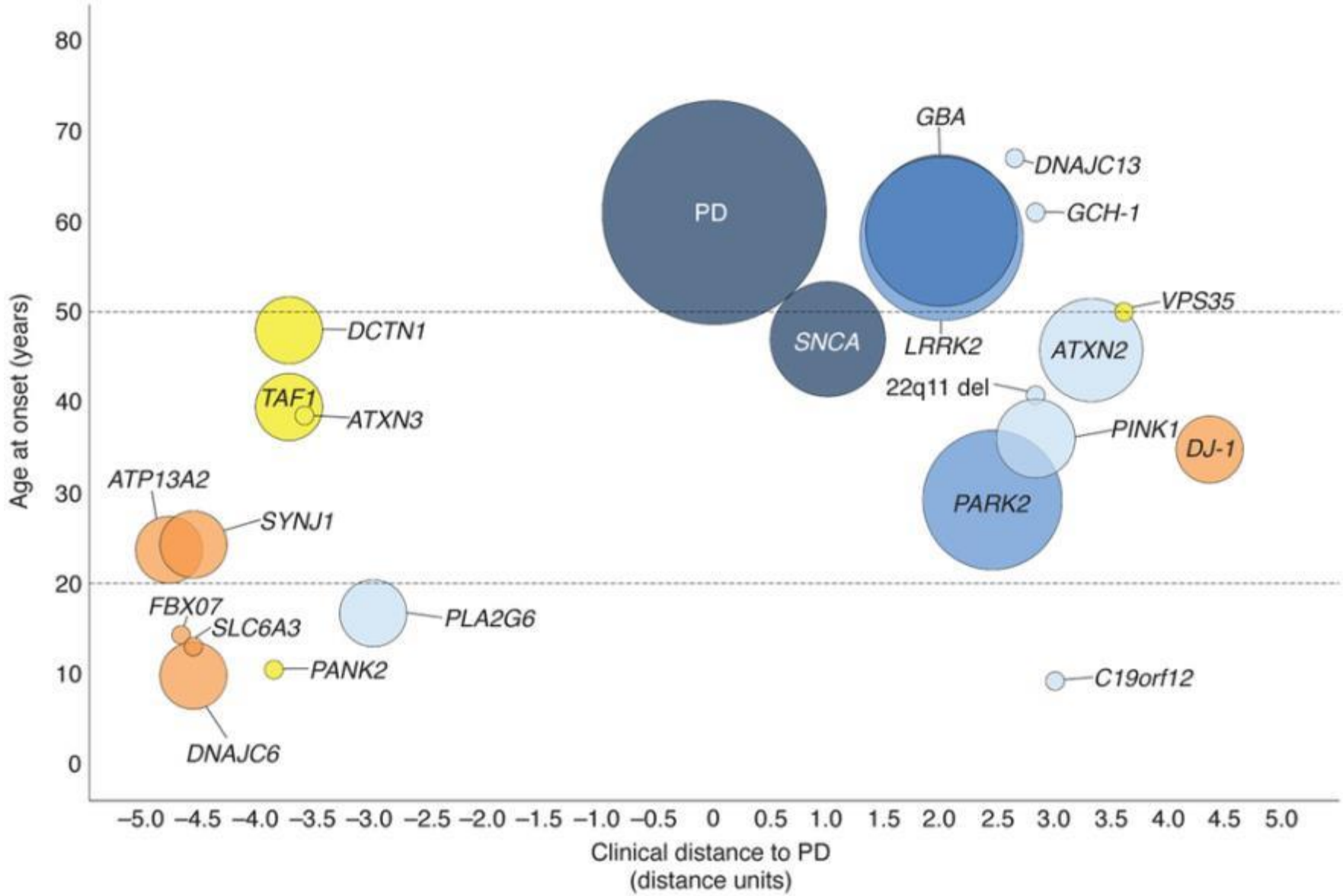
Parkinsonism has many causes…

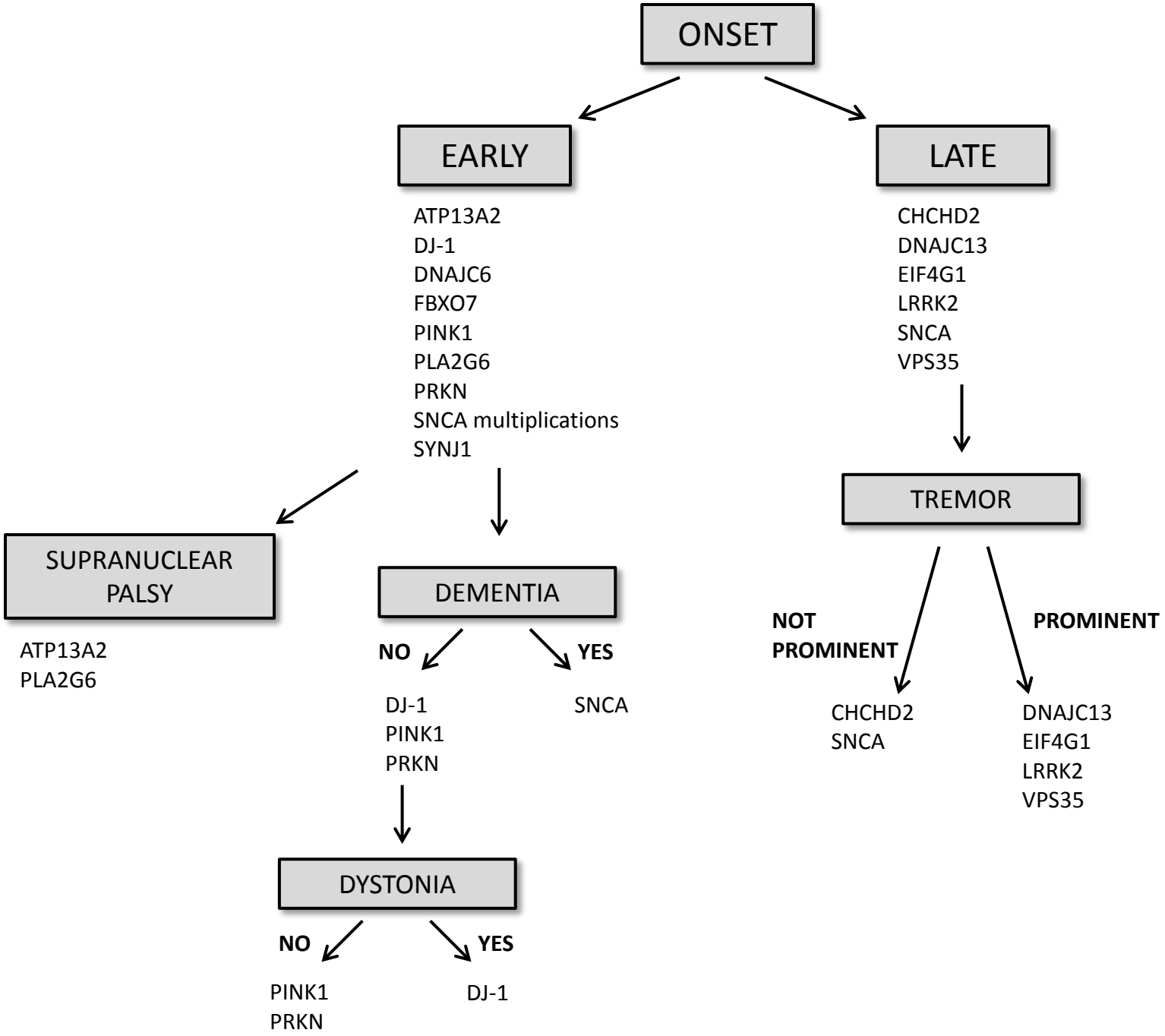


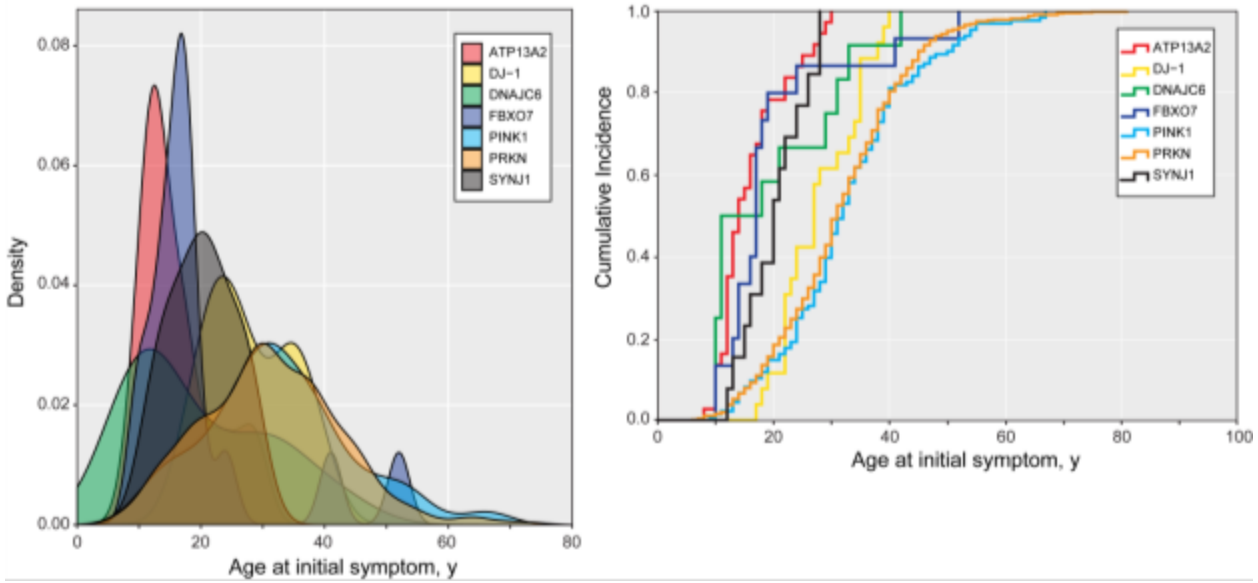
Illustrations of Parkinson's disease by William Richard Gowers.

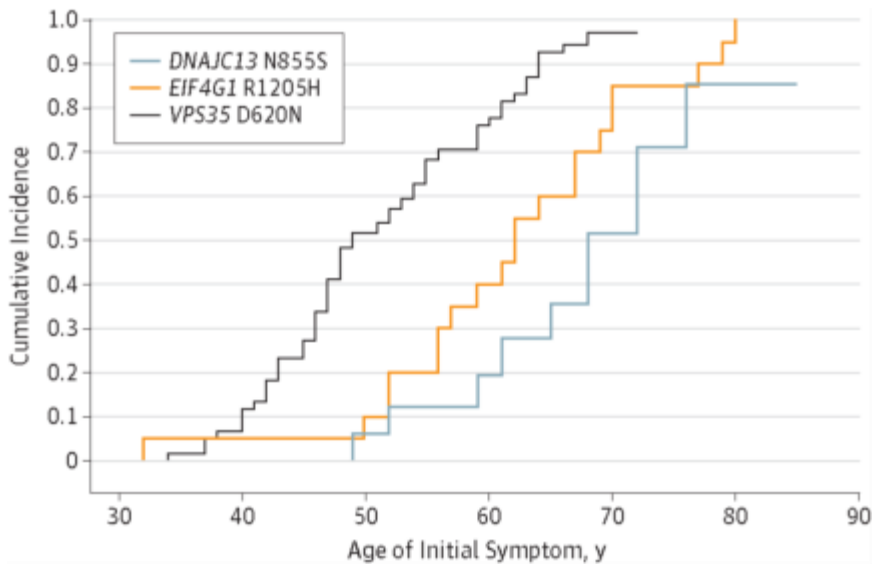
Primary Parkinsonism	Parkinson disease (sporadic, familial)
Secondary Parkinsonism	Drug-induced: dopamine antagonists and depletors
	Hemiatrophy-hemiparkinsonism
	Hydrocephalus: normal pressure hydrocephalus
	Hypoxia
	Infectious: postencephalitic
	Metabolic: parathyroid dysfunction
	Toxin: Mn, CO, MPTP, cyanide
	Trauma
	Tumor
	Vascular: multi-infarct state
Parkinson-plus Syndromes	Cortical-basal ganglionic degeneration
	Dementia syndromes: Alzheimer disease, diffuse Lewy body disease, frontotemporal dementia
	Lytico-Bodig (Guamanian Parkinsonism-dementia-ALS)
	Multiple system atrophy syndromes: striatonigral degeneration, Shy-Drager syndrome, sporadic olivopontocerebellar degeneration (OPCA), motor neuron disease-parkinsonism
	Progressive pallidal atrophy
	Progressive supranuclear palsy
Familial Neurodegenerative Diseases	Dystonia-parkinsonism: DYT5a/b, DYT12
	Hallervorden-Spatz disease
	Huntington disease
	Lubag (X-linked dystonia-parkinsonism)
	Mitochondrial cytopathies with striatal necrosis
	Neuroacanthocytosis
	Neurodegeneration with brain iron accumulation
	Wilson disease

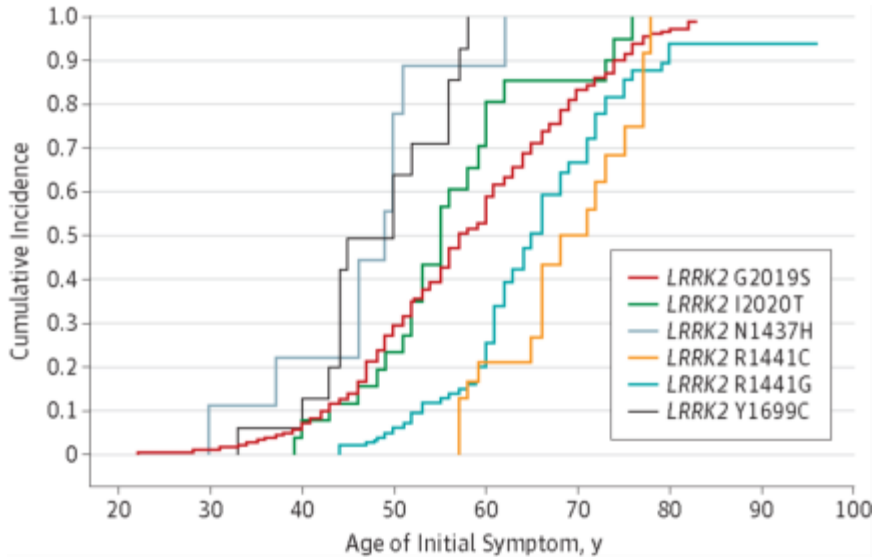

Gene
ATP1A3, GCH1, SPR, TAF1, TH, PRKRA
AT6AP2, ATP7B, ATXN2/3, PPP2R2B, RAB39B, TBP
GBA
DNAJC13, LRRK2, VPS35, PINK1, PRKN
SNCA
DCTN1
MAPT
ATP13A2, DNAJC6, DJ-1, FBXO7, PLA2G6, SYNJ1, VPS13C
Clinical phenotype
Dystonia-parkinsonism
Combined dystonia
GLUT1-DS, SCA, Wilson disease, XPDS, X-linked ID with parkinsonism
Neurological disease with parkinsonism
PD
DLB
PSP
PS
MSA
Juvenile-onset parkinsonism
Parkinsonism and related disorders
ALS
FTD
ALS-FTD
FTD-ALS spectrum

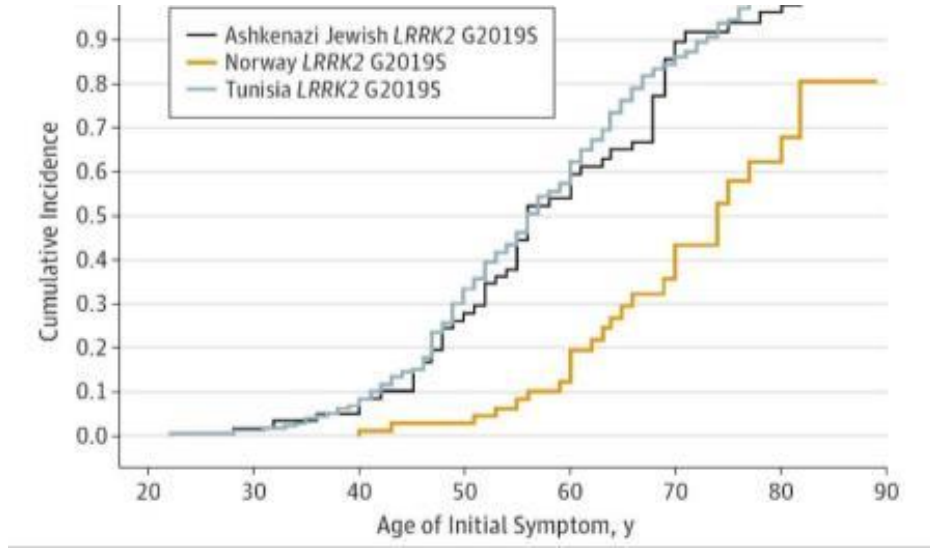

Age at onset (years)
80
70
60
50
40
30
20
10
0
PD
GBA
DNAJC13
GCH-1
SNCA
LRRK2
VPS35
ATXN2
22q11 del
PINK1
DJ-1
PARK2
DCTN1
TAF1
ATXN3
ATP13A2
SYNJ1
FBX07
SLC6A3
PLA2G6
PANK2
C19orf12
DNAJC6
–5.0 –4.5 –4.0 –3.5 –3.0 –2.5 –2.0 –1.5 –1.0 –0.5 0 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0
Clinical distance to PD
(distance units)


ONSET
EARLY
ATP13A2
DJ-1
DNAJC6
FBXO7
PINK1
PLA2G6
PRKN
SNCA multiplications
SYNJ1
LATE
CHCHD2
DNAJC13
EIF4G1
LRRK2
SNCA
VPS35
SUPRANUCLEAR PALSY
ATP13A2
PLA2G6
DEMENTIA
NO
YES
DJ-1
PINK1
PRKN
SNCA
DYSTONIA
NO
YES
PINK1
PRKN
DJ-1
TREMOR
NOT PROMINENT
PROMINENT
CHCHD2
SNCA
DNAJC13
EIF4G1
LRRK2
VPS35


Density
Age at initial symptom, y
ATP13A2
DJ-1
DNAJC6
FBXO7
PINK1
PRKN
SYNJ1
Cumulative Incidence
Age at initial symptom, y
ATP13A2
DJ-1
DNAJC6
FBXO7
PINK1
PRKN
SYNJ1


Cumulative Incidence
DNAJC13 N855S
EIF4G1 R1205H
VPS35 D620N
Age of Initial Symptom, y

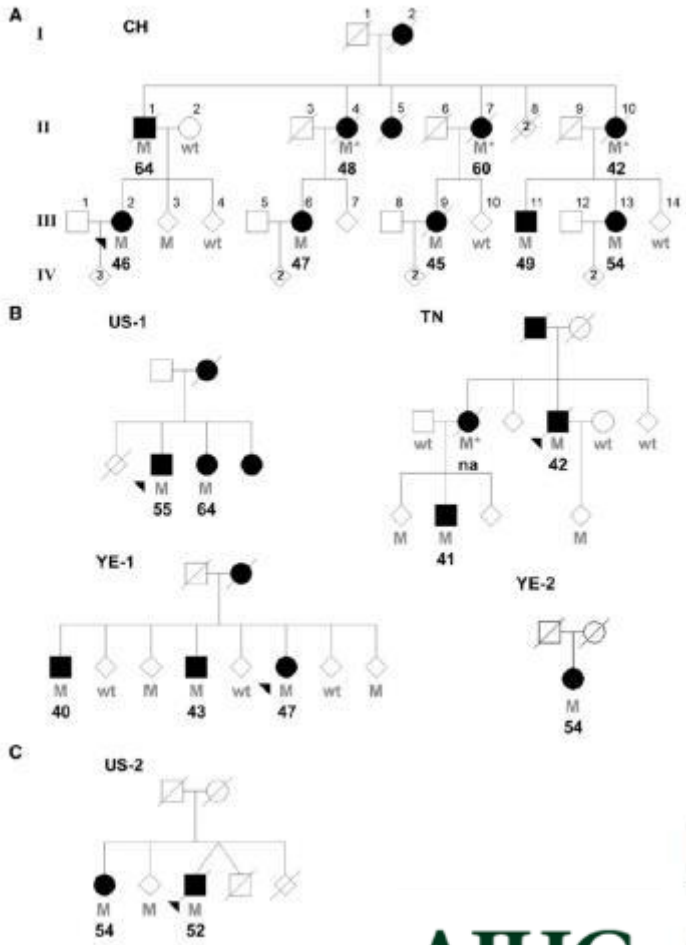

Cumulative Incidence
LRRK2 G2019S
LRRK2 I2020T
LRRK2 N1437H
LRRK2 R1441C
LRRK2 R1441G
LRRK2 Y1699C
Age of Initial Symptom, y

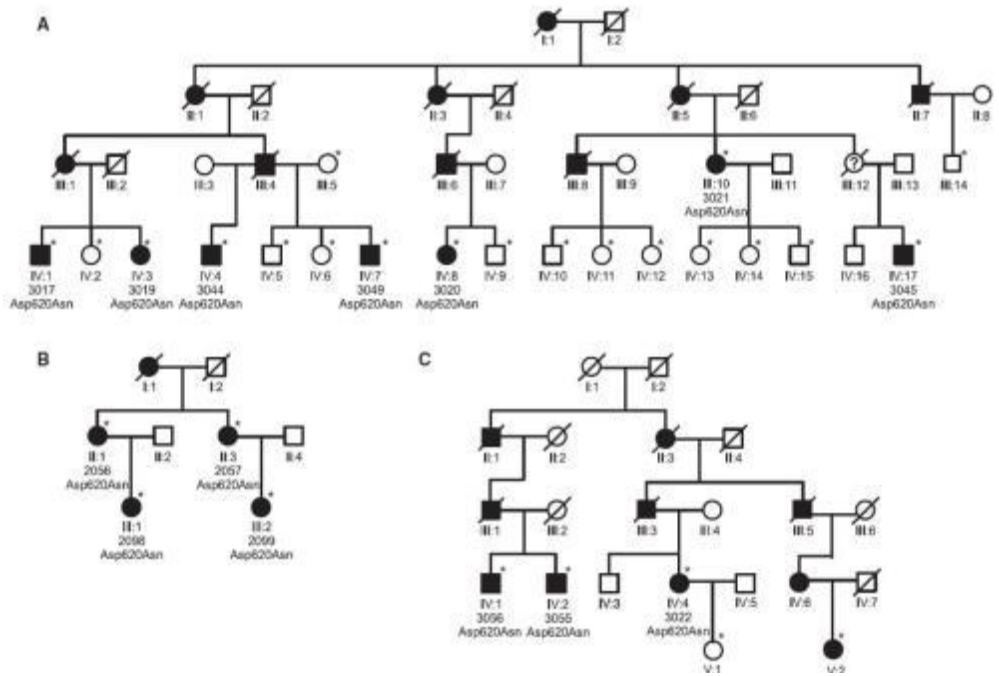

Cumulative Incidence
Ashkenazi Jewish LRRK2 G2019S
Norway LRRK2 G2019S
Tunisia LRRK2 G2019S
Age of Initial Symptom, y

REPORT

AJHG 2011

VPS35 Mutations in Parkinson Disease





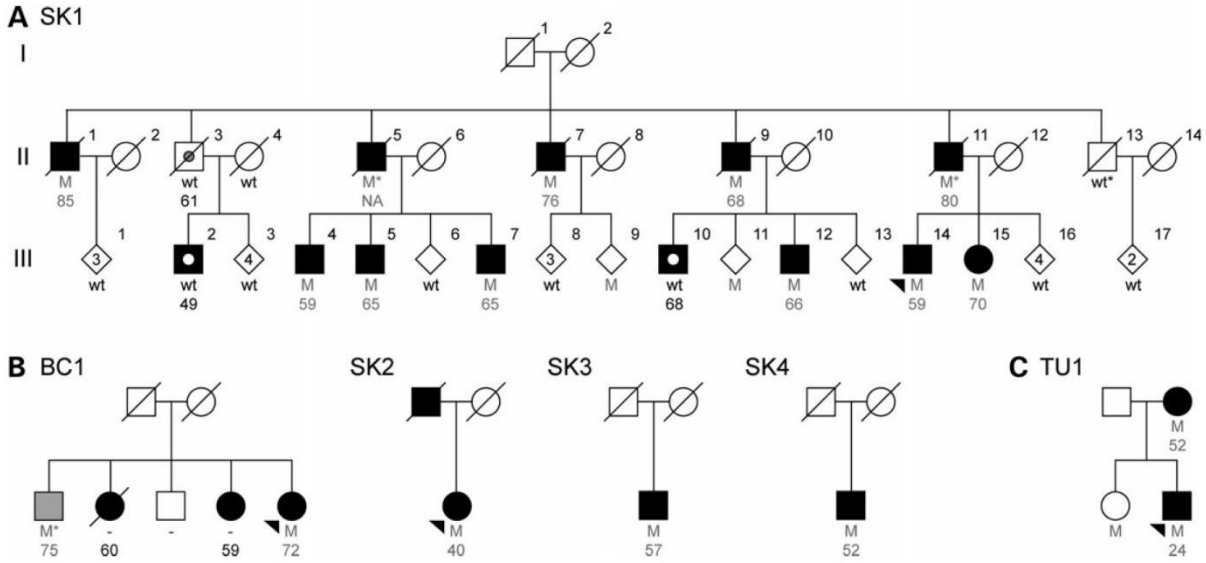
REPORT

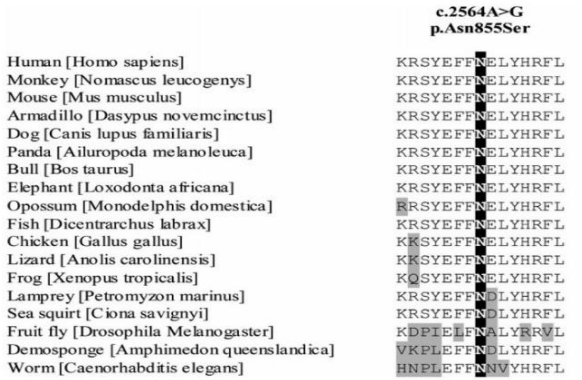
AJHG 2011

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

DNAJC13 (RME-8) parkinsonism

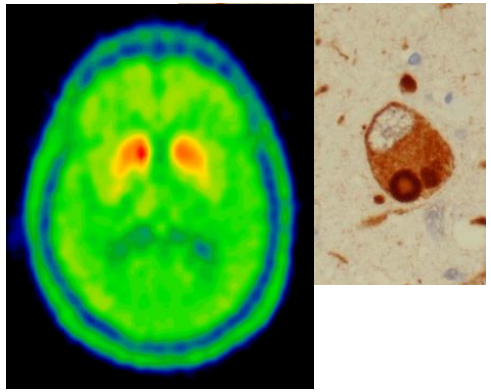
Human Molecular Genetics 2014

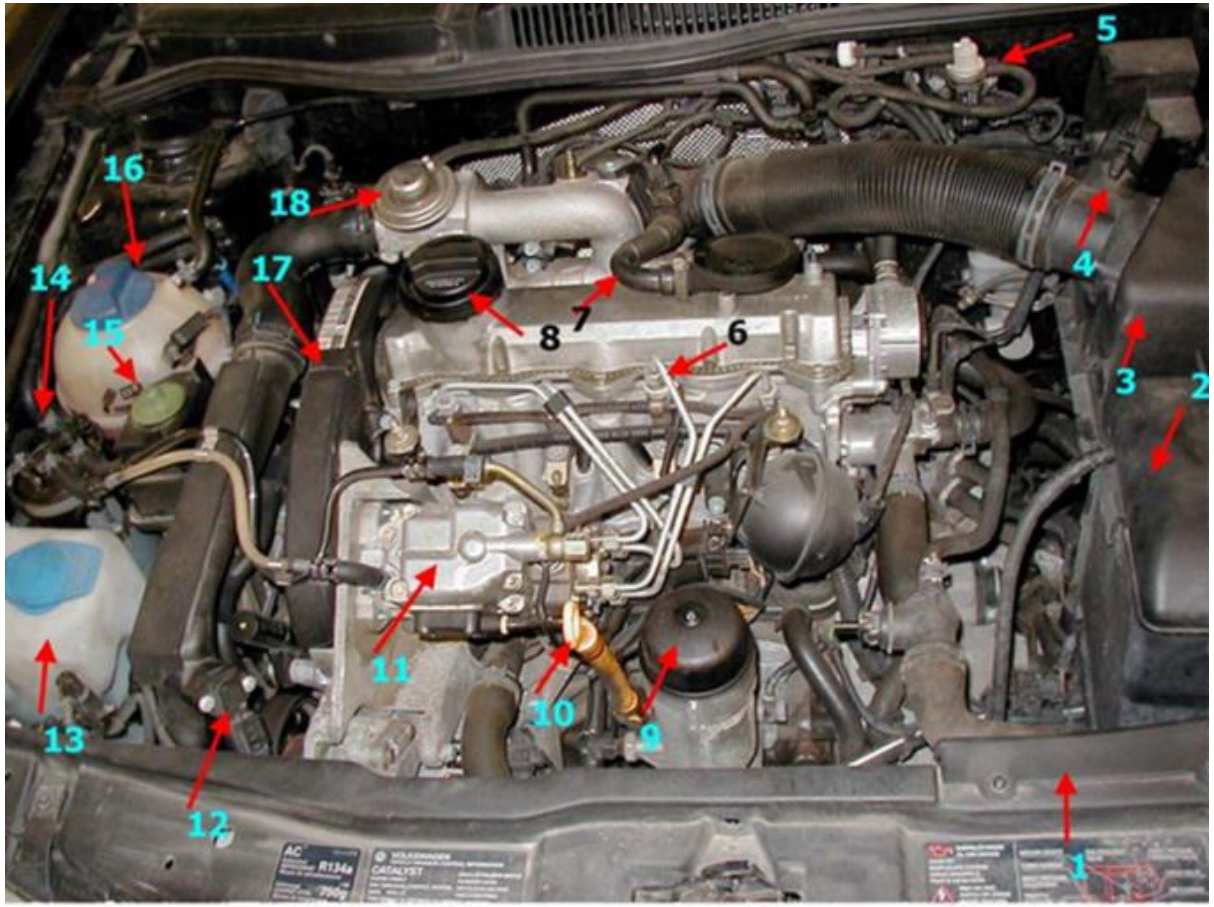




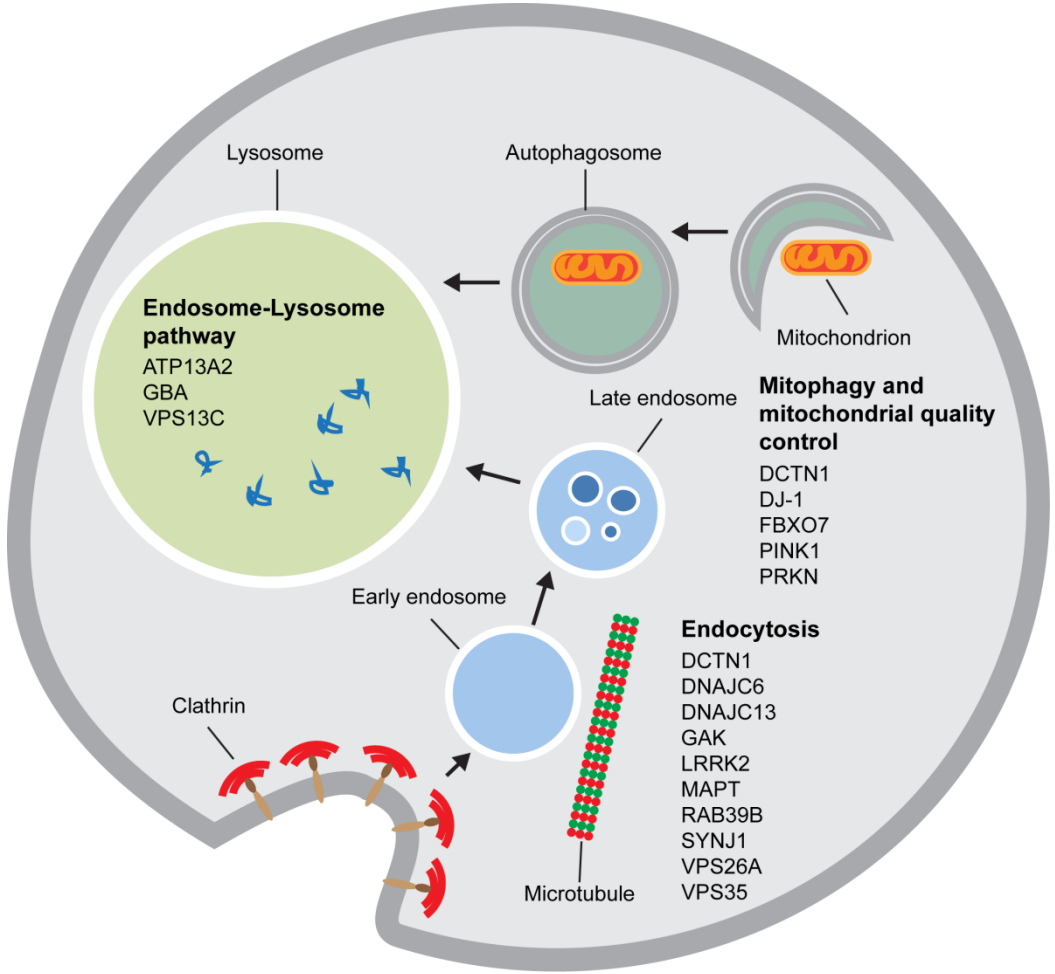
Clinical, Positron Emission Tomography, and Pathological Studies of DNAJC13 p.N855S Parkinsonism

Movement Disorders 2014

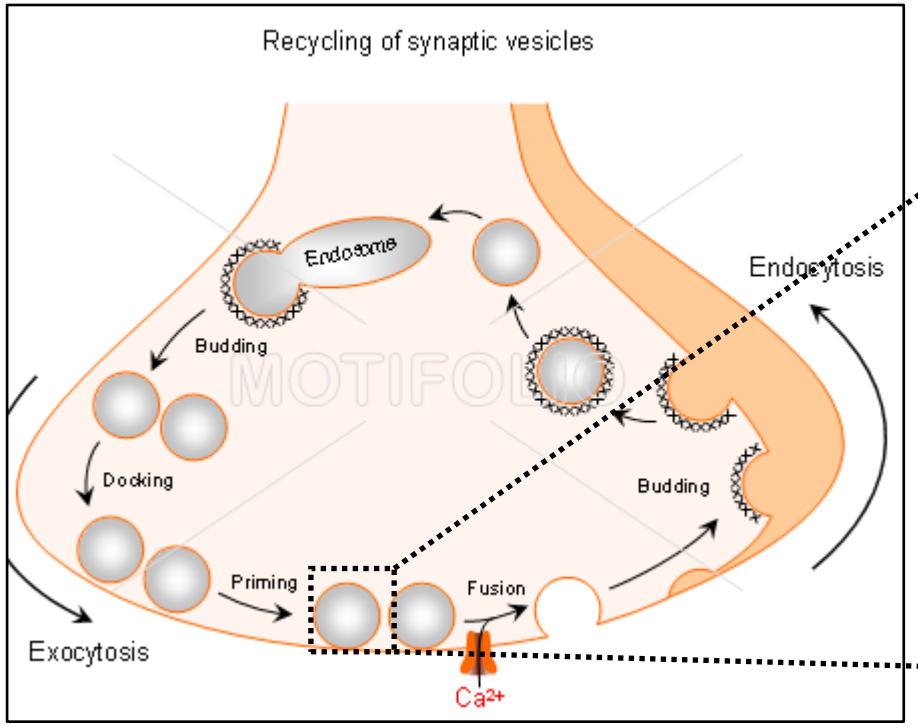


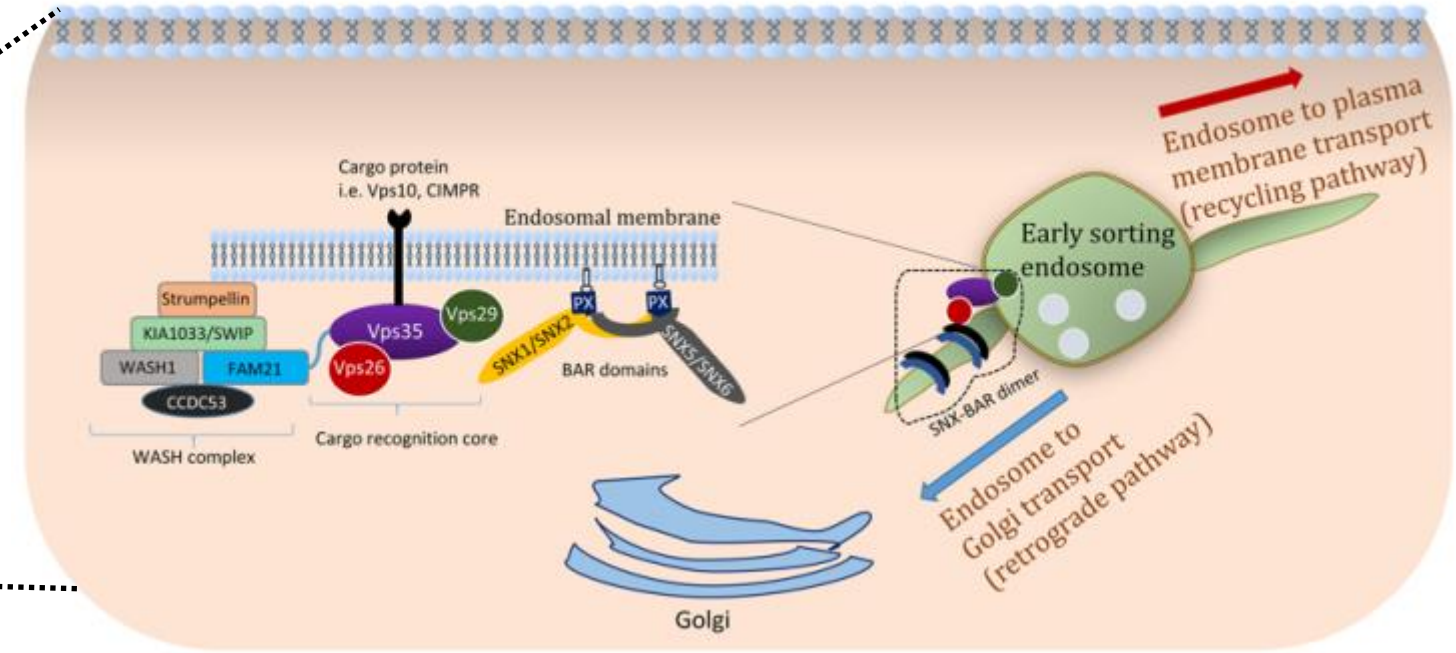


1. Air Intake
2. Battery
3. Air Filter Box
4. MAF
5. Vacuum Switch
6. Fuel Injector
7. CCV
8. Oil Filler Cap
9. Oil Filter Cap
10. Dipstick
11. Fuel Injection Pump
12. IAT
13. Washer Fluid
14. Fuel Filter
15. Powers Steering Fluid
16. Coolant Tank
17. Timing Belt Cover
18. EGR Valve



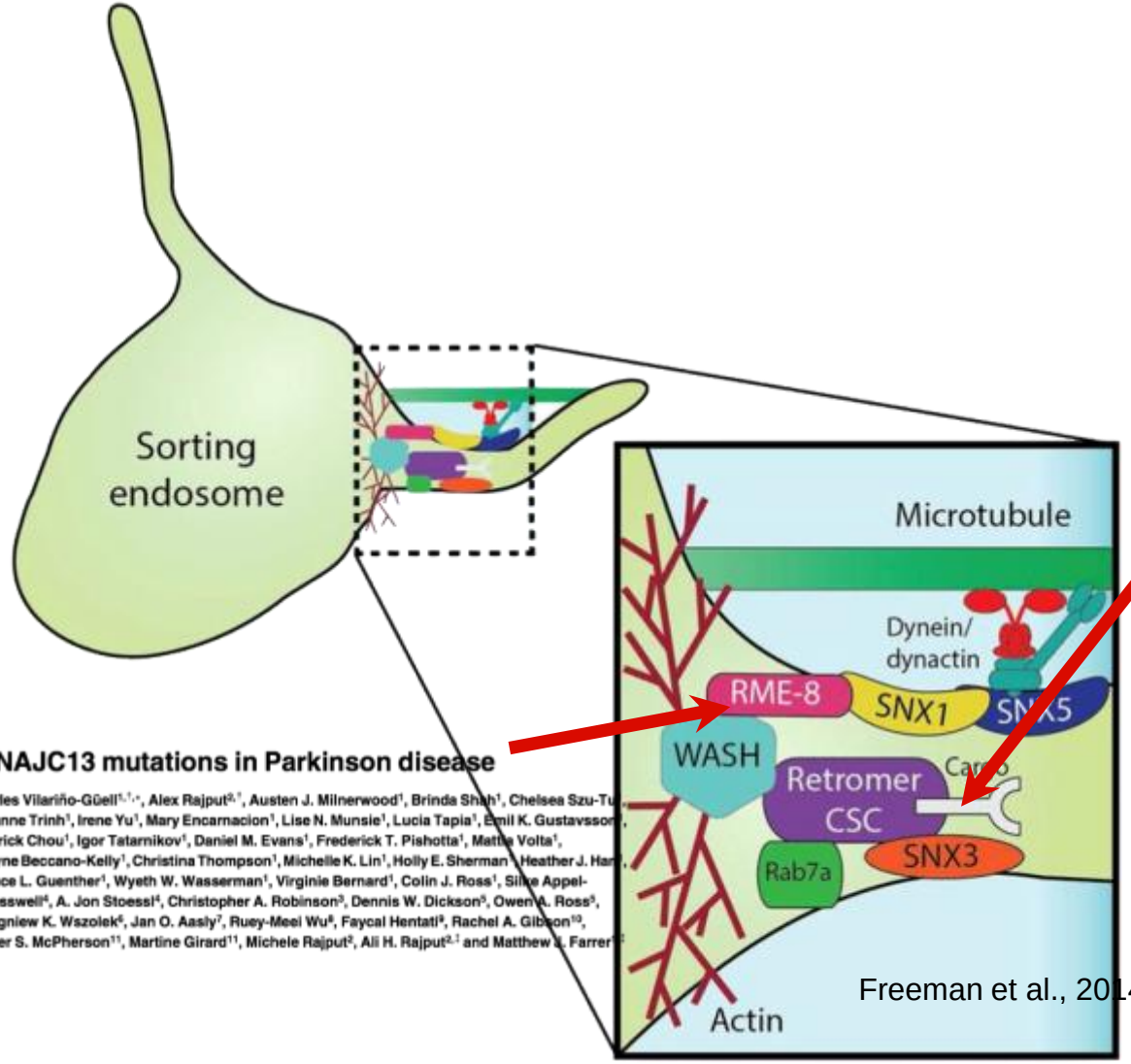
Membrane compartments regulate synaptic transmission





- Membrane movement and recycling is the basis of synaptic transmission
- Membrane is recycled to allow “re-filling” of vesicles depleted of neurotransmitter
- Impairing this recycling process disrupts neurotransmission
- Several known regulates of membrane trafficking are implicated in Parkinson’s disease

Mutations in endosomal proteins cause Parkinson's disease



DNAJC13 mutations in Parkinson disease

Carles Vilariño-Güell[5,7,*], Alex Rajput[5,7], Austen J. Milnerwood[1], Brinda Shah[1], Chelsea Szu-Tu[1], Joanne Trinh[1], Irene Yu[1], Mary Encarnacion[1], Lise N. Munsie[1], Lucia Tapia[1], Emil K. Gustavsson[1], Patrick Chou[1], Igor Tatarnikov[1], Daniel M. Evans[1], Frederick T. Pishotta[1], Mattia Volta[1], Dayne Beccano-Kelly[1], Christina Thompson[1], Michelle K. Lin[1], Holly E. Sherman[1], Heather J. Han[1], Bruce L. Guenther[1], Wyeth W. Wasserman[1], Virginie Bernard[1], Colin J. Ross[1], Silke Appel-Cresswell[4], A. Jon Stoessl[4], Christopher A. Robinson[5], Dennis W. Dickson[5], Owen A. Ross[5], Zbigniew K. Wszolek[6], Jan O. Aasly[7], Ruey-Meei Wu[8], Faycal Hentati[9], Rachel A. Gibson[10], Peter S. McPherson[11], Martine Girard[11], Michele Rajput[2], Ali H. Rajput[2,†] and Matthew J. Farrer[1,3]

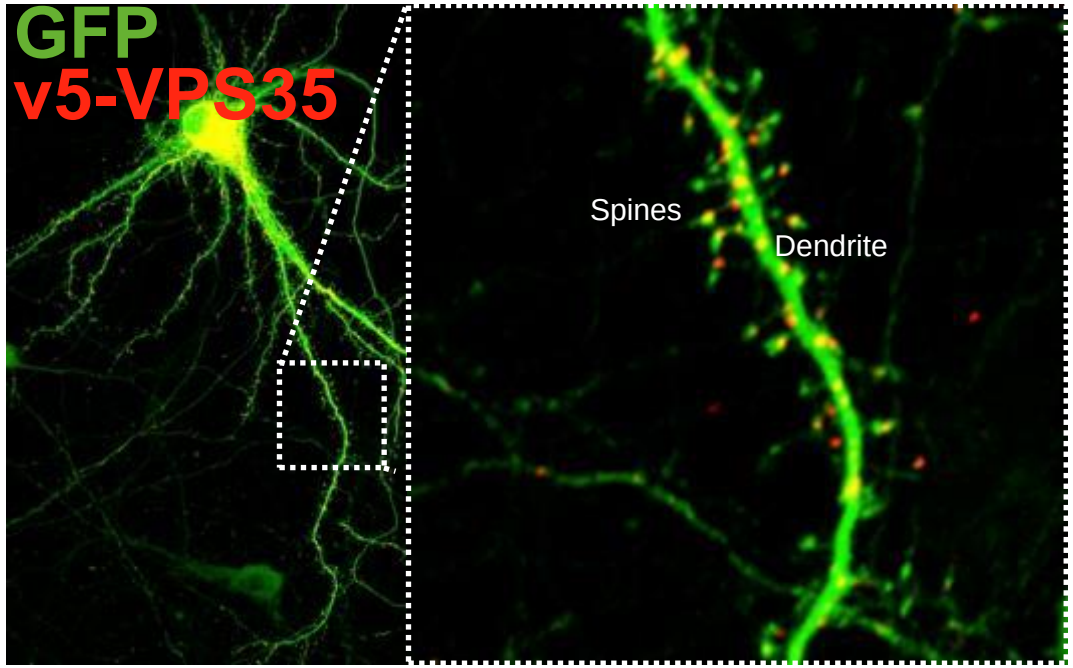
Freeman et al., 2014

REPORT

VPS35 Mutations in Parkinson Disease

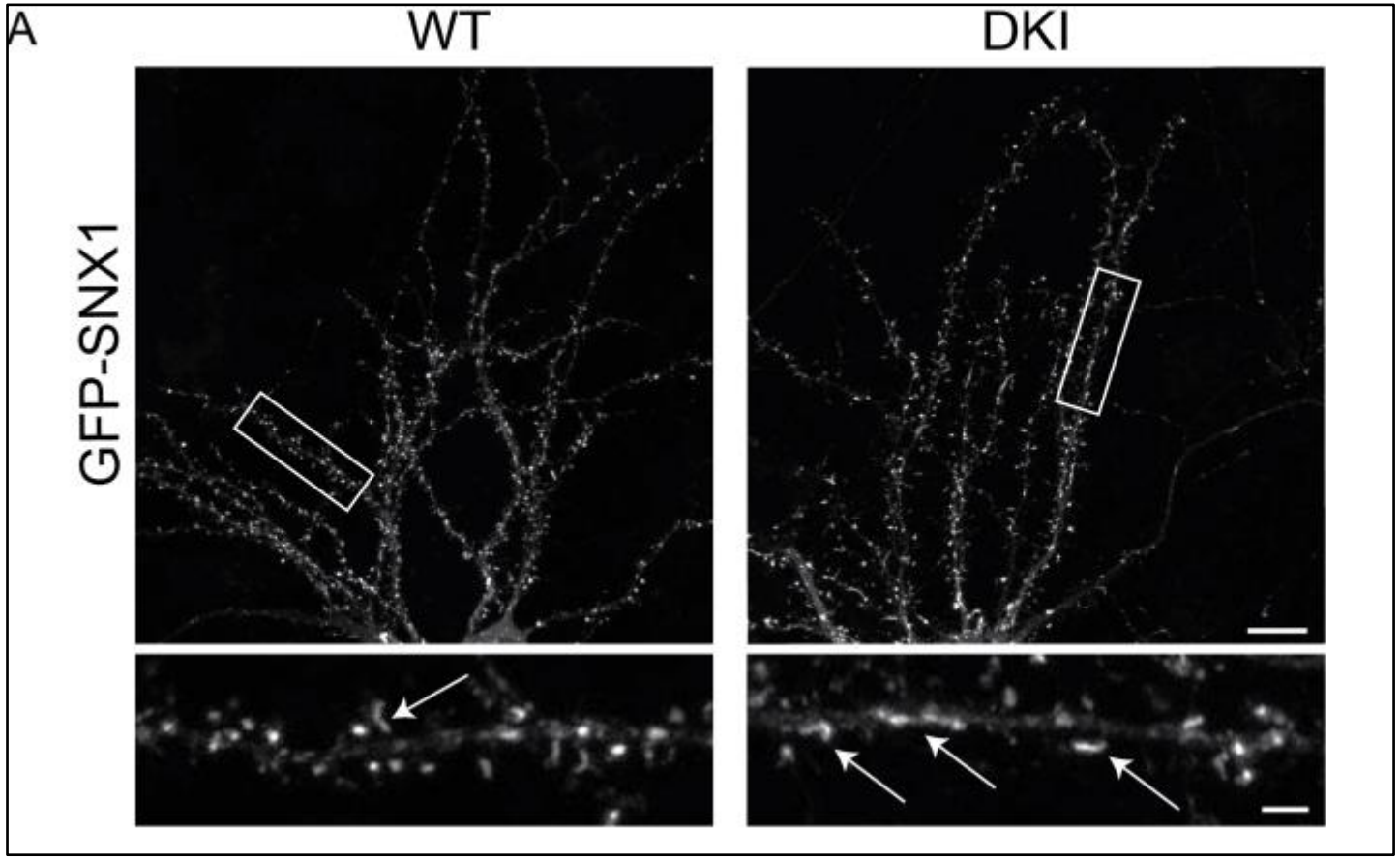
Carles Vilariño-Güell,[1,15,*] Christian Wider,[2,15] Owen A. Ross,[3] Justus C. Dachsel,[3] Jennifer M. Kachergus,[3] Sarah J. Lincoln,[3] Alexandra I. Soto-Ortolaza,[3] Stephanie A. Cobb,[3] Greggory J. Wilhoite,[3] Justin A. Bacon,[3] Bahareh Behrouz,[3] Heather L. Melrose,[3] Emna Hentati,[3] Andreas Puschmann,[3,4] Daniel M. Evans,[1] Elizabeth Conibear,[1] Wyeth W. Wasserman,[1] Jan O. Aasly,[5] Pierre R. Burkhard,[6] Ruth Djaldetti,[7] Joseph Ghika,[2] Faycal Hentati,[8] Anna Krygowska-Wajs,[9] Tim Lynch,[10,11] Eldad Melamed,[7] Alex Rajput,[12] Ali H. Rajput,[12] Alessandra Solida,[2] Ruey-Meei Wu,[13] Ryan J. Uitti,[14] Zbigniew K. Wszolek,[14] François Vingerhoets,[2] and Matthew J. Farrer[1,3]

VPS35 Cell Biology

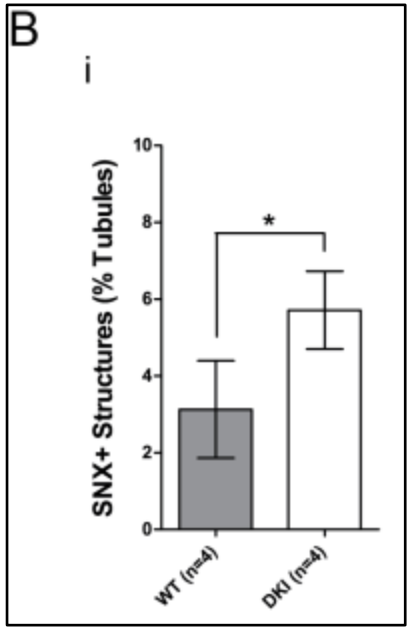


Mouse Cortical Neurone at 23 days *in vitro* GFP V5-VPS35. L.N. Munsie, CAN.

DNAJC13 p.N855S expression impedes vesicle trafficking

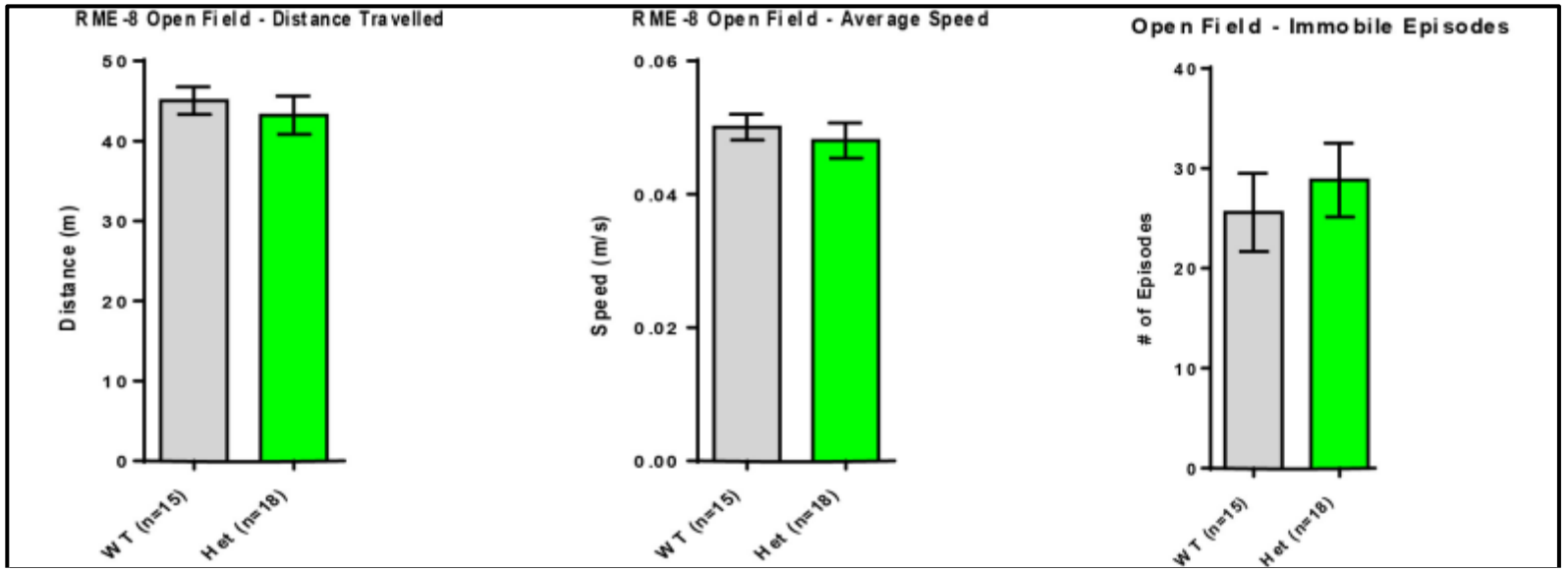


Mouse Cortical Neuron at 21 days *in vitro* expressing GFP-SNX1. DNAJC13 p.N855S causes extended tubule-like structures.

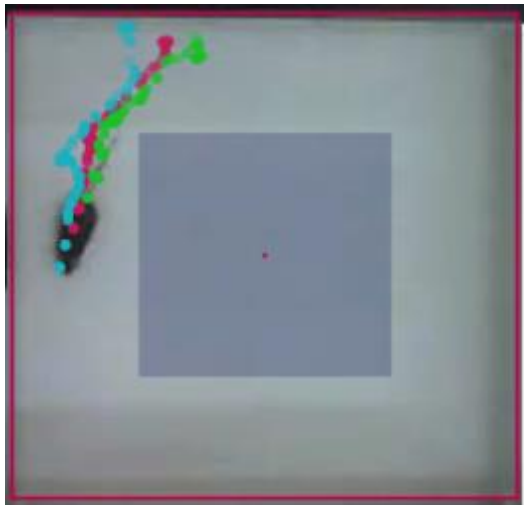


- DNAJC13 p.N855S expression increases the percentage of SNX1+ tubules
- Tubules are necessary membrane formations needed to transport material and make new vesicles

DNAJC13 p.N855S mice show age-dependent decline in basic behavioral tasks



3 months of age



- Motor testing of DNAJC13 p.N855S mice at 3 months of age show no impairments

Article | OPEN | Published: 21 August 2018

Altered dopamine release and monoamine transporters in Vps35 p.D620N knock-in mice

Stefano Cataldi, Jordan Follett, Jesse D. Fox, Igor Tatarnikov, Chelsie Kadgien, Emil K. Gustavsson, Jaskaran Khinda, Austen J. Milnerwood ✉ & Matthew J. Farrer ✉

npj Parkinson's Disease **4**, Article number: 27 (2018) | Download Citation

Hum Mol Genet. 2016 Oct 15;25(20):4507-4517. doi: 10.1093/hmg/ddw279.

Impaired striatal dopamine release in homozygous Vps35 D620N knock-in mice.

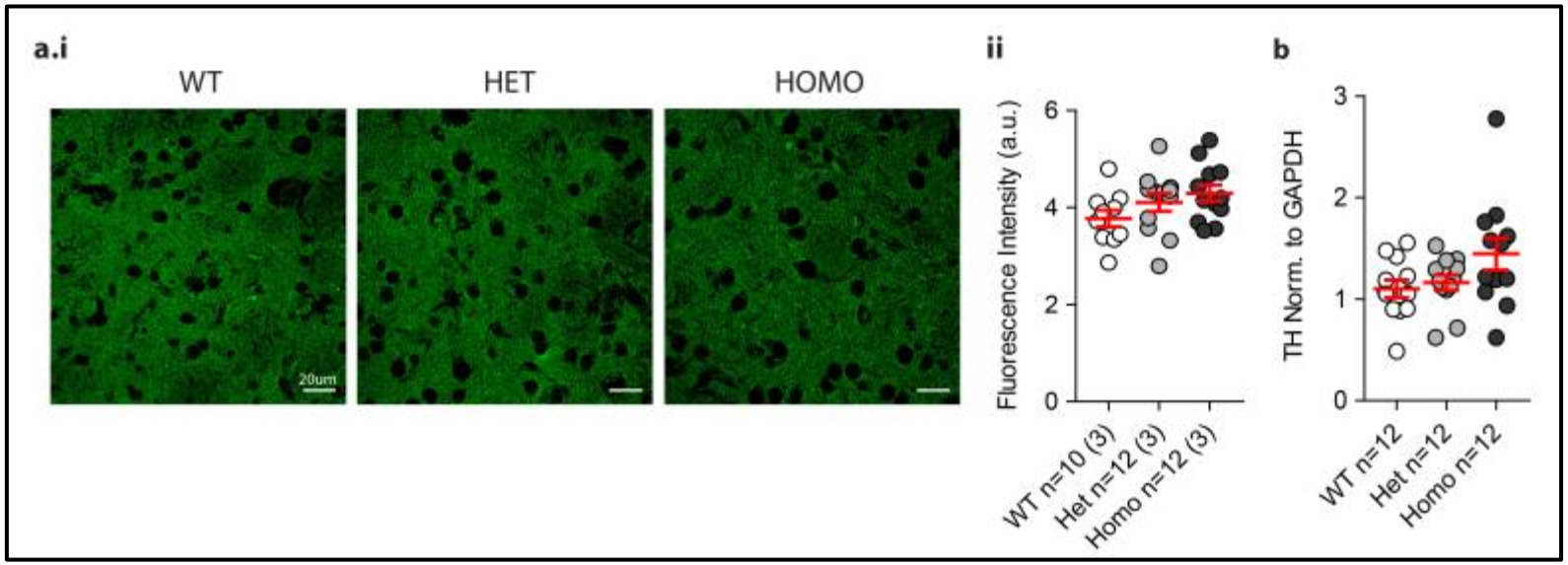
Ishizu N[1,2], Yui D[1], Hebisawa A[3], Aizawa H[4,5], Cui W[4,5], Fujita Y[6], Hashimoto K[6], Ajioka I[7], Mizusawa H[8], Yokota T[1], Watase K[7].

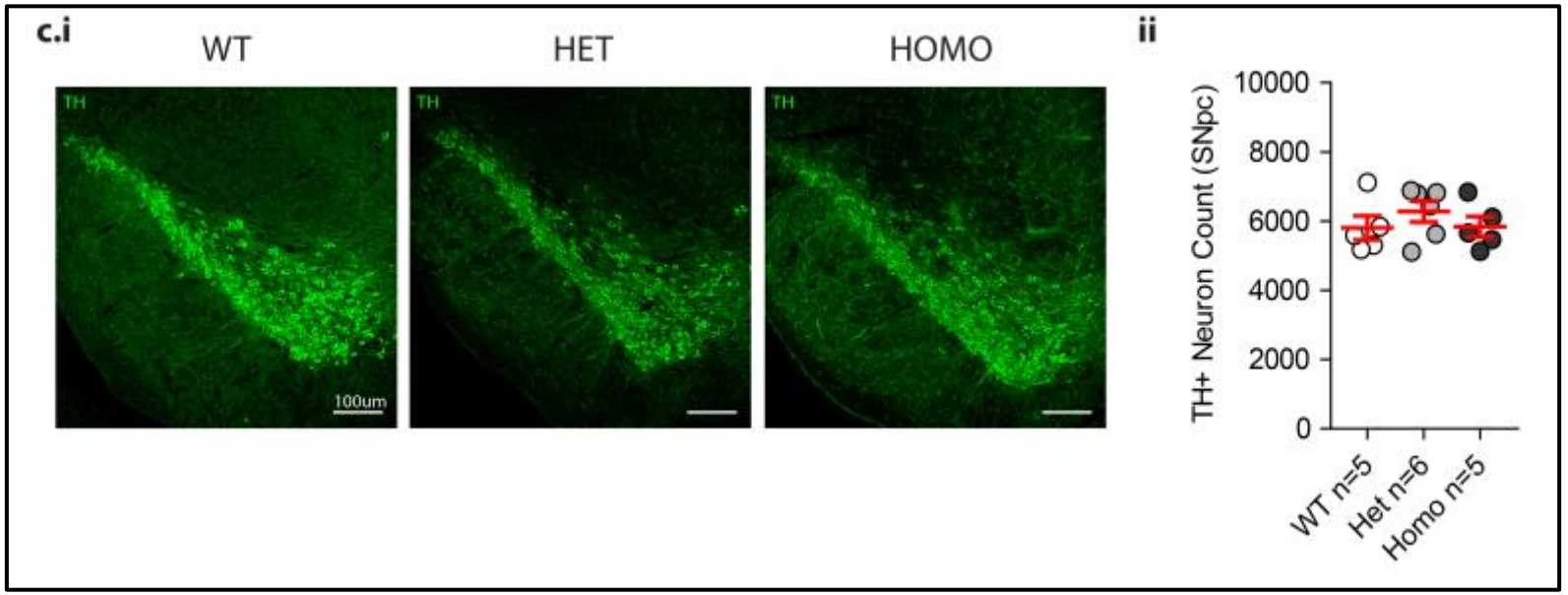
Author information

Abstract

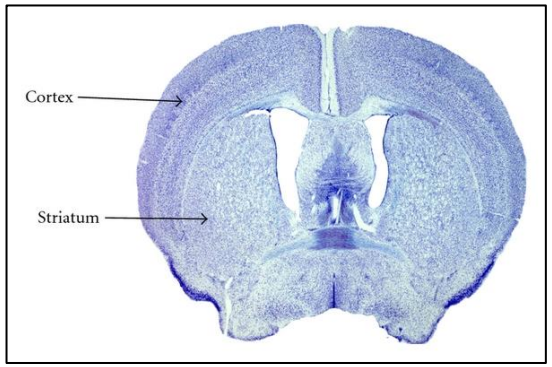
Point mutations in the vacuolar protein sorting 35 gene (VPS35) have been associated with an autosomal dominant form of late-onset Parkinson disease (PARK17), but there has been considerable debate over whether it is caused by a loss- or gain-of-function mechanism and over the intracellular target site of neurotoxicity. To investigate the pathogenesis of PARK17 in vivo, we generated Vps35 D620N knock-in (KI) mice, expressing the homologous mutant protein with endogenous patterns of expression, simultaneously with Vps35 deletion 1 (Del1) mice, which carry 1bp deletion in the exon15 of Vps35, by CRISPR/Cas9-mediated genome engineering. Neither homozygous nor heterozygous Vps35 D620N KI mice suffered from premature death or developed clear neurodegeneration up to 70 weeks of age. Vps35 Del1 allele appeared to be a null or at least severely hypomorphic allele and homozygous Vps35 Del1 showed early embryonic lethality. Heterozygous crossings between Del1 and D620N knock-in mice revealed that the D620N/Del1 compound heterozygous mice, but not heterozygous Del1 mice, suffered from survival disadvantage. In vivo microdialysis showed that DA release evoked by 120 mM potassium chloride was significantly reduced in the caudate putamen of adult homozygous Vps35 D620N KI mice. Taken together, these results suggest that Vps35 D620N allele is a partial-loss-of-function allele and that such a genetic predisposition and age-related alterations in the nigrostriatal dopamine system cooperatively influence the pathogenesis of PARK17.

No difference in the density of Dopamine producing neurons in p.D620N mice





Cataldi et al, 2018

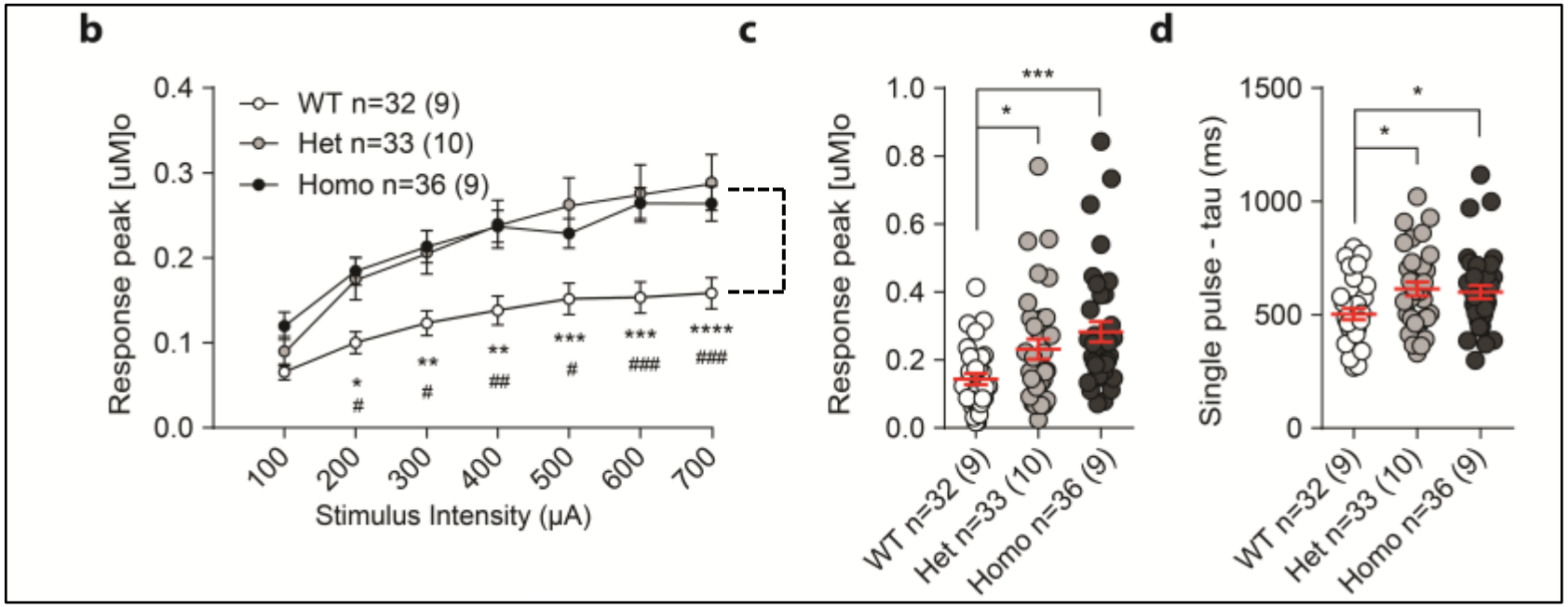


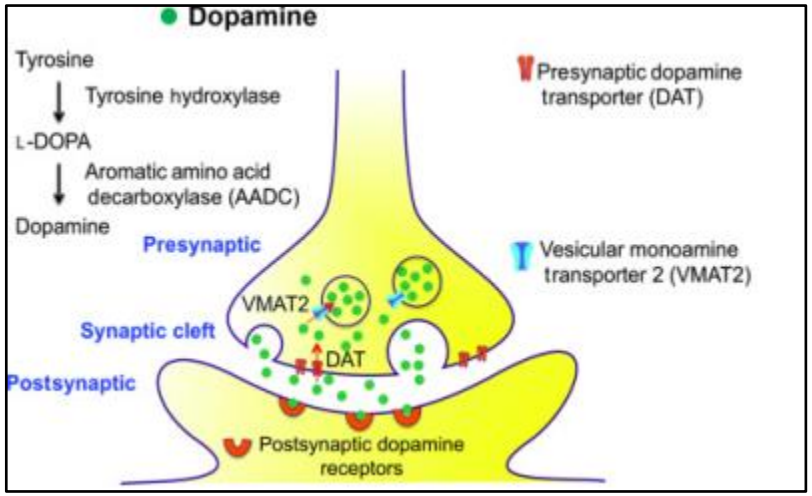
The integrity of the nigrostriatal system (striatum) and substantia nigra pars compacta (SNpc)] by immunohistochemical staining of the dopamine synthesis enzyme tyrosine hydroxylase (TH).

No significant differences were observed between VKI and WT littermates in the intensity of TH signal produced by nigral terminals in the dorsolateral striatum.

Healthy PD

Vps35 p.D620N mice have increased dopamine release at 3 months of age

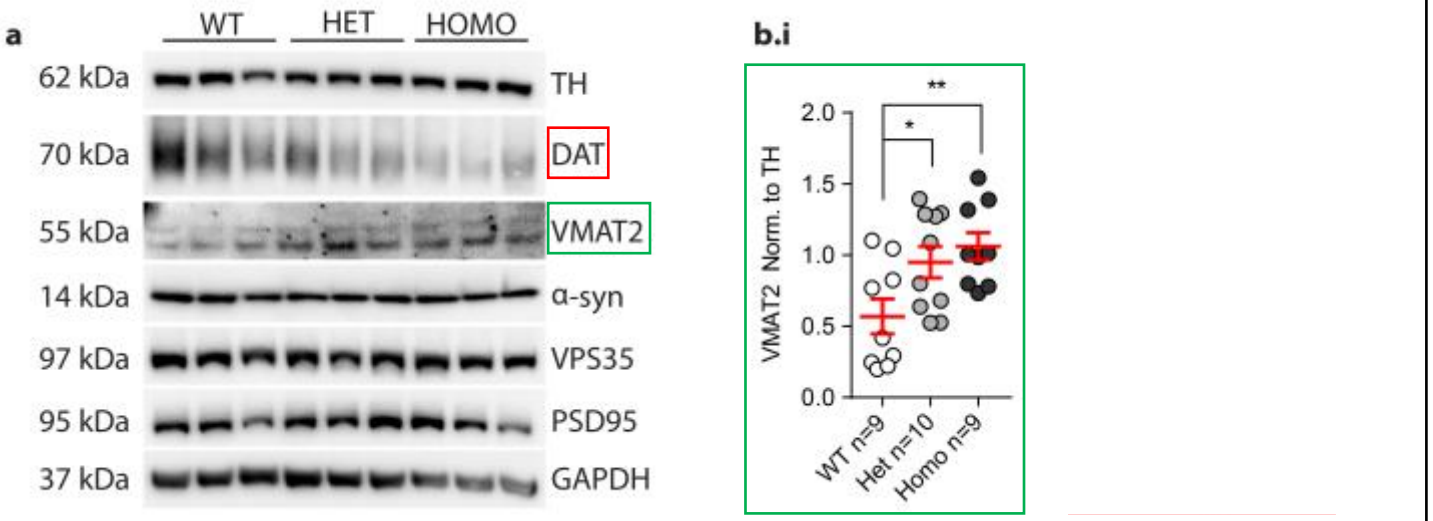


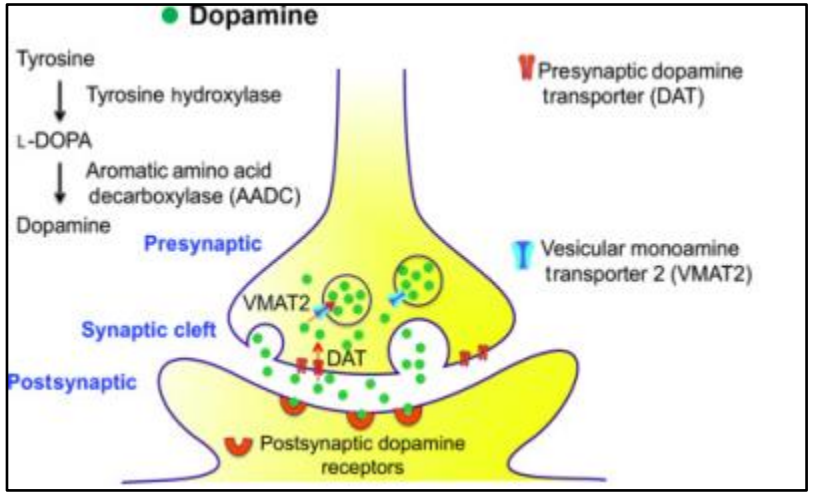


Cataldi et al, 2018

- Vps35 p.D620N mice have increased dopamine release at 3 months of age
- The increase in dopamine release in mutant mice is also complicated by impaired re-uptake/clearance

Synaptic protein markers that transporter dopamine are altered in Vps35 p.D620N mice



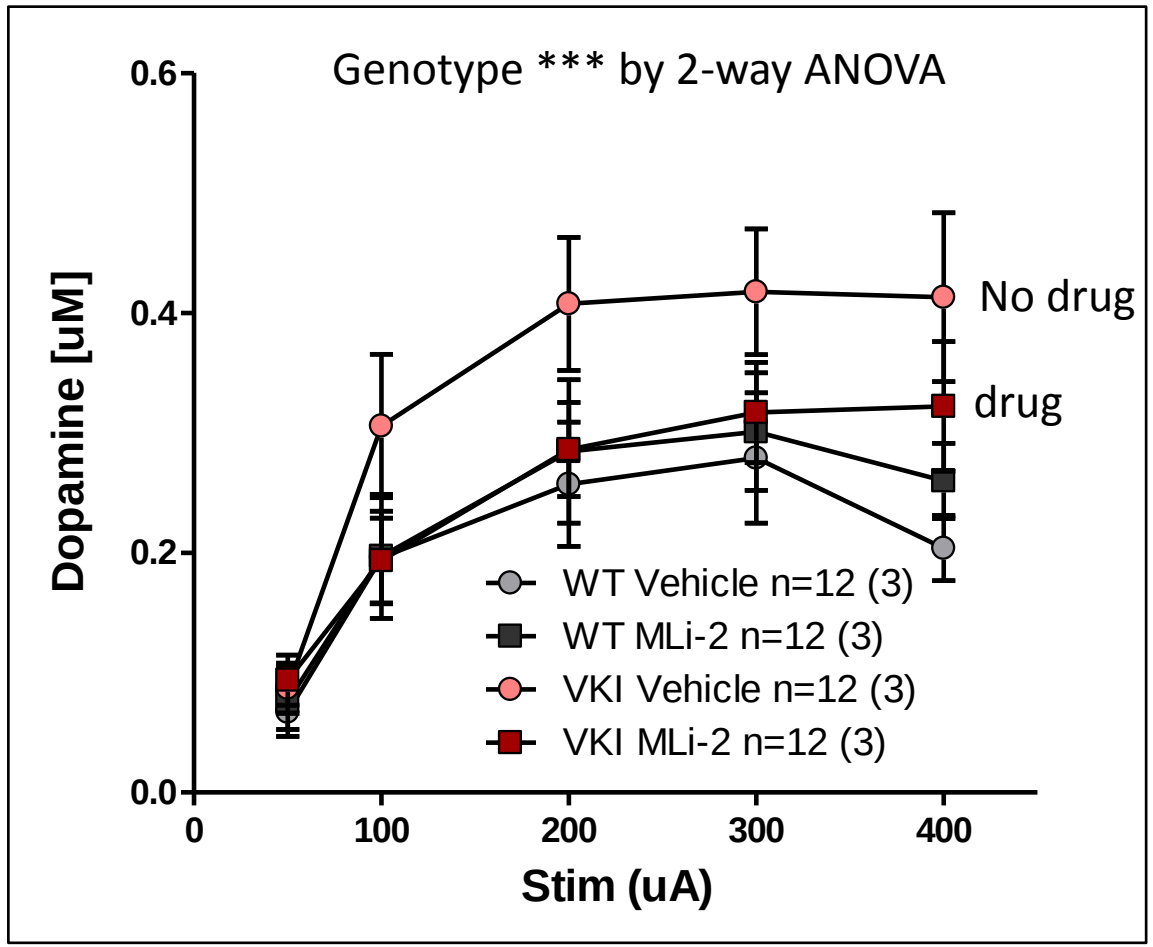


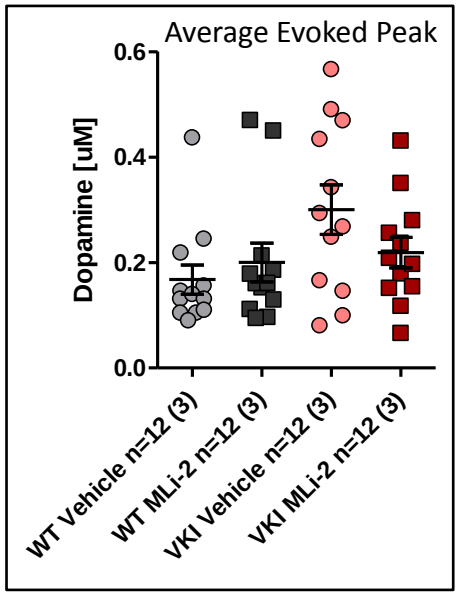
Cataldi et al, 2018

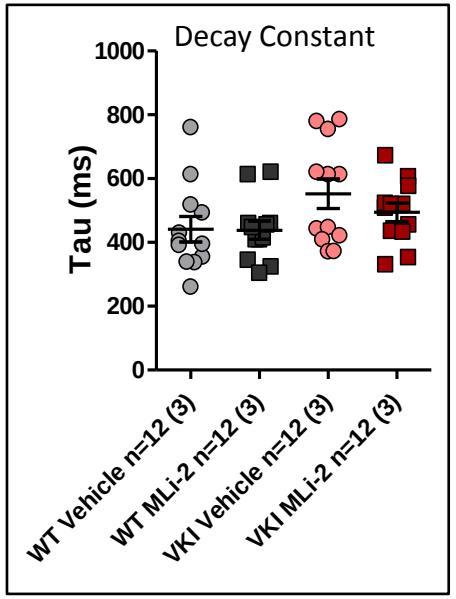
Vps35 p.D620N mice show a loss of dopamine transporter (DAT) and an increase in VMAT2

DAT is needed for the re-uptake of dopamine and VMAT2 is needed to package it into vesicles

Increased dopamine release in Vps35 p.D620N mice is rescued by LRRK2 kinase inhibitors







- Vps35 p.D620N mice receive Mli-2(drug) or placebo (control) for a period of 7 days.
- On day 7, dopamine levels are measured in mice
- Vps35 p.D620N mice that receive Mli-2 for a total of 7 days have reduced Dopamine release and now mirror non-mutant mice
- Ongoing studies are investigating changes in localization of synaptic machinery needed for DA transport

Igor Tatarnikov, Masters student at UBC

PERSPECTIVE | NEURODEGENERATION

LRRK2 kinase in Parkinson's disease

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RESEARCH ARTICLE | PARKINSON'S DISEASE

LRRK2 activation in idiopathic Parkinson's disease

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Small-Molecule Inhibitors of LRRK2.

Hatcher JM[1], Choi HG[2], Alessi DR[3], Gray NS[4].

⊕ Author information

Abstract

Mutations in the leucine-rich repeat kinase 2 (LRRK2) protein have been genetically and functionally linked to Parkinson's disease (PD). The kinase activity of LRRK2 is increased by pathogenic mutations; therefore, modulation of LRRK2 kinase activity by a selective small-molecule inhibitor has been proposed as a potentially viable treatment for Parkinson's disease. This chapter presents a historical overview of the development and bioactivity of several small-molecule LRRK2 inhibitors that have been used to inhibit LRRK2 kinase activity in vitro or in vivo. These compounds are important tools for understanding the cellular biology of LRRK2 and for evaluating the potential of LRRK2 inhibitors as disease-modifying PD therapies.

Exp Neurol. 2017 Dec;298(Pt B):236-245. doi: 10.1016/j.expneurol.2017.07.019. Epub 2017 Jul 29.

Achieving neuroprotection with LRRK2 kinase inhibitors in Parkinson disease.

West AB[1].

⊕ Author information

Abstract

In the translation of discoveries from the laboratory to the clinic, the track record in developing disease-modifying therapies in neurodegenerative disease is poor. A carefully designed development pipeline built from discoveries in both pre-clinical models and patient populations is necessary to optimize the chances for success. Genetic variation in the leucine-rich repeat kinase two gene (LRRK2) is linked to Parkinson disease (PD) susceptibility. Pathogenic mutations, particularly those in the LRRK2 GTPase (Roc) and COR domains, increase LRRK2 kinase activities in cells and tissues. In some PD models, small molecule LRRK2 kinase inhibitors that block these activities also provide neuroprotection. Herein, the genetic and biochemical evidence that supports the involvement of LRRK2 kinase activity in PD susceptibility is reviewed. Issues related to the definition of a therapeutic window for LRRK2 inhibition and the safety of chronic dosing are discussed. Finally, recommendations are given for a biomarker-guided initial entry of LRRK2 kinase inhibitors in PD patients. Four key areas must be considered for achieving neuroprotection with LRRK2 kinase inhibitors in PD: 1) identification of patient populations most likely to benefit from LRRK2 kinase inhibitors, 2) prioritization of superior LRRK2 small molecule inhibitors based on open disclosures of drug performance, 3) incorporation of biomarkers and empirical measures of LRRK2 kinase inhibition in clinical trials, and 4) utilization of appropriate efficacy measures guided in part by rigorous pre-clinical modeling. Meticulous and rational development decisions can potentially prevent incredibly costly errors and provide the best chances for LRRK2 inhibitors to slow the progression of PD.

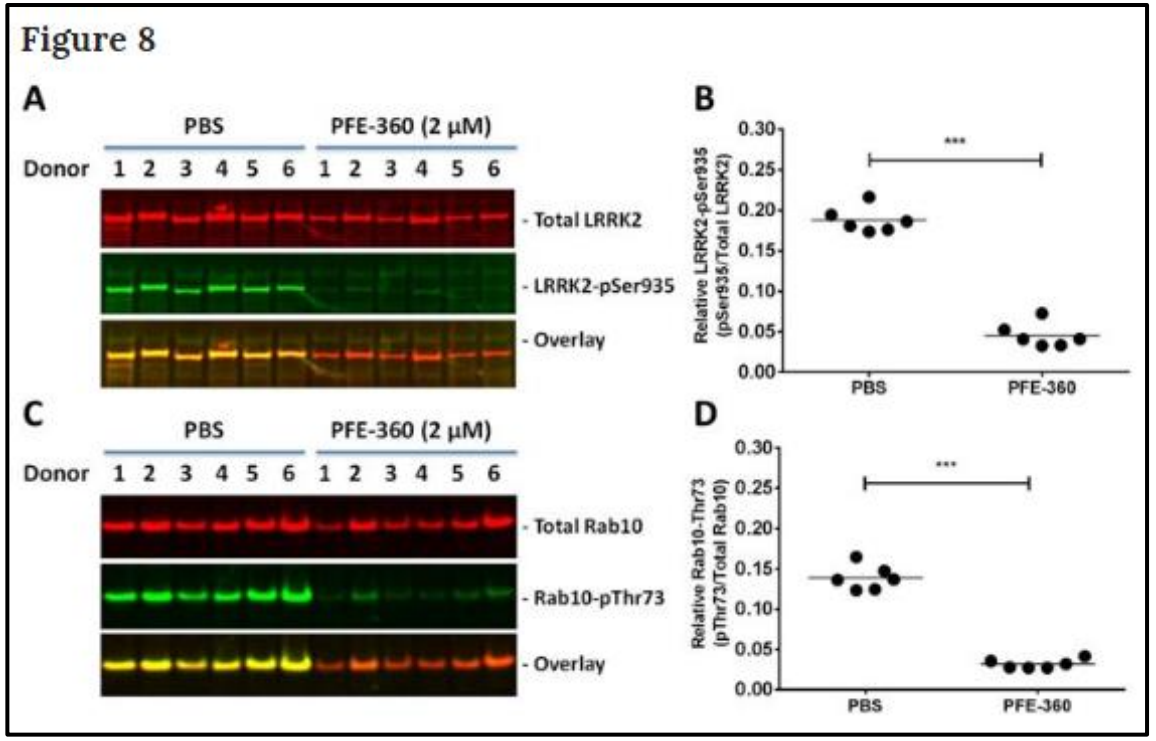
Blood collection as a biomarker for kinase activity

Article | OPEN | Published: 31 August 2017

Selective LRRK2 kinase inhibition reduces phosphorylation of endogenous Rab10 and Rab12 in human peripheral mononuclear blood cells

Kenneth Thirstrup, Justus C. Dächsel, Felix S. Oppermann, Douglas S. Williamson, Garrick P. Smith, Karina Fog & Kenneth V. Christensen

Scientific Reports **7**, Article number: 10300 (2017) | Download Citation



- human peripheral mononuclear blood cells (PBMCs) treated with the LRRK2 inhibitor
- These cells can be grown in a culture dish and tested repetitively with varying concentrations and drugs (ie personalisation of medical treatment)

In the near future, there will be a high demand for central and peripheral markers to monitor LRRK2 kinase activity in clinical trials aiming at evaluating the potential of LRRK2 inhibitors as disease-modifying treatment for Parkinson's disease

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