Mutation analysis of *CHCHD10* in neurodegenerative diseases, including Parkinson's disease

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Objective is to update on recent genetic findings related to *CHCHD10 & CHCHD2*

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CHCHD10 is novel ALS/FTD gene

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A mitochondrial origin for frontotemporal dementia and amyotrophic lateral sclerosis through *CHCHD10* involvement

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FTD & ALS: genetic, clinical & histopathology data

[Hardy J & Rogaeva E, Experimental Neurology, 2013]

Genetics of ALS and/or FTD			Continuum based on:		
Gene	Frequency in familial cases	Type of mutations	Clinical presentation	Brain Pathology*	Likely pathological effect
SOD1	~20%	mainly missense	ALS	SOD1/p62	Toxic aggregation
FUS	~5%	mainly missense, & in-frame small	ALS	FUS/p62	DNA/RNA metabolism
TARDBP (TDP43)	~3%				sm
C9orf72	~30%			p62/repeat-dipeptides, UBQLN2	Toxic aggregation (?) Low C9orf72 expression (?)
VCP	Rare	missense	FTD, ALS, IBMPFD	TDP43/p62	Autophagy
SQSTM1 (p62)	~3%	missense and nonsense	FTD, ALS, PDB	TDP43/p62	Autophagy
OPTN	Rare	missense and nonsense (haploinsufficiency)	ALS/FTD, glaucoma PDB (by GWAS)	TDP43/p62	Autophagy
UBQLN2	Rare	missense	ALS, FTD, SP, MS	TDP43/p62, UBQLN2, FUS, OPTN	Autophagy
GRN	~10%	nonsense (haploinsufficiency)	FTD, CLN11	TDP43/p62	Autophagy/lysosomal pathway
CHMP2B	Rare	C-terminal truncation of the CHMP2B	FTD	p62	Autophagy/lysosomal pathway
MAPT	~10%	missense and splicing of exon 10	FTD	abnormal tau filaments (tangles)	Toxic aggregation (defect in neuronal cytoskeleton)

Novel disease genes:

MATR3 (RNA/DNA-binding protein): ALS [Johnson et al, Nature Neur, 2014]

CHCHD10 (mitochondrial protein): ALS/FTD [Bannwarth et al., Brain, 2014]

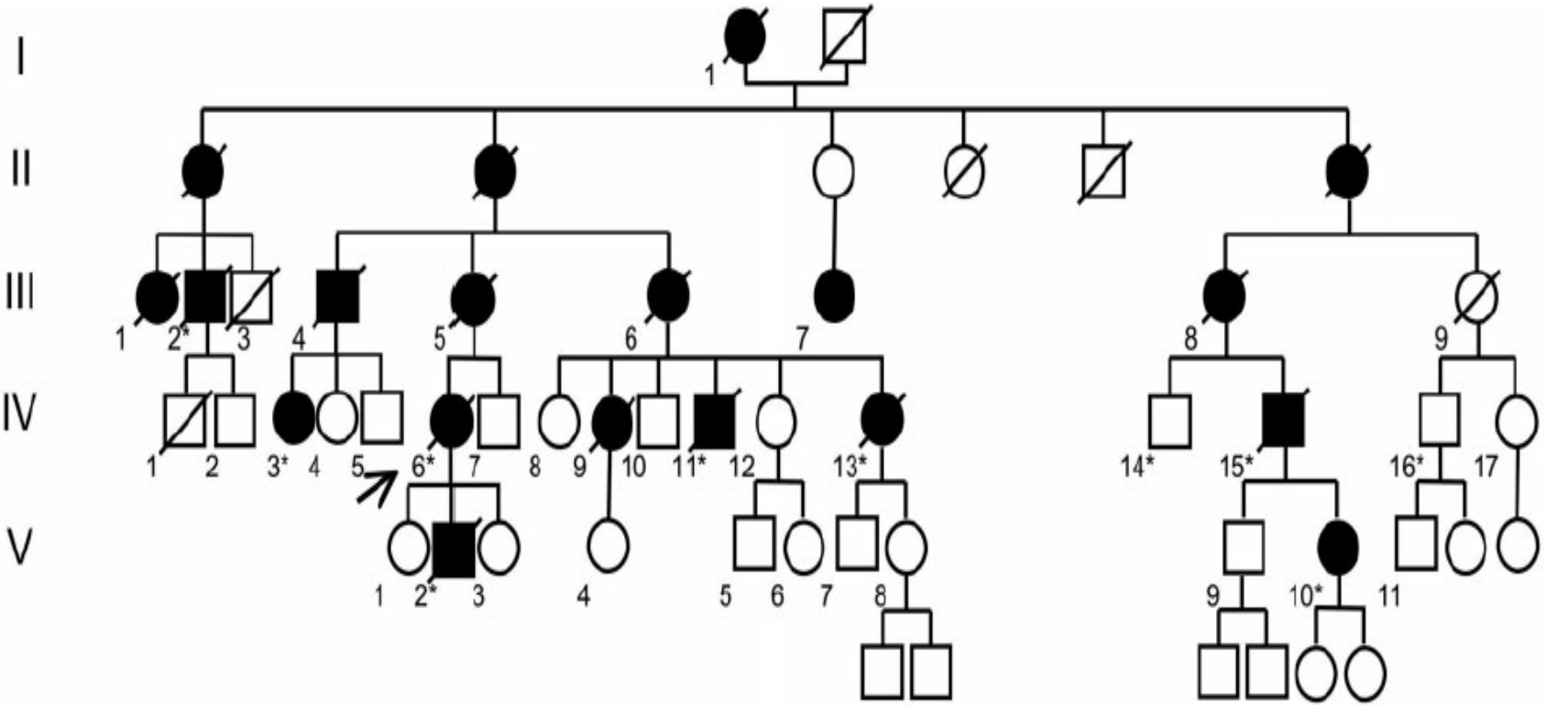


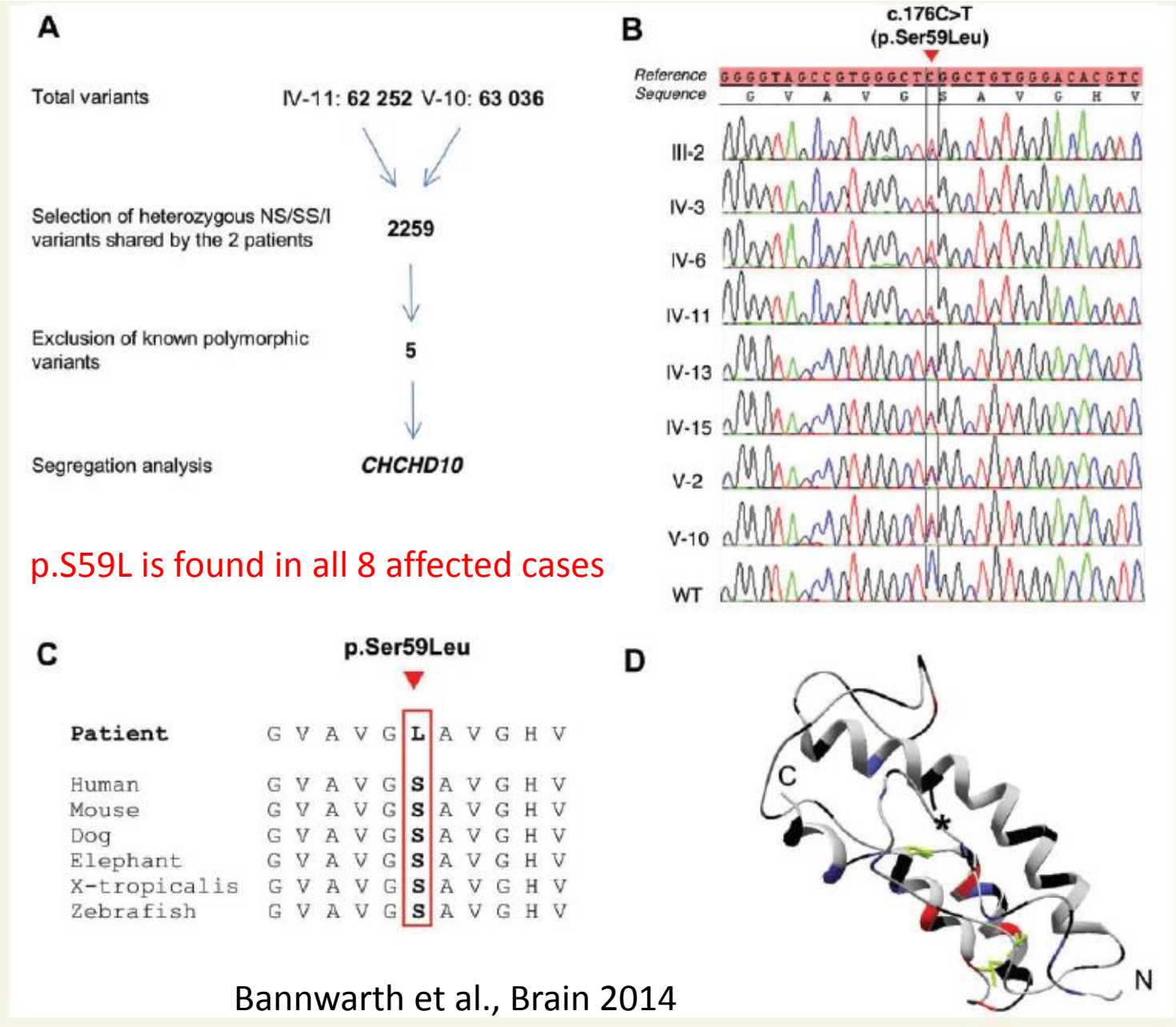
Figure 1 Pedigree of the first family. Solid symbols represent clinically affected individuals. Asterisk corresponds to individuals tested for segregation analysis.

Patients of the French family presented with a complex phenotype, including:

- ALS (main)
- ALS/FTLD
- mitochondrial myopathy
- cerebellar ataxia
- parkinsonism

Bannwarth et al., Brain 2014

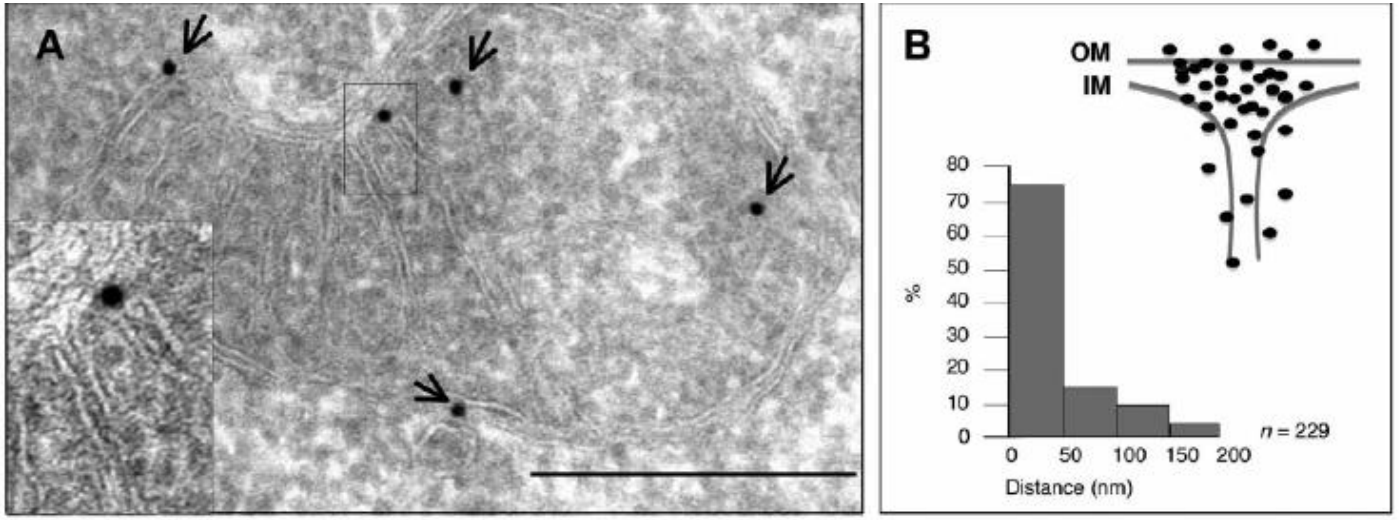
Result of whole exome sequencing of 2 affected family members



Bannwarth et al., Brain 2014

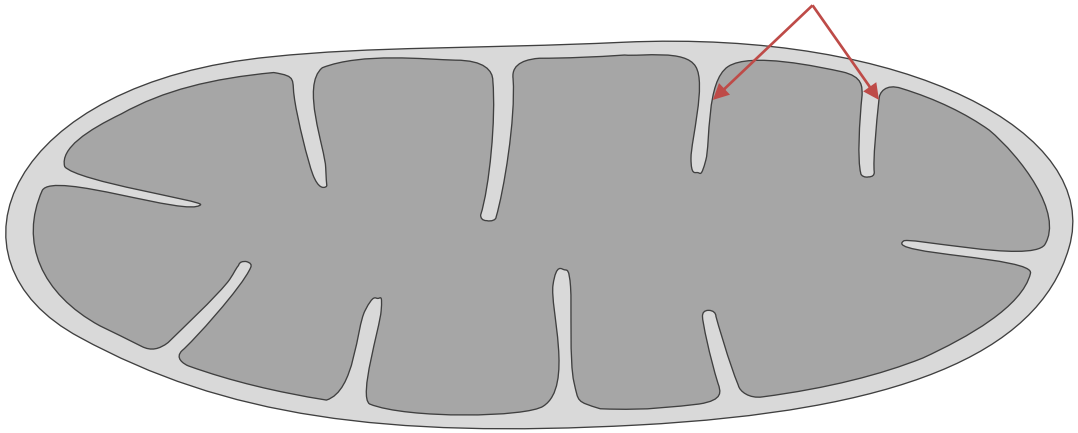
CHCHD10 is located in mitochondrial intermembrane space

Bannwarth et al., Brain 2014



Immunoelectron microscopy of CHCHD10

CHCHD10 protein is enriched at cristae junctions



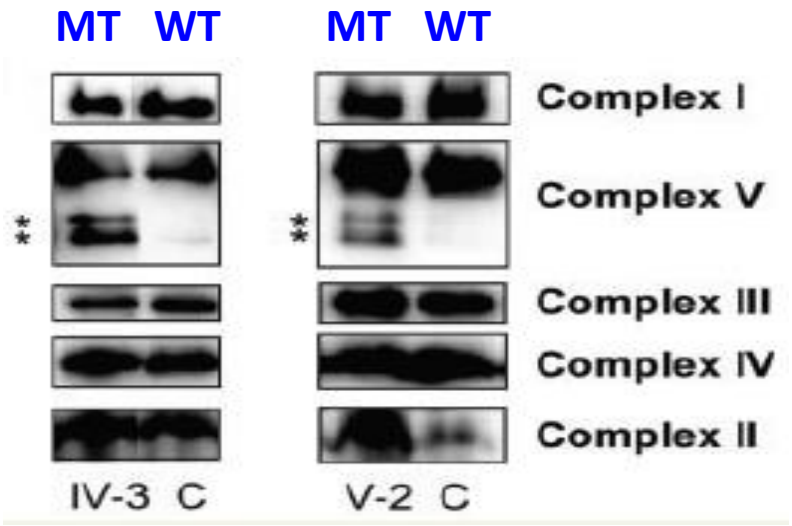
mitochondria

Destruction of the mitochondrial network in *CHCHD10* patients

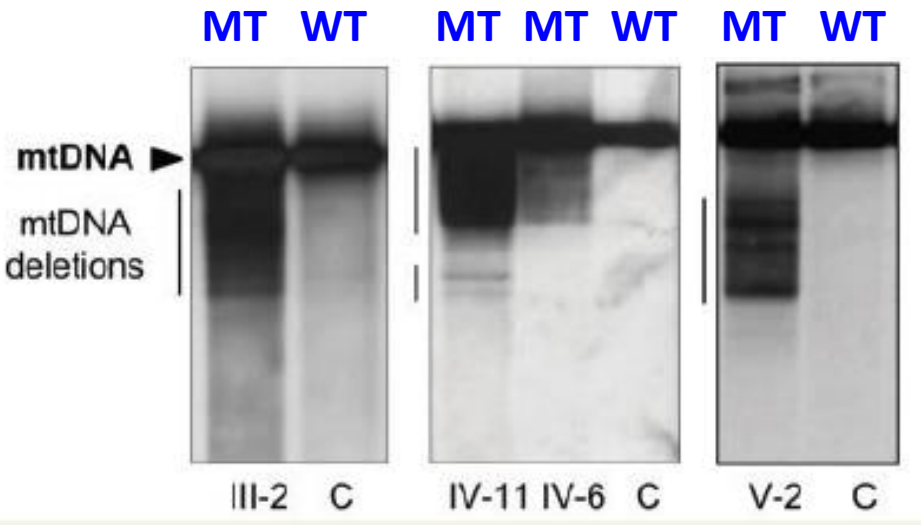
Bannwarth et al., Brain 2014

Muscle biopsy shows respiratory chain deficiency

Defect in assembly of mitochondrial Complex V



Deletions in mitochondrial DNA



Brain pathology in mutation carriers is unknown

CHCHD10 is confirmed as ALS gene: novel p.R15L in 3 ALS familes

doi:10.1093/brain/awu265 Brain 2014: Page 1 of 2 | e1

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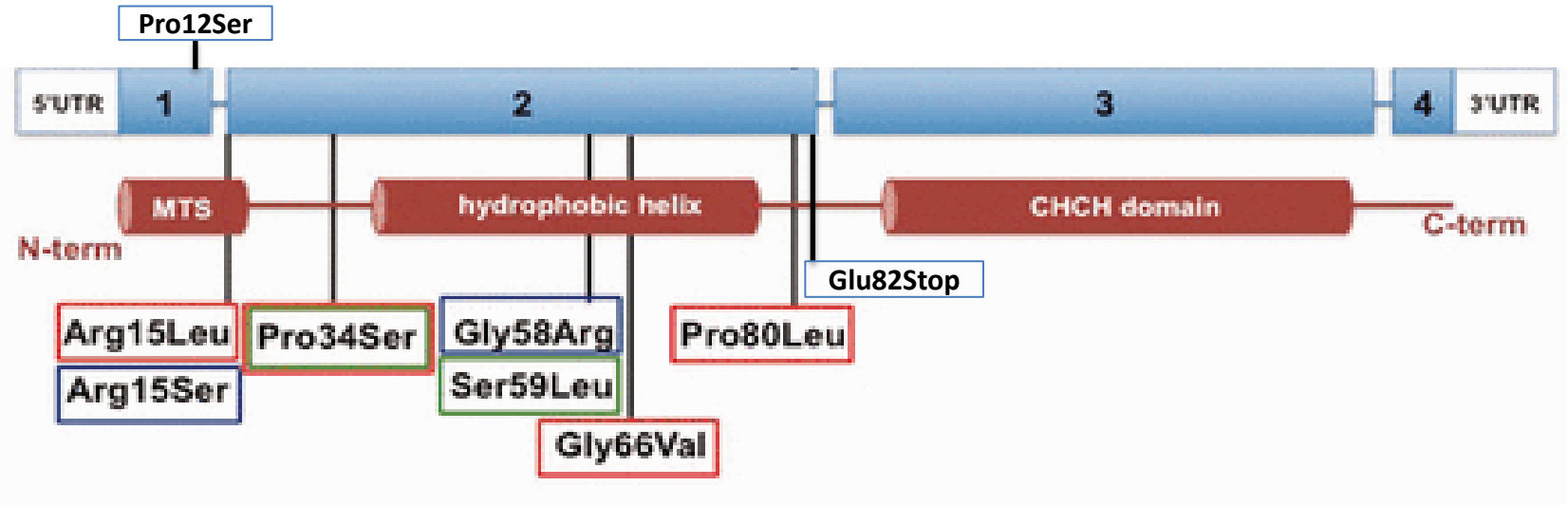
LETTER TO THE EDITOR

Mutations in the *CHCHD10* gene are a common cause of familial amyotrophic lateral sclerosis

Janel O. Johnson,[1] Shannon M. Glynn,[1] J. Raphael Gibbs,[2] Mike A. Nalls,[3] Mario Sabatelli,[4] Gabriella Restagno,[5] Vivian E. Drory,[6] Adriano Chiò,[7] Ekaterina Rogaeva[8] and Bryan J. Traynor[1]

- By WGS we detected a p.R15L mutation segregating with ALS (6 patients/family).
- It was observed in 2 other familial ALS patients.

CHCHD10 structure: exon 2 is a mutation hotspot



[Modified from Ronchi et al., 2015 Brain]

Color for phenotypes: ALS, FTD-ALS, mitochondrial myopathy

Mitochondrial targeting sequence (MTS): protein localization
Hydrophobic helix: protein-protein interaction

Are there any *CHCHD10* mutations in related diseases (ALS, FTD, AD, PD)?

doi:10.1093/brain/awv082 BRAIN 2015: Page 1 of 4 | e1

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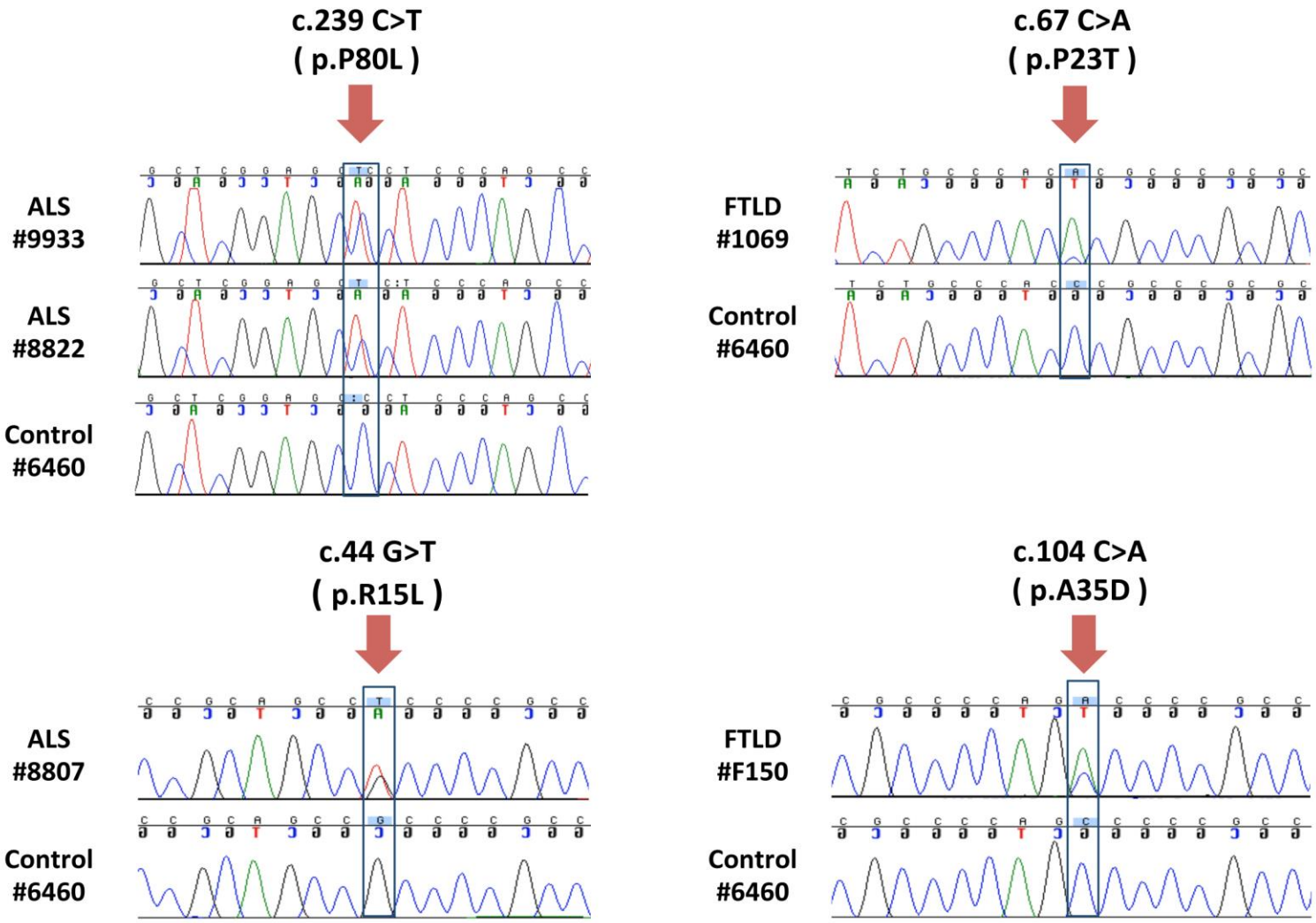
LETTER TO THE EDITOR

Mutation analysis of *CHCHD10* in different neurodegenerative diseases

Ming Zhang,[1,*] Zhengrui Xi,[1,*] Lorne Zinman,[2] Amalia C Bruni,[3] Raffaele G Maletta,[3] Sabrina A. M. Curcio,[3] Innocenzo Rainero,[4] Elisa Rubino,[4] Lorenzo Pinessi,[4] Benedetta Nacmias,[5] Sandro Sorbi,[5] Daniela Galimberti,[6] Anthony E. Lang,[7,8] Susan Fox,[7,8] Ezequiel I. Surace,[9] Mahdi Ghani,[1] Jing Guo,[1] Christine Sato,[1] Danielle Moreno,[1] Yan Liang,[1] Julia Keith,[2] Bryan J. Traynor,[10] Peter St George-Hyslop[1,8,11] and Ekaterina Rogaeva[1,8]

Cohorts	Age at onset, y, mean (SD)
ALS (n=204)	58.9 (13.2)
FTD (n=158)	64.0 (8.9)
PD (n=153)	51.3 (12.2)
AD (n=141)	74.3 (13.4)
Controls (n=497)	>65 y.o.

We detected 4 *CHCHD10* mutations that are not found in controls

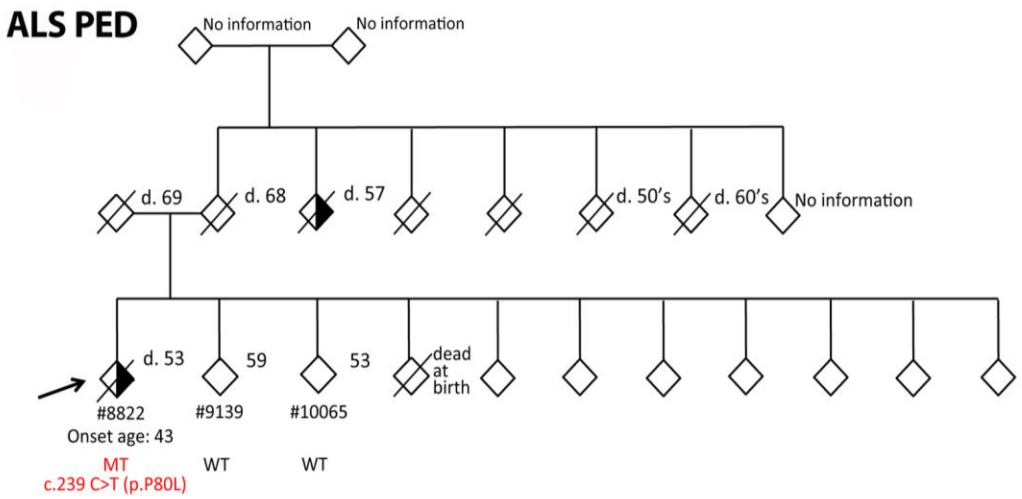


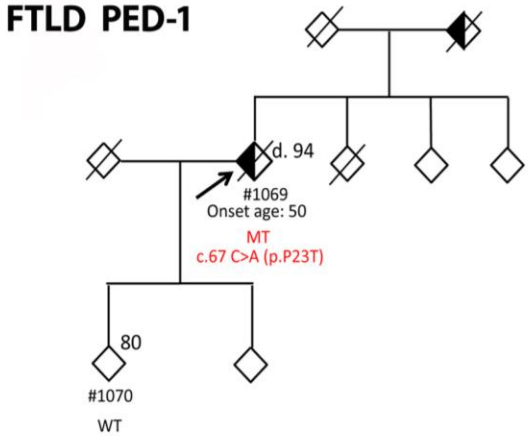
Clinical information of *CHCHD10* missense variant carriers

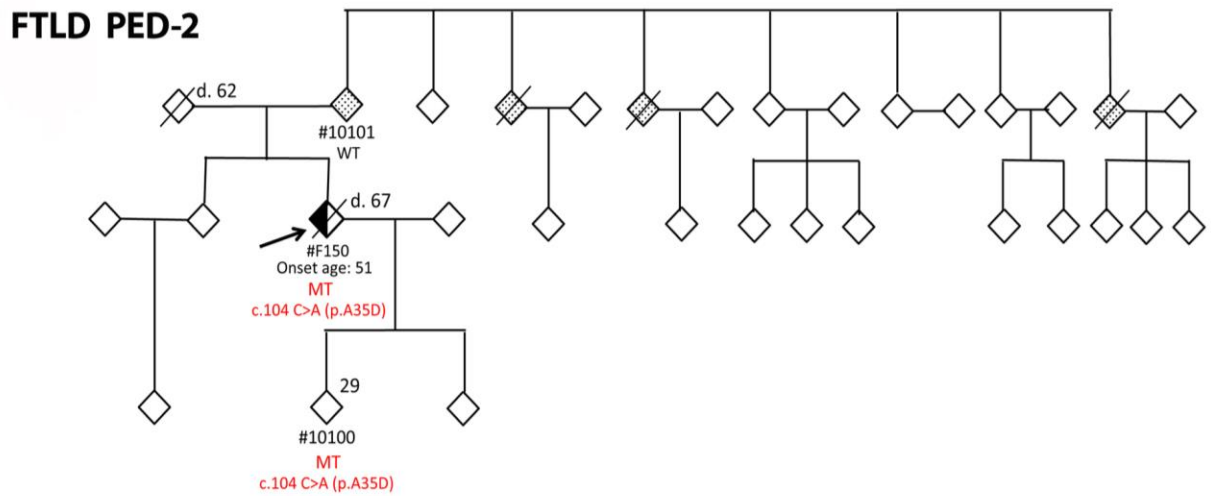
CRND #	Diagnosis	Familial	Sex	Age at onset	Duration (years)	Site of Onset	*CHCHD10* exon2 mutation
8807	ALS	No	M	54	on going	Upper limb (began at the right, weakness)	c.44G>T (p.R15L)
8822	ALS	Yes	F	43	9	Upper limb (began in left arm)	c.239C>T (p.P80L)
9933	ALS	No	F	58	on going	Bulbar	c.239C>T (p.P80L)
1069	FTLD	Yes	F	50	44	NA	c.67C>A (p.P23T)
F150	bvFTLD	No	F	51	16	NA	c.104C>A (p.A35D)

Our patients had slow disease progression (e.g. in [Muller *et al.*, 2014]).

The result of segregation analysis in *CHCHD10* families







MT = mutant allele

WT = wild type allele

Conservation analysis of the CHCHD10 protein across species: regions flanking the mutations

p.P80L (ALS)

Patient	G	G	S	S	E	**L**	S	Q	P	A	V
Human	G	G	S	S	E	**P**	S	Q	P	A	V
Rhesus	G	G	S	S	E	**P**	S	Q	P	A	A
Mouse	G	G	N	S	E	**P**	A	Q	P	A	V
Dog	G	G	S	S	E	**P**	A	Q	P	A	V
Cat	G	G	S	S	E	**P**	A	Q	P	A	T
Elephant	G	G	S	S	E	**P**	A	Q	P	A	V
X-Trpicalis	G	G	S	S	E	**P**	S	K	P	V	A
Zebrafish	G	G	S	S	S	E	A	P	K	P	A

p.R15L (ALS)

Patient	R	P	-	A	S	**L**	P	A	A	P	S
Human	R	P	-	A	S	**R**	P	A	A	P	S
Rhesus	R	P	-	A	S	**R**	P	A	A	P	S
Mouse	R	P	-	V	S	**R**	P	A	P	P	P
Dog	R	P	-	A	S	**R**	P	A	A	P	S
Cat	R	P	-	A	S	**R**	T	A	A	P	S
Elephant	R	S		A	R	**E**	H	G	H	P	P
X-Tropicalis	R	T		A	S	**S**	H	A	S	S	P
Zebrafish	P	A	P	A	S	**A**	P	A	P	S	Y

p.P23T (FTD)

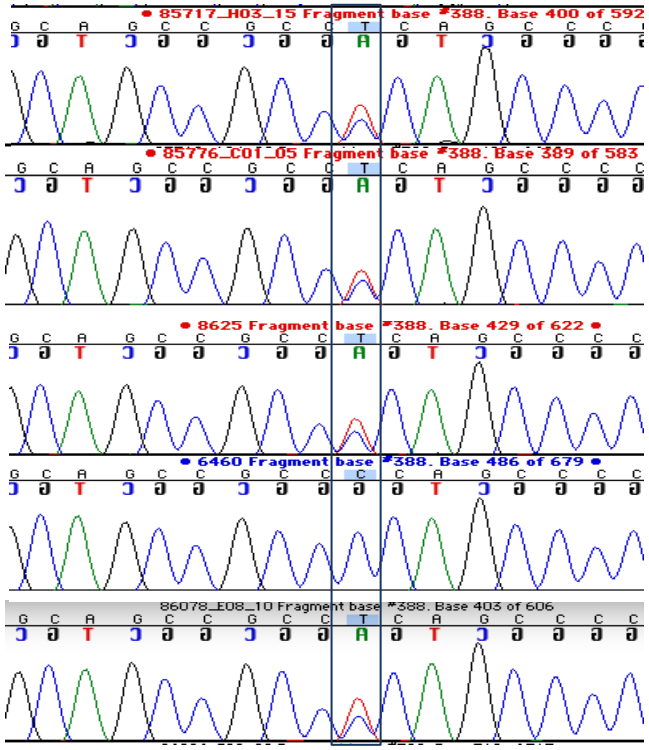
Patient	A	P	S	A	H	**T**	P	A	H	P	P
Human	A	P	S	A	H	**P**	P	A	H	P	P
Rhesus	A	P	S	A	H	**P**	P	A	H	P	P
Dog	A	P	S	A	A	**P**	P	A	P	P	P
Cat	A	P	S	V	H	**P**	P	A	H	P	P
Elephant	H	P	P	G	R	**P**	-	-	-	-	-
Mouse	P	P	P	A	-	-	-	-	H	P	P
X-Tropicalis	S	S	P	A	P	**A**	P	A	N	P	P
Zebrafish	P	S	Y	A	P	**A**	P	A	A	P	P

p.A35D (FTD)

Patient	A	A	-	-	P	**D**	P	A	P	S	G
Human	A	A	-	-	P	**A**	P	A	P	S	G
Rhesus	A	A	-	-	P	**A**	P	A	P	S	G
Mouse	-	-	A	A	P	**A**	P	A	A	P	G
Dog	A	A	A	A	A	**A**	A	A	P	S	G
Elephant	A	C	G	H	S	**A**	P	C	S	S	G
X-Tropicalis	-	-	L	A	S	**A**	P	A	P	S	Q
Zebrafish	-	A	V	A	P	**A**	A	A	Q	P	K
Cat	A	A	-	-	P	**A**	P	T	P	S	G

The p.P34S variant was detected in control, PD & AD subjects

c.100 C>T
(p.P34S)

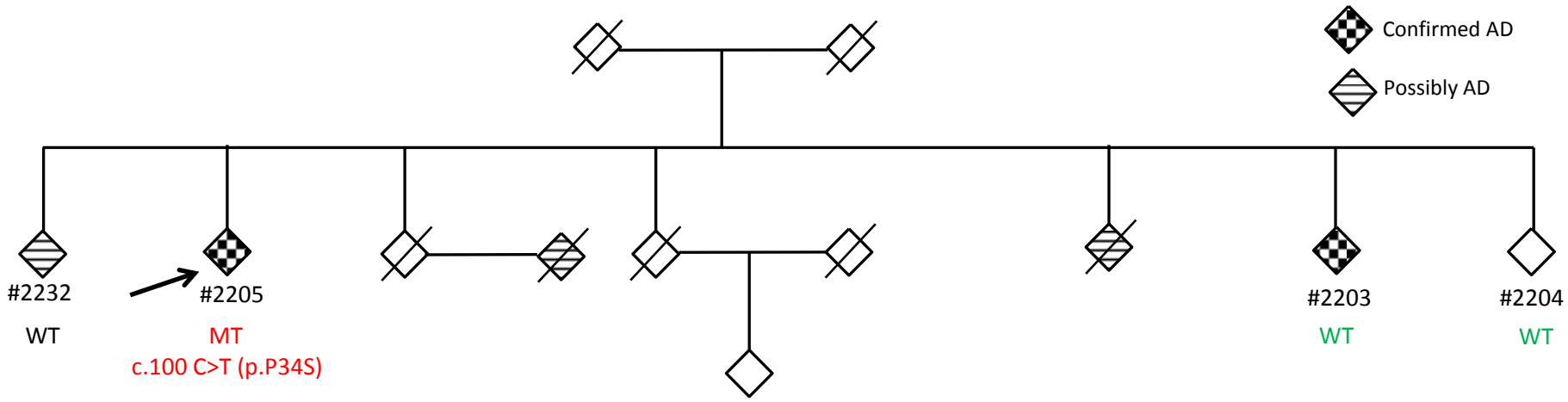


c.100 C>T
(p.P34S)

Patients	P	S	A	A	A	**S**	A	P	A	P	S
Human	P	S	A	A	A	**P**	A	P	A	P	S
Rhesus	P	S	A	A	A	**P**	A	P	A	P	S
Mouse	P	S	A	A	A	**P**	A	P	A	A	P
Dog	P	S	A	A	A	**P**	A	A	A	P	S
Elephant	P	S	A	A	A	**P**	A	P	A	A	S
Zebrafish	P	M	A	V	A	**P**	A	A	A	Q	P
X-Tropicalis	P	S	A	L	A	S	A	P	A	P	S

It affects a conserved amino acid.

The p.P34S variant does not segregate with AD phenotype (PD family was not available for study)



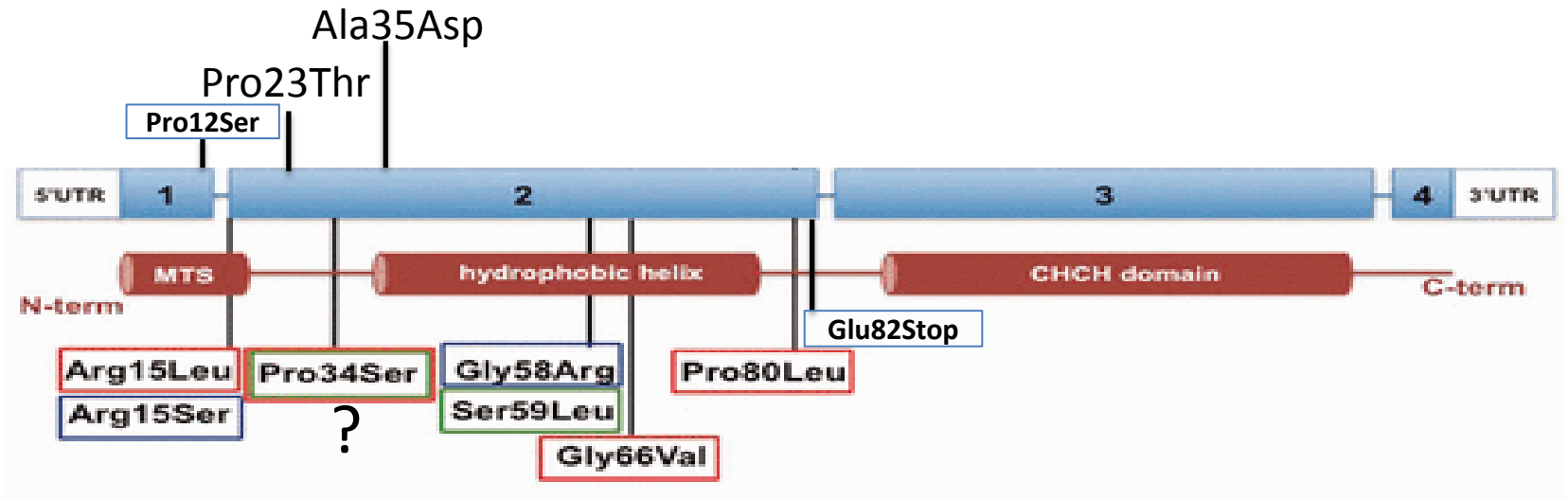
	Public Database			In house		
	NHLBI Exome sequencing project	1000 Genome project (European cohort)	ExAC (European cohort)	Controls	PD	AD
p.P34S frequency	0	0.001	0.003	0.007	0.003	0.007

- Our study did not support the pathogenic nature of the p.P34S variant, in contrast to the reports that did not avaluated sufficient number of controls (Chaussenot et al., 2014; Ronchi et al., 2015; Chiò et al., 2015).
- Dols-Icardo et al. 2015 confirmed our conclusion in Spanish study

Scoop from all CHCHD10 reports (modified from Bannwarth et al., Brain 2015)

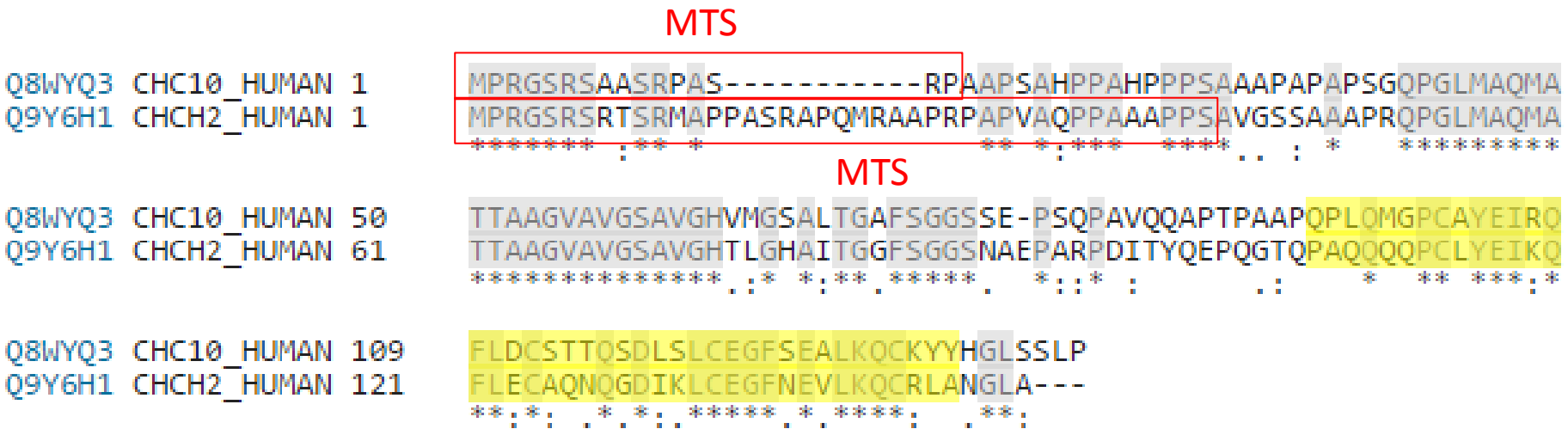
	Bannwarth et al., 2014	Chaussenot et al., 2014	Muller et al., 2014	Johnson et al., 2014	Ajroud-Driss et al., 2015	Rochi et al., 2015	Kurzweily et al., 2015	Chiò et al., 2015	Zhang et al., 2015	Oriol et al., 2015
Cases no.	8	3	5	6	10	3	4	3	5	8
Myopathy	8	-	-	-	10	-	-	-	-	-
FTD	-	-	-	-	-	-	-	-	2	5
ALS/FTD	6	3	-	-	-	-	-	-	-	-
ALS	-	-	5	6	-	3	4	3	3	3
Cerebellar sign	5	-	-	-	-	-	1	-	-	-
parkinsonism	1	-	-	-	-	-	-	-	-	1
Age at onset	49-65	59-67	35-73	?	?	25-75	41-73	44-69	43-58	58
Disease duration	1-27	4-8	6-17	?	>30	2-8	2-15	1.3-7	9-44	11, >3
Muscle biopsy	RRF, COX	-	-	-	RRF	RRF, COX	-	-	-	
Mutations	**S59L**	**P34S** **S59L**	**G66V** **R15L**	**R15L**	**R15S** **G58R**	**P34S** **P80L**	**R15L**	**P34S**	**P34S** **P80L** **R15L** **P23T** **A35D**	**P34S** **P12S** **Q82X**

Summary of CHCHD10 study



- Our study supports the causal role of *CHCHD10* in ALS & FTLD, but not in AD & PD:
  - 2 FTLD patients with novel mutations (p.P23T & p.A35D)
  - 3 ALS patients with known mutations (p.P80L & p.R15L)
  - the pathogenic nature of the p.P34S variant is questionable
- The mutation frequencies in our dataset:
  - 3% for familial & 1% for sporadic ALS
  - 2% for familial & 1% for sporadic FTD
- Carriers are characterized by long duration (9 y. of ALS, 16-44 y. of FTLD)
- Ongoing proteomics studies in fibroblasts from carriers of the p.P80L and p.R15L

Would other structurally similar proteins contribute to Neurodegenerative diseases?



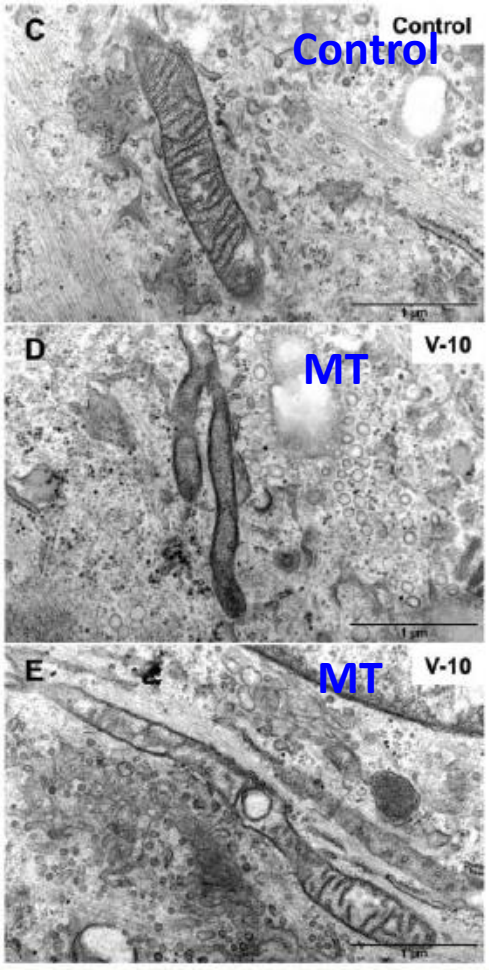
MTS (N-terminal), CHCH domain (C-terminal), share 55% amino acids sequence.

- Mutations in *CHCHD2* were very recently reported to cause PD in Japanese patients (Funayama et al. 2015, Lancet Neurology).

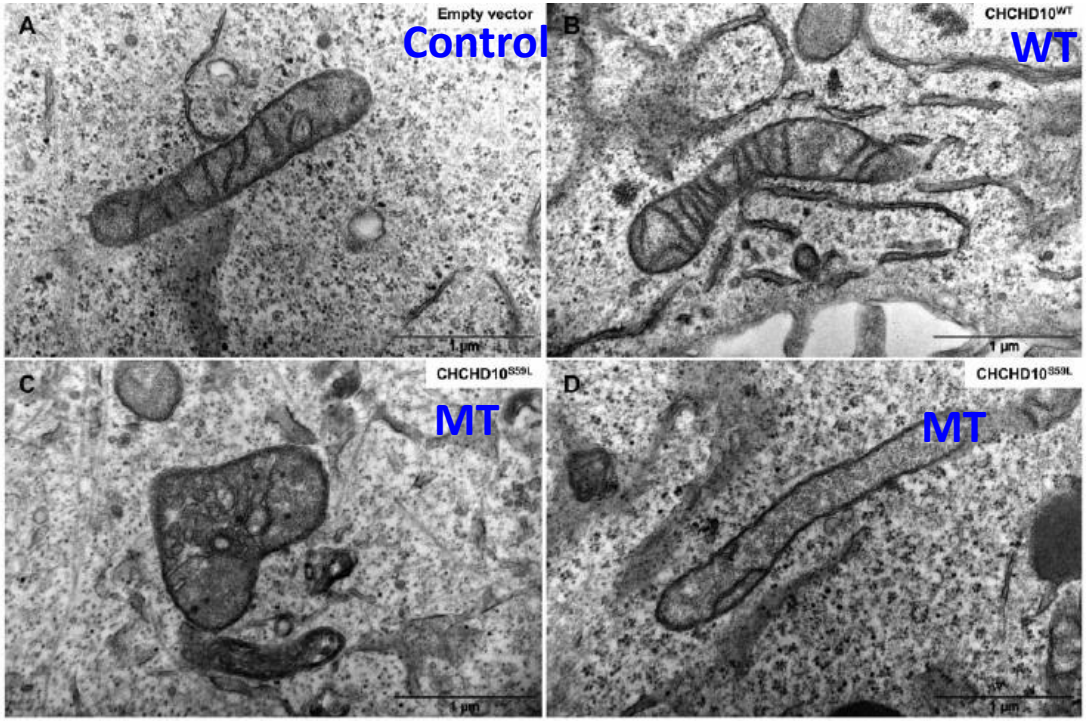
- Both *CHCHD2* and *CHCHD10* are linked to the mitochondrial pathway like several known PD genes (e.g. *PINK1* and *PARK2*).

- Mutant CHCHD10 leads to fragmentation of the mitochondrial network
- Similar functional studies are needed for CHCHD2…

Fibroblast



Hela cell



Bannwarth et al., Brain 2014

CHCHD2 and PD [Funayama et al. 2015]

- A **Thr61Ile** mutation was identified in 13 cases from two autosomal dominant Japanese PD families.
- Two other familial PD cases had different mutations: **Arg145Gln** and **300+5G>A** (in SH-SY5Y cells it caused exon 2 skipping).
- Two variants (**-9T>G & p.P2L**) were associated with sporadic PD.

A pilot study of *CHCHD2* in Canadian PD patients

- No mutations were found in 156 independent familial PD patients.

Scoop from all *CHCHD2* reports

	Funayama et al. 2015	Jansen et al. 2015	Puschmann et al. 2015	Liu et al. 2015 (Meta)	Foo et al. 2015
Mutation carriers	15	4	0	0	0
PD cases	857	8170	4	13708	809
AD cases	-	-	-	25580	-
Ethnicity	Japan	European	Sweden	European	Chinese
Thr61Ile	13	0	0	-	0
Arg145Gln	1	0	0	-	0
300+5G>A	1	0	0	-	0
rs10043	p=0·0004	-	-	-	-
Rs142444896 (p.P2L)	P=0.002	P>0.05	-	-	P=0.02
rs816411	P=0.22	-	-	P>0.05	-
Other variants	-	Ala32Thr Pro34Leu	-	-	-

Conclusion:

- ***CHCHD2* mutations are mainly play a role in Japanese population.**
- **The support for pathogenic nature of the reported mutations is critical in the utility of genetic screening in patient care.**

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