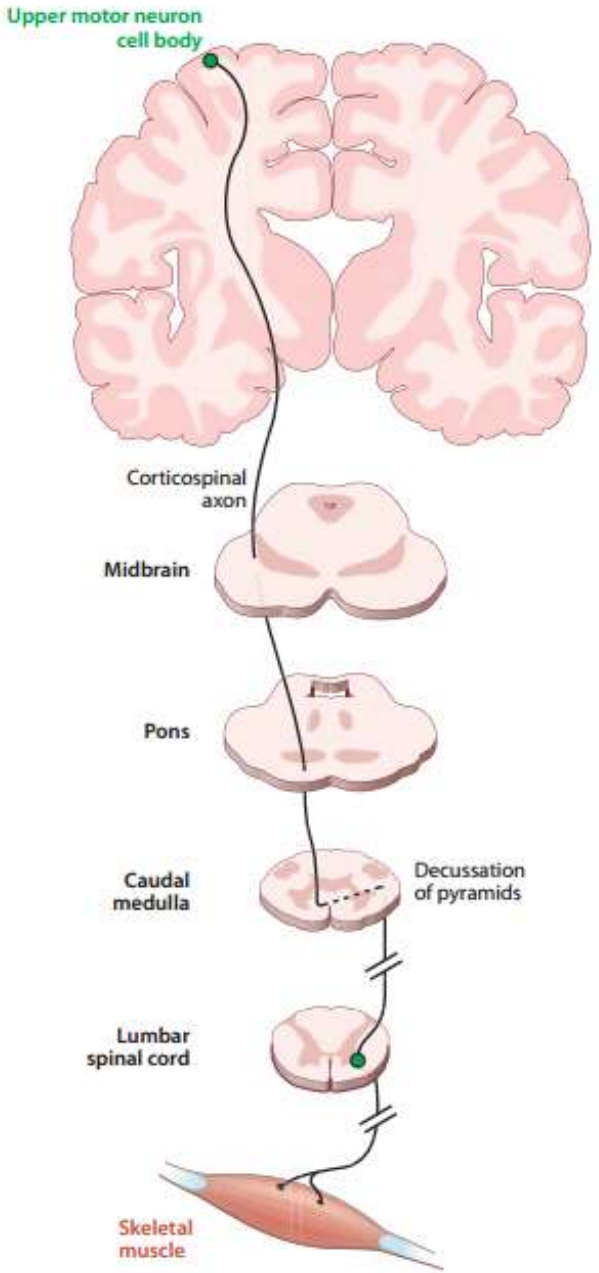
Euro HSP Annual Meeting
17/6/2017

Hereditary Spastic Paraplegia: **the complex form SPG9**

Emanuele Panza, PhD

Medical Genetics Unit
Department of Medical and Surgical Sciences
University of Bologna



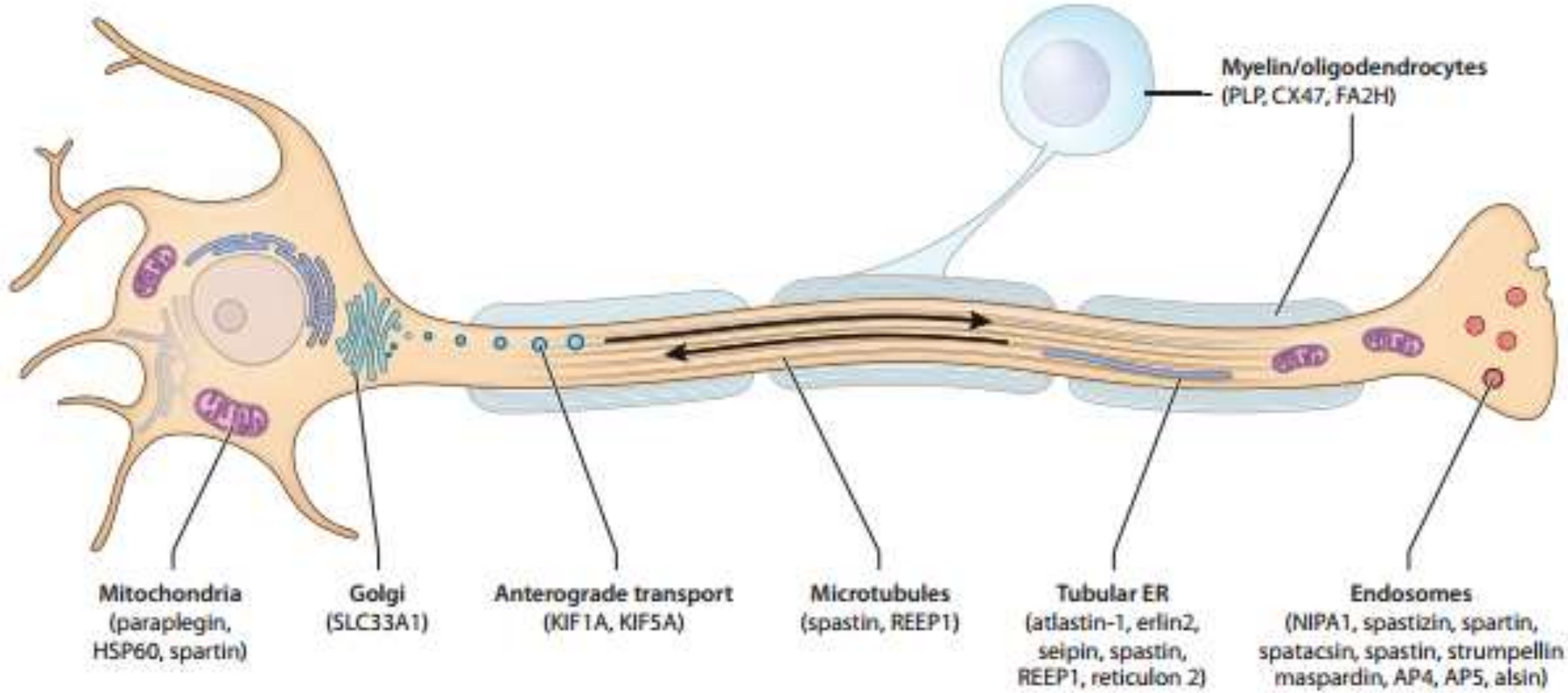
COMMON FEATURES OF HEREDITARY SPASTIC PARAPLEGIA

- Hereditary neurodegeneration disease
- Clinically and genetically heterogeneous group of disease
- Main neurological signs: spasticity and wakness of the lower limbs
- 80 forms reported (Mendelian inheritance and non)
- Common pathological feature:

  retrograde distal axonopathy of the long discending motor fibers
- Several intracellular pathogenetic mechanisms
- No therapies available, only symptomatic treatments

Blackstone et al., Ann Rev Neurosci 2012

Common Pathogenetics Themes in HSPs



Blackstone, Annu. Rev. Neurosci. 2012

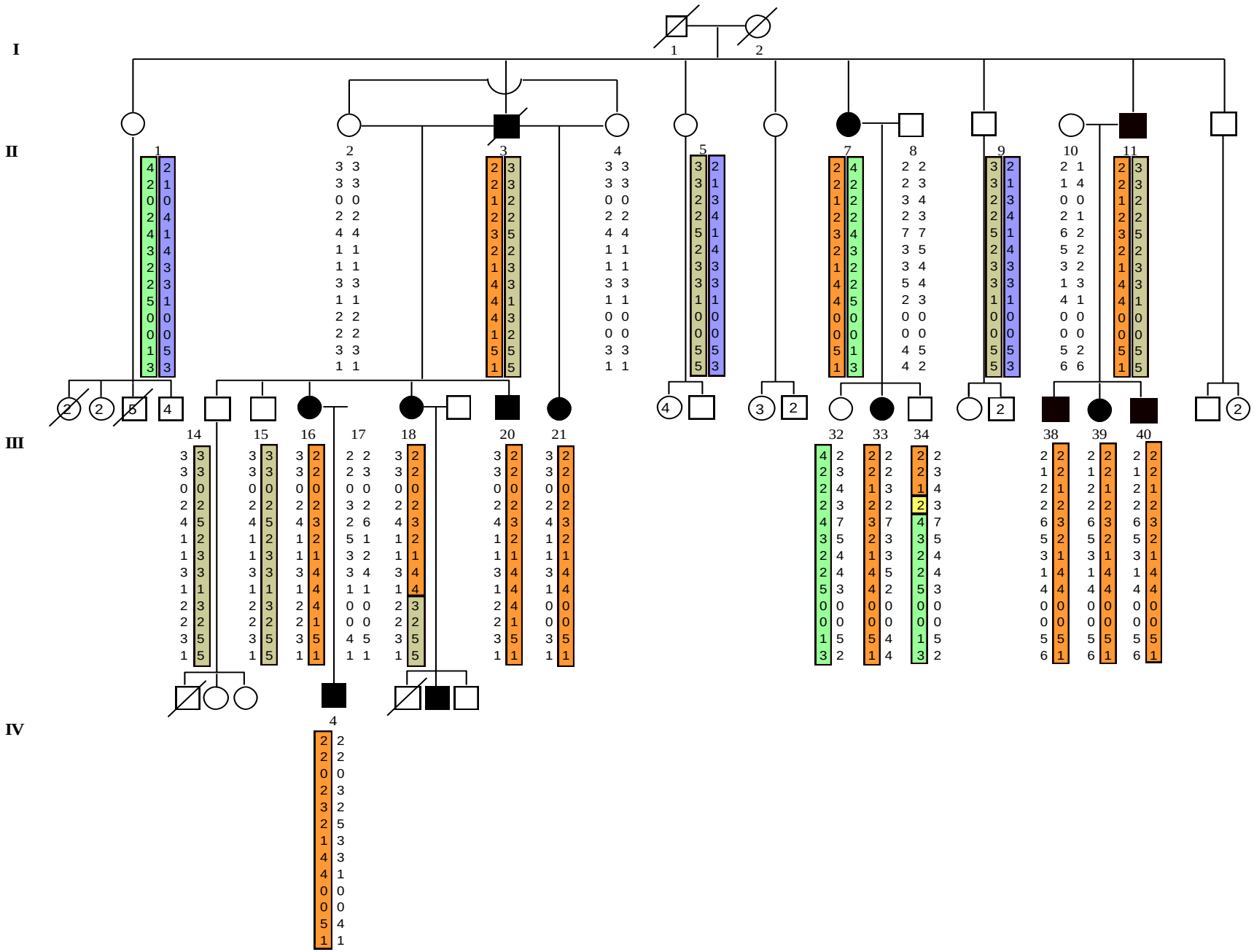
Genetic Mapping to 10q23.3-q24.2, in a Large Italian Pedigree, of a New Syndrome Showing Bilateral Cataracts, Gastroesophageal Reflux, and Spastic Paraparesis with Amyotrophy

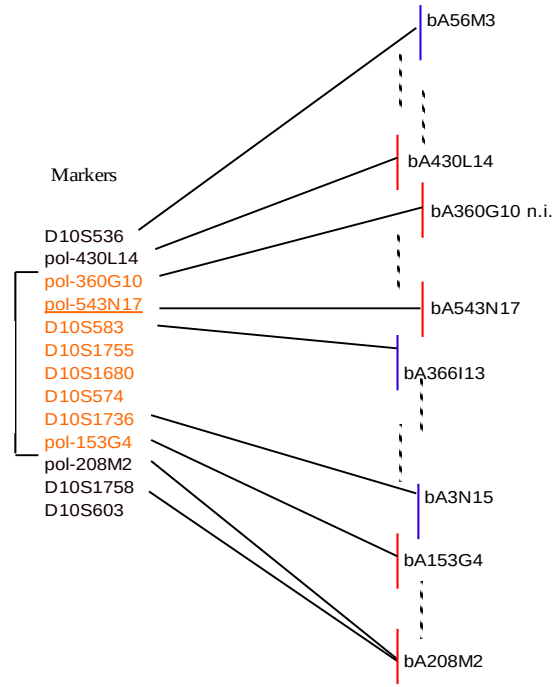
Am. J. Hum. Genet. 64:586-593, Seri Marco et al.,1999.

Main Clinical Features:

- spastic paraplegia with incomplete dominance and/or variable expressivity
- early bilateral cataracts
- persistent vomiting
- autosomal dominant inheritance
- spastic paraplegia arises in the first and third decade of life

Redefinition of the SPG9 locus





SPG9 critical region
4.8 Mb

The SPG9 critical region contains 52 genes

*138250

ALDEHYDE DEHYDROGENASE 18 FAMILY, MEMBER A1; ALDH18A1

Alternative titles: symbols

1-PYRROLINE-5-CARBOXYLATE SYNTHETASE; PYCS

P5CS

GLUTAMATE GAMMA-SEMIALDEHYDE SYNTHETASE; GSAS

HGNC Approved Gene Symbol: ALDH18A1

Cytogenetic location: 10q24.1 ***Genomic coordinates (GRCh38): 10:95,605,928-95,656,809*** (from NCBI)

*604619

LEUCINE-RICH GENE, GLIOM

Alternative titles: symbols

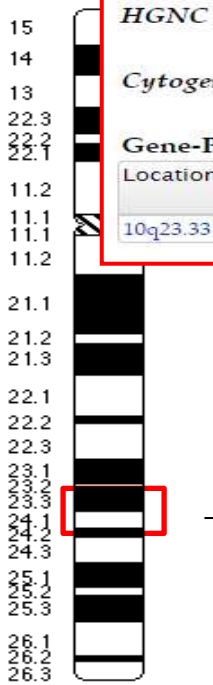
EPITEMPIN; EPTP

HGNC Approved Gene Symbol: LGI1

Cytogenetic location: 10q23.33 ***Genomic coordinates (GRCh38): 10:93,757,769-93,798,173*** (from NCBI)

Gene-Phenotype Relationships

Location	Phenotype	Phenotype MIM number	Inheritance (in progress)	Phenotype mapping key
10q23.33	Epilepsy, familial temporal lobe, 1	600512	AD	3



LOC387703
FER1L3
C10orf3
GPR120
RBP4
PDE6C
C10orf4
LGI1
TMEM20
PSMD4P2
PLCE1
C10orf117
TBC1D12
HELLS
LOC441573
CYP2C18

C10orf129
PDLIM1
SORBS1
LOC439999
PYCS
C10orf61
ENTPD1
FLJ34077
LOC387706
C10orf130
CCNJ
ZNF518
LOC399804
BLNK
DNTT
TMEM10
TLL2
SMBP
PIK3AP1
LOC283340
LOC387709
MLR2

NCBI Build 36.2

© 2000 Oxford University Press
Human Molecular Genetics, 2000, Vol. 9, No. 19 2853–2858

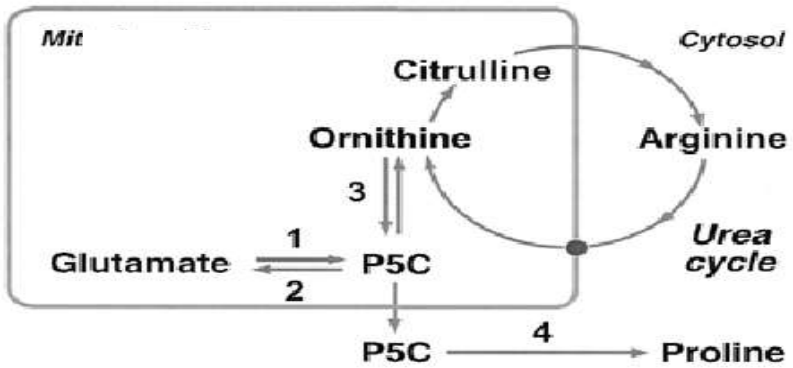
Hyperammonemia with reduced ornithine, citrulline, arginine and proline: a new inborn error caused by a mutation in the gene encoding Δ^1-pyrroline-5-carboxylate synthase

Matthias R. Baumgartner[1], Chien-an A. Hu[1], Shlomo Almashanu[2], Gary Steel[2], Cassandra Obie[2], Bernard Aral[3], Daniel Rabier[3], Pierre Kamoun[3], Jean-Marie Saudubray[3] and David Valle[1,2,+]

OMIM 138250

iperammonemia, ipoornitinemia, ipocitrullinemia, ipoarginemia, ipoprolinemia

Deficit Δ^1-pyrrolin-5-carbossilato-sintasi



Eur J Pediatr (2005) 164: 31–36
DOI 10.1007/s00431-004-1545-3

ORIGINAL PAPER

Matthias R. Baumgartner · Daniel Rabier
Marie-Cécile Nassogne · Jean-Louis Dufier
Jean-Paul Padovani · Pierre Kamoun · David Valle
Jean-Marie Saudubray

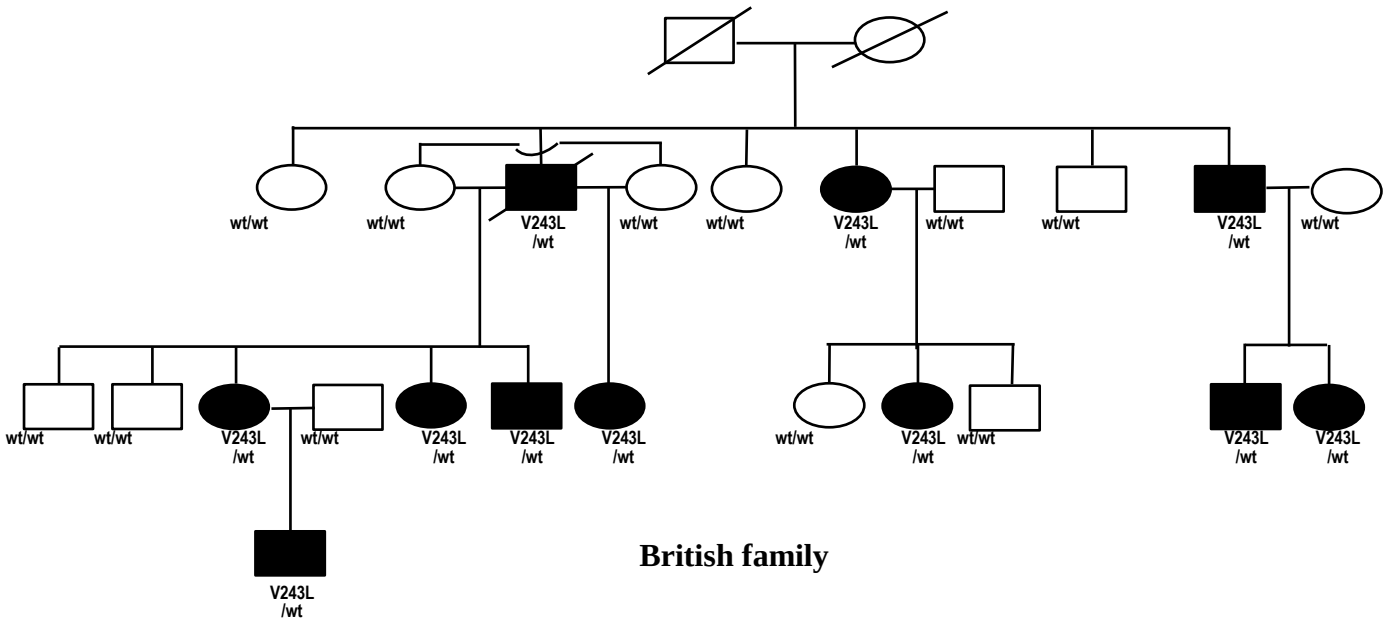
Δ^1-pyrroline-5-carboxylate synthase deficiency: neurodegeneration, cataracts and connective tissue manifestations combined with hyperammonaemia and reduced ornithine, citrulline, arginine and proline

SPG9		Deficit Δ^1-pyrrolin-5-carbossilato-sintetasi
Persistent vomiting, gastroesophageal reflux	.....	Chronic vomiting (due to gastro-esophageal reflux)
Skeletal abnormalities, Short stature	.....	Joint laxity resulting in severe pes planus and dislocated hips. Slight dysmorphic features (short neck long fingers and toes) Hyperelasticity of the skin
Learning disabilities	.....	Mental deterioration and abnormal behaviour
Bilateral cataracts	.....	Bilateral zonular cataracts
Amyotrophy	.....	Severe hypotonia, muscular wasting of the limbs,dystonia of the hands and feet
Motor system disorder Spastic Paraparesis	...	Pyramidal syndrome and peripheral, predominantly axonal, neuropathy

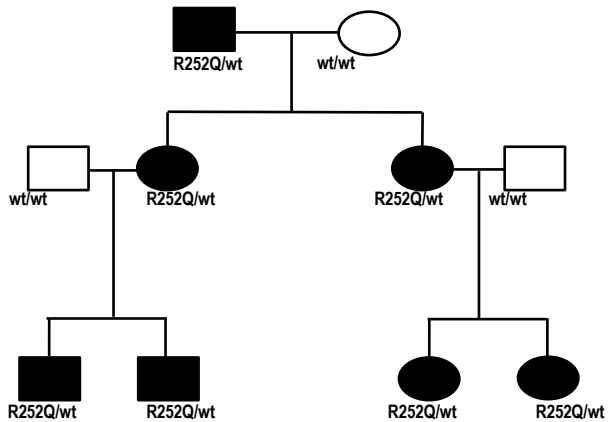
Sanger sequencing and NextGen sequencing confirm the presence of the mutation p.V243L in the Italian family and the mutation p.R252Q in a second English family.

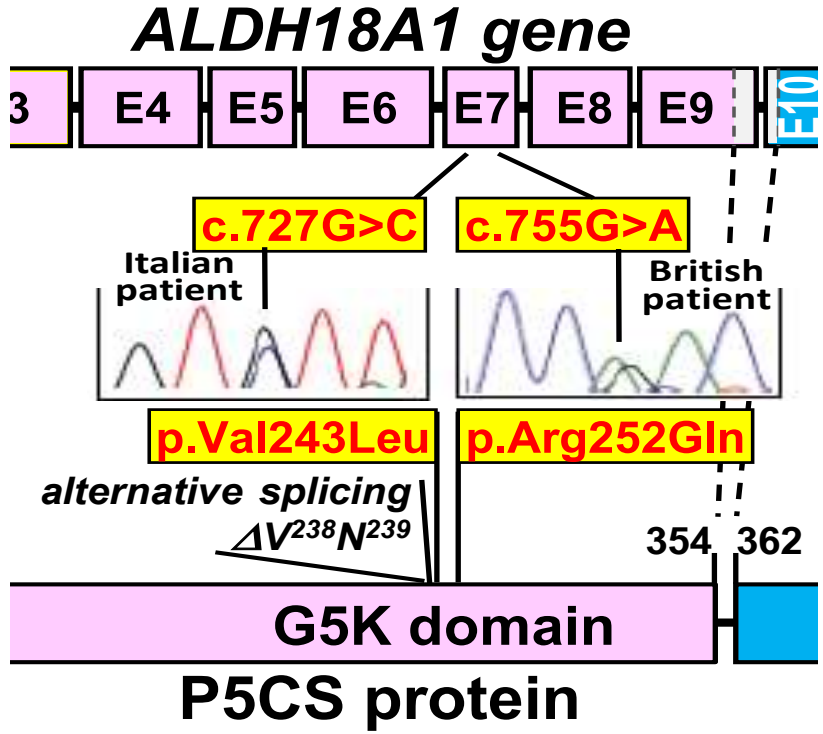
The mutations segregate only in affected patients of the families and are not present in a panel of 466 chromosomes Geographically matched, or in polymorphisms databases (dbSNPs, 1000 Genome Project, Exac).

Italian family

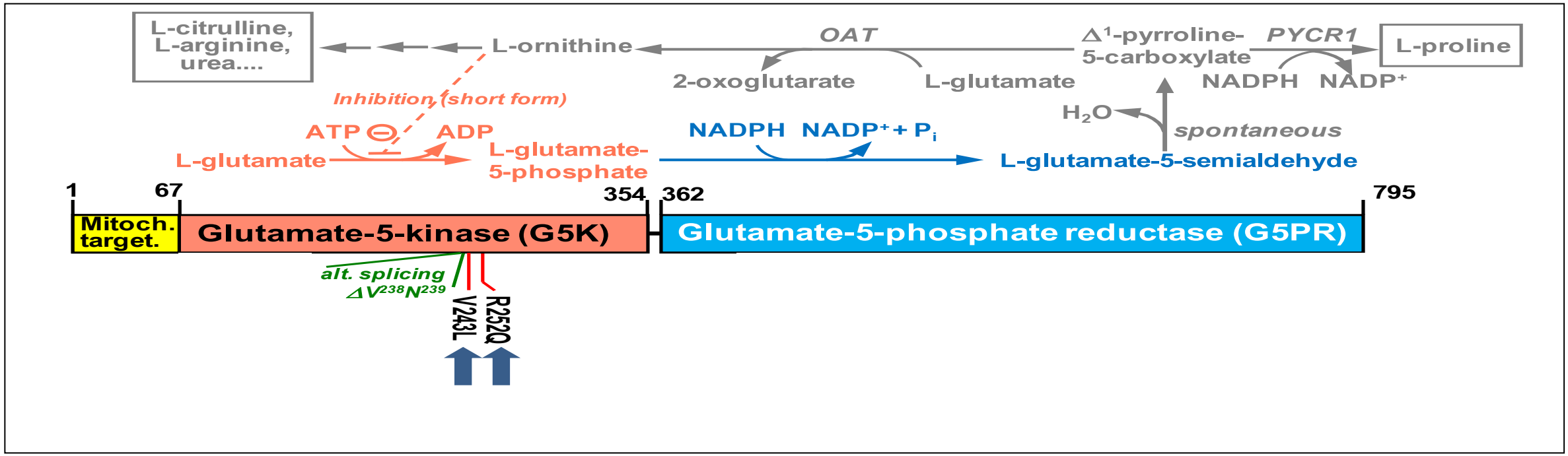


British family

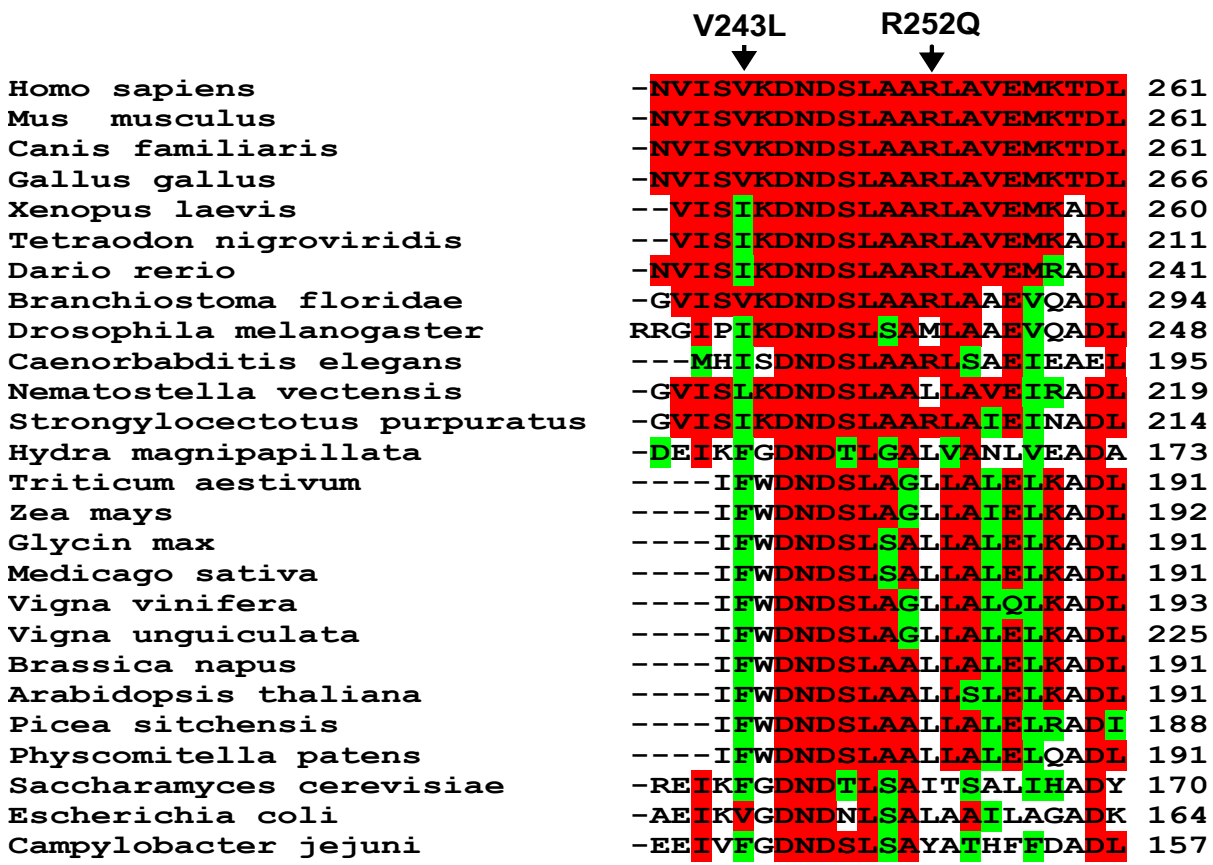




P5CS is a byfunctional enzyme catalyzing the first two steps of de novo syntheisis of proline, ornithine, citrulline and arginine



Protein sequence of the mutated region in comparison with the same region in other species



Mutations in SPG9 cause «Loss of Function»

Plasma levels of selected aminoacids in SPG9 patients

Table 1 Plasma levels of selected amino acids in SPG9 patients

Analyte	Levels in patient and corresponding reference range (in parenthesis) (μmol/l)					
	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Ornithine	72 (41–119)	50 (41–119)	63[a] (63–133)	63[a](63–133)	37[b] (63–133)	49[b] (63–133)
Citrulline	17[a] (17–53)	13[b] (17–53)	20 (15–57)	21 (15–57)	14[b] (15–57)	8[b] (15–57)
Arginine	87 (38–135)	81 (38–135)	101 (36–172)	139 (36–172)	131 (36–172)	80 (36–172)
Proline	186 (117–332)	150 (117–332)	144 (50–350)	215 (50–350)	199 (50–350)	160 (50–350)
Hydroxyproline	5[b] (16–53)	14[b] (16–53)	12 (0–50)	12 (0–50)	15 (0–50)	Not determined

Patients belong to the Italian pedigree and were heterozygotes for the mutation V243L.

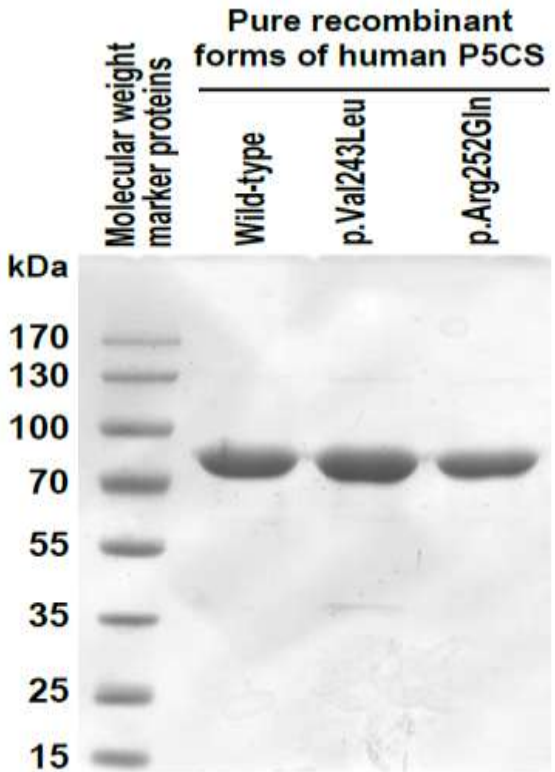
[a]Levels are at the lower limit of the reference range.

[b]Levels are below the reference range.

da Panza et al., Brain 2016

Mutations in SPG9 cause «Loss of Function»

Mutations in the Human recombinant protein P5CS abolish the activity of the mutated G5K domain



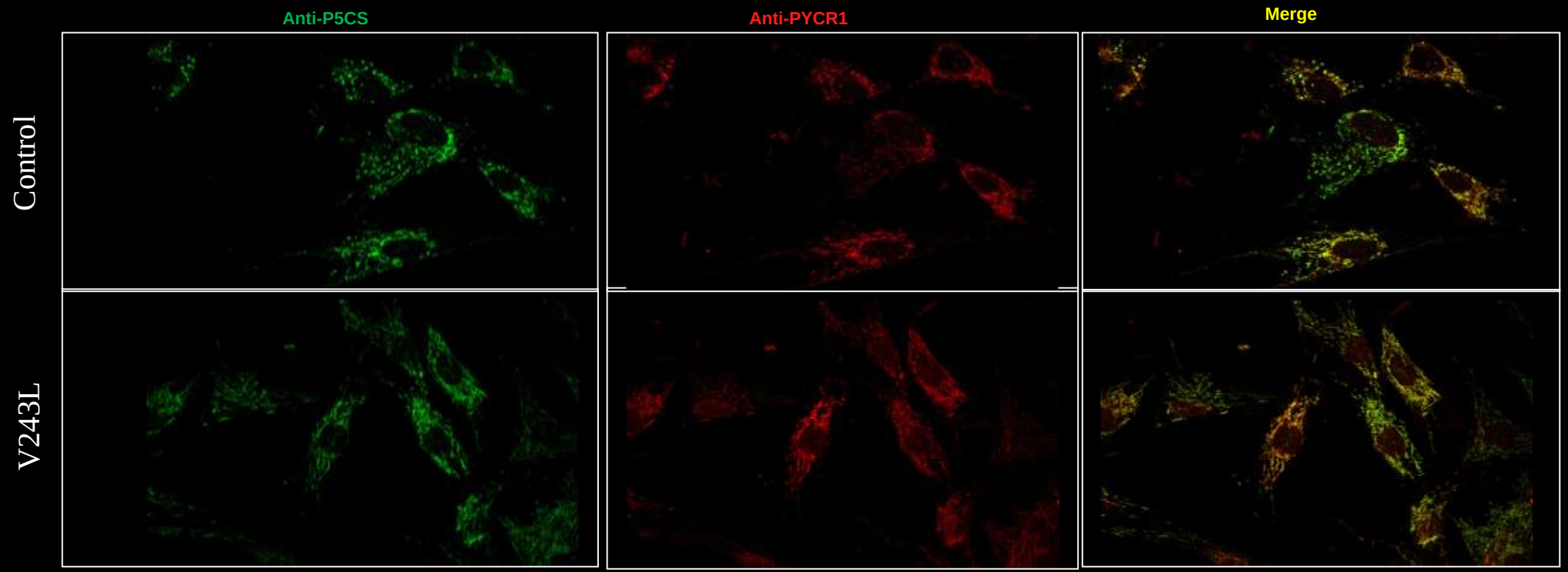
Enzyme activities of purified recombinant P5CS wild-type and mutant forms

Assay of	Activity U/ mg (% of wild-type)		
	Wild-type	p.Val243Leu	p.Arg252Gln
Total P5CS activity	2.11±0.11 (100)	< 0,07 (< 3.5%)	< 0,07 (< 3.5%)
Partial activities			
G5K	18.3±1.2 (100)	0.018±0.001 (0.1%)	0.027±0.003 (0.15%)
G5PR	2.55±0.07 (100)	1.78±0.01 (70%)	1.94±0.02 (76%)

For details on the assays, see Panza et al. (2016) Brain 139:e3

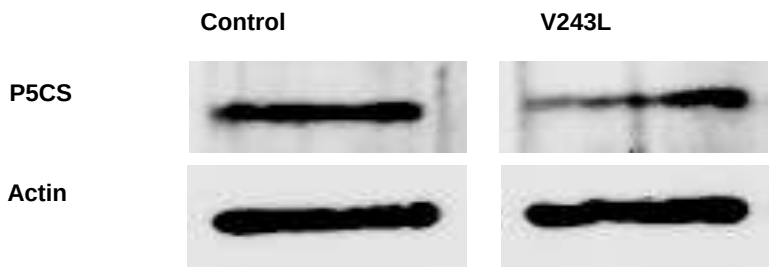
da Panza et al., Brain 2016

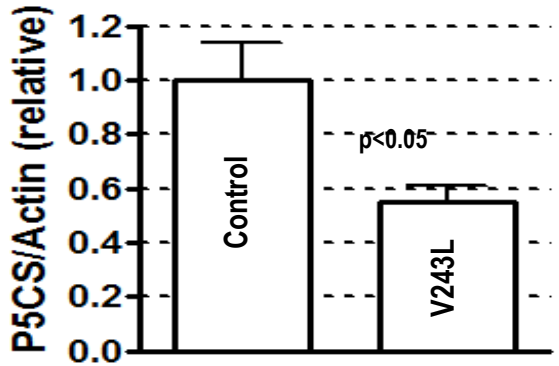
Immunofluorescence:
Patient’s fibroblasts in a patient of the Italian family bearing the p.V243L mutation



Mutant cDNAs are expressed with a pattern similar to the Wild type
and are not associated with abnormalities in the localization nor a premature degradation

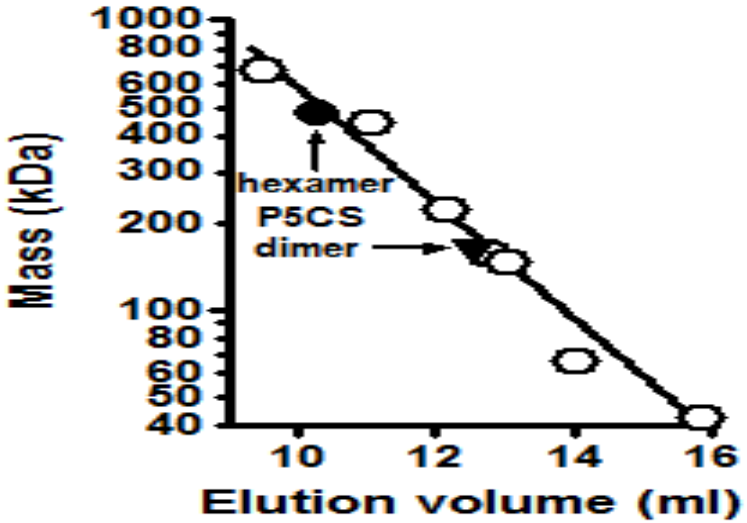
Western blot on patient’s fibroblasts and controls show a roughly 45% reduction of P5CS in p.V243L cells

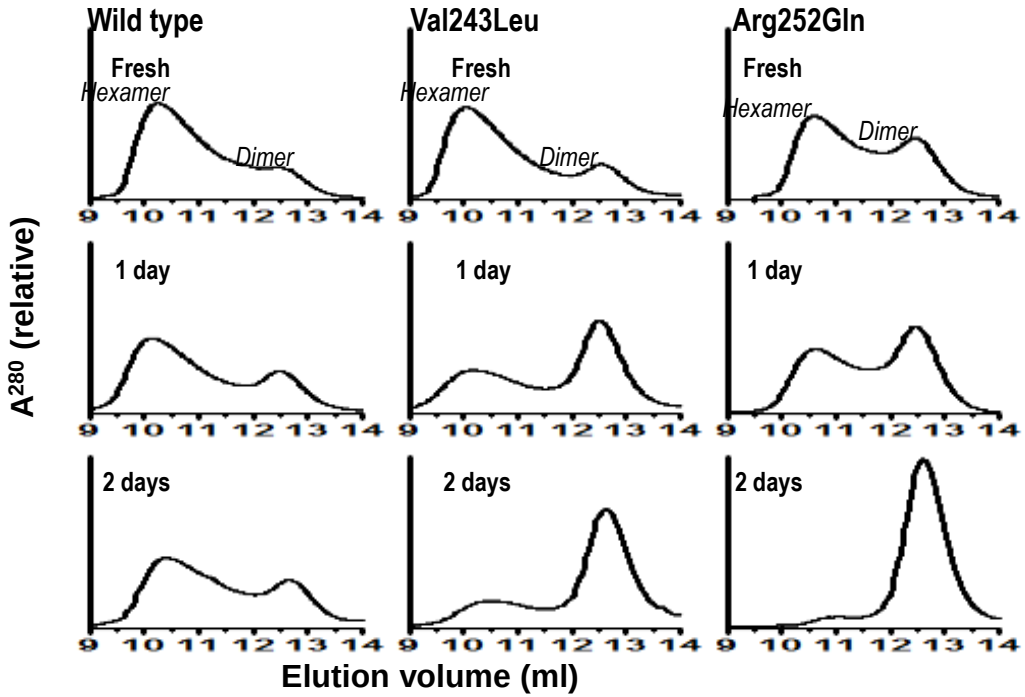




da Panza et al., Brain 2016

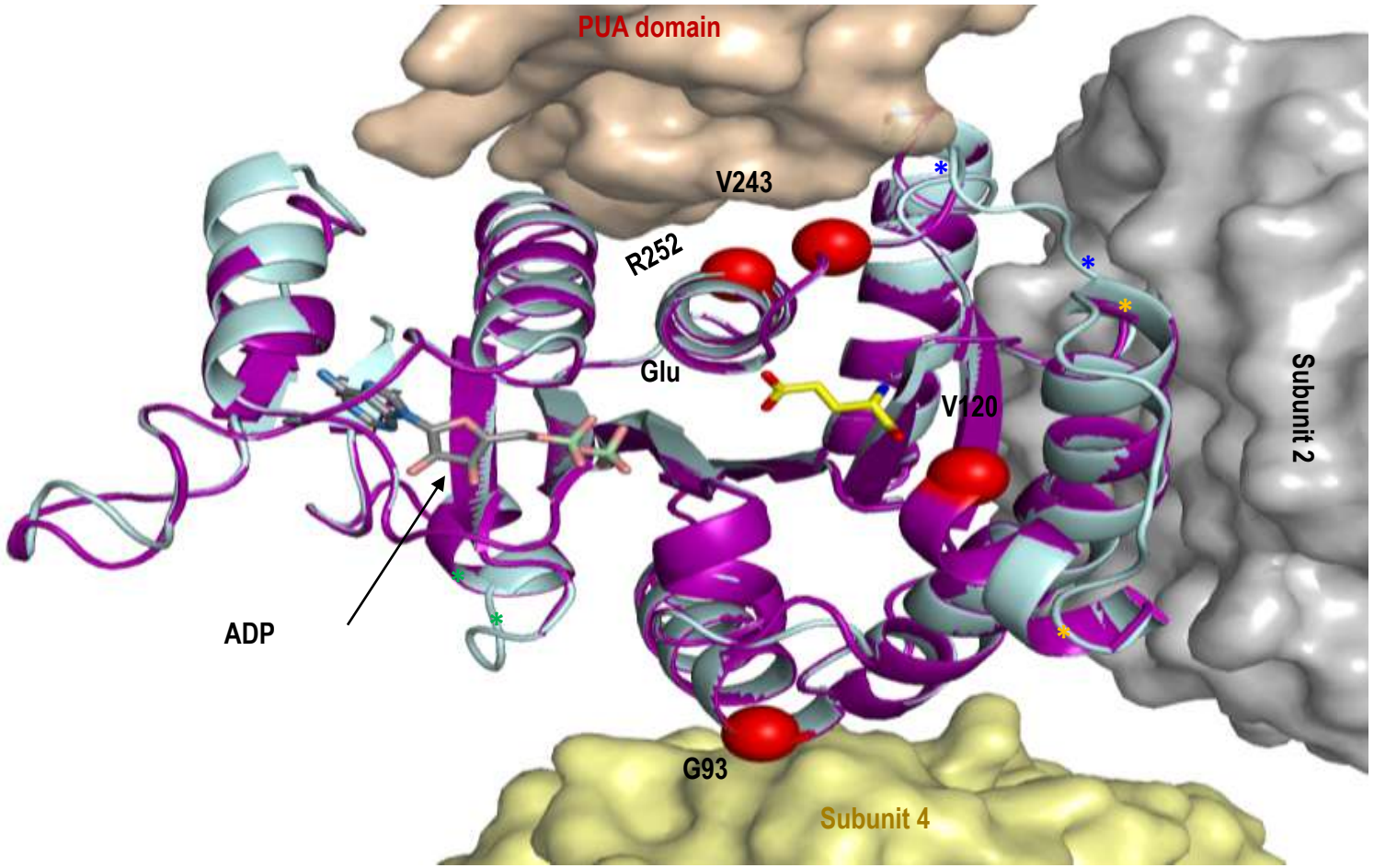
Why a dominant inheritance for «Loss of Function» mutations?





da Panza et al., Brain 2016

Why dominant and recessive mutations exist?



da Panza et al., Brain 2016

Recessive and dominant mutations in *ALDH18A1* have been identified in forms of HSP and of cutis laxa

ARCL3A OMIM 219150

ELSEVIER

Molecular Genetics and Metabolism

Volume 112, Issue 4, August 2014, Pages 310–316

Severe congenital cutis laxa with cardiovascular manifestations due to homozygous deletions in *ALDH18A1*

Björn Fischer[a, b], Bert Callewaert[c], Phillipe Schröter[a], Paul J. Coucke[c], Claire Schlack[a], Claus-Eric Ott[a, b], Manrico Morroni[d], Wolfgang Homann[e], Stefan Mundlos[a, b, f], Eva Morava[g], Anna Ficcadenti[h], Uwe Kornak[a, b, f]

ADCL3 OMIM 616603

AJHG

Cell PRESS

Volume 97, Issue 3, 3 September 2015, Pages 483–492

Report

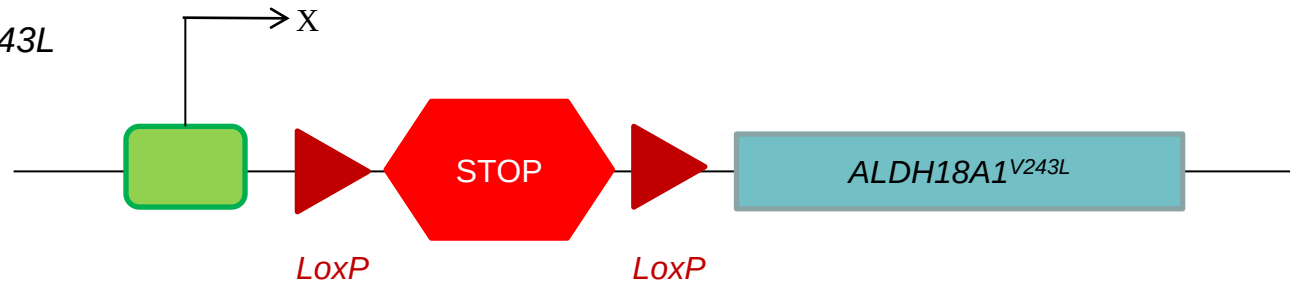
Recurrent De Novo Mutations Affecting Residue Arg138 of Pyrroline-5-Carboxylate Synthase Cause a Progeroid Form of Autosomal-Dominant Cutis Laxa

Björn Fischer-Zirnsak[1, 2, 25], Nathalie Escande-Beillard[3, 25], Jaya Ganesh[4], Yu Xuan Tan[3], Mohammed Al Bughaili[1], Angela E. Lin[5], Inderneel Sahai[5], Paulina Bahena[6], Sara L. Reichert[4], Abigail Loh[7], Graham D. Wright[3], Jaron Liu[3], Elisa Rahikkala[8], Eniko K. Pivnick[9], Asim F. Choudhri[10, 11, 12, 13], Ulrike Krüger[1], Tomasz Zemojtel[1, 14], Conny van Ravenswaaij-Arts[15], Roya Mostafavi[9], Irene Stolte-Dijkstra[15], Sofie Symoens[16], Leila Pajunen[8], Lihadh Al-Gazali[17], David Meierhofer[18], Peter N. Robinson[1, 2, 19],

Towards the generation of a mouse model for «*ALDH18A1-related* disease»

- To investigate the pathogenetic mechanism of this disease
- To test new therapies

Rosa26 ALDH18A1^{V243L}



Generated in Mario Capecchi Laboratory

SPF SPASTIC PARAPLEGIA FOUNDATION, INC.
Research / Education / Support

Summary

SPG9 is due to the non-synonimous single nucleotide changes c.727G>C or c.755G>A in exon 7 of the ALDH18A1 gene that affect proximate amino acids of the G5K domain (p.Val243Leu and p.Arg252Gln, respectively).

These mutations do not prevent production nor cause cellular mislocalization of P5CS.

They are loss-of function mutations as evidenced by plasma amino acid analysis and by enzyme activity studies in recombinantly produced human P5CS.

They selectively inactivate the domain where they map (G5K).

P5CS is a high oligomer (possibly an hexameric trimer of dimers).

SPG9 mutations disturb the architecture of the P5CS oligomer, making it prone to dissociate to dimers.

In silico structural analysis suggests that P5CS mutations can be dominant or recessive depending on whether they affect or not residues involved in intersubunit or interdomain interactions, disturbing or not disturbing the architecture of the oligomer, thus supporting a dominant negative disease-causing mechanism

Recessive and dominant mutations have been identified in HSP (SPG9A MIM601162, SPG9B MIM616586) and in forms of cutis laxa (ADCL3 MIM616603, ARCL3A MIM219150)

The generation of a mouse model will be essential to dissect the pathogenetic mechanisms of SPG9 and to test new therapies

Ackwnoledgments

Professor Marco Seri
Unità di Genetica Medica
Azienda Universitaria Ospedaliera di Bologna

Professor Vicente Rubio Zamora
Instituto de Biomedicina de Valencia of the CSIC, Valencia, Spain Group 739
Centro para Investigacio´n Biome´dica en Red sobre Enfermedades Raras
CIBERER-ISCIII, Valencia-Spain

Professor Rocco Liguori
IRCCS Istituto delle Scienze Neurologiche di Bologna
Department of Biomedical and NeuroMotor Sciences, University of Bologna

Professor Giuseppe de Michele
Department of Neurosciences and Reproductive and Odontostomatologic Sciences
University Federico II - Napoli

Professor Jane Hurst
Department of Clinical Genetics, Great Ormond Street Hospital, London, UK.

Professor Mario Capecchi
Howard Hughes Medical Institute, University of Utah School of Medicine
Department of Human Genetics
Salt Lake City Utah, USA

SPF SPASTIC PARAPLEGIA FOUNDATION, INC.
Research / Education / Support