Hereditary Cerebral Small Vessel Diseases

Main clinical, neuroimaging and genetic features

E. Tournier-Lasserve

Genetics Lab, Hôpital Lariboisière

Inserm / Paris7 University Research Unit UMR-S1161

Paris, France

2015 Annual meeting of the American Association of Neuropathologists

Hereditary Cerebral Small Vessel Diseases

- An heterogeneous and rapidly expanding group of diseases
- Age at clinical onset: 0 - 80 years old !
- Stroke, epilepsy, vascular dementia, extra-neurological symptoms …
- Various inheritance patterns / Several SVD genes already identified
- Challenges and opportunities in the era of High Through Put genomics +++
- Gene identification benefits:
    - Diagnostic tools
    - Possibility to create animal models to explore mechanisms
    - Identification of signaling pathways and mechanisms +++
    - Understanding of non hereditary stroke and vascular dementia

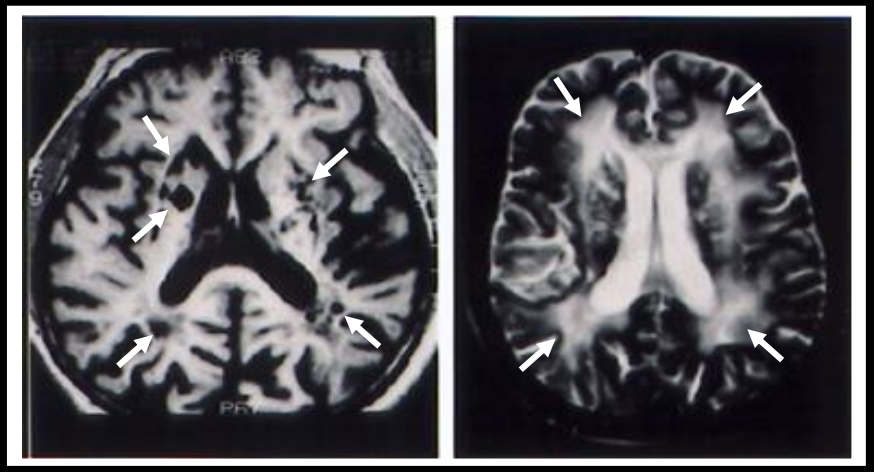
Adult Onset Hereditary SVD

- CADASIL: autosomal dominant (AD), NOTCH3
- CARASIL: autosomal recessive (AR), HTRA1
- HERNS / CRVL (AD), TREX1
- COL4A1/ COL4A2 angiopathies / (AD), COL4A1/COL4A2
- AD Cerebral Amyloid Angiopathies: APP / GSN / TTR / BRI

- SVD linked to Chr 20q13 (AD), gene ?
- PADMAL / Pontine Leukoencephalopathy (AD), gene ?
- LCC / Leucoencephalopathy Calcifications Cysts (AR), gene ?
- SVD reported in unrelated families : Swedish, French…, genes ?

CADASIL, Clinical and MRI Features

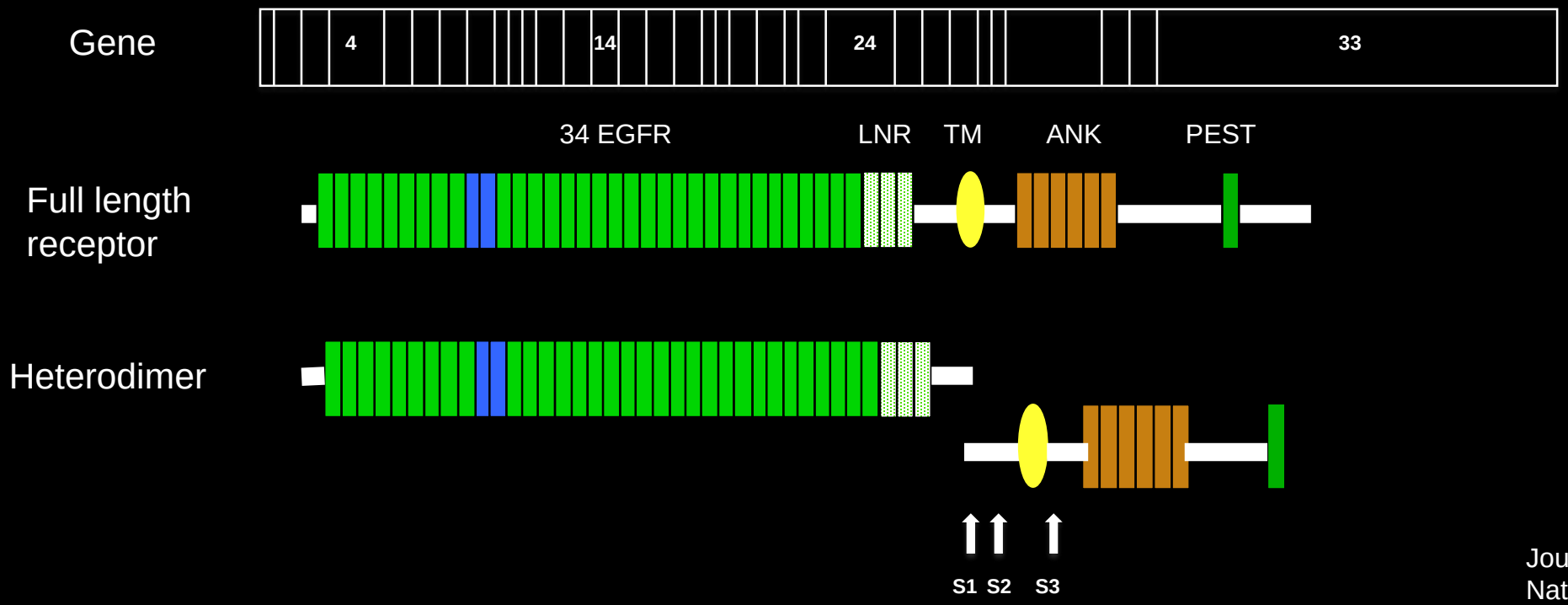
- Cerebral Autosomal Dominant Arteriopathy Subcordtical Infarcts & Leukoencephalopathy
- > 650 known families / The most common hereditary SVD
- Autosomal dominant
- Age at onset ~ 45 yo (long asymptomatic phase)
- Migraine with aura, recurrent lacunar infarcts, mood disorders, apathy, cognitive impairment > dementia > death ~ 65 yo
- MRI : diffuse leukoaraiosis and deep lacunar infarcts
- Specifc electronic microscopy marker in vascular smooth muscle cells : GOM



Tournier-Lasserve et al, ,Nature Genet 1993
Joutel et al, Nature 1996
Chabriat et al, Lancet 2009

NOTCH3, gene and protein

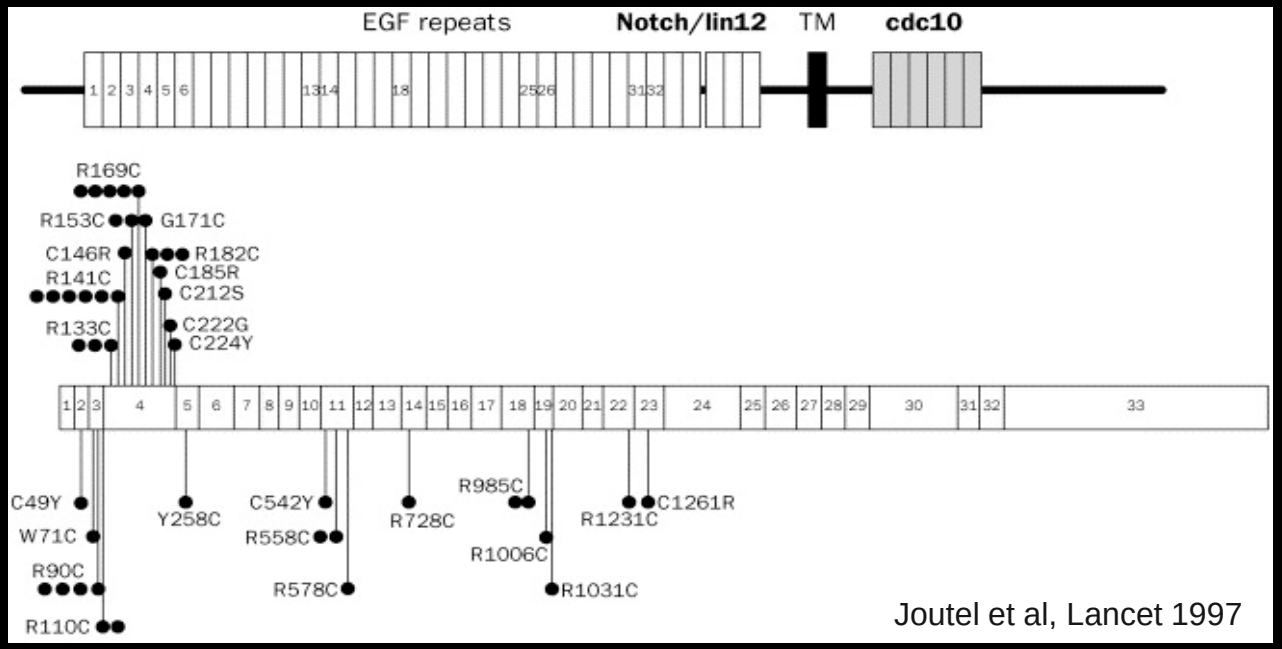
- NOTCH3 gene / 33 Exons encoding a 2321 aminoacids single pass transmembrane receptor
- Furin cleavage at S1 site
- Large extra cellular domain including 24 EGF-like motifs, each containing 6 cystein residues
- Membrane tethered intracellular domain



Joutel et al, Nature, 1996

NOTCH3 mutations in CADASIL

Highly stereotyped mutations leading to an odd number of cysteines



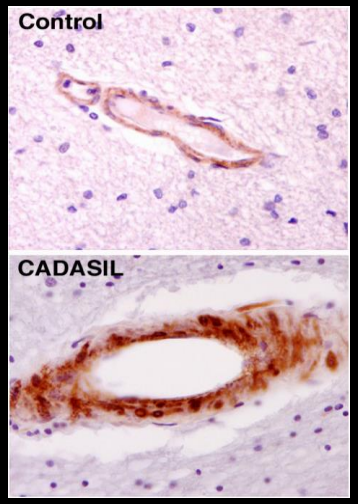
Joutel et al, Lancet 1997

Lancet 1997

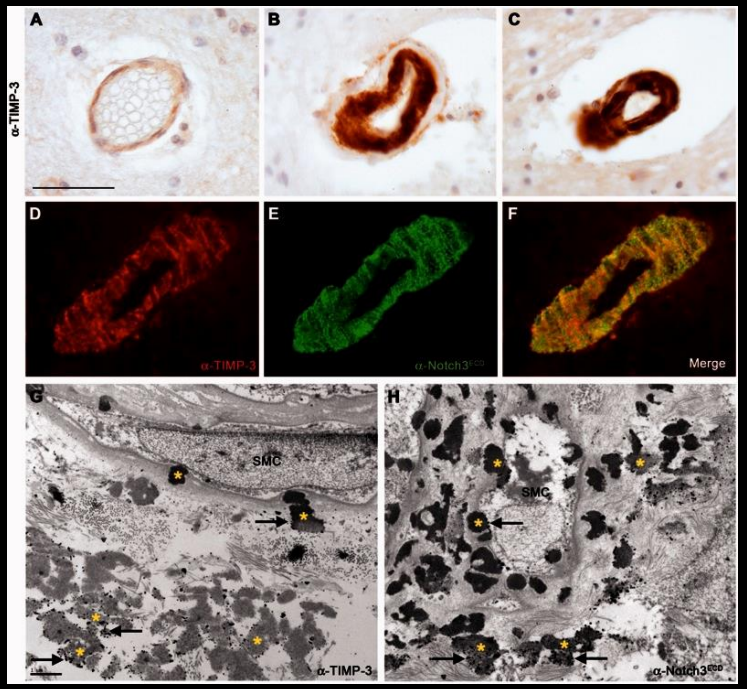
- Our group in 1997: 50 families either linked to ch19 or biopsy proven (GOM) / Mutation identified in 45 / in all cases: odd number of cystein residues
- Confirmation by Dichgans' group and Kalimo's group in > 120 biopsy proven patients for each group

CADASIL mechanisms

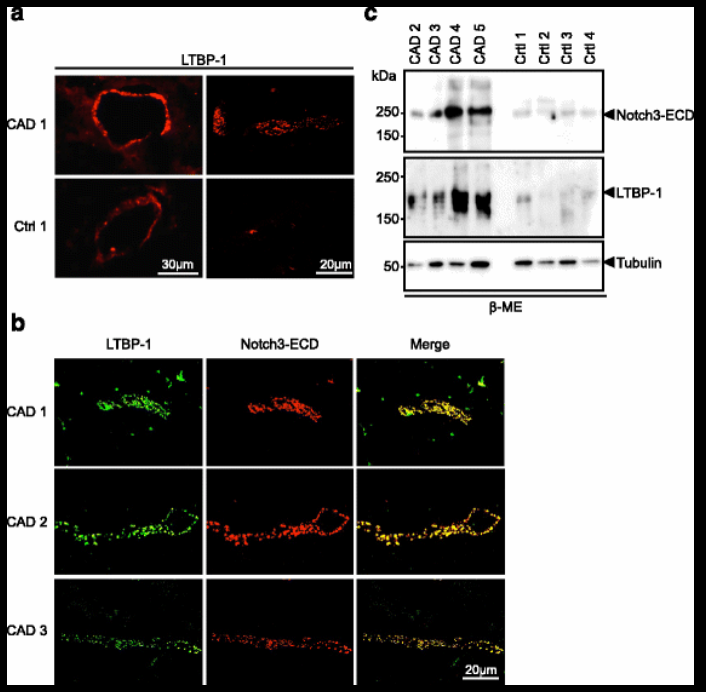
Abnormal aggregation of Notch3^{ECD} leading to a sequestration of ECM proteins



Notch3 ECD accumulation in VSMC



TIMP3 and Notch3 ECD are incorporated in GOM



LTBP1, a key regulator of TGF-beta availability within the ECM, co-fractionates with Notch3 ECD

- CADASILpatients & transgenic animal models tissues. Biochemical, proteomics, immunohisto approaches.
- Accumulation of Notch3^{ECD} , the starting point of pathological events leading to the abnormal recruitment and potential dysregulation of various functionally important ECM proteins leading to multifactorial toxicity.

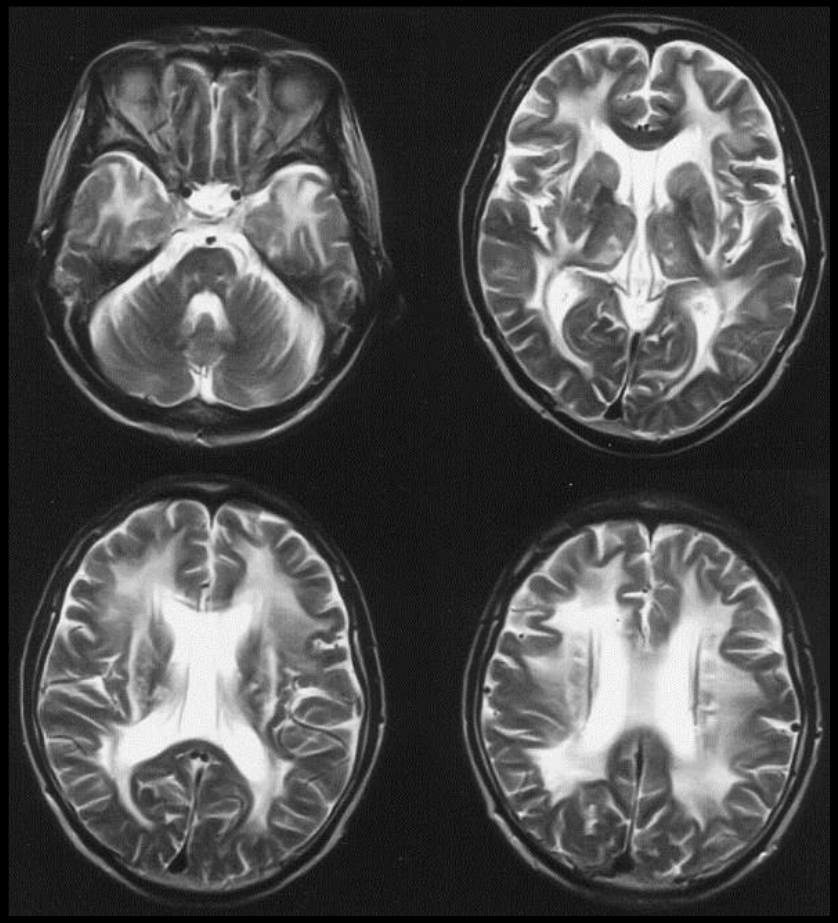
Monet-Lepretre et al, Brain 2013
Kast et al, Acta neuropath 2014

CARASIL, Clinical and MRI Features

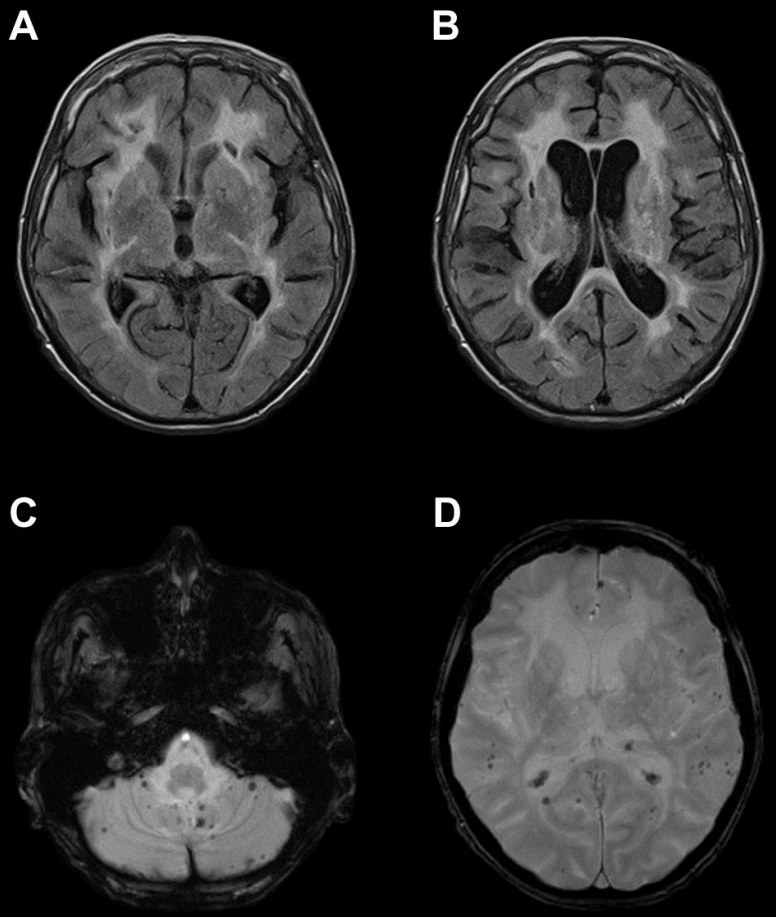
- Cerebral Autosomal Recessive Arteriopathy Subcordtical Infarcts Leukoencephalopathy
- Very rare SVD reported initially in Japan (Maeda et al, 1976)
- 2015: 13 mutated families so far, including 6 non Japanese families
- Autosomal recessive
- Early onset stroke (~ 32 yo) and early onset cognitive impairment (~ 33 yo)
- Very early onset alopecia and spondylosis deformans +++
- Severe disease leading to an early death
- MRI: deep lacunar infarcts + diffuse leukoencephalopathy. MRI phenotype very similar to CADASIL MRI phenotype

Hara et al, 2009
Nozaki et al, 2014

CARASIL / MRI Features

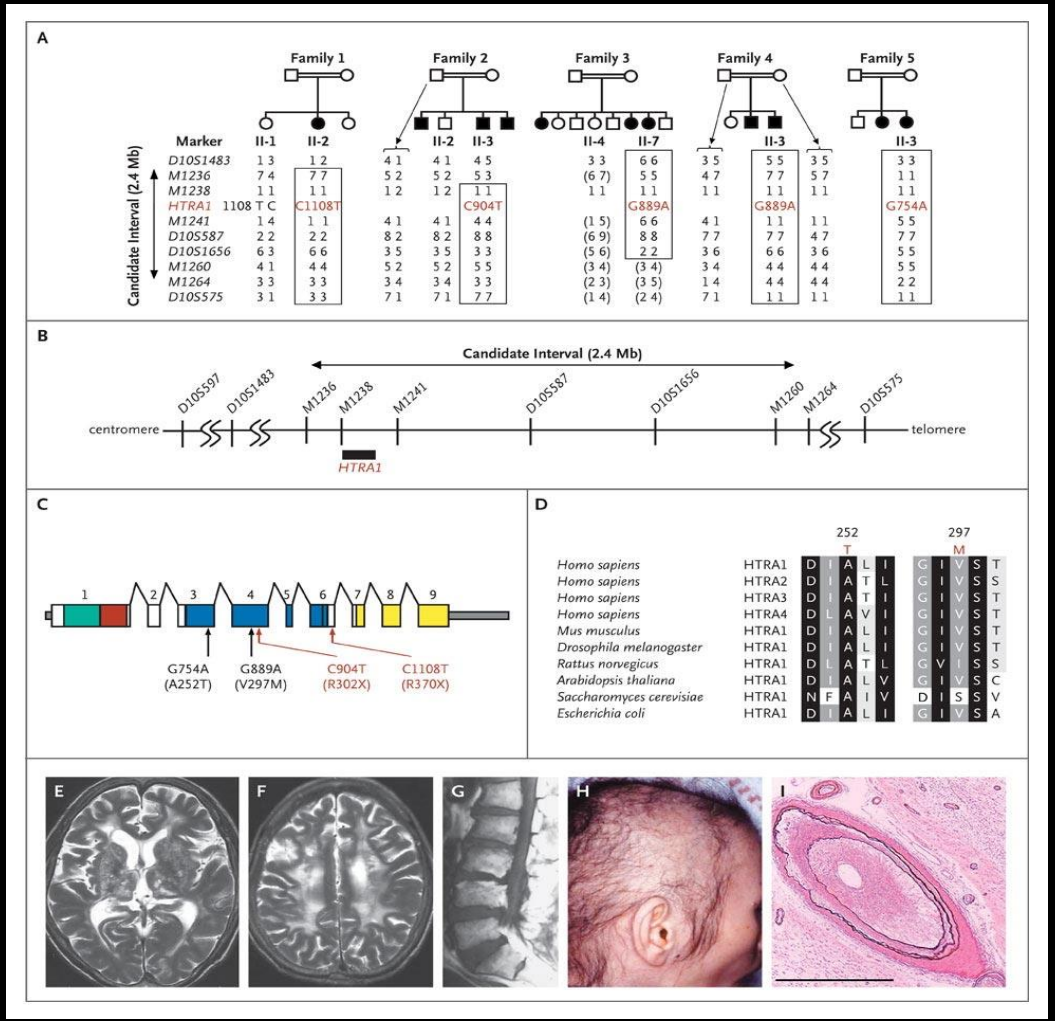


Brain T2-weighted MRI in in a ~40 yo CARASIL patient . Greatly increased signal intensity in an extensive area of the cerebral white matter in thalamus and pons.
Yanagawa et al, Neurology 2002



Unpublished CARASIL proven French female patient Flair and T2* images showing a diffuse vascular leukoencehalopathy and multiple microbleeds

HTRA1 Loss of function mutations in CARASIL



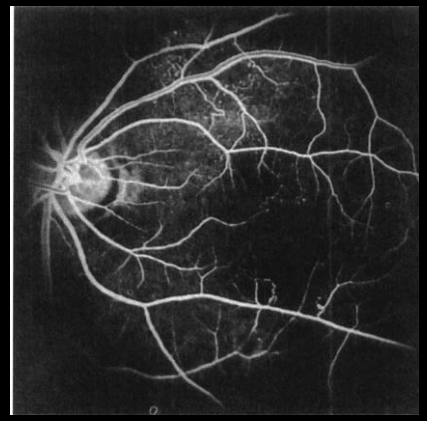
- HTRA1 encodes a serine protease
- Patients have biallelic loss of function mutations leading to loss of activity of this enzyme
- Loss of HTRA1 leads to increased TGF beta activity
- Mechanisms leading to this increased TGF beta activity yet to be deciphered
- However, recent data suggesting that HTRA1 may facilitate TGF beta activity
- Additional work to be done on HTRA1 within brain vessels

Hara K et al. NEJM 2009
Shiga et al, HMG 2011
Beaufort et al, 2015

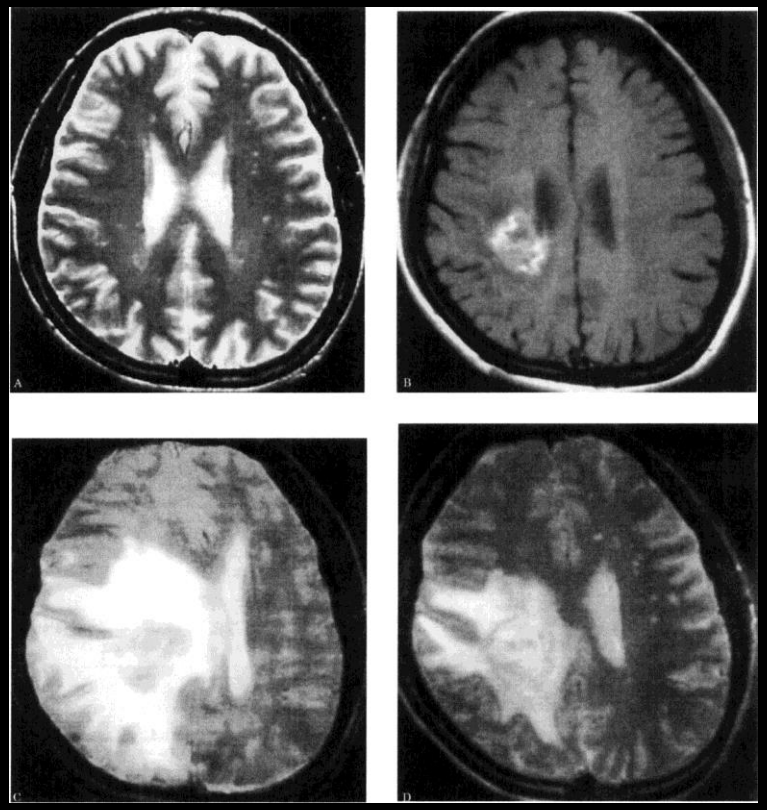
HERNS / CRVL

Hereditary Endotheliopathy with Retinopathy, Nephropathy & Stroke
Cerebro Retinal Vasculopathy & Leukodystrophy

- Very rare autosomal dominant SVD
- Systemic SVD: eye, kidney, brain
- Age of onset: 30-40 yo
- Visual loss
- Migraine like headaches, stroke, neurocognitive impairment
- Early death



- Macular edema, capillary drop out
- Telangiectatic capillaries, shunts

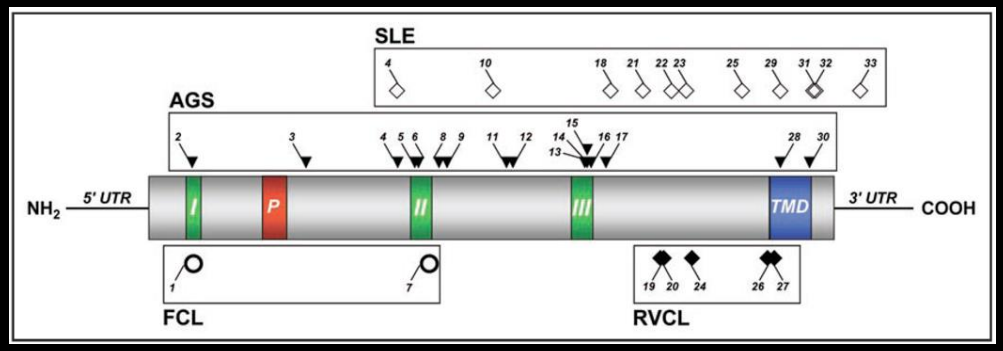


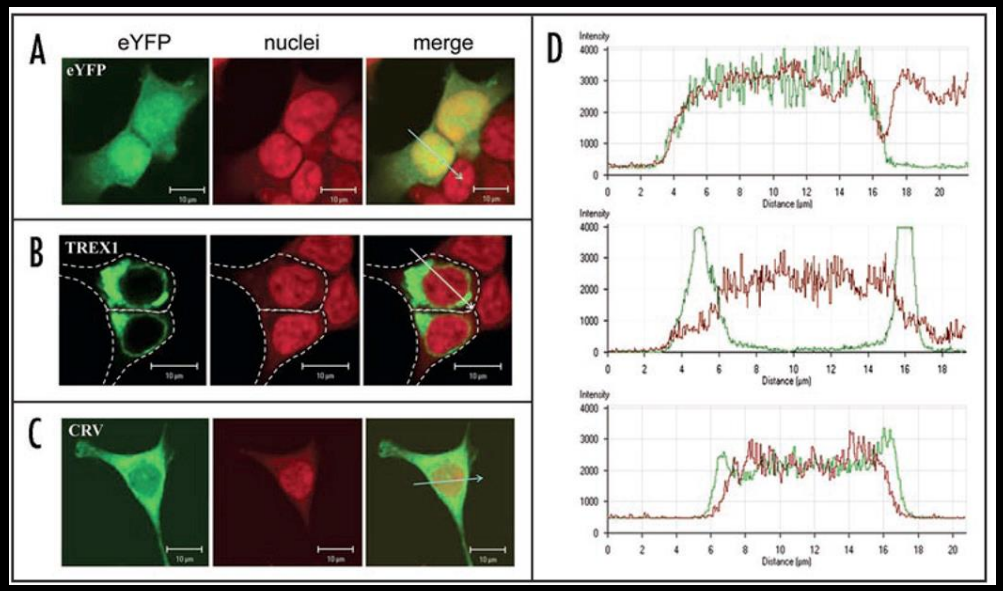
- T2 hypersignals
- Contrast enhancing lesions / oedema

Grand et al 1988, ,Jen et al, 1997
Richards et al, 2007

HERNS / CRVL

Heterozygous mutations in the TREX1 exonuclease

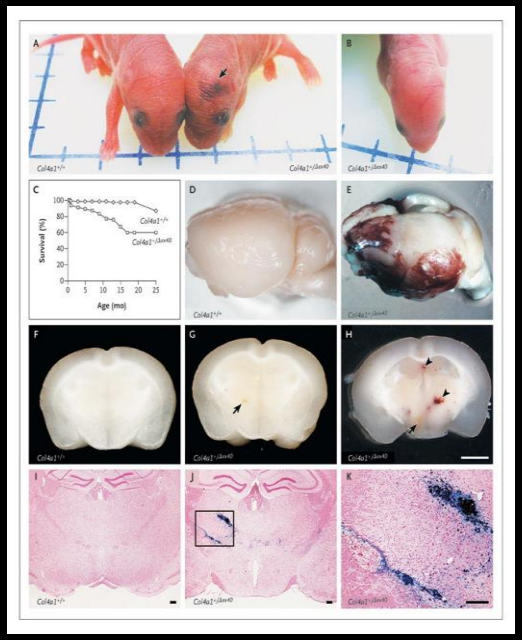




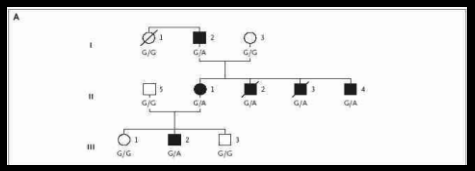
- TREX1 encodes a 3'-5' exonuclease
- Stop codons in the TREX1 gene
- Mutations located in the C-terminal region
- CRVL mutations modify Trex1 protein intra-cellular localisation
- This gene is mutated in at least 3 other diseases: Aicardi-Goutières/AGS (AR), Chilblain lupus (AD) and Systemic Lupus
- In AGS & Chilblain lupus: loss of activity
- Role of Trex1 in immune control
- In HERNS / RCVL, mechanisms of vascular lesions ?

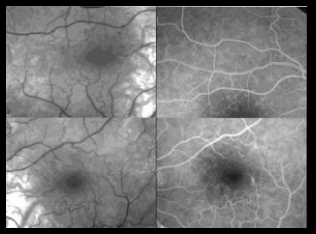
Kavanagh et al, 2008

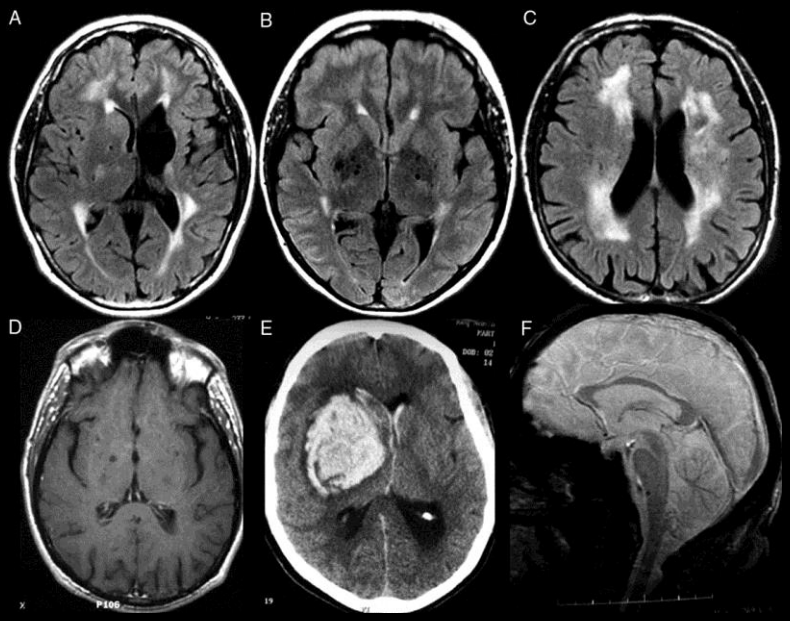
COL4A1 angiopathy
Clinical & MRI Features in mouse & man



- Random mutagenesis in mouse
- Perinatal hemorrhagic stroke
- Porencephaly in survivors
- Col4A1 gene mutation



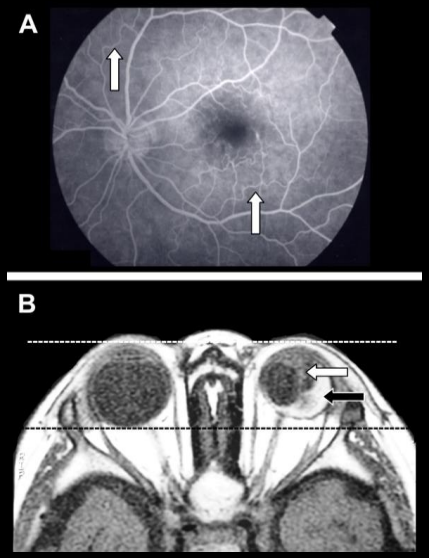


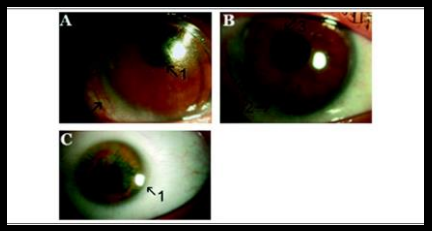


- Infantile hemiparesis
- Retinal arteriolar tortosities
- Hemorrhagic stroke & Leukoencephalopathy
- G562E Col4A1 mutation

Vahedi et al, 2003
Gould et al, Science 2005, NEJM 2006

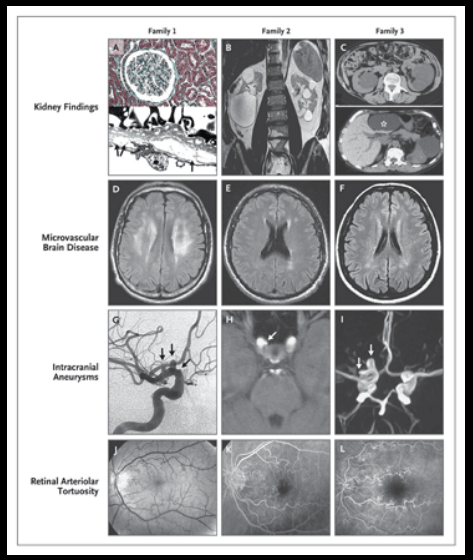
Neurological and extra neurological associated manifestations
Eye, Kidney, Muscle, Small and Large vessels…





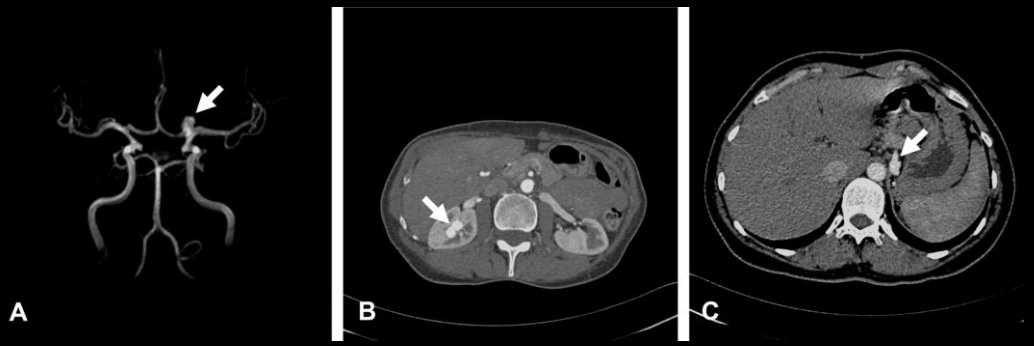
EYE

- Early onset cataract (30 % families)
- Microcornea, corneal opacity, microphtalmia, glaucoma
- Retinal arteriolar tortuosities (30 %)



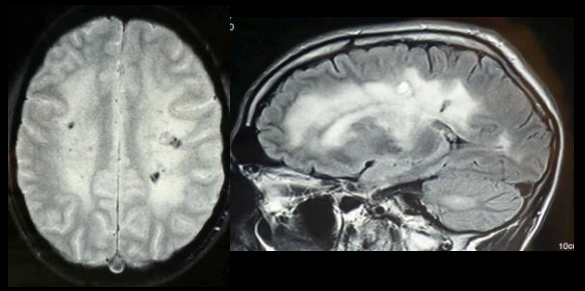
KIDNEY

- Hematuria
- Renal cysts
- Carotid aneurysms
- Cramps
- HANAC



intra and extra cranial large vessels

- Intra and extra cranial aneurysms and stenoses
- Intracranial aneurysms: 30 % families, mostly asymptomatic



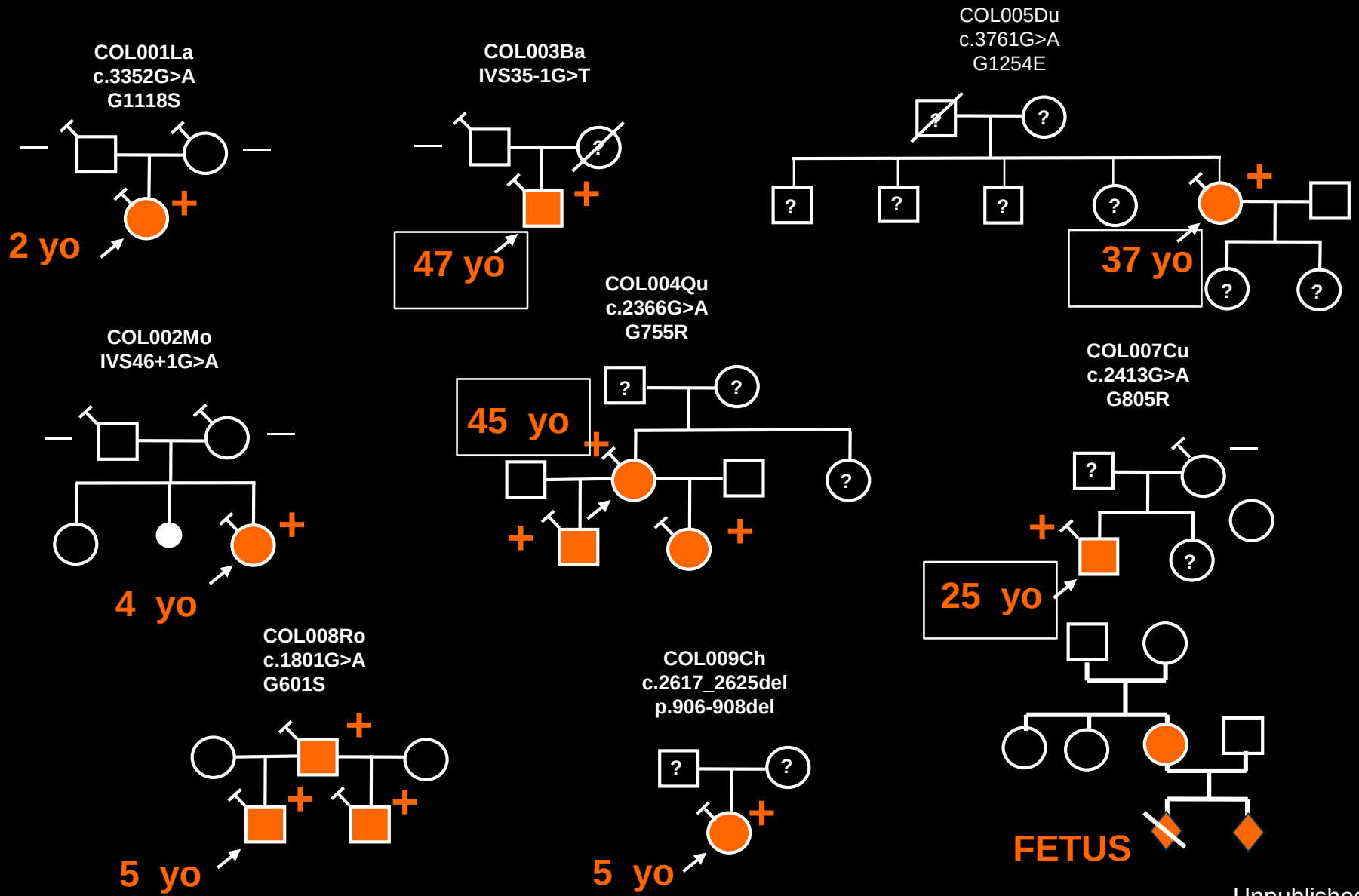
No clinically overt ICH or stroke

- Leukoencephalopathy and microbleeds plus …….

Plaisier et al 2007 / Sibon et al, 2007, Alamowitch et al, 2009,
Rouaud et al, 2010 / Mine, Buffon et al, in prep

Clinical onset: from fetal to adult life

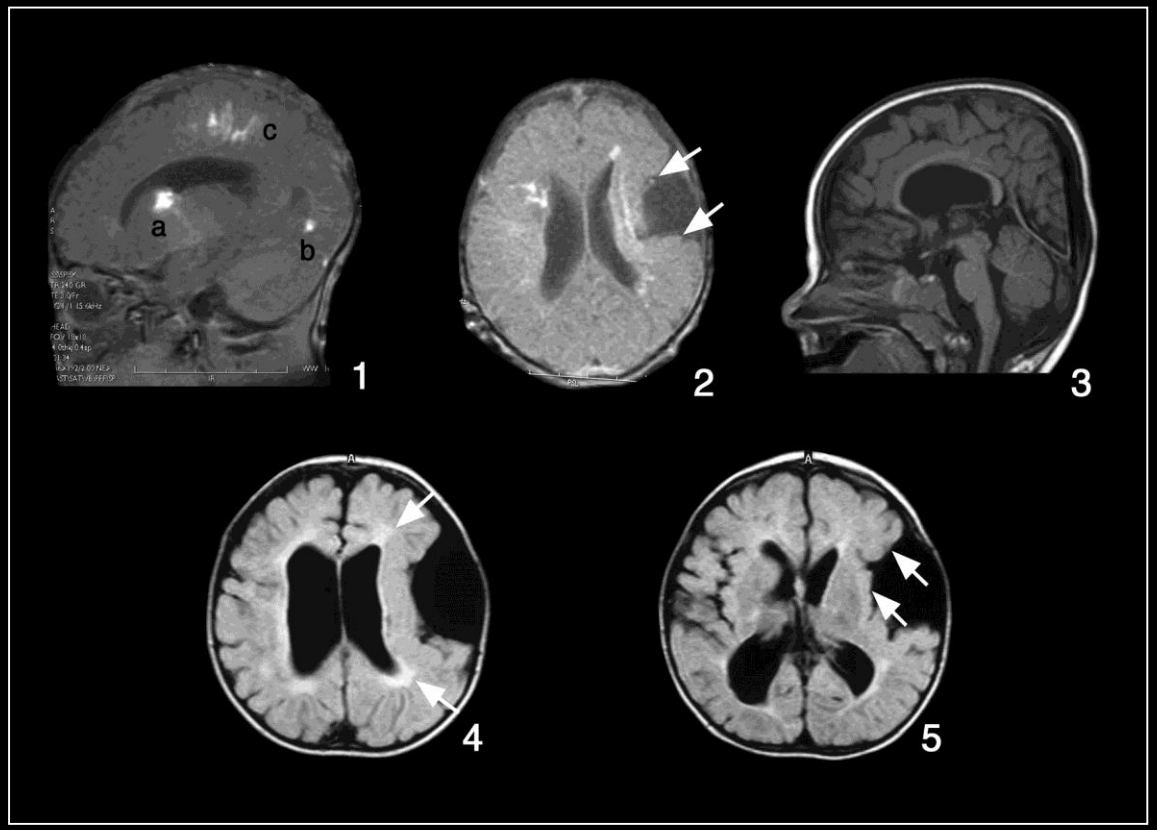
2009-2014: ~ 75 COL4A1 mutated families identified in Lariboisière genetics lab



Unpublished data

Peri / prenatal Hemorrhagic Stroke in infants

- **Patient Col 001**: First child of non consanguineous healthy parents
- Born at 41 weeks of gestation (forceps)
- Birth weight: 2.5 kg / 50cm; Apgar score and normal clinical examination



- **Day 8** brain MRI: several **hemorrhagic lesions** and an enlarged and polygyric left sylvian scissure

- **12 months** :
  - myoclonic seizures and moderate developmental delay
  - bilateral cataract
  - MRI: ventricles dilatation, porencephaly

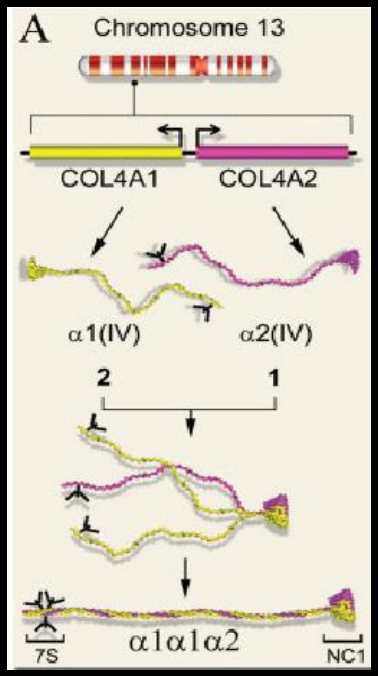
Sporadic case
de novo G1118S Col4A1 mutation

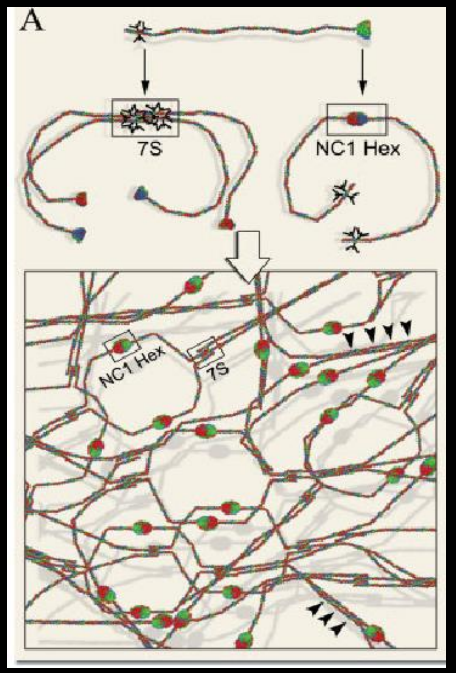
Moutard et al, unpublished
Trousseau hospital, Paris

Col4A1 / COL4A2 « basalopathies »

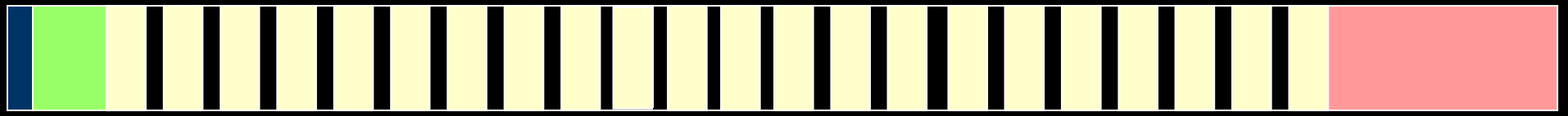
- Highly variable age of onset, severity, clinical features
- Risk of antenatal / perinatal / post natal life cerebral bleeding
- Increased risk of bleeding for mutated infants during vaginal delivery
- Similar phenotypes in COL42 mutants
- Importance of genetic counseling +++
- For all those reasons, molecular screening is important +++ including for fetuses with suggestive neuropathological lesions

Col4A1, gene and protein





Signal peptide



7S domain
N-terminal
Cross-link of 4 triple helices

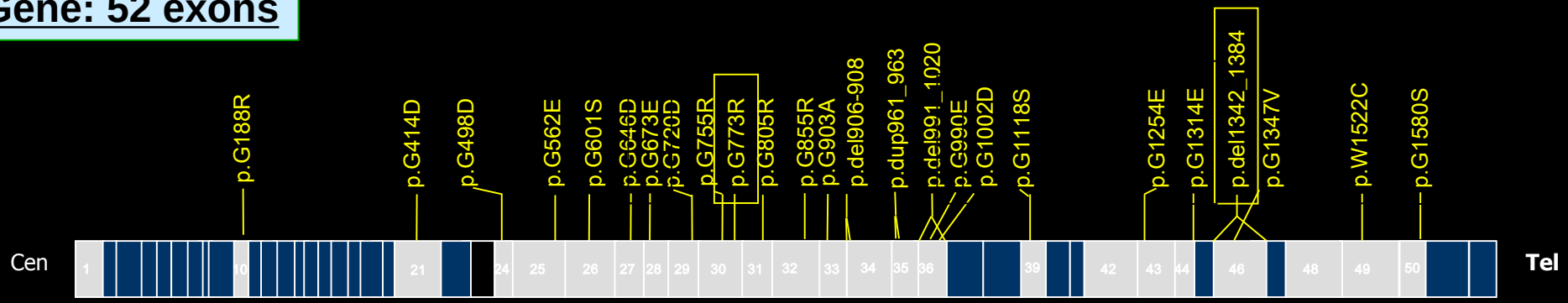
Triple helix (1413 aa)
- 22 *Gly-X-Y* motifs
- 21 interruptions
  - flexibility
  - interchain linkage

NC1 domain
C-terminal (229 aa)
(12 cystein residues)
Molecular assembly

Col4A1 mutation spectrum

Experience of Lariboisière Genetics laboratory

Gene: 52 exons



Protein

- **Most mutations are located in the triple helical domain**
  - **Glycin missense mutations in the triple helix of COL4A1 ++++**
  - **Rare missense mutations in the NC1 domain**
  - **Rare mutations leading to a premature stop codons**

Mine et al, Ann Neurol 2009
Renard et al, 2014
Mine, Prieto-Morin et al unpublished data

Pathophysiological mechanisms ?

- Mechanisms leading to multiple types of vascular lesions ?
- Mechanisms of the leucoencephalopathy ?
- Mechanisms of extra-neurological lesions (eye, kidney…)
- Mechanisms controlling the variability of the phenotype ?

- No autopsy material available so far in adult patients
- Skin and kidney biopsies / Fetus autopsies

- Several COL4A1 mouse models currently analyzed by various groups
- Various hypotheses:
    - intracellular trapping of the mutated molecules,
    - Endoplasmic Reticulum stress,
    - Expression of mutated COL4A1 trimers in the ECM

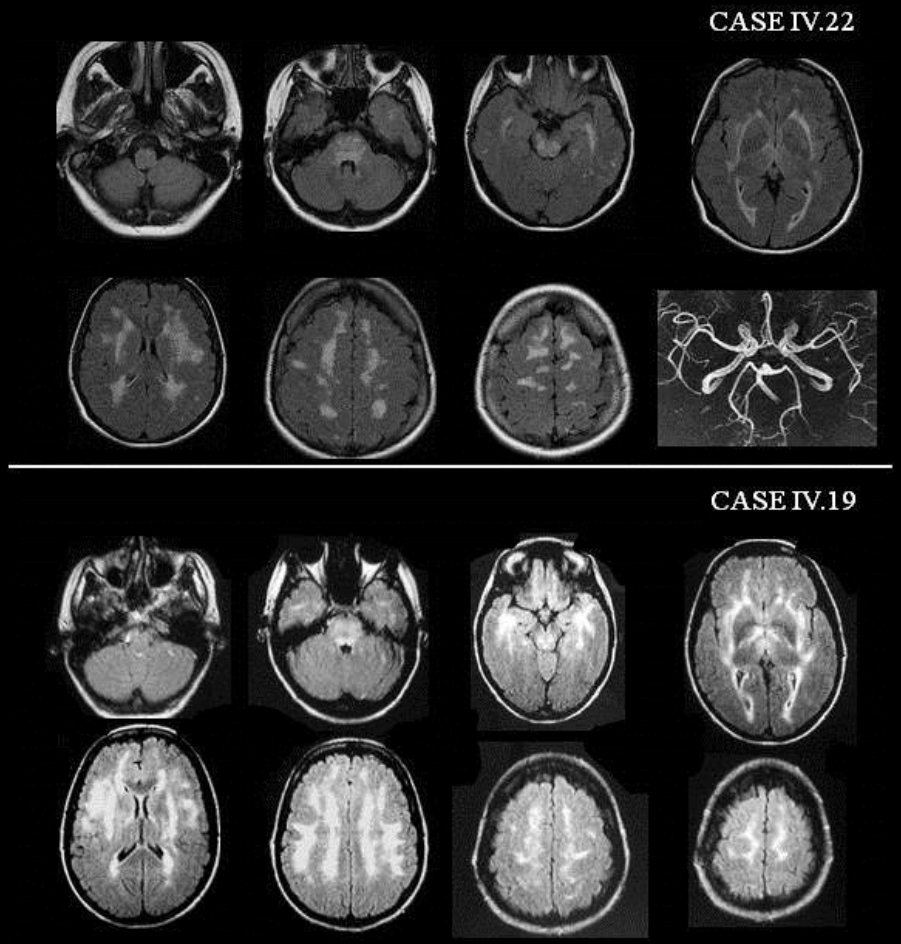
A new concept emerging in SVD pathophysiology
Cerebral SVD, a consequence of brain vessel matrisome / ECM anomalies

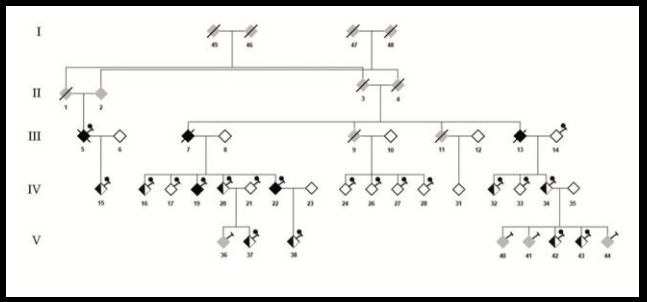
Joutel et al, 2015
Haffner et al, 2015

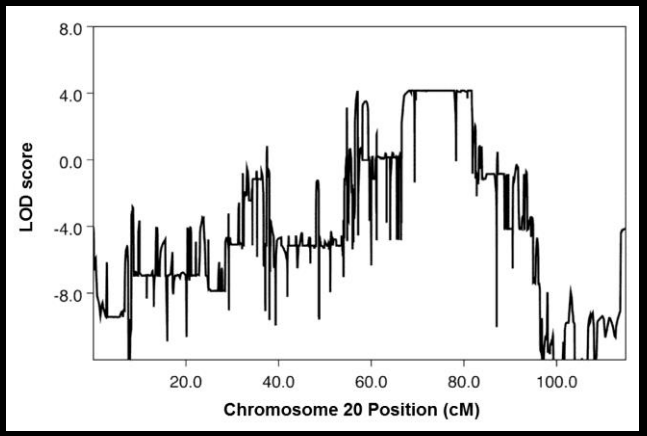
Familial SVD with unknown causative mutations

A novel autosomal dominant SVD

Linkage of this SVD to chromosome 20q13







- Progressive, age related, hemispheric and brainstem leukoencephalopathy
- Contrasting with the paucity and late onset of clinical symptoms in most patients
- The gene is located on chromsome 20. Gene identification is ongoing.

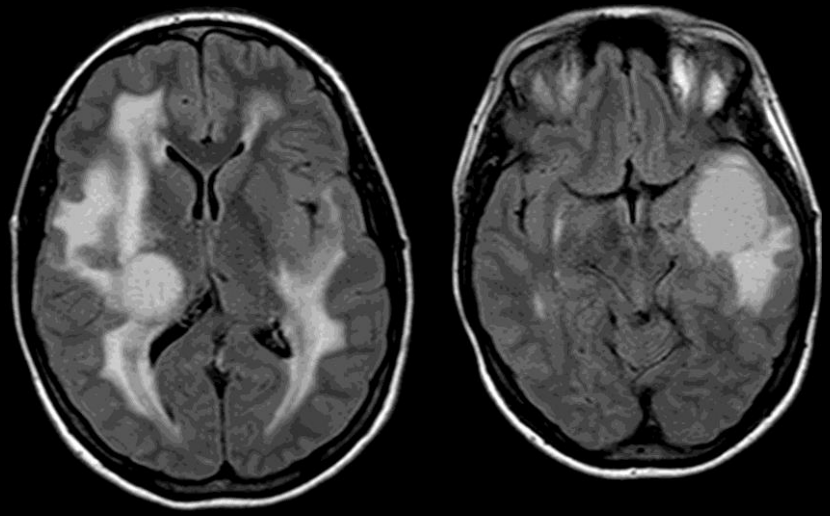
Hervé et al, Neurology, 2012

LCC

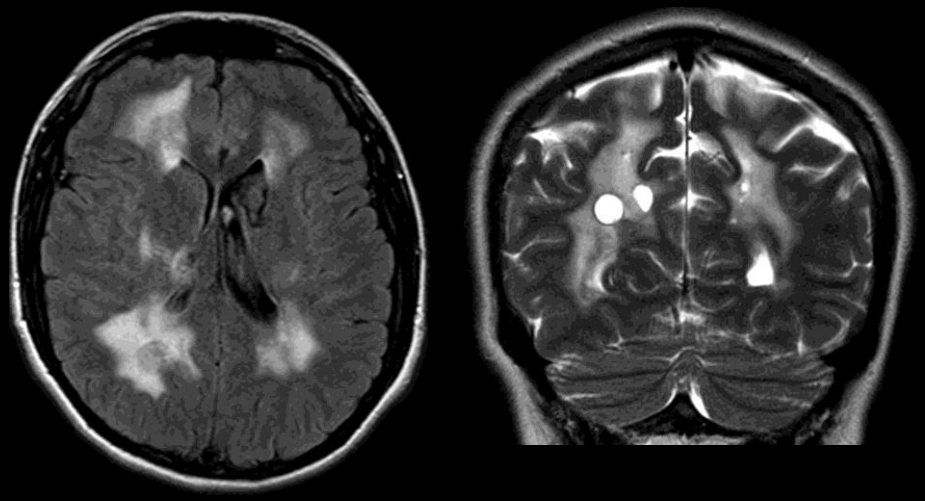
Leukoencephalopathy with **C**alcifications and **C**ysts

A severe, autosomal recessive, microangiopathy

LCC in 2 adult patients



- Woman born in 1975
- Healthy parents and relatives
- Nothing until 2005
- **Headache, Intra Cranial Hypertension**
- CT scan: calcifications, cysts
- MRI: **Calcifications Cysts and leukoaraiosis**
- All investigations (parasites etc…) negative
- New epidode of severe headache in 2006
- Recurrent neurosurgery for cysts



- Woman born in 1975
- Healthy parents
- Nothing until 2006
- **Right hemiparesia of sudden onset**
- CT scan: calcifications, cysts
- Cerebral MRI:
  - **Calcifications Cysts and leukoaraiosis**
  - Recent infarct in the corona radiata

  etc…)

LCC: A strongly suggestive neuroimaging phenotype

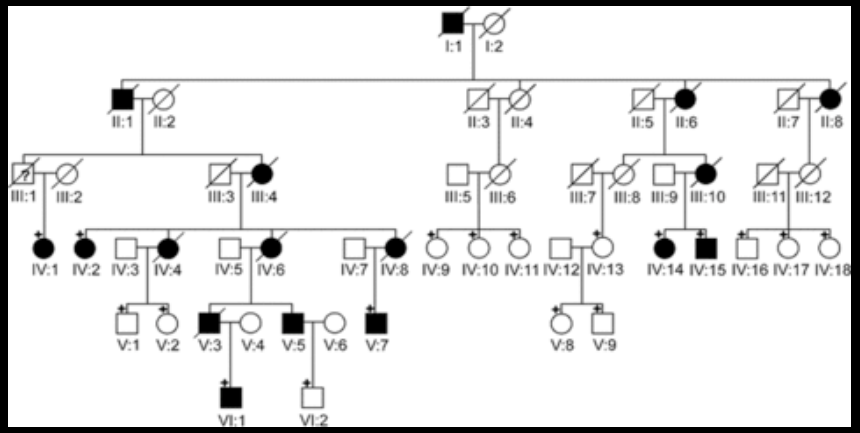
LCC

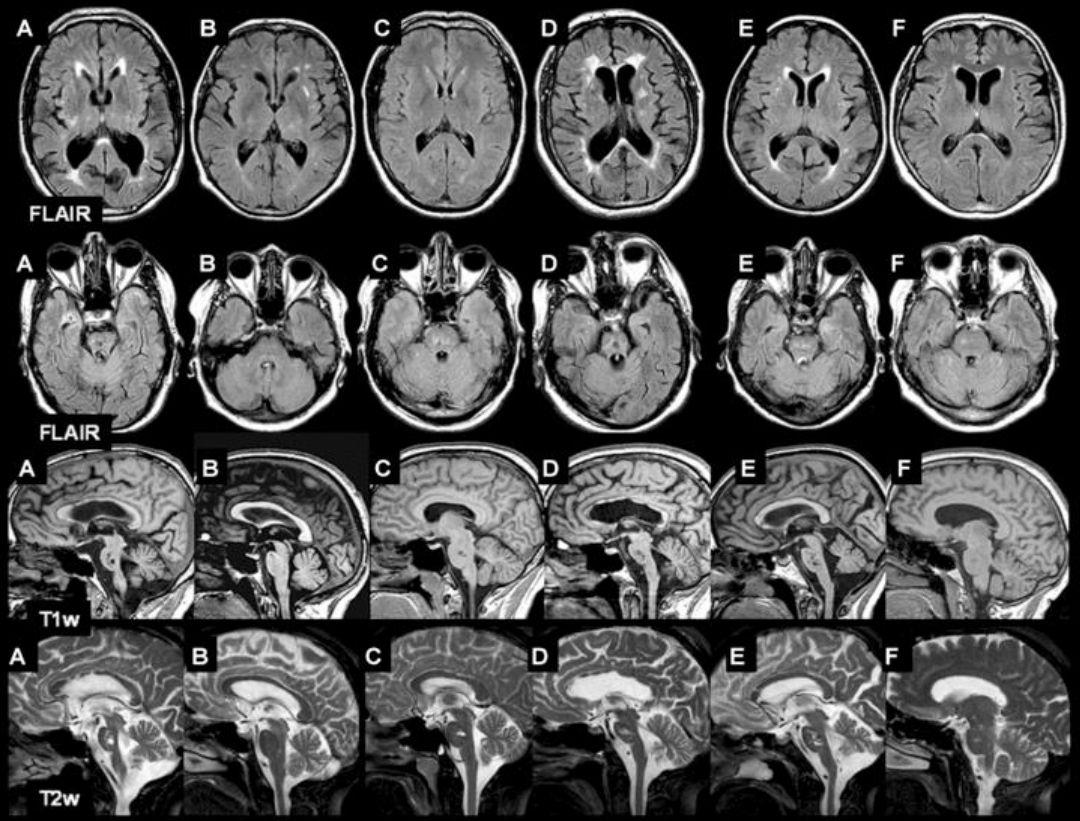
Mechanisms of leukoaraiosis and cysts formation ?

Ongoing identification of the gene(s) involved in this disease

PADMAL

Pontine Autosomal Dominant Microangiopathy & Leukoencephalopathy



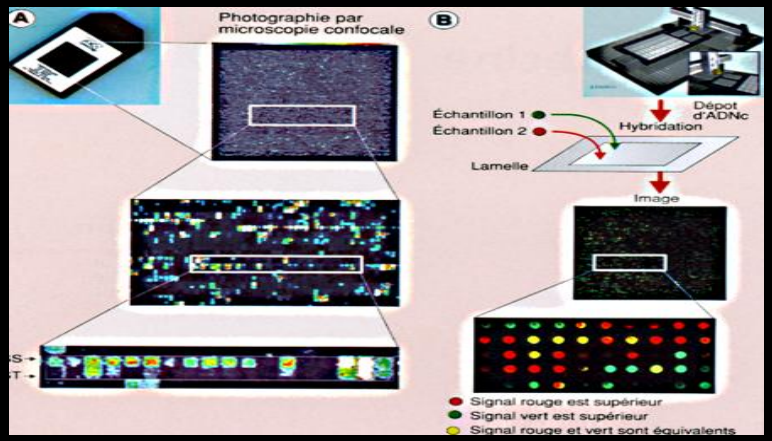


- AD SVD in a family from Hamburg
- Clinical onset: 20-50 yo
- Duration of the disease: around 12 years
- Recurrent lacunar infarcts, dementia
- High frequency of pons infarcts
- Shared features with the « Swedish » family
- Notch3 excluded
- Ongoing gene identification

Hagel et al, 2004; Ding XQ et al, 2007

Novel SVD Genes in the era of high-throughput Sequencing

2015 Challenges and Very Exciting opportunities for hereditary SVD



High density DNA chips
Very fast linkage analysis

High Throughput sequencing
Whole Exome Sequencing

- Unique small families and even sporadic cases now tractable for gene identification using Whole Exome Sequencing
- Collaboration between clinicians, geneticists and neuropathologists to identify novel phenotypes and novel genes +++

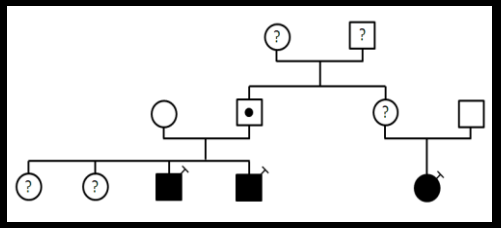
A novel autosomal dominant SVD of unknown etiology

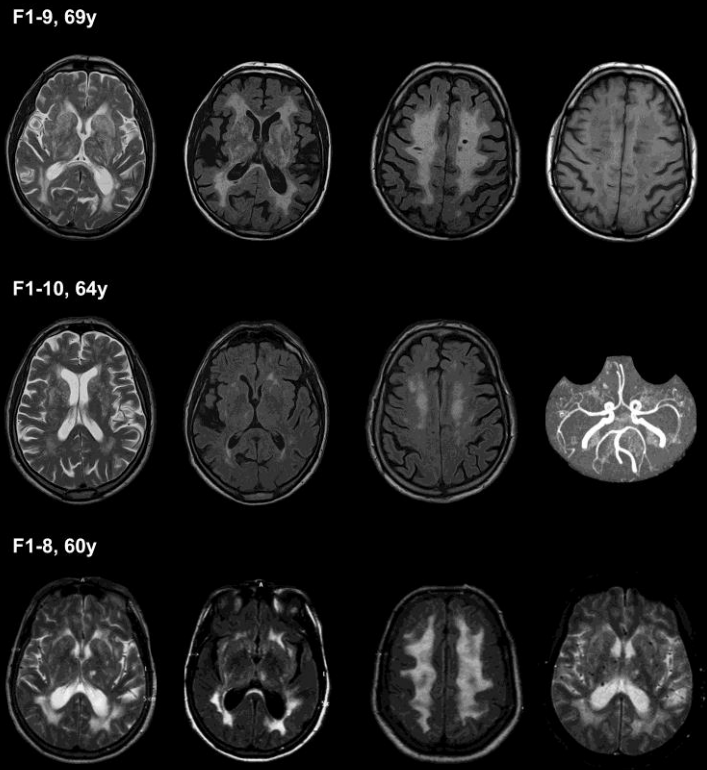
- Clinical onset around 60 y
- Lacunar infarcts and cognitive impairment
- No extra neurological manifestation

- 3 cousins affected
- Autosomal dominant inheritance

- No mutation within NOTCH3 and APP

- Whole exome sequencing of the 3 patients
- Identification of 5 candidate genes showing an heterozygous mutation absent from all databases,

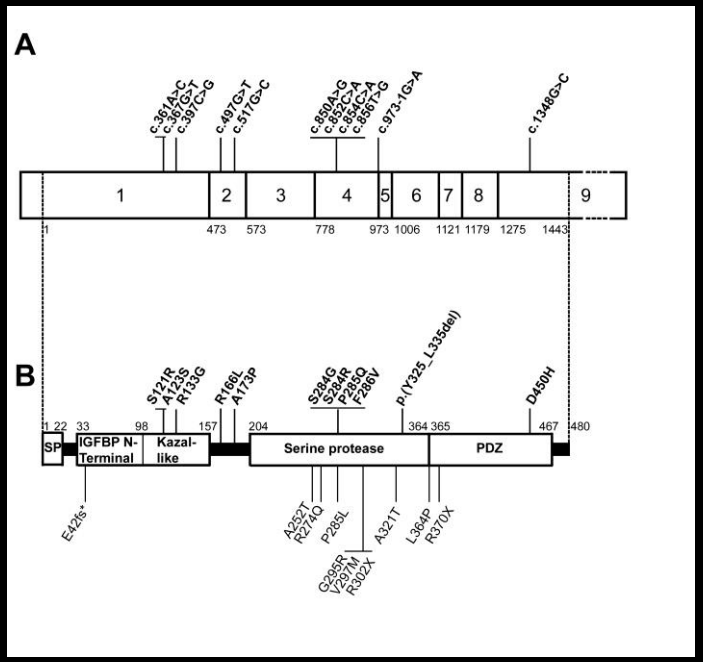


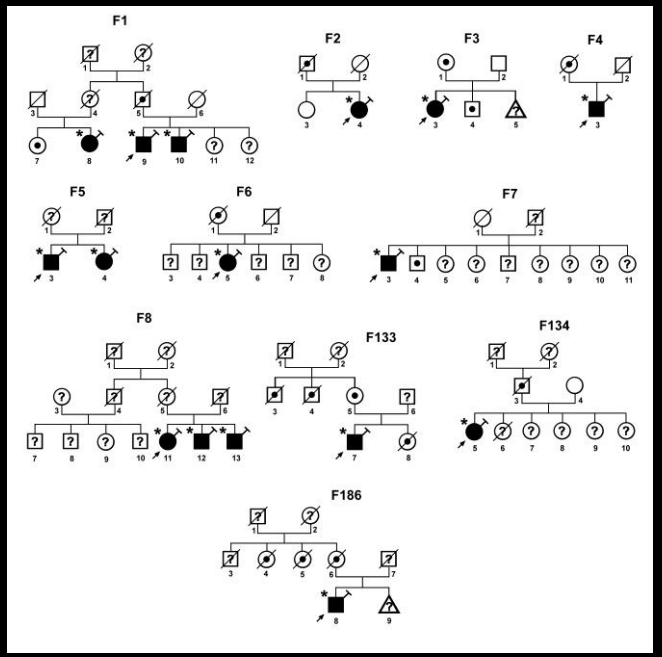


Verdura et al, Brain in press

Heterozygous HTRA1 mutations lead to an AD small vessel disease very similar to common sporadic SVD

- NGS sequencing of these 5 candidate genes in 200 index SVD familial cases
- Identification of 10 additional families showing an heterozygous mutation in one of these 5 candidate gene, namely HTRA1 (p= 4.2x10-6)
- Heterozygous HTRA1 mutations lead to a dominant SVD, with a much later age of onset (60 yo) than CARASIL (35 yo), no extra neurologiqal manifestations, which is very similar to common SVD
- 5 % of familial SVD are herozygous HTRA1 mutation carriers

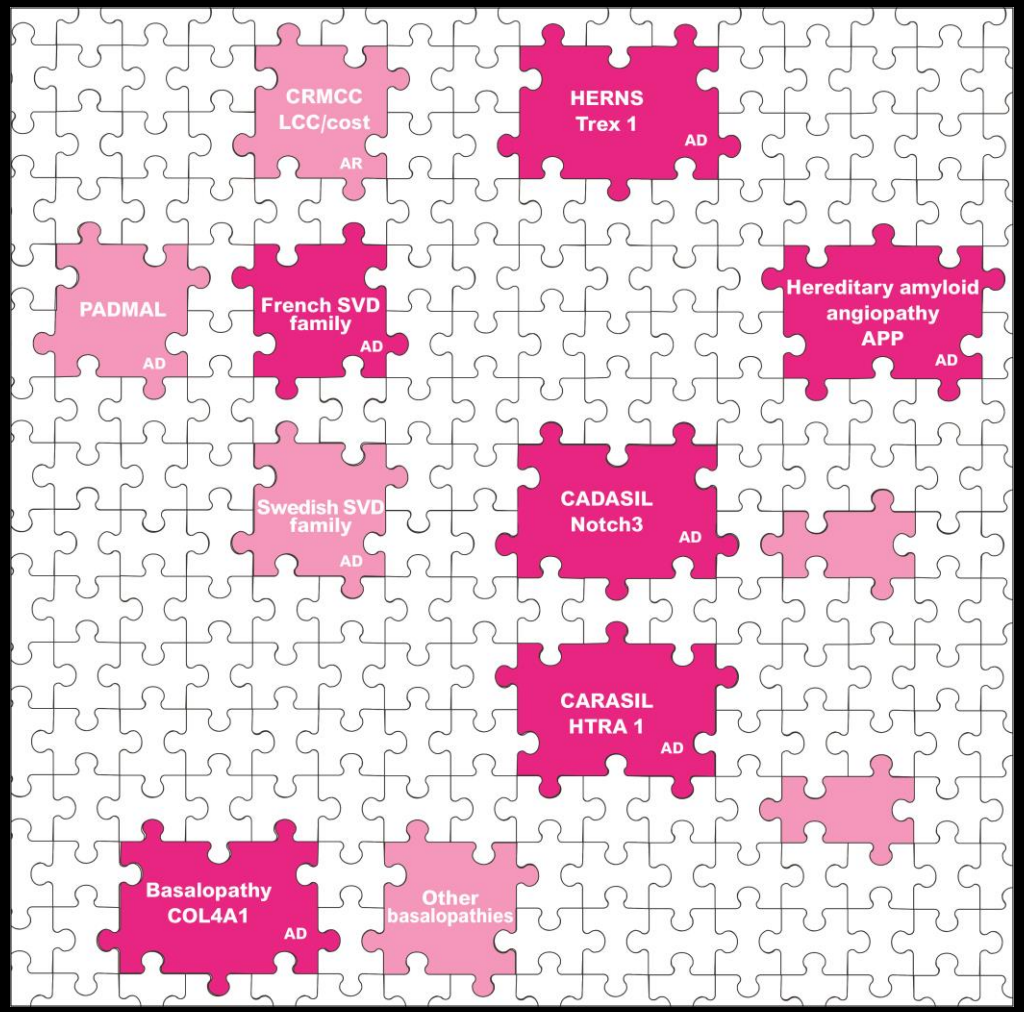




Verdura et al, Brain in press

Genetics of Cerebral Small Vessel Diseases

Advancing the « Next generation » of stroke / Vascular dementia research



- Identification in the very next future of many additional genes involved in stroke pathophysiology

- Multiple genes / Similar phenotypes / Common pathway ?

- The next challenge will then be to bridge the gap between genes and pathophysiology

- Importance of neuropathological investigations on brain autopsic material +++

Genetics of Ischemic and Hemorrhagic Small Vessel Diseases
Acknowledgements

- **Institutions:** INSERM U1161 / Paris7 University, AP-HP Lariboisière hospital genetics lab / CERVCO
- **Funding**: INSERM, PHRC, Leducq Foundation

- **Lariboisière hospital Genetics lab and Inserm U1161:**

  - M. Mine, C. Prieto-Morin, M.D Amador, F. Buffon, A. Delaforge, ETL (SVD diagnostic),

  - E. Verdura, A. Corlobé, MA Dalloz, F. Bergametti, M. Arnould, ETL (SVD gene identification / NGS)

  - A. Joutel's team (CADASILmechanisms project)

- **Collaborations :**

- - D. Hervé, K. Vahedi, MG Bousser, H. Chabriat (Neurology Dpt, Lariboisière Hospital)

  - ML Moutard ,D. Rodriguez (Neuropediatrics Trousseau Hospital)

  - V. Krivosic, P. Massin, A. Gaudric (Ophtalmology, Lariboisière)

  - F. Chapon (Neuropathology, Caen) & F. Gray (Neuropathology, Lariboisière hospital)

  - P. Labauge, C. Denier, L. Guyant-Marechal, F. Rouanet, C. Goizet, E. Berger, M. Sevin, B. Guillon,