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Niemann-Pick Disease Type C

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Disclosures

- **Actelion : member of scientific advisory boards, speaker at sponsored meetings**
- **Genzyme : member of a scientific advisory board**
- **Shire : member of a DSMB and a scientific advisory board**
- **VTesse/ Sucampo: member of a scientific advisory board**

Niemann-Pick diseases
= two distinct diseases

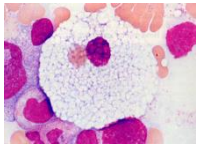
- **Acid Sphingomyelinase deficiency (ASMD)**
  - **Primary Sphingolipidosis**
  - **Typical lysosomal disease / enzyme deficiency/ *SMPD1***
    Niemann-Pick A (neuro) and B (systemic)

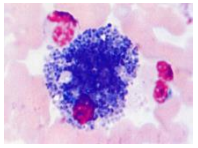
- **Niemann-Pick disease type C (NPC)**
  - **A complex lipidosis also involving sphingolipids**
  - **not an enzyme deficiency**
  - **Lipid trafficking disorder**

Similarities and Historical Distinction

Crocker's types A, B, C [1959, 1961]

- **Similar storage cells (bone marrow, systemic organs)**
- **Hepatosplenomegaly**
- **Lung involvement**





- **Sphingomyelin storage in tissues**
  - Liver: +++ in NP-A and NP-B ; + in NP-C
  - Brain: ++ in NP-A; - in NP-C
- **Neurovisceral or only systemic disease**
  - Early infantile neurological onset, rapid death: NP-A [Niemann, 1914]
  - Only visceral disease: NP-B
  - Later neurological onset, slow progression: NP-C
- **1965-1968: sphingomyelinase deficiency: only NP-A & NP-B**
- **1984-1990: NPC block in intracellular traffic of cholesterol**

(P. Pentchev et al)

NPC: Difficulties in clinical diagnosis

- **Extreme clinical variability**
  - Age at onset : from perinatal period to >50 years
  - Age at death : perinatal to >60 years
- **Classically: a neurovisceral disease**
  - But not always
    - Visceral disease only
      - Perinatal period, possibly very severe leading to early death
      - Preceding onset of neurological symptoms
    - Purely neurological forms (not only in adults)
- **Transversal medical specialties**
  - Neonatal hepatology, general pediatrics, pediatric onco-hematology, neuropediatrics
  - Psychiatry, adult neurology

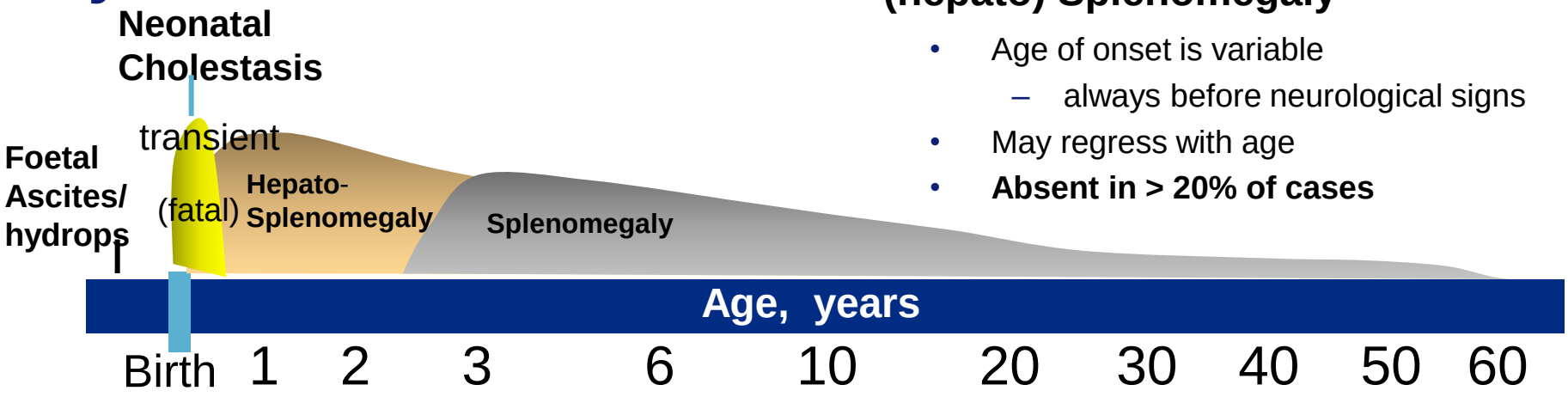
NPC : General features

- The neurological involvement is what defines the severity of the disease in close to 90% of the patients
  - ***Neurological onset never in the very first months of life*** *[hypotonia when severe systemic disease not really NPC-specific]*

- The initial symptom is often systemic
  - ***Neurological onset can be very protracted in relation to the systemic involvement***
    - *Don't exclude NPC in absence of neurological symptoms*
    - *Important to ask for history in neonatal period and infancy*

Neurovisceral disease

1.Systemic involvement



(hepato) Splenomegaly

- Age of onset is variable
  - always before neurological signs
- May regress with age
- **Absent in > 20% of cases**

2. Neurological involvement …

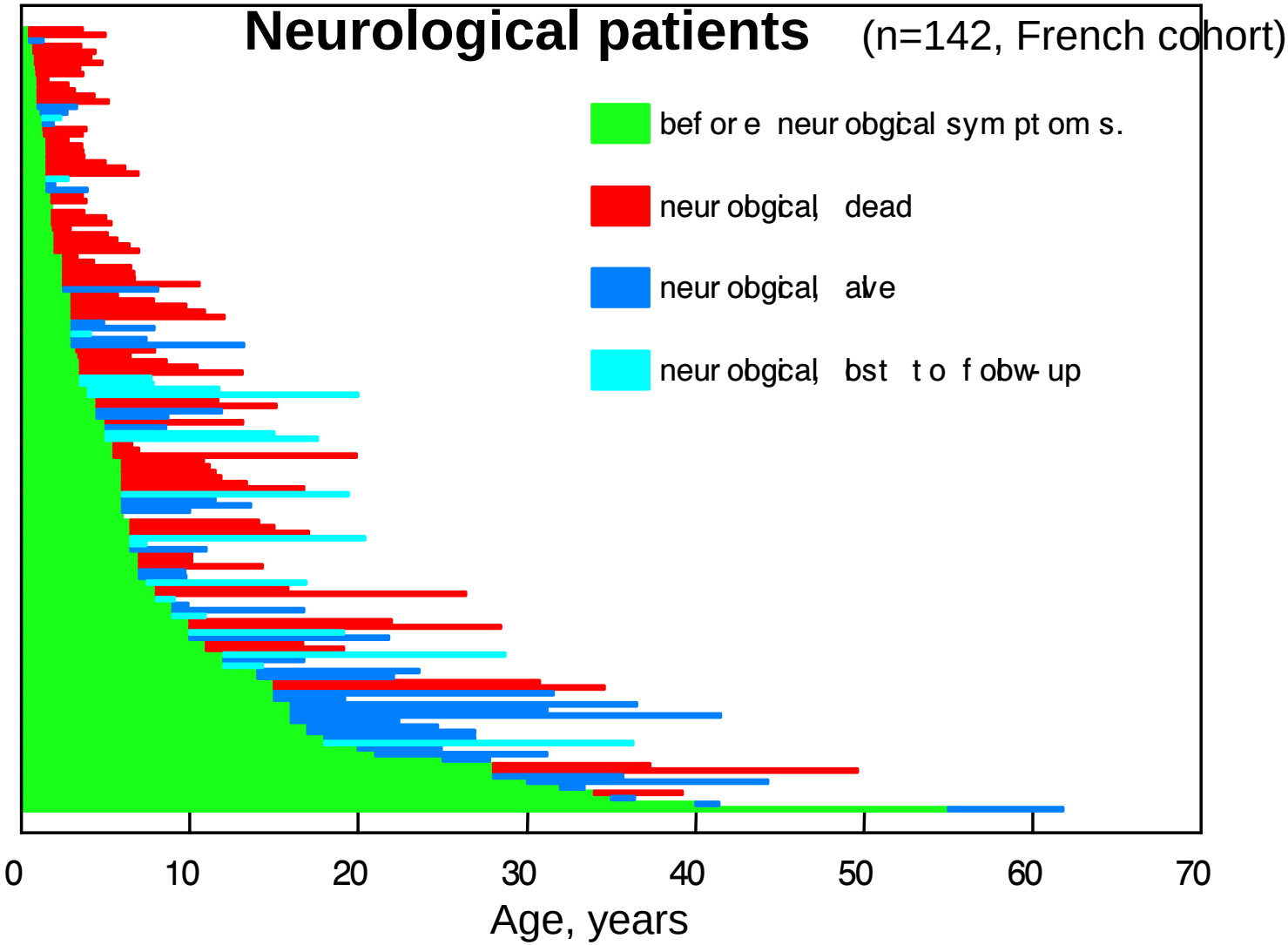
Modified from Vanier, Orphanet J Rare Dis (2010) 5:16

J Inherit Metab Dis. (2015)38:187

Period of onset Duration

Neurological involvement

Variable age at onset – defines clinical forms

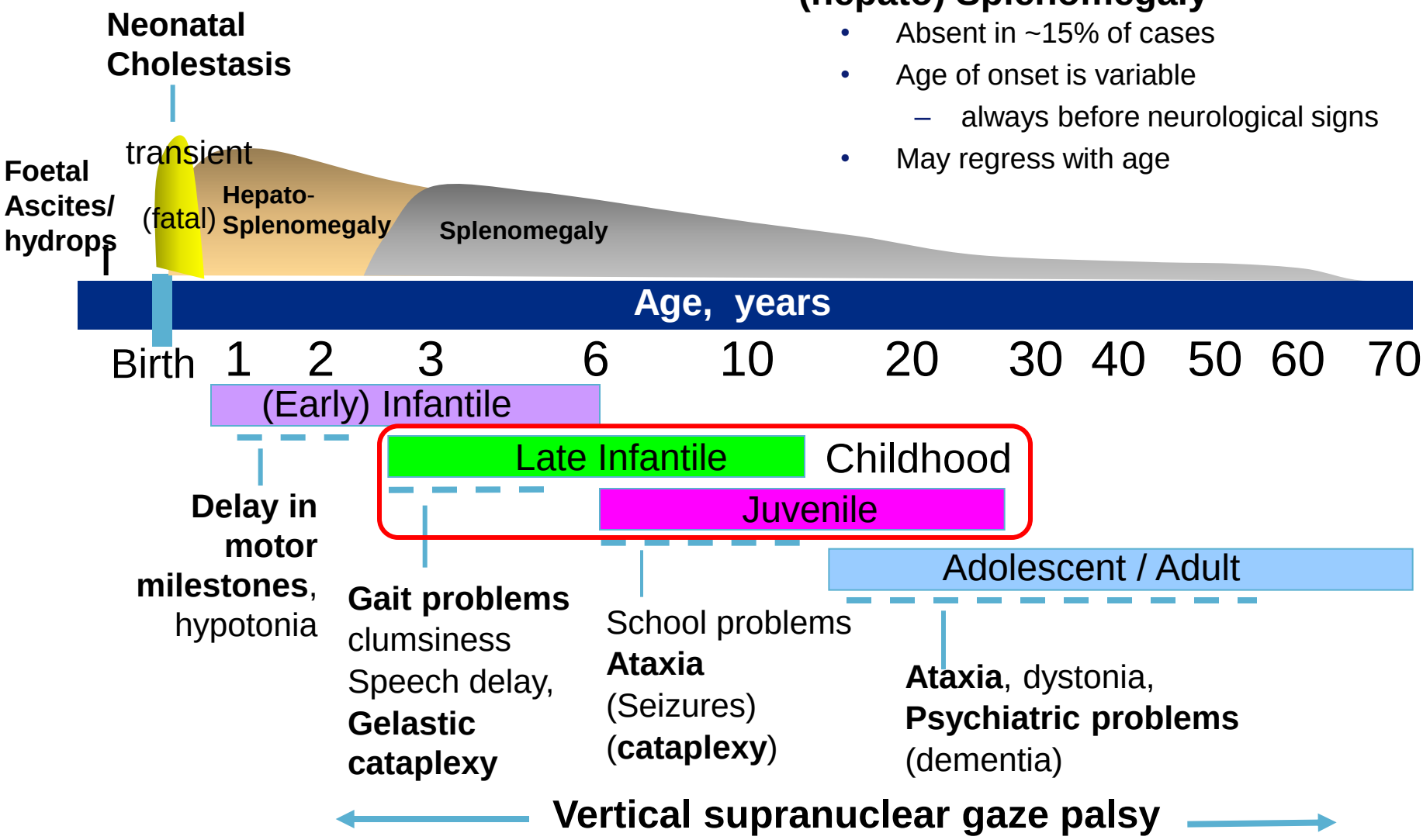


Modified from Orphanet J Rare Dis (2010) 5:16

Systemic involvement

(hepato) Splenomegaly

- Absent in ~15% of cases
- Age of onset is variable
  - always before neurological signs
- May regress with age



Neurological involvement

Period of onset — Duration

Vanier *J Inherit Metab Dis* (2015)38:187

Vertical supranuclear gaze palsy

- Gaze initiation failure in the vertical plane
  most often downwards
- Often missed because in the beginning **slow pursuit is conserved**
- **Study the voluntary saccades (ask patient to rapidly look up and down)**

Main Disabilities and Progression

Ambulation	Ataxia	Dystonia		Spasticity
	Falls	assisted walking		wheelchair
Manipulation	Ataxia	Dysmetria	Dystonia	
	Impairment of Fine Movements, progressive worsening			
Language	Dysarthria		non verbal	no communication
Swallowing	Dysphagia	occasional	daily	G-tube
Seizures		occasional	controlled	uncontrolled
Ocular movements		slow saccades	VSGP	complete

Mental Status: very variable motor status often worse than mental one
Evolution towards dementia is common

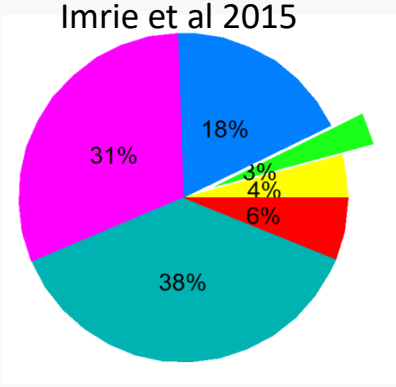
NP-C: Broad clinical heterogeneity

Sorry, illustrative photos of patients had to be deleted

Distribution of clinical forms in cohorts from different European countries

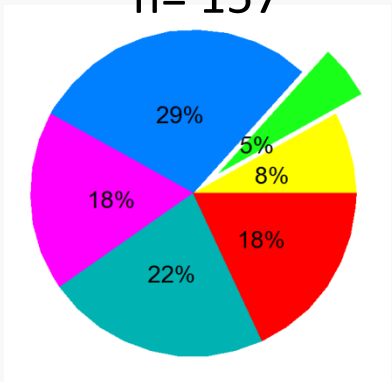
UK n=146

Imrie et al 2015



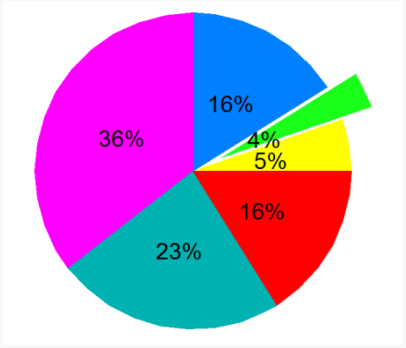
France 1990- end 2014

n= 157



Czech R n= 56

Jahnova et al 2014



Perinatal systemic fatal

Systemic only

Adult neuro onset

Juvenile neuro onset

Late infantile neuro onset

Early infantile neuro onset

Incidence at birth close to 1/100,000

Global prevalence much lower (~ 1.3×10^6 in France?)

Niemann-Pick C Disease

- **Two causative genes (*NPC1 or NPC2*)**
  - **>95% of patients worldwide have mutations in the *NPC1* gene**
  - NP-C2 also panethnic - with variable frequency
    - Seems higher in North Africa, Turkey, Italy…very low UK, USA, Spain…
- **Same disease, whichever gene is defective**
  - From studies in patients AND in *Npc1*$^{-/-}$ and Npc2$^{-/-}$ mouse models
- **Full function of corresponding proteins only partially known**
  - **Cooperate in a sequential manner**
    - Npc1/Npc2 double ko mouse Sleat et al PNAS (2004)
  - **Intracellular trafficking of cholesterol** (P. Pentchev et al, work between 1982-1997)
  - possibly still unclear other functions

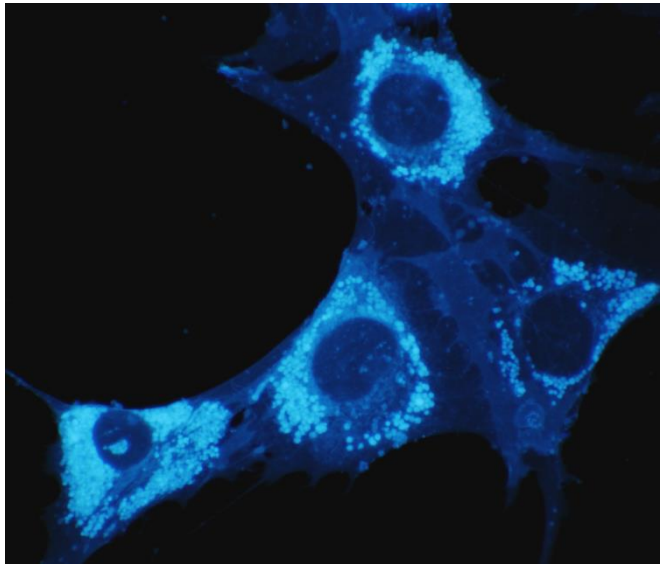
NPC

Block in intracellular trafficking of cholesterol

- **Most evident abnormality in peripheral organs/cells:**
  - *unesterified cholesterol accumulates in the late endosomal/lysosomal compartment*
  - *Can be visualized by filipin staining* *(used as diagnostic test)*

First filipin study: J Biol Chem (1986)

Cellular hallmark of NP-C

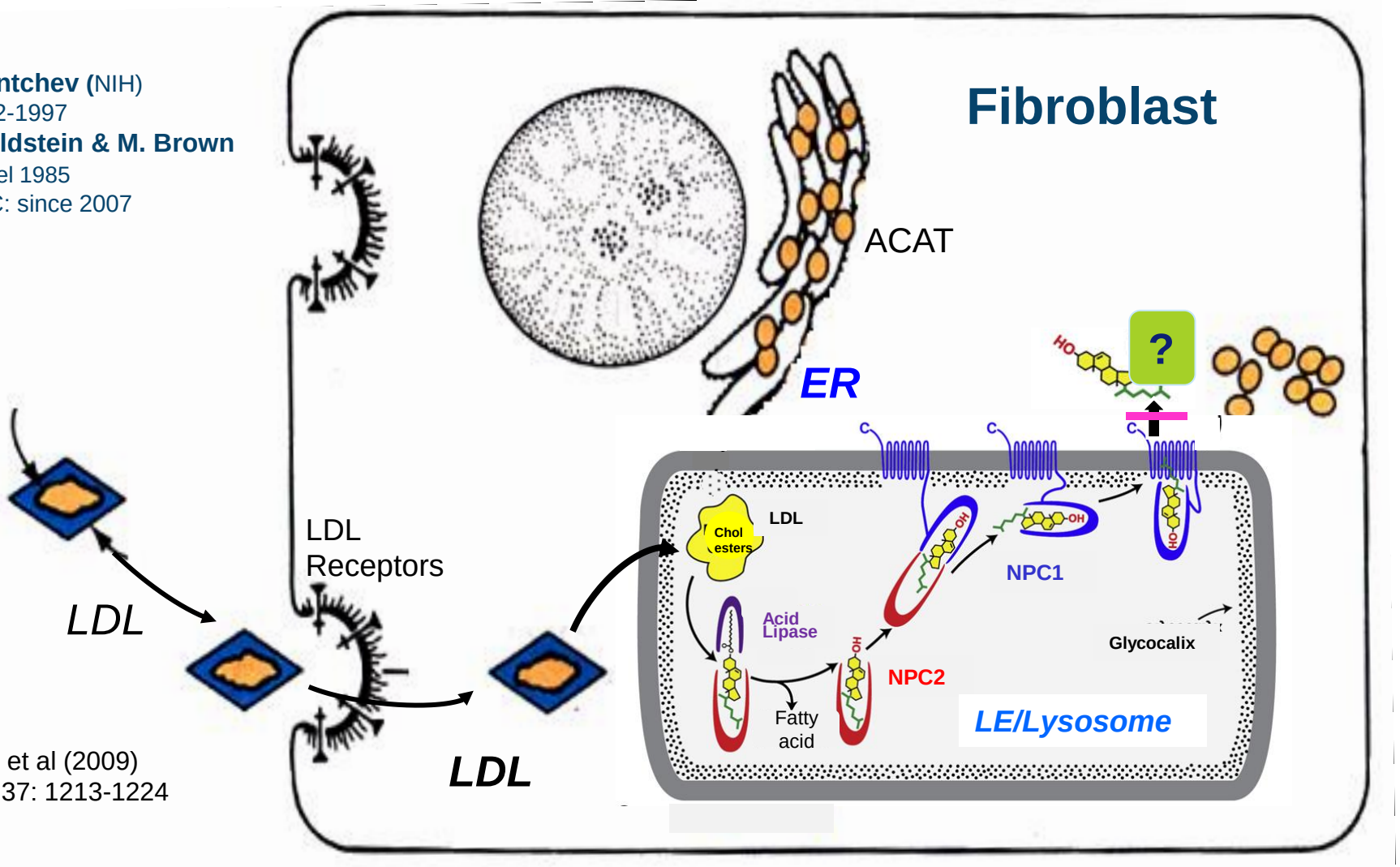


Cultured fibroblasts

Filipin staining

NP-C
Block in lysosomal egress of cholesterol

- **P. Pentchev (**NIH)
  - 1982-1997
- **J. Goldstein & M. Brown**
  - Nobel 1985
    NPC: since 2007

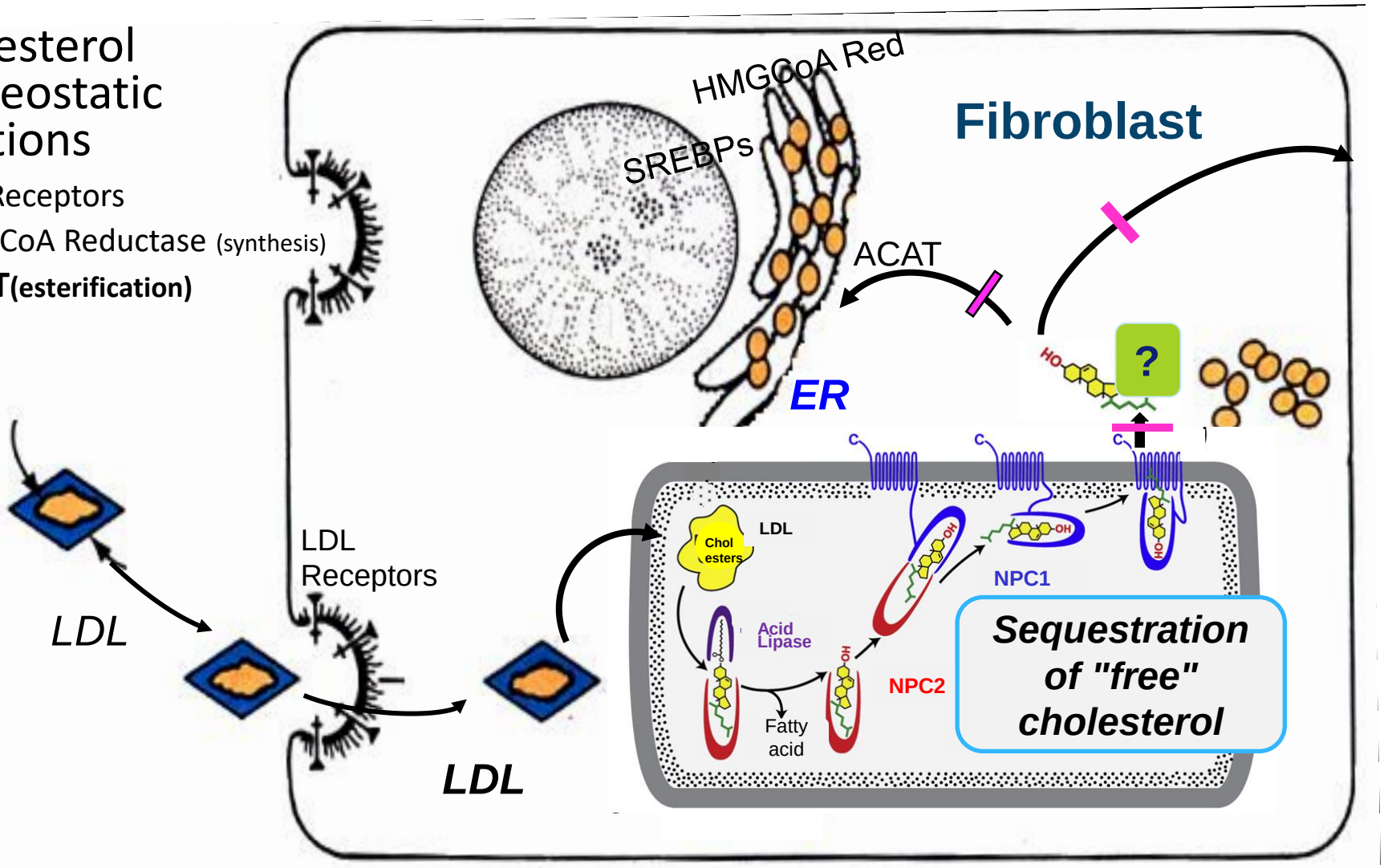


Kwon et al (2009)
Cell 137: 1213-1224

NPC: Block in lysosomal egress of cholesterol secondarily impairs homeostatic reactions

Cholesterol Homeostatic reactions

- ↓ LDL Receptors
- ↓ HMGCoA Reductase (synthesis)
- ↑ **ACAT(esterification)**

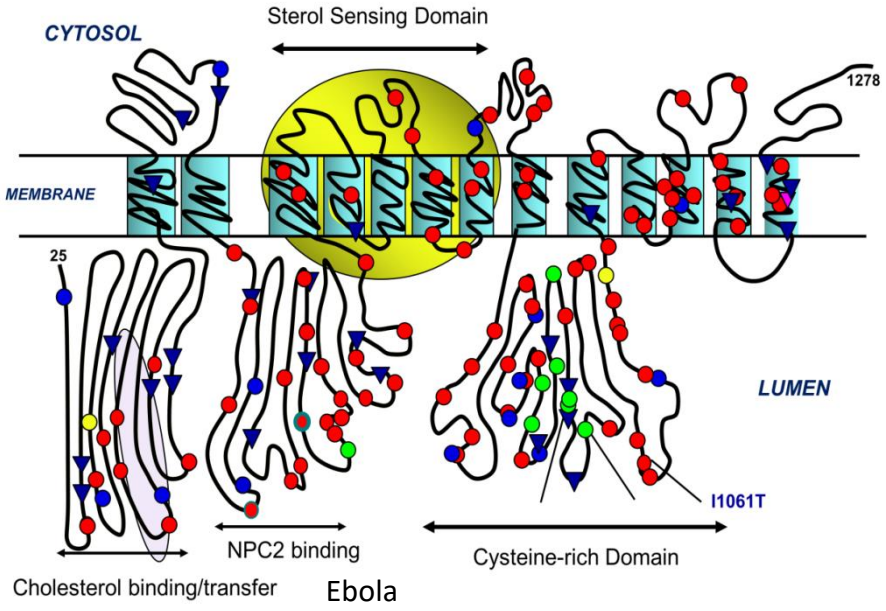


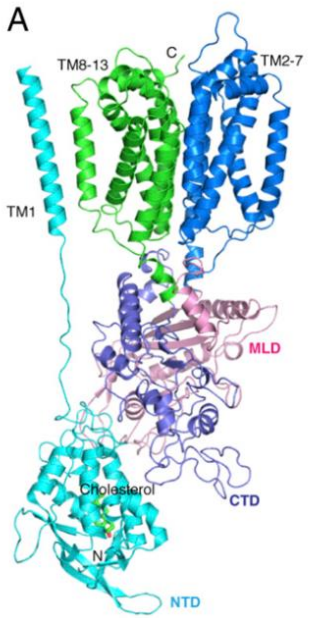
NPC1 Protein

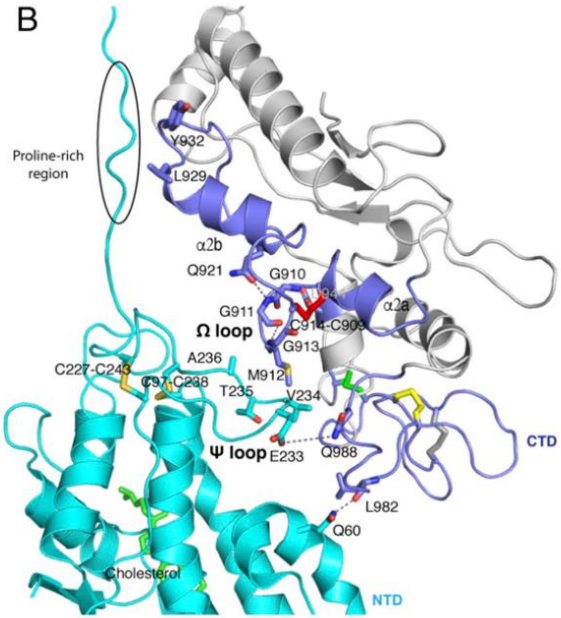
- ***NPC1* gene: 1997**
- **NPC1: 1278 aa (mature 1256)**
  - Mostly resident of LE (coloc Rab7)
  - Role of AP-1 & LL in LE/Lys targeting
  - 13 transmembrane helices [3-7:SSD]
  - 3 luminal domains

- **Recent structural studies**
  - Near-atomic X-ray structure: SSD can accomodate a cholesterol molecule (Li et al PNAS 2016)
  - Cryo-EM at 4.4 A (Gong et al Cell 2016)
  - Complex NPC1-MLD/NPC2 (Li et al PNAS 2016)
  - 3.3 A structure 314-1278 (Li et al PNAS 2017)
    - Docking into full-length cryo-EM NPC1 reveals interface between CTD and NTD

2-D: Davies & Ioannou (2000)

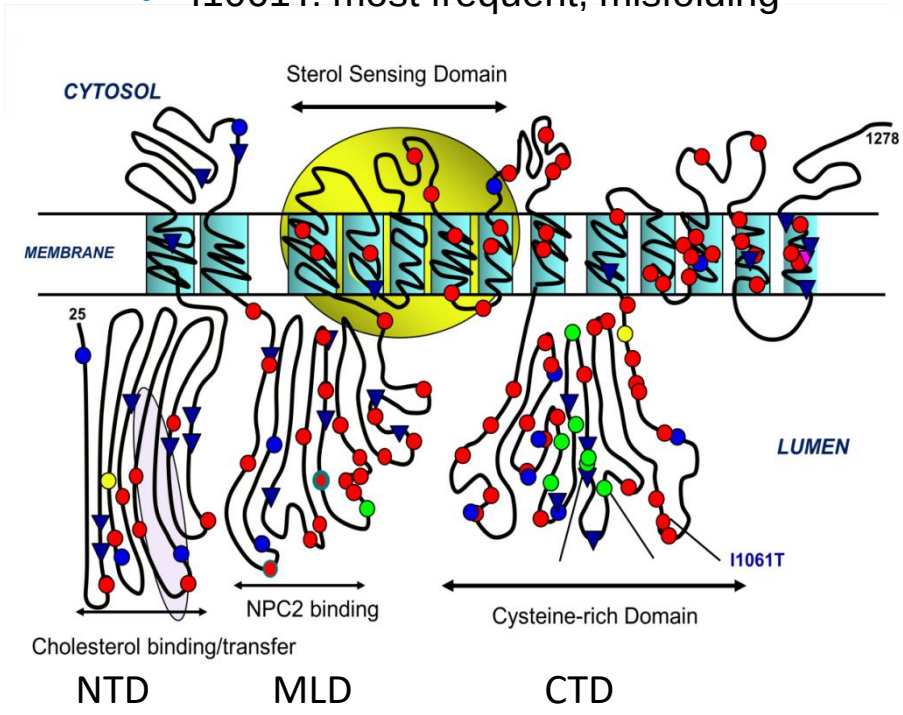




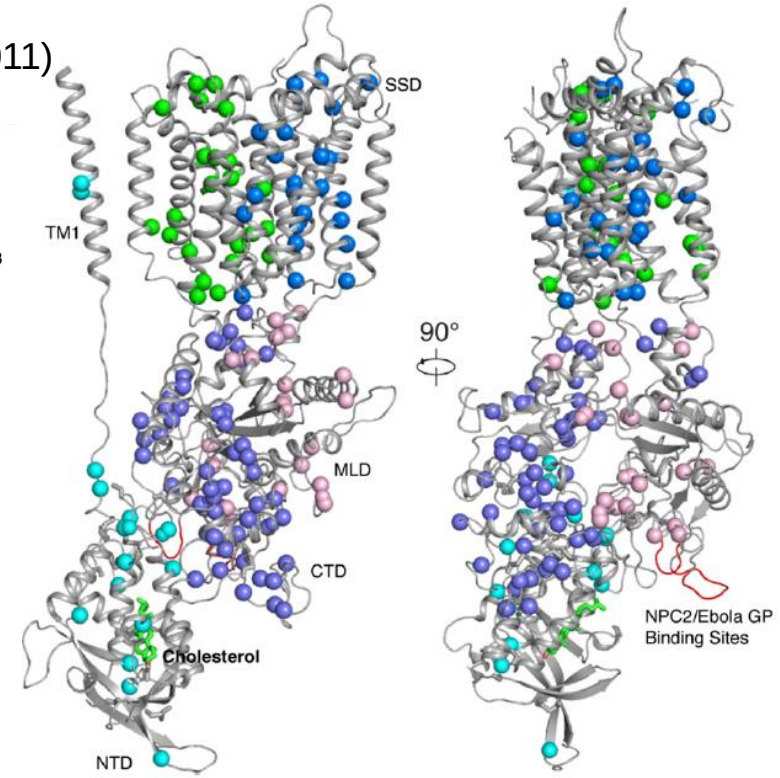


Distribution of NPC1 mutations

- **Close to 400 pathogenic *NPC1* variants known**
  - Mapping NPC-causing mutations (particularly missense ones) can help to understand how they alter protein function –also knock-in mice
    - P202-F203: chol binding (Xie et al 2011)
    - R518Q: NPC2 binding (Deffieu Pfeffer 2011)
    - I1061T: most frequent, misfolding



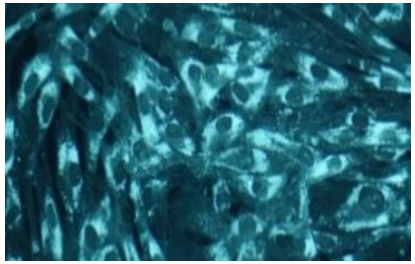
Mutations from the French cohort
(data from the CET-NP)



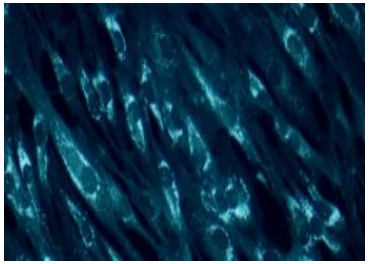
Mutations from the literature
Li et al PNAS 2017

Missense mutations may have a different impact on cellular cholesterol trafficking

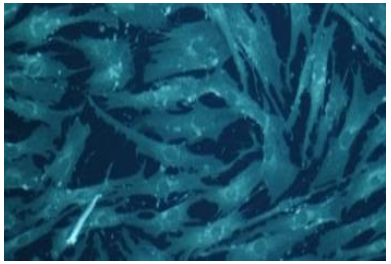
- **Evident by filipin staining**



CLASSIC NP-C
Prototype
p.**I1061T**



VARIANT NP-C
Prototype
p.**P1007A**

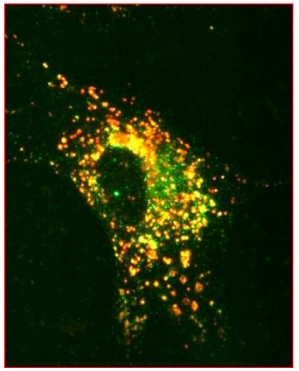


NORMAL CELLS

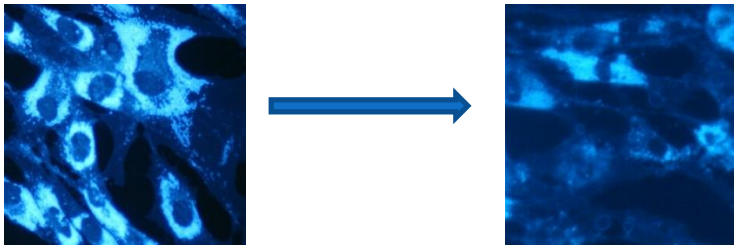
- **Cells from adult onset patients may show either variant or classic pattern. Not well understood**
- **Has been a problem in diagnostic testing**

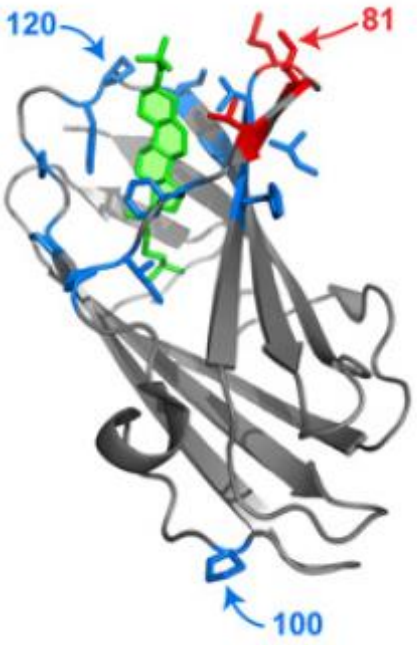
NPC2 protein

- **NPC2 gene: 2000 [=HE1]**



- **NPC2: 151aa (mature 132)**
  - Small, soluble, colocalizes with cathepsin D
  - Tranport to Lys via the M6P receptor
    - Glycosylation Asn58
  - Binds cholesterol: 1:1 ratio and very high affinity – - binding domain/site known
  - Transfer activated by BMP/LBPA, inhibited by SM
    Ko et al, Friedland et al, 2003; Wang et al 2010
  - Role in cholesterol transfer to mitochondria
  - NPC2 deficient cells: NPC2 protein added to the medium can correct cholesterol accumulation

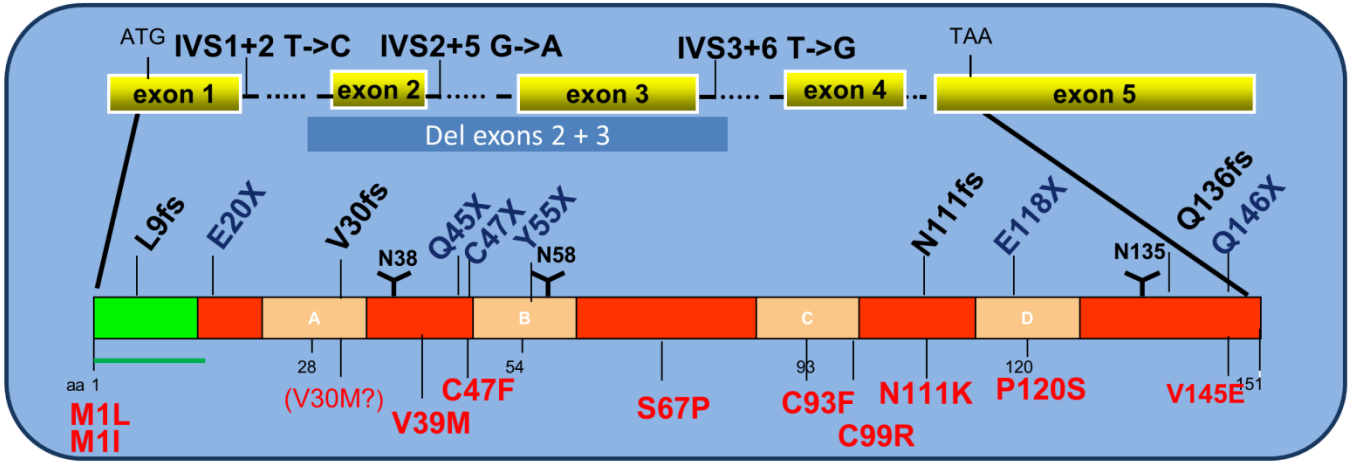




  - Also other described functions of NPC2

Distribution of NPC2 mutations

- **26 mutations described**
  - Many severe mutations (frameshift, stop, one large deletion)
  - Several patients with p.P120S (affects cholesterol binding site)



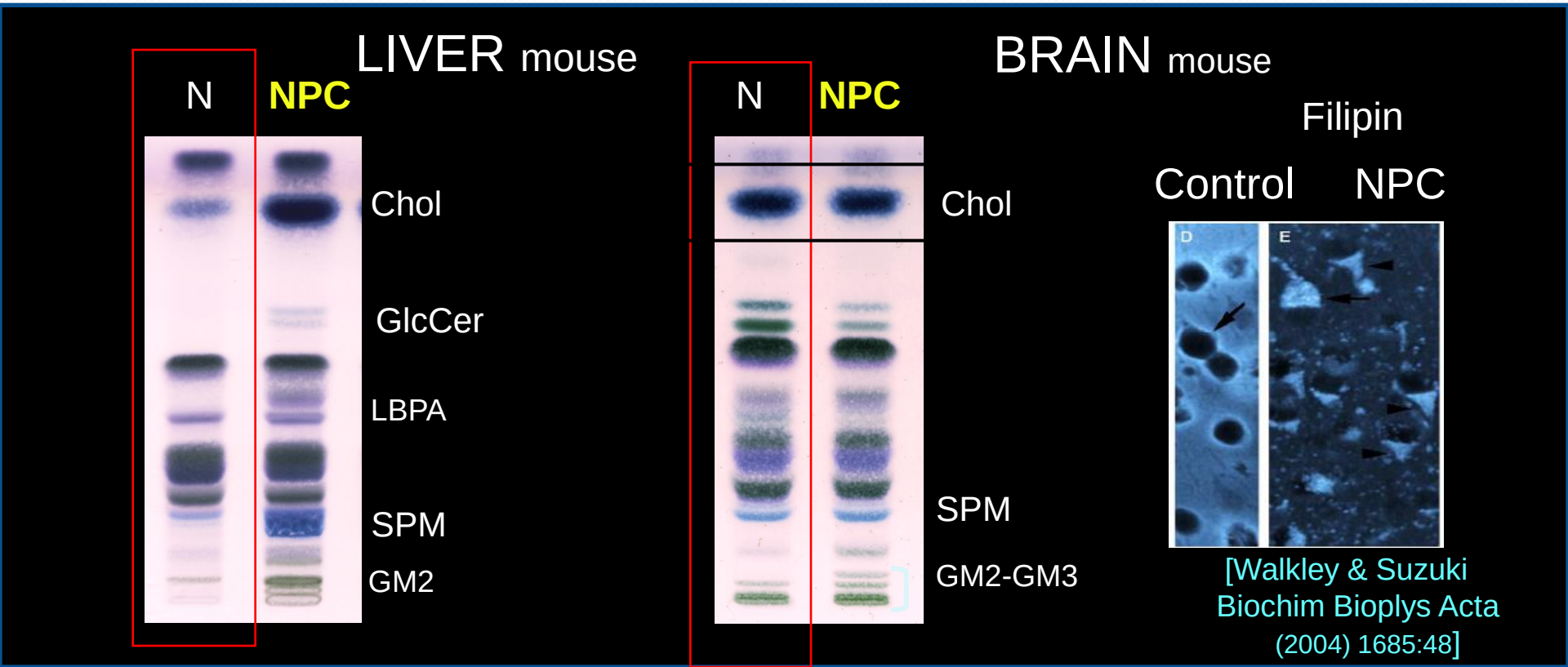
NPC1 and *NPC2* mutations correlate with the neurological form but not with the systemic involvement

- **Multiplex families** with one sibling dying from systemic disease in the neonatal period
  and another sibling suffering from neurological disease with variable age of onset
- **Siblings usually belong to the same global neurological form**
  - Generally true for the early- and late-infantile forms
  - Much more heterogeneity between siblings within the juvenile and adult onset forms

Tissue Main Lipids Storage Differences CNS vs visceral tissues

- **Liver/spleen : cholesterol and sphingomyelin storage**
- **Brain: no net increase of cholesterol or sphingomyelin**
  - but cytochemically, cholesterol storage in neurons

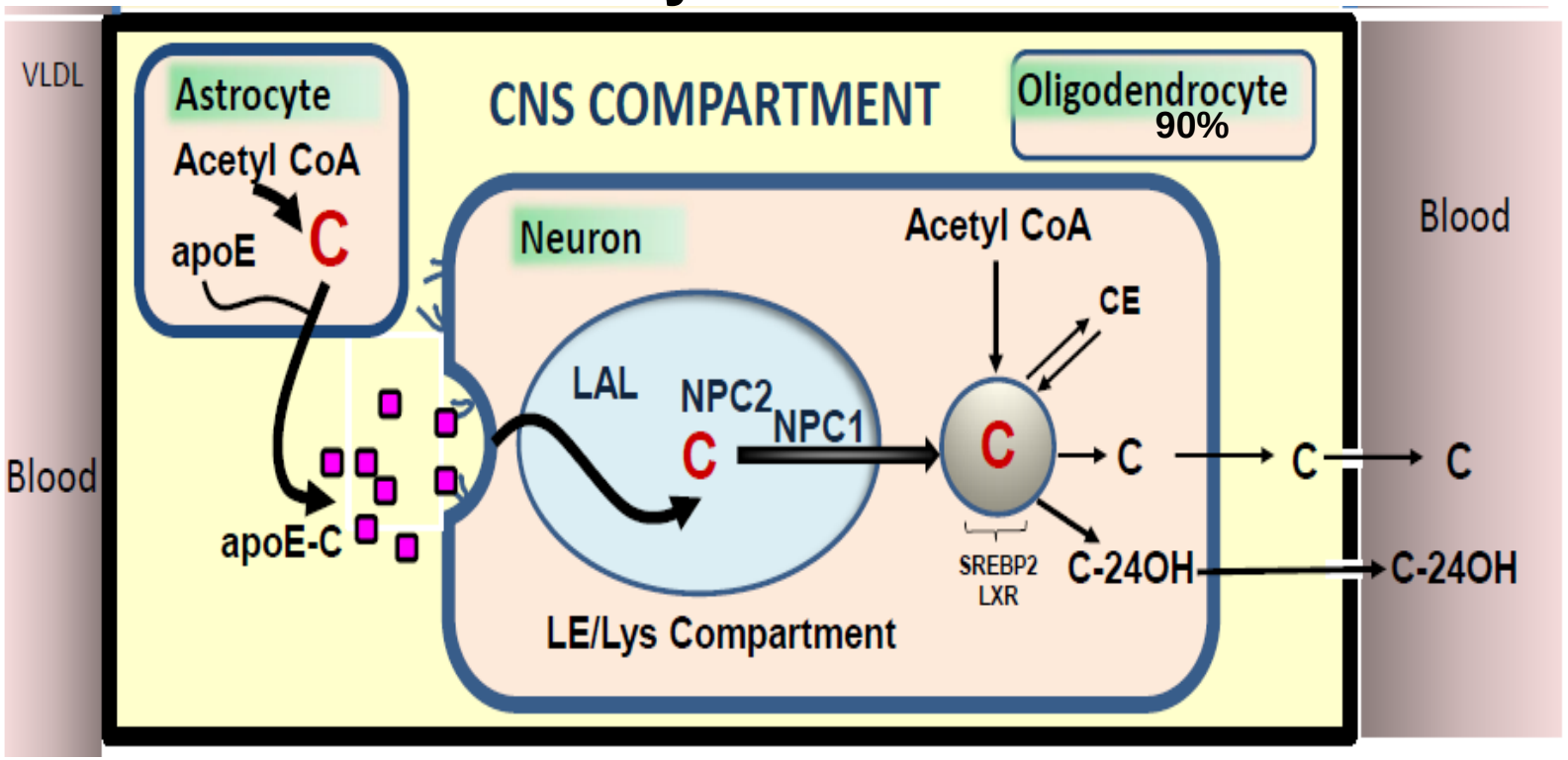
Vanier J Inherit Metab Dis. 2015;38:187



Different situation in brain

Adapted from Aqul et al J Neurosci, 2011 in Vanier J Inherit Metab Dis. (2015)38:187

The brain synthesizes the cholesterol it needs
No exogenous source (BBB)
But astrocyte to neuron……

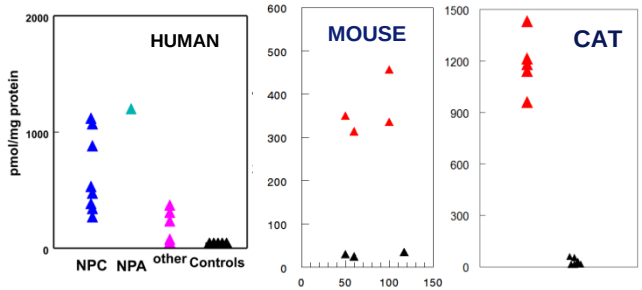


Tissues Lipid Storage Profiles
Minor sphingolipids

NON-NEURAL TISSUES

Spleen, Liver …

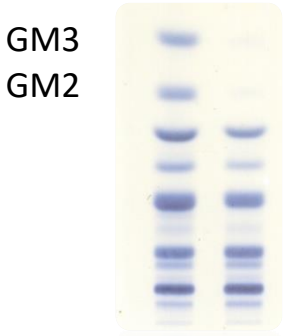
- **Glc-Cer**, **Lac-Cer**, Gb3
- GM3 (human, cat)
- **Free Sphingosine, liver: x30-50**



- **Sphingosine, fibroblasts**
  - Also elevated (variable level) BBA 1994
  - PKC inhibition Biochem J 1997;

BRAIN

- Large increase of **gangliosides GM2 and GM3**



  - Originate from recycling pathway
  - Cholesterol and SM trigger 2^{ry} storage of GM2
- Also Glc-Cer, Lac-Cer
- **Free sphingosine: x2-3**

Reviewed in Vanier J Inherit Metab Dis. 2015;38:187

Sphingosine storage and its potential role in pathophysiology of NP-C

- **NP-C cells, recent studies with « caged » sphingosine:**
  - Localization in LE/Lys, impaired transport out of lysosomes

    Höglinger et al 2016 Elife 4:e10616; 2017 PNAS 214:1566
- **Further work on PKC in NP-C** (Y Ioannou's group)
  - (2009) Inhibition of PKC, hypophosphorylation of vimentin, Rab9 entrapment: can be mimicked by sphingosine
  - (2013) PKC activation restores subcellular cholesterol transport
- **Lysosomal Ca^{++} is impacted in NP-C** (F Platt's group)

  Lloyd-Evans et al Nat Med 2008 14:1247
  - Which Ca++ channels are impacted and how - still a matter of debate **– TPC1, TPC2,** NAADP mediated signaling…
  - **This can be mimicked by sphingosine**;
  - No general agreement to the authors' postulate that sphingosine is the primary storage compound in NP-C, but sphingosine very likely plays a significant role in pathophysiology of the disease

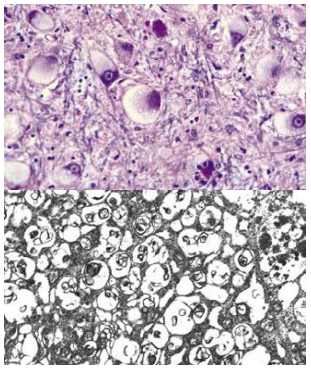
NP-C: Pathogenic events

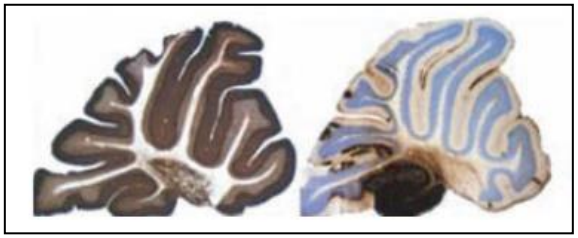
- **Impaired Ca^{++} filling of LE/Lys and reduced Ca^{++} release** Lloyd-Evans & Platt Cell Calcium (2011)50:200
  - **or reduced release by inhibition of a TRP channel** Chen et al Nature Commun (2012) 3:731
- **Impaired autophagic flux**
  defective amphisome formation – failure in SNARE machinery
  [Jaenisch's group] Cell Rep 2013
- **apoptosis**
- **ER stress**
- **Neuroinflammation**
- **Mitochondrial abnormalities**

See also Vance & Karten J Lipid Res (2014)55:1600; Vanier J Inherit Metab Dis. (2015)38:187

NP-C Neuropathology

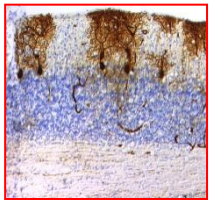
- **Neuronal storage**
- **Neuronal loss:**
  - Death of Purkinje cells
- **Alzheimer-like changes**
  - Neurofibrillary tangles
  - Tauopathy
- **Meganeurites and ectopic dendritogenesis**
- **Neuroaxonal dystrophy**
- **Inflammation**

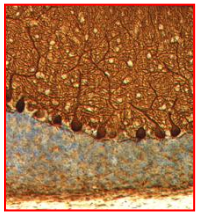


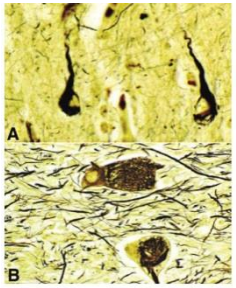


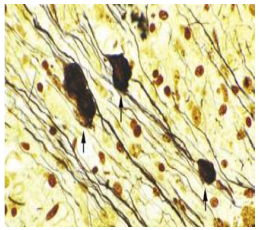
npc mouse cerebellum

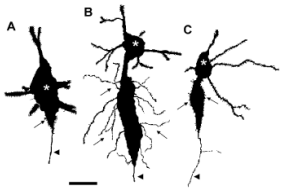
Mutant Wild type











Potential CNS Biomarkers in CSF

- **Proteins markers of neuronal damage**
  - Calcium-binding protein D (**Calbindin D**)
    - High level in cerebellum, localized in Purkinje cells
    - neuronal damage : elevation in CSF in NP-C

      Bradbury et al (2016) J Pharmacol Exp Ther 358:254
  - Fatty acid binding protein 3 (**FAB3**)

    Cologna et al (2012) PlOs ONE 7: e47845
- **Proteins that are altered in Alzheimer disease**
  - Tau

    Mattson et al (2011) Neurology 76:366
  - Amyloid β (Aβ) (γ-secretase dependent altered in NP-C)
- **Markers of inflammation**
  - Various cytokines

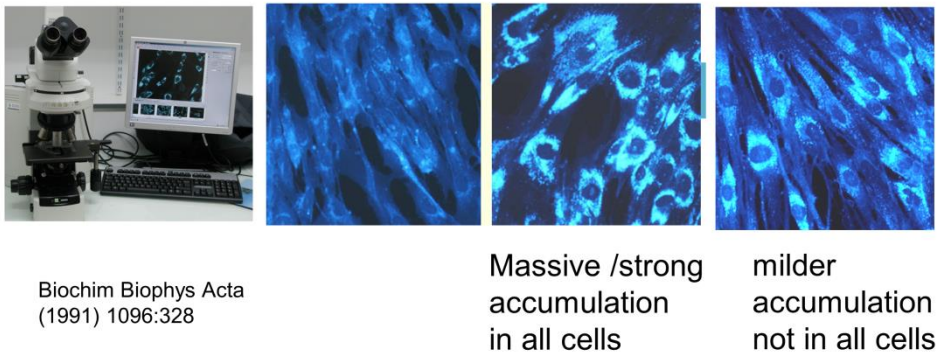
Diagnosis and Treatment

Laboratory Diagnosis

The Classical Tools

Cell Biology

Filipin staining in cultured fibroblasts



- Skin biopsy, cell culture, expert lab
  - >7weeks turn around time
- Problem of « variant » pattern (15% of families)
  - More frequent in adult onset patients
- Interpretation can be difficult (15% of cases)

Molecular Genetics

gDNA Sanger sequencing, NGS

NPC1/*NPC2* exons and junctions

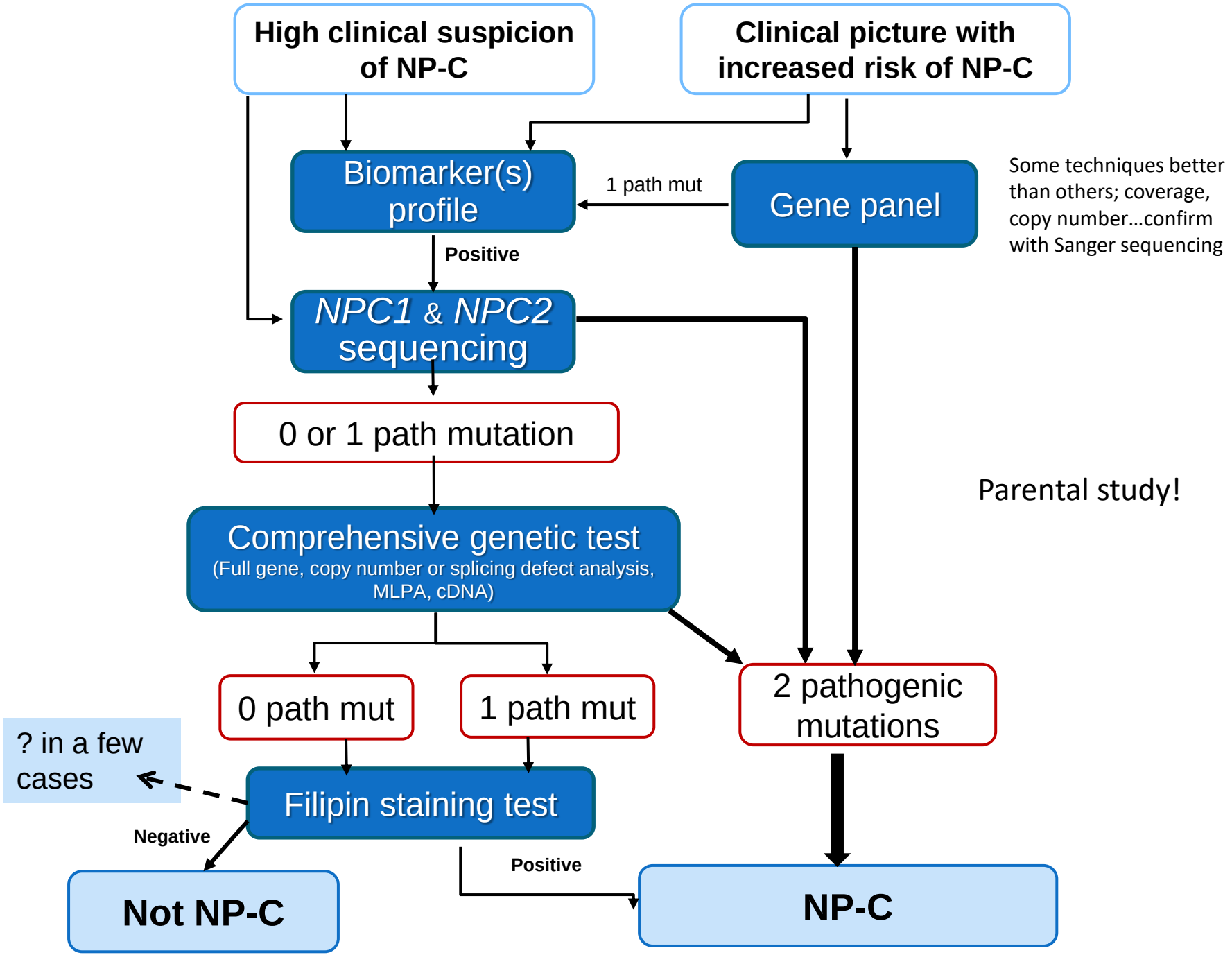
NPC1=25 exons; *NPC2*=5 exons

- Pathogenicity of new variants
- Not all mutations can be detected
- More refined testing can be needed
  - MLPA, cDNA…

New Tools for first line screening of NP-C Plasma biomarker profiles

- **"Oxysterols"** (initial study: Porter et al Science Trans Med 2010)
  - **Cholestane-3β,5α,6β-triol**
  - (7-ketocholesterol)
- **Lysosphingomyelin and analogues**
  - **LysoSM-509** (Giese et al ORJD 2015; Polo et al Clin Chem Lab Med 2016…)
  - **Lysosphingomyelin** (Welford et al PlosOne 2014; Polo et al Clin Chem Lab Med 2016
- **Bile acid metabolites:** Jiang et al Sci Transl Med 2016
  Mazzacuva et al FEBS Let 2016
  - N-(3β,5α,6β-trihydroxy-cholan-24-oyl)glycine

Technical limitation : access to sensitive LC- MS/MS machine


High clinical suspicion of NP-C
Clinical picture with increased risk of NP-C
Biomarker(s) profile
1 path mut
Gene panel
Some techniques better than others; coverage, copy number…confirm with Sanger sequencing
Positive
NPC1 & NPC2 sequencing
0 or 1 path mutation
Parental study!
Comprehensive genetic test
(Full gene, copy number or splicing defect analysis, MLPA, cDNA)
0 path mut
1 path mut
2 pathogenic mutations
? in a few cases
Filipin staining test
Negative
Positive
Not NP-C
NP-C

Current management of NP-C patients

- **Symptomatic therapy**
  - Cataplexy, **epilepsy,** spasticity, **dysphagia**…
- **One single drug currently approved** [not in USA] **for NPC** (neurological disease)**: miglustat**

  (iminosugar, inhibitor of glucosylceramide-synthase)
  - **Mode of action in NP-C appears more complex than substrate reduction** Stein et al 2012 J Neuropathol Exp Neurol 71:434-448.
  - Clinical trials + a number of published studies: **miglustat can slow down neurological progression of the disease**
  - **no effect on systemic symptoms**
  - GI problems: low disaccharide diet S. boulardii? (OJRD)

Treatment with Miglustat: Actelion Registry

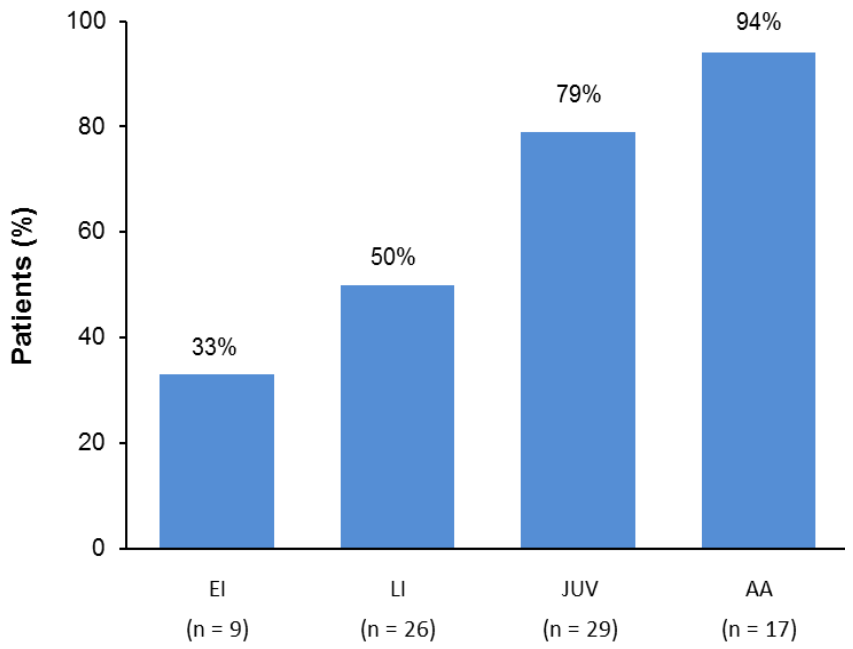
86 patients treated continuously for >1y

Proportion with stable/ improved disability

Observation period : 2 ± 0.7 years
A number already treated at enrolment

Clear correlation with age at neurological onset

- Works best in juvenile and adult neurological onset forms
- Relatively poorly in early infantile neurologic onset form



Patterson et al OJRD (2015) 10:65

Experience of miglustat in France

- **>70 patients treated since 2006**
  - Slightly more adults than children
  - Variable length of treatment, some ~10 y
- **Patients with later neurological onset globally much better responders (but not all)**
  - The disease continues to progress, but with a lower rate
  - Best results when start of treatment at early stage of neurological disease (we do not treat before neurological onset)
- **No long term effect on early infantile neurological form**

Clinical trials

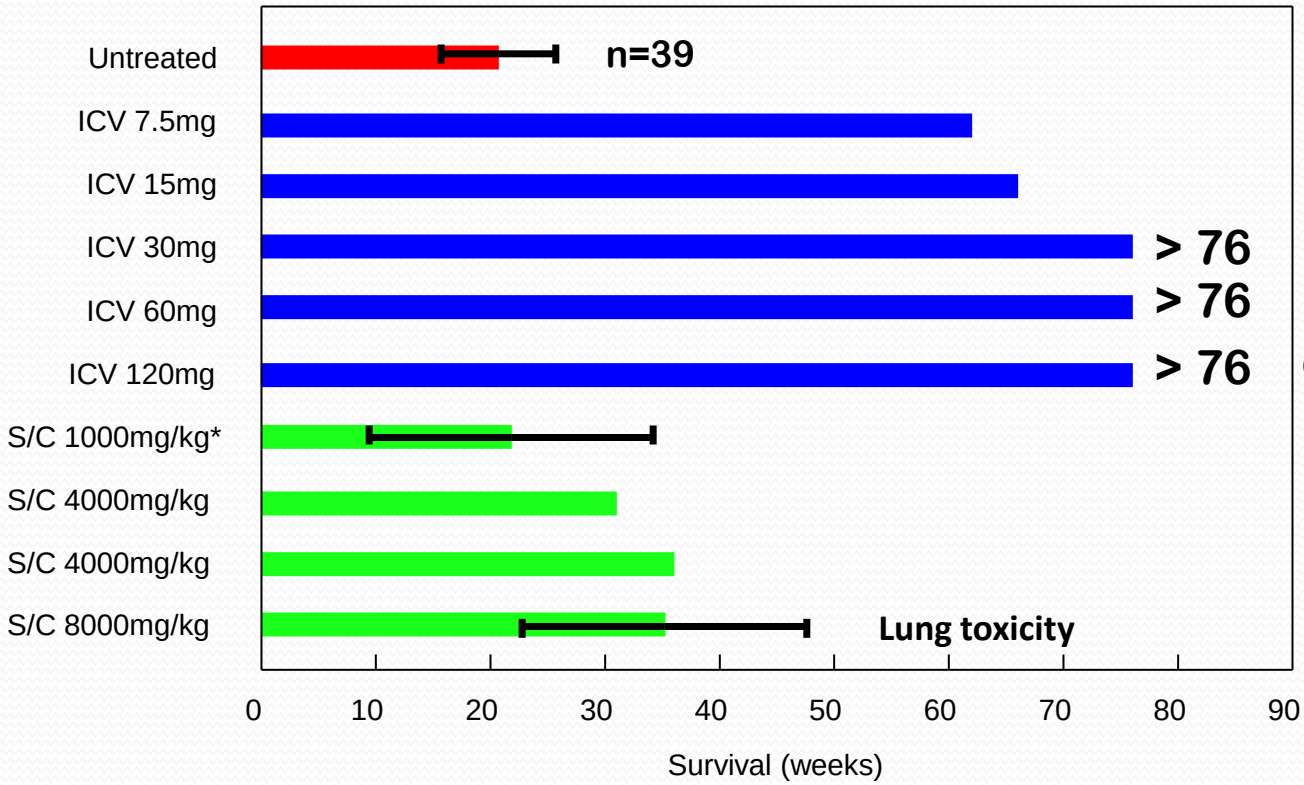
- **2 Compounds**
  - **2-HP-β–Cyclodextrin**: cyclic oligosacccharide with a hydrophobic core , widely used as pharmaceutical excipient (also food, cosmetics, Febreze…)
    - – detailed preclinical data in NPC mouse and cat
    - – does not significantly cross the BBB
  - **Arimoclomol** = Hydroxylamine derivative – **Hsp70/90co-inducer** in cells under stress

    limited preclinical data on NPC mouse model and cells
- **3 Ongoing Trials**
  - **2-HP-β-cyclodextrin** (Kleptose HPB) **intrathecal** (currently via LP)
    - **VTesse/Sucampo: VTS-270** : phase 2b-3
  - **2-HP-β-cyclodextrin** (Trappsol) **IV**
    - **CTD-Holdings**
  - **Arimoclomol (per os)**
    - **Orphazyme: AIDNPC-002:** phase 2-3

HPßCD in the cat model

Presymptomatic Treatment Groups

Survival Study



Oldest still alive at 95 wks

Oldest still alive at 121 wks

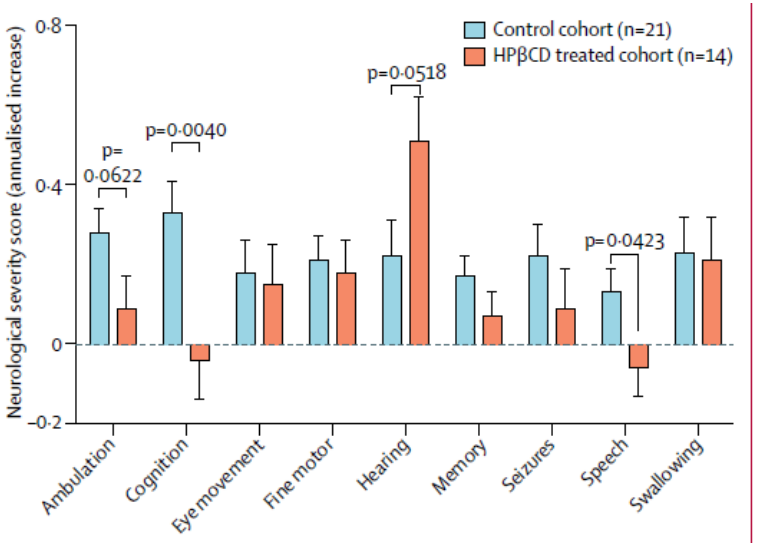
*+allopregnanolone

Vite et al Sci Transl Med (2015) 7:276ra26

2-HP-ß-CD

- **Promising results (by ICV) in the *Npc1* cat model**
  Vite et al (2015) Sci Transl Med
  - Reduces multiple lipid accumulation in brain (neurons), improves Purkinje cells survival, prolongs life significantly even if already symptomatic at initiation of therapy - Safety issue: ototoxicity

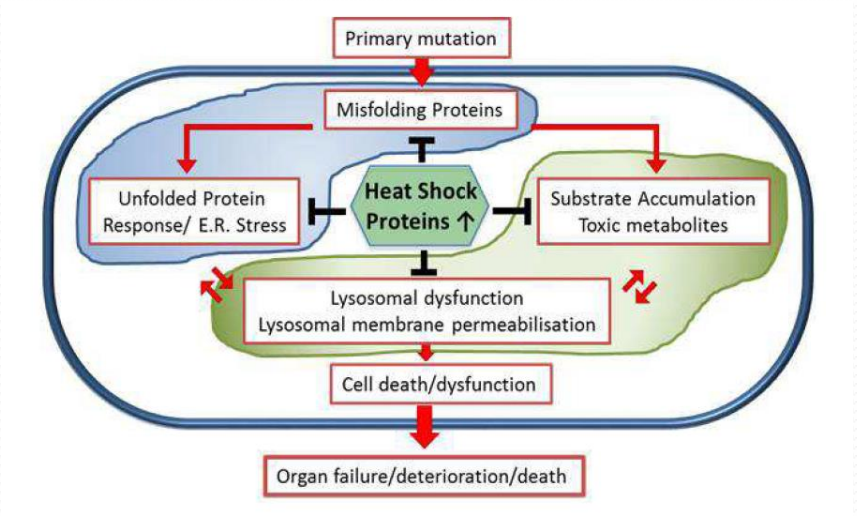
- **VTS-270 [KleptoseHPB] intrathecal [+/- miglustat]**
  - **Phase 1/2a**
    - dose escalation (50-1200mg)
    - Results Ory et al (2017) Lancet Neurol
      - Safety: ototoxicity (high frequencies)
      - Severity scores



  - **Phase 2b/3 ongoing**
    - **international, multicentric,**
      - Patients aged 4-21 years (neuro onset <15y) 1/3 sham + SoC – 12 months
      - Open label extension

Arimoclomol

Known protective role of hsp70 and hsp40



- Some experimental work on the potential role of certain heat shock proteins in NPC pathology - Nakasone et al J Biol Chem 2014

- **Clinical trial: Sponsor: Orphazyme – Europe and USA**
  - **Patients with NPC: aged 2-18 years** **[+/- miglustat]**
  - **Interventional period: 12 months**

    2/3 arimoclomol + SoC **1/3 Placebo+ SoC** (escape route)
  - **Open-label extension**

Other experimental paths explored by different teams

- **Histone deacetylase (HDAC) inhibitors**
  - Vorinostat ameliorates cholesterol storage in NP-C fibroblasts with certain missense mutations (p.I1061T)
  - Vorinostat, phase 1 trial in adult NP-C patients completed
    - Problem: Vorinostat does not cross the BBB
  - In Npc1 mouse ^{D1005G}: Combination of Vorinostat/HPBCD/PEG by IP route in D1005G mouse: doubles lifespan….
    - Alam et al Sci Transl Med 2016
- **Gene therapy in NP-C1 :**
  - at least 3 teams are trying in mouse and cat models
  - In theory very difficult (NPC1 not secreted not recaptured) but some interesting preliminary results

Only for NP-C2: HSCT

- **NPC2 protein is a soluble protein, transported to the LE/Lys compartment through the Mann-6-P pathway**
  - Secreted, recaptured
  - Good rationale for HSCT
- **Only one case with known follow-up**
  - only half successful

Future

- **Likely combinatorial therapies**
  - Directed toward different levels of the pathogenic cascade
    - Defective protein (proteostasis modulators, chaperones)
    - Lysosomal storage
    - ER stress
    - Impaired autophagic flux
    - Impaired lysosomal Ca^{++} homeostasis
    - Immunomodulators

Beyond the disease… NP-C as a model

- **NP-C has led to the discovery of important players - NPC2 and NPC1 - in homeostasis and trafficking of cellular cholesterol**
- **Also to a better understanding of the interplay between cholesterol and sphingomyelin**
- **useful in the study of Ca++ and K+ channels**
- **Recent work reveals a coupling of cholesterol trafficking through the lysosome to regulation of cellular growth signaling implicating an NPC1-SLC38A9 complex and mTORC1**

Science (2017) 355: 1306

- **And more……..**