A Niemann-Pick C betegség biomarkerei

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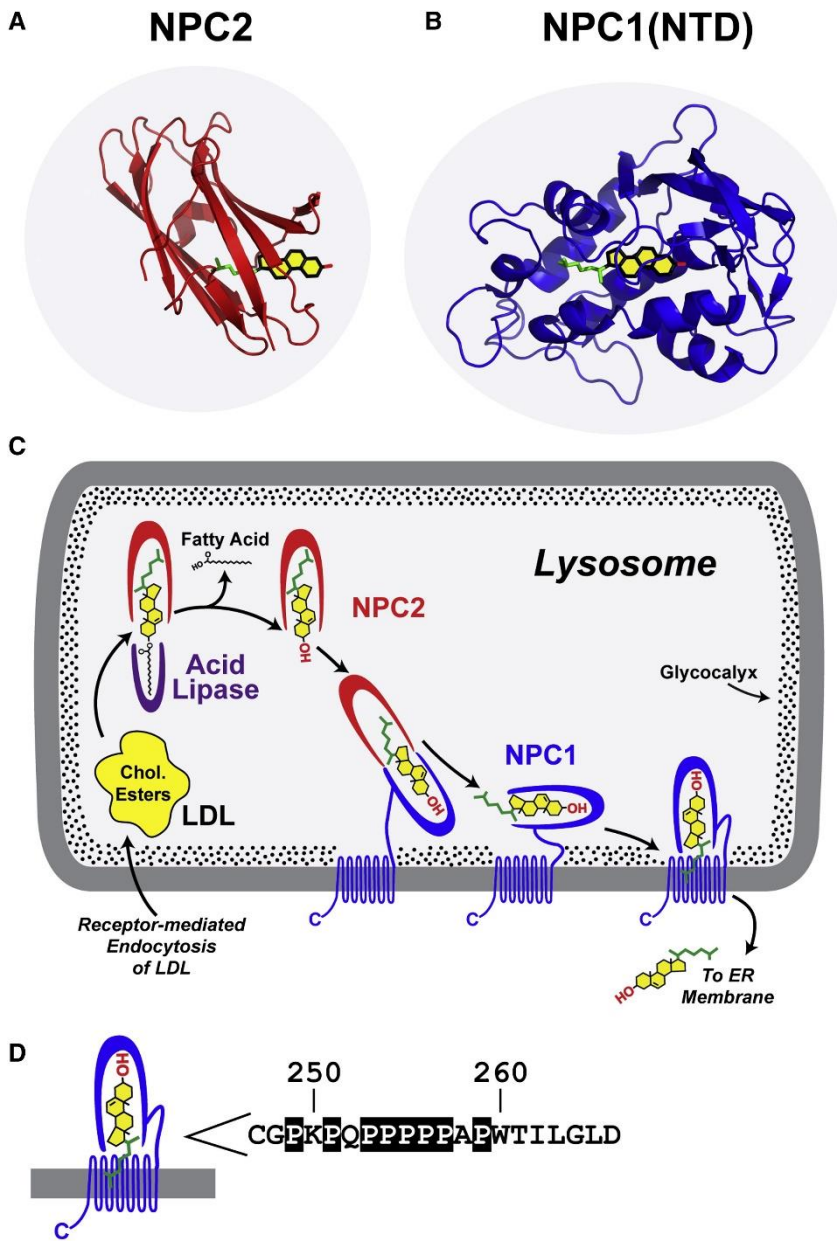
SEMMELWEIS EGYETEM LABORATÓRIUMI MEDICINA INTÉZET

TÖMEGSPEKTROMETRIAI RÉSZLEG

BIONIKAI INNOVÁCIÓS KÖZPONT NONPROFIT KFT.

Rövid összefoglaló

- A Niemann-Pick C típusú betegség autoszomális recesszív öröklődésű, progresszív, irreverzibilis lizoszomális tárolási rendellenesség;
- prevalenciája kb. 1:120 000 → Magyarországon 60-80 beteg lehet;
- felismerésére bármely életkorban van esély: Reunert et al. a betegek 40%-át 20 éves kor felett azonosították (Reunert J et al. EBioMedicine 2016;4:170-5);
- a tünetek enyhítésére, az életminőség javítására elérhető gyógyszer (miglustat), de gyógyítására, a progresszió késleltetésére egyelőre nincs mód;
- az esetek 95%-ában az NPC1, 4%-ában az NPC2, kb. 1%-ban egyéb, azonosítatlan gén mutációja áll a hátterében.



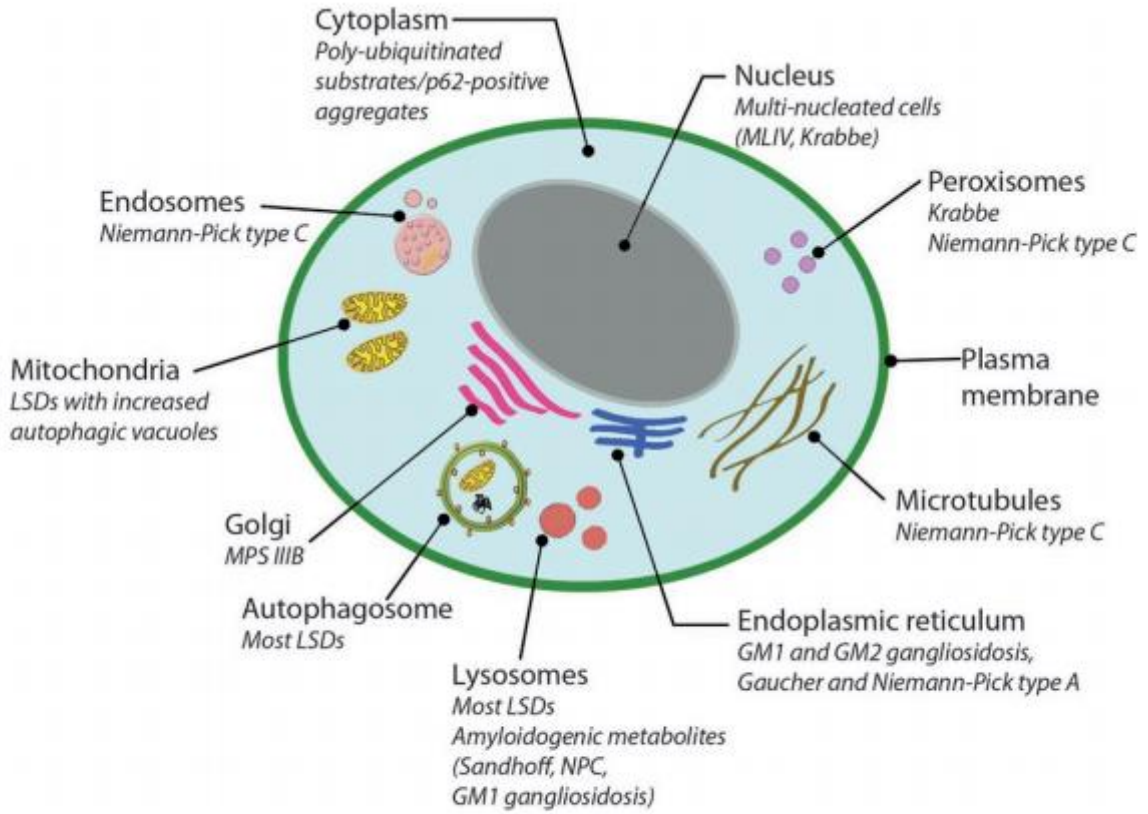


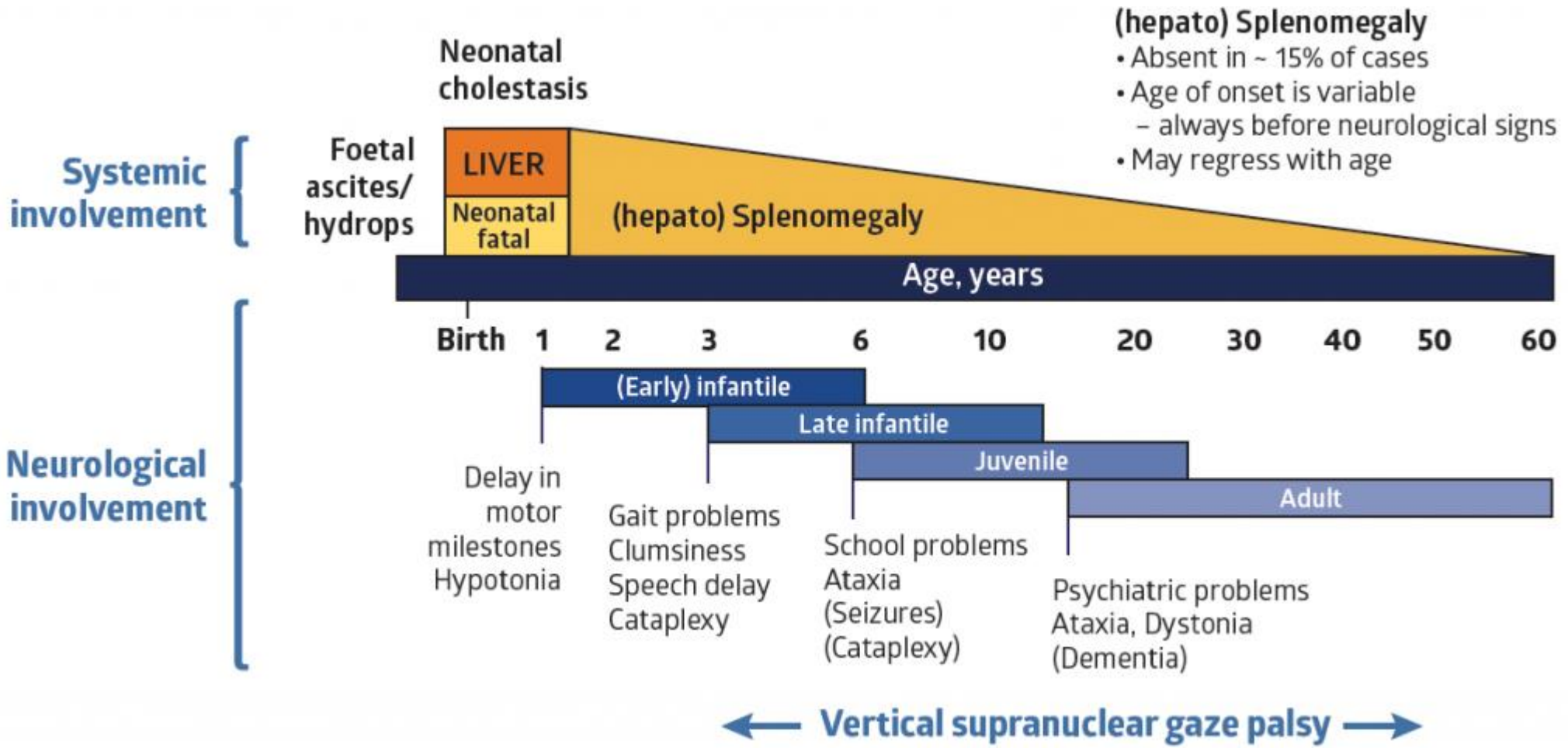
Figure 3. **Summary of organelles affected in LSDs.** Also shown are selective examples of LSDs. See Table 1 and main text for details.

Platt FM et al. J Cell Biol 199 (2012):723.

Az NPC tünetegyüttese

- **Zsigeri tünetek:** hepatomegalia, splenomegalia, elhúzódó újszülöttkori sárgaság, hydrops foetalis, foetalis ascites, aspirációs pneumonia, alveolaris lipidosis, tüdő kötőszöveti elváltozásai
- **Neurológiai tünetek:** vertikális supranuclearis tekintet bénulás, gelasztikus kataplexia, ataxia, dystonia, dysarthria, dysphagia, hypotonia, nehézkes mozgás, fejlődési zavarok, görcsök, halláskárosodás
- **Pszichiátriai tünetek:** fejlődési zavarok, korai kognitív hanyatlás, szervi pszichózis, viselkedés zavarok, terápia rezisztens pszichiátriai tünetek progresszív kialakulása

Mengel E et al. Orphanet J Rare Dis 8 (2013):166.
Faludi G et al. Neuropharmacol Hung 15 (2013):95.



http://www.niemann-pick-c.com/HCP/Symptoms/Symptomatic-manifestations-of-Niemann-Pick-type-C-disease

	Visceral	Neurological	Psychiatric	Family History
Very Strong		★ Gelastic cataplexy ☐ ★ Vertical supranuclear gaze palsy ☐		★ Parent or sibling with NP-C ☐
Strong	★ Isolated unexplained splenomegaly (historical and/or current) with or without hepatomegaly ☐ ★ Prolonged unexplained neonatal jaundice or cholestasis ☐		★ Pre-senile cognitive decline or dementia ☐	★ Cousin with NP-C ☐
Moderate		Dystonia ☐ Dysarthria and/or dysphagia ☐ Ataxia, clumsiness or frequent falls ☐	★ Psychotic symptoms (hallucinations, delusions and/or thought disorder) ☐	
Weak		Acquired & progressive spasticity ☐	Other psychiatric disorders ☐ Treatment-resistant psychiatric symptoms ☐	
Ancillary	Sibling with foetal ascites ☐ Hydrops foetalis ☐	Hypotonia ☐ Myoclonus ☐ Seizures (partial or generalised) ☐ Delayed developmental milestones ☐	Disruptive or aggressive behaviour in adolescence or childhood ☐	

http://www.npc-si.com/tools/np-c-si4y-tool/

Megváltozott lipid metabolom: **szfingolipidek**

monohexozil-ceramidok

szfingoid bázisok

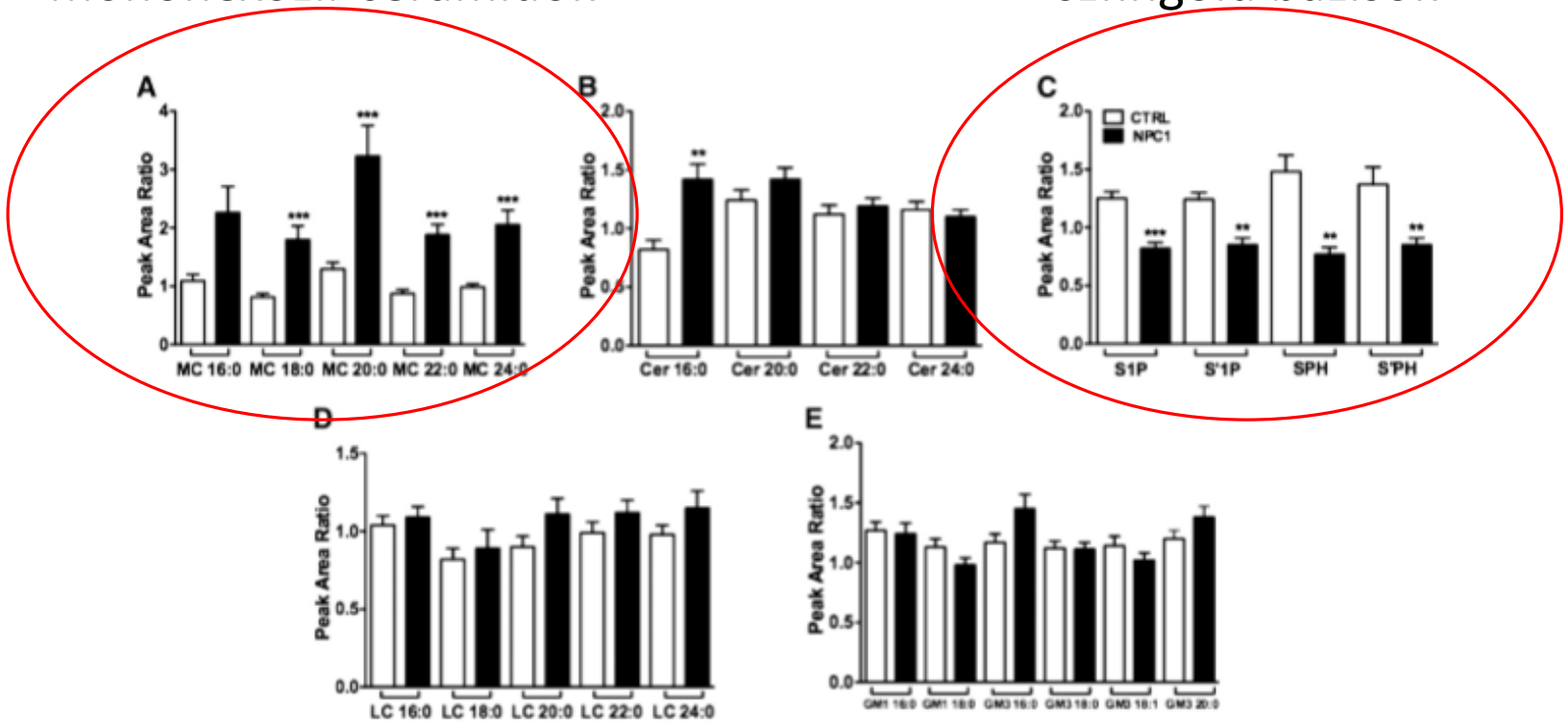


Fig. 4. Plasma sphingolipid profiles in NPC1 subjects. Plasma concentrations (peak area ratios) are shown for MCs (A), ceramides (Cer) (B), sphingoid bases (C), lactosylceramides (LC) (D), and gangliosides (E) for age-matched control and NPC1 subjects (n = 56/group). Data are shown as mean ± SEM; $^{**}P < 0.01$, $^{***}P < 0.001$.

Fan N et al: J Lipid Res 54 (2013):2800.

Megváltozott lipid metabolom: **szterolok**

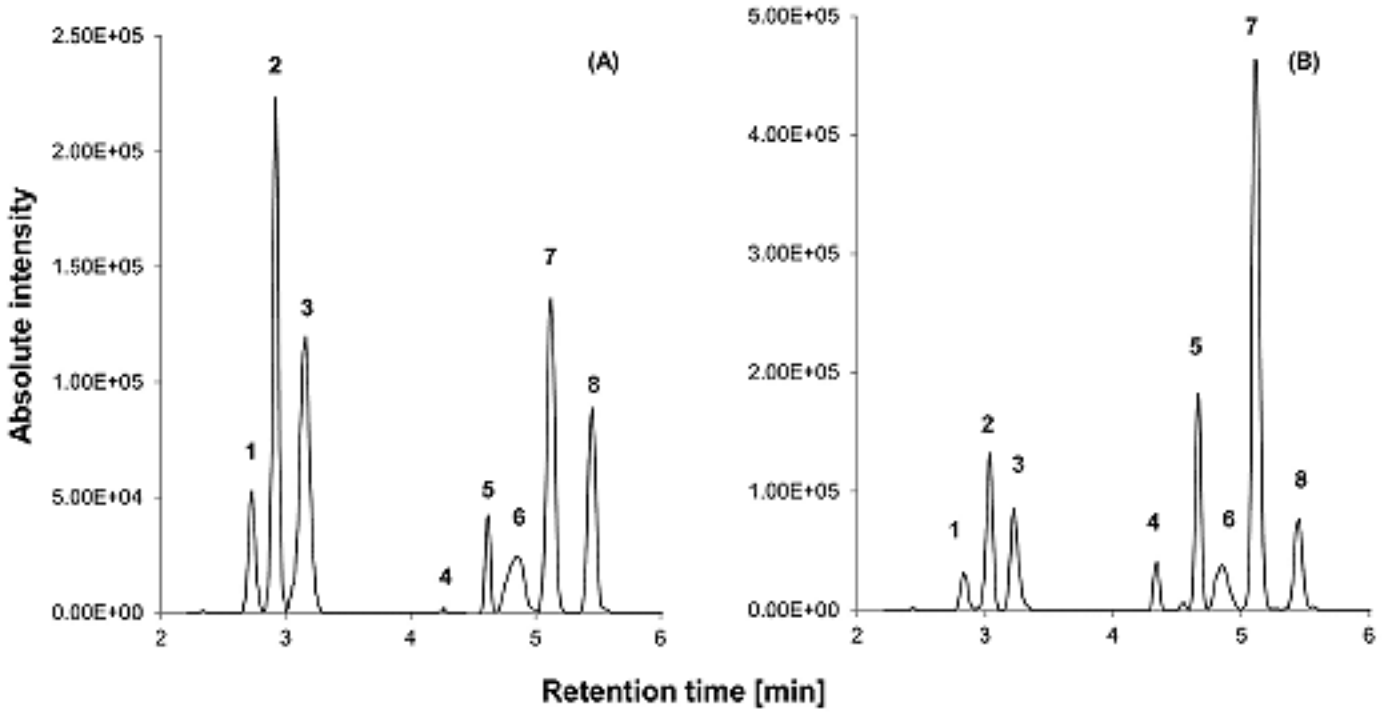


Fig. 4. Selected PRM chromatograms for the separation of oxysterol derivatives in NPC patients. (**A**) Heterozygous family member of the patient; (**B**) Niemann–Pick type C patient; elution order: (**1**) 25-hydroxycholesterol-DMG; (**2**) 24(S)-hydroxycholesterol-DMG; (**3**) 27-hydroxycholesterol-DMG; (**4**) cholestan-3β,5α,6β-triol-DMG; (**5**) 7β-hydroxycholesterol-DMG; (**6**) 7α-hydroxycholesterol-DMG; (**7**) 7-ketocholesterol-DMG; (**8**) 4β-hydroxycholesterol-DMG; for chromatographic conditions see Section 2.3.

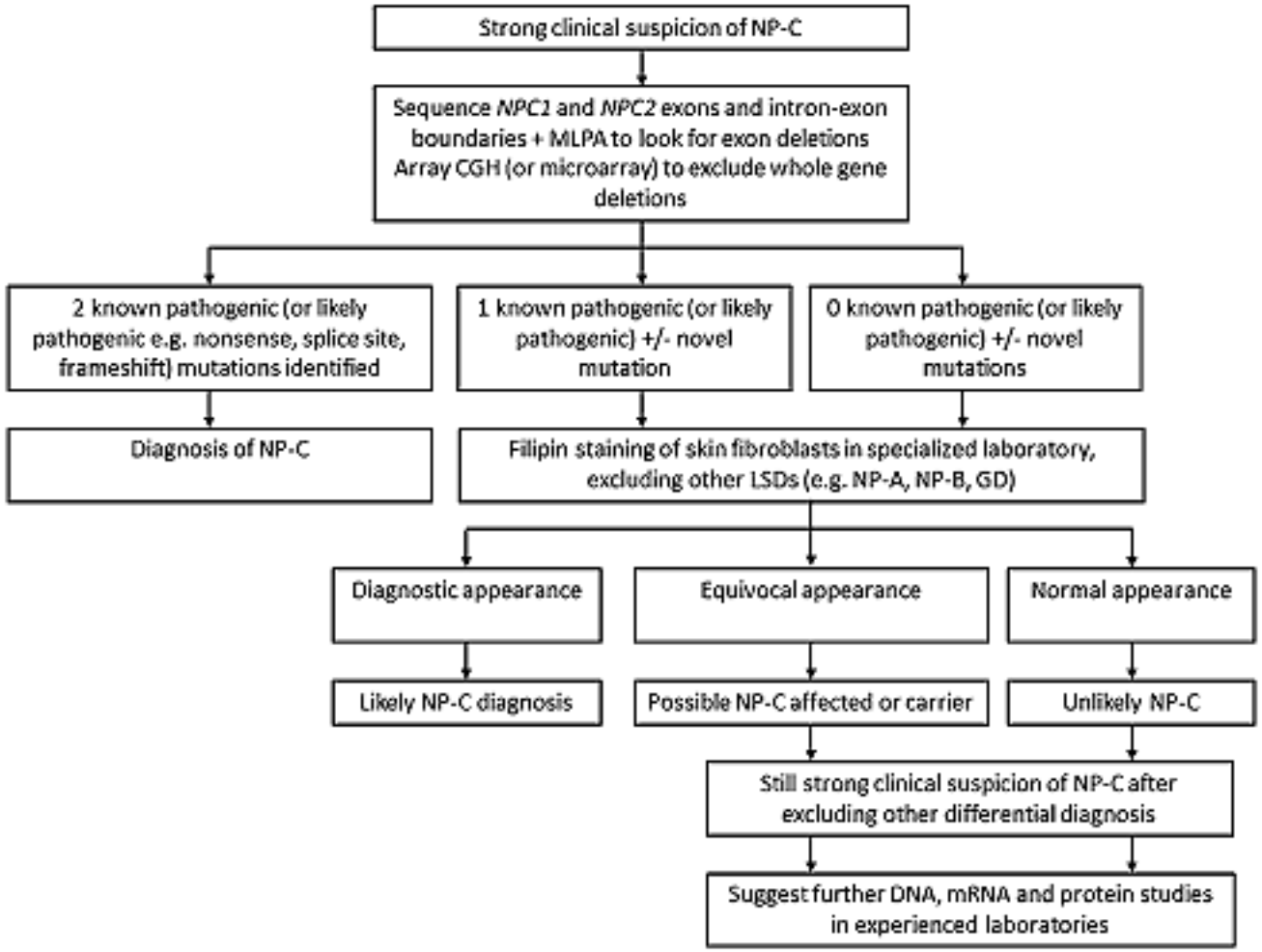
Pataj Z et al. J Chromatogr A 1439 (2016):32.

Az NPC diagnózisának jelenlegi irányai

- **„emerging biomarkers”:**
  - oxysterolok
  - szfingolipidek
  - epesav metabolitok

- **kiforrott vizsgálati lehetőségek:**
  - genetikai vizsgálat
  - fibroblasztok festése (pl. Filipin-teszt)

- **célzott metabolom vizsgálatok**



KcKay Bounford K, Gissen P. J Neurol 261 (2014):S569.

A Filipin-festési eljárás

bőr biopsziával fibroblasztokat nyernek

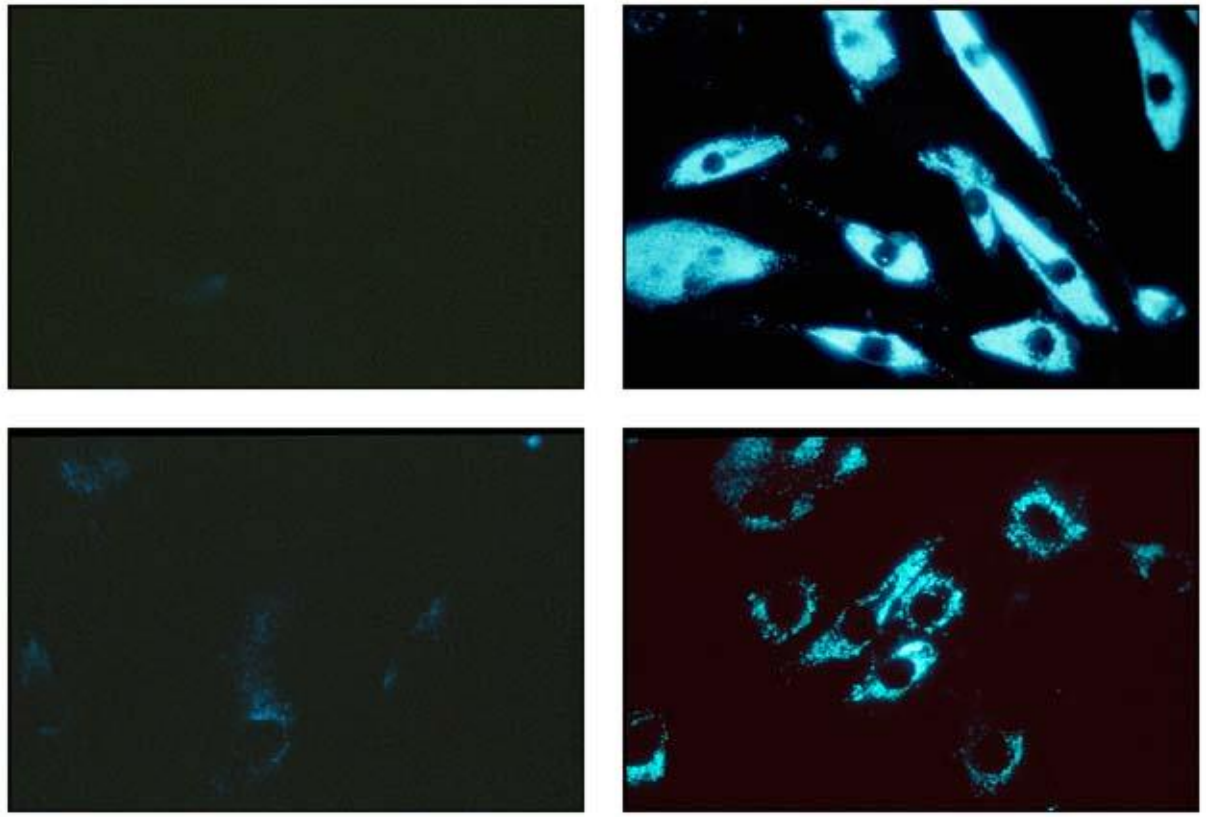
mosást követően a koleszterint filipinnel festik

a mintát fluoreszcens mikroszkóp alatt vizuálisan vizsgálják

létezik kvantitatív eljárás is

egészséges személy mintája

NPC-vel diagnosztizált személy mintája



http://www.tufts.edu/~lliscum/ResProj.html

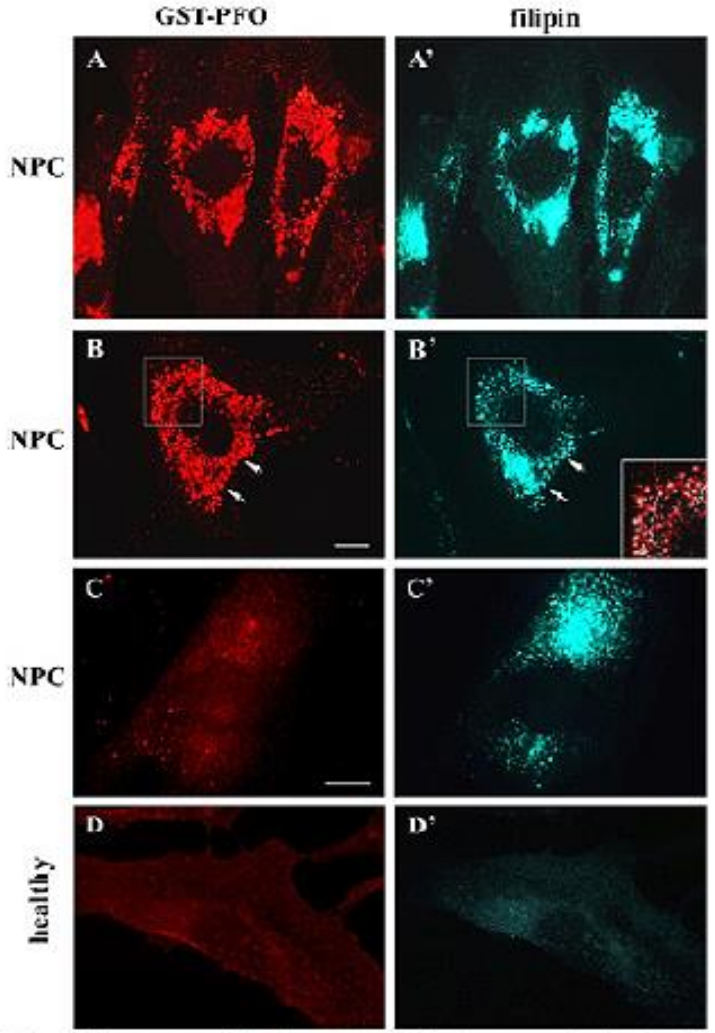


Figure 2 GST-PFO labels cholesterol deposits in NPC fibroblasts. (A-C) NPC fibroblasts were cultured in DMEM/10% FBS (**A, A'**) or in DMEM supplemented with delipidated 10% FBS for 72 h (**B-C'**). Inset in (**B'**) shows an enlargement of a merged image of an area marked in (**B, B'**). Colocalization of GST-PFO and filipin staining is in white. Colocalization of the labels is also seen in vesicles marked by arrows in (**B, B'**). (**C, C'**) Omitting GST-PFO during labelling of permeabilized NPC cells yields only traces of non-specific staining with secondary antibodies (**C'**). In these conditions, filipin detects cholesterol accumulated in the cells (**C'**). (**D-D'**) Fibroblasts from a healthy donor were cultured in DMEM/delipidated 10% FBS. Cells were fixed, permeabilized with 0.05% Triton X-100 and incubated with 5 μg/ml GST-PFO followed by secondary antibodies

Kwiatowska K et al. Orphanet J Rare Dis 9 (2014):64.

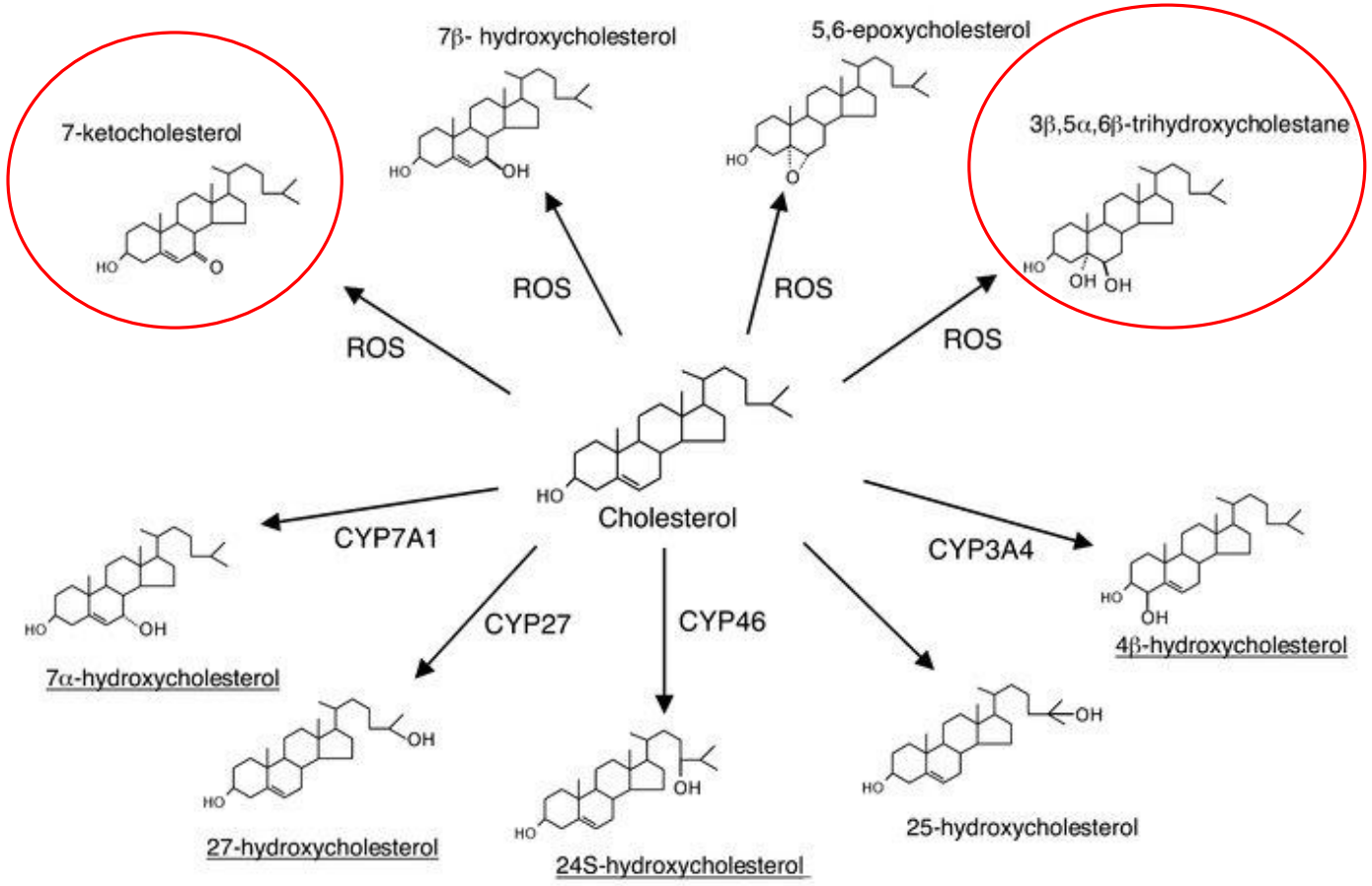
Table 2
Plasma and urinary diagnostic biomarkers in NP-C: an overview of advantages and limitations.

Technique	Advantages	Limitations
Plasma oxysterol testing (LC-MS/MS or GC–MS; quantitative)	• Elevated in the majority of patients with NP-C (NP-C1 and NP-C2) • Minimally-invasive – small volume of frozen EDTA plasma or serum • Rapid analysis – results often available within 1 week • Able to identify the majority of adult patients • Suitable for analysis of a large number of samples • Cost savings *versus* filipin testing	• Unspecific – 7-KC and C-triol are elevated in other diseases. Even the more specific C-triol does not distinguish NP-C from NP-A/B, acid lipase deficiency and some other conditions (including CTX and some causes of neonatal cholestasis) • Possibility of false positive results due to incorrect handling or storage of samples • Limited knowledge of range values in heterozygotes • Not applicable to dry blood spots
Plasma lysosphingolipids (LC-MS/MS; quantitative)	• Minimally-invasive – small volume of blood • Potential for fewer pre–analytical problems *versus* oxysterol testing • Concomitant study of lyso-SM-509 and SPC differentiates NP-C • High throughput • Multiplex assay can identify other LSDs – meet demands; faster turnaround time • Cost savings *versus* filipin testing	• Lyso-SM-509 does not clearly distinguish NP-C from NP-A/-B • SPC alone does not have sufficient sensitivity as an NP-C biomarker • Experience in clinical laboratory setting is still limited
Bile acid metabolites plasma; dried blood spots; urine (LC-MS/MS; quantitative)	• Non- or minimally-invasive • Current data indicate good discriminatory power • Cost savings *versus* filipin testing	• Also elevated in NP-A/B • Possibly other diseases? • Experience in clinical laboratory setting still lacking

C-triol: cholestane-3β,5α,6β-triol; CTX: cerebrotendinous xanthomatosis; GC: gas chromatography; 7-KC: 7-ketocholesterol; LC: liquid chromatography; LSDs: lysosomal storage disorders; Lyso-SM-509: lysosphingomyelin-509; MS: mass spectrometry; NP-A, -B, -C: Niemann-Pick disease types A, B, C; SPC: sphingosylphosphorylcholine (lysosphingomyelin).

Vanier MT et al. Mol Genet Metab 118 (2016):244.

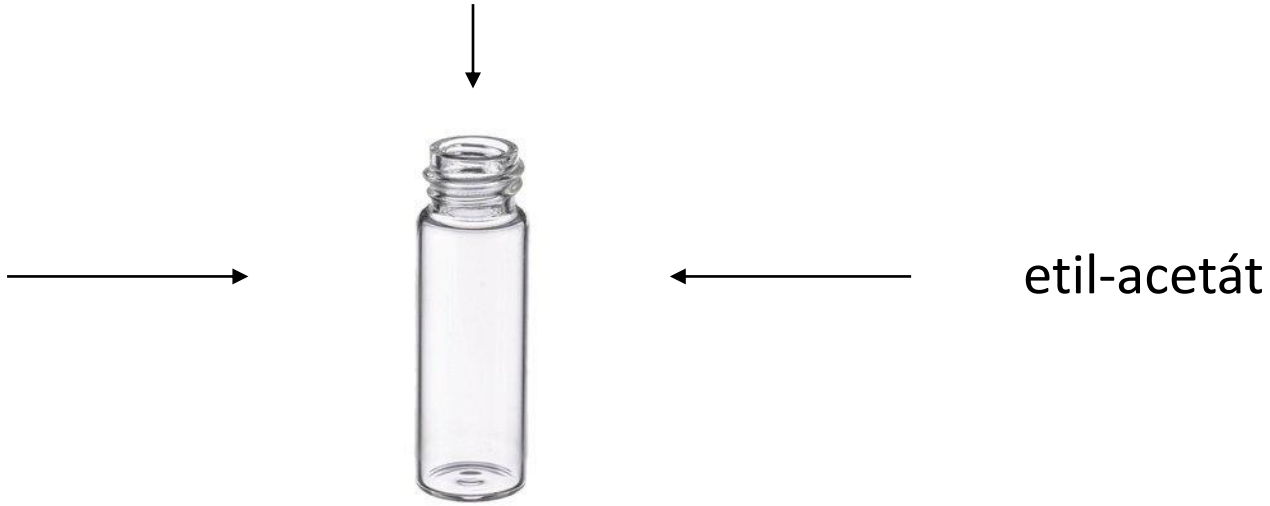
Oxysterol vegyületek keletkezése



Björkhem I: J Clin Invest 110 (2002):725.

Az oxysterol vizsgálat menete (Boenzi után): deuterált belső standard mintához adása, folyadék-folyadék extrakció

D7-C-triol, D7-ketokoleszterin

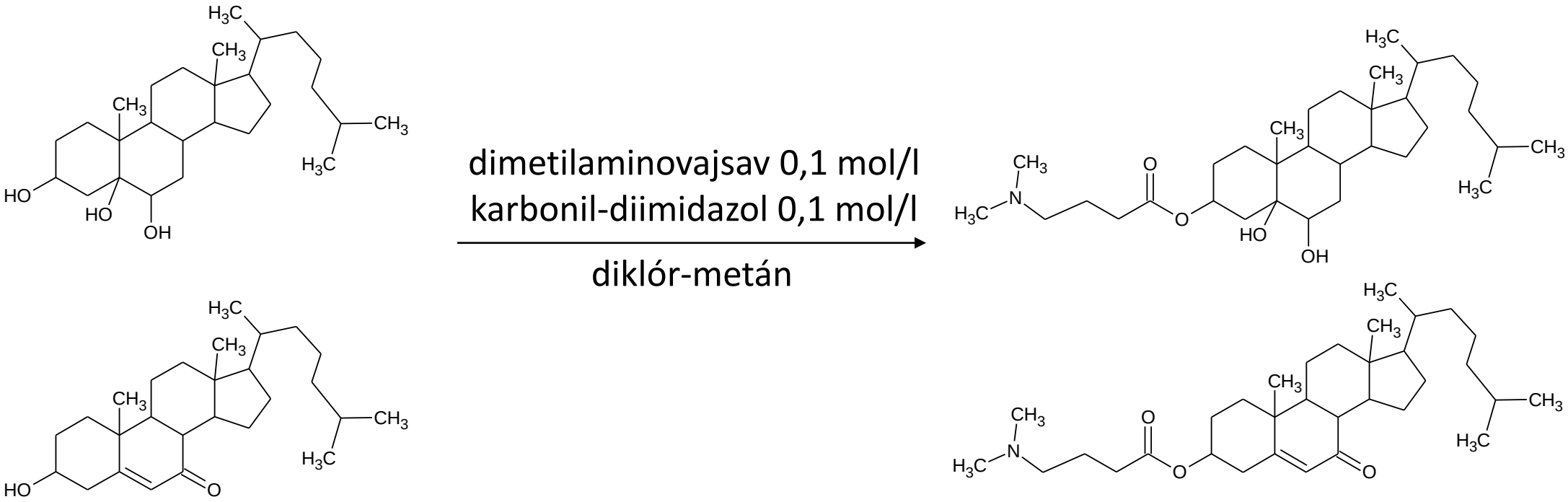


etil-acetát

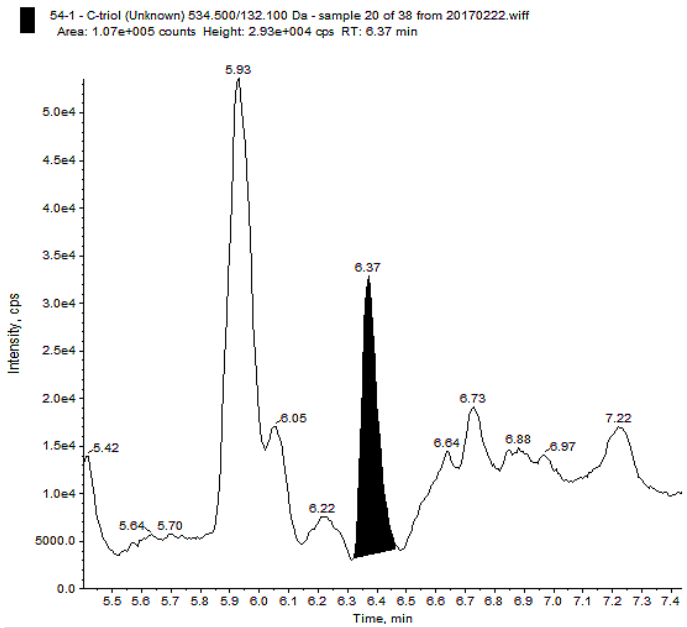
A mintavétel után a plazmát azonnal el kell választani, és le kell fagyasztani.

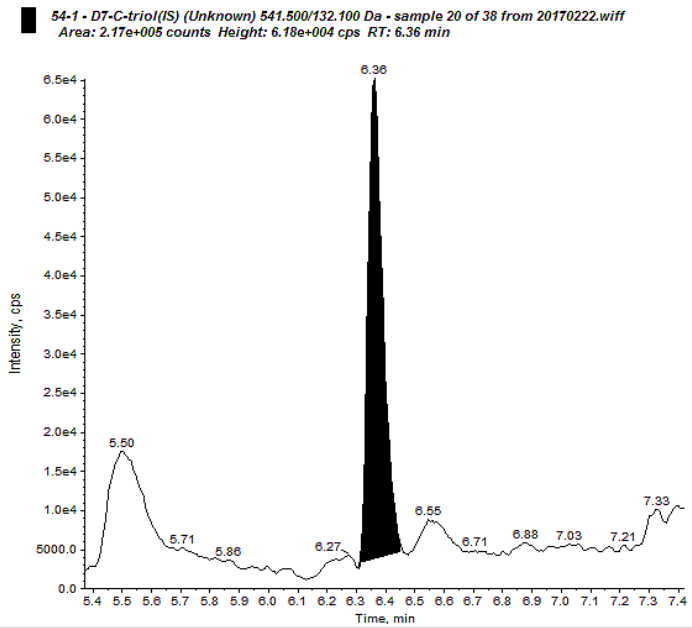
Boenzi S et al: Clin Chim Acta 437 (2014):93.

Az oxysterol vizsgálat menete: dimetilaminobutirát származékok képzése



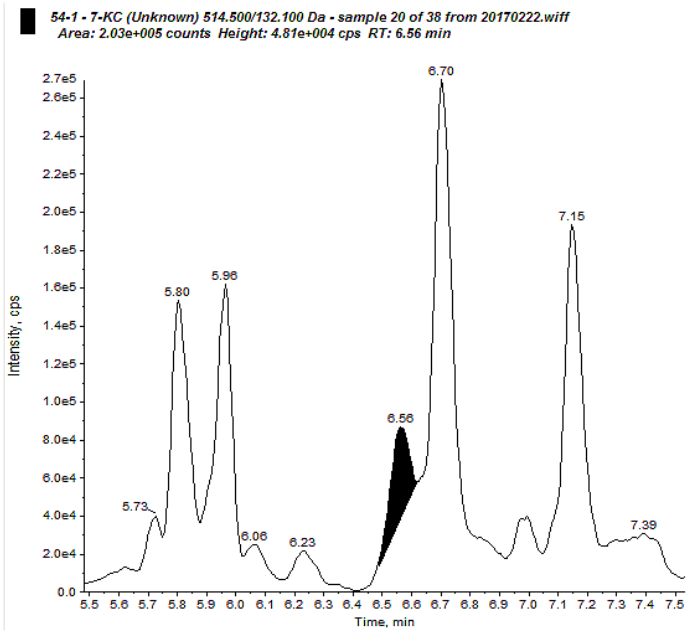
C-triol
(36,3 ng/ml)

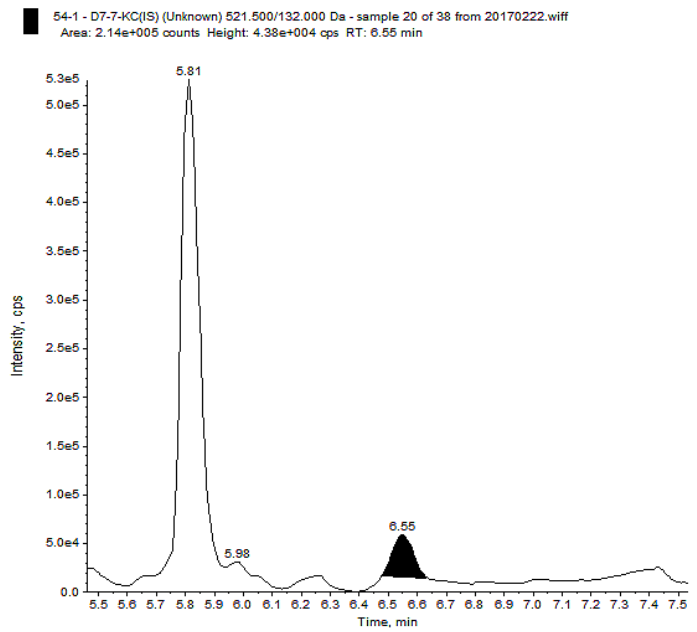




D7-C-triol
(100 ng/ml)

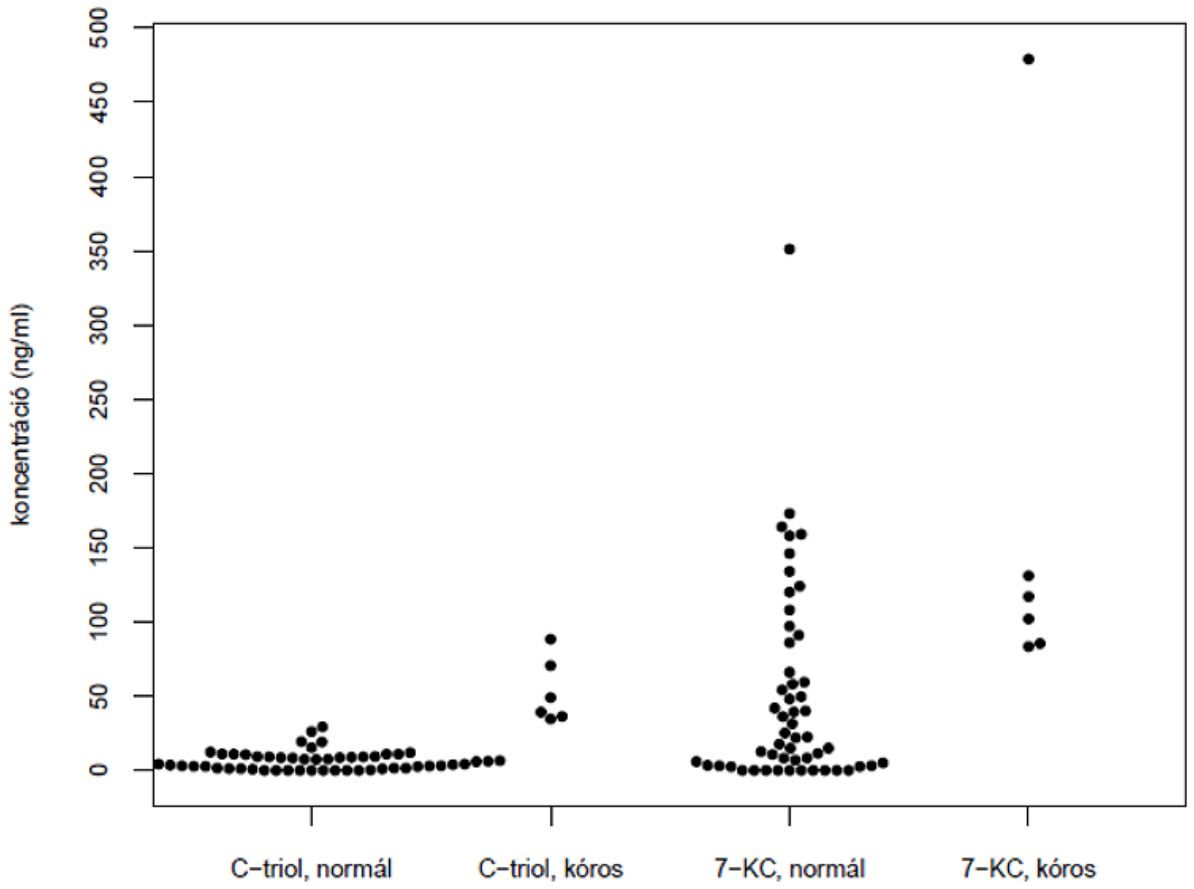
7-ketokoleszterin
(85,4 ng/ml)

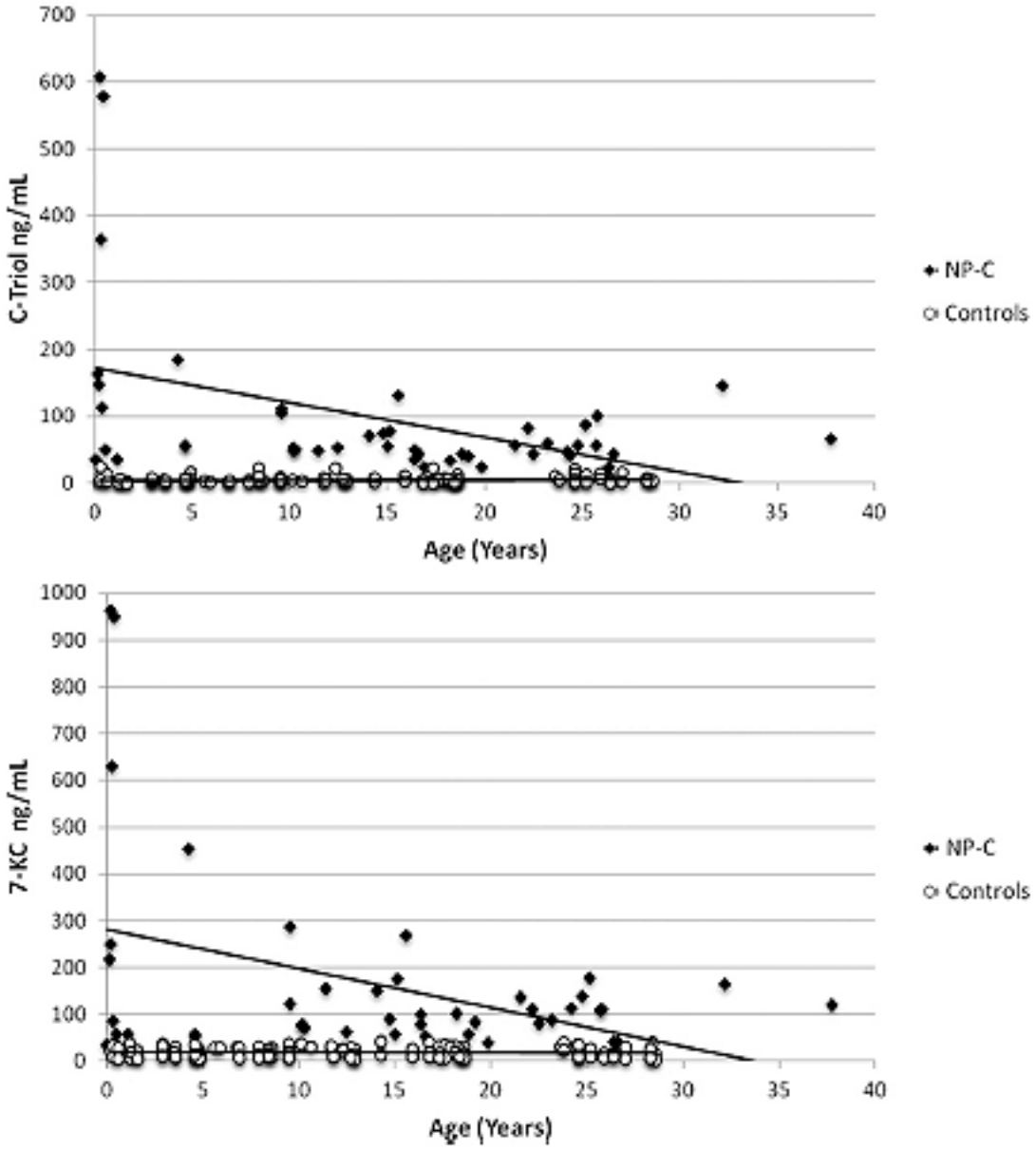




D7-ketokoleszterin
(100 ng/ml)

C-triol és 7-ketokoleszterin szintek egészséges és kóros mintákban





Boenzi S et al: J Lipid Res 57 (2016):361.

Javasolt referencia tartományok az oxysterol vizsgálathoz

Hivatkozás	7-KC	C-triol
Jiang X et al	< 47,8 ng/ml	< 38,0 ng/ml
Boenzi S et al	3,7 – 21,8 ng/ml	12,8 – 38,8 ng/ml
Wu et al	nem vizsgálják	8,1 – 37,7 ng/ml
Polo et al	7,5 - 85,5 ng/ml	3,0 - 35,0 ng/ml

LAUNCH OF NEW OXYSTEROL ASSAY FOR FASTER AND EASIER DIAGNOSIS OF NIEMANN-PICK C

Willink Biochemical Genetics Laboratory, Central Manchester University Hospitals NHS FT

We are pleased to announce that as from April 2014 we are now offering a service for Oxysterol analysis for the investigation and diagnosis of Niemann-Pick C (NP-C).

We believe this test will prove to be a big step forward in improving the ease and speed at which NP-C can be diagnosed, since it only requires a blood sample rather than cultured fibroblasts. We would like to encourage all to think about and test for NP-C at a much earlier stage than has previously occurred for this underdiagnosed disorder. Jaundice / hepatosplenomegaly shortly after birth and later ataxia, dystonia, dysarthria, epilepsy, intellectual decline and the characteristic vertical supranuclear gaze palsy should prompt testing. In late presenting adult cases psychiatric problems and dementia may be prominent. It would certainly be appropriate to consider requesting oxysterols as a complementary test alongside the lysosomal enzyme screen when considering the lysosomal storage disorders as a differential diagnosis.

Oxysterol analysis will now be the standard first line test for NP-C in our laboratory although filipin staining will still be available for confirmation of cases of NP-C or for further investigation of patients with results in the equivocal range.

The specific oxysterol metabolite measured is cholestane-3β, 5α, 6β-triol. Interpretation and any suggestions for further testing or follow up will be given with the report.

Sample requirements: 1-2 ml EDTA plasma separated and frozen on same day of sampling.

Storage and Shipping requirements: Unless EDTA plasma sample can be delivered to the Willink Lab on the same or next day of sampling, the sample must be sent frozen (preferred)

Turnaround time: 15 days

Reference Range: **Control (n=70):** **8.1-37.7** ng/ml (95% CI **9.6-37.0**)

NPC1 (n=15): **35.3-1170** ng/ml (95% CI **39.3-811.9**)

Cost: £87

Oxysterol képződés a tárolt mintákban

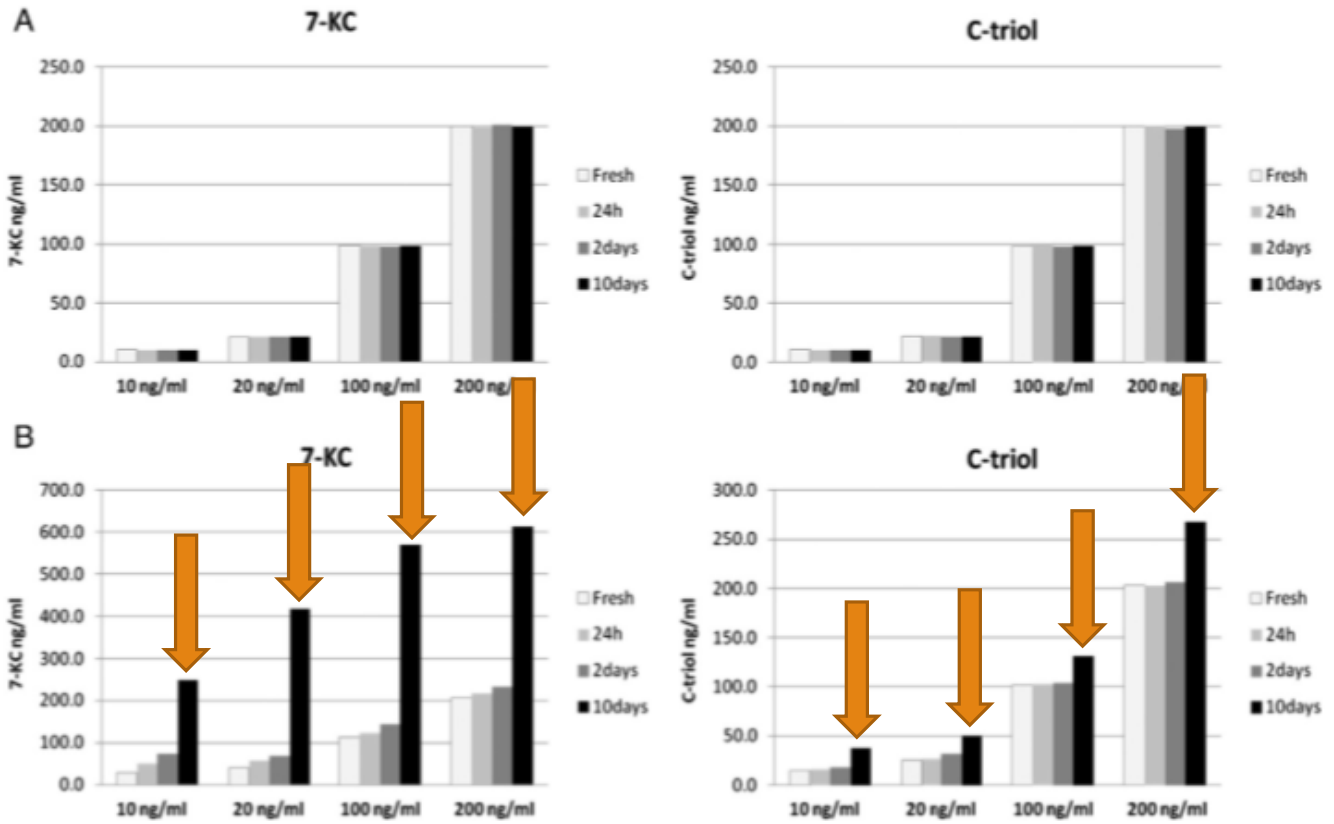


Fig. 5. Post-preparative stability of the two oxysterol DMAB-derivatives in the autosampler at room temperature at different times: 0 h, 24 h, 48 h, 10 days. The two oxysterol DMAB-derivatives were measured both in standard solutions (panel A) and in plasma samples (panel B) at low concentrations (pooled plasma + 10 and 20 ng/mL) and at high concentrations (pooled plasma + 100 and 200 ng/mL).

Boenzi S et al: Clin Chim Acta 437 (2014):93.

Lizoszfingomielinek vizsgálata

- Lyso-SM-509 és Lyso-SM-465 kombinált vizsgálata
- elvégezhető szérumban, plazmában, teljes vérben és szárított vércseppben
- szenzitivitás: 100%
- specifikusság: 98,15%

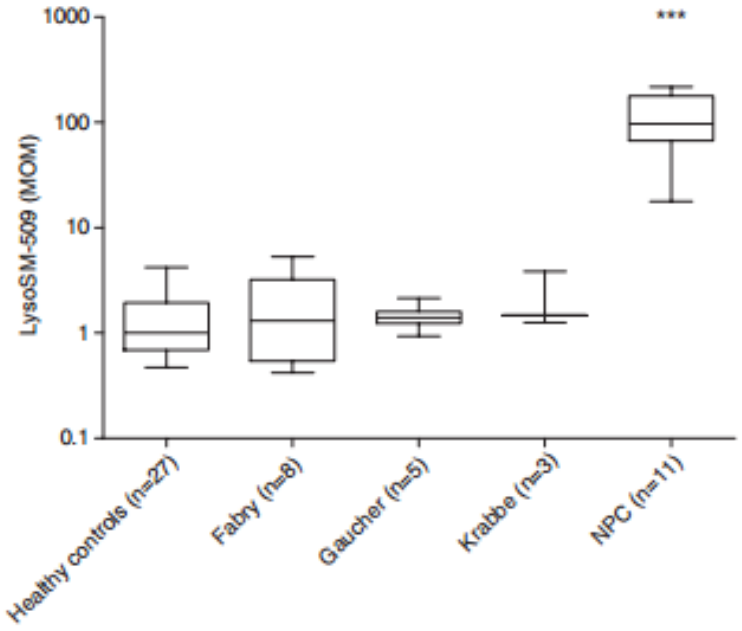
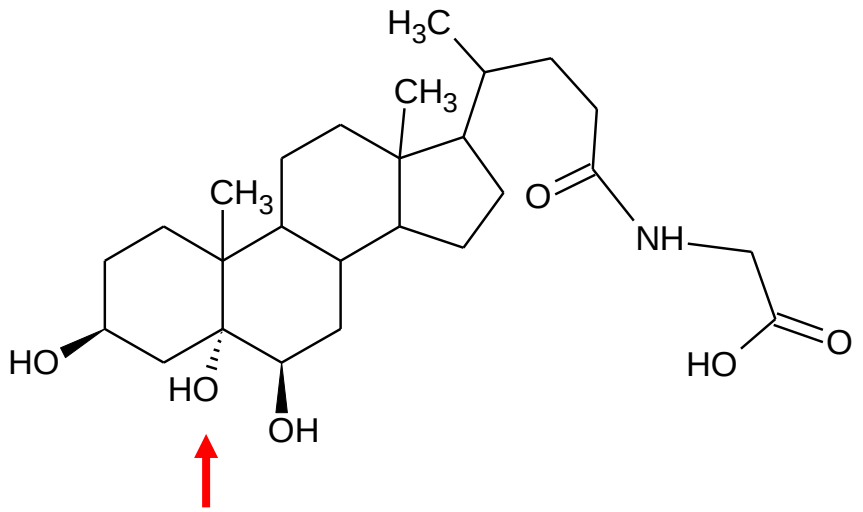


Figure 5: LysoSM-509 plasma levels expressed as MOM in healthy controls, Fabry, Gaucher, Krabbe and Niemann-Pick type C disease patients (Log10 scale).
Box 25–75th percentile, line: median, box whiskers: minimum and maximum. ***p-value ≤ 0.001; FD, Fabry disease; GD, Gaucher disease; KD, Krabbe disease; NPC, Niemann Pick disease type C.

Polo G et al: Clin Chem Lab Med 55 (2017):403.
Cozma C et al. Mol Gen Metab 120 (2016):S17.

Új eljárás az NPC diagnózisára az újszülöttkori anyagcsere szűrés keretében

- 100% szenzitivitás és specifikusság 4992 egészséges és 44 NPC-vel diagnosztizált újszülött vizsgálata alapján
- analit: 3β,5α,6β-trihidroxikolánsav glicin konjugátuma
- prekurzora a C-triol oxidációs terméke
- az epesavak 5α-hidroxilált metabolitjai Niemann-Pick specifikus termékeknek tűnnek

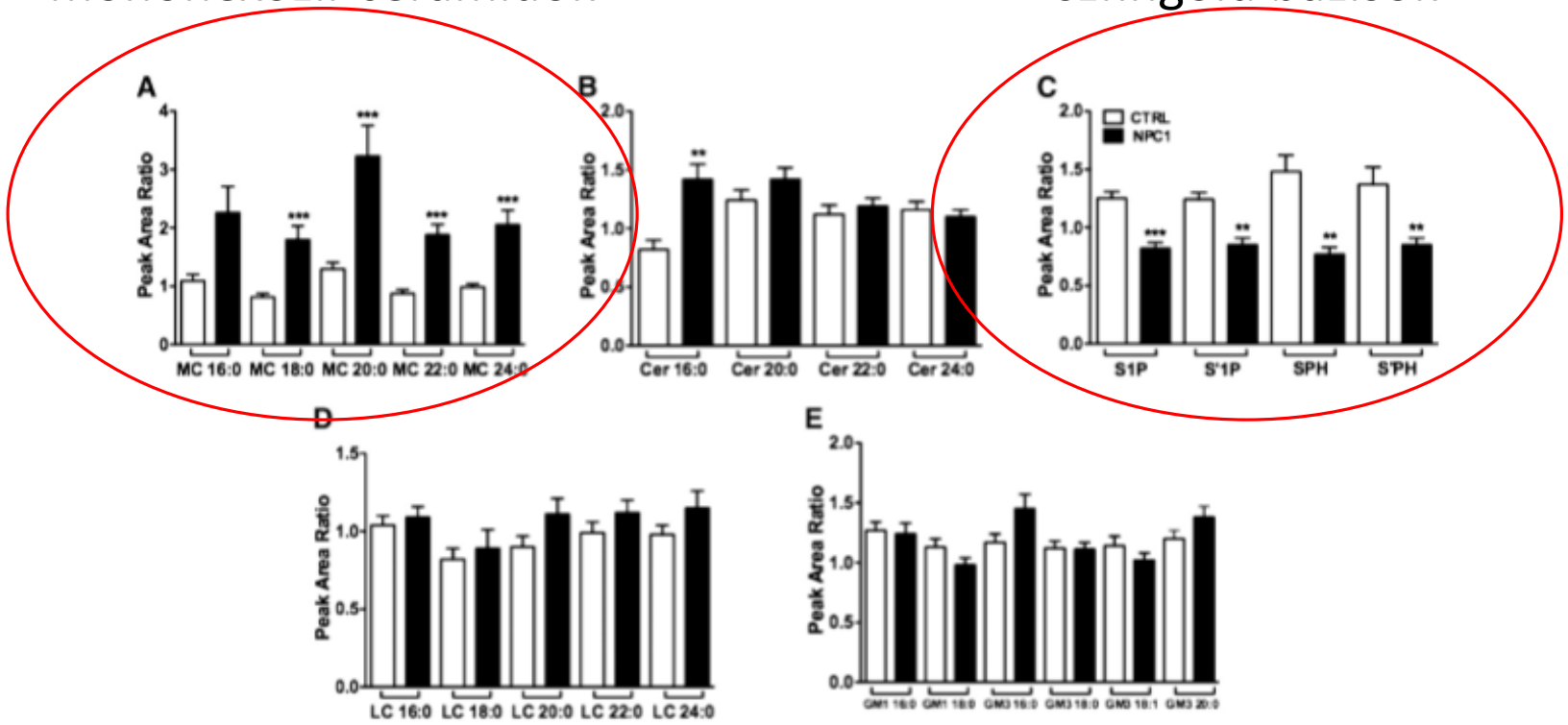


Jiang X et al: Sci Transl Med 8 (2016):337ra63.

Megváltozott lipid metabolom: **szfingolipidek**

monohexozil-ceramidok

szfingoid bázisok



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Fan N et al: J Lipid Res 54 (2013):2800.

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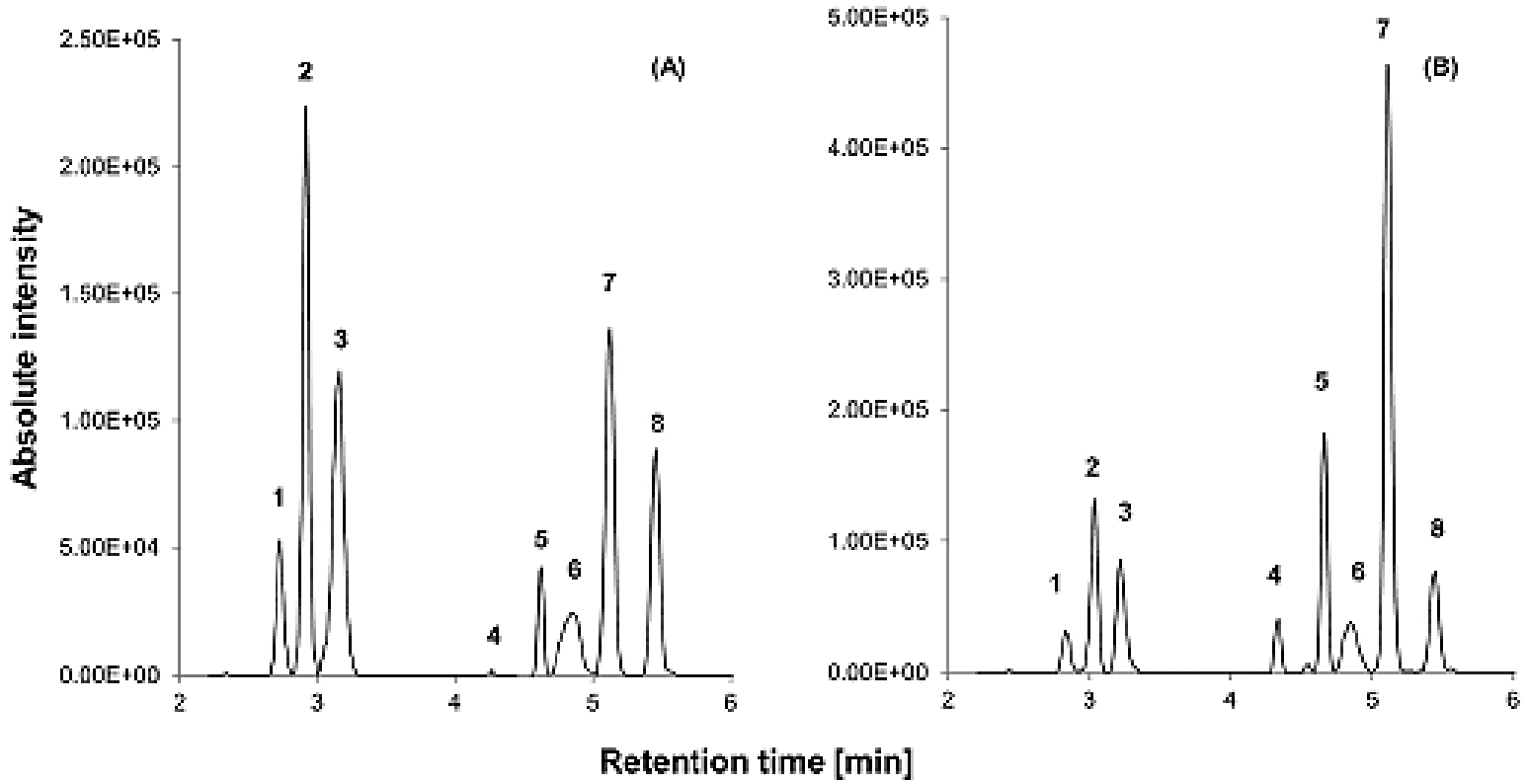

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Pataj Z et al. J Chromatogr A 1439 (2016):32.

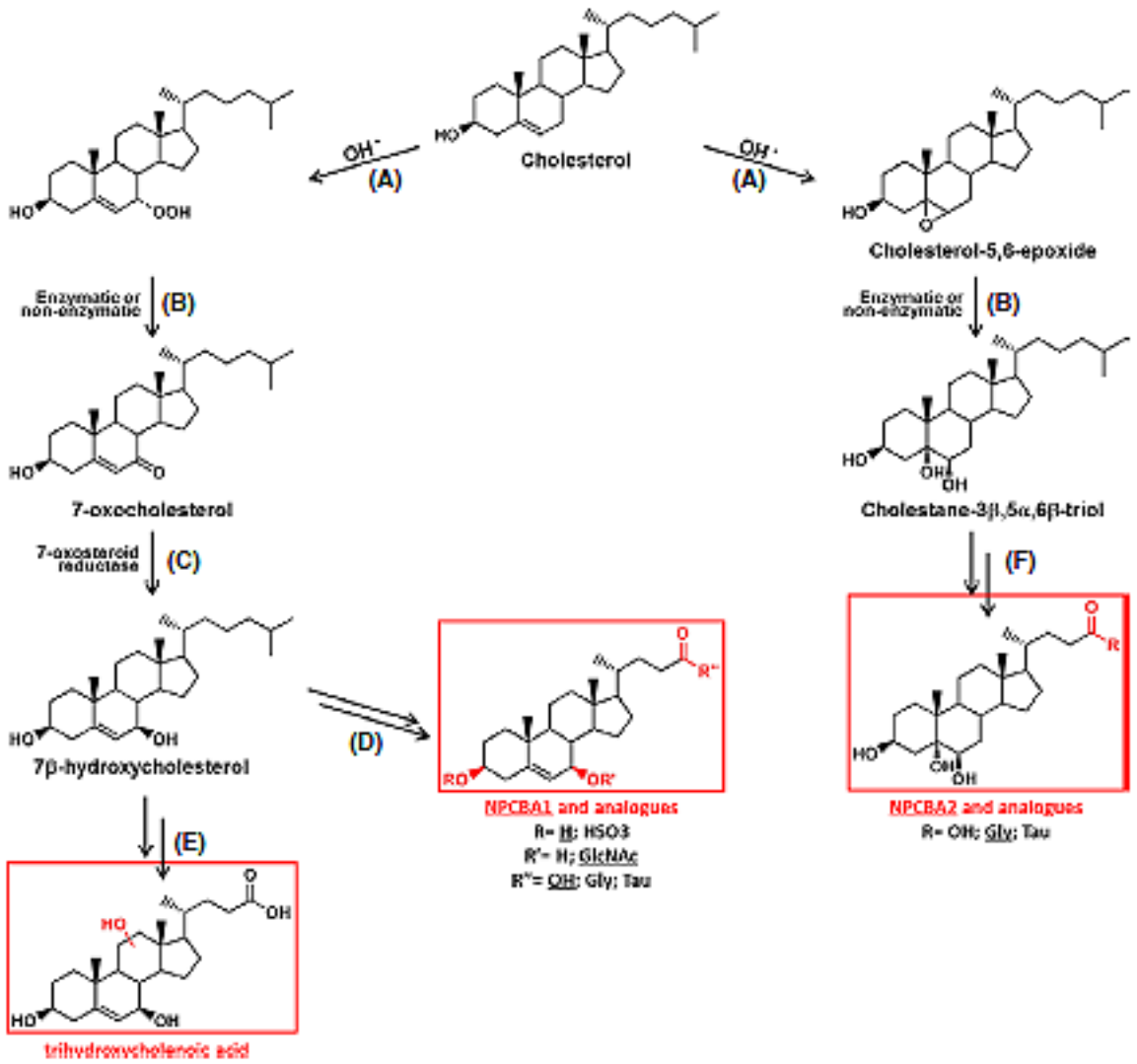

Fig. 2. Proposed pathway for the hepatic synthesis of 'NPC bile acids'. Hepatic formation of NPCBA1, NPCBA1 analogues (left pathway) and NPCBA2 (right pathway). Endolysosomal accumulation of unesterified cholesterol, associated with cellular oxidative stress (A,B), leads to the production of oxysterol molecules (7-oxocholesterol and cholestane-3β,5α,6β-triol). The keto group of the 7-oxocholesterol can be reduced by 7-oxosteroid reductase (C) forming 7β-hydroxycholesterol which, upon further enzymatic modifications, can lead to the series of NPCBA1 (D) or, alternatively to the formation of a trihydroxycholenoic acid (E). In a similar way, NPCBA2 series can be produced, via enzymatic reactions (F), from cholestane-3β,5α,6β-triol.

Mazzacuva F et al. FEBS Letters 590 (2016):1651.


KÖSZÖNÖM A MEGTISZTELŐ FIGYELMET.