MCAD defekt; en lang rejse

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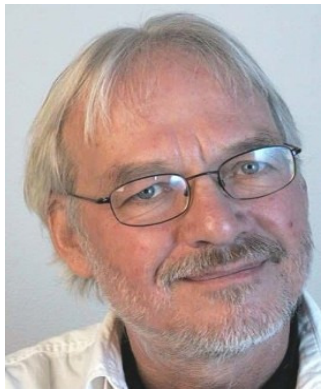
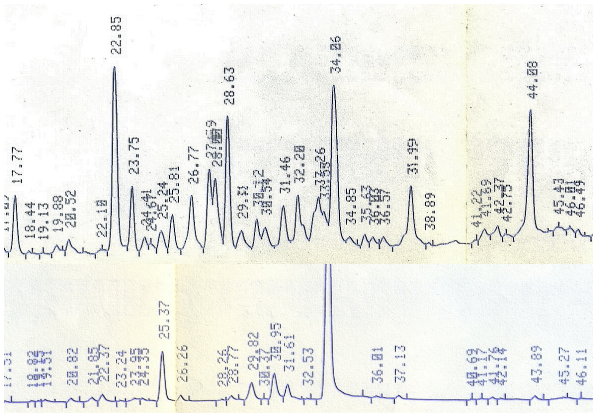
Molekylær Medicinsk Forskningsenhed

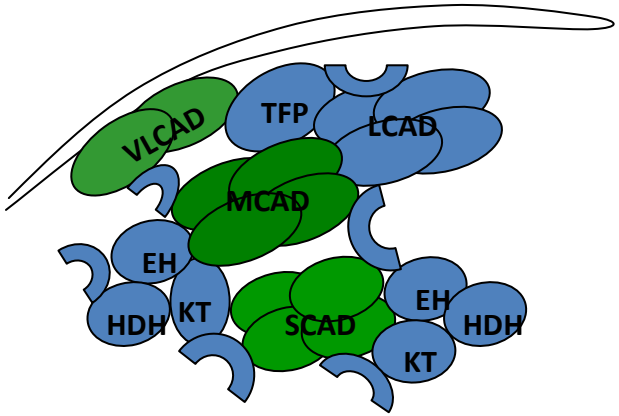
Klinisk Institut, Aarhus Universitet

og

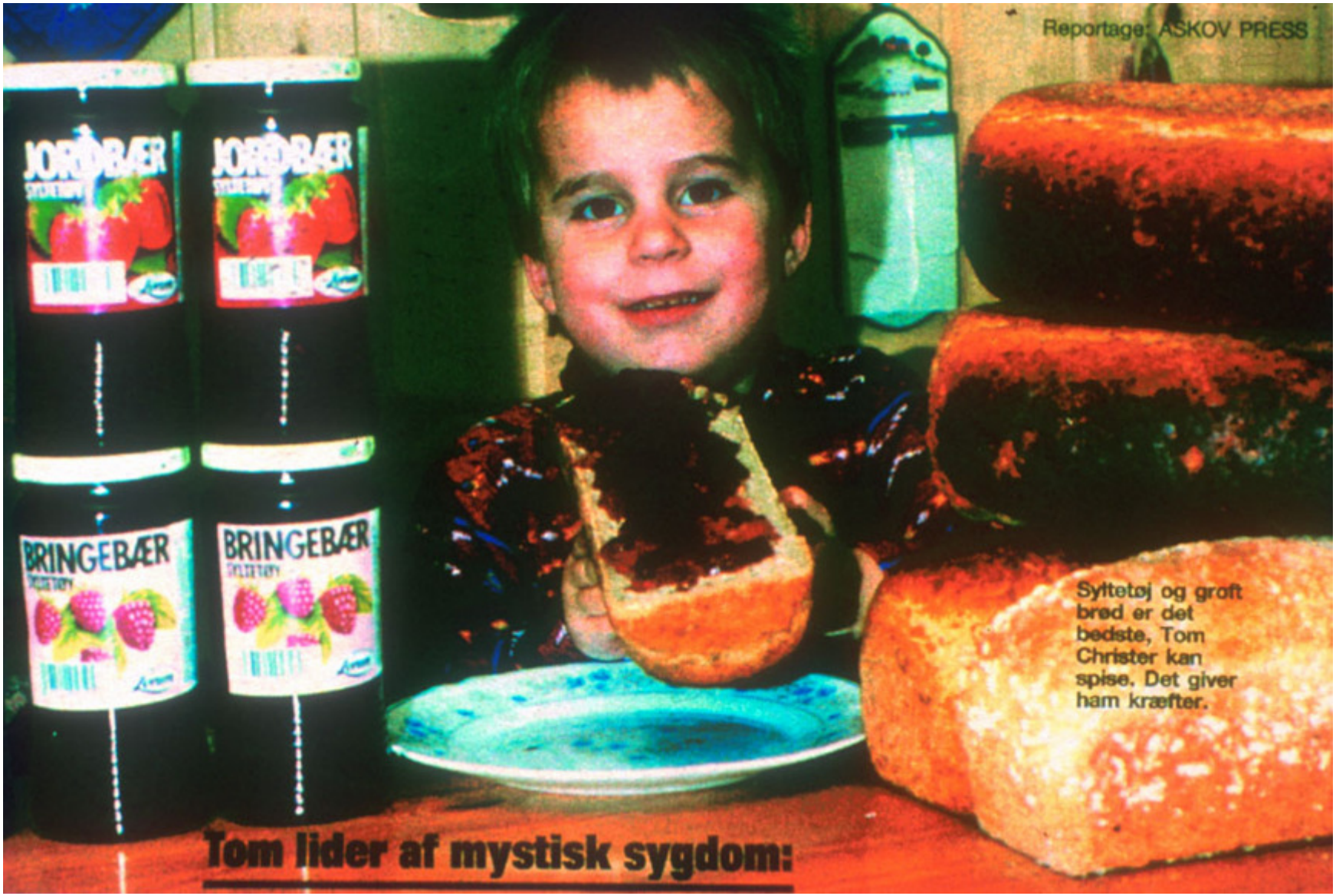
Århus Universitetshospital, Skejby







MCAD defekt: En defekt i omsætningen af fede syrer

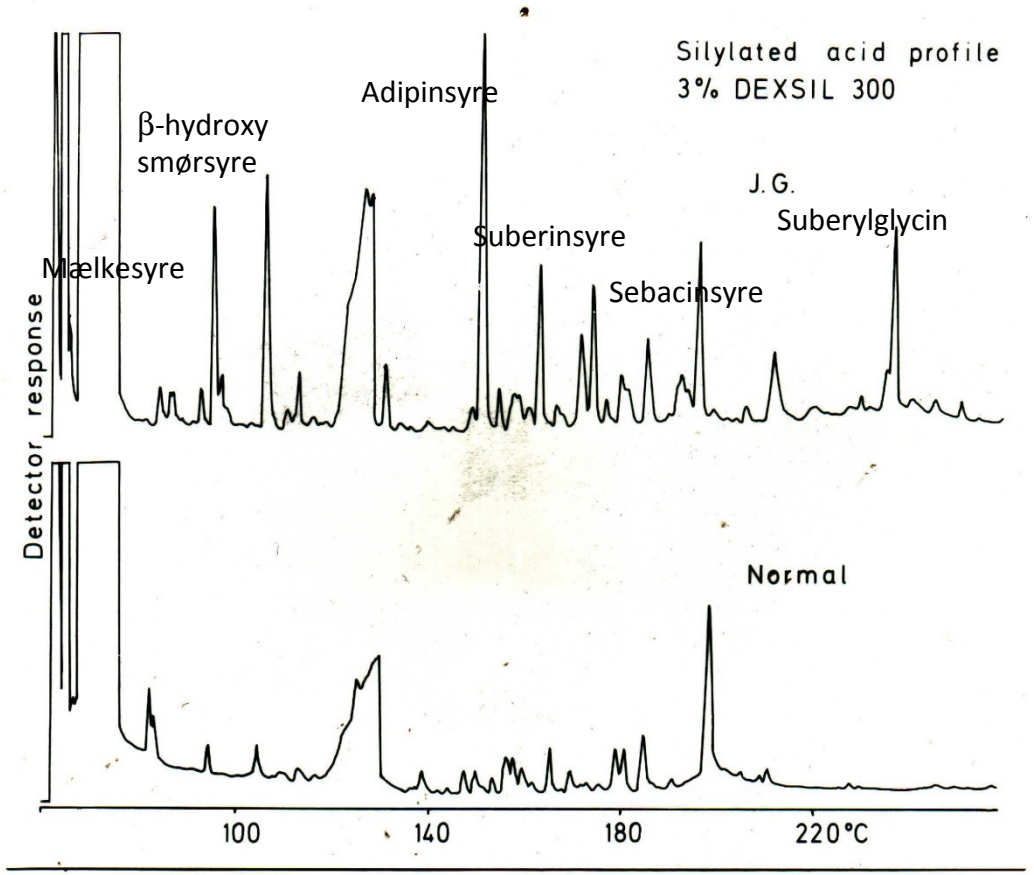


Urinundersøgelse for organiske syrer ved gaskromatografi-massespektrometri (GC-MS)

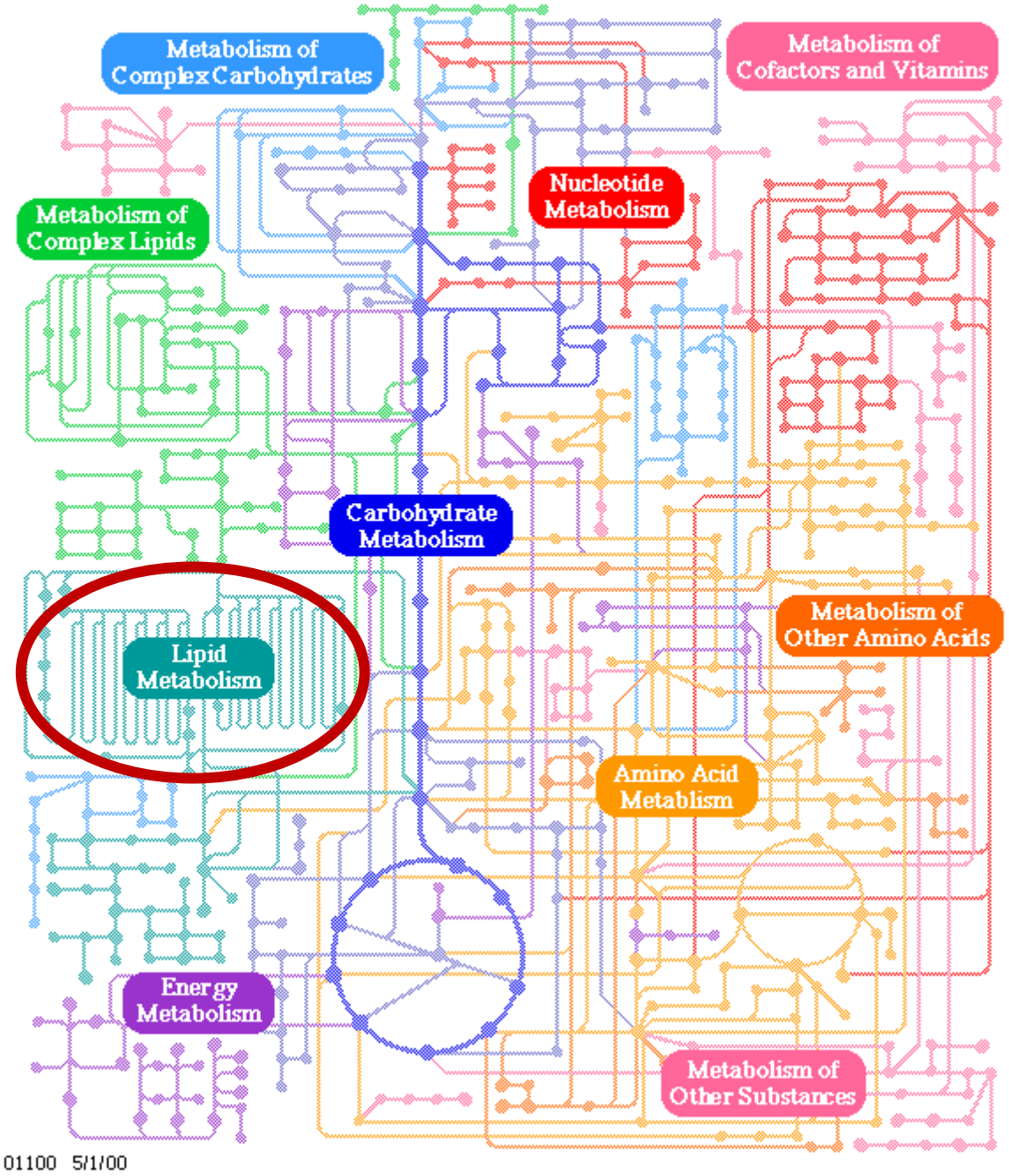
2-årig dreng
3. anfald
Døsighed udviklet til bevistløshed
Hypoketotisk hypoglykæmi
Acidose
Forhøjede leverenzymer

Seks anfald i alderen
2, 20, 25, 27, 28 og 30 mdr.

GC-MS urinundersøgelse

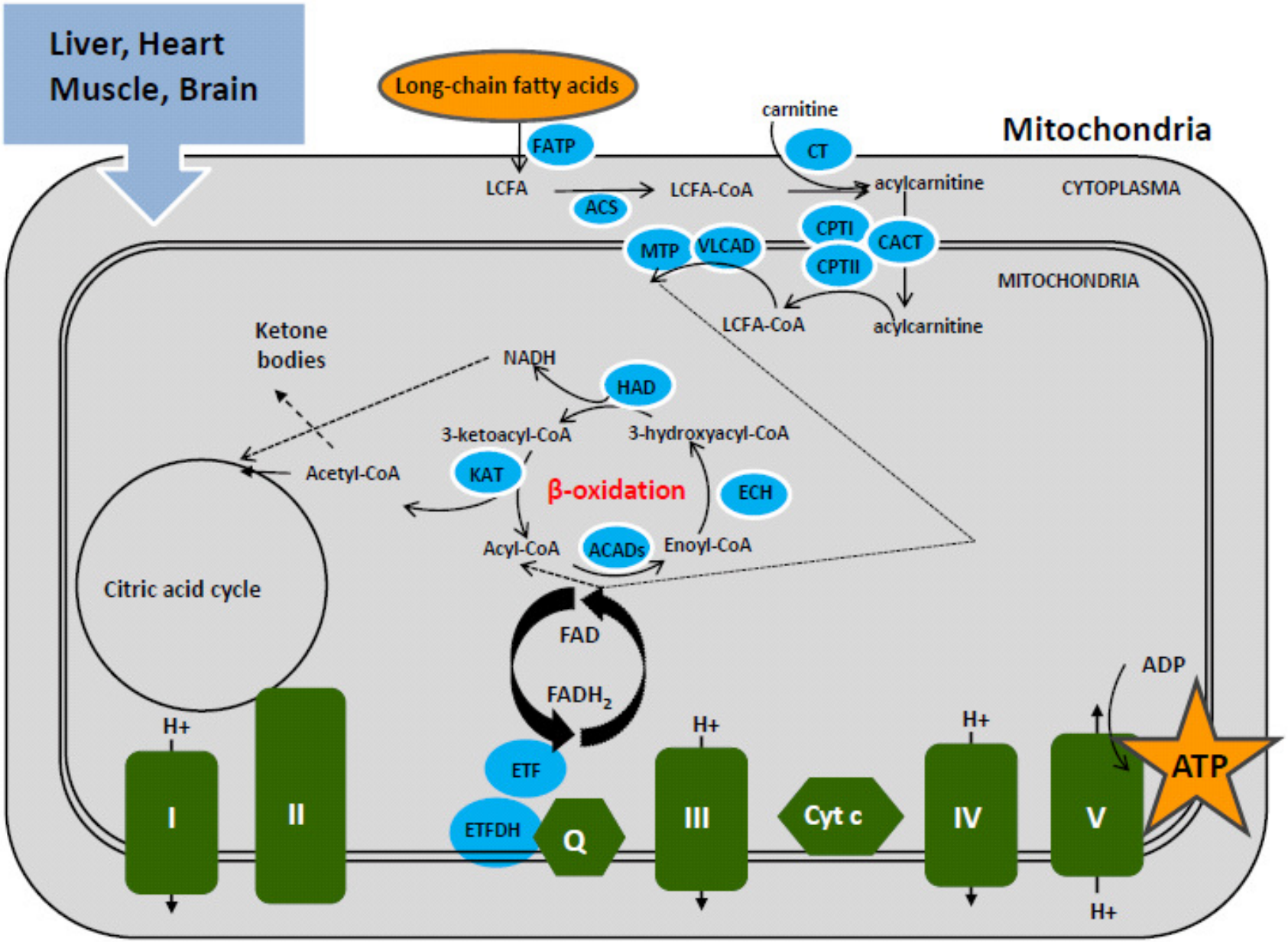


METABOLIC PATHWAYS



01100 5/1/00

Fedtsyreoxidationen (β-oxidationen) i mitokondrierne



Fedtsyreoxidationen (β-oxidationen)

LK-fedt acyl-CoA

↓ *VLCAD*

Dekanoyl-CoA

↓ *MCAD*

Oktanoyl-CoA

↓ *MCAD*

Hexanoyl-CoA

↓ *MCAD*

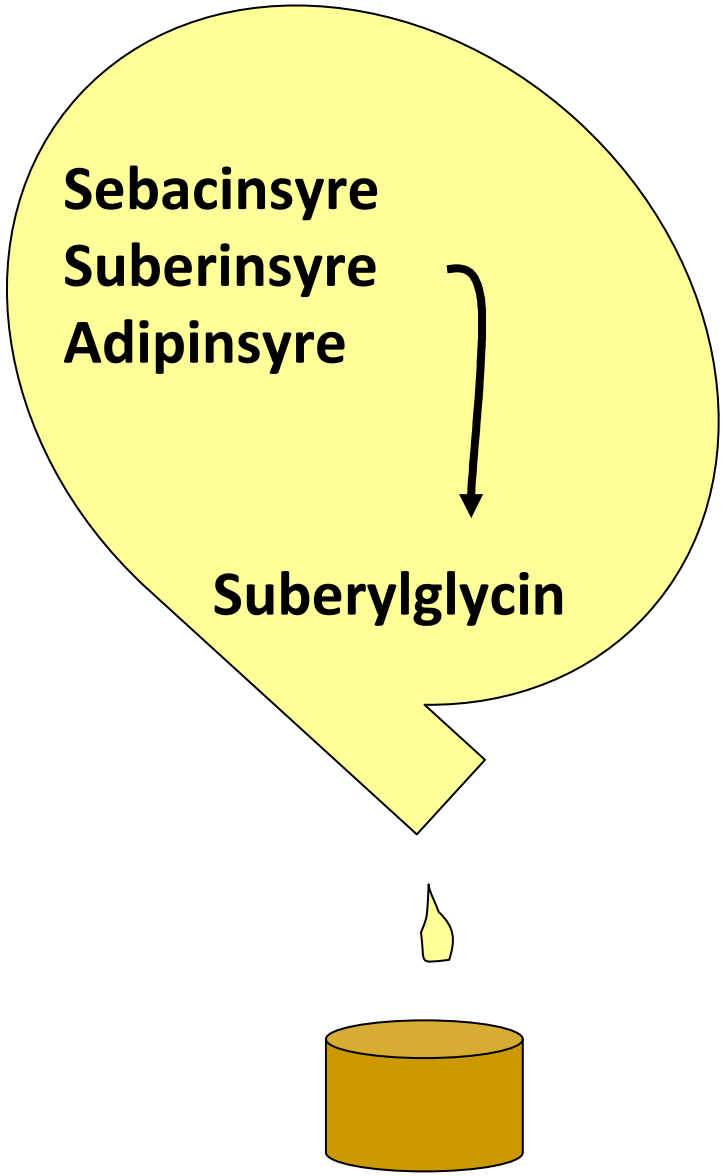
Butyryl-CoA

↓ *SCAD*

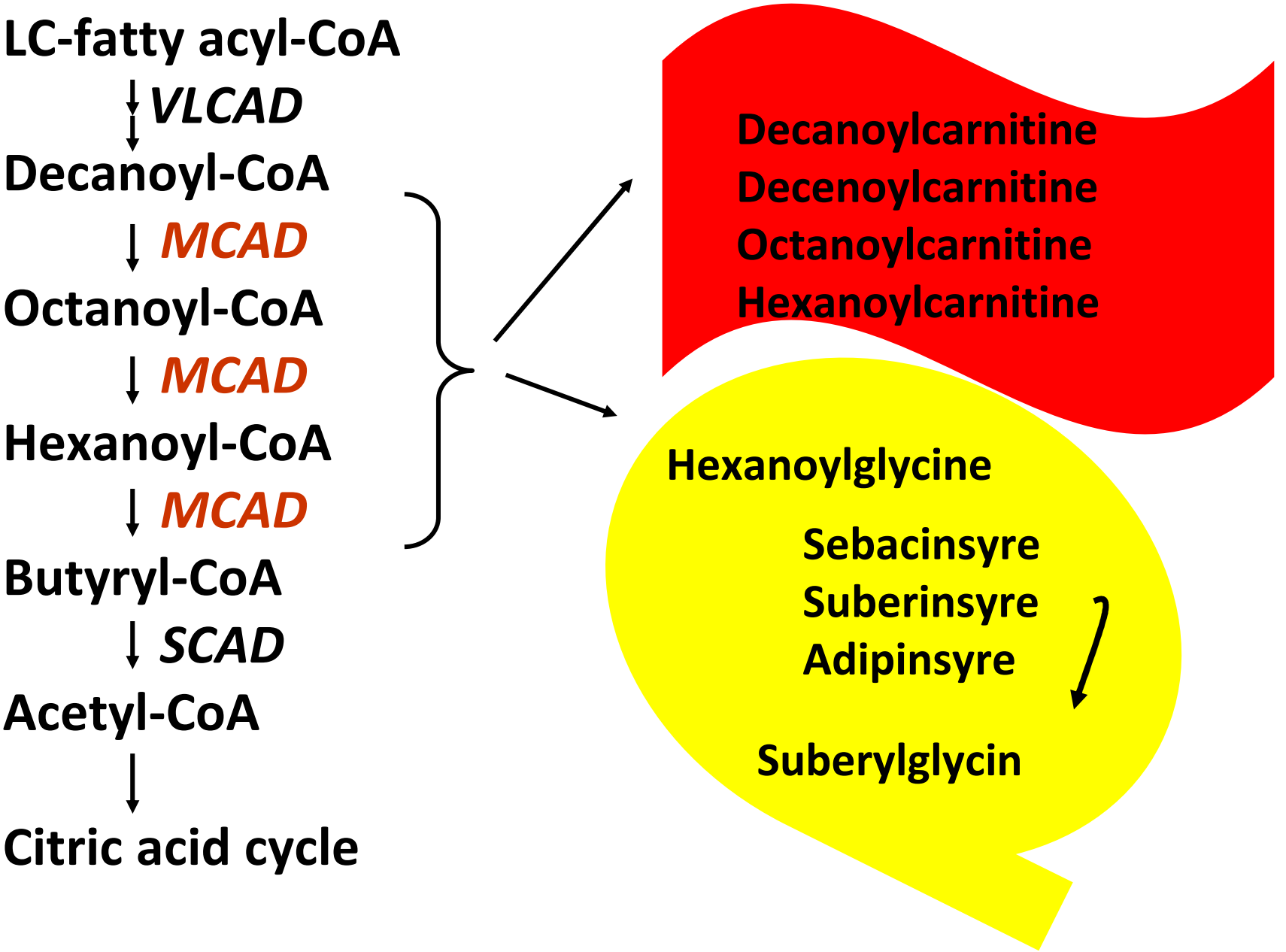
Acetyl-CoA

↓

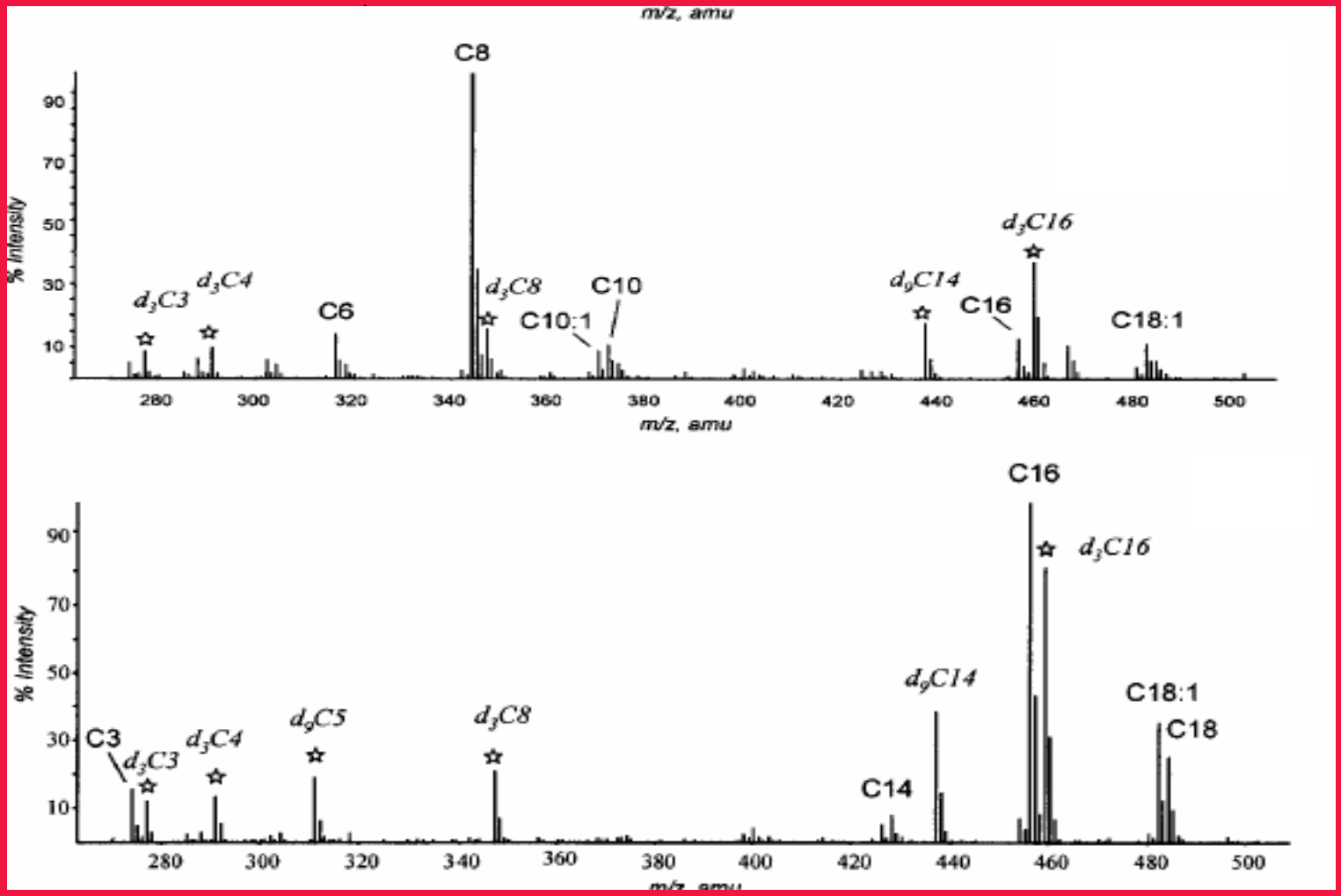
Citronsyrecyklus



Metabolitter ved MCAD mangel



Tandem masssespektrometri af acylcarnitiner i blod fra patient med MCAD mangel



Fra patient til gen og tilbage

Kliniske symptomer hos patienten

Unormale metabolitter i urin og blod

1976

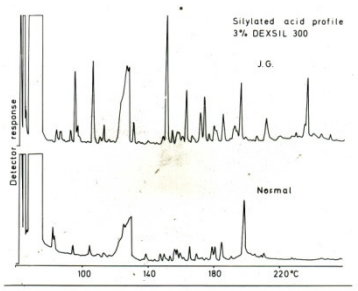
Nedsat enzymaktivitet

1982

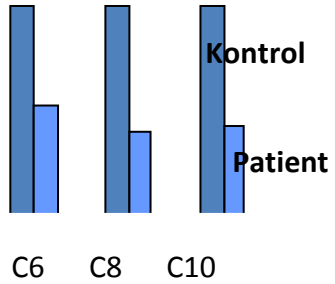
Mutation i gen

1990

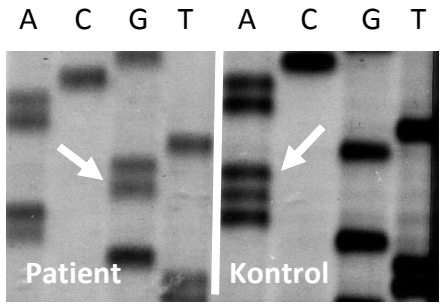
Urin metabolitter

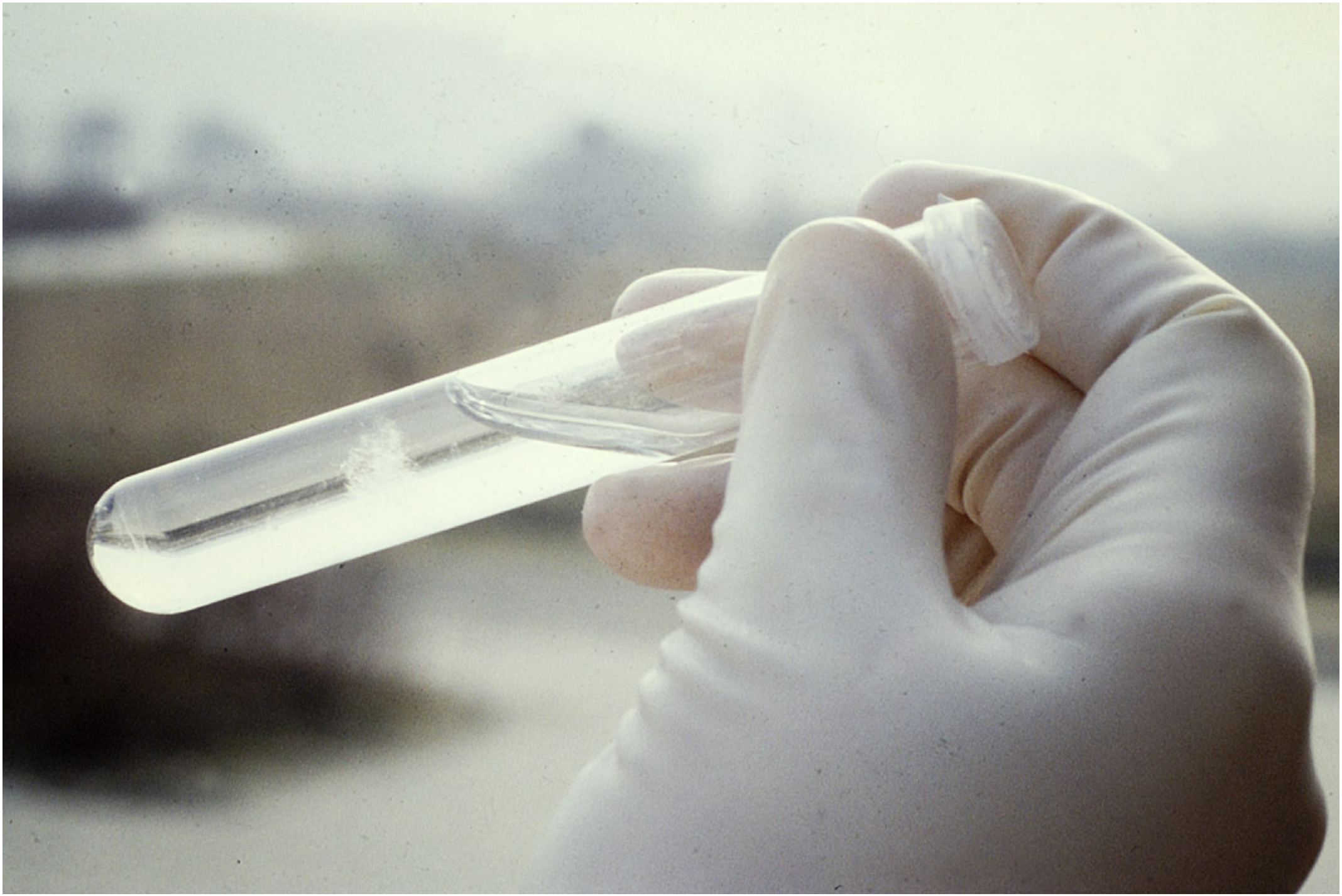


ACAD aktivitet

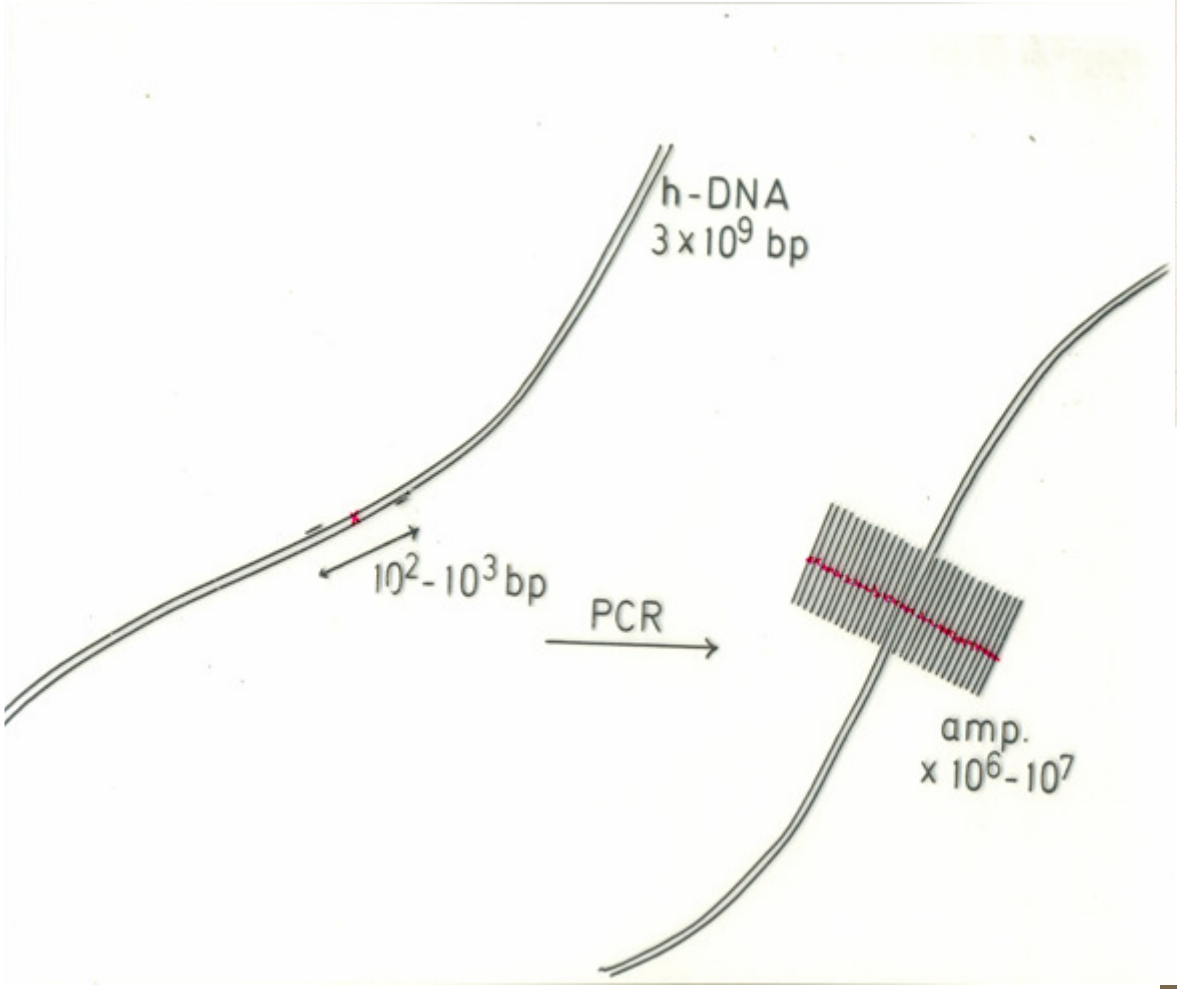


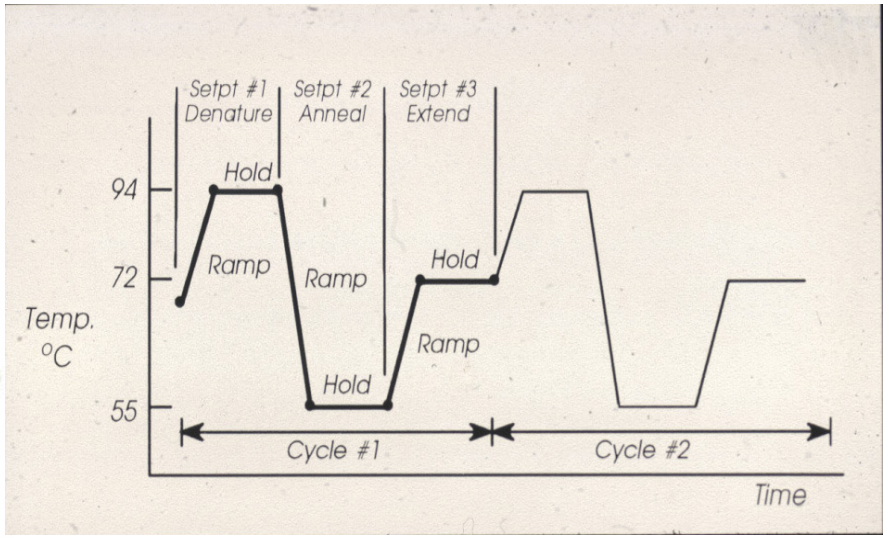
MCAD gensekventering





PCR


h-DNA
3 x 10^9 bp
10^2 - 10^3 bp
PCR
amp.
x 10^6 - 10^7

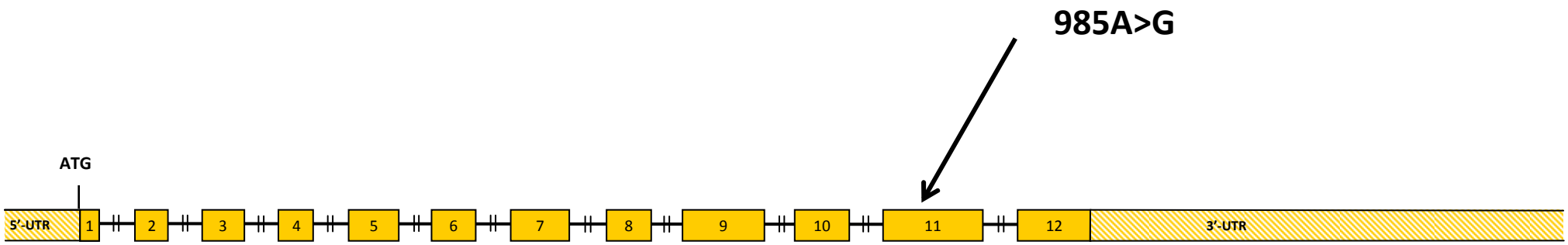

Setpt #1
Denature
Setpt #2
Anneal
Setpt #3
Extend
Hold
Ramp
Hold
Ramp
Hold
Ramp
94
72
55
Temp.
°C
Cycle #1
Cycle #2
Time


94°C
5min
HETO


22°C
1min
HETO


53°C
6min
HETO

MCAD genet

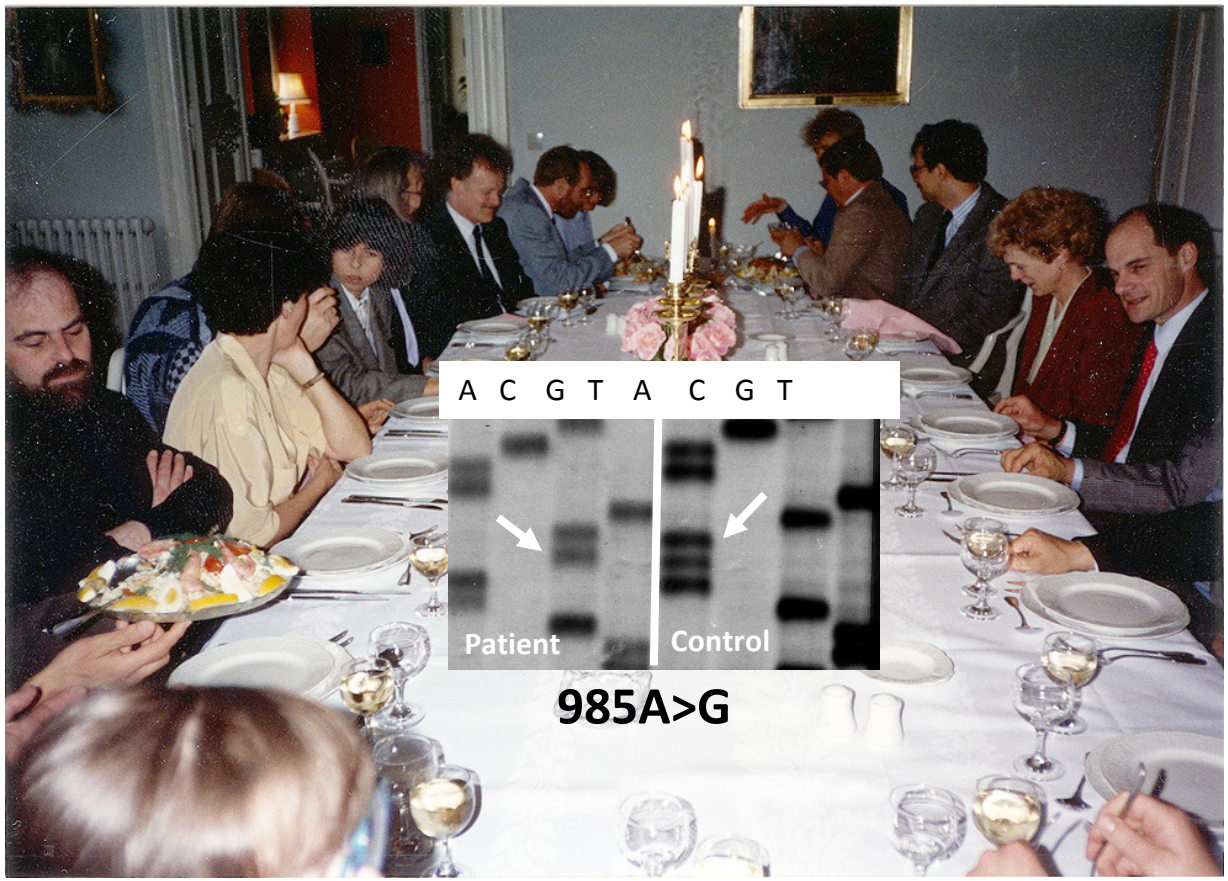


Fatty acid oxidation workshop. Sandbjerg May 1990

Matsubara Y, Narisawa K, Miyabayashi S, Tada K, Coates PM, Bachmann C, ElsasLJ, Pollitt RJ, Rhead WM, Roe CR. Biochem Biophys Res Comm 171; 1990: 498-505

Gregersen N, Andresen BS, Bross P, Winter V, Rüdiger N, Engst S, Christensen E, Kelly D, Strauss AW, Kølvraa S, Bolund L, Ghisla S. Hum Genet 86; 1991: 545-551.

Yokota I, Indo Y, Coates PM, Tanaka K. J Clin Invest 86;1990:1000-1003.



Kelly DP, Whelan AJ, Ogden ML, Alpers R, Zhang ZF, Bellus G, Gregersen N, Dorland L, Strauss AW.. Proc Natl Acad Sci (USA) 87, 1990: 9236-9240.

MCAD deficiency

985A>G prevalence: Philadelphia 1991

Genotypering af 172 patienter med MCAD defekt for 985A>G

Total	G/G	G/non-G	non-G/non-G
172	138	30	4
	80%	18%	2%

Coates PM, Chen YT , Curtis D, Gregersen N, et al. Prog Clin Biol Res 1992; 375:499-506

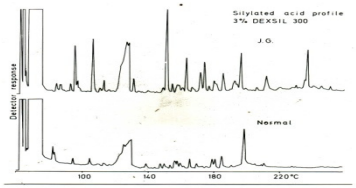
Tanaka K, Yokota I, Coates PM, Strauss AW, Kelly DP, Zhang Z, Gregersen N, Andresen BS, Matsubara Y, Curtis D, Chen YT. Hum Mut 1; 1992. 271-299.

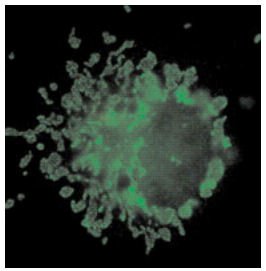
Translatorisk medicin cyklus

Treatment

Patients

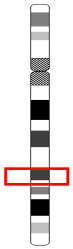
Clinical, Biochemical and/or genetic tests



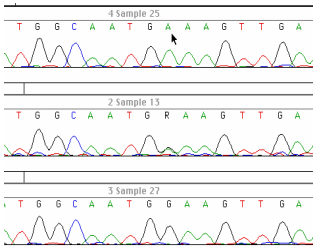


Molecular cell pathology

Indications of genes involved



Molecular genetic investigations

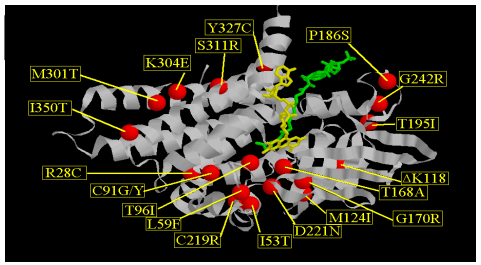


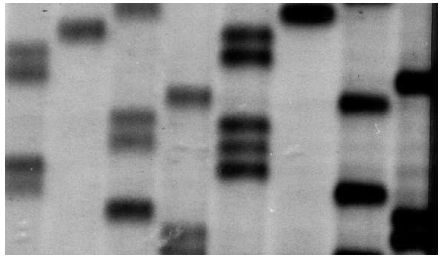
Identification of gene variations

Cell and animal models

Molecular pathogenesis

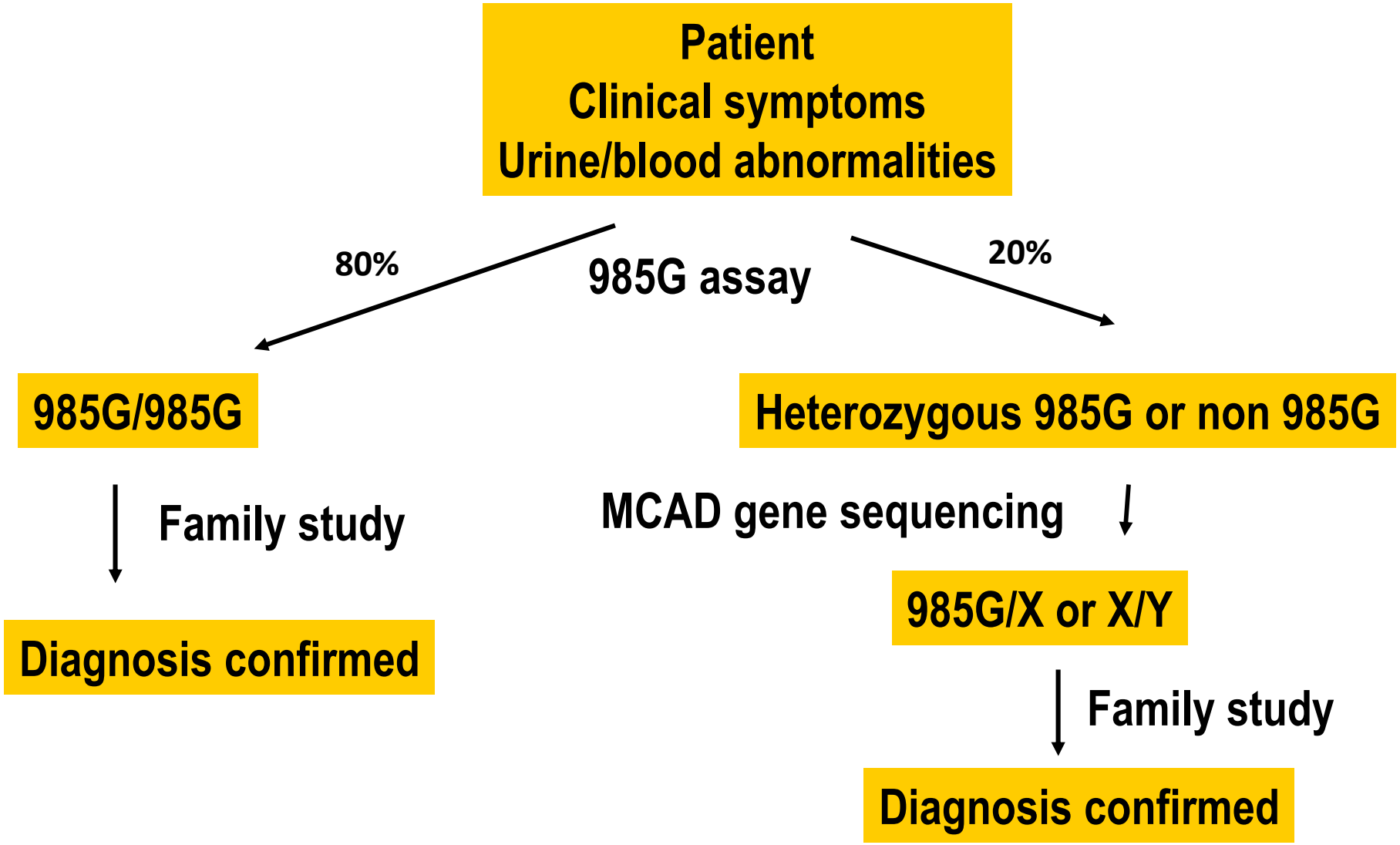
Gene variation(s)





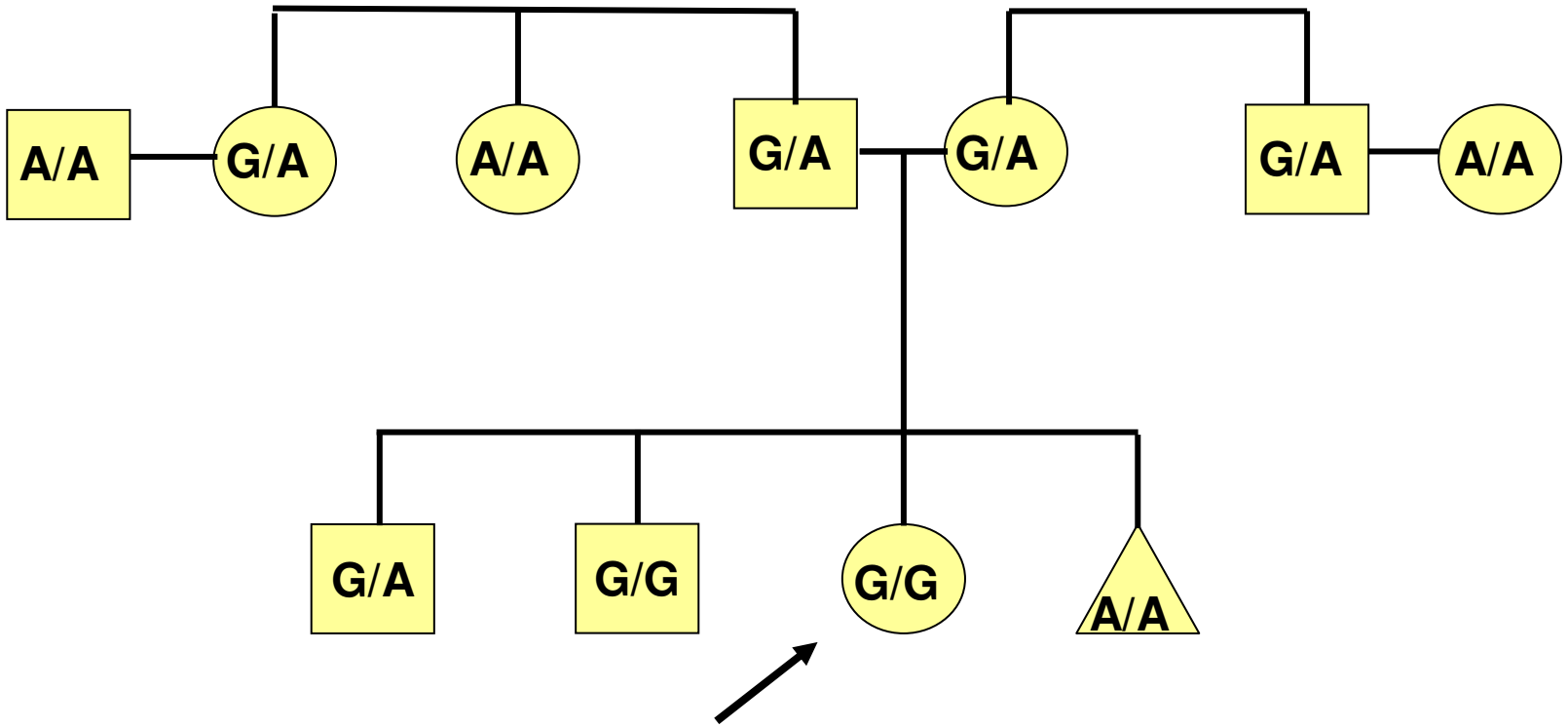
Functional studies

Diagnostisk strategi ved mistanke om MCAD defekt

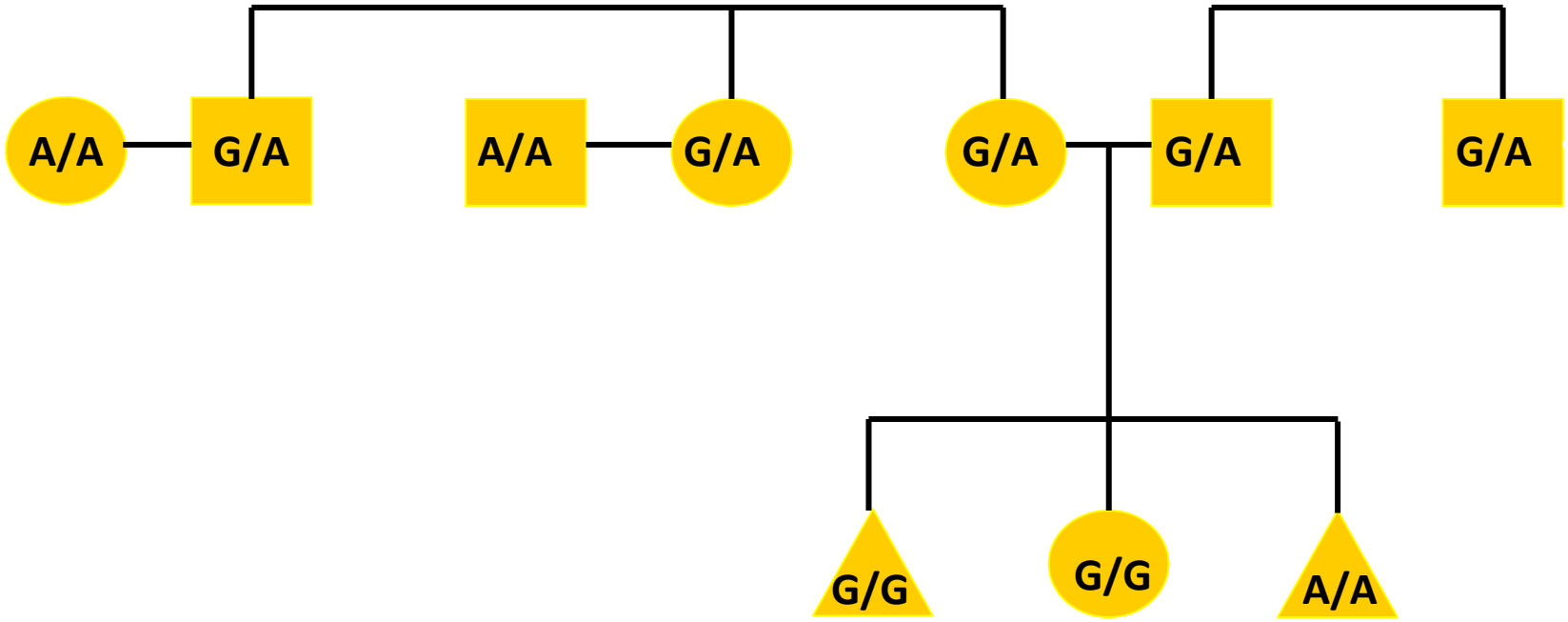


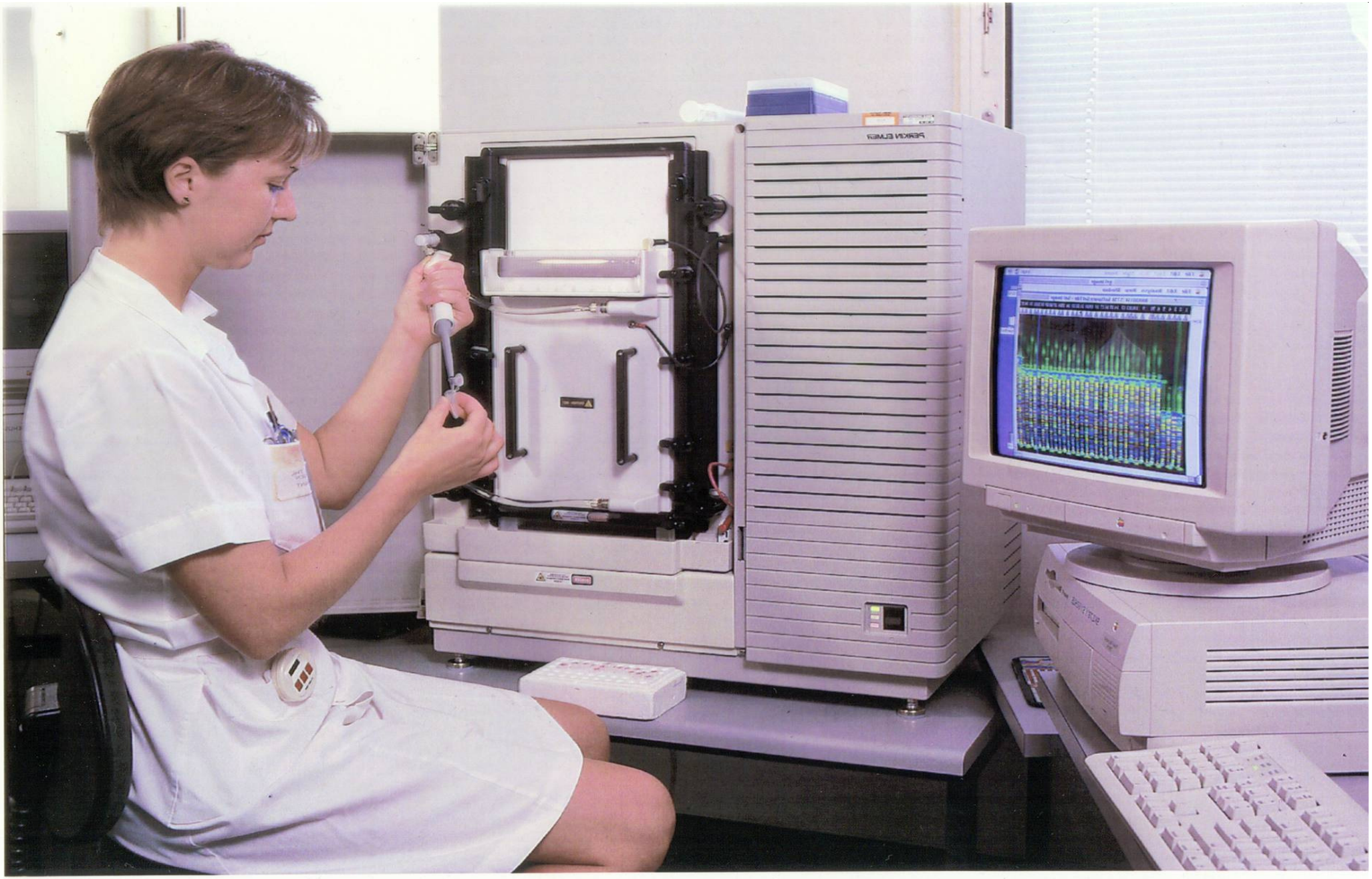
Genetisk Diagnostik

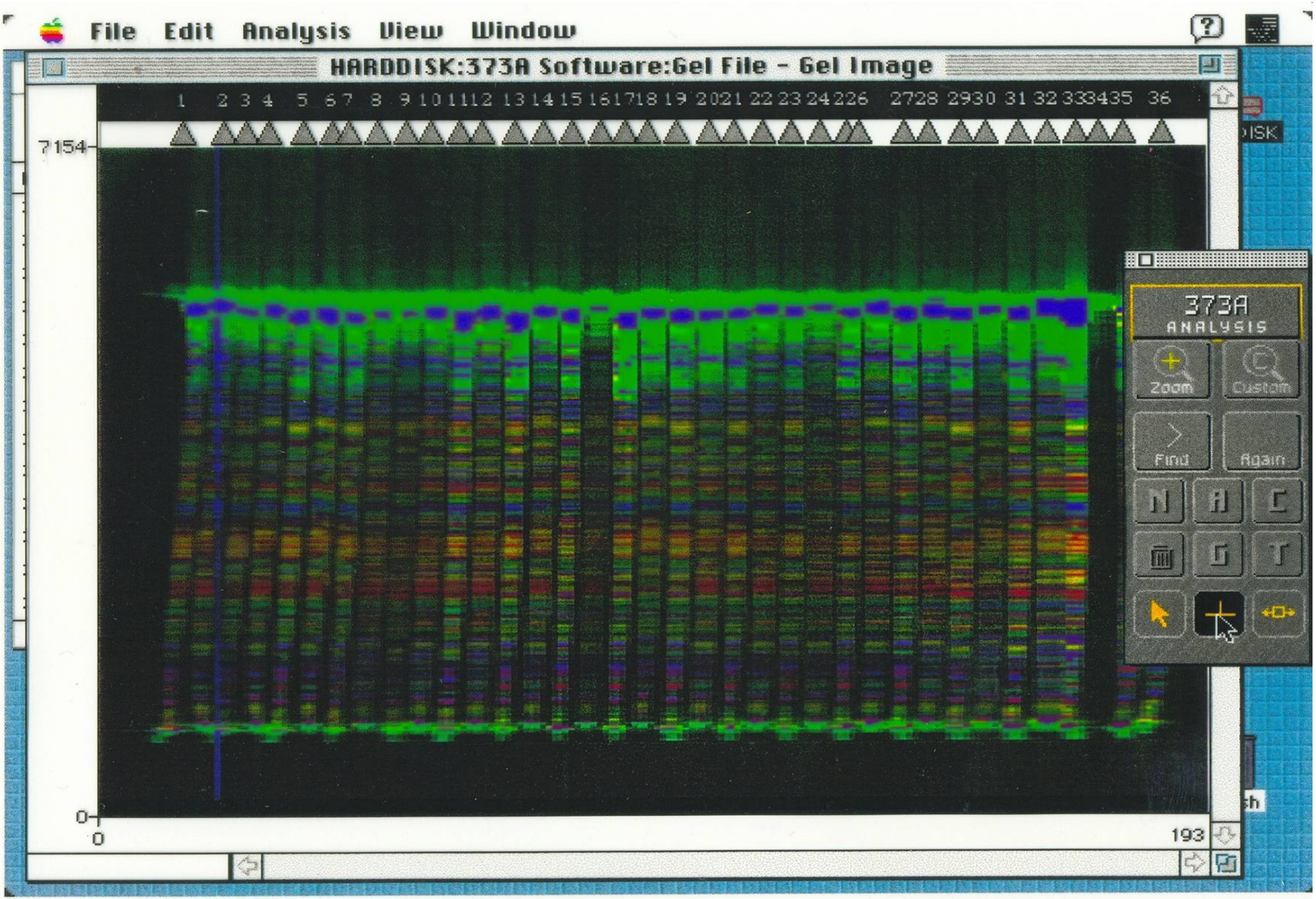
Genotypering af 985A>G MCAD mutationen

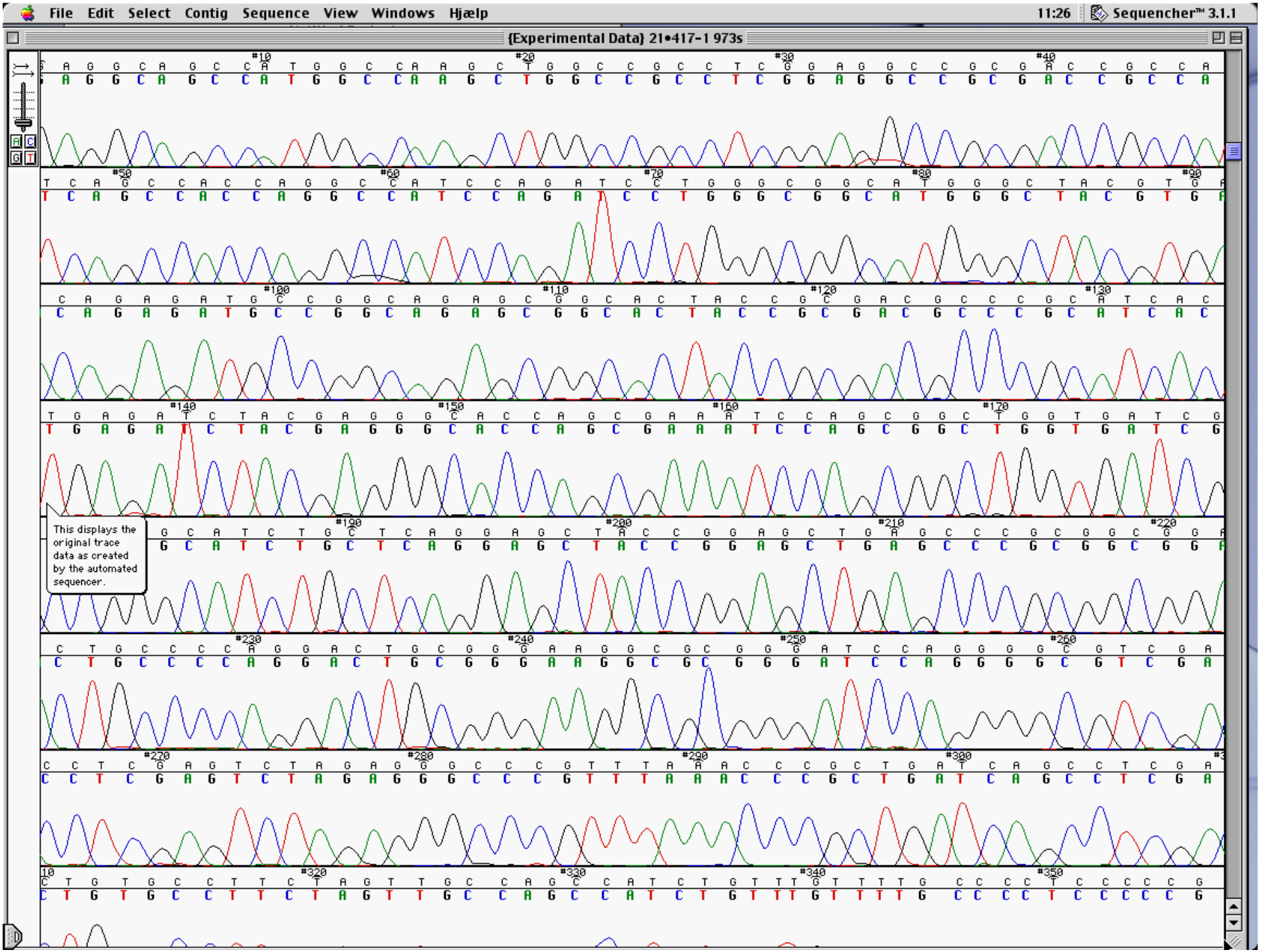


Diagnostisk brug af variationsspecifikt assay
MCAD 985A>G






File Edit Analysis View Window
HARDDISK:373A Software:Gel File - Gel Image
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 26 27 28 29 30 31 32 33 34 35 36
7154
0
0
193
373A
ANALYSIS
Zoom
Custom
Find
Again

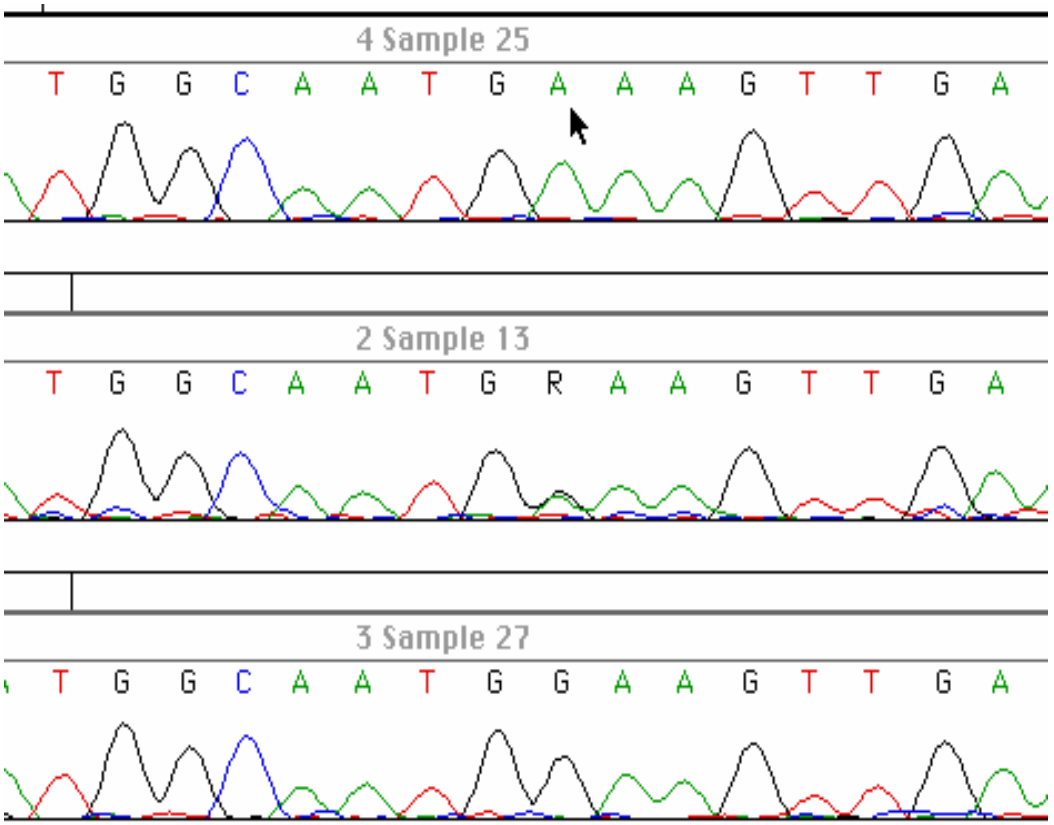

File Edit Select Contig Sequence View Windows Hjælp
11:26
Sequencher™ 3.1.1
{Experimental Data} 21•417-1 973s
This displays the original trace data as created by the automated sequencer.

Diagnostisk sekventering af exon 11

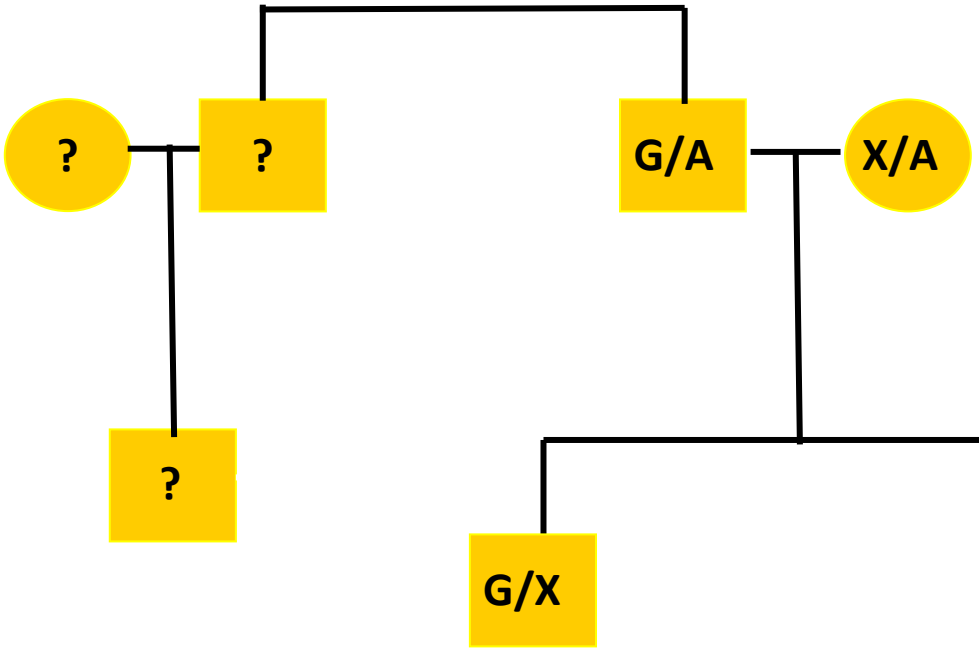
Kontrol

Heterozygot

Homozygot



Mild og alvorlig MCAD gene variation



G: 985A>G
A: normal
X: mild variation
558T>A

Fundet ved screening efter nedsættelse af cut-off

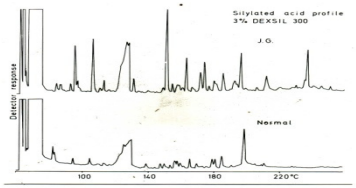
Fundet ved screening ved 'normal ' cut-off.

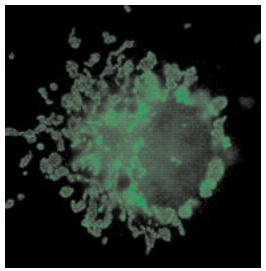
Translatorisk medicin cyklus

Treatment

Patients

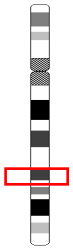
Clinical, Biochemical and/or genetic tests





Molecular cell pathology

Indications of genes involved



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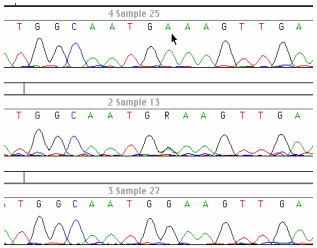

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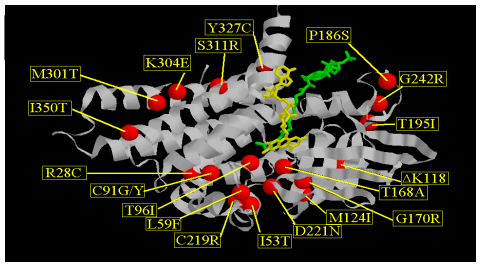
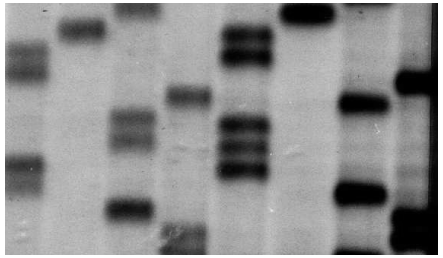




Functional studies

Fra patient til gen og tilbage

Kliniske symptomer hos patienten

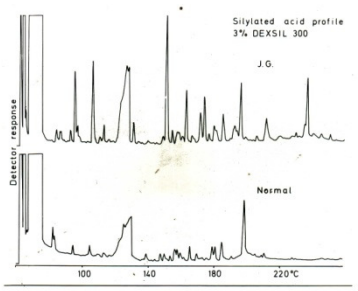
Unormale metabolitter i urin og blod — 1976

Nedsat enzymaktivitet — 1982

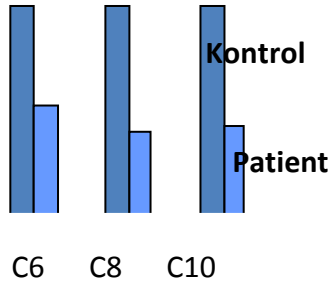
Mutation i gen — 1990

Genfrekvens — 1991

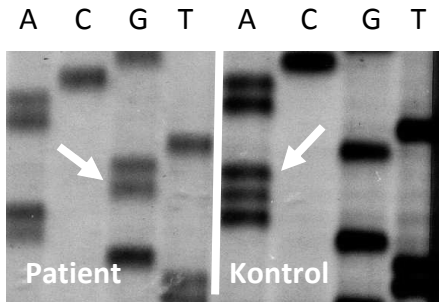
Urin metabolitter



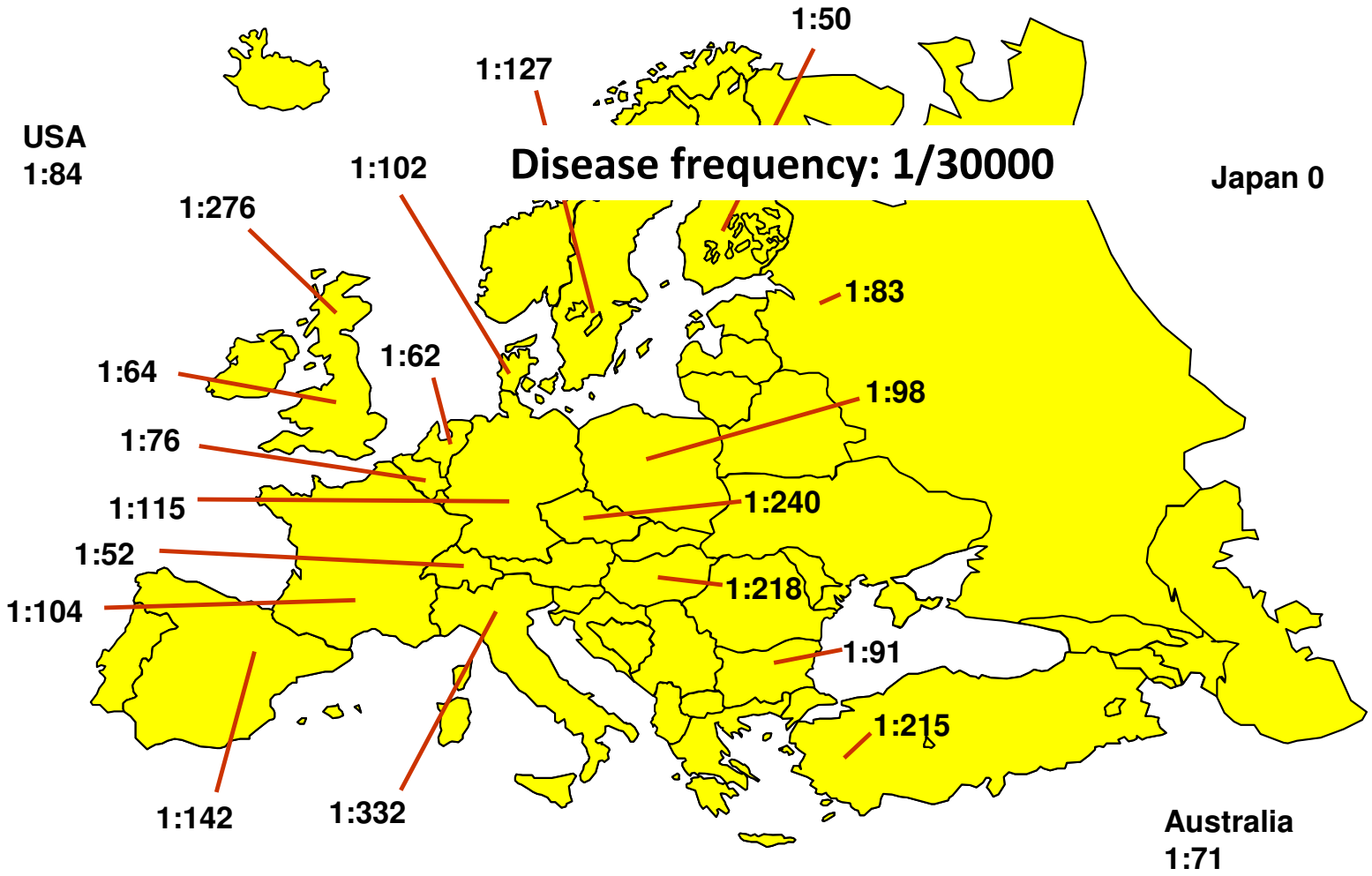
ACAD aktivitet



MCAD gensekventering



Frequency of MCAD 985A>G



Tanaka K, Gregersen N, et al. Pediat Res 1997;41:201-209

Fra patient til gen og tilbage

Kliniske symptomer hos patienten

Unormale metabolitter i urin og blod — 1976

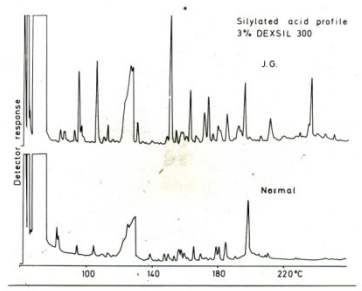
Nedsat enzymaktivitet — 1982

Mutation i gen — 1990

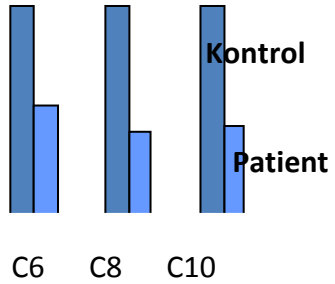
Genfrekvens — 1991

Screening af nyfødte — 2002

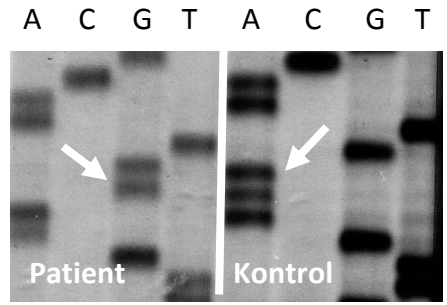
Urin metabolitter



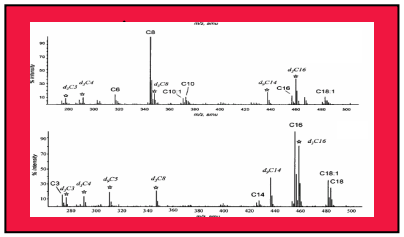
ACAD aktivitet



MCAD gensekventering



Blodmetabolitter



Translatorisk medicins cyklus

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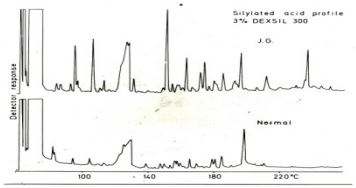
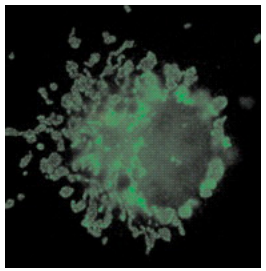
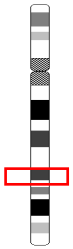


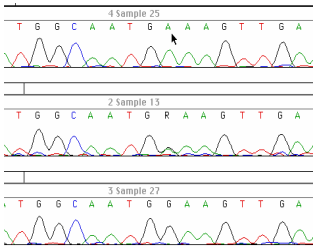


Molecular cell pathology

Indications of genes involved

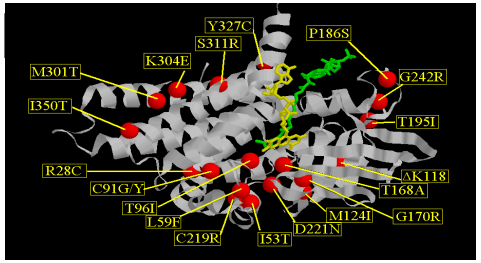


Molecular genetic investigations



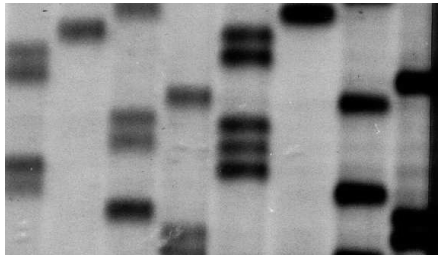
Identification of gene variations

Cell and animal models



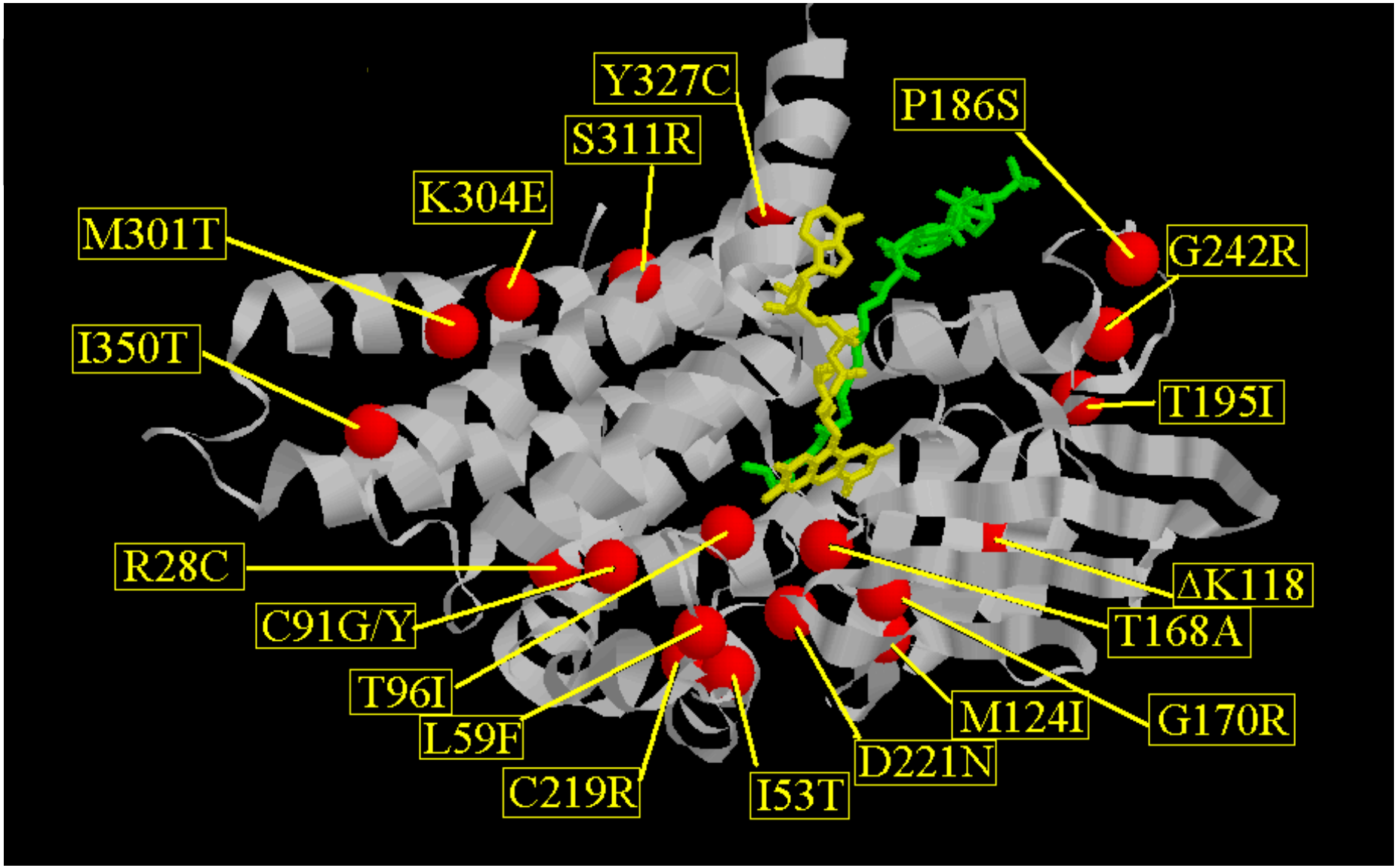
Molecular pathogenesis

Gene variation(s)

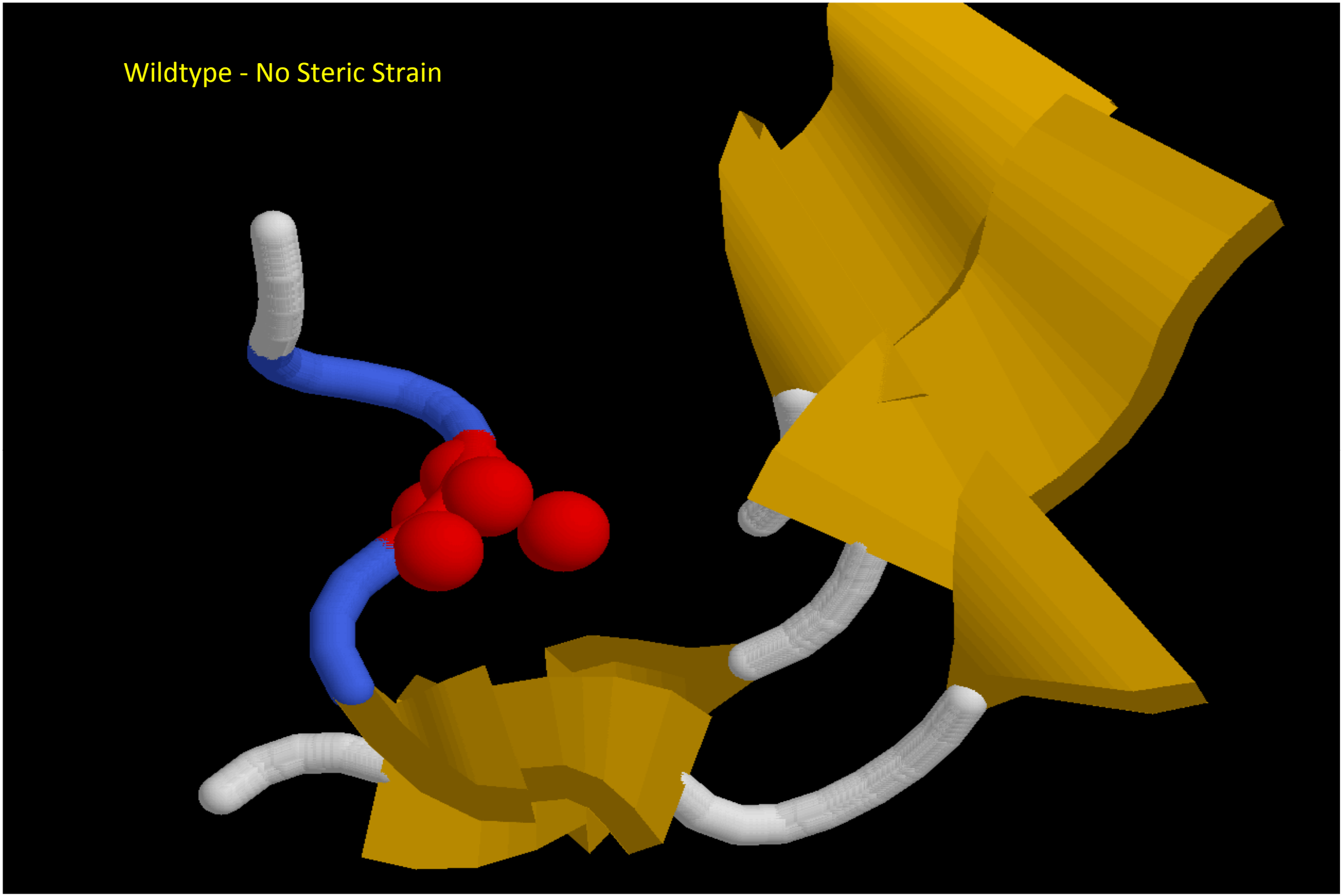


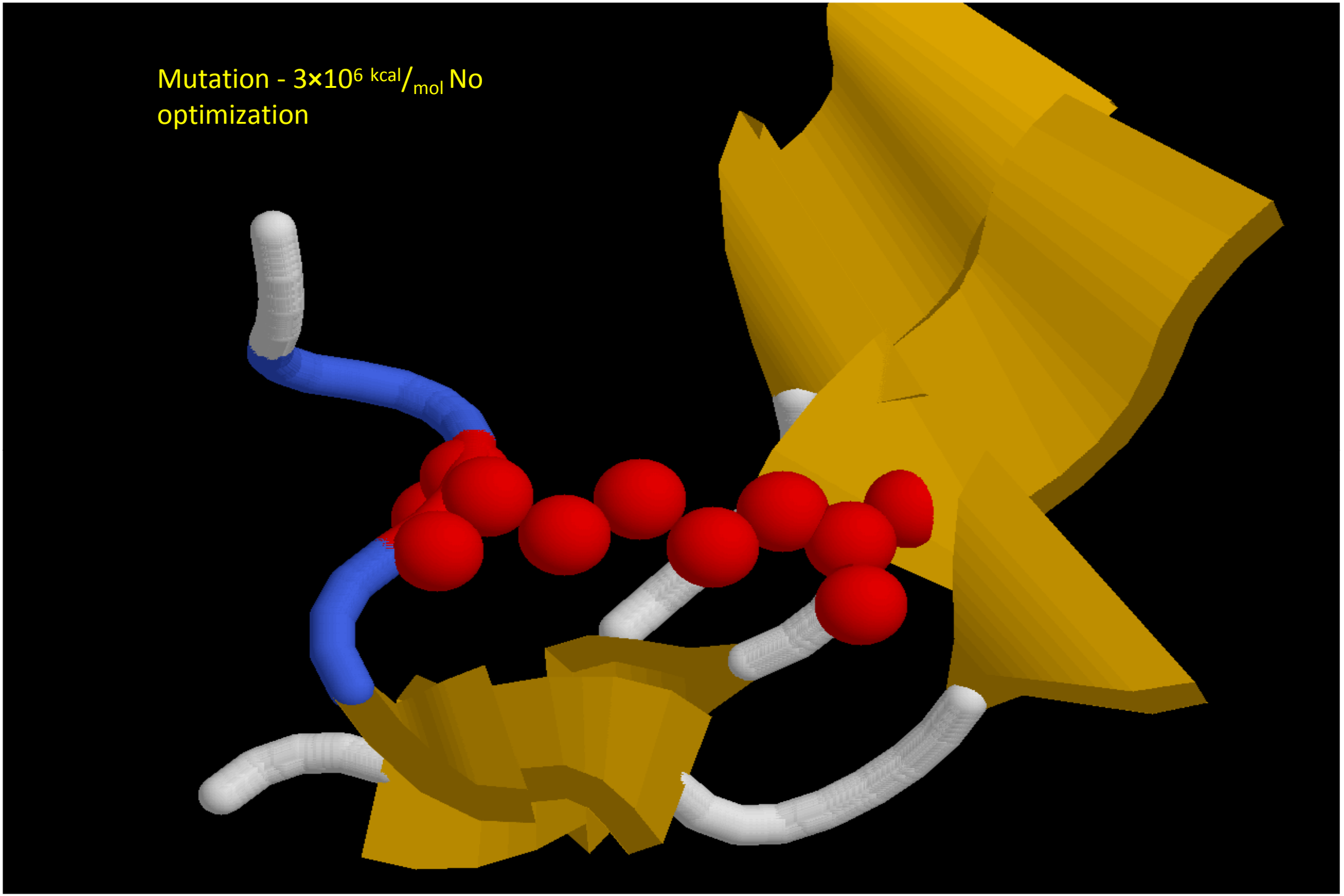
Functional studies

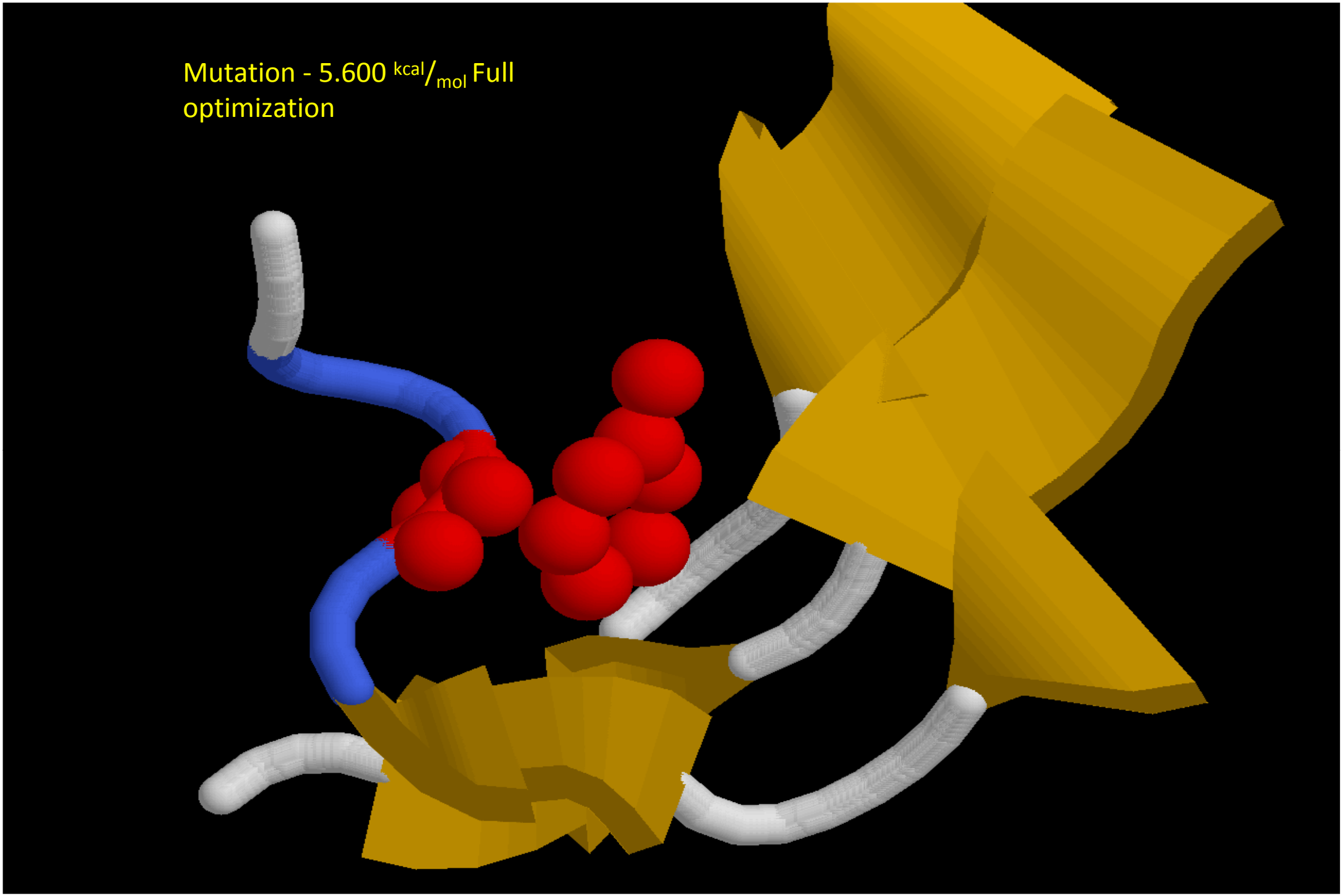
MCAD missense mutationer




Sterically Forbidden Mutation
G170R


Wildtype - No Steric Strain


Mutation - 3×10^{6} $^{kcal}/_{mol}$ No optimization


Mutation - 5.600 kcal/mol Full optimization

Sygdomsmekanisme ved MCAD mangel

LC-fatty acyl-CoA

↓ *VLCAD*

Decanoyl-CoA

↓ *MCAD*

Octanoyl-CoA

↓ *MCAD*

Hexanoyl-CoA

↓ *MCAD*

Butyryl-CoA

↓ *SCAD*

Acetyl-CoA

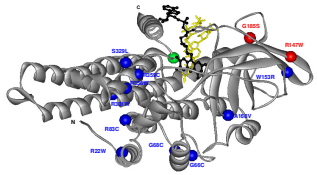
↓

Citric acid cycle

Oktansyre

Energi mangel

Nedbrydning



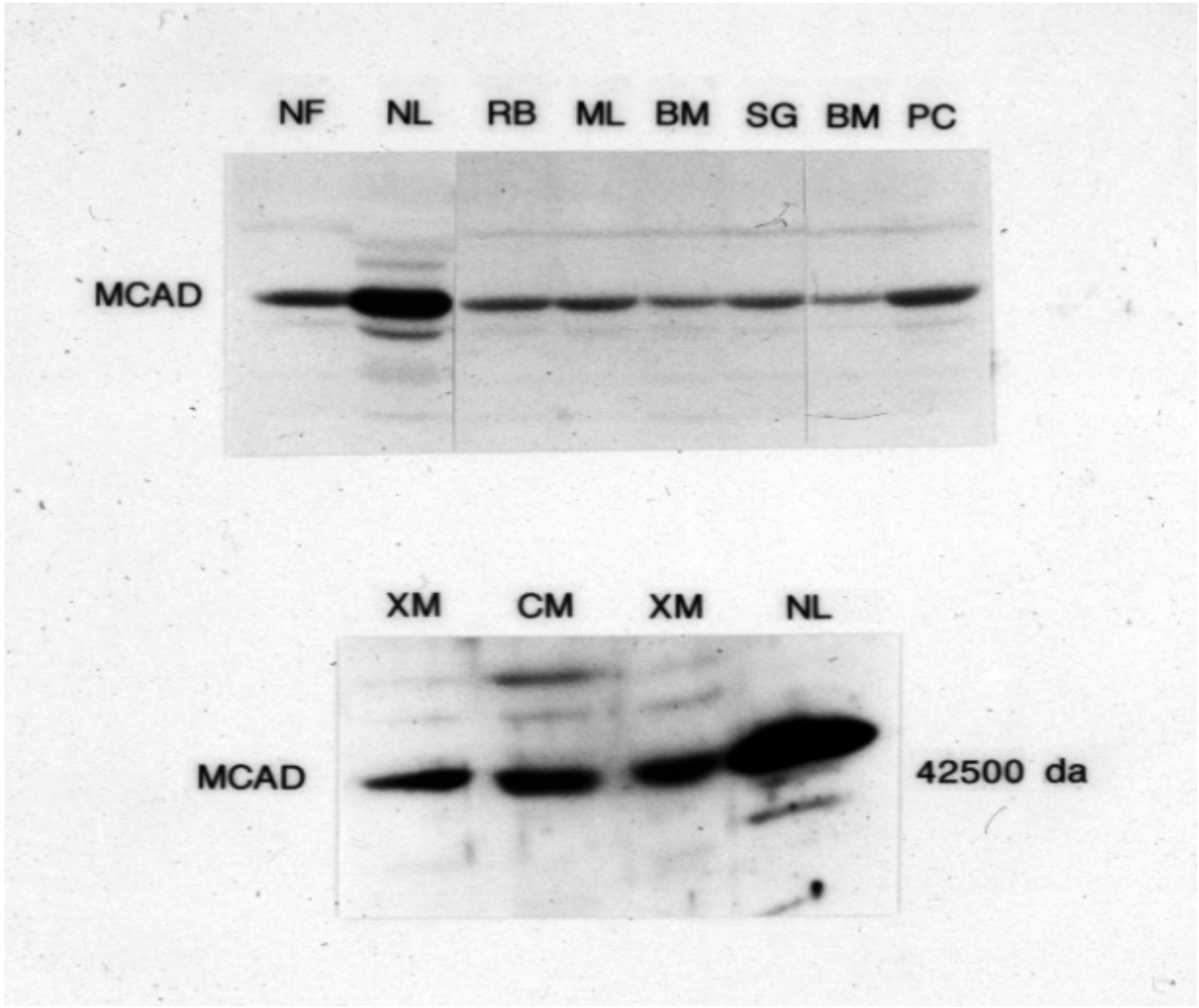
Ophobning

Loss-of-function

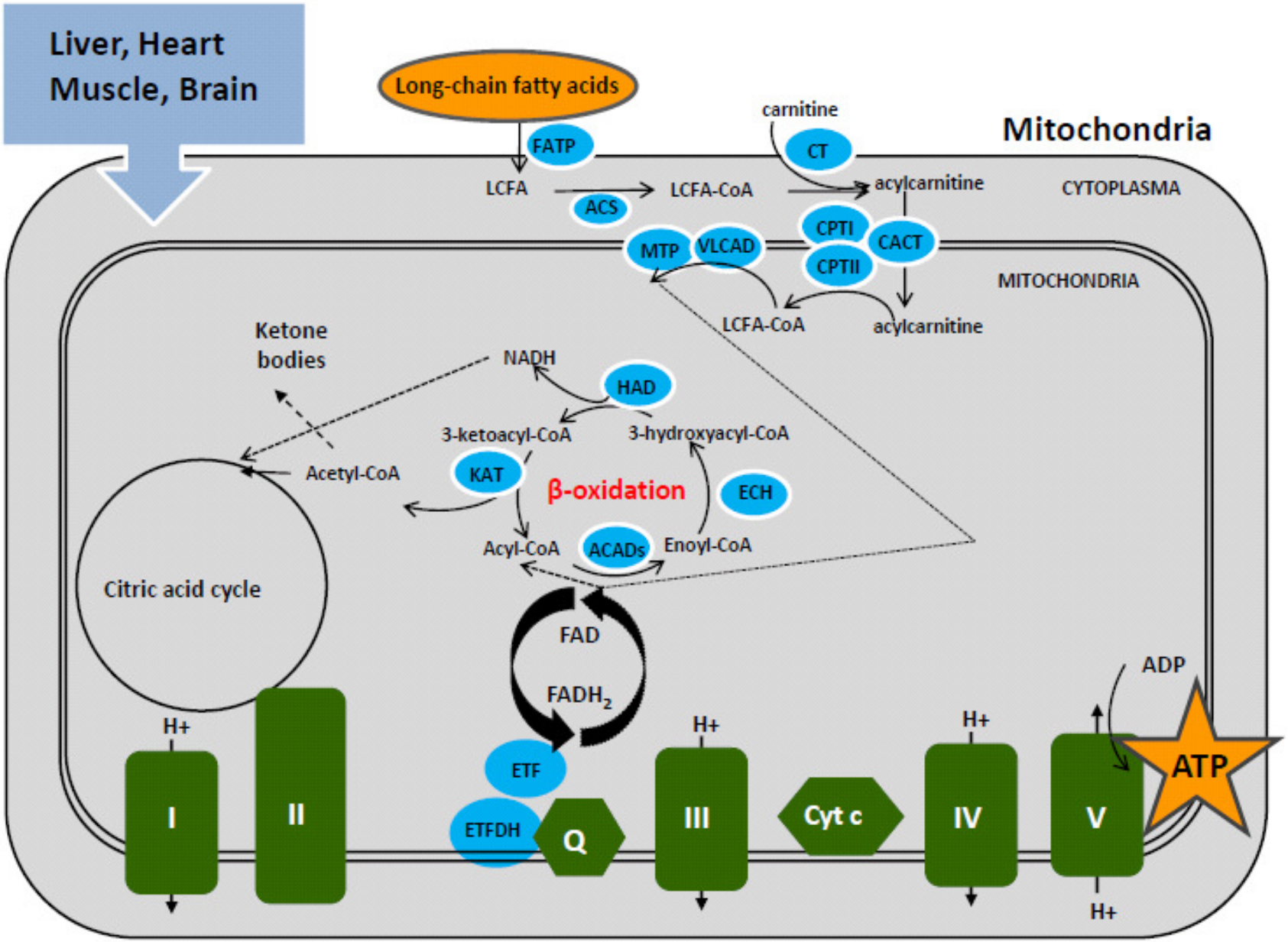
Gain-of-function

MCAD Protein (Western blot med anti-MCAD antistof)

1989



Fedtsyreoxidationen (β-oxidationen) i mitokondrierne



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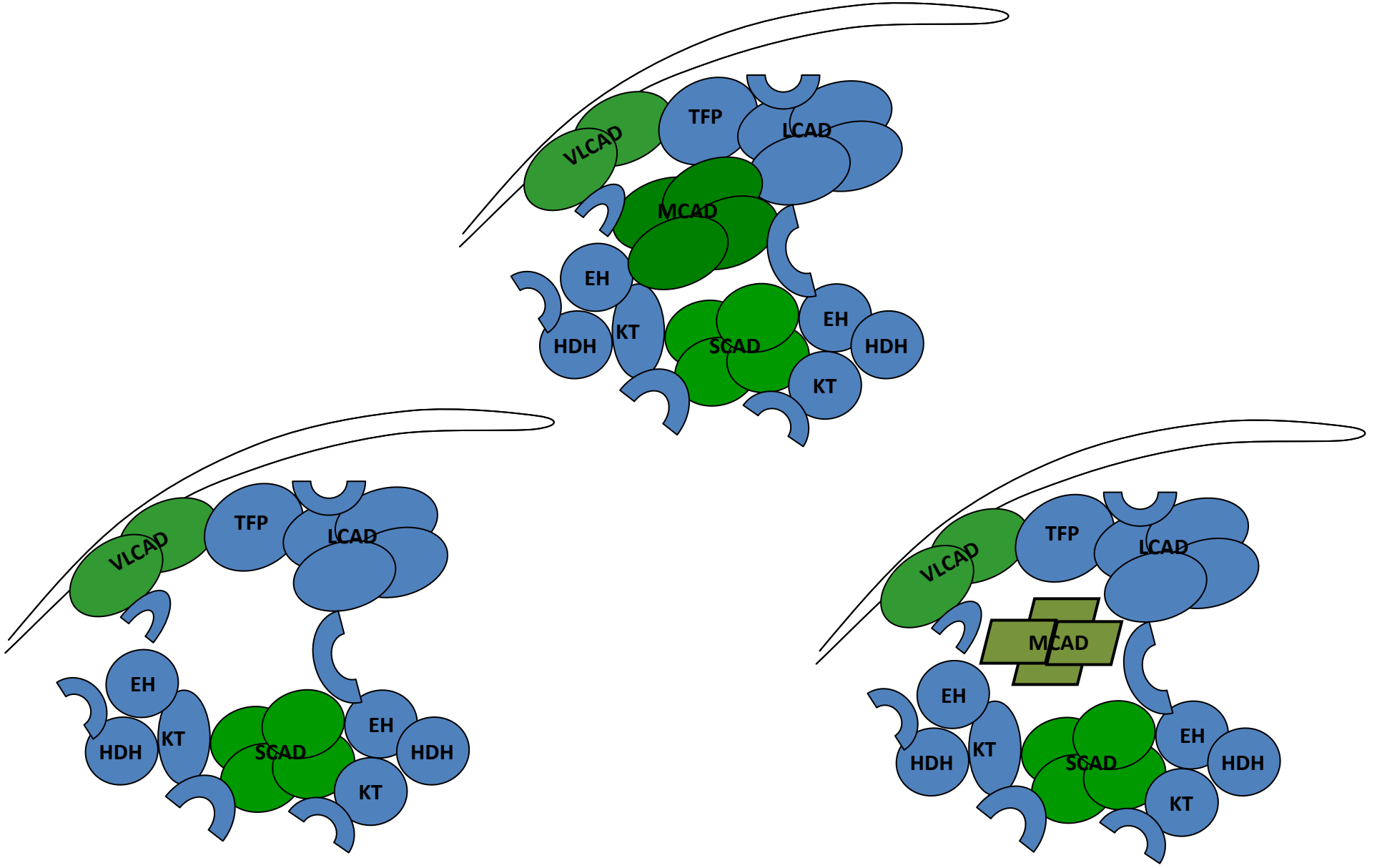
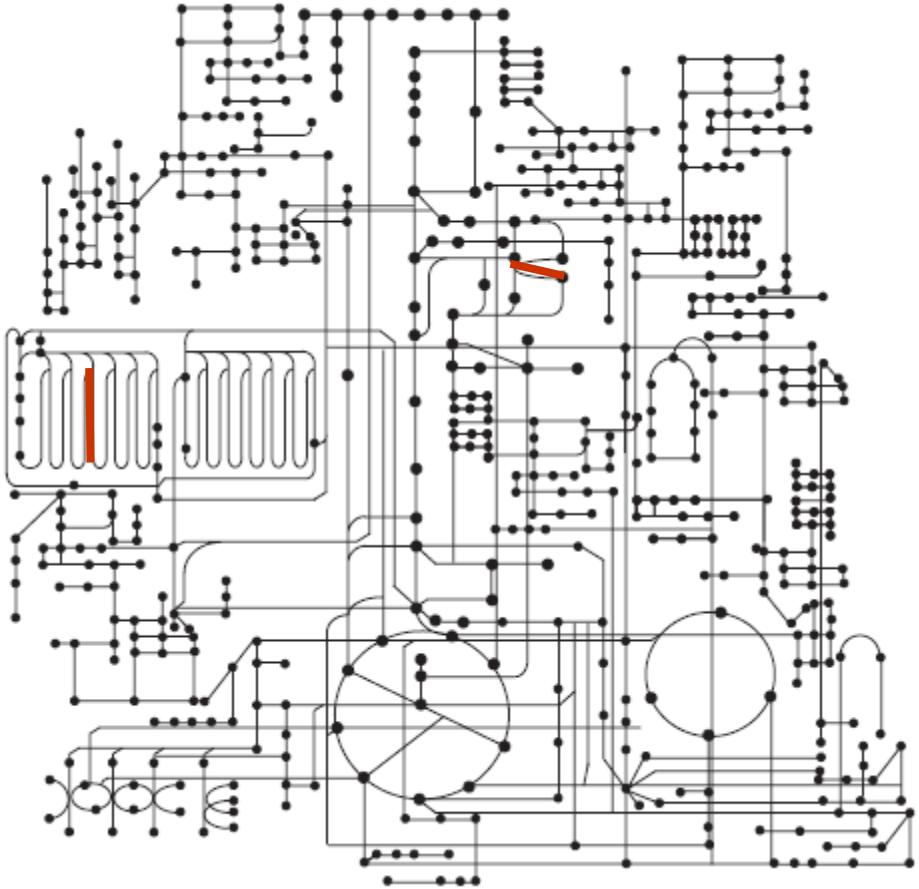
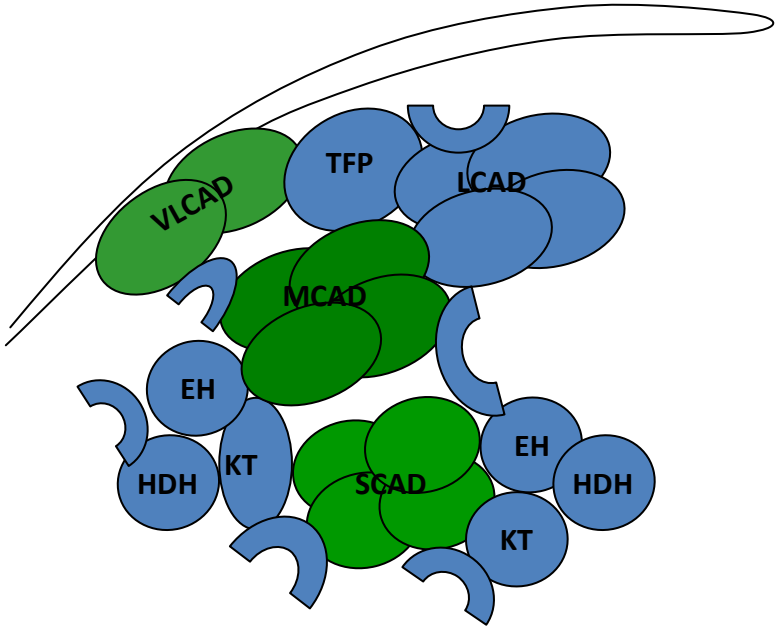
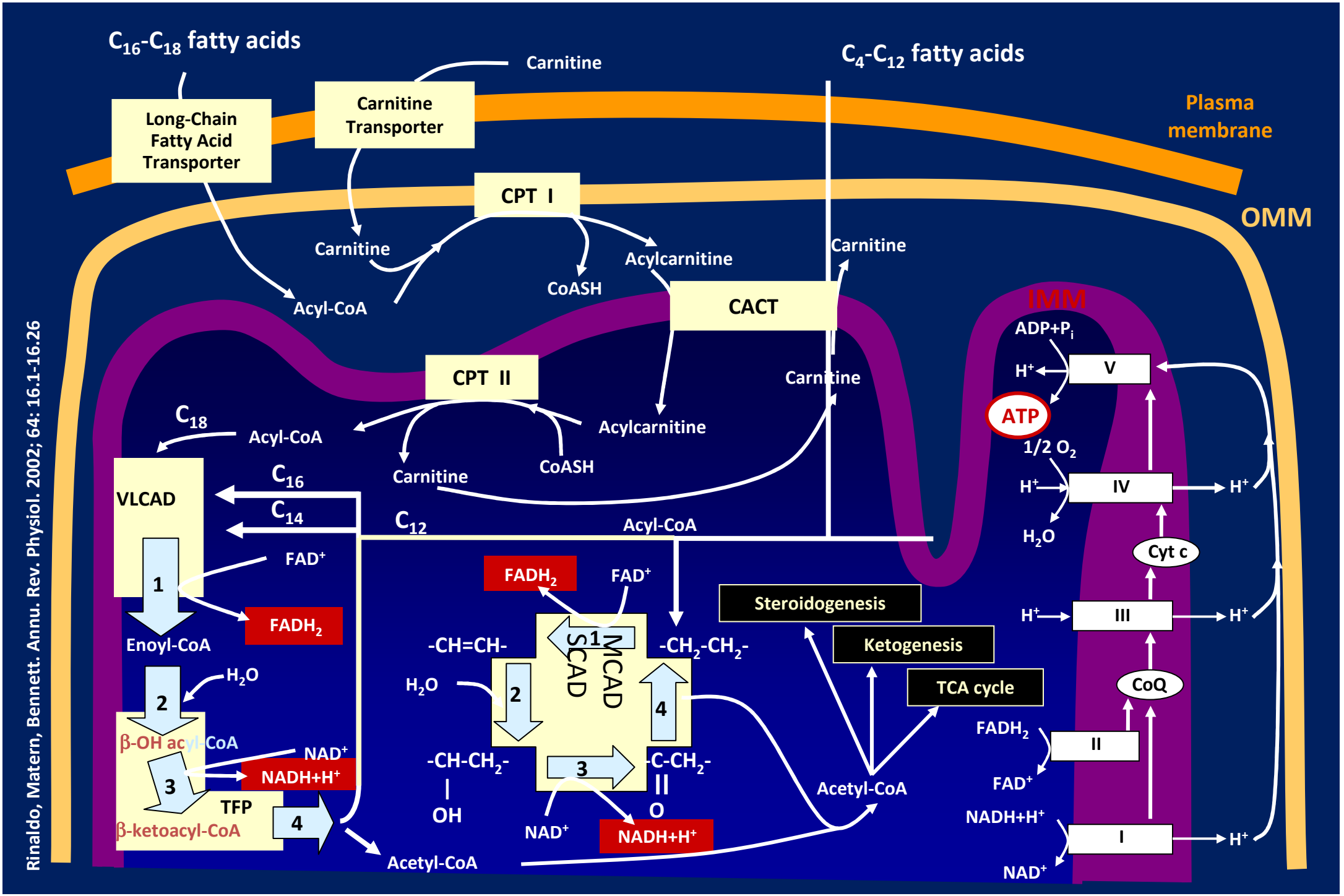


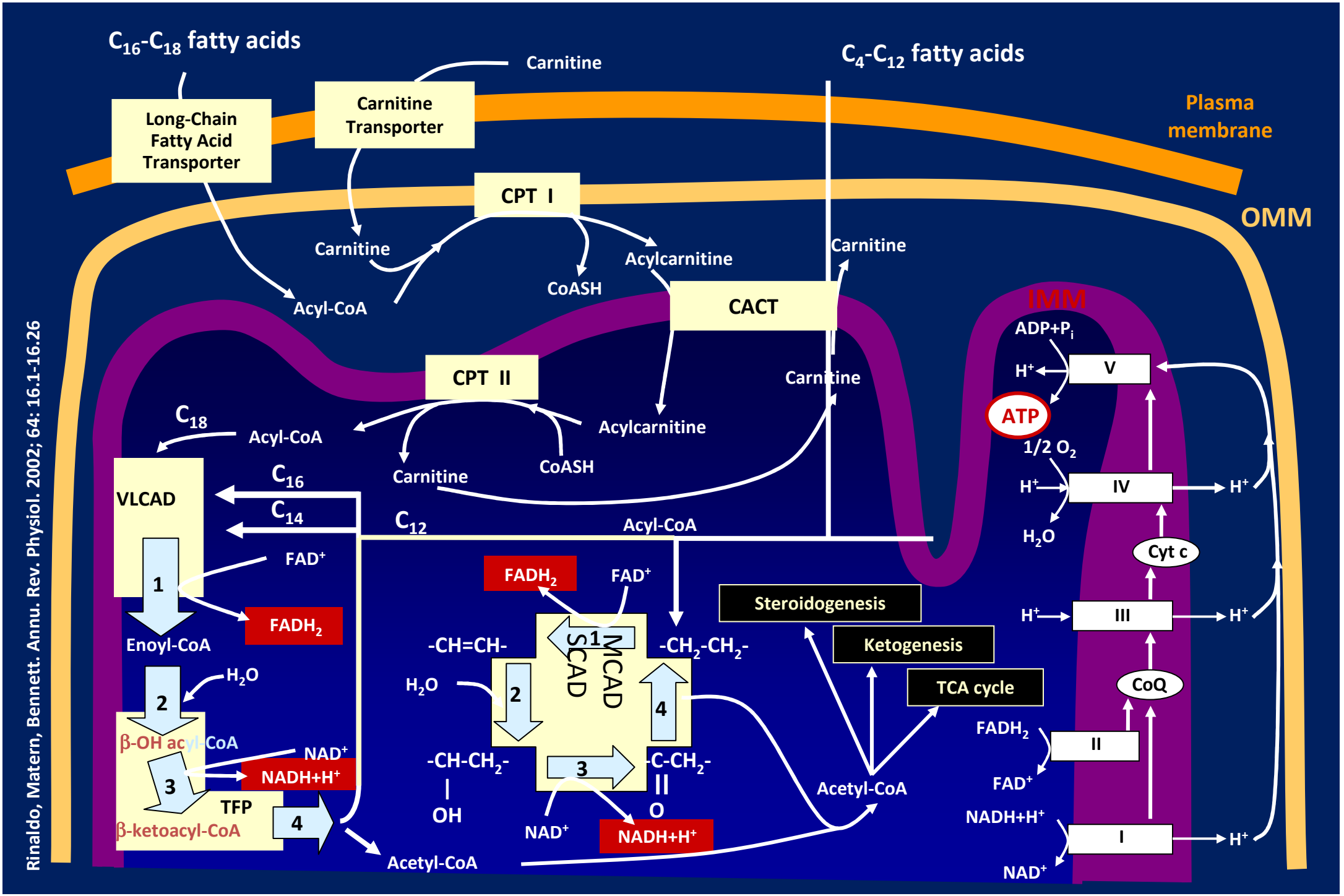
MCAD Mangel

Grunden til den store variation i alvorligheden af sygdommen kenden vi ikke i dag. Det må skyldes andre genetiske variationer og ydre påvirkninger, så som feber og stess. Det er det vi prøver at finde ud af.






C_{16}-C_{18} fatty acids
Carnitine
C_4-C_{12} fatty acids
Long-Chain Fatty Acid Transporter
Carnitine Transporter
Plasma membrane
CPT I
OMM
Carnitine
Acylcarnitine
CoASH
Acyl-CoA
CACT
Carnitine
IMM
CPT II
ADP+P_i
H^+
V
ATP
C_{18}
Acyl-CoA
Carnitine
Acylcarnitine
CoASH
Carnitine
1/2 O_2
H^+
IV
H^+
H_2O
VLCAD
C_{16}
C_{14}
C_{12}
Acyl-CoA
Cyt c
FAD^+
$FADH_2$
$FADH_2$
FAD^+
Steroidogenesis
H^+
III
H^+
1
Enoyl-CoA
-CH=CH-
SCAD
MCAD
$-CH_2-CH_2-$
Ketogenesis
H_2O
2
H_2O
2
4
CoQ
TCA cycle
β-OH acyl-CoA
NAD^+
$FADH_2$
II
3
$NADH+H^+$
$-CH-CH_2-$
3
$-C-CH_2-$
FAD^+
TFP
OH
O
Acetyl-CoA
$NADH+H^+$
β-ketoacyl-CoA
4
NAD^+
$NADH+H^+$
I
H^+
Acetyl-CoA
NAD^+
Rinaldo, Matern, Bennett. Annu. Rev. Physiol. 2002; 64: 16.1-16.26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Steroidogenesis
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1
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Rinaldo, Matern, Bennett. Annu. Rev. Physiol. 2002; 64: 16.1-16.26