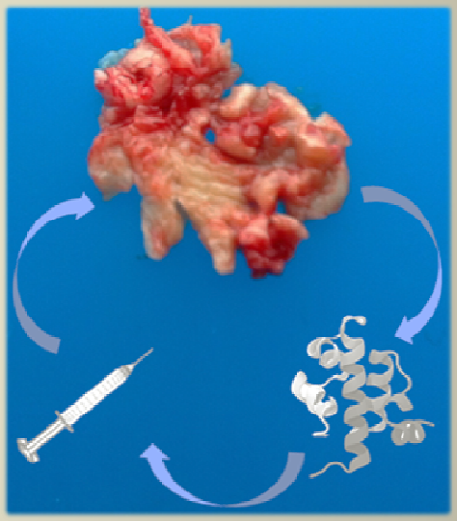
Clinical and Proteomic Characterization of Cholesteatoma

Nye teorier for patogenesen af Cholesteatom baseret på proteomanalyse.

Opsummering af PhD projekt



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Clinical and Proteomic Characterization of Cholesteatoma

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Background & Aims

Clinical characteristics



Molecular characteristics



Clinical and Proteomic Characterization of Cholesteatoma

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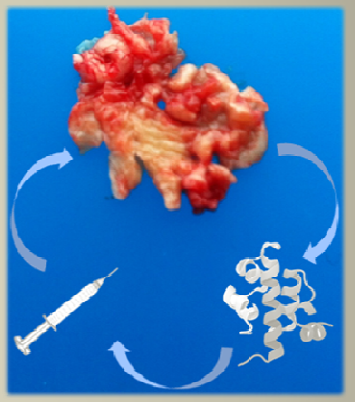
Aims

Translational analysis of Cholesteatoma

..from clinical problem to molecular process and back



Studies:

I Prognostic factors for the recidivism rate

II Protein profile (Proteome)

III Cytokine profile

Clinical and Proteomic Characterization of Cholesteatoma

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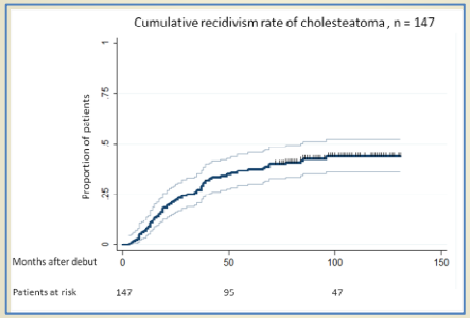
Study I

Recidivism Rate and Prognostic Factors for Cholesteatoma



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Study I

Recidivism Rate and Prognostic Factors for Cholesteatoma

Background

- Very high variation in reported outcomes
- Very few control for co-variates

Aims

- Recidivism rate of debuting cholesteatoma (Kaplan – Meier)
- Strongest independent clinical prognostic factors (Cox regression)
- Link to molecular processes?..
- Hearing outcomes

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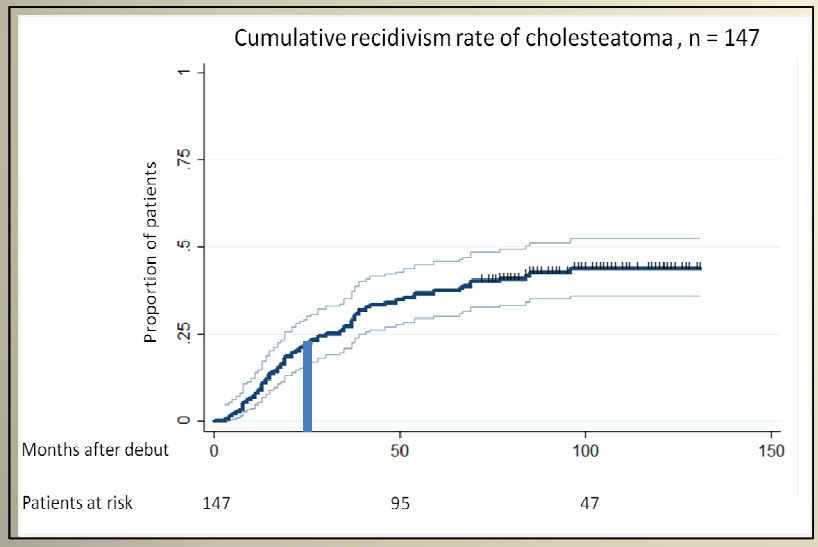
Study I

Recidivism Rate and Prognostic Factors for Cholesteatoma

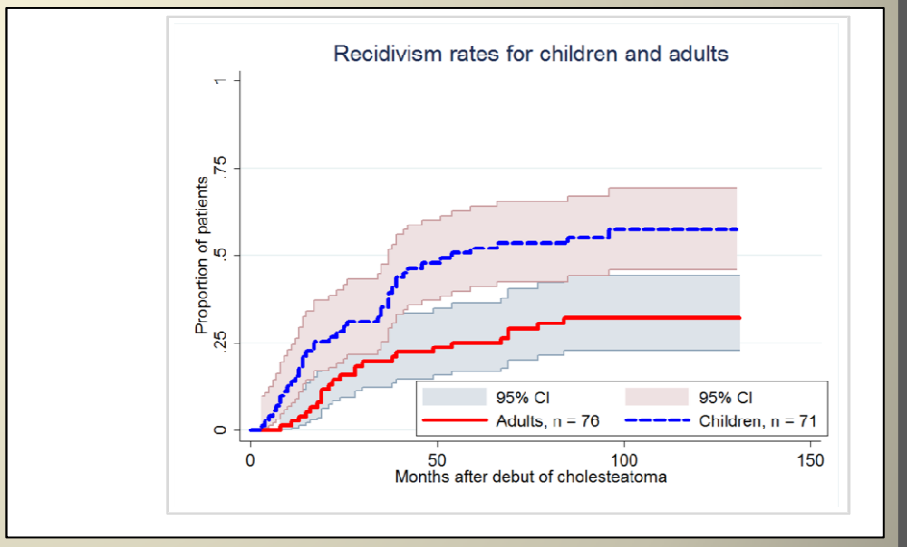
Recidivism rates (Kaplan – Meier time to event)

n = 147 , median follow-up time 8.5 years

Rates at five and ten years



Overall	0.38	0.44



Children	0.52	0.57
Adults	0.25	0.32

Study I

Recidivism Rate and Prognostic Factors for Cholesteatoma

Prognostic factors for recidivism

n = 147 , 64 events

Univariate log-rank tests for correlation with recidivism

p< 0.25	Covariate	p –value
➡	Age below 15 years	0.002
➡	Male gender	0.11
	Chol. limited to tymp.cavity	0.41
➡	Chol. In the mastoid	0.01
	Mastoidectomy	0.73
➡	Ossicular erosion, all	0.03
	Malleus	0.8
➡	Stapes	0.01
➡	Incus	0.04
➡	Additional bone involvement (ABI), all	0.04
	ABI Iatrogenic	0.63
➡	ABI Spotaneous	0.06
	Ossiculoplasty	0.58
	Attic type	0.31
	Sinus type	1.17
	Tensa type	0.42
➡	Operator (surgeon)	0.02

Multivariate cox regression analysis of independent predictors
Stratified by operator

Combined hazard ratio ≈ 14

Study I

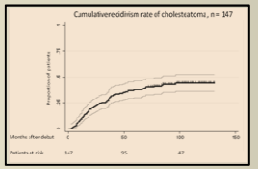
Recidivism Rate and Prognostic Factors for Cholesteatoma

Conclusion

Recidivism rates

0.38 five year

0.44 ten year



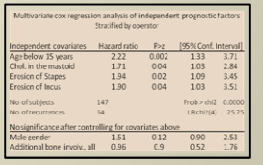
Prognostic factors

Age

Mastoid involvement

Ossicular erosion

- Incus
- Stapes



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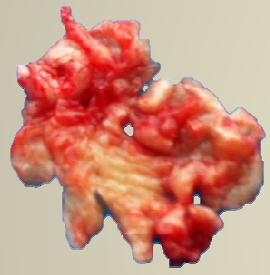
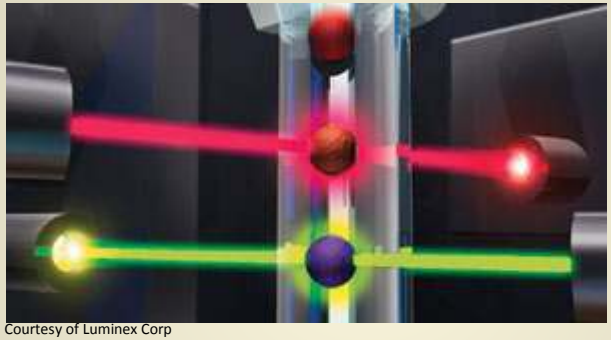
Study III

44-Plex Cytokine Profile of Cholesteatoma





Courtesy of Luminex Corp

Inflammation?

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Study III

44-Plex Cytokine Profile of Cholesteatoma

Background

- Signaling molecules - important in inflammatory diseases
- Challenging to analyze
- Direct drug targets

Aims

- Profile of cytokines
- Explanation for the behavior
- Drug targets for cholesteatoma?

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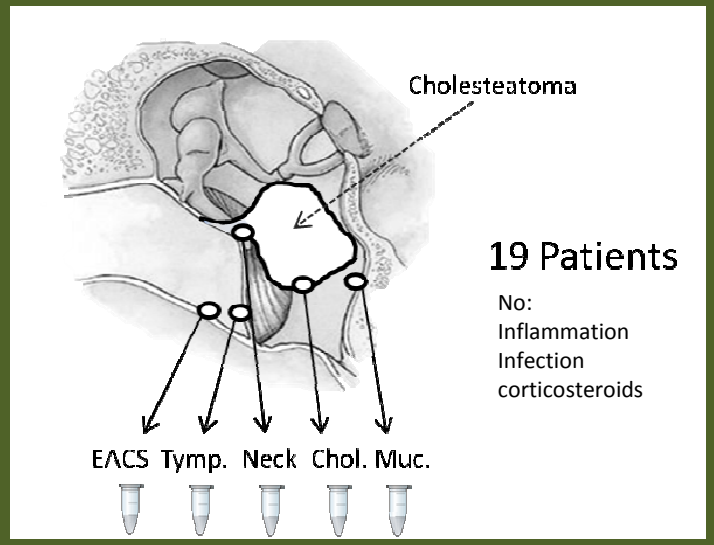
Study III

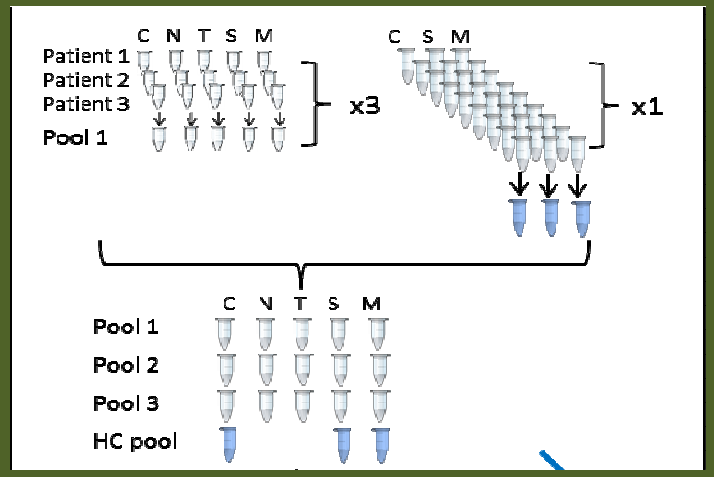
44-Plex Cytokine Profile of Cholesteatoma

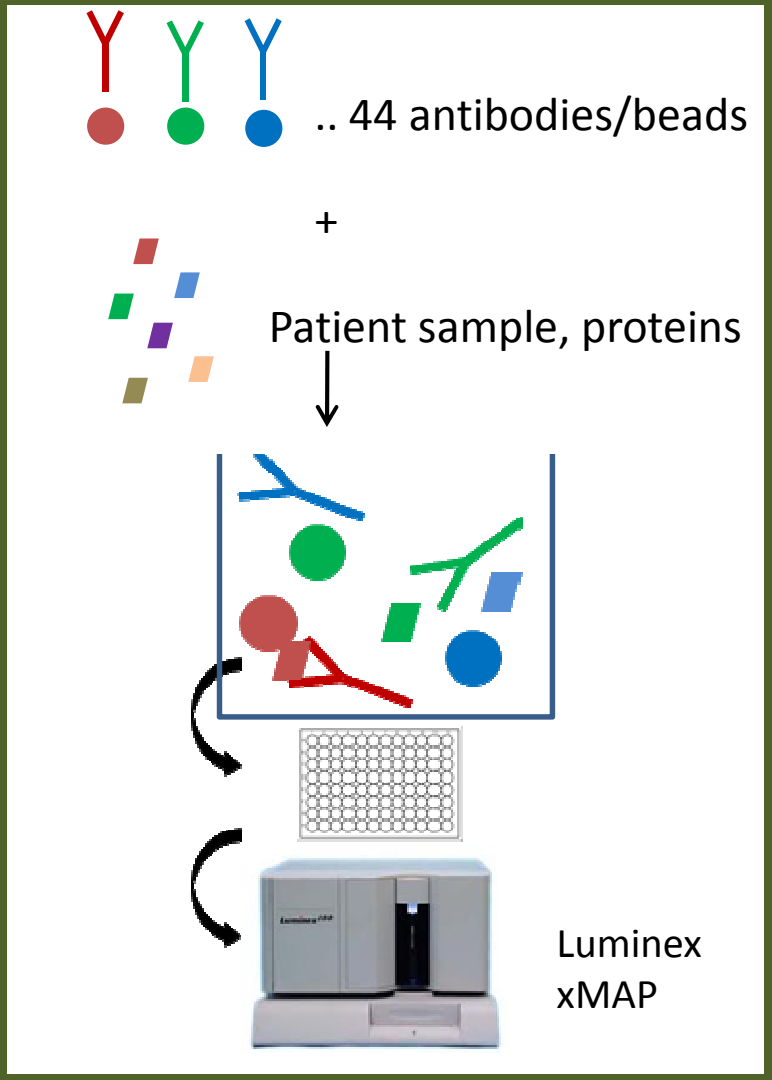


19 Patients

No:
Inflammation
Infection
corticosteroids

Pooling





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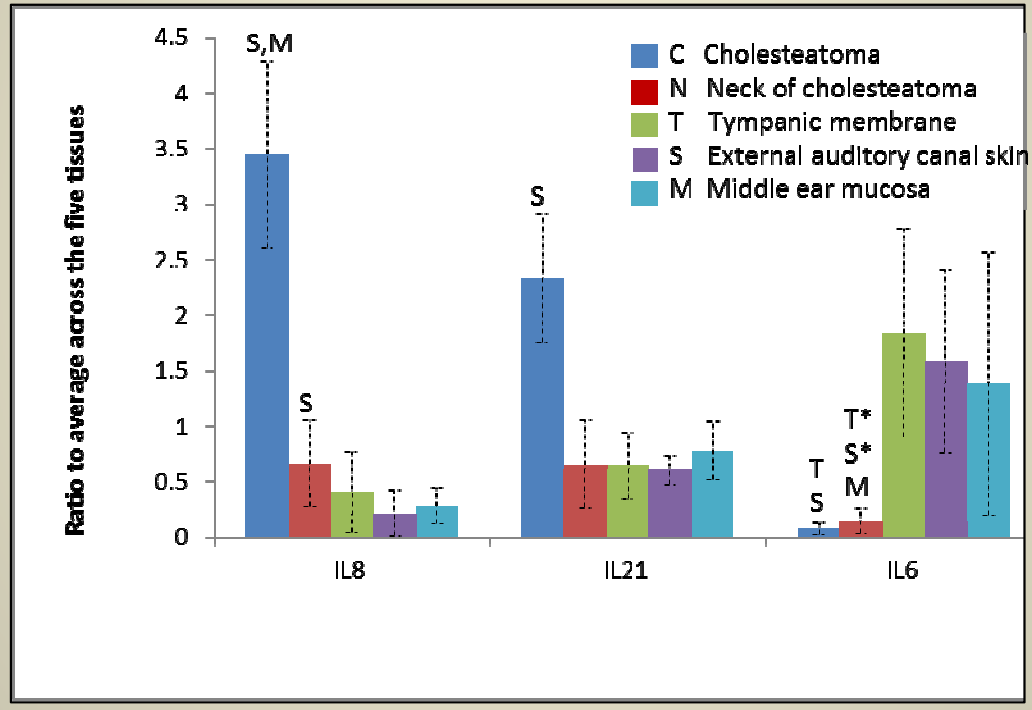
Study III

44-Plex Cytokine Profile of Cholesteatoma

Results



Letters over standard deviation bars indicate the tissues compared to which significant differences ($p < 0.05$, Students t-test on log -ratios) were found in the pairwise comparisons. *(non-significant p-value, but single ratios all greater than 2xSEM

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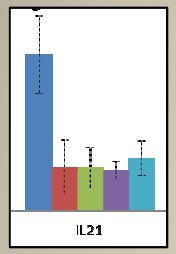
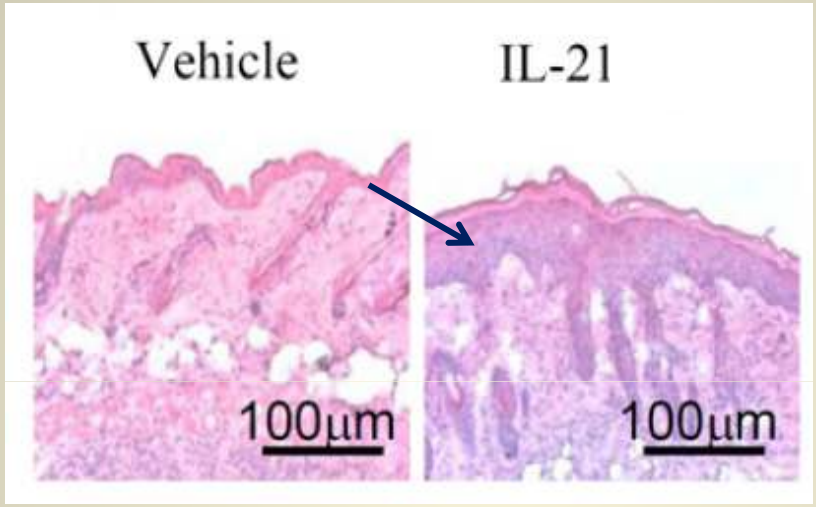
IL-21

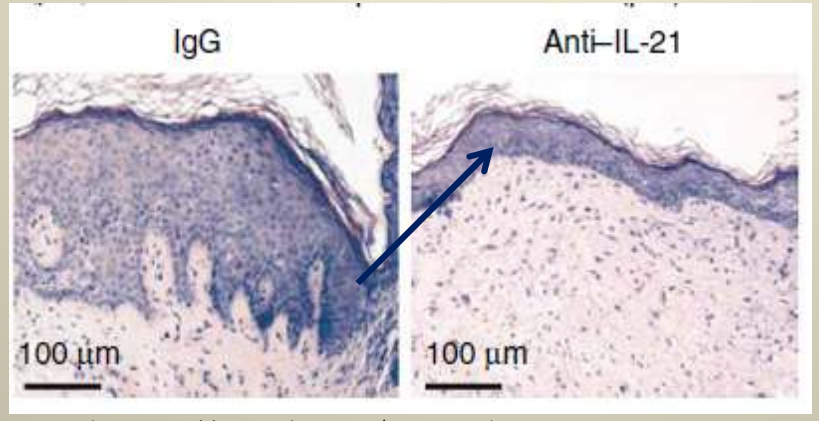


IL-21 Wild type mice



Sarra et al, J Immunol. 2011 May 1;186(9):5435-42. doi: 10.4049/jimmunol.1003326. Epub 2011 Mar 25.

IL-21 block Psoriasis mice



Nat Med. 2009 Sep;15(9):1013-5. doi: 10.1038/nm.1995. Epub 2009 Aug 16.

Ongoing clinical trials on IL-21 blockers..

(Topical) drug target for cholesteatoma?

Clinical and Proteomic Characterization of Cholesteatoma

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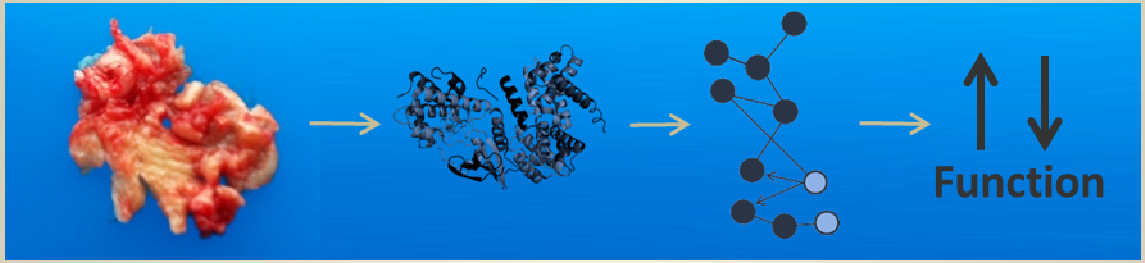
Study II

Large Scale Proteomic Analysis of Cholesteaoma



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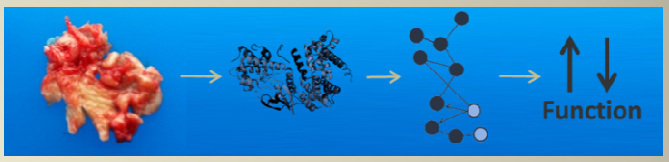
Study II

Large Scale Proteomic Analysis of Cholesteatoma

Background



- Focused analyses – no breakthrough
- Difficult to combine analyses
- Unfocused screening analysis!

Aims

- Investigate the proteome
- Characterize the biology
- Identify key elements for the pathogenesis

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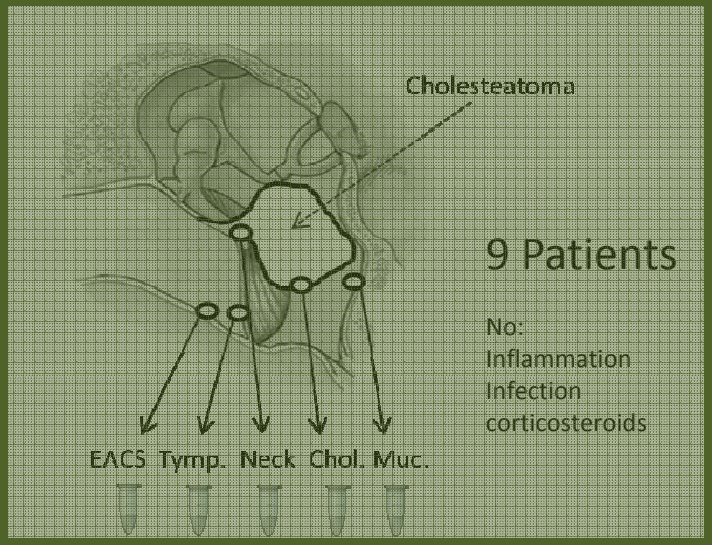
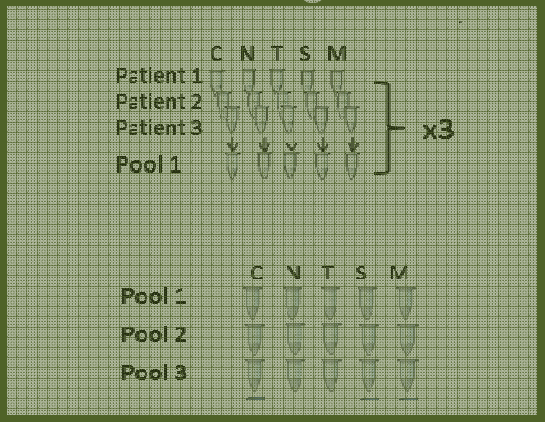
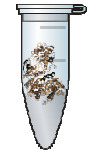


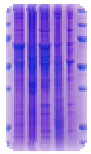
Pooling



150 analyses



Extraction



SDS-PAGE



Trypsin cleavage



nano-LC



Label free
LTQ Orbitrap MS/MS



Identification
Bioinformatics

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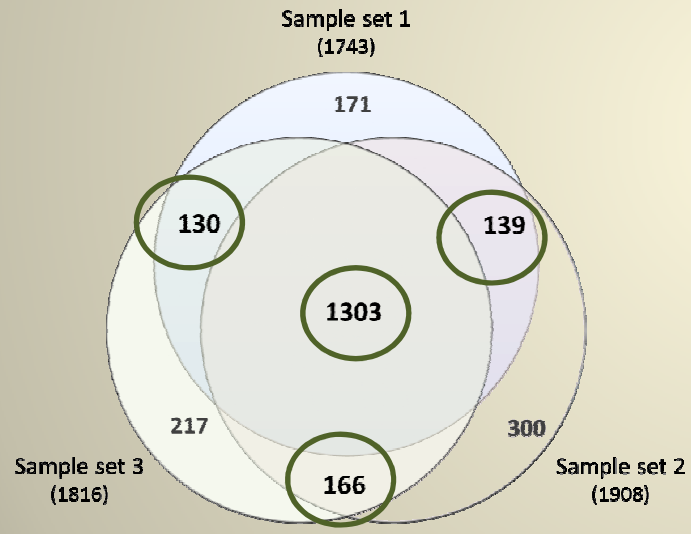
Study II

Large Scale Proteomic Analysis of Cholesteatoma

Results

> 2400 unique proteins



Sample set (pool)= C N T S M

Tissue comparison

- 1738 proteins
- 98 % Overlap - Cholesteatoma sack vs. other

Cutoff:

- Mean ratio > 2 x SEM ≈ Faktor 3 - 8
- P < 0.05

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Large Scale Proteomic Analysis of Cholesteatoma

Results

Differential level proteins

Up-regulated

Gene name	Accession #	Protein description	Tissue comparison and ratio: Cys N	Cys T	Cys S
RNASE7	Q9H1E1	Ribonuclease 7	9.59	6.00 a	7.08 a
KRT4	P19013	Keratin 4	4.88 a	4.42	40.4 b(t)
S100A7A	Q86SG5	Protein S100-A7A, Koebnerisin	179 b(t)	242	963 b(t)
CEACAM6	P40199	Carcinoembryonic antigen-related cell adhesion molecule 6	3.81	14.2 a	225 b
SERPINB10	P48595	Serpin B10	23.0	6.99 a	102 b
STS	P08842	Steryl-sulfatase	3.40	45.0 a	35.0 b
APOA2	P02652	Apolipoprotein A-II	16.6 b	10.90	30.0 b
NPC2	P61916	Epididymal secretory protein E1	4.18	6.81 a	6.82 b
MPO	P05164	Myeloperoxidase	1.85	1.59	13.5 a
KRT7	P08729	Keratin 7	0.99	1.56	10.3 a
ELANE	P08246	Neutrophil elastase	1.23	2.19	9.74 a
ASAH1	Q13510	Acid ceramidase	2.47	3.14	4.84 a
SERPINB12	Q96P63	SERPINB12 protein	2.77	3.89 a	3.81
KRT19	P08727	Keratin, type I cytoskeletal 19	1.85	0.73	2.87 a
HRNR	Q86YZ3	Hornerin	14	142 a(t)	140
ECM1	Q16610	Extracellular matrix protein 1	2.64	33.2 a(t)	10.6
PRTN3	P24158	Myeloblastin	1.12	2.58	15.7 b(t)
LCN2	P80188	Neutrophil gelatinase-associated lipocalin	1.24	1.45	14.5 b(t)
LTF	P02788	Lactotransferrin	0.98	1.50	11.5 b(t)
KRT8	P05787	Keratin 8	1.63	0.62	5.92 b(t)

Down-regulated

Gene name	Accession #	Protein description	Tissue comparison and ratio: Cys N	Cys T	Cys S
SBDS	Q9Y3A5	Ribosome maturation protein SBDS	0.083 b(t)	0.050 a	0.042 a
SYCP1A	Q14683	Structural maintenance of chromosomes protein 1A	0.027 b	0.010 a	0.008 b
SULT1A1	P50225	Sulfotransferase 1A1	0.112 b	0.025 a	0.072 b
PPBP	P02775	Platelet basic protein	0.080 b	0.062 a	0.041 b
COL18A1	P39060	Collagen alpha-1(XVIII) chain	0.100 b	0.064 a	0.043 b
ANPEP	P15144	Aminopeptidase N	0.060 b	0.051 a	0.048 b
PFN2	P35080	Profilin-2	0.392	0.026 a	0.026 a
FMOD	Q06828	Fibromodulin	0.157 a	0.173	0.088 a
POLR2E	P19388	DNA-directed RNA polymerases I, II, and III subunit RPABC1	0.598	0.170 a	0.209 a
HINT1	P49773	Histidine triad nucleotide-binding protein 1	0.303	0.171 a	0.247 a
CRYAB	P02511	Alpha-crystallin B chain	36.6	0.312 a	0.244 a
AK2	P54819	Adenylate kinase 2, mitochondrial	1.24	0.299 a	0.378 a
COCH	O43405	Cochlin	0.134	0.100 a(t)	0.078 b(t)
VWA1	Q8IPC0	von Willebrand factor A domain-containing protein 1	0.404	0.116 a(t)	0.135 b(t)
LUC7L2	O95232	Putative RNA-binding protein Luc7-like 2	0.542	0.007 a	0.045 b
AEBP1	Q8IUX7	Adipocyte enhancer-binding protein 1	0.203	0.018 a	0.016 b
HIST1H2AE	P04908	Histone H2A type 1-B/E	0.014 b	0.082 a	0.517
NID2	Q14112	Nidogen-2, Entactin	0.109	0.022 a	0.016 b
ABI3BP	Q7Z7G0	Target of Nesh-SH3	0.112 b	0.322	0.021 b
TNXB	P22105	Tenascin-X	0.084 b	0.312	0.027 b

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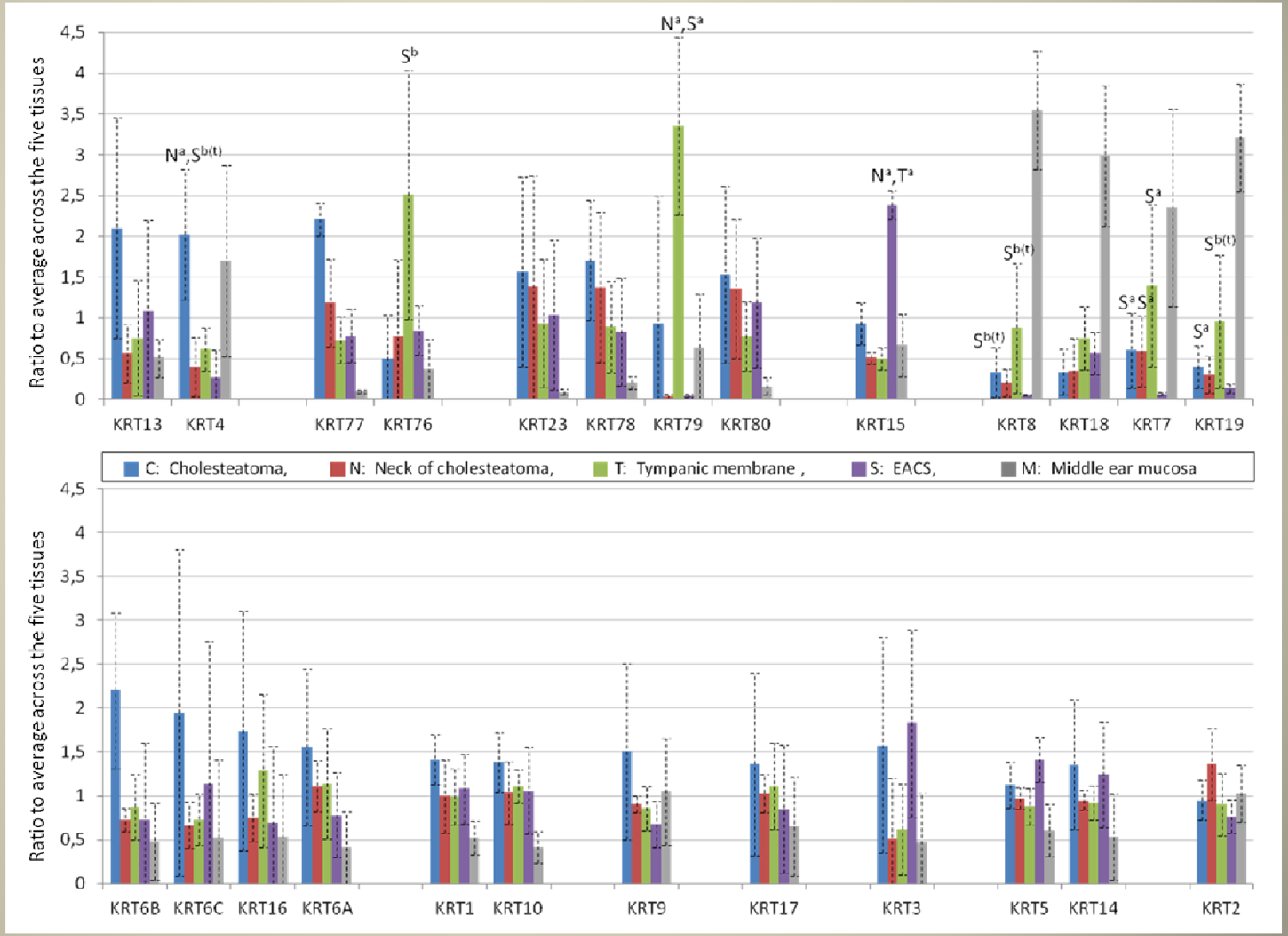
Cytokeratins



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Study II

Large Scale Proteomic Analysis of Cholesteatoma

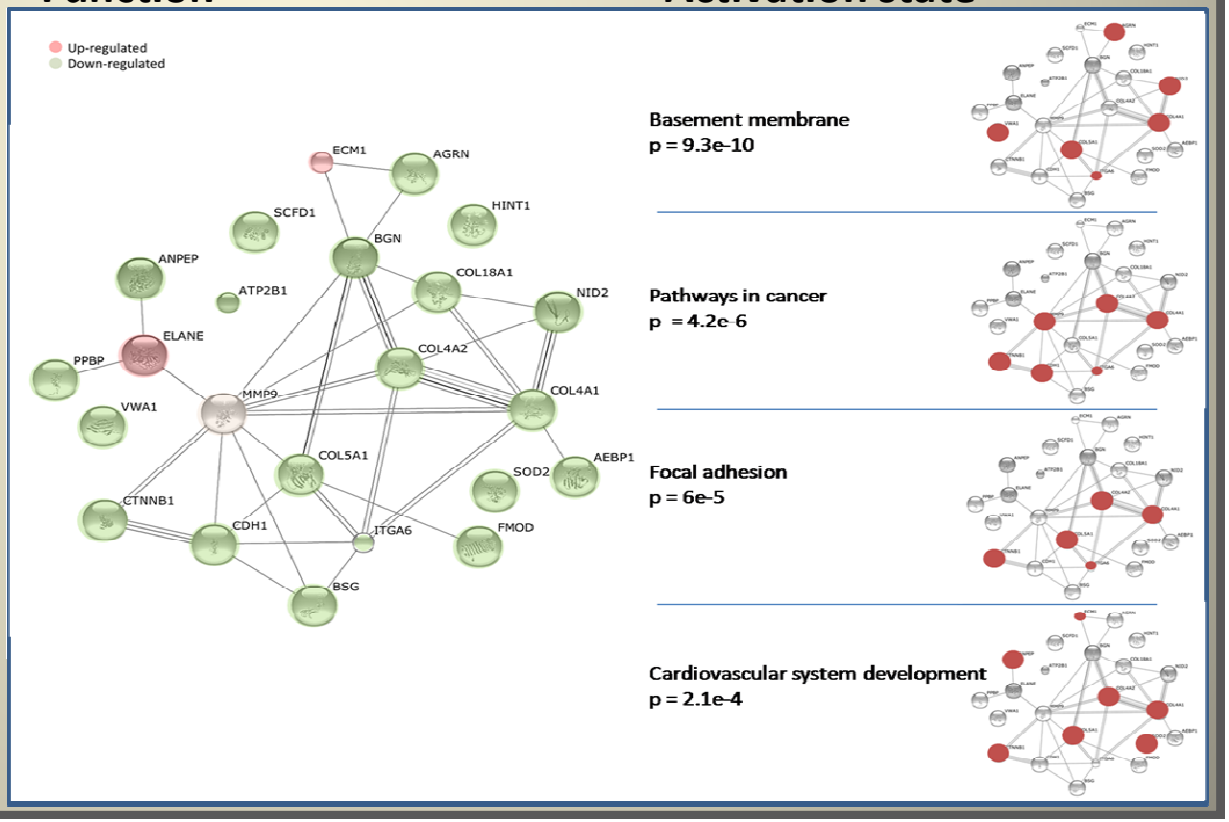
Results

Bioinformatics

Experimental data
+
Biological information
+
Statistics

Ingenuity Pathway Analysis
STRING
Panther

Function **Activation state**



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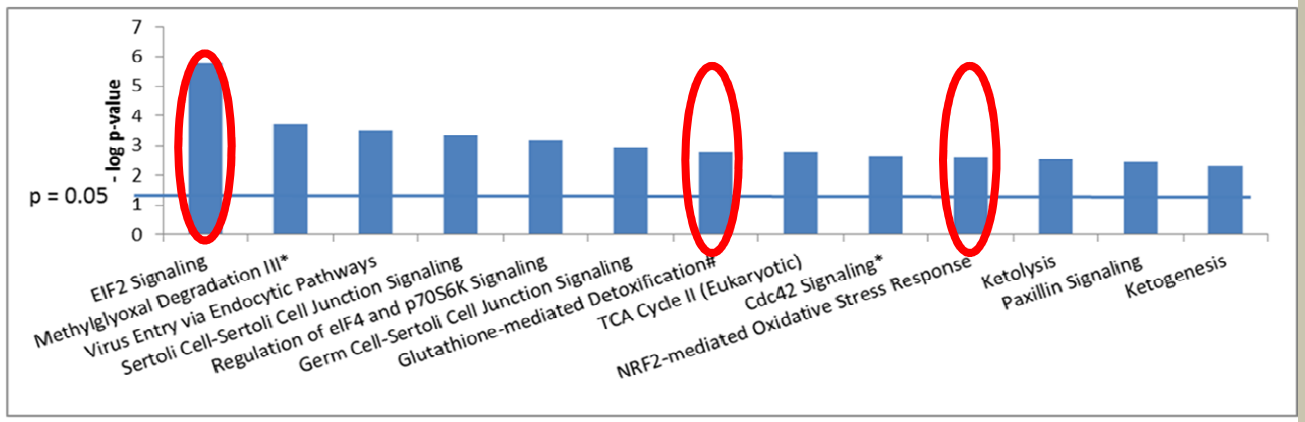
Large Scale Proteomic Analysis of Cholesteatoma

Cellular stress "coping" pathways

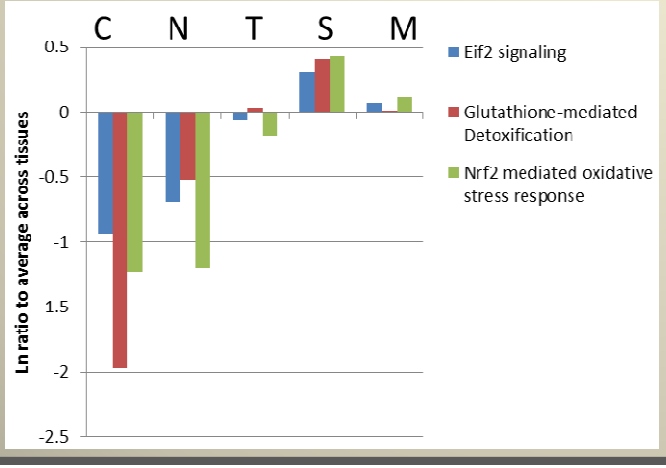
A

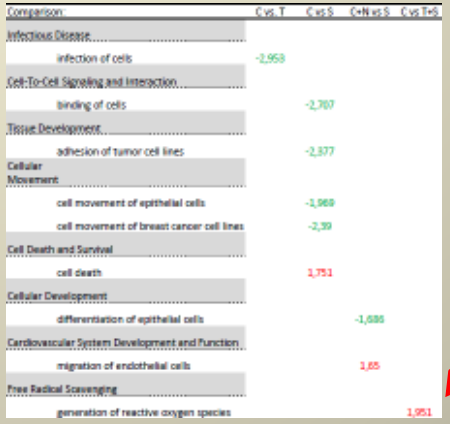


B

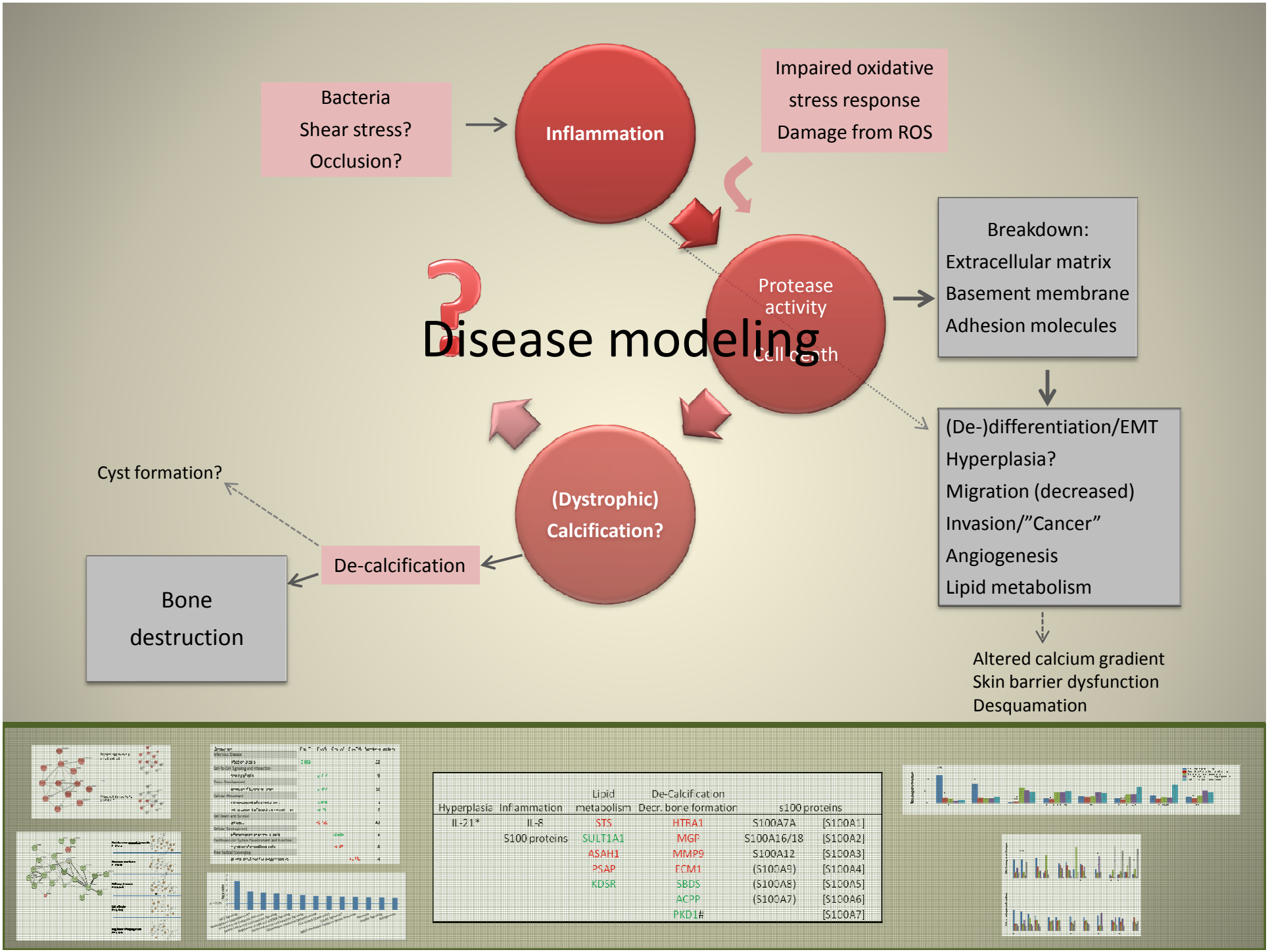
Canonical pathway	Tissue comparison	p - value	Proteins (gene names)
EIF2 Signaling	Chol. vs. EACS	1.70E-06	RPL15,NRAS,RPL18A,RPL10,RPS9,RPS8,RPL35A,RPL9,RPL18,EIF3K,RPS12
Sertoli Cell-Sertoli Cell Junction Signaling	Chol. vs. EACS	4.57E-04	SPTBN2,CDH1,NRAS,MAPK14,CLDN1,ITGA2,CTNNB1,RAB8B
Glutathione-mediated Detoxification#	Chol. vs. EACS	1.66E-03	GSTT1,GSTM3,ANPEP
NRF2-mediated Oxidative Stress Response	Chol. vs. EACS	2.45E-03	CUL3,GSTT1,NRAS,MAPK14,GSTM3,DNAJB1,SOD3

Impaired response to oxidative stress





Generation of reactive oxygen species

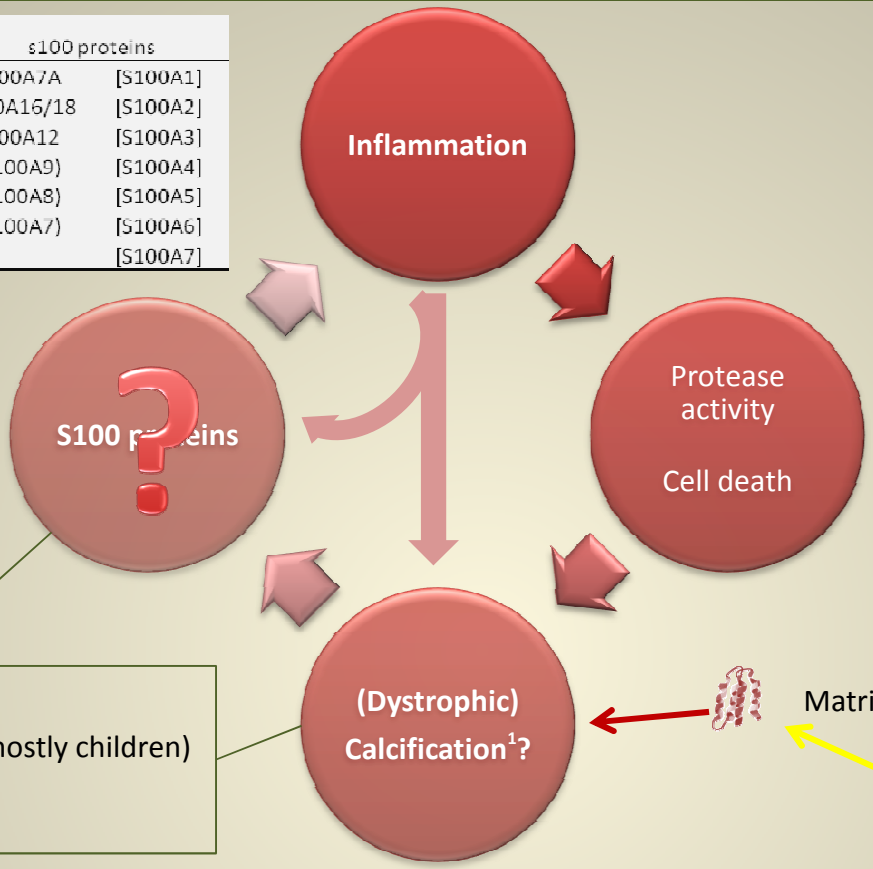


Hyperplasia	Inflammation	Lipid metabolism	De-Calcification Decr. bone formation	s100 proteins	
IL-21*	IL-8	STS	HTRA1	S100A7A	[S100A1]
	S100 proteins	SULT1A1	MGP	S100A16/18	[S100A2]
		ASAH1	MMP9	S100A12	[S100A3]
		PSAP	ECM1	(S100A8)	[S100A4]
		KDSR	SBDS	(S100A8)	[S100A5]
			ACPP	(S100A7)	[S100A6]
			PKD1#		[S100A7]

Self perpetuating inflammatory cycle

Extremely up-regulated
Pro-inflammatory
Calcium binding
- •Polymerize
- •Stabilize
- •..corpora amylacea

s100 proteins	
S100A7A	[S100A1]
S100A15/18	[S100A2]
S100A12	[S100A3]
(S100A9)	[S100A4]
(S100A8)	[S100A5]
(S100A7)	[S100A6]
	[S100A7]



Inflammation
Connective tissue disorders (mostly children)
Local milieu
Atherosclerosis

Atherosclerosis Signaling p = 0.038
COL1A2,LYZ,CD36,MMP9,GLG1

Matrix Gla Protein[2]
- •Highly up-regulated (x 45)
- •Clears calcification
- •Vitamin K dependent
- •Lower In children*

Inflammation
+
Rantes/CCL5
Fibromodulin
Biglycan
De-Calcification
Decr. bone formation
HTRA1
MGP
MMP9
ECM1
SBDS
ACPP
PKD1#

…Myringoslcerosis?

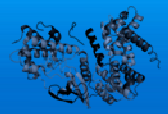


1 Whyte MP, Extraskeletal (Ectopic) Calcification and Ossification , American Society for Bone and Mineral Research
2 Schurgers LJ, Cranenburg EC, Vermeer C. Matrix Gla-protein: the calcification inhibitor in need of vitamin K. Thromb Haemost 2008 October;100(4):593-603.

Clinical and Proteomic Characterization of Cholesteatoma

AARHUS UNIVERSITY
DEPARTMENT OF CLINICAL MEDICINE
1ST DEPARTMENT OF OTO-RHINO-LARYNGOLOGY

Joint conclusion and perspective

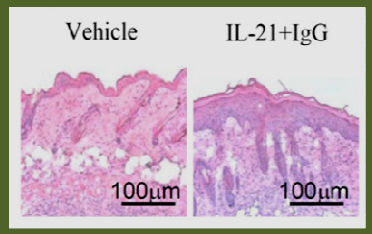




Mastoid involvement

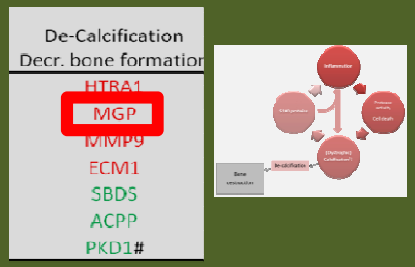
Ossicular erosion

Age

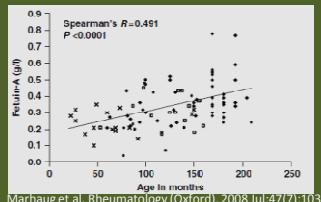
Size/hyperplasia



De-calcification



Anti calcifying capacity



Marhaug et al, Rheumatology (Oxford). 2008 Jul;47(7):1031-7

Children with Connective Tissue disorders → calcification

IL-21 block




Vitamin K

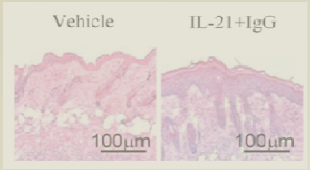
Serum levels
Risk groups – JIA?

Grow up!..

Thank you!

Mastoid involvement

Size/hyperplasia?



IL-21 block



Ossicular erosion

De-mineralization



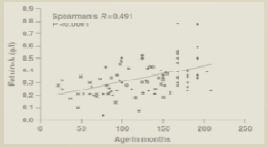


Vitamin K

..EDTA?

Age

Anti calcifying capacity



Children with Conn. Tissue disorder

Serum levels
Risk groups – JIA?

Grow up!

Supported by:

Danaflex, Ørelæge Hans Skouby's og Hustru Emma Skouby's Fond, Poul Traun-Pedersens Mindelegat, Region Midtjyllands Forskningsfond, Overlærer Svend Hansens legat, Institut for Klinisk Medicin - AU, DSOHH.