Slide 1

Interim Findings of the Research Project on Sebaceous Adenitis (SA)

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Sebaceous Adenitis (SA) disease in Akitas

...Changing Our World Through
Canine Health Research
-A New Perspective

Slide 3

The Background Story of SA

Etiology of the Disease:

- Male and female dogs are affected
- First signs monitored: Age of 2 years
- Not coat-colour depended
- Different forms of SA:
  1. Fast form
  2. Silent form
- Mostly triggered by stress: eg. pregnancy
- No medical treatment available to restore the destroyed sebaceous glands

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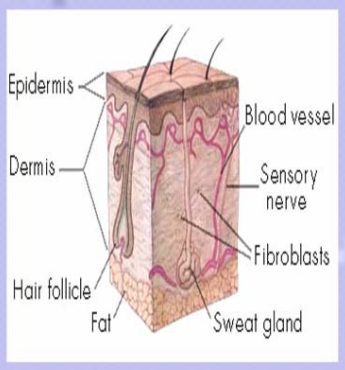
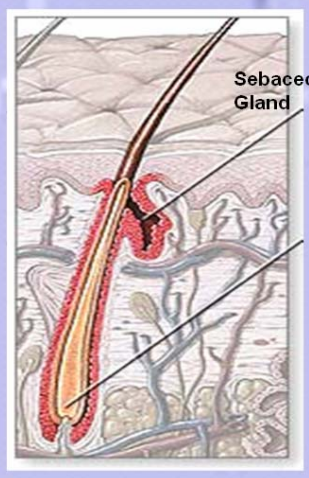
The Background Story of SA

Skin:

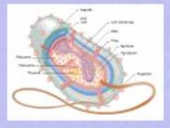


Sebaceous Gland:

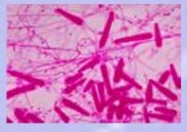


Reasons:

~~Virus~~



~~Bacteria~~



~~Fungi~~

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The Background Story of SA

Histopathology

1. Situation in healthy Akitas:
   - Sebozytes within the sebaceaous glands

2. Situation in SA-affected Akitas:
   - Massive infiltration of antigen-presenting cells (APC) in the Sebaceous Glands:
   - Macrophages
   - T-Lymphocytes
   - Dendritic Cells

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The Background Story of SA

Histopathology

- Sebozytes were attacked by APC-Cells
- No virus; No bacterial infection or fungi
- The recognition process between "self" and "non- self" is running out of control
- "Autoimmune-mediated" disease

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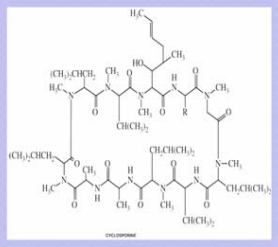
The Background Story of SA

Observed Facts:

- Immunosuppressive Medication, like Cyclosporine (Glucocorticoide) , inhibit the autoimmune reaction



- If the medicine is set down, the cells were de novo attacked

Question:

- What kind of body control mechanism is out of order ?

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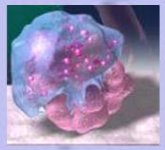
The Background Story of SA

The immunesystem localized in the blood

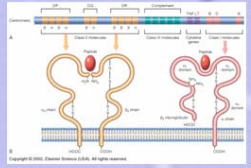
Very complex interaction of different components:

Antibodíes

Immune-Cells



MHC-System



Key role in autoimmune recognition: MHC

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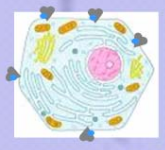
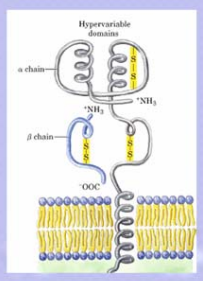
MHC: Responsible for autoimmune defects

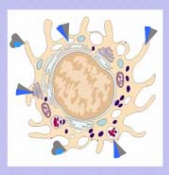


Body-Cell

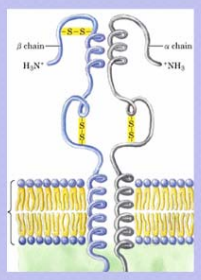


MHC- class I molecule

- Proteins localized on the surface of all cells of the body
- Antigenpresentation for T-Lymphozytes



Macrophage



MHC- class-II molecule

- Proteins only localized on APC-Cells

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The Background Story of SA

The basic reaction of
immunity and autoimme-controle is a

„KEY – LOCK – PRINCIPLE“

In the body different molecules participate in this process

Modifications within this molecules lead to disorder

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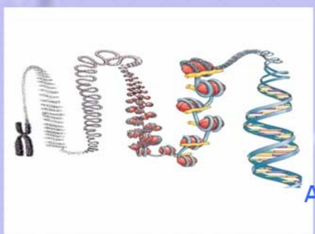
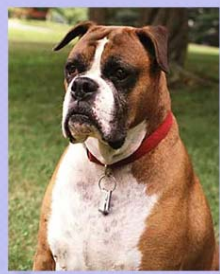
Candidate - Gene - Approach

- Loci with *a priori* propability, influencing the trait of interest
- Canine Genome since 2003 deposited in GenBank (NCBI)



2.8 x 10^9 bp
(2.8 billion basepairs)



Tasha

Primerdesign: Gen DLA-DRA1

C

C A A A C C A C A G A A T T A G C A A

TTTGTTTATTTTGCTATTTTTATTTGATTATATGTATGAGGTGTTTTTTCTTTTTCTTTTCTTTTCTTTTCTTTTCTTTTCTTTTTCTTTCTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTATTAAGAGC
TTTACTTGTTTACCTGACCCTTTGACAAGAGCTCTTCCCTTAGCAACAGATGTATCATCTCAAAAGTTTTTCTGATTGGCTTCAGCTCAACTTATTTAAATTTCAATCAGTCAAACTCTAT
C
ATACCATACATTCTCTCTTTTTTTTTAAATCCGTGTCAGTTATACCCTACTCTTTAGCTCTACTCGACCTCCCAACAAATCACAGAAGAAA**ATGACCATAAGTGGAGTCCCAGTGCTAGG**
ATTTTTCATCATGGCTTTCCTGATGGGTCCTCAGGAATCATGGGCTGTCAAAGGTAAGTGCTGAGAGAAACAAAACTTGGGATAGAAATTCTGAGAGCATTGGAGAGATAAATTGTAA
ATATTTGCAAGACTGTCTTTGAAGTGAAGGAAAAGTGTGGCAGGCATCGTGTGGTCTAAATCTGAAAGTATTAAAAAATTGTAGTTTGGAGGAGTTCTGATCTTATAAATTGAAGTCAG
T
CACATTTAGTTGAGAGGAACAAAAAATGGTATGGATTAGTACCATTGACTTTGAGATAAGGCTGACTTGAAGTGAAAATCTTTTCTGAGAGGTATTAACAAGCTATCCTCTGGGCTTTA
A
TTTCTTTTTCTGGAAAATTAGTAGATTTTGAAATCTCACATCATTTTGTGGGG

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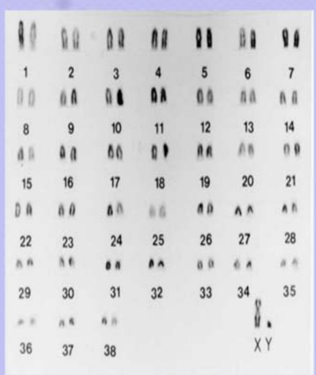
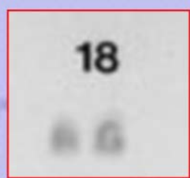
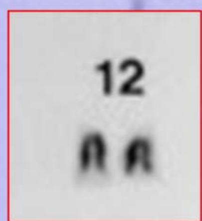
MHC-Complex Candidate-Gene-Screen



MHC Class I Genes	Chromosome
DLA-88	12
DLA-79	18
DLA-12	12
DLA64	12

MHC Class II Genes	Chromosome
DLA-DRA1	12
DLA-DRB1	12
DLA-DQA1	12
DLA-DQB1	12





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Material and Methods

Optimization of the reaction

- Each pair of primers was tested for best results.
- We were looking for only one, clear and dark band within expected size.

gelelectrophoresis.exe

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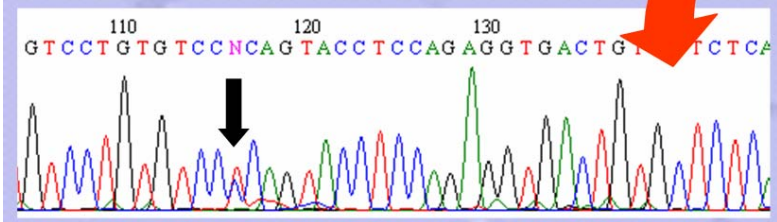
MHC-Complex Candidate-Gene-Screen

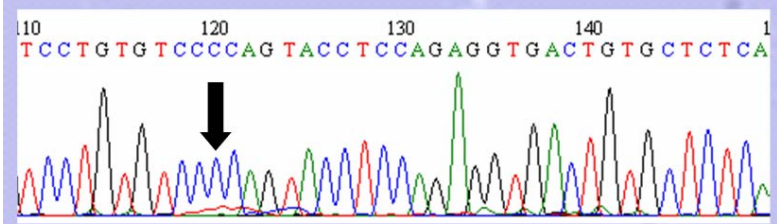
ABIprism 3100 genetic Analyzer



Heterozygot

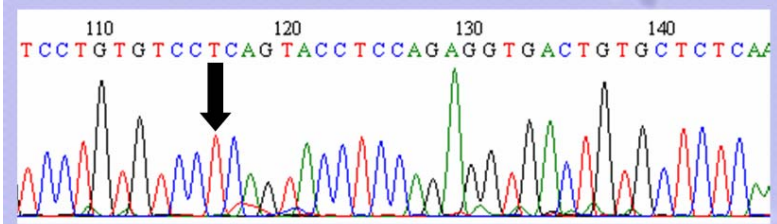
C/T

Sequencing



Homozygot

C/C



Homozygot

T/T

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MHC-Complex Candidate-Gene-Screen

MHC Class I Genes	Status	PCR-Products	Primers
DLA 88	Complete	1 (850bp)	New
DLA 79	Complete	7 (4833bp)	New
DLA 12	Complete	6 (4663bp)	New
DLA 64	Complete	4 (3178bp)	New

MHCKlasse II Gene	Status	PCR-Produkte	Primer
DLA-DRA1	Complete	4 (1762bp)	New
DLA-DRB1	Complete	1 (545bp)	
DLA-DQA1	Complete	1(581bp)	
DLA-DQB1	Complete	1(1018bp)	

29 Healthy Akitas
13 Affected Akitas

732.060bp

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MHC-Complex Candidate-Gene-Screen

Results:

- **An association between one MHC-candidate gene and SA susceptibility in Akita dogs was observed. The positive trend is very promising for SA diagnostics. (Publication is in preparation)**

- **The first documentation of canine MHC1 class candidate gene haplotypes was successful performed: Tools for large scale screening are now available to investigate the relevance of other canine diseases in MHC I class genes. (Publication is in preparation)**

- **Several canine MHC I genes demonstrate an unexpected high degree of variance**

- **A high degree of homozygousity was measurable within Akita dogs**

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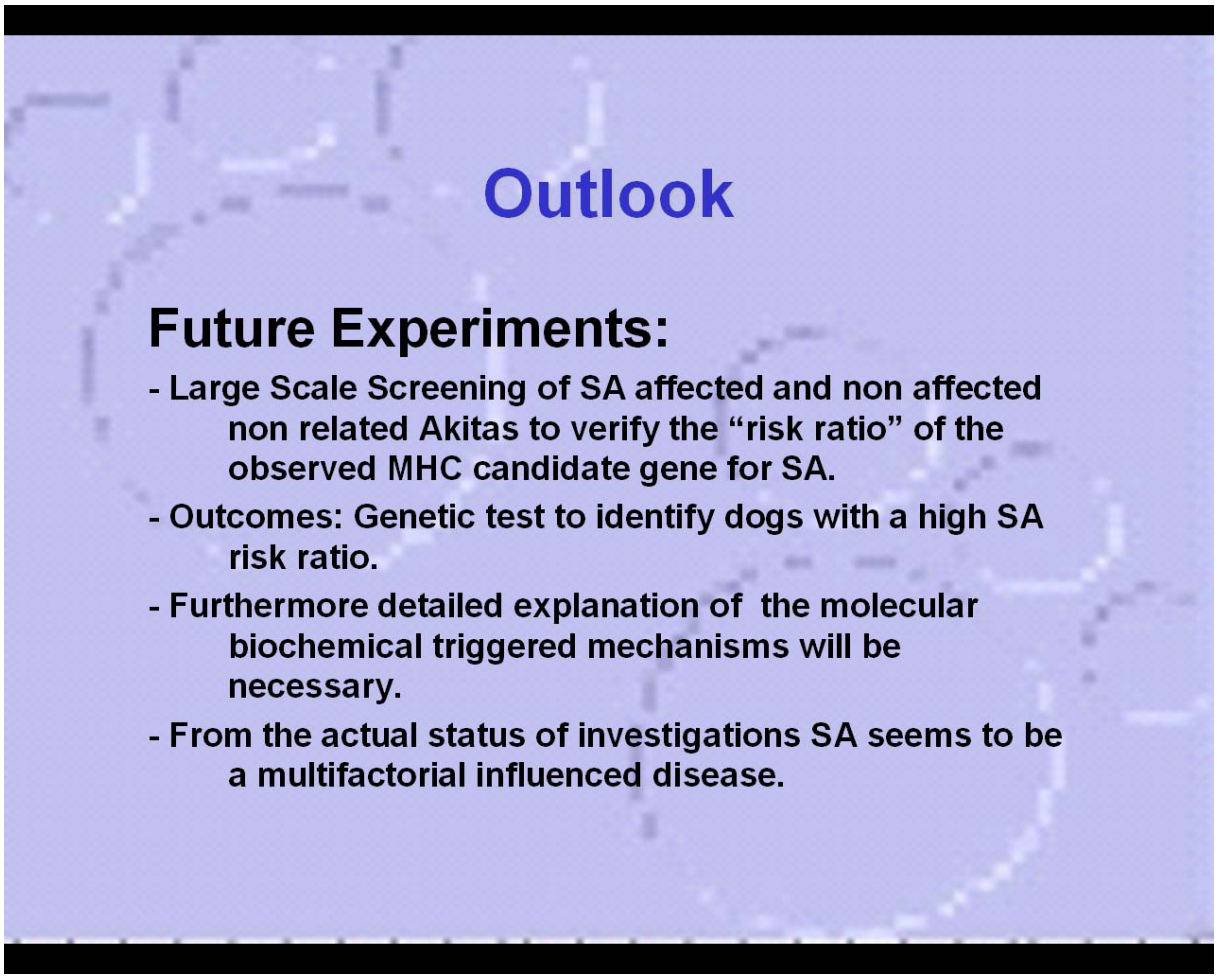
Outlook
Future Experiments:
- Large Scale Screening of SA affected and non affected non related Akitas to verify the "risk ratio" of the observed MHC candidate gene for SA.
- Outcomes: Genetic test to identify dogs with a high SA risk ratio.
- Furthermore detailed explanation of the molecular biochemical triggered mechanisms will be necessary.
- From the actual status of investigations SA seems to be a multifactorial influenced disease.