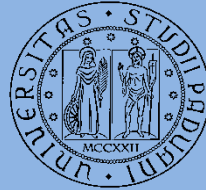




Dipartimento di biomedicina  
comparata e alimentazione



UNIVERSITÀ  
DEGLI STUDI  
DI PADOVA



Equine Patavium Hospital

# Gene expression profiles of the immuno-transcriptome in IAD and RAO affected horses

Dr. Elisa Padoan

# INTRODUCTION

IAD (Inflammatory Airways Disease)

Acute flogosis

Neutrophilic infiltrate

Mast cells infiltrate

6-8 years old

RAO (Recurrent Airways Obstruction)

Chronic flogosis

Neutrophilic infiltrate

12-15 years old

# IMPORTANCE IN EQUINE VETERINARY PRACTICE...

- Poor knowledge about pathogenesis
- Limited pharmacological success
- Extending time to go back to sport activities in IAD-horses
- Poor life quality in RAO patients
- Huge economic losses

# AIM OF THE STUDY

IMMUNO-RELATED GENE EXPRESSION IN THE  
BRONCHO-ALVEOLAR LAVAGE FLUID IN

IAD

RAO

HORSES

- **FIRST RESEARCH**

Real time RT-PCR analysis of inflammatory mediators expression in RAO-affected horses

- 13 Horses
  - ✓ 7 Controls
  - ✓ 6 RAO (T0 e T1)
  - ✓ any pharmacological treatment
- Real time RT-PCR 10 immuno-related genes

- **SECOND RESEARCH**

Gene expression profiles of the immuno-related genes in respiratory system of IAD and RAO affected horses

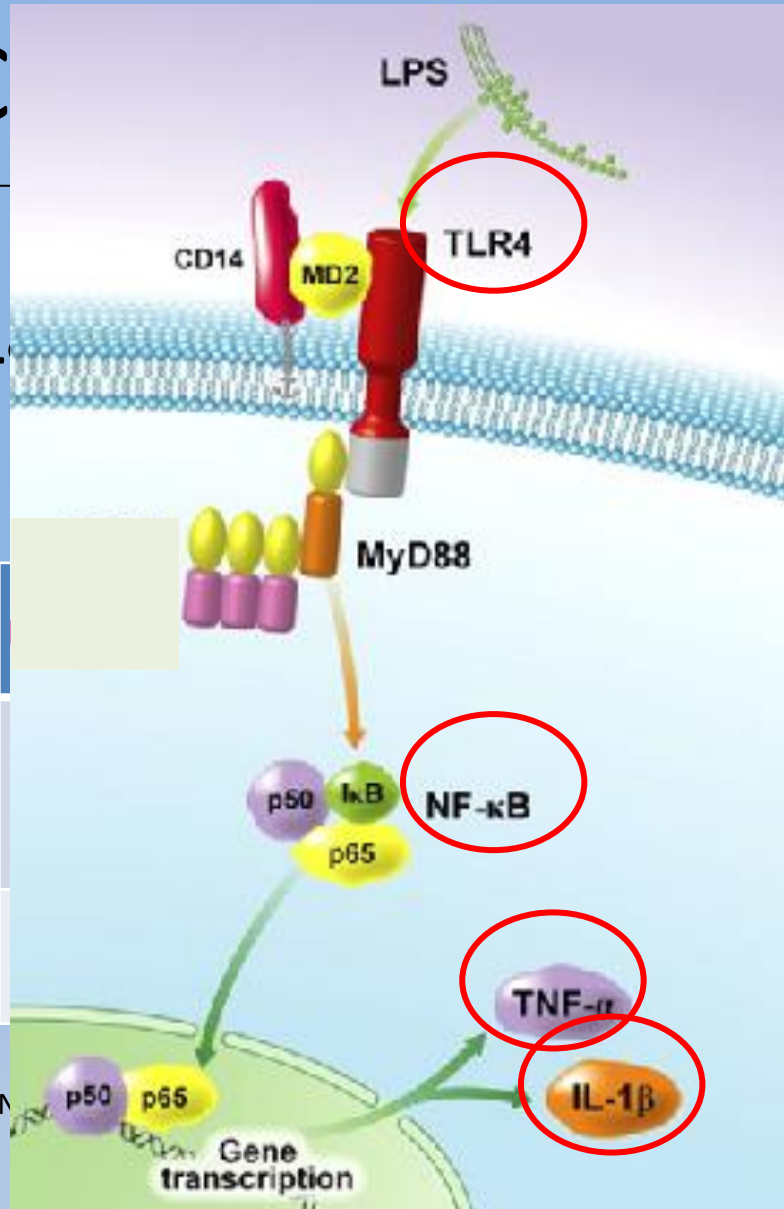
- 54 Horses
  - ✓ 8 Controls
  - ✓ 20 IAD
  - ✓ 26 RAO
- DNA-Microarray

# RAO vs C

# e RT-PCR

IL1 $\beta$ , IL6, IL

1  $\beta$ , NF- $\kappa$ B,



GENE	<i>IL1<math>\beta</math></i>
<i>Fold-change</i>	11,9
<i>P-value</i>	<0,05

<i>TNF<math>\alpha</math></i>	<i>INF<math>\gamma</math></i>
2,9	2,3
<0,01	<0,05

“REAL TIME RT-PCR ANALYSIS OF IN  
E. Padoan, S. Ferraresso, S. Pegolo,

OBSTRUCTION-AFFECTED HORSES”  
path. Submitted)

# CLINICAL EVALUTATIONS

CLINICAL EXAMINATION AND ENDOSCOPY

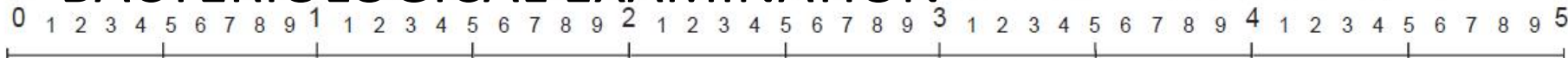


BRONCHO-ALVEOLAR  
LAVAGE



CITOLOGICAL EVALUTATION

BACTERIOLOGICAL EXAMINATION

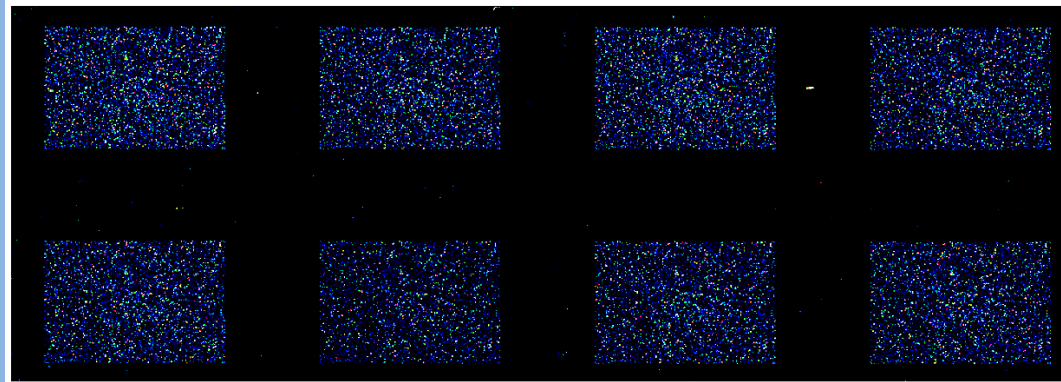


0	1	2	3	4	5	6	7	8	9	1	2	3	4	5	6	7	8	9	2	1	2	3	4	5	6	7	8	9	3	1	2	3	4	5	6	7	8	9	4	1	2	3	4	5	6	7	8	9	5
None	Little					Moderate					Marked					Large					Extreme																												
Clean	singular	Multiple small globes				Larger globes				Confluent				Stream-forming				Pool-forming				Profuse amounts																											

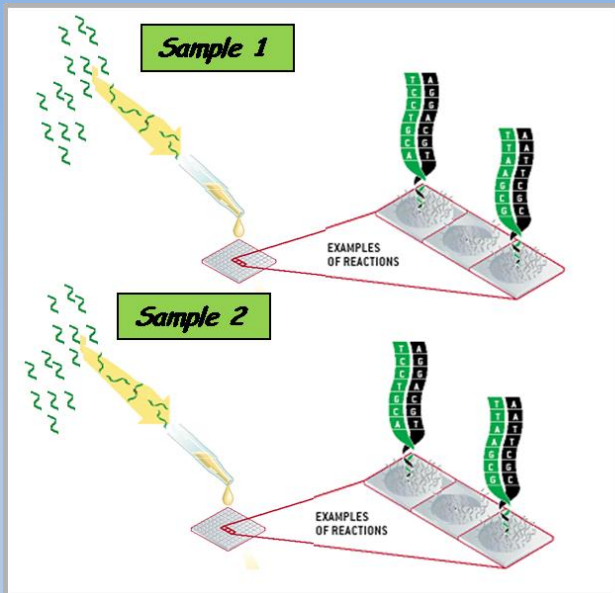
## GENES EXPRESSION ANALYSIS

“Endoscopic scoring of mucus quantity and quality: observer and horse variance and relationship to inflammation, mucus viscoelasticity and volume» V. Gerber, *Equine vet. J.* (2004) 36 (7) 576-582

# DNA-MICROARRAY



It allows to measure the expression level of thousands of genes simultaneously



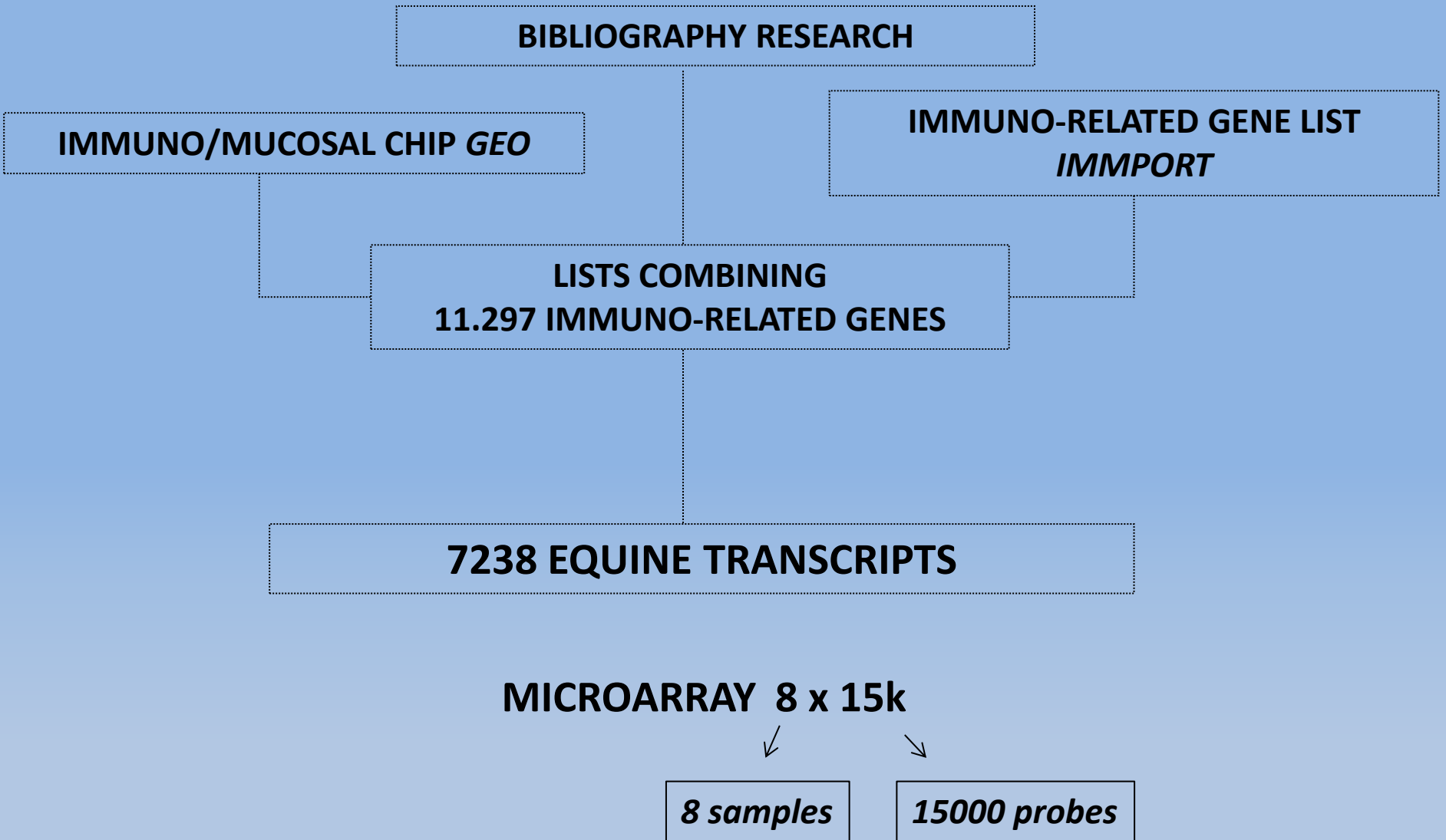
The samples of different categories are all labeled with the same fluorophore (Cy-3)



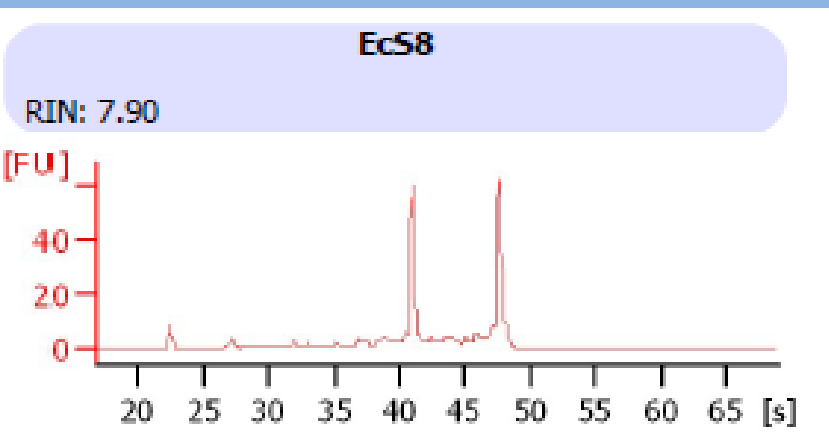
The expression level of each gene is measured from the fluorescence emitted by the corresponding probe



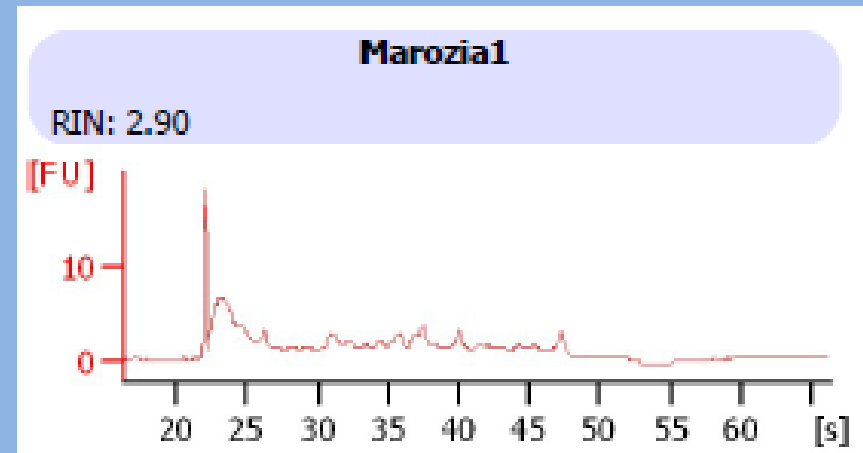
# TRANSCRIPT SELECTION AND ARRAY DESIGN



# RNA Integrity Number



*RNA intact*



*RNA degraded*

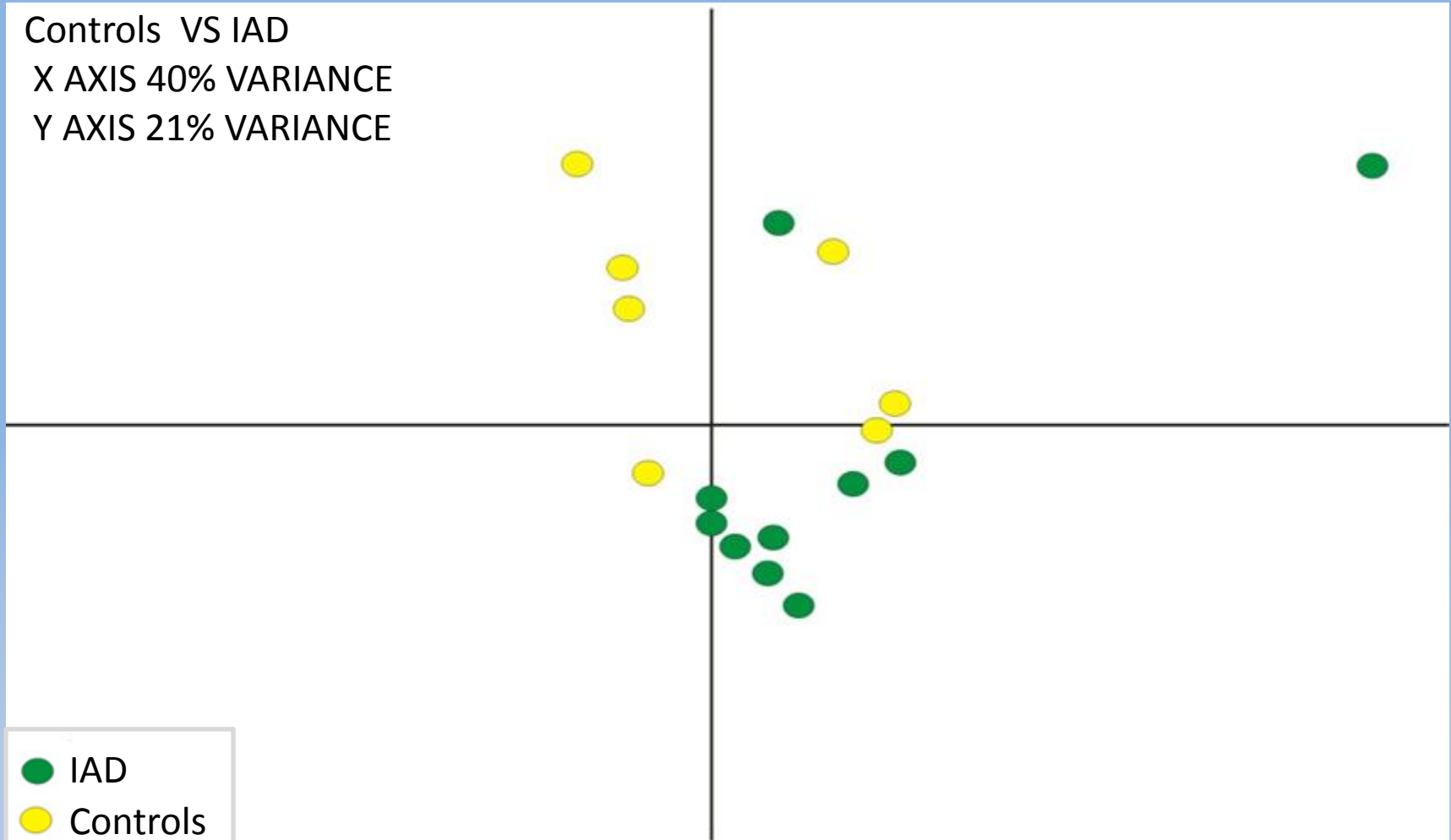
7 Controls

10 IAD

8 RAO

# Principal Component Analysis

Controls VS IAD  
X AXIS 40% VARIANCE  
Y AXIS 21% VARIANCE

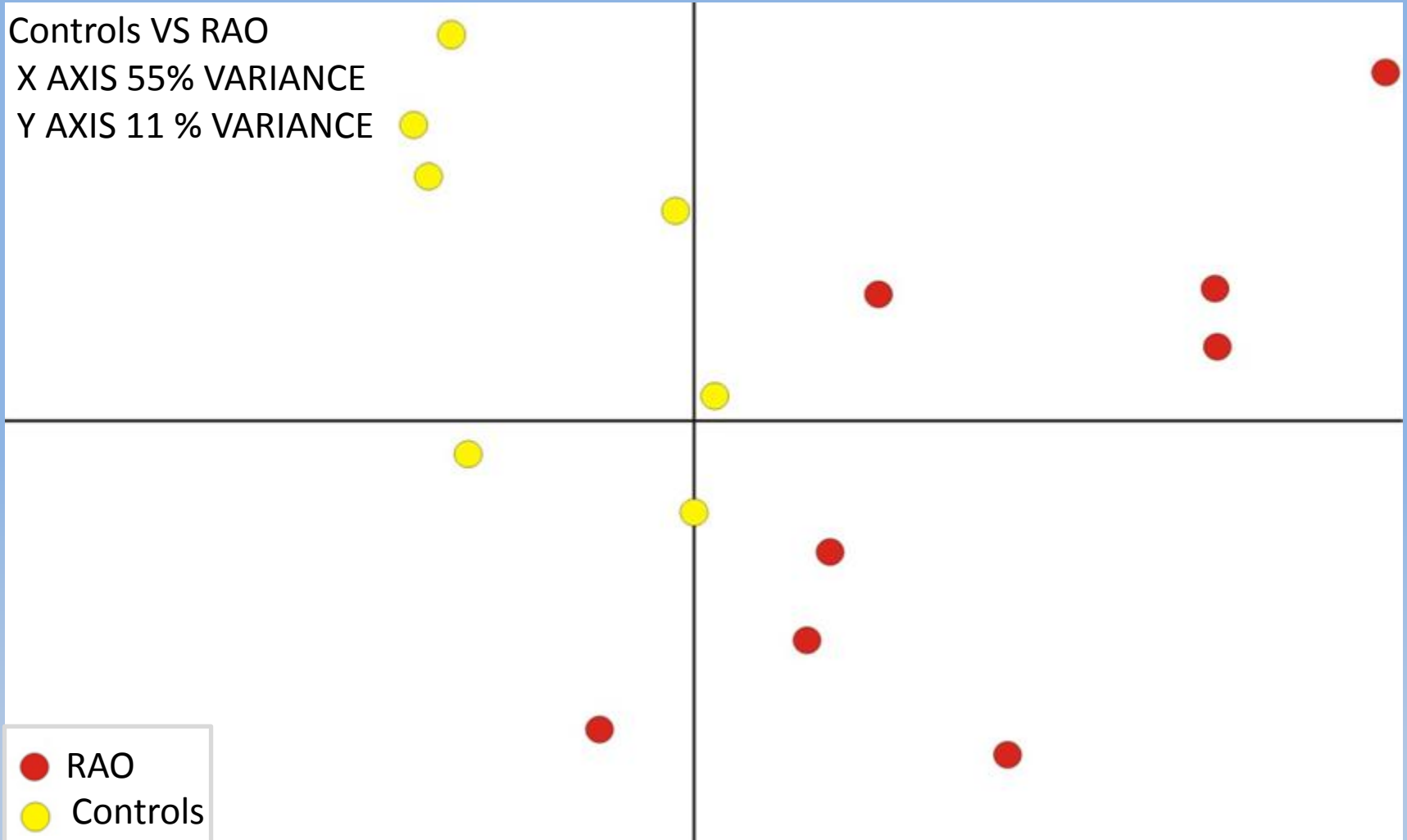


# Principal Component Analysis

Controls VS RAO

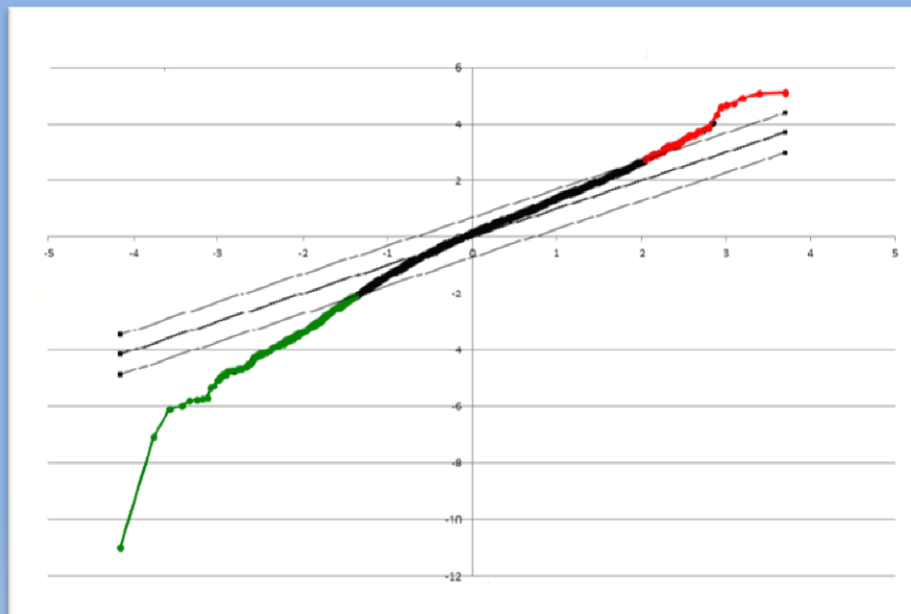
X AXIS 55% VARIANCE

Y AXIS 11 % VARIANCE



# IAD

## Significance Analysis Microarray



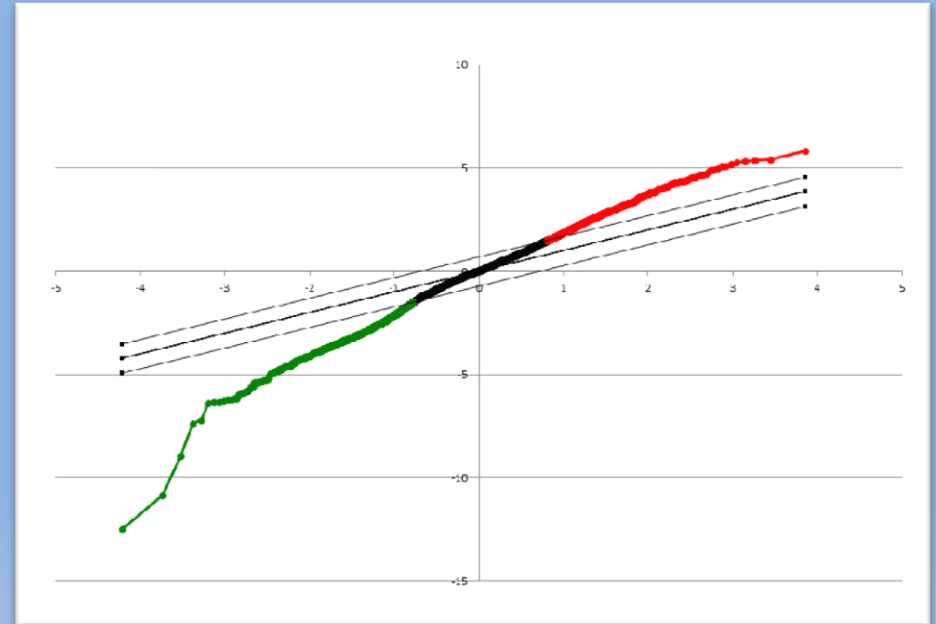
379 Transcripts (6,3 %)

55 Over-regulated (0,9%)

324 Down-regulated (5,4%)

# RAO

## Significance Analysis Microarray



1763 Transcripts (29,2%)

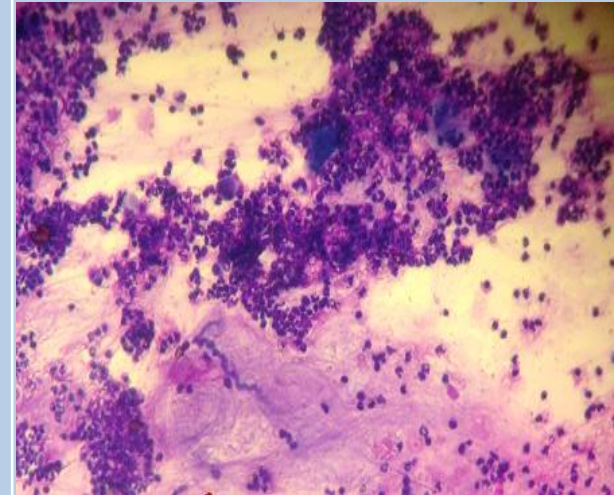
903 Over-regulated (14,9%)

860 Down-regulated (14,3%)

# IAD UP-REGULATED

KATA2A K(lysine) acetyltransferase 2A  
LENG8 LRC member 8  
SYNJ1 Synaptojanin1  
TSNEN15 tRNA splicing endonuclease 15 homolog  
TRIM23 Tripartite motif containing 23  
NAT10 N-acetyltransferase 10  
PRPF39 Pre-mRNA processing factor 39 homolog

## *Cellular metabolism*



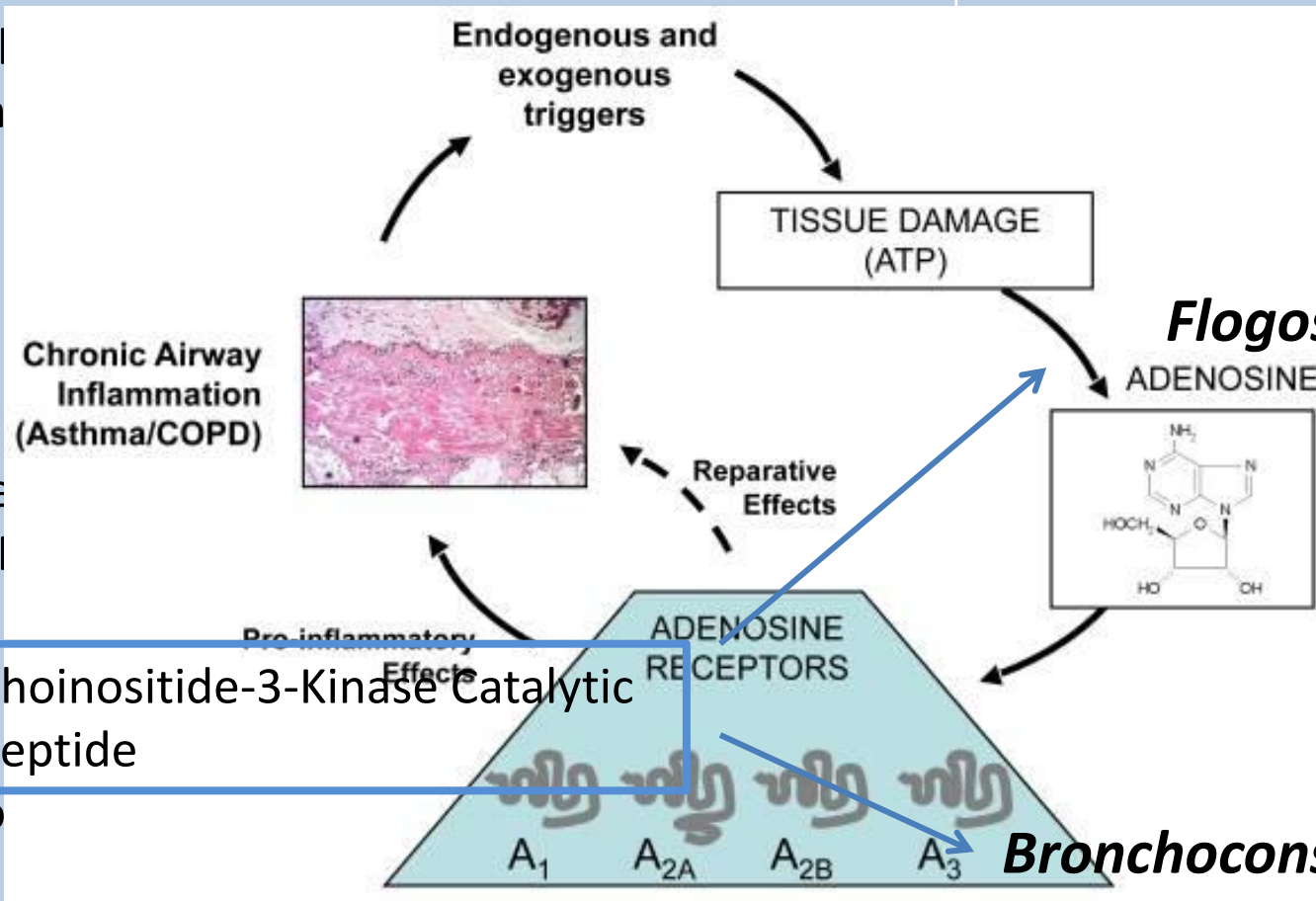
IL10RA Interleukin 10 receptor, alpha

## *Regulation*

# RAO UP-REGULATED

CCL17 Chemokine  
 ARHGAP27 Rho GTPase  
 ARHGAP15  
 ARHGAP26  
 ARHGAP9  
 ARHGAP25  
 ARHGAP40  
 ADORA2A Adenosine Receptor  
 TLR4 Toll Like Receptor

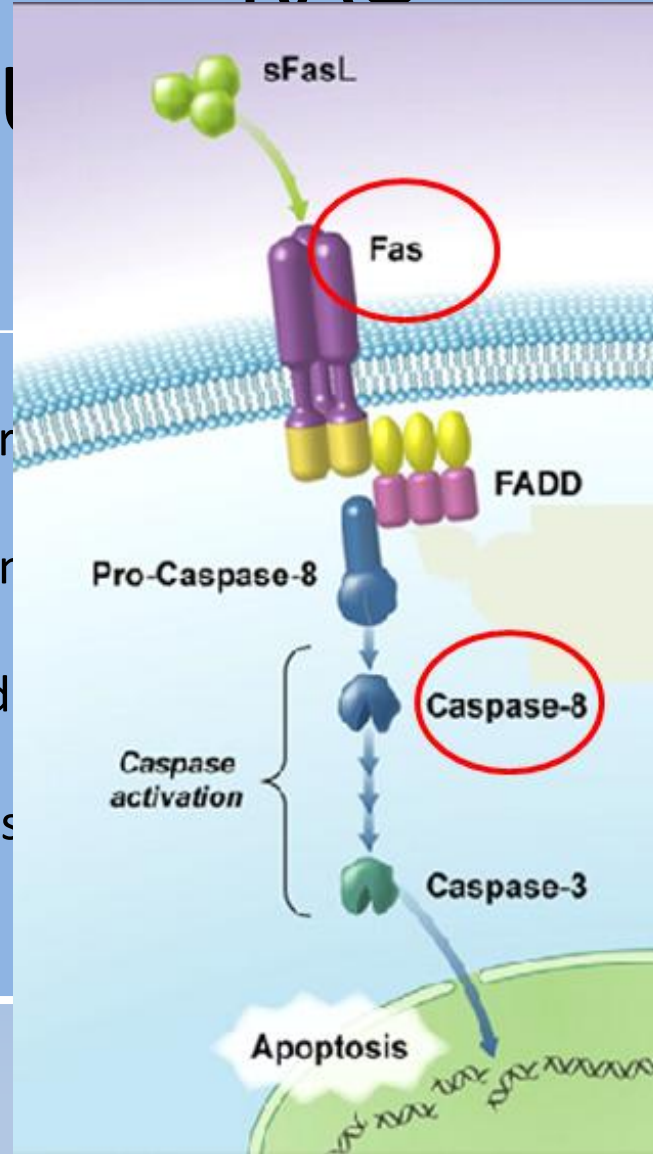
PIK3CG Phosphoinositide-3-Kinase Catalytic  
 Gamma Polypeptide  
 TGFβ1 Transforming Growth Factor  
 MMP9 Matrix Metalloproteinase



**Bronchoconstriction**

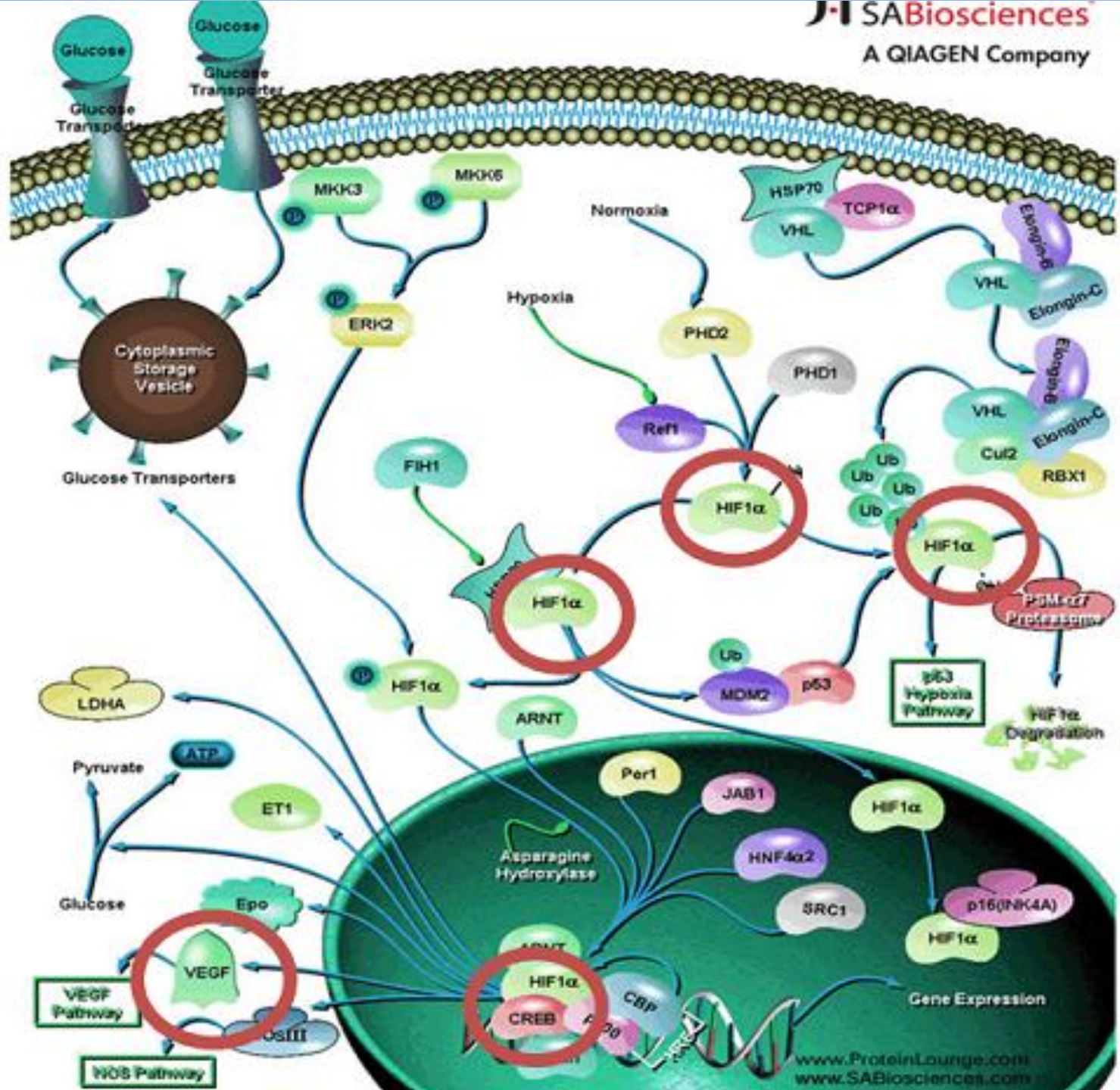


# RAO



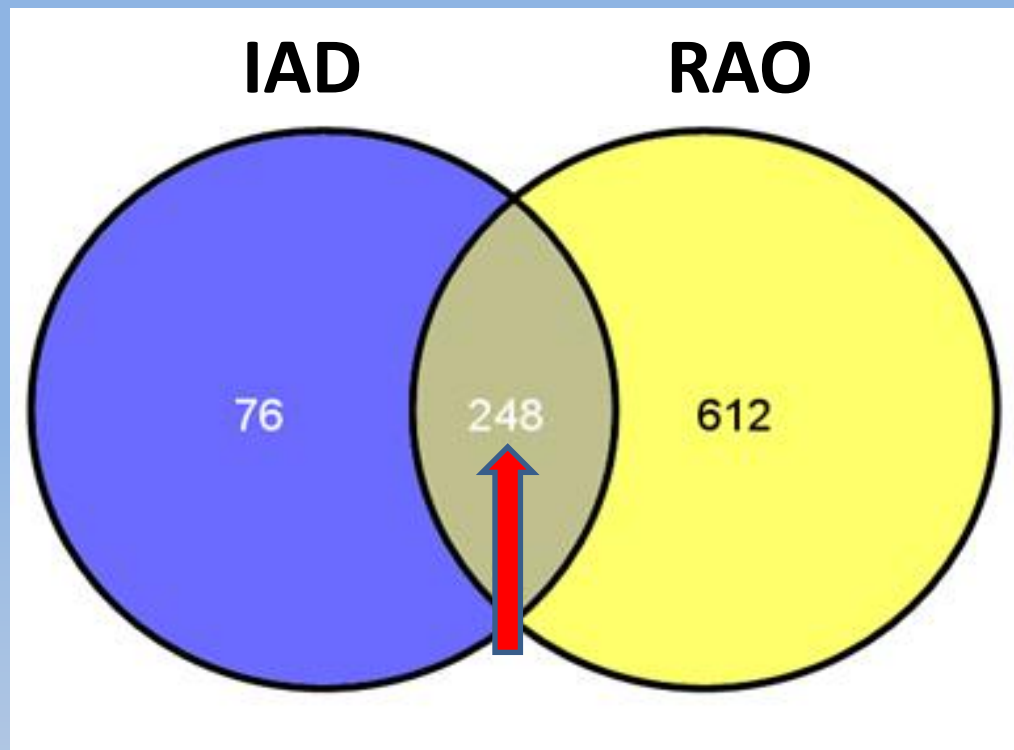
TFPT TCF3 (E2A) fusion part  
forkhead box P3  
FAS TNF Receptor Superfamily  
6 (FC 2,03)  
MAPK8 Mitogen-Activated  
Kinase 3  
CASP 8 Caspase 8, Apoptosis  
Cysteine Peptidase

***Apoptosis***



MMP1  
MBP M  
HIF1AN  
subunit  
VEGF V  
CREBBF

# DOWN-REGULATED IAD & RAO



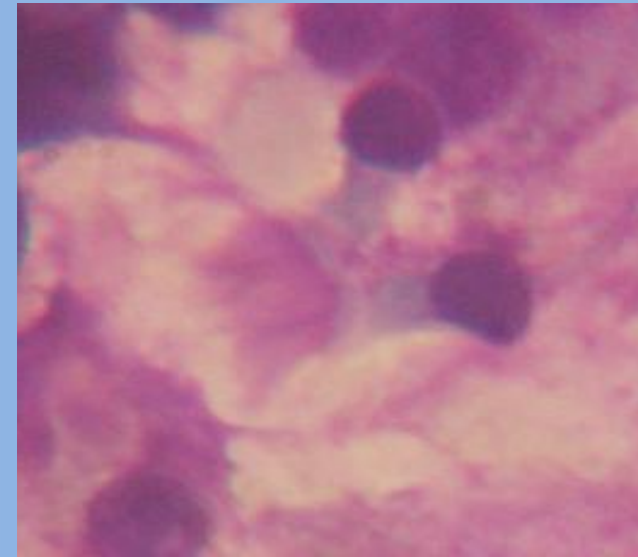
# DOWN-REGULATED IAD & RAO

RSPH9 Radial Spoke Head 9

IFT57 Intraflagellar Transport 57, 74, 27, 871, 88, 20,  
52, 122, 140, 80, 172

NEK3 Never in Mitosis Gene Related Kinase 3, 4, 11

***Muco-ciliary  
Clearance Reduction***



SERPINA 1 Serpin Peptidase Inhibitor, Clade A

***Extracellular Matrix  
Remodelling***

IGHG4 Immunoglobulin Heavy Constant Gamma 4

IGLC1 Immunoglobulin Lambda Constant 1

***Antibodies Release  
Reduction***



# Comparative Analysis



Differentially expressed RAO genes

IRAK3 interleukin-1 receptor-associated kinase 3  
(FC 3,09)



*Pathway* bronchitis, bronchiectasia,  
enphysema, asthma, COPD

SUMMARIZING...

DNA MICROARRAY



PATHOGENESIS

# SUMMARIZING...

Reduced muco-ciliary clearance vs mucus hypersecretion (IAD and RAO)

Extracellular matrix remodelling/  
Bronchoconstriction (IAD and RAO)

Hypoxia markers (RAO)

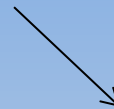
# ...PROSPECTIVES

PROTEIC EXPRESSION



**DNA**

**MICROARRAY**



CLINICAL SIGNS



ANIMAL MODEL

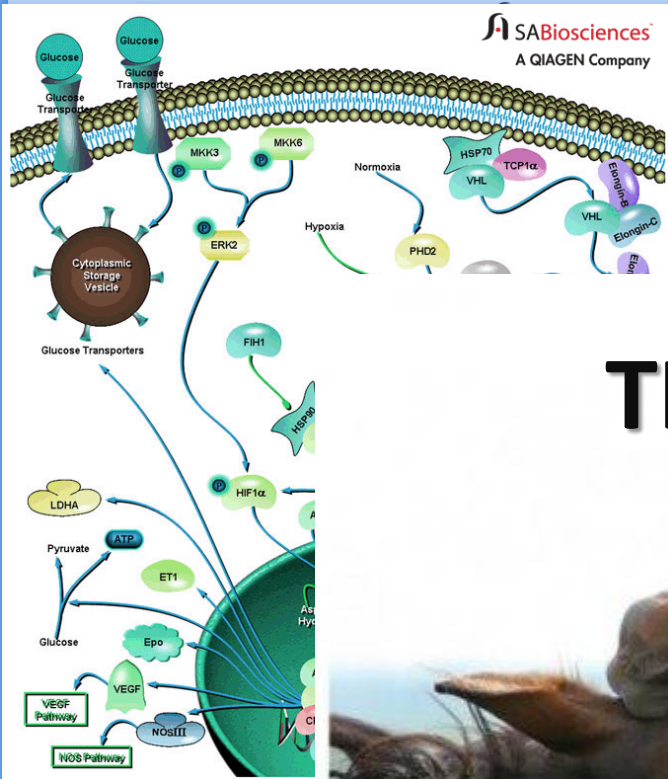
PIK3CG

CCR7

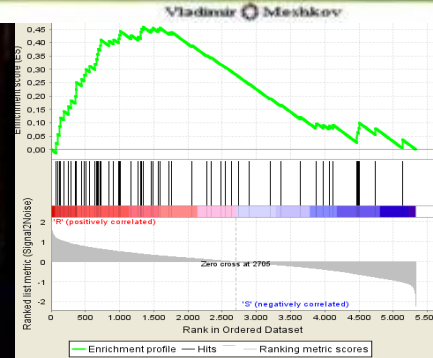
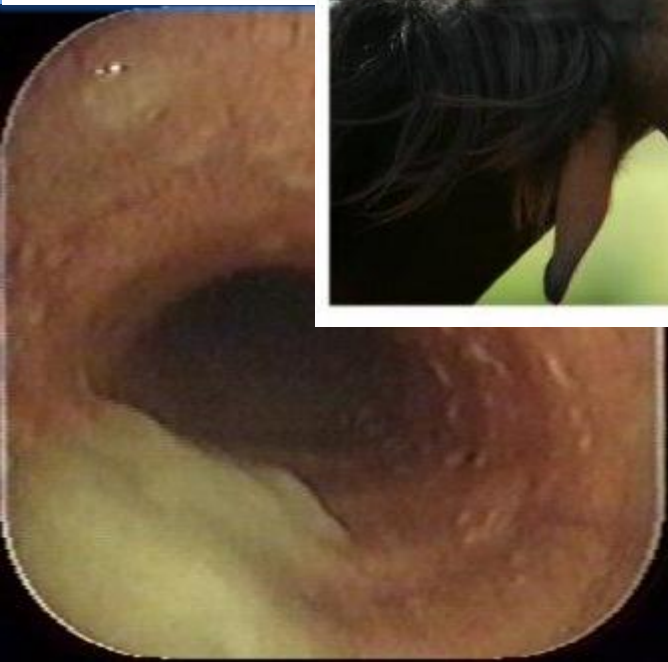
ADORA2A

IL2





# THANK YOU



# TO MY DAD