

WP3 - UNISR

5-6.03.2015



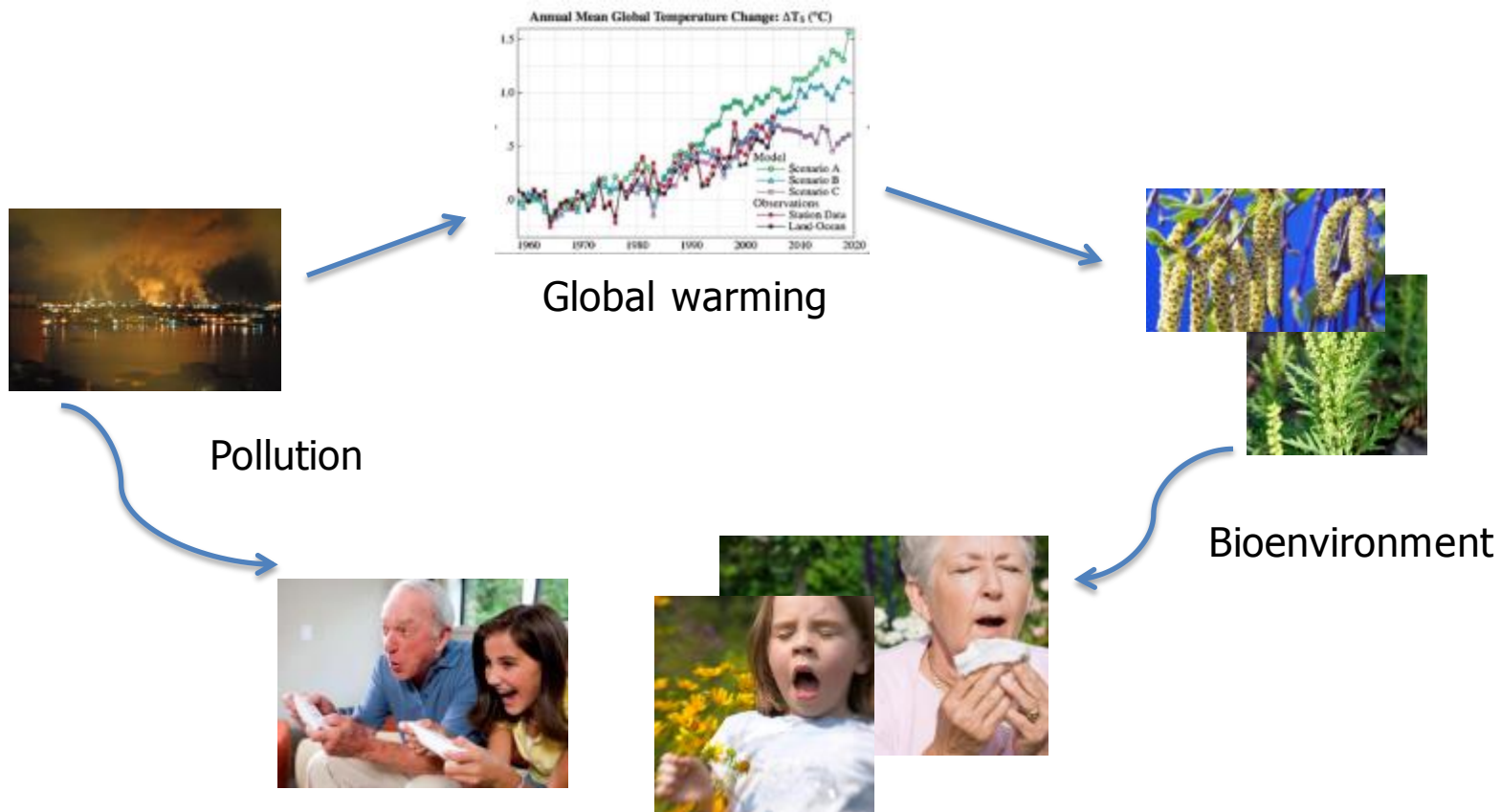
atopica[®]
atopic diseases in changing
climate, land use & air quality

Genomic profiles of young and aged ragweed allergic patients

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atopica is a project funded by the
European Commission under the 7th FP





Predisposition to develop atopy

Genes with variants associated with atopy (Malacard):

- | | |
|---------------------------|---------------------------|
| • IL4R | • RNASE3 |
| • HAVCR1 | • CYSLTR1 |
| • SPINK5 | • FCER2 |
| • MS4A2 | • MICU1 |
| • PLA2G7 | • IGES |
| • SELP | • IGHE |
| • SART1 | • IL5 |
| • IL9 | • LALBA |
| • LTC4S | • PRG2 |
| • IL13 | • IL13RA1 |
| • CMA1 | • SLC11A1 |
| • CYSLTR2 | • CCL17 |
| • TSLP | • CCL22 |
| • EPX | • TBXA2R |
| • CD14 | • CCL11 |
| • IL4 | • CCR3 |
| | • IL5RA |
| | • IRF1 |
| | • LTA |

- Sudden rise in frequency of atopy among population
- Discrepancies of parental risk (FceR1-b)
- Some animal model suggest transgenerational inheritance

4%-10% of hereditability

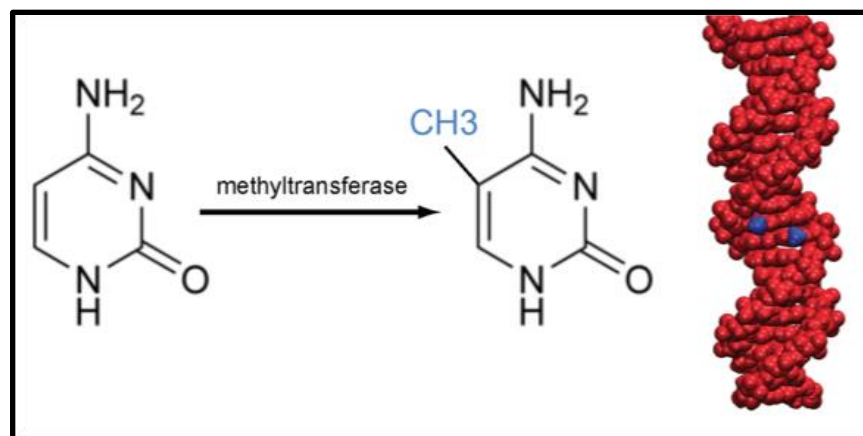
Why we need to look more into epigenetics:

Epigenetics *involves genetic control by factors other than an individual's DNA sequence.*

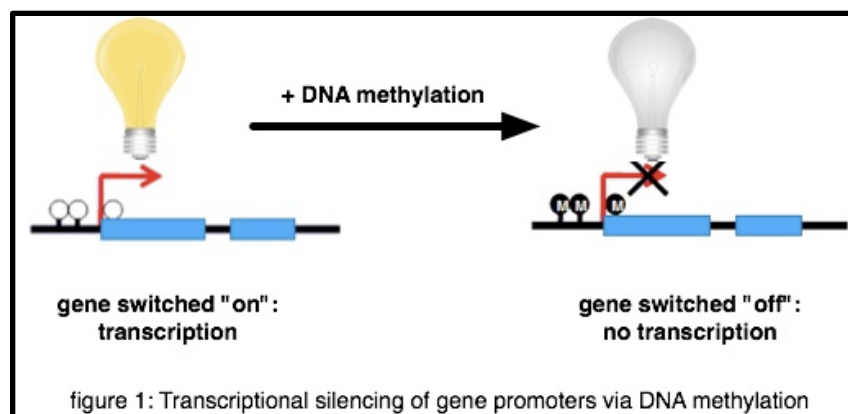
- Shapes the physical structure of genome, creating a second layer of information
- Regulates which sets of genes are active or not, defining cell and phenotype identity
- Responds dynamically to environment stimuli , real time response
- Epigenetics+genetics at same DNA loci might provide better stratification between subjects
- If environmental pressure is present can be transmitted and amplified through generations but at the same time if selection pressure stop, epigenetically determined phenotype can be reverted to normal

Epigenetic control :

- DNA Methylation
- Histone Modifications
- RNA-Associated Silencing



How DNA methylation works?



Hypothesis: Adaptive response of the genome during the life

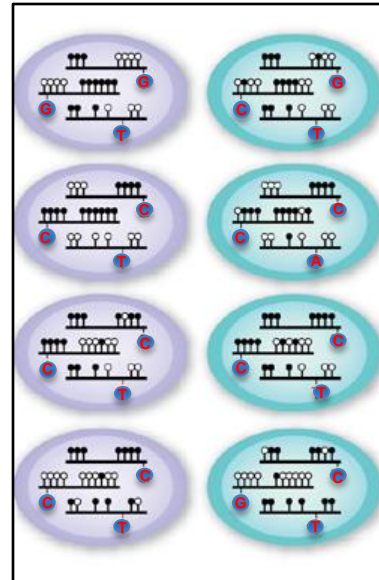
Bioenvironment
(food)

Physical environment
(temperatura, chemicals)

Social environment
(stress, abuse)

Signalling pathways

Genotype



DNA Methylation

Phenotype

Non-sensitised <-Health challenges-> sensitised

Aim of project

Targets:

- investigate the role of DNA methylation in shaping the allergic response in the two populations of different age
- identification of predictive biomarkers
- **Collection** of 3.2k blood/DNA samples
 - establishment of a Biobank (UULM, CHS)
- **Whole genome methylation analysis:**
 - 120 subjects from CHS **pediatric** cohort(Slavonia region)
 - 120 subjects form UULM **elderly** cohort(Ulm University)
- **Bioinformatic analysis:**
 - **DMR identification** between sensitized and healthy subjects



methylation analysis

Methods to study DNA methylation

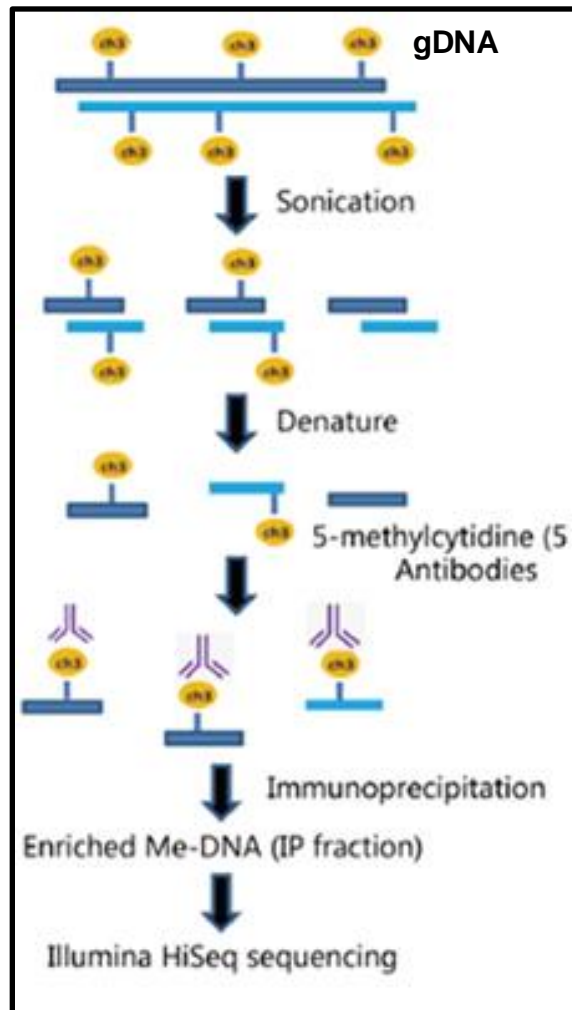
Array based:

- DNA methylation arrays (Human Methylation 27K, 450K bead chip)

Sequenced based:

- **Sodium bisulfite conversion:**
 - WGBS-Seq
 - RRBS-Seq
 - Sequence-specific enzyme digestion
- **Enrichment methods:**
 - **MeDIP-seq (anti-5mC Ab)**
 - MBD-seq (methyl-binding protein)

MeDIP:



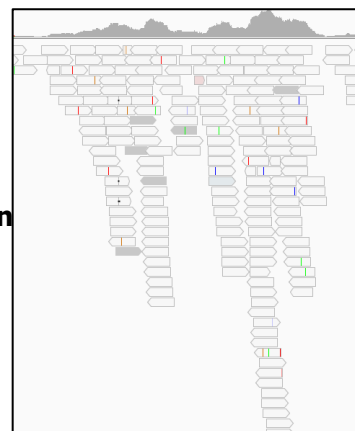
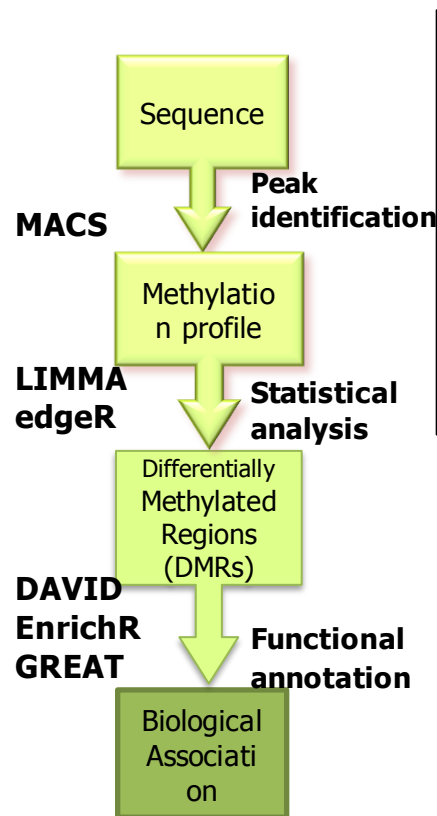
Advantage:

- Genome-wide ,high resolution
- Fast, cost effective, high-throughput
- Discriminate 5-mC from 5-hmC
- High correlation with BS-Seq data
- Low input DNA needed

Disadvantage:

- Non single base resolution
- Laborious and time consuming validation process

DMR identification and gene association



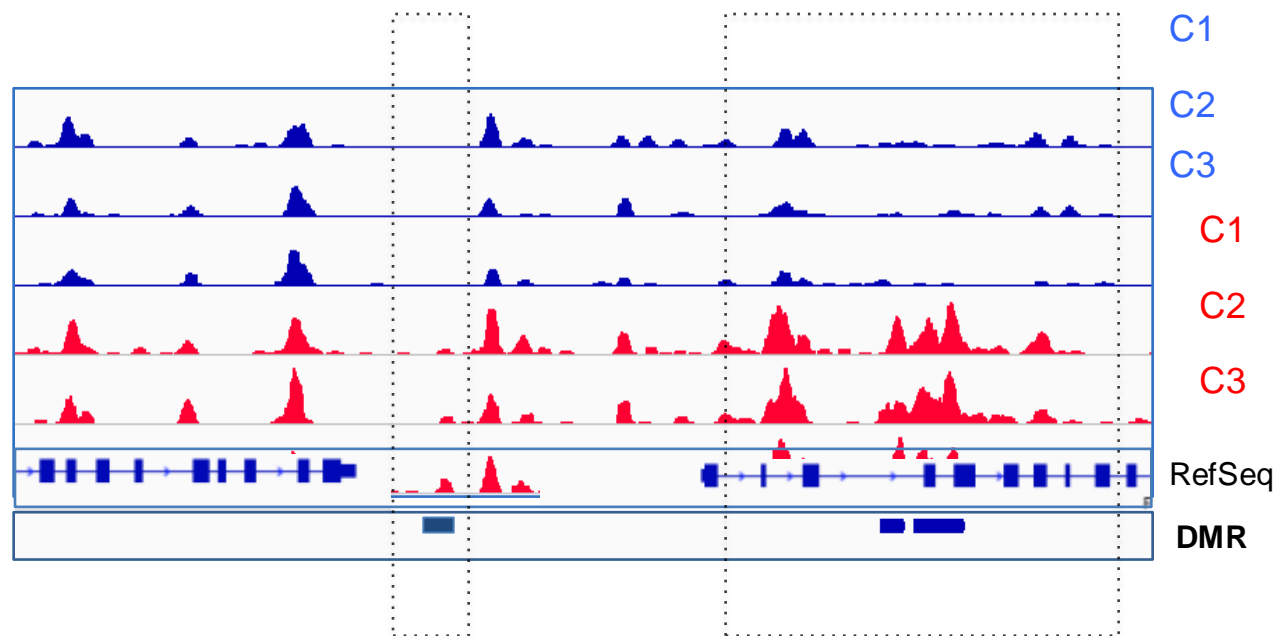
MACS
peak
caller

Peaks position

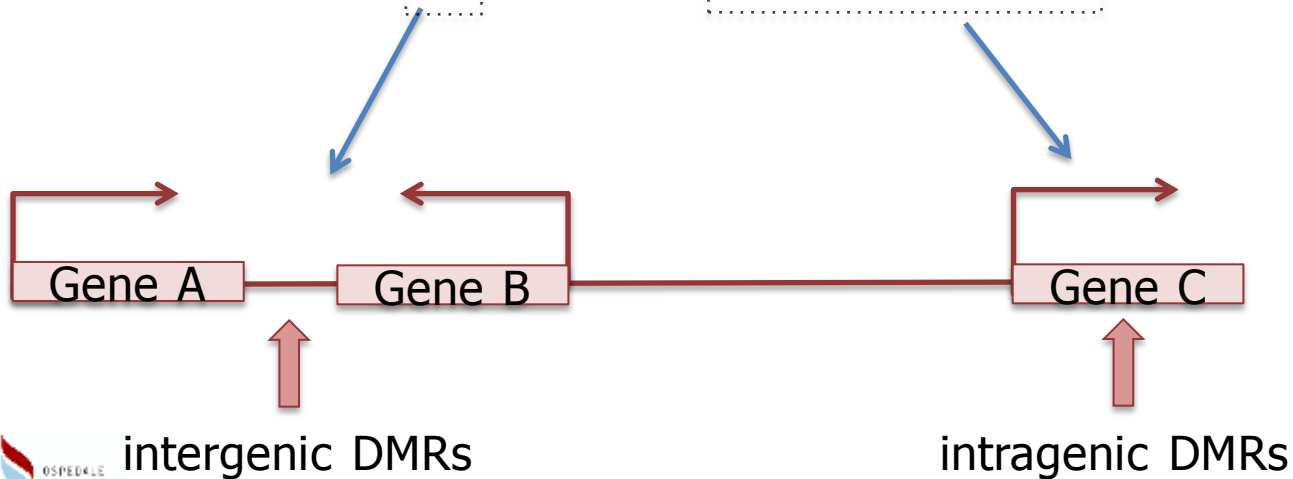
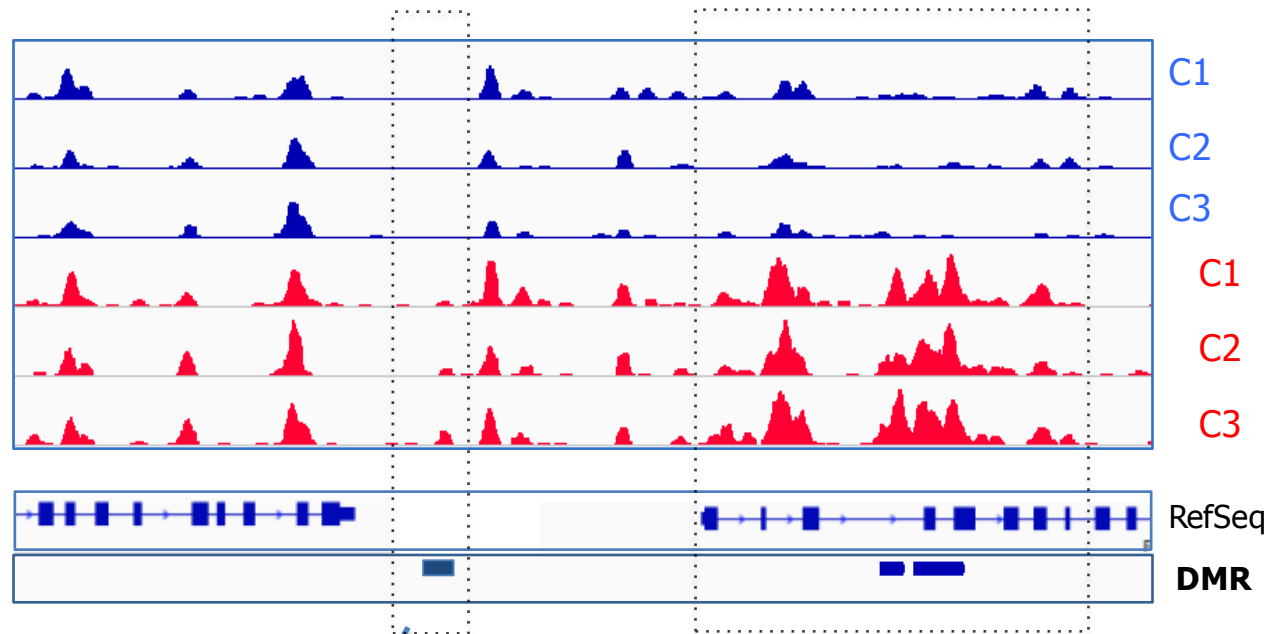


| | | |
|------|----------|----------|
| chr1 | 38960462 | 38961340 |
| chr1 | 38961864 | 38962302 |

We generate peaks position for each sample and then we merge all obtained regions in order to compare different samples



DMR identification and gene association



Methylation analysis: CHS cohort

Selection criteria

Slavonia children selected for methylation analysis; age: 4 to 10 years.

Data available:

- skin prick test response (SPT)

| | |
|-------------|----------------|
| Birch | Dog Hair |
| Hazel | cat dander |
| Grasses mix | D. Pterossynus |
| Ragweed | Cladosporium |

- Clinical data (questionnaire)
- IgE levels

Groups were defined by SPT response:

Sensitized: SPT positive to any allergen

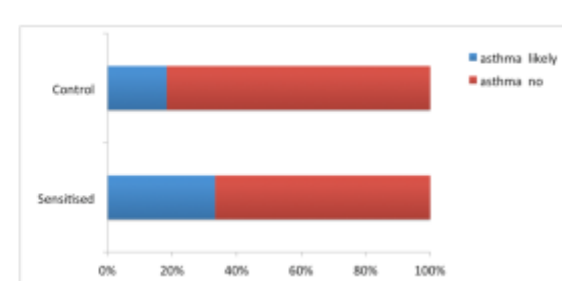
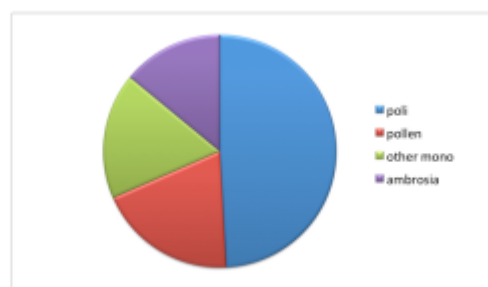
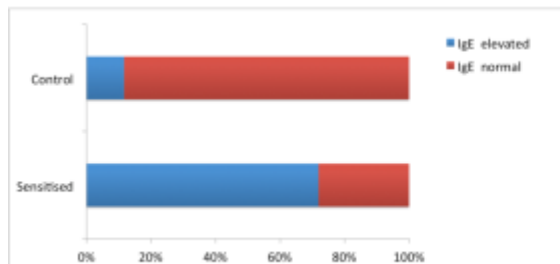
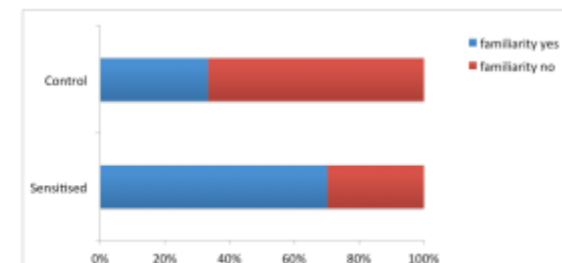
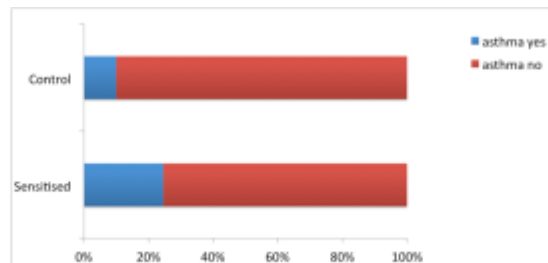
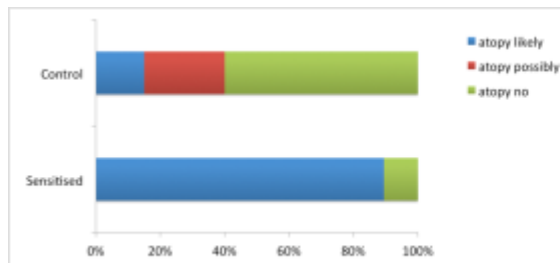
Controls: SPT negative to any allergen

Association between clinical data and prick test

Groups were defined by SPT response:

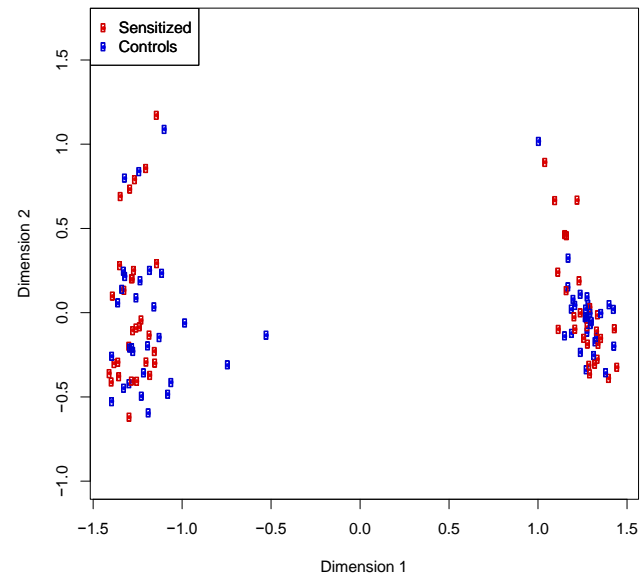
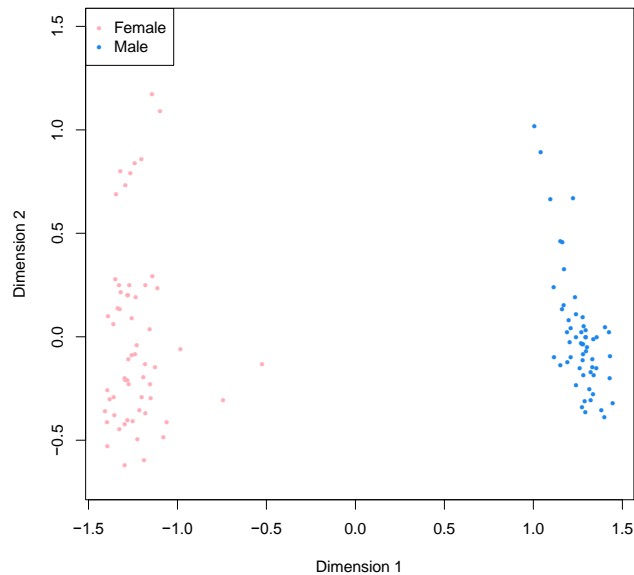
Sensitized: SPT positive to any allergen

Controls: SPT negative to any allergen

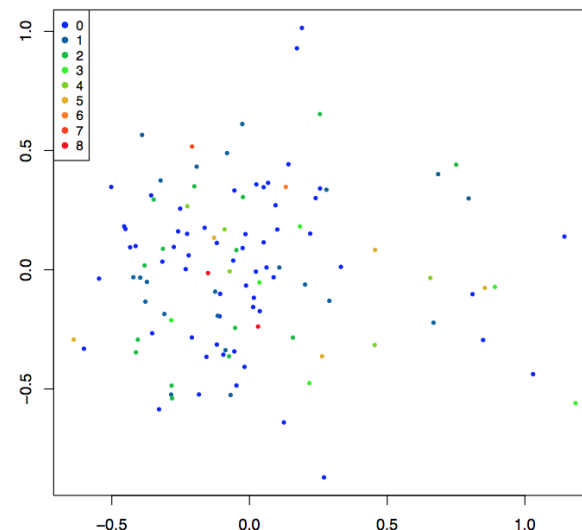
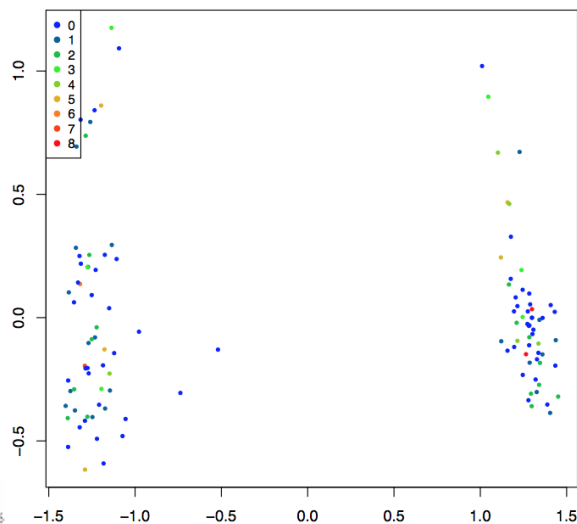


Methylation analysis: CHS cohort

MDS-positive skin prick test

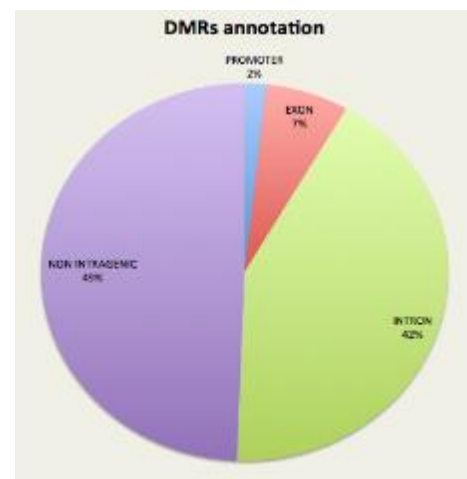


MDS – Number of positive skin prick tests



Methylation analysis: CHS cohort

- We compared methylation profiles of sensitized patients with those of Not Sensitized patients.
- We applied a **GLM using sex, pool and age information as covariates**.
- We selected **587 DMRs** with nominal p-value < 0.0005
 - Hypermethylated in Sensitized: 296 DMRs
 - Hypomethylated in Sensitized: 291 DMRs
- DMRs have been associated to **814 genes**.
- Distribution of DMR along a genome:
 - Promoter-2%
 - Exon-7%
 - Intron-42%
 - Intergenic-49%



Methylation analysis: CHS cohort

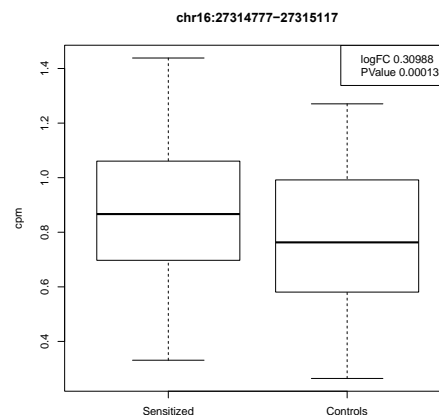
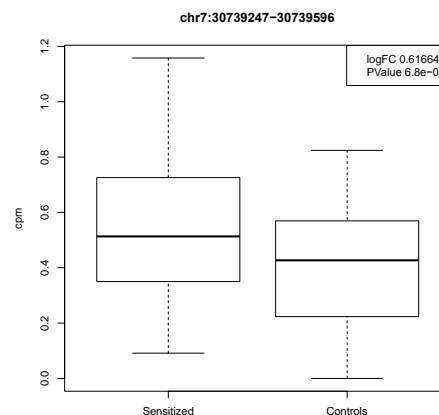
DMR-associated genes: insights

CRHR2

- Corticotropin-releasing hormone (CRH) receptor 2
- CHR is the central regulating hormone of the hypothalamic-pituitary-adrenal axis.
- Already related to stress and asthma and bronchodilator response

IL4-R

- ILR4 can bind interleukin 4 and interleukin 13 to regulate IgE production.
- promote differentiation of Th2 cells.
- inhibit IL4-mediated cell proliferation and IL5 upregulation by T-cells.



Association of DMR with known genes linked with atopy:

- **Intersection with GWAS data**

- AUTS2
- BCAS3
- C11orf74
- CNTN5
- CNTNAP5
- CSMD1
- CTNNA3
- EDIL3
- EPS15
- IRX1
- LTBP1
- NPY
- RAB6B
- TENM1
- TENM2
- THEMIS
- XKR6

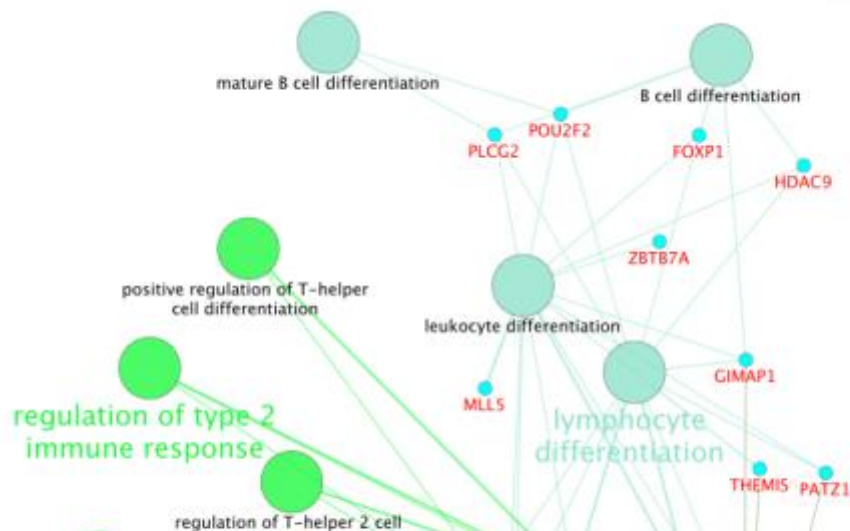
- **Intersection with Malacards associated genes**

- CYSLTR2
- FNDC3A
- **IL4R**
- SART1

Pathway analysis:

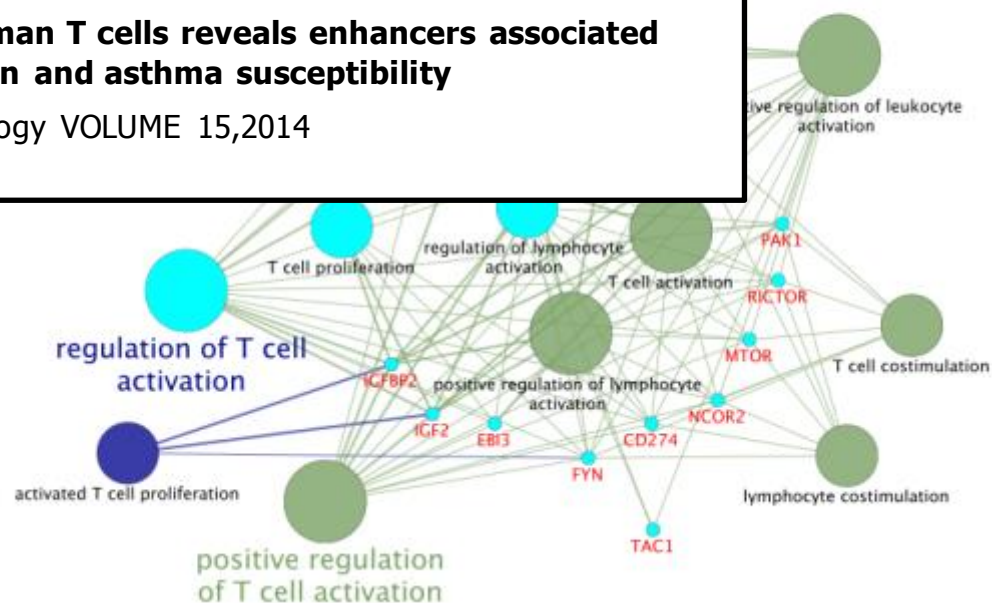
In **sensitized subjects**
differential methylation
affects relevant pathways
related to:

- **immune system regulation**
(Th₂ cell differentiation, B cell differentiation)
- **signaling and cell adhesion**
(cadherin binding, B cell receptor and integrin-mediated signaling pathways)



Epigenomic analysis of primary human T cells reveals enhancers associated with Th₂ memory cell differentiation and asthma susceptibility

Grégory Seumois, et al. Nature immunology VOLUME 15, 2014



atopic diseases in changing climate, land use and air quality



Methylation analysis: Ulm cohort

Methylation analysis: Ulm cohort

Samples were collected by Ulm hospital (center 1) and selected for methylation analysis. age: 60 to 80.

Allergens tested:

| | |
|----------------------|----------------------------|
| Trees | Birch |
| house dust mite I/II | ambrosia ALK / Bencard/HAL |
| Cats | nuts |
| Mugworth | celery |
| | Melon |

Groups were defined by SPT response:

- **Sensitized:** SPT positive to any allergen
- **Controls:** SPT negative to any allergen

(NB: different method of evaluation of skin prick test results between ULM and CHS)

Sensitized patients in **males and females separately.**

Methylation analysis: Ulm cohort

To identify DMRs we applied a **GLM(general linear model)** using **pool and age information as covariates**

Summary:

FEMALES

22 DMRs with $FDR < 0.1$

252 DMRs with $P.value < 0.0001$

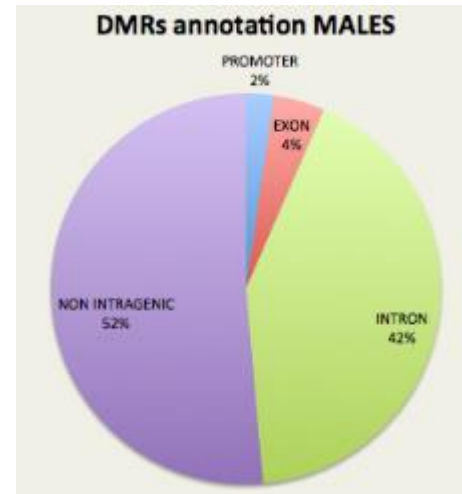
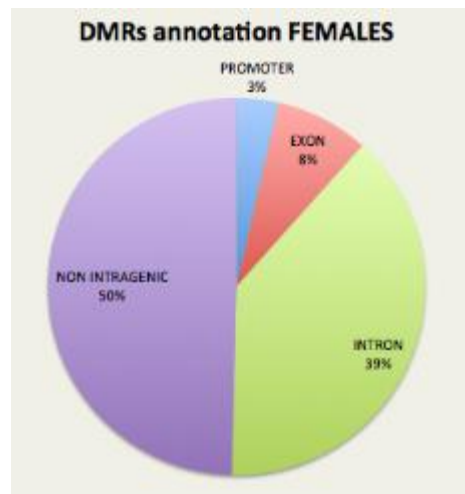
DMRs have been associated to 364 genes.

MALES

8 DMRs with $FDR < 0.1$

217 DMRs with $P.value < 0.0001$

DMRs have been associated to 308 genes.



Association of DMR with known genes :

F;22 DMRs associated to 29 genes:

- CCNA1
- CCNT1
- **CD84, Leucocyte differentiation antigen CD84**
- CNTNAP2
- COMMD1
- DEFB107A
- FAM161A
- GALR1
- GPR63
- **IL33, Interleukin 33**
- **IRF2, Interferon regulatory factor 2**
- KATNB1
- LOC728175
- **MBP, Myelin basic protein**
- OR8D1
- PAPOLA
- PEX2
- **POMT1, Protein-O-Mannosyltransferase 1**
- PPP4R4
- R3HCC1L
- SCAI
- SERTM1
- SLC24A3
- SLC6A16
- TPD52L3
- TTC13
- UFL1
- VRK1
- ZMYND8

M;8 DMRs associated to 10 genes:

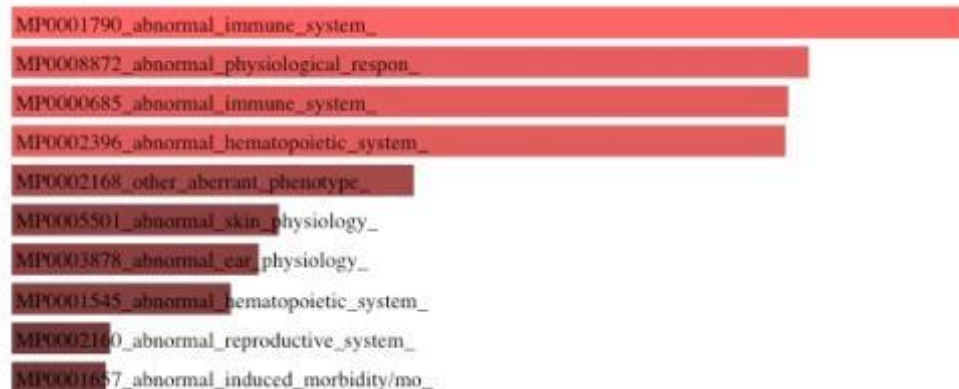
- DENND1A
- DTX2P1-UPK3BP1-PMS2P11
- FLJ31104
- **IL6ST, Interleukin 6 signal transducer**
- **KIR2DS3, Killer cell immunoglobulin-like receptor, two domains, short cytoplasmic tail, 3**
- **KIR2DS5, Killer cell immunoglobulin-like receptor, two domains, short cytoplasmic tail, 5**
- LINC00620
- MCTP2
- USP17L30
- ZNF726

Pathway analysis:

males
GO Biological process

| Index | Name | P-value |
|-------|---|----------|
| 1 | positive regulation of inflammatory response (GO:0050729) | 0.005714 |
| 2 | positive regulation of tyrosine phosphorylation of Stat3 protein (GO:0042517) | 0.005714 |
| 3 | positive regulation of adaptive immune response (GO:0002821) | 0.006232 |
| 4 | positive regulation of osteoblast differentiation (GO:0045669) | 0.006750 |
| 5 | regulation of cytokine-mediated signaling pathway (GO:0001959) | 0.006750 |
| 6 | positive regulation of anti-apoptosis (GO:0045768) | 0.007785 |
| 7 | positive regulation of T cell proliferation (GO:0042102) | 0.009337 |

females
MGI Mammalian Phenotype



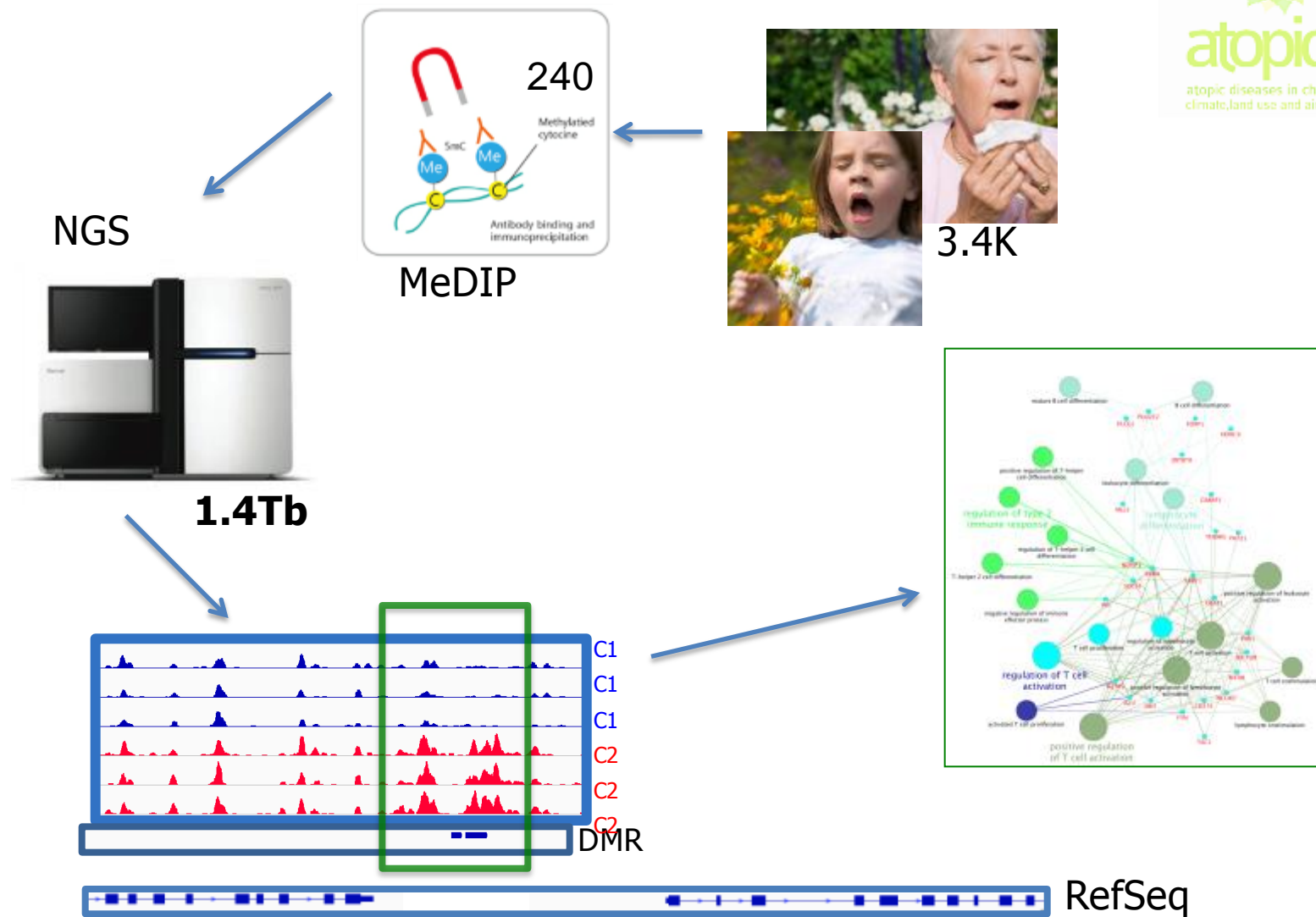
GO Molecular function

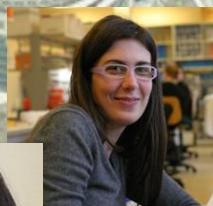
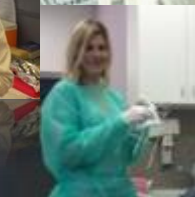
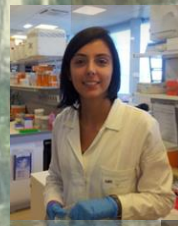
| Index | Name | P-value |
|-------|---|----------|
| 1 | MHC class I receptor activity (GO:0032393) | 0.006008 |
| 2 | cytokine receptor activity (GO:0004896) | 0.01375 |
| 3 | transmembrane receptor activity (GO:0004888) | 0.01408 |
| 4 | hematopoietin/interferon-class (D200-domain) cytokine receptor binding (GO:0005126) | 0.01550 |
| 5 | growth factor binding (GO:0019838) | 0.02180 |
| 6 | receptor activity (GO:0004872) | 0.02262 |
| 7 | cytokine binding (GO:0019955) | 0.02633 |
| 8 | phospholipid binding (GO:0005543) | 0.02702 |
| 9 | signal transducer activity (GO:0004871) | 0.03922 |
| 10 | calcium ion binding (GO:0005509) | 0.04465 |

Conclusions:

- We present the whole genome methylation dataset made on large scale, starting from a patients with atopy
- Preliminary results underline a differential methylation region associated with genes involved immunological pathways
- Data we obtained strongly suggest important role of epigenetics in atopy pathogenesis, before onset of disease and during
- Taking together our data and data obtained by climatic/pollen model we can hypothesize that a number of the patients will arise at least for the next one –two generations independent of air quality

Summary





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