DNA Repair in risk associated with genotoxic exposure

DNA REPAIR ENZYME SIGNATURE USING A MULTIPLEXED REPAIR ASSAY

CHARACTERIZATION OF A COHORT OF 100 HEALTHY INDIVIDUALS

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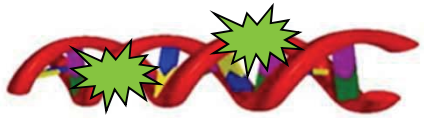
DNA REPAIR

Genotoxic Exposure

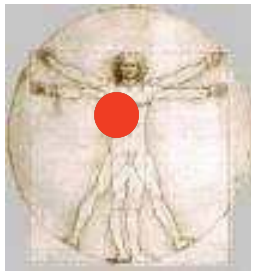
DNA Repair



Risk

Hypersensitivity reaction, cancer, premature aging

DNA REPAIR



DNA Repair

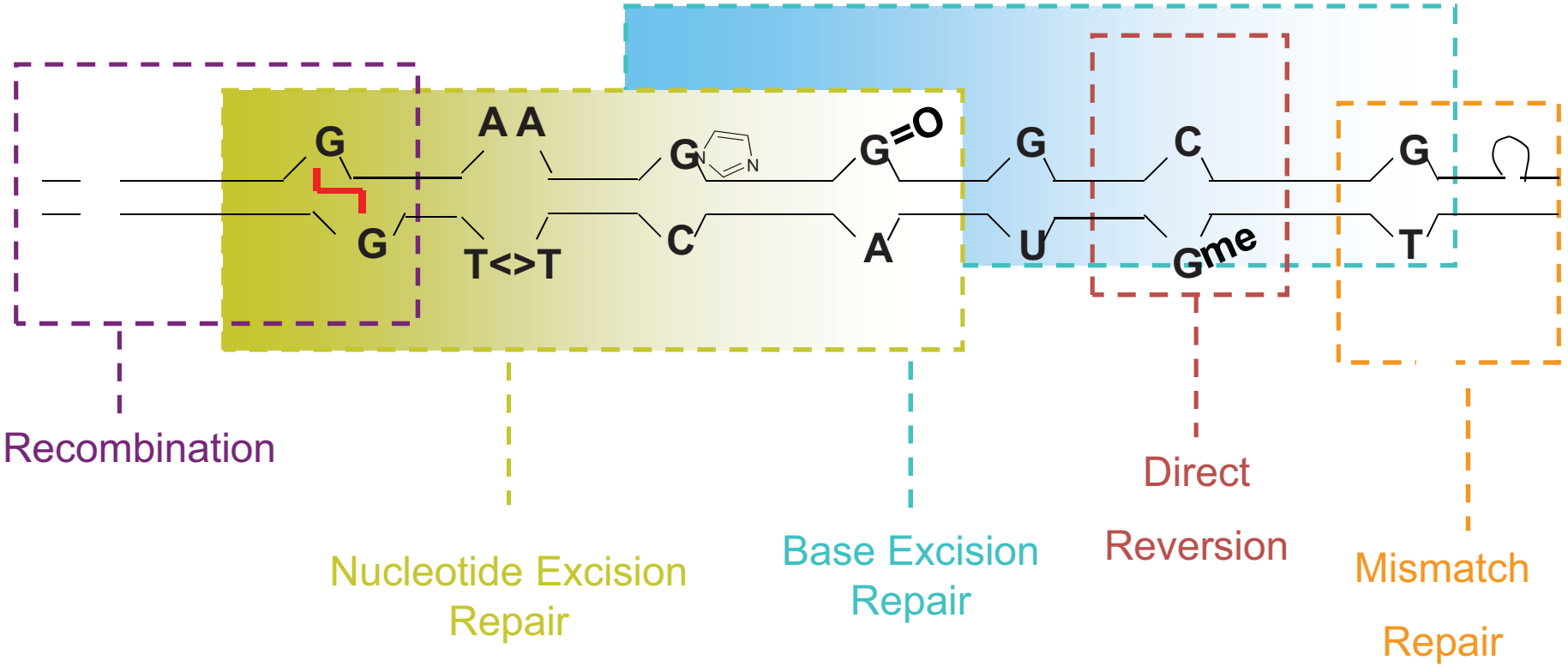
Chemo- and radio- resistance

Therapeutic failure

A diversity of DNA repair pathways to handle a diversity of DNA lesions

Genotoxic agents :

UV, HAP, oxidative stress, ionizing radiations, drugs, etc



WHY ?






- To take in charge individuals and populations exposed to genotoxic compounds (industrial and environmental hazards)

- To assure the follow up of persons at risk for cancer due to genetic susceptibility factors

→ need to identify
- **Biomarkers** of **exposure**
- **Biomarkers** of **risk**

DNA repair capacity (DNA Repair Enzymes): good biomarker candidate

→ Integrates the consequences of genetic factors (+epigenetic) x genotoxic exposure history (age, life style, smoking, genotoxic exposure history)

→ DNA repair is inducible (+/- specific)

HOW ?

Measuring Peripheral Blood Mononuclear Cells (PBMCs)
DNA Repair capacity

- Differentiated from bone marrow stem cells
- Lifetime: few days, up to several years
- Sensors of infection, inflammation, oxidative stress, systemic response (trigger DNA Damage Response ? DNA Repair ?)

Reflects individuals'
genetic background x genotoxic exposure history

Getting blood samples : minimally invasive
Cells (PBMCs) are handled easily

As a first step toward this goal, it is required to know the

AIM

- Distribution of DNA Repair activities in normal healthy population

- Impact of factors possibly interfering with the DNA Repair baseline activity

To start with:

Age, Gender

Life style (sport practice, hormone intake, etc).

Study/Assay Criteria (suitable for Population studies) :

- Fast – Simple - Limited number of steps
- Informative - Amenable to automation – Robust

- 1 parameter not sufficient → comprehensive multiplexed approach

100 healthy individuals

- Non smokers, no cancer
- Questionnaire
  (life style, medication)
- Study approval by Ethical Committee

5 Age Groups

AG1	m 25 y
AG2	m35 y
AG3	m 45 y
AG4	m 55 y
AG5	m 65 y

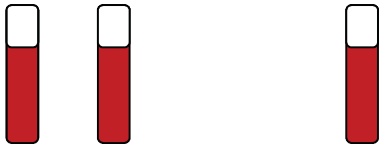
COHORT

- 10 males
- 10 females

by Age Group

3 blood samples / subject

D1 D+7



a b c

PBMCs

↓

Whole Cell Extract

↓

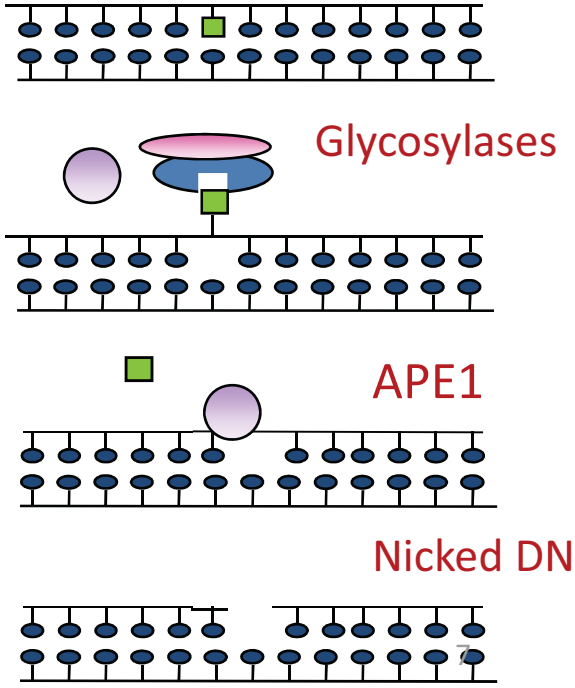
Protein concentration

↓

Repair Assay

Blind study

Base Excision Repair

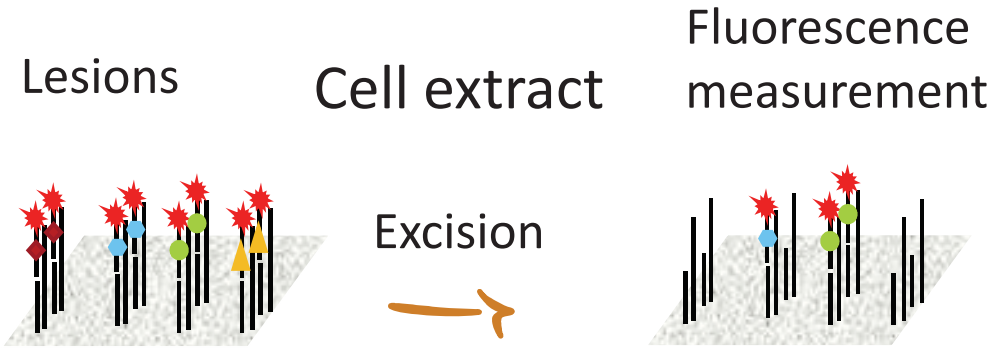


DNA Repair Enzyme Signature (functional assay)

ASSAY

Multiplexed oligonucleotide cleavage assay on Biochip

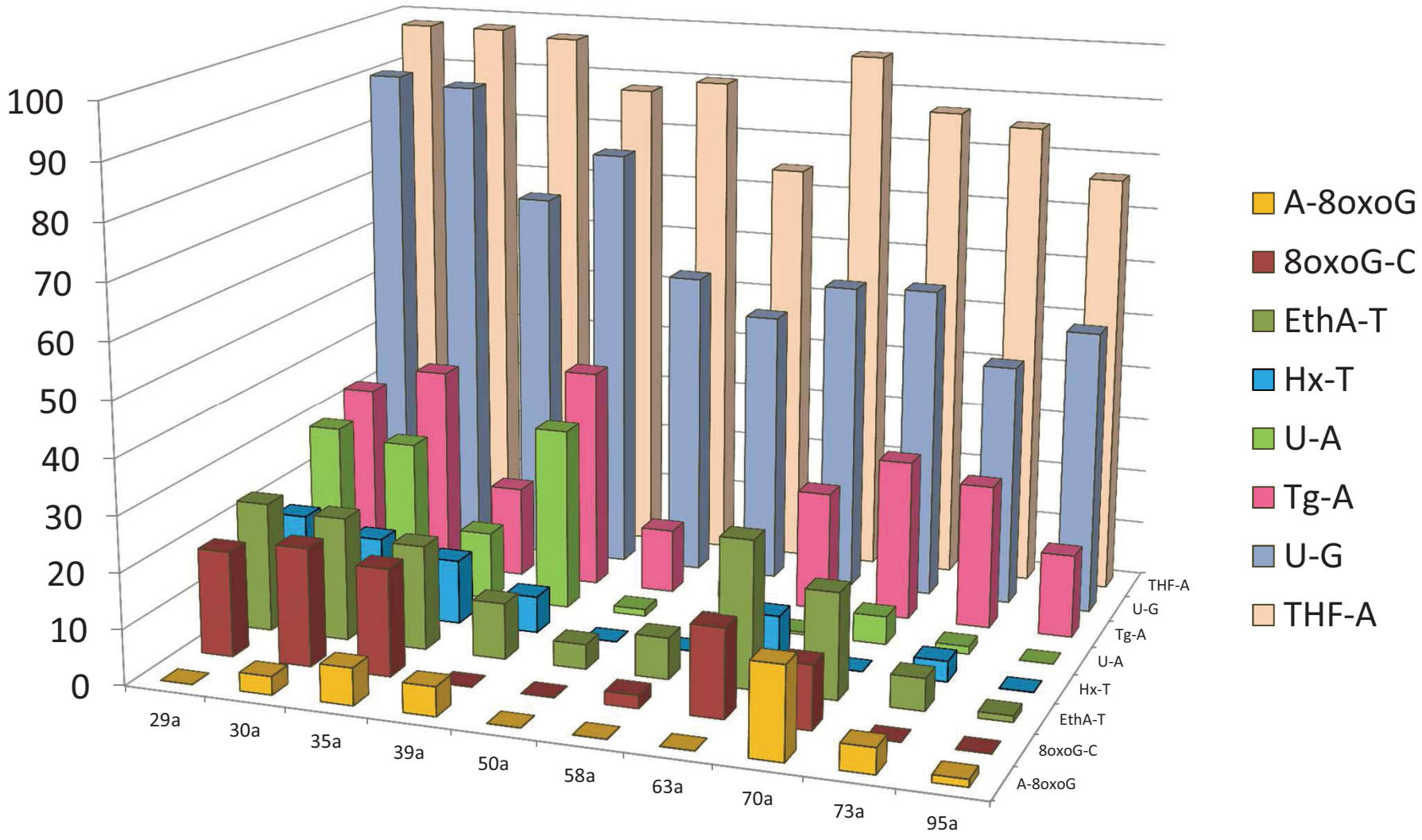
(takes into account the DNA Repair complexity/redundancy)



Brevet CEA
Sauvaigo *et al*, 2004, Pons *et al,* 2010,
Candéias *et al* 20110

Substrate	Human Enzyme
8-OxoG (opp C)	hOGG1
Thymine Glycol	NTH1, NEIL1
U (opp G)	SMUG1
U (opp A)	SMUG1, UNG2
A (opp 8-oxoG)	MUTYH
Hypoxanthine (Inosine)	MPG/AAG
EthenoA	MPG/AAG
Abasic sites	APE1

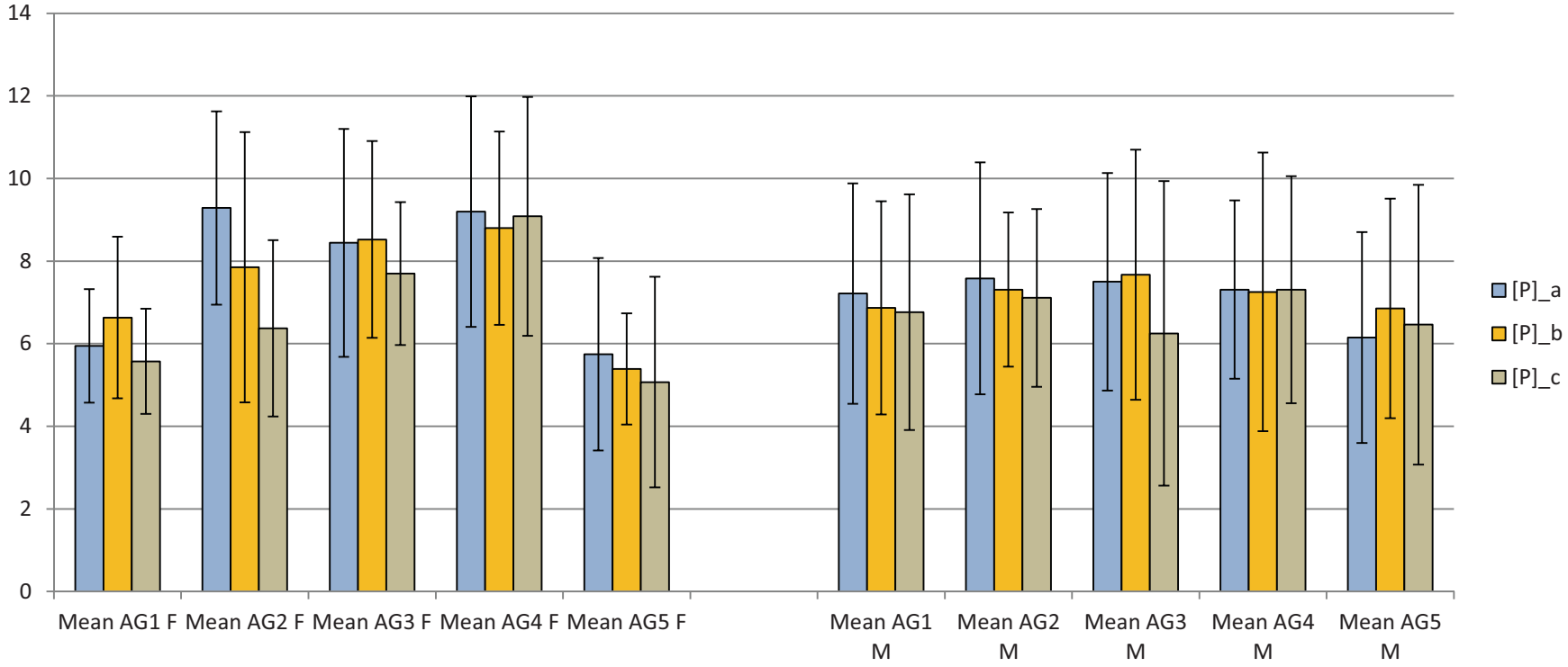
Example: 10 subjects, same AG, same Gender



Excision activities expressed as *Percentage of cleavage*

Protein concentration (mg/mL)
Effect of Age - Effect of Gender

[PROTEIN]



F mean = 7.2 mg/mL +/- 2.6 **M**

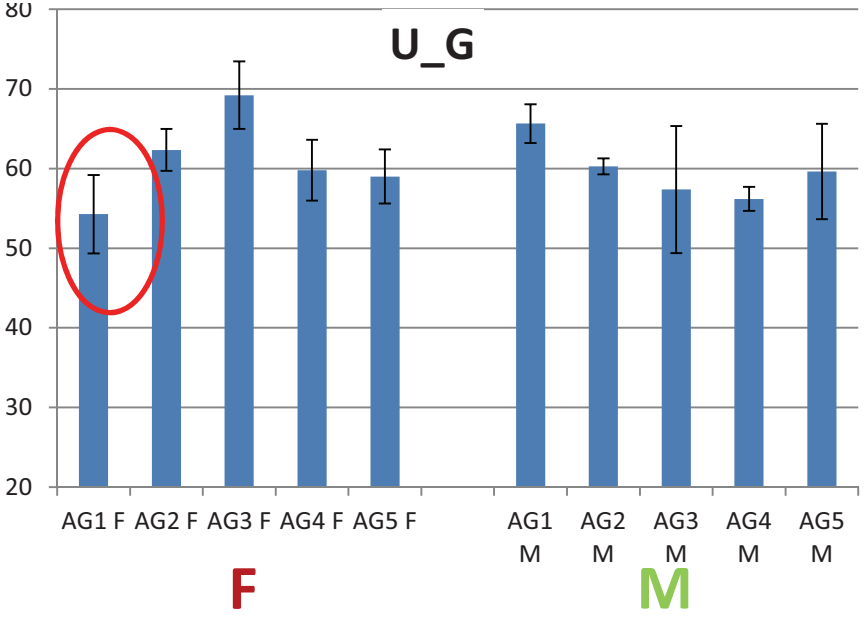
Gender specific effect* of age on extract protein concentration

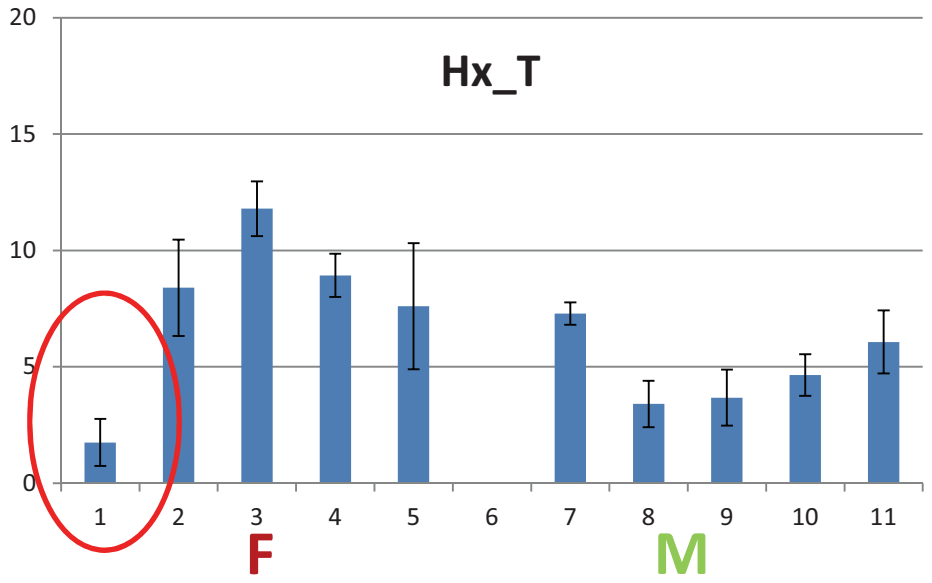
Female Group: bell shape curve

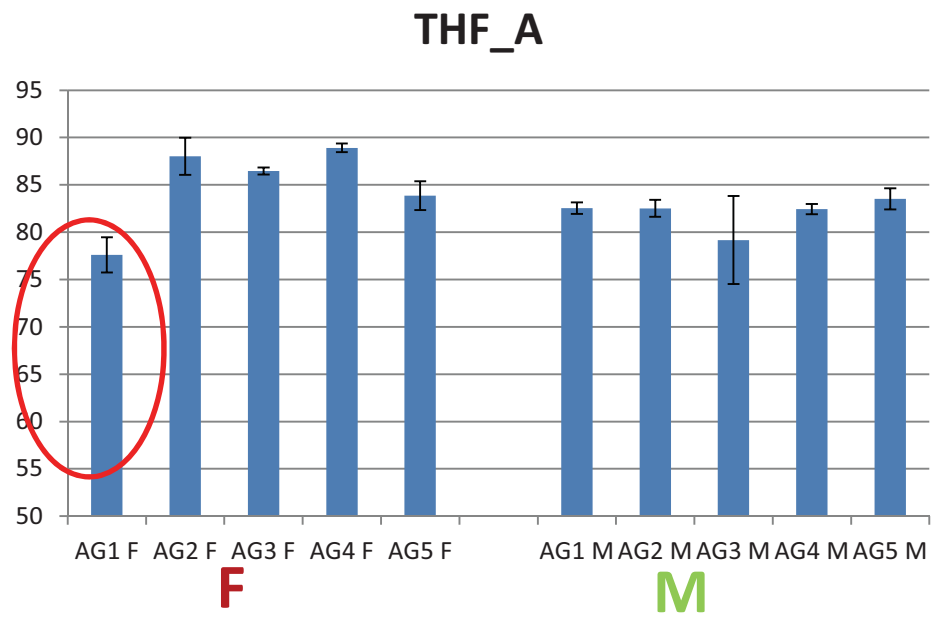
Male Group : constant over time

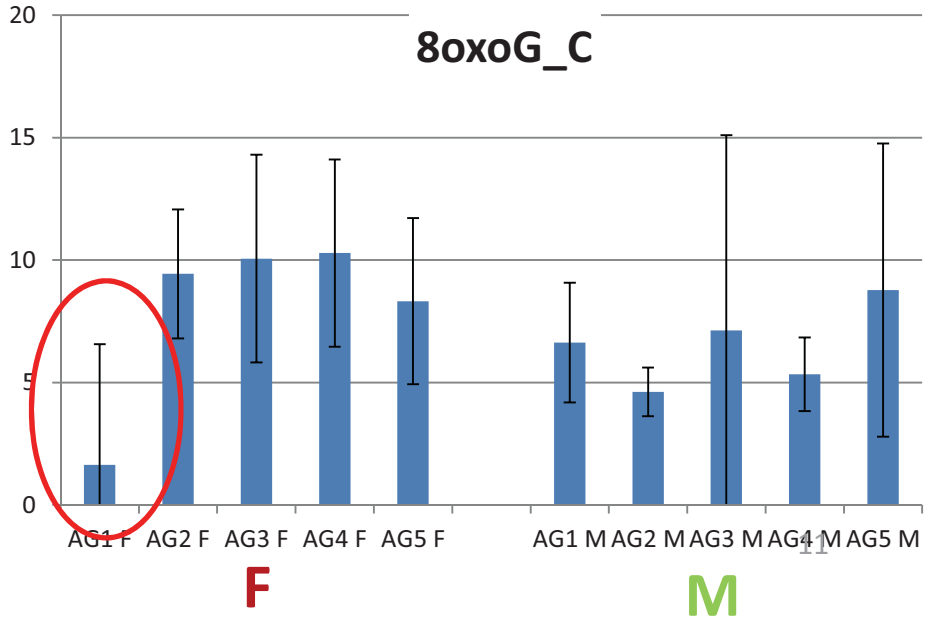
*Statistical significance to be calculated

Distibution of excision activities among Groups (Percentage of cleavage)









Means (a, b and c) and SD by Age Group and by Gender

→ For Females:
Excision activities are somehow related to the extracts protein concentration (AG1)
Hypothesis: Hormonal status, pregnancy influence ?

Remark: Less cells in AG5

→Need to remove unreliable data
→Statistical significance will then be calculated

! Small groups (9-10 subjects)

Variability of the procedure: sampling, extract, assay (a,b)
Variability of DNA Repair as a biological parameter (a,e) and (b,e)

Lesion	Correlation Coefficient (a et b)	Correlation Coefficient (a et e)	Correlation Coefficient (b et e)
A-8oxoG	0.668	0.373	0.538
U-A	0.697	0.245	0.362
EthA-T	0.757	0.547	0.669
Tg-A	0.750	0.569	0.660
Hx-T	0.626	0.598	0.674
THF-A	0.948	0.698	0.680
U-G	0.774	0.591	0.583
8oxoG-C	0.741	0.511	0.544

There is a biological intra-subject variability (decrease of the CC)

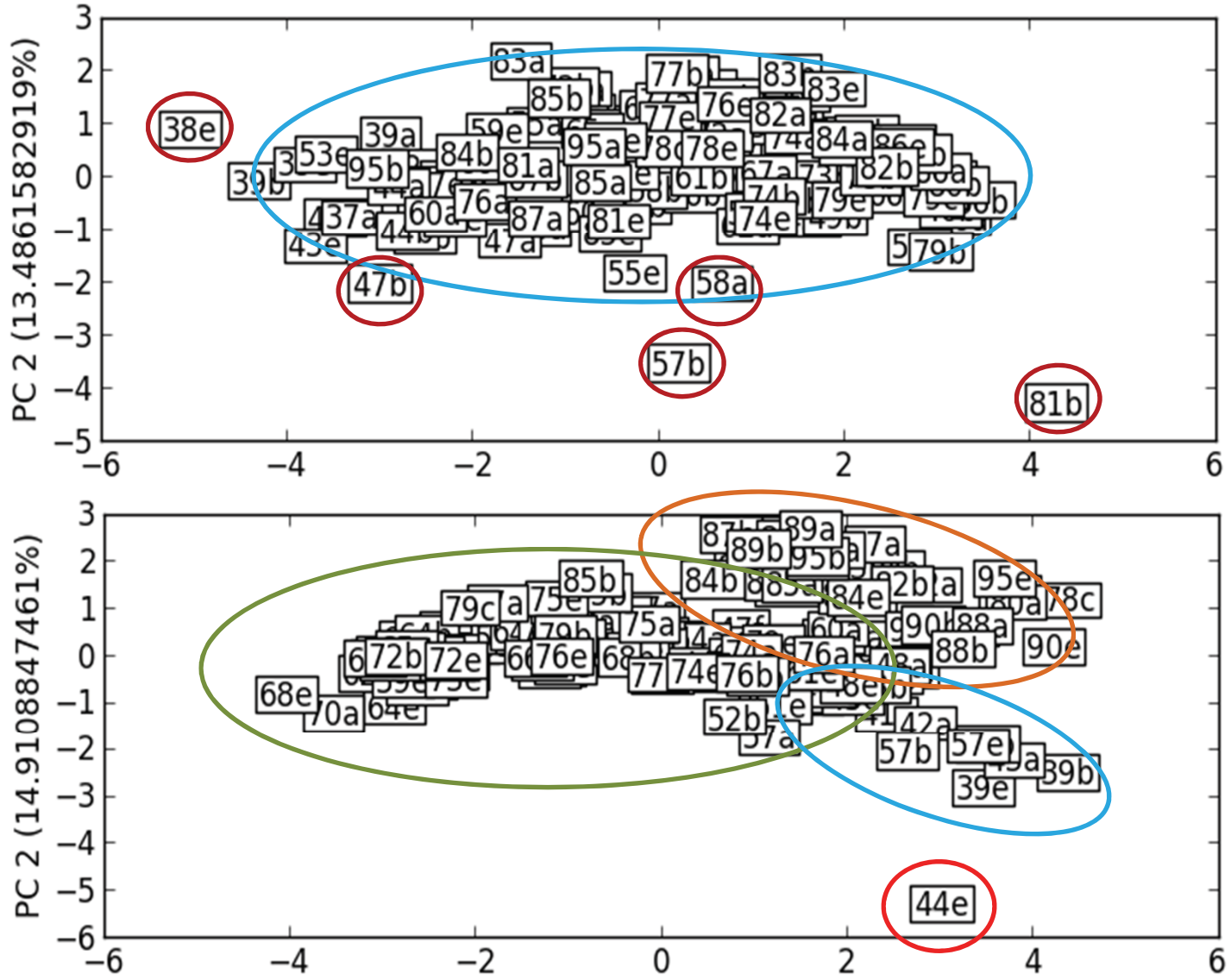
Meaning of the variability = biomarker of something ?

What are the most relevant lesions ?

Identification of Sub-Groups and Atypical Subjects using Principal Component Analysis

M

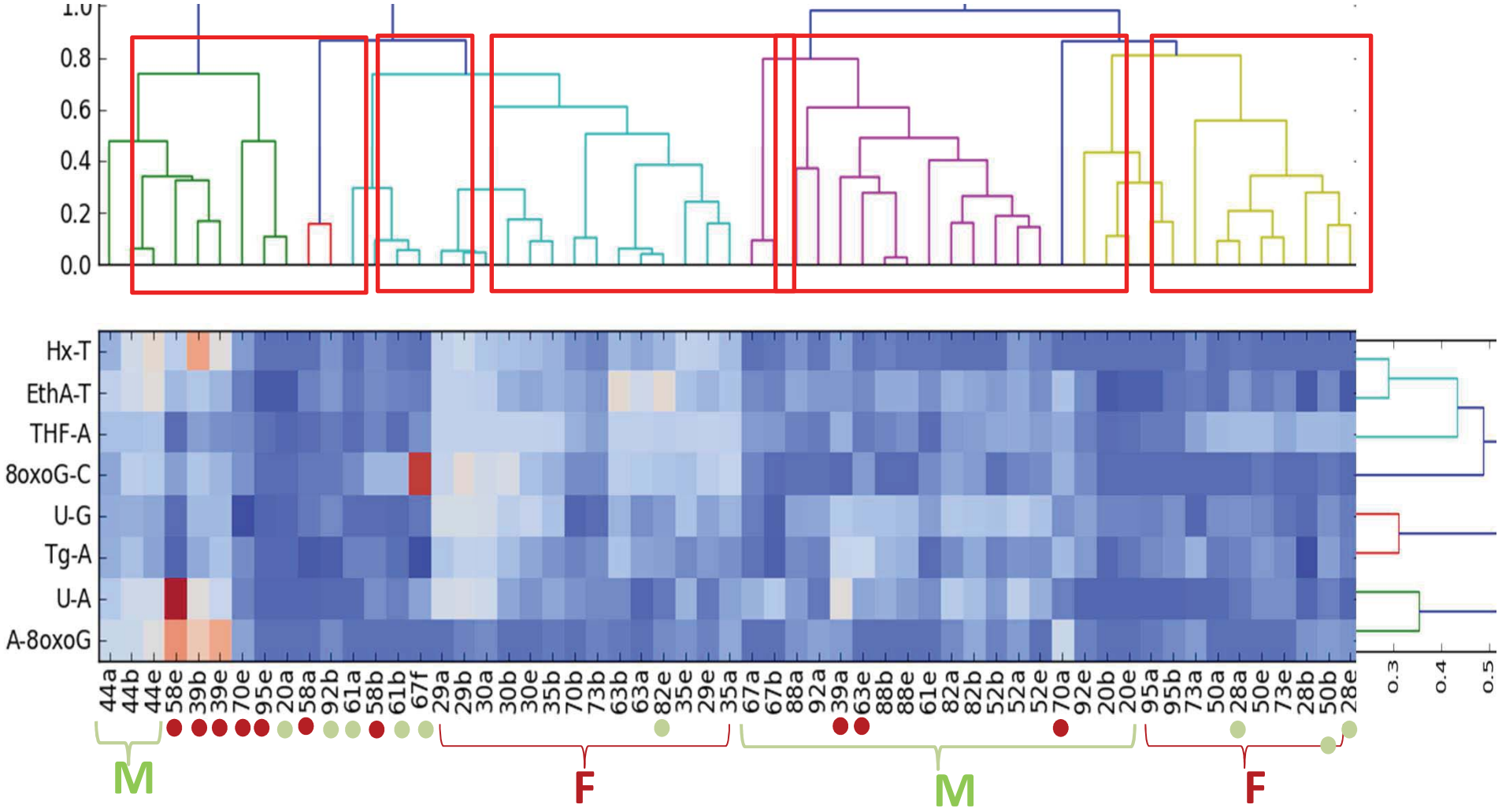
F



Characteristics of the Sub-Groups ? What are the differences related to ?

Similarities across subjects and across DNA Repair activities (HC)

- Cluster subjects by DNA Repair profile similarities (AG2, 3 series)
- Identify correlations between repair pathways



Are the specific correlations between some DNA Repair Activities influenced by age, by gender, by life style ?

CONCLUSION

(preliminary results)

- Sort data, integrate life style factors to the study
- Statistical significance of the results to be determined

- There are differences between Males and Females

  Females: Age dependent DNA Repair Signature

  Male: no obvious relationship

  → But to be investigated for each lesion and at the level of the whole DNA Repair Signature

Multiplex approach = combination of factors

PCA and clustering: similarities across subjects and across pathways

→ powerful for biomarkers identification

PERSPECTIVES

Hypersensitivity reaction risk and cancer susceptibility risk biomarkers

- DNA Repair Enzyme Signature as Biomarker of radiosensitivity (radiotherapy)

- Compare DNA Repair enzyme Signature between exposed and non exposed individuals (smokers : exposure dose and time)
- Identify pulmonary cancer risk factors associated with DNA Repair

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