Claudio Franceschi
University of Bologna, Italy

INFLAMM-AGING

V Norwegian Congress of Geriatrics , OSLO, April 22nd 2013

Guidelines and Priorities

"*Increased longevity without quality of life is an empty prize".*

WHO declaration

"Horizon 2020"

has identified

HEALTHY AGEING

as a major priority

The critical importance of having reliable biomarkers of aging

Prospects & Overviews

Problems & Paradigms

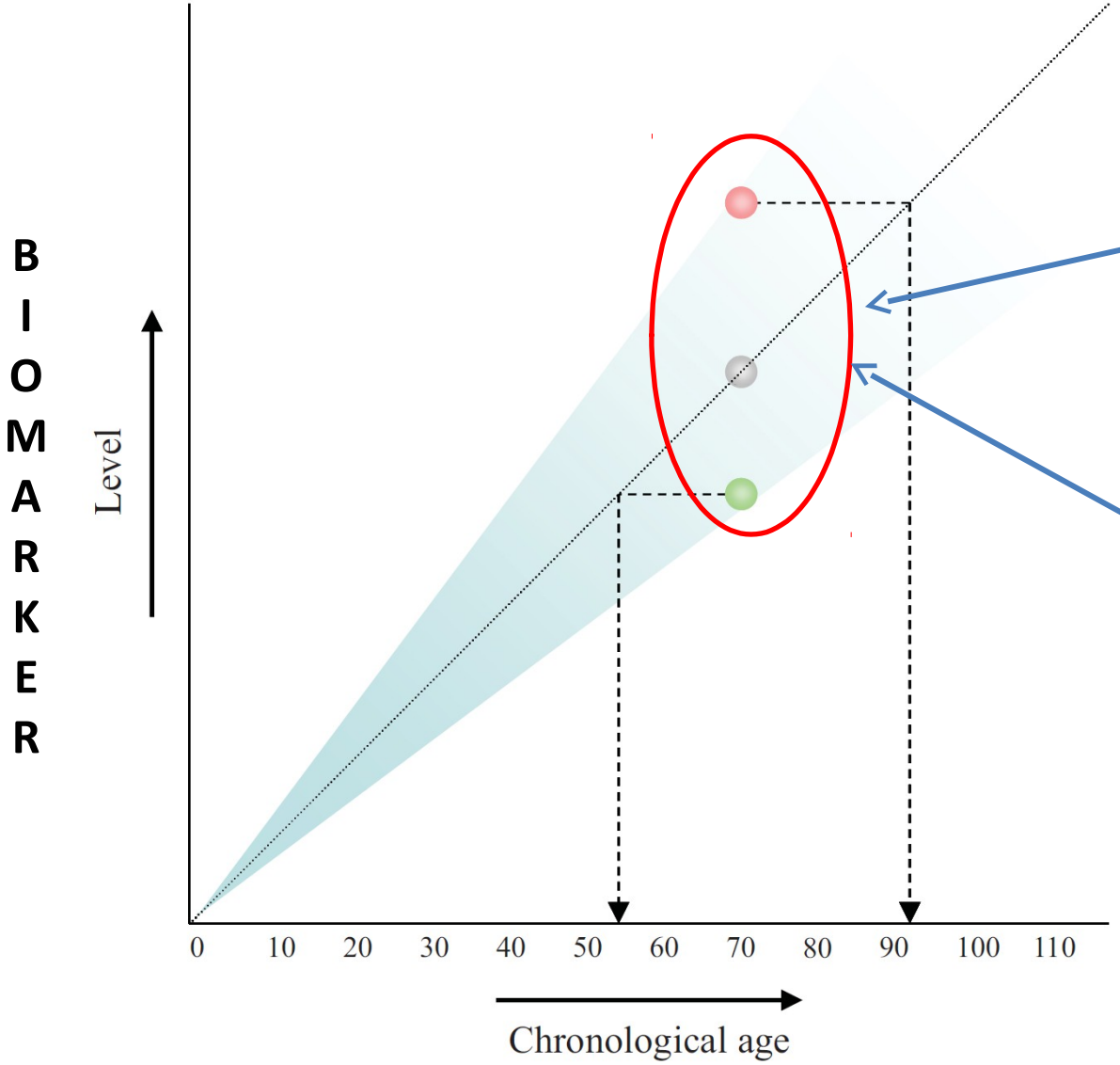
Identifying the genomic determinants of aging and longevity in human population studies: Progress and challenges

Joris Deelen[1)2)], Marian Beekman[1)2)], Miriam Capri[3)], Claudio Franceschi[3)] and P. Eline Slagboom[1)2)]*

[1)] Section of Molecular Epidemiology, Leiden University Medical Center, Leiden, The Netherlands
[2)] Netherlands Consortium for Healthy Ageing, Leiden University Medical Center, Leiden, The Netherlands
[3)] Department of Specialized, Diagnostic and Experimental Medicine, University of Bologna, Bologna, Italy

BioEssays 2013

Biomarkers of aging
chronological *vs* biological age



Individual variability within a population

Different rates of aging in different individuals

BioEssays 2013

Biomarkers of aging

We have recently identified two powerful biomarkers of aging:

1. a glycomic biomarker (related to inflamm-aging)

2. an epigenetic biomarker

Both validated within the EU Large Project MARK-AGE

GLYCOMICS and aging

in collaboration with Valerie Vanhooren

Department for Molecular Biomedical Research, VIB, Department of Biomedical Molecular Biology, Ghent University, Technologiepark 927,

B-9052 Gent-Zwijnaarde, Belgium

Vanhooren *et al*., Rejuvenation Res., 2007

Vanhooren *et al*., Mech Ageing Dev., 2009

Vanhooren *et al*., Exp. Gerontol., 2010

GLYCOMICS

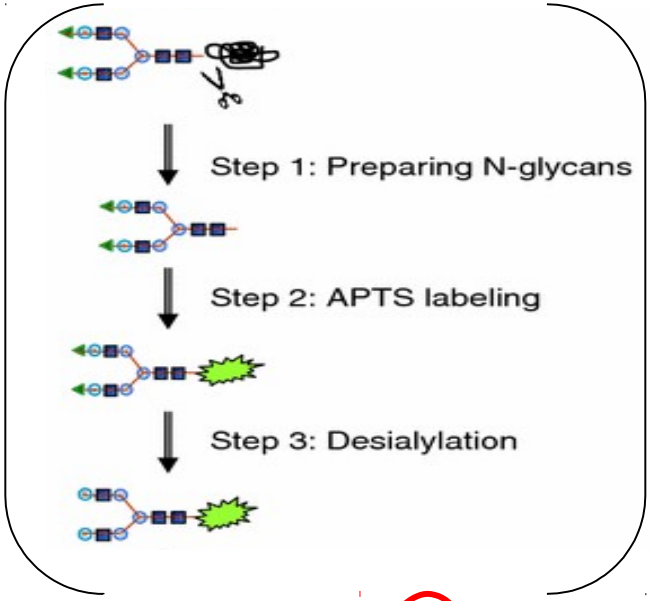
N-glycans profiling appears to be one of the more robust biomarker of biological age

N-Glycomic biomarkers of biological ageing and longevity: a link with inflamm-ageing

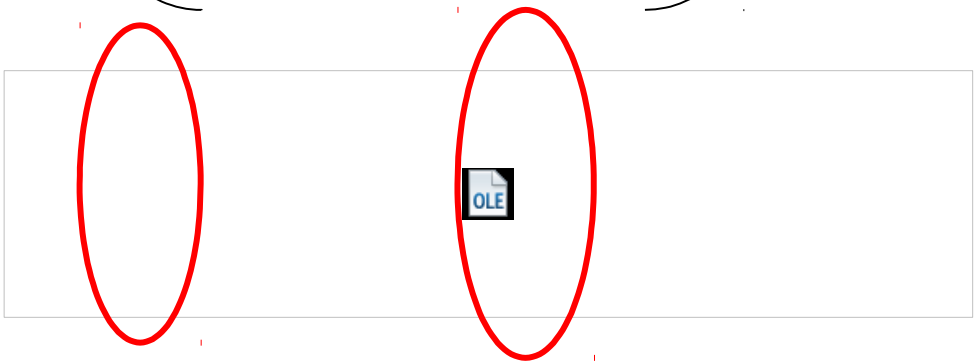
Fabio Dall'Olio, Valerie Vanhooren, Cuiying C Chen, P. Eline Slagboom, Manfred Wuhrer & Claudio Franceschi

Ageing Research Reviews 2012

1. N-glycan labeling

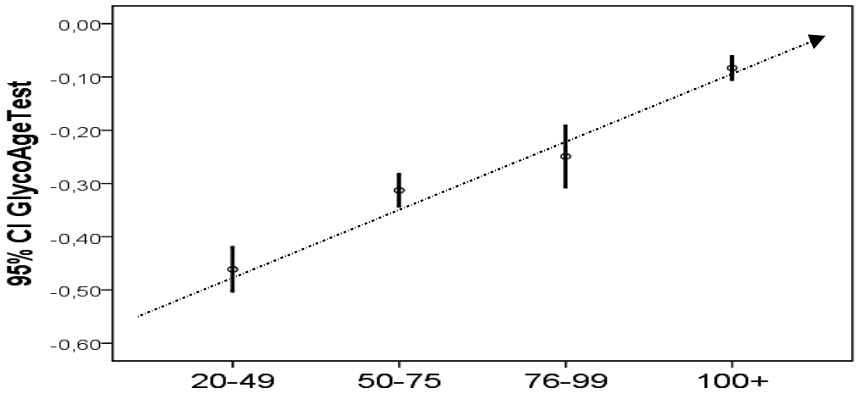


2. N-glycan profiling Using DNA-sequencer



3. GlycoAgeTest

log(P1/P6)



Peak 1 (an **a**galactosylated core-α-1,6-fucosylated biantennary; NGA2F) **increased gradually with age**

Peak 6 (a **bi**galactosylated, core-α-1,6-fucosylated biantennary; NA2F) **decreased gradually with age** .

The log ratio of NGA2F (peak1) and NA2F (peak6) can be considered **a new ageing biomarker**, named **GlycoAgeTest**

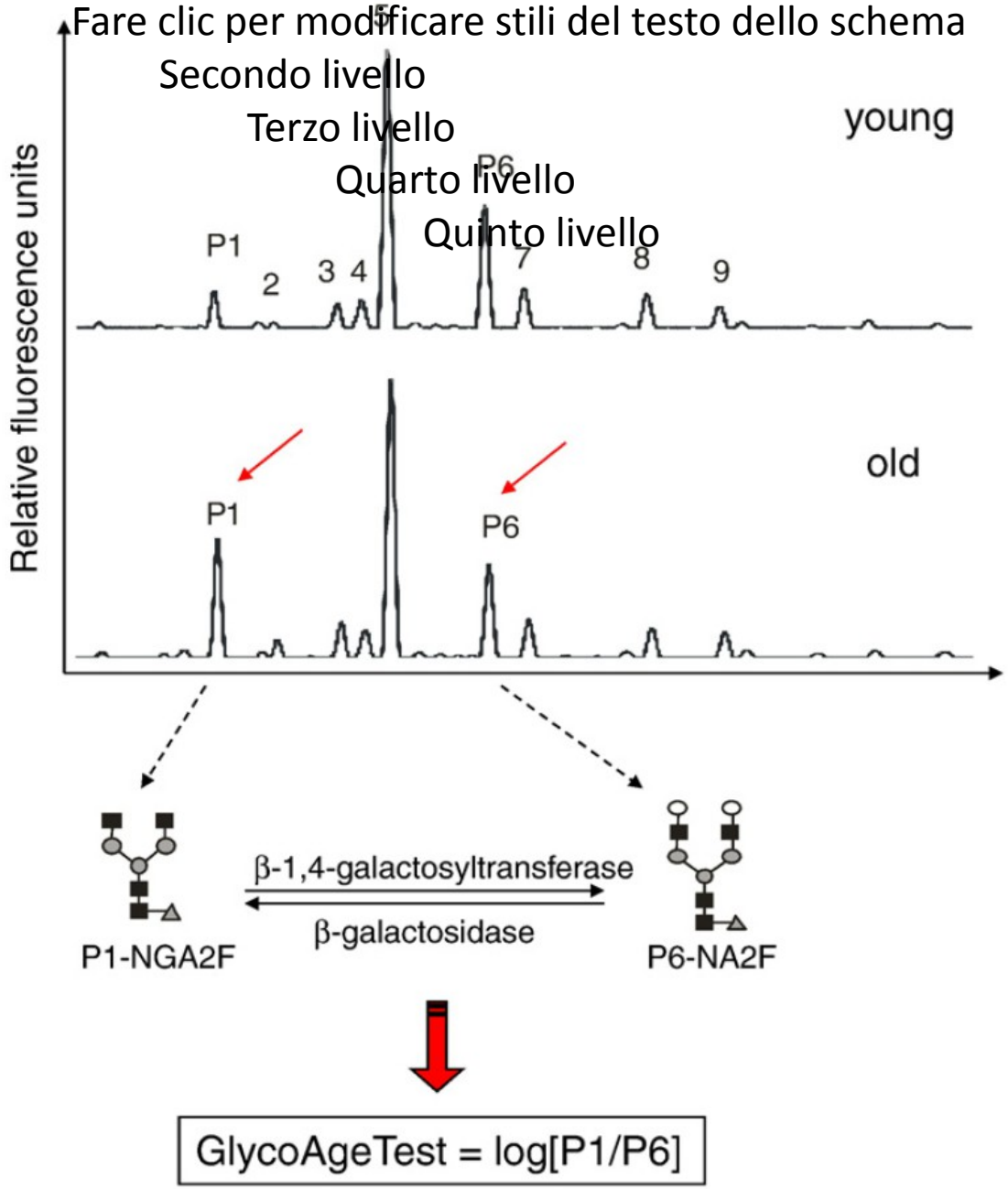
Fare clic per modificare stili del testo dello schema

Secondo livello

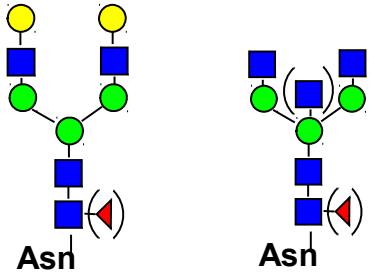
Terzo livello

Quarto livello

Quinto livello



Increase of agalactosylated N-glycan structures as biomarker of biological age



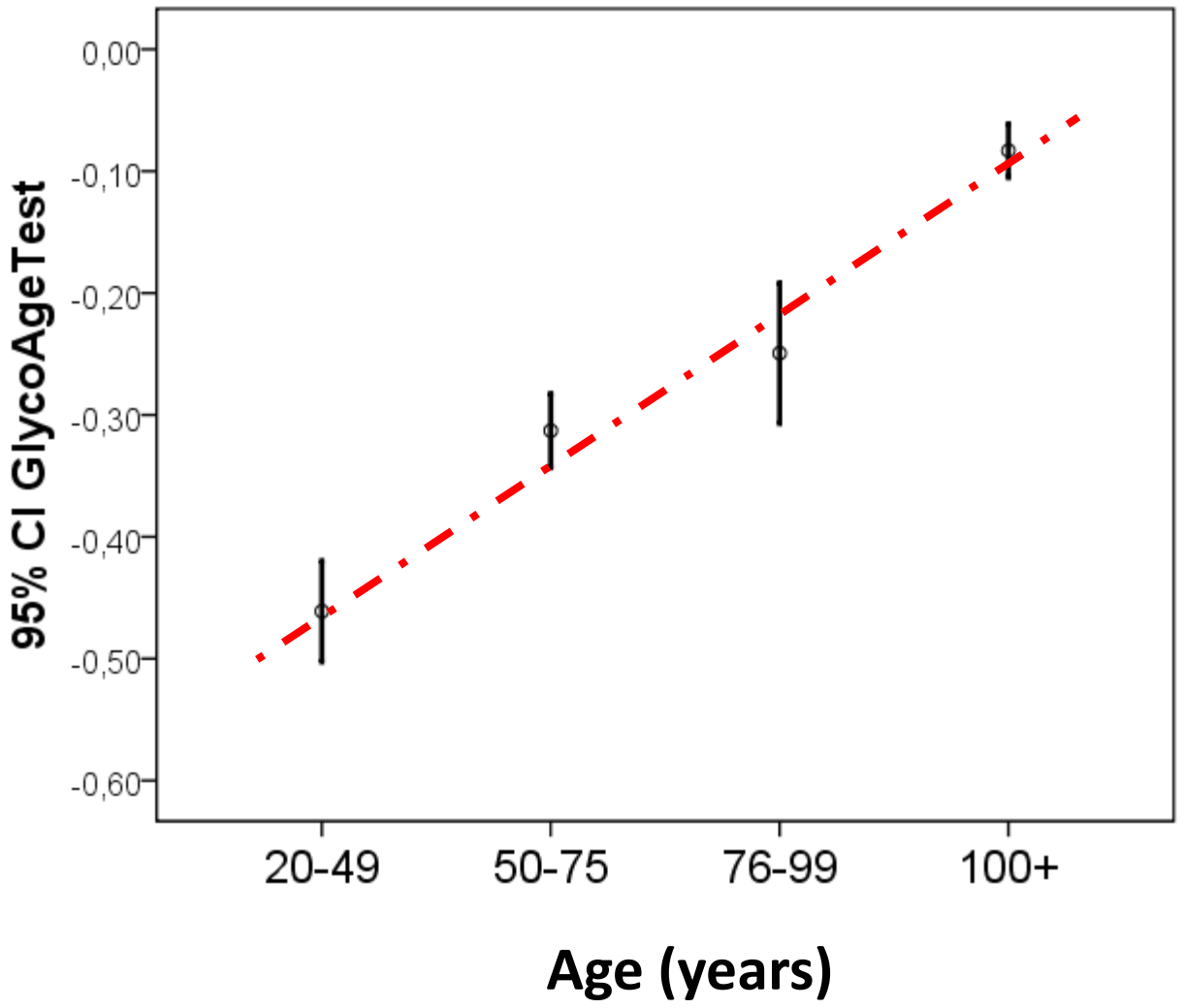
Human conditions

- Age below 60 — High — Low
- Age above 60 — Low — High
- Centenarians — Low — High
- Centenarian Offspring — High — Low
- Cockayne syndrome — Low — High
- Werner syndrome — Low — High
- Down syndrome — Low — High

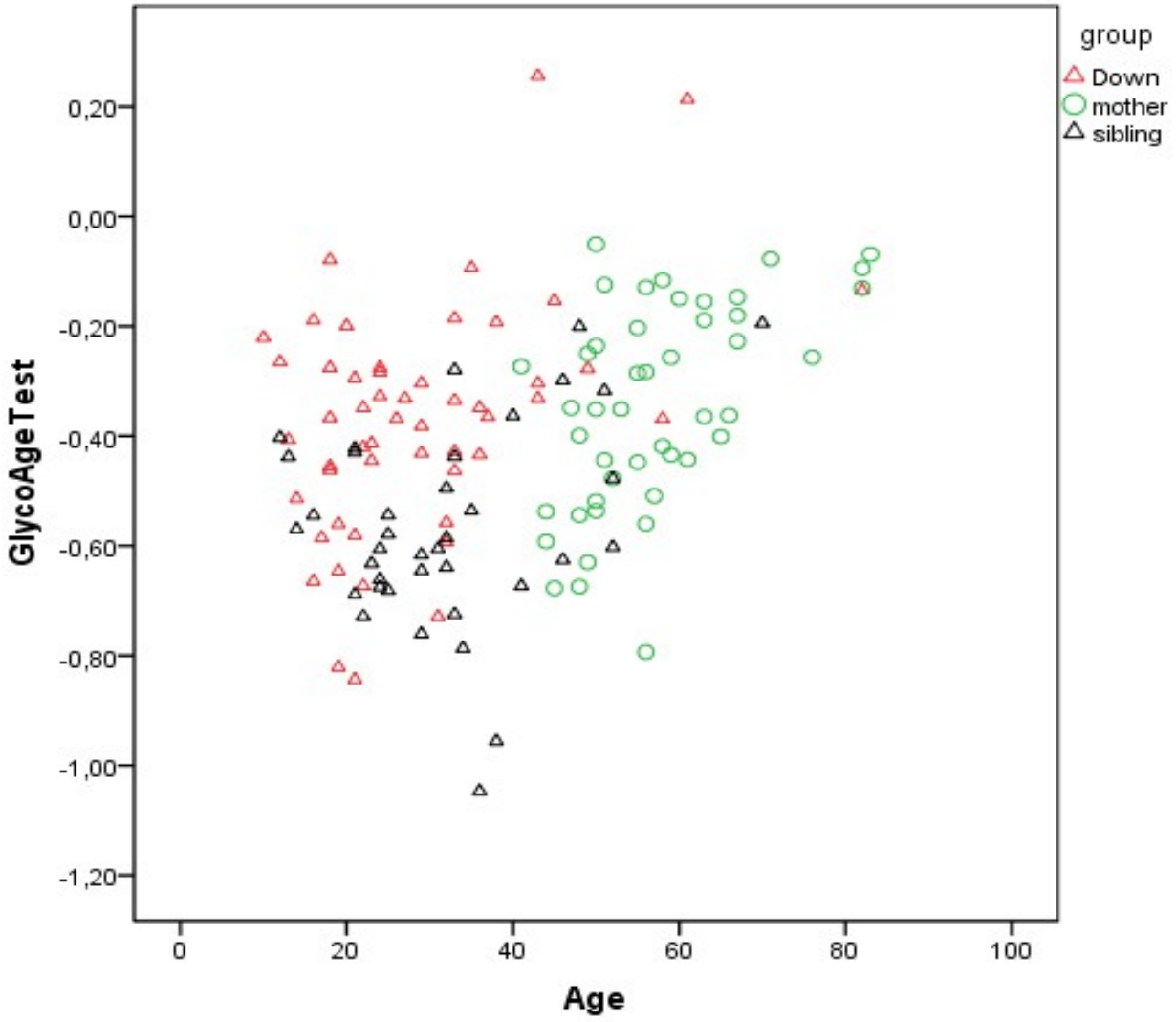
Animal models

- Young mice — High — Low
- Old mice — Low — High
- Mice caloric restricted — High — Low

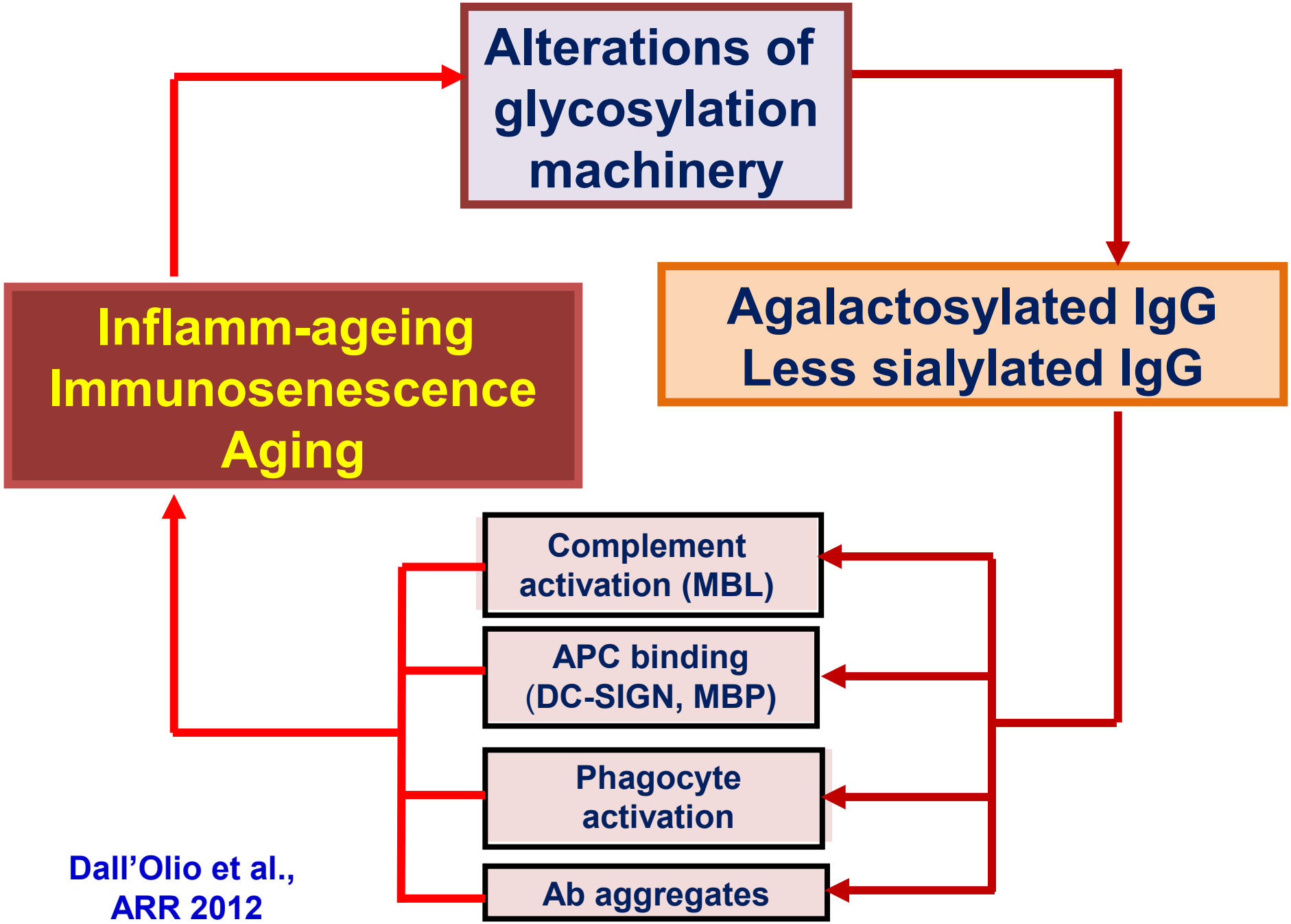
The GlycoAgeTest value increases gradually during ageing and reaches the highest level in centenarians (N=84)



The value of GlycoAgeTest increases faster in Down' Syndrome subjects than in their unaffected siblings and is similar to that of their mothers



unpublished results



Dall'Olio et al., ARR 2012

Different methylated regions (DMR) between 32 mothers and their unaffected offspring using the Illumina *Infinium* HumanMethylation450 BeadChip (480,000 CpG/genome)

The top 5 loci mapped within the CpG islands of 3 genes:

ELOVL2 (cg16867657), ***FHL2*** (cg06639320, cg22454769, cg24079702) and ***PENK*** (cg16419235).

The CpG islands of *ELOVL2*, *FHL2* and *PENK* resulted **hypermethylated in mothers respect to the offspring cohort** with no sex- or family-associated differences in methylation levels.

***Garagnani et al.*, AGING CELL 2012**

DMR between 32 mothers and their unaffected offspring

Spearman's correlation analysis showed

striking correlation values between methylation levels and age:

ELOVL2 **(cg16867657, ρ= 0.91; cg21572722, ρ= 0.89; cg24724428, ρ= 0.85)**

FHL2 **(cg06639320, ρ= 0.91; cg22454769, ρ= 0.92; cg24079702, ρ= 0.86)**

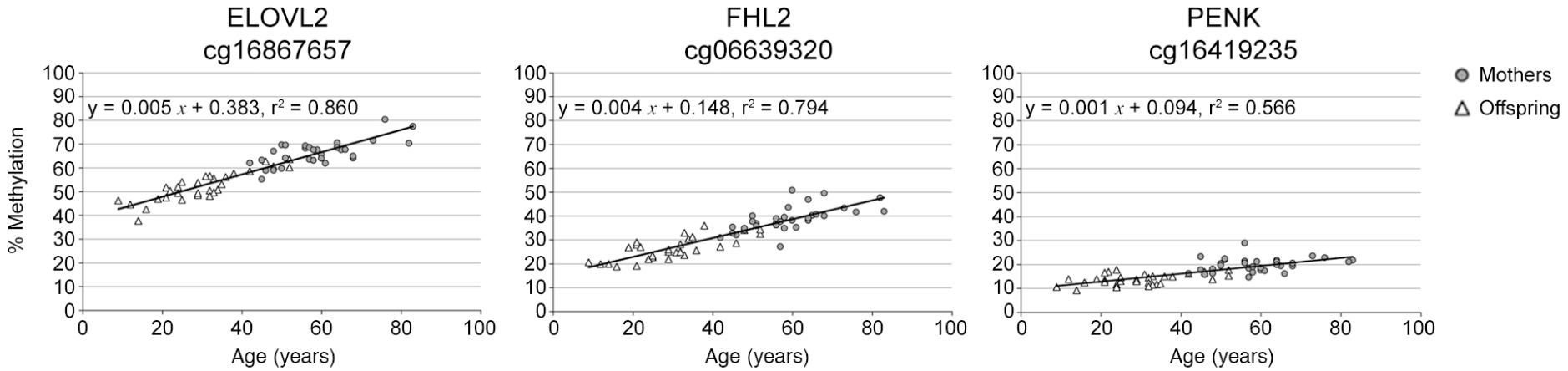
PENK **(cg16419235, ρ= 0.76; cg16219603, ρ= 0.62; cg12877723, ρ= 0.63).**

ELOVL2: elongation of omega 3 and omega 6 FA

FHL2: transcriptional co-activator or co-repressor, important in development and cell differentiation

Gonçalves, AGING CELL 2012

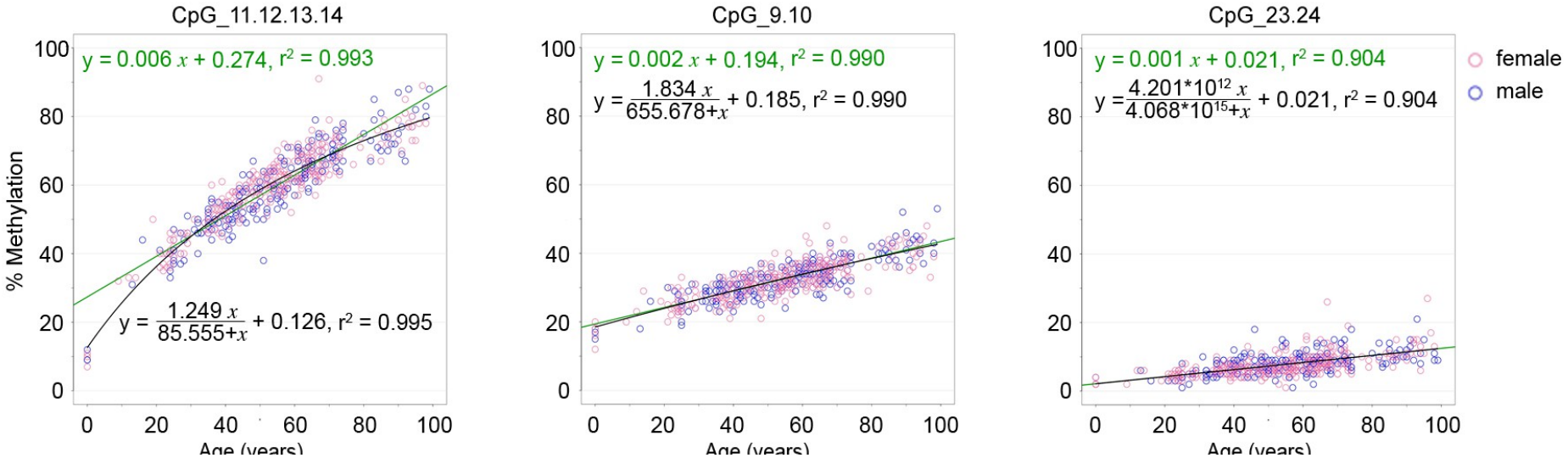
DMR between mothers and offspring in *ELOVL2, FHL2, PENK*



The linear regression curve and the corresponding equation are reported.

***Garagnani et al.*, AGING CELL 2012**

Methylation of *ELOVL2*, *FHL2* and *PENK* CpG islands in 501 healthy subjects from newborns to centenarians (Sequenom EpiTYPER)



the most powerful biomarker of aging (epigenetic)

Garagnani *et al*., AGING CELL 2012

INFLAMM-AGING

encompassing:

- aging and longevity
- successful and unsuccessful aging

Elena S., 106 years

United Nations, Department of Economic and Social Affairs, Population Division (2011)
World Population Prospects: The 2010 Revision

	80+	100+	TOTAL POP
• China 2010	18 211 404	13 514	1341 M
• Italy 2010	3 545 982	12 756	60 M
• China 2012	20 448 968	15 104	1353 M
• Italy 2012	3 761 061	15 543	61 M
• China 2050	98 338 685	262 487	1295 M
• Italy 2050	7 659 229	97 995	59 M

Centenarians have major advantages

- They live **20-30 years more** than the members of their demographic cohort
- Most of them **avoided or postponed**

  (> 20 years) all major age-related diseases
  - **They die in few days !**

Accordingly, they can be considered the best example (model) of healthy aging in humans

Italian Centenarian Study 2008

Franceschi, Bologna, Coordinator ; D Monti, Florence; D Mari, Milan; C Caruso, Palermo

	Centenarians n=156	100+ Offspring n=267	Offspring of non long-lived parents n=107
Age (mean) yrs (min-max)	100.8 (98-111)	70.2 (54-88)	71.1 (50-86)
Male, n (%)	34 (21.8)	109 (40.8)	56 (52.3)
Female, n (%)	122 (78.2)	158 (59.2)	51 (47.7)

Within the Italian National Project "Role of Epigenetics and mtDNA Genetics on Longevity: a study on subjects over 105 years of age" we are recruiting 105+ and 110+ subjects and their offspring in Emilia-Romagna, Lombardia and Calabria

Centenarian offspring are in good shape

they have a better health than offspring of non long living parents

Centenarians offspring
on most cases both parents long lived

Lower	Weight	Higher
Lower	Waist circumference	Higher
Lower	BMI	Higher
Lower	N° of drug assumed	Higher
Lower	N°of subjects taking drugs	Higher
Lower	N°of subjects with lipid-lowering therapy	Higher
Lower	Myocardial Infarction	Higher
Lower	Stroke, cerebral thrombosis	Higher
Lower	Cancer	Higher
Lower	Irregular heart failure	Higher
Lower	Hypertension	Higher
Lower	Osteoporosis	Higher
Lower	More than 2 pathologies	Higher
Lower	IGF-1	Higher
Higher	Handgrip strength	Lower
Higher	N°of subjects able to perform chair stand	Lower
Higher	N°of subjects able to climb stairs w/o aid	Lower

Offspring of non long-lived parents
both parents short living

The phenotype of centenarians is unexpectedly complex being a unique mixture of

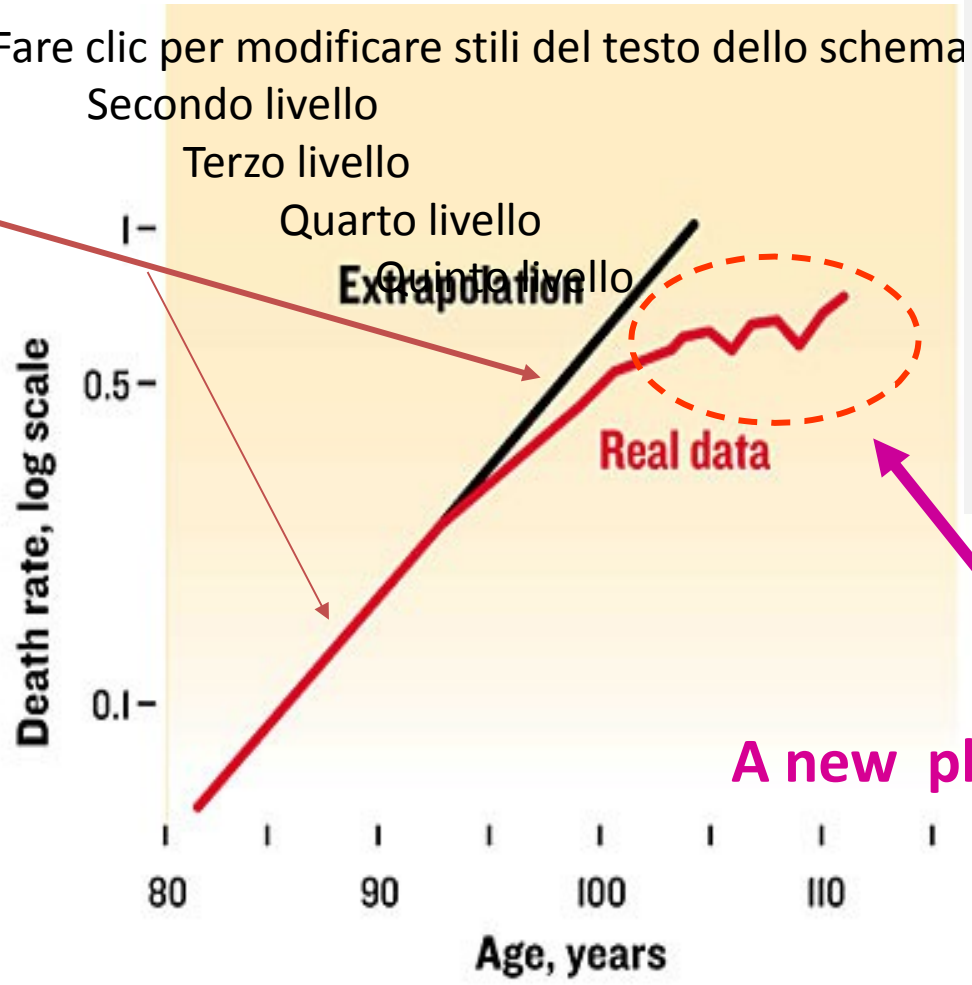
1. adaptive Robustness & accumulating Frailty

2. Protective & Risk Factors

concomitantly present in the same individuals

mortality deceleration in the oldest old
& increasing adaptive robustness

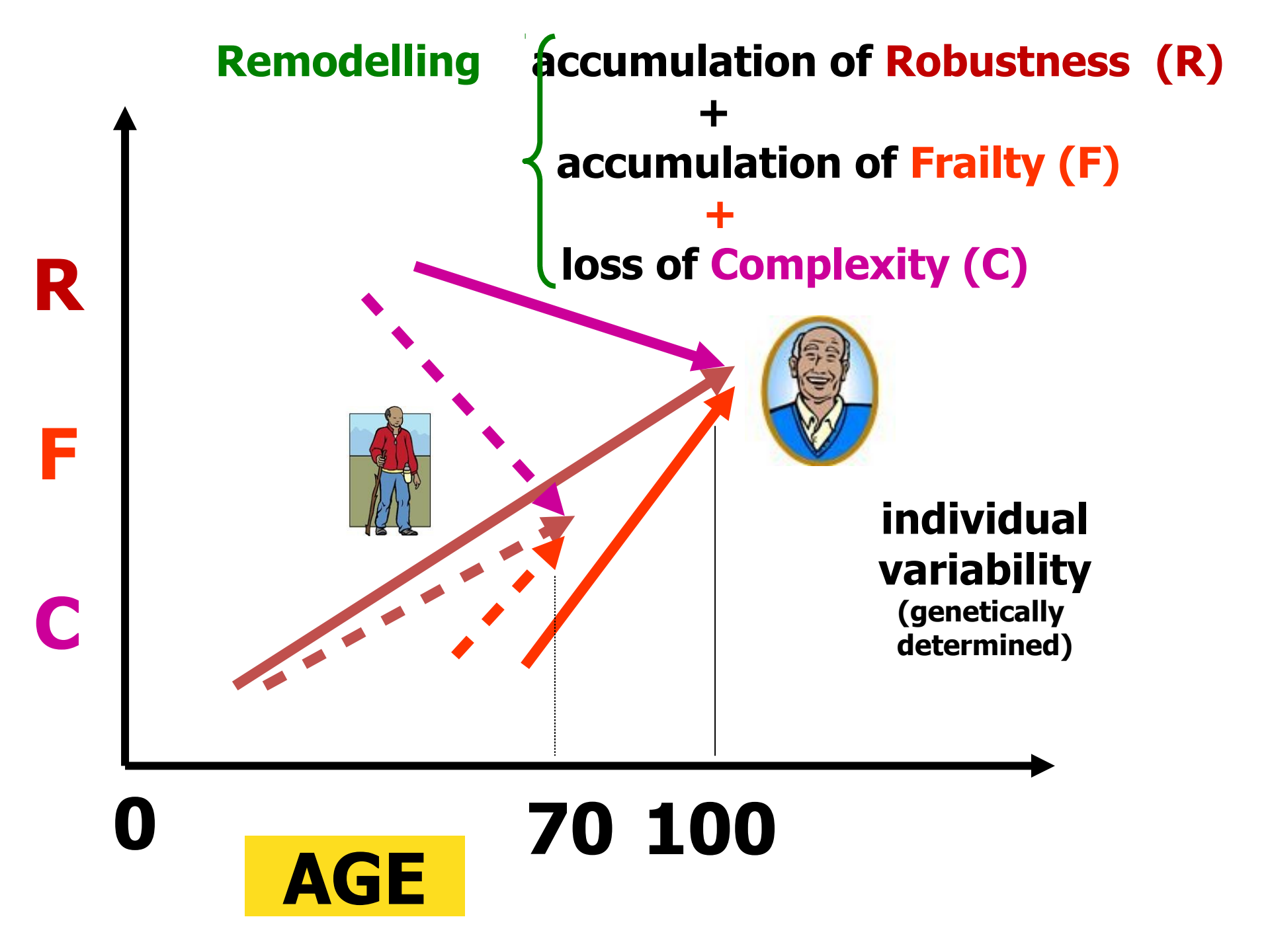
Gompertz curve
semilog plot



100+ were likely robust since the beginning but they became even more robust with aging

A new phase of life ?

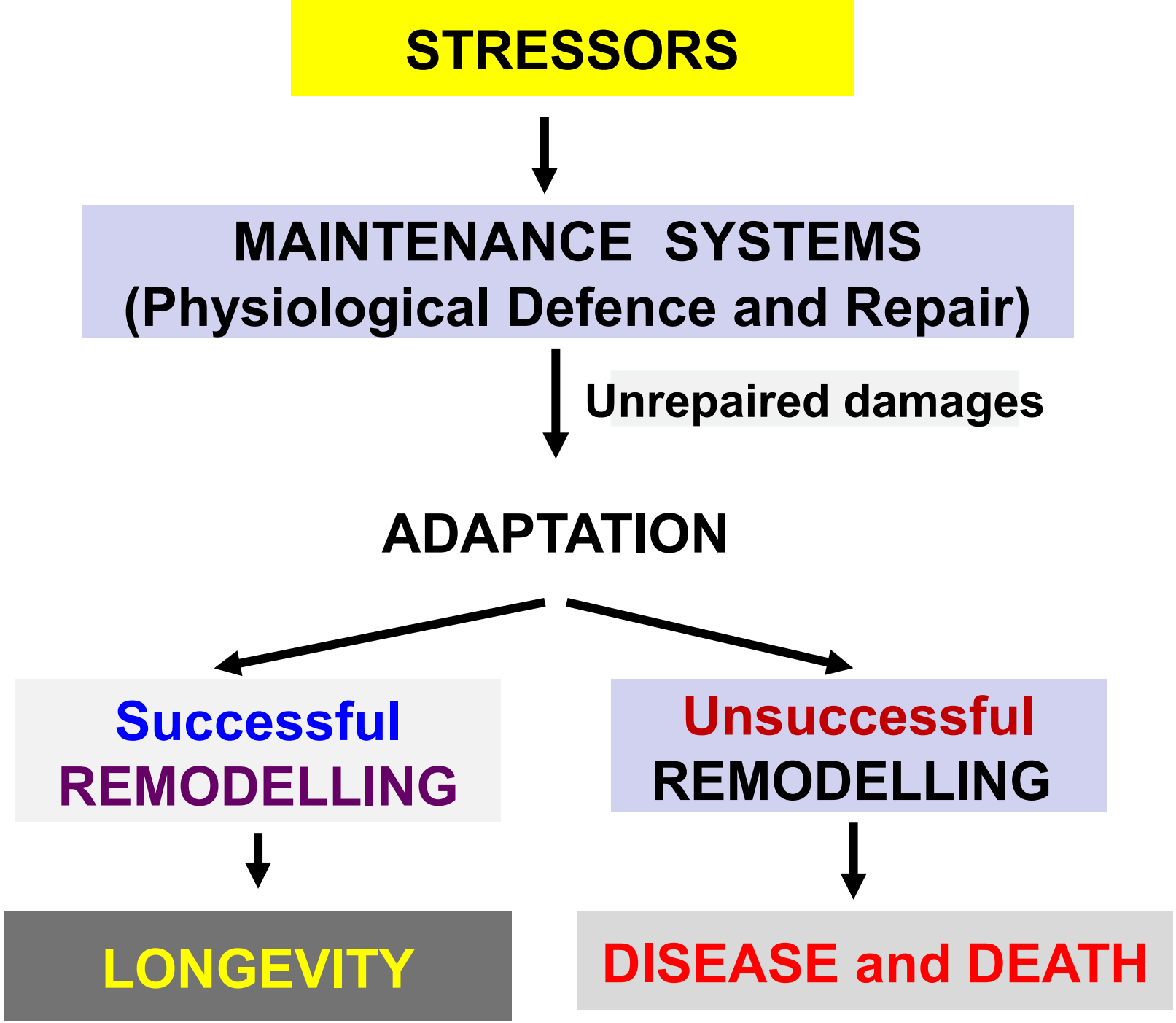
Inspired by Gavrilov and Gavrilova


Remodelling
accumulation of Robustness (R)
+
accumulation of Frailty (F)
+
loss of Complexity (C)
R
F
C
individual variability
(genetically determined)
0
70
100
AGE

“the remodelling theory of aging” (*Franceschi 1995, 2000*)

The ageing phenotype is the result of two major process:

- **the accumulation of unrepaired damages at all levels of the biological organization (molecules, cells, tissues, organs)**
- **the capability of the body to react/adapt/neutralize such damages by activating a variety of physiological molecular and cellular repair and defence responses/pathways**
- **a systemic approach is needed to address such a complexit**


STRESSORS
MAINTENANCE SYSTEMS
(Physiological Defence and Repair)
Unrepaired damages
ADAPTATION
Successful
REMODELLING
Unsuccessful
REMODELLING
LONGEVITY
DISEASE and DEATH

The remodelling theory of aging
(*Franceschi 1995, 2000*)

Such beneficial, physiological responses may turn detrimental in the post-reproductive period of life, largely unpredicted by evolution (*antagonistic pleiotropy*)

An example is INFLAMMATION

The Inflammatory Theory of Aging (an example of remodelling)

Fare clic per modificare stili del testo dello schema
Secondo livello
Terzo livello
Quarto livello
Quinto livello

Inflamm-aging

An Evolutionary Perspective on Immunosenescence

CLAUDIO FRANCESCHI,[a,b,e] MASSIMILIANO BONAFÈ,[a] SILVANA VALENSIN,[a] FABIOLA OLIVIERI,[b] MARIA DE LUCA,[d] ENZO OTTAVIANI,[c] AND GIOVANNA DE BENEDICTIS[d]

[a]*Department of Experimental Pathology, University of Bologna, Bologna, Italy*

[b]*Department of Gerontological Research, Italian National Research Center on Aging (INRCA), Ancona, Italy*

[c]*Department of Animal Biology, University of Modena and Reggio Emilia, Modena, Italy*

[d]*Department of Cell Biology, University of Calabria, Calabria, Italy*

Ann. N.Y. Acad. Sci., 908, 244-254, 2000

The Inflammatory Theory of Aging

Inflamm-aging fits the new emerging hypothesis that

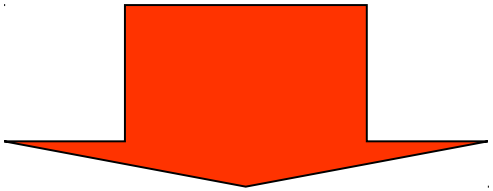
AGING IS QUASI-PROGRAMMED

being a continuation of developmental programs which in the post-reproductive period of life loose their strict control

- **loss of regulatory control mechanisms (?)**
- **lack of a maintenance program (?)**

LIFELONG ANTIGENIC/INFLAMMATORY LOAD

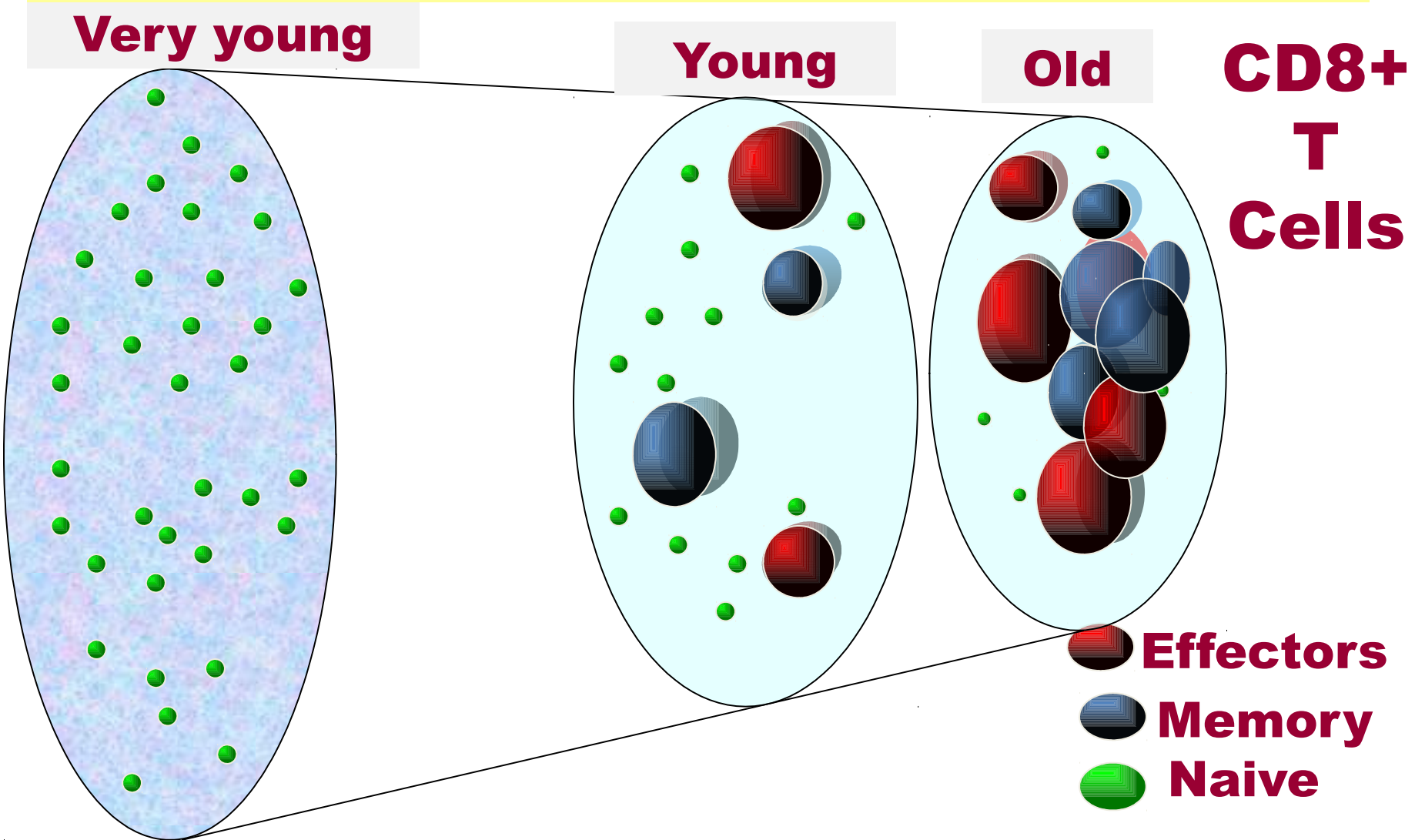
***e.g.* persistent infections (CMV)**



INFLAMM-AGING

IMMUNO-BIOGRAPHY

The filling of the IMMUNOLOGICAL SPACE



The complex role of CMV infection in the elderly

Fare clic per modificare stili del testo dello schema
Secondo livello
Terzo livello
Quarto livello
Quinto livello

Massive Load of Functional Effector CD4$^+$ and CD8$^+$ T Cells against Cytomegalovirus in Very Old Subjects[1]

Rosanna Vescovini,* Claudia Biasini,* Francesca Fagnoni,† Anna Rita Telera,* Luca Zanlari,* Mario Pedrazzoni,* Laura Bucci,‡ Daniela Monti,‡ Maria Cristina Medici,§ Carlo Chezzi,§ Claudio Franceschi,¶ and Paolo Sansoni[2]*

The Journal of Immunology, 2007, 179: 4283–4291.

The complex role of CMV infection in the elderly

Intense Antiextracellular Adaptive Immune Response to Human Cytomegalovirus in Very Old Subjects with Impaired Health and Cognitive and Functional Status

Rosanna Vescovini,* Claudia Biasini,* Anna Rita Telera,* Manuela Basaglia,* Adriano Stella,* Francesca Magalini,* Laura Bucci,[†,‡] Daniela Monti,[§] Tiziana Lazzarotto,[¶] Paola Dal Monte,[¶] Mario Pedrazzoni,* Maria Cristina Medici,[‖] Carlo Chezzi,[‖] Claudio Franceschi,[†,‡] Francesco F. Fagnoni,[#] and Paolo Sansoni*

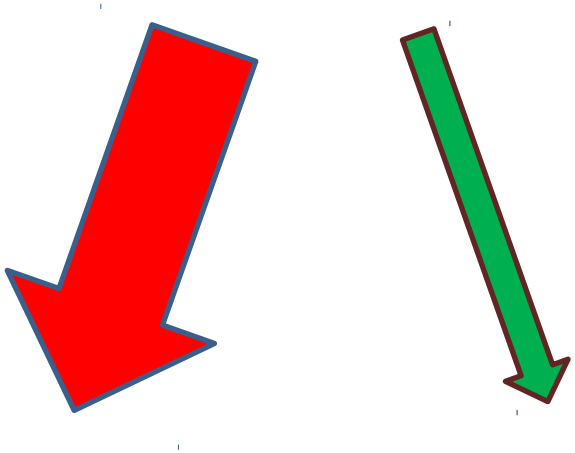
J Immunol 2010;184;3242-3249

Prevalence of CMV positivity among Centenarians, Centenarians'offspring and Offspring of non long-lived parents

	IgG anti-CMV negative	IgG anti-CMV positive
Centenarians, n (%)	7 (5.3)	125 (94.7)
Centenarians'offspring n (%)	22 (9.0)	223 (91.0)
Offspring of non long-lived subjects, n (%)	6 (5.9)	95 (94.1)

CMV infection in 100+ families

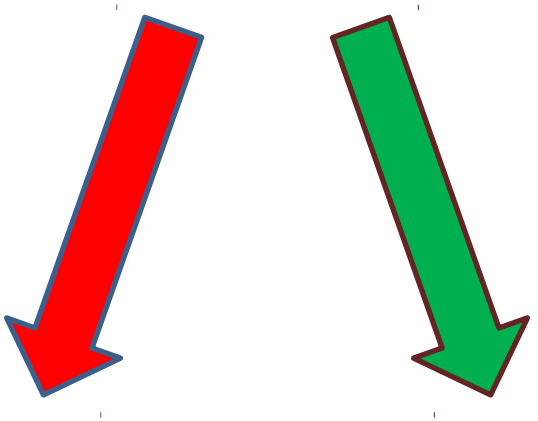
CMV+ Centenarians, n=97



CMV+ offspring
N= 91 (93.8%)

CMV- offspring
N= 6 (6.2%)

CMV - Centenarians, n=7



CMV+ offspring
N= 4 (57.1%)

CMV- offspring
N= 3 (42.9%)

Centenarians, n=132

CMV seropositivity does not influence the inflammatory status

	Seronegative for CMV Antibodies	Seropositive for CMV IgG Antibodies		
		Low Antibody Groups	High Antibody Group	P-value
CENTENARIANS, n (%)	7 (5.30)	89 (67.43)	36 (27.27)	
Male/Female, n	3/4	17/72	8/28	
IL-6 (pg/ml)	39.80 (141.80)	32.45 (372.50)	31.50 (28.90)	ns
IL-10 (pg/ml)	1.70 (5.70)	2.55 (3.63)	1.70 (2.0)	ns
TNF alpha (pg/ml)	9.30 (29.20)	11.0 (44.80)	9.10 (12.80)	ns
Leptin (ng/ml)	10.92 (23.84)	7.98 (22.43)	12.29 (25.54)	ns
Resistin (ng/ml)	17.85 (8.12)	18.07 (11.29)	19.90 (11.73)	ns
Fibrinogen (mg/ml)	7.61 (4.02)	4.44 (2.43)	4.22 (3.16)	ns
PAI-1 act (ng/ml)	2.70 (5.1)	1.50 (2.2)	2.80 (3.1)***	ns
VWF Ag (%)	256 (221)	255 (104)	256 (106)	ns
Adamts-13 Ag (%)	38.80 (14.8)	45.50 (9.1)	42.40 (10.8)	ns
Adiponectin (ug/ml)	45.39 (24.54)	57.67 (51.83)	44.02 (38.85)***	ns
ASAA (ug/ml)	174.73 (1305.92)	211.43 (502.83)	320.25 (770.92)	ns
TGF-beta1 (ng/ml)	3 (4.30)	3.82 (3.21)	3.18 (2.70)	ns

P value was derived by Kruskal Wallis test; differences between groups were analysed by Man-Whitney U test;
* p<0.05 between negative and low CMV-IgG;
** p<0.05 between negative and high CMV-IgG;
*** p<0.05 between low and high CMV-IgG.

CMV+ and CMV- Centenarian offspring

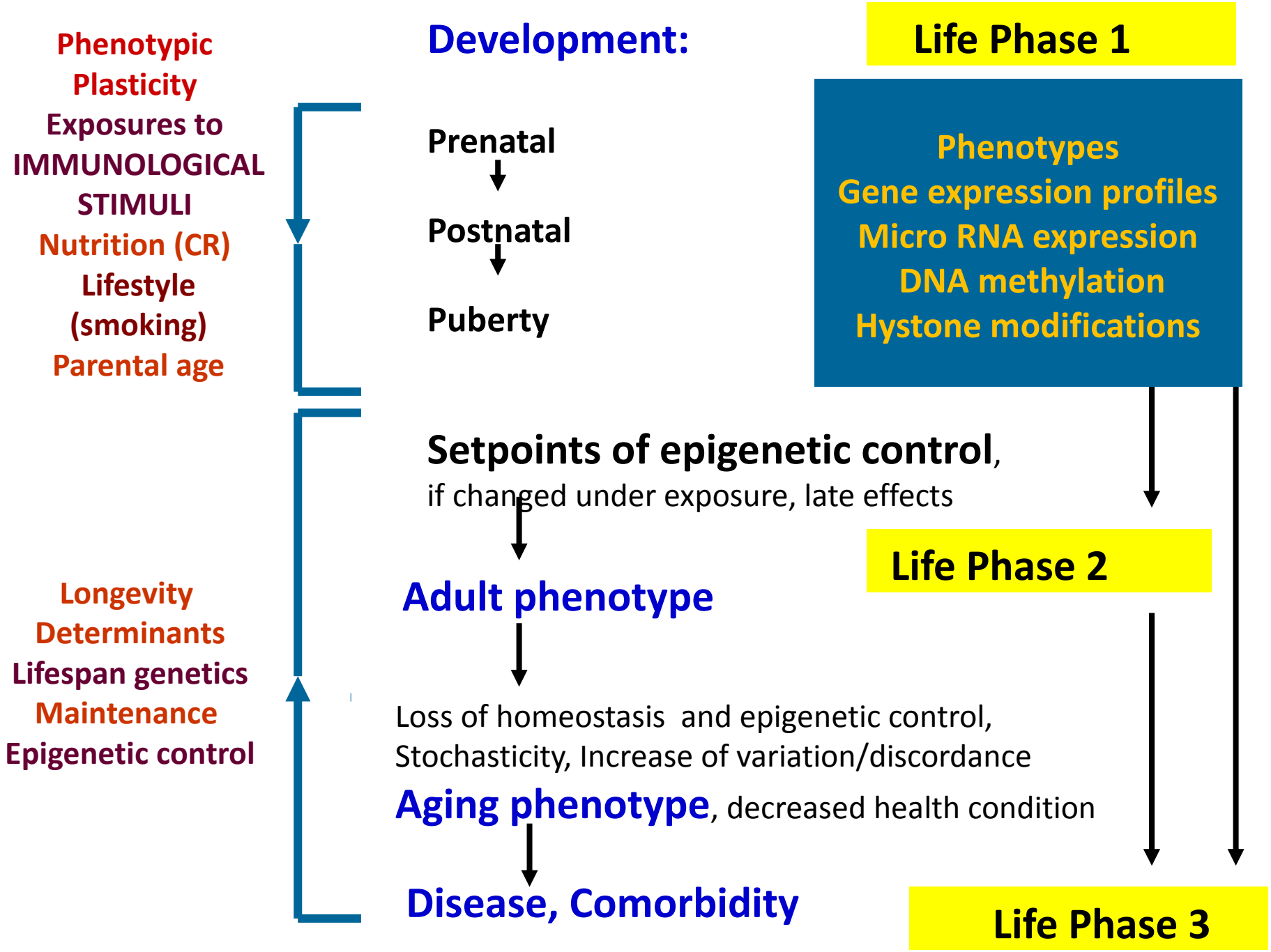
Inflammatory mediators, hemato-biochemical paramenters and the prevalence of past and current diseases were not different between CMV+ and CMV- Centenarian offspring

IMMUNO-BIOGRAPHY

and the hypothesis of

" the fetal origins of adult diseases"

the EU Large Project "IDEAL": particular emphasis on epigenetic changes (long term effects of early events)


Phenotypic Plasticity
Exposures to IMMUNOLOGICAL STIMULI
Nutrition (CR)
Lifestyle (smoking)
Parental age
Development:
Prenatal
Postnatal
Puberty
Life Phase 1
Phenotypes
Gene expression profiles
Micro RNA expression
DNA methylation
Hystone modifications
Setpoints of epigenetic control,
if changed under exposure, late effects
Life Phase 2
Adult phenotype
Longevity Determinants
Lifespan genetics
Maintenance
Epigenetic control
Loss of homeostasis and epigenetic control,
Stochasticity, Increase of variation/discordance
Aging phenotype, decreased health condition
Disease, Comorbidity
Life Phase 3

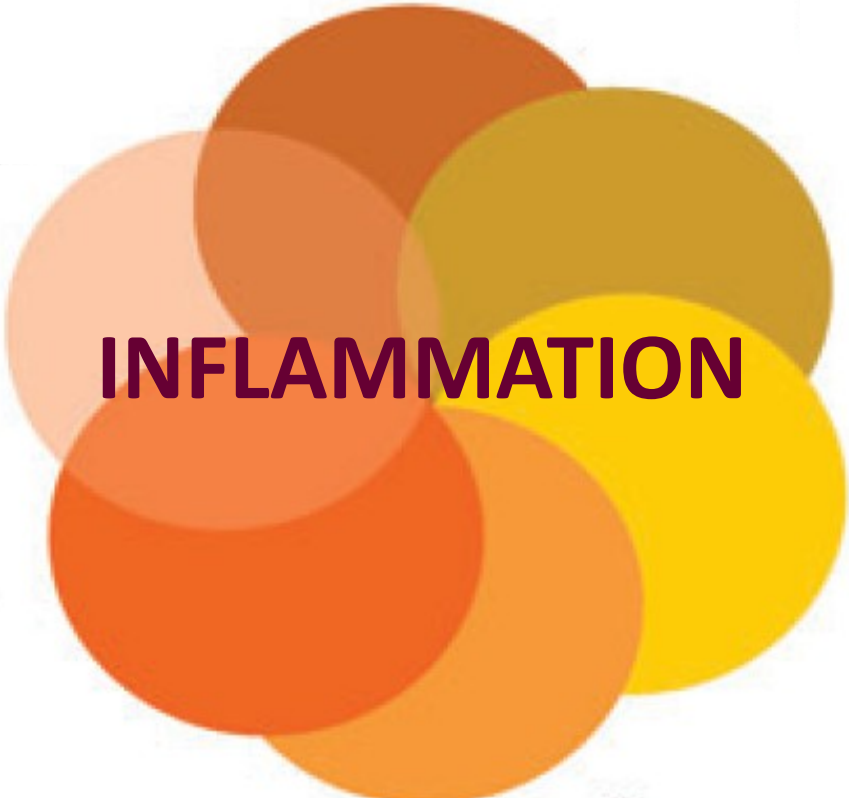
the fetal origins of adult diseases

long term "Inflammatory Memory"

The Inflammatory Theory of Aging

Obesity
Metabolic syndrome
Type 2 Diabetes

Cancer



Cardiovascular diseases

Alzheimer dementia

Sarcopenia
Frailty

Depression

Inspired by Pedersen, 2010

Prospects & Overviews

Review essays

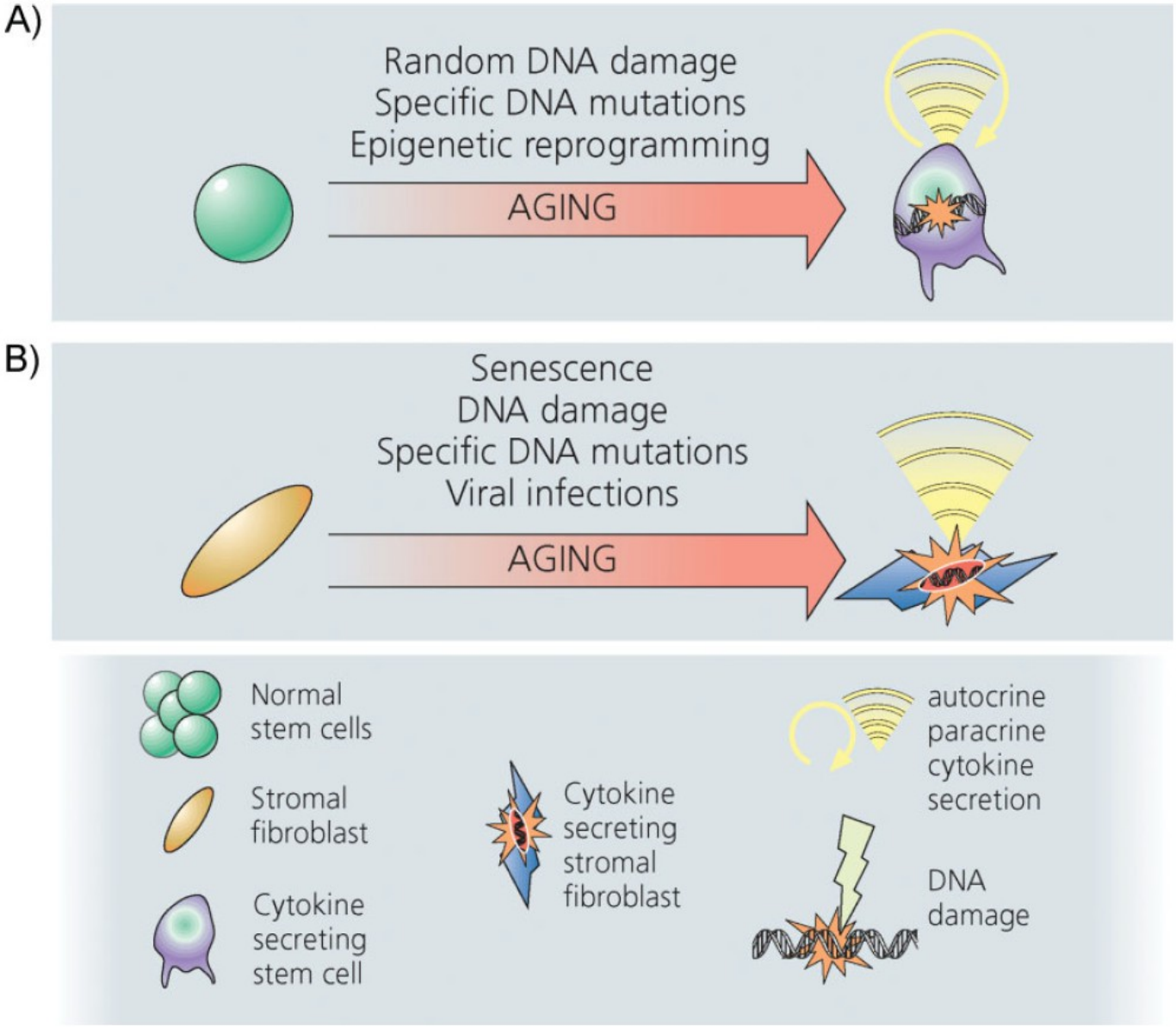
Inflamm-aging of the stem cell niche: Breast cancer as a paradigmatic example

Breakdown of the multi-shell cytokine network fuels cancer in aged people

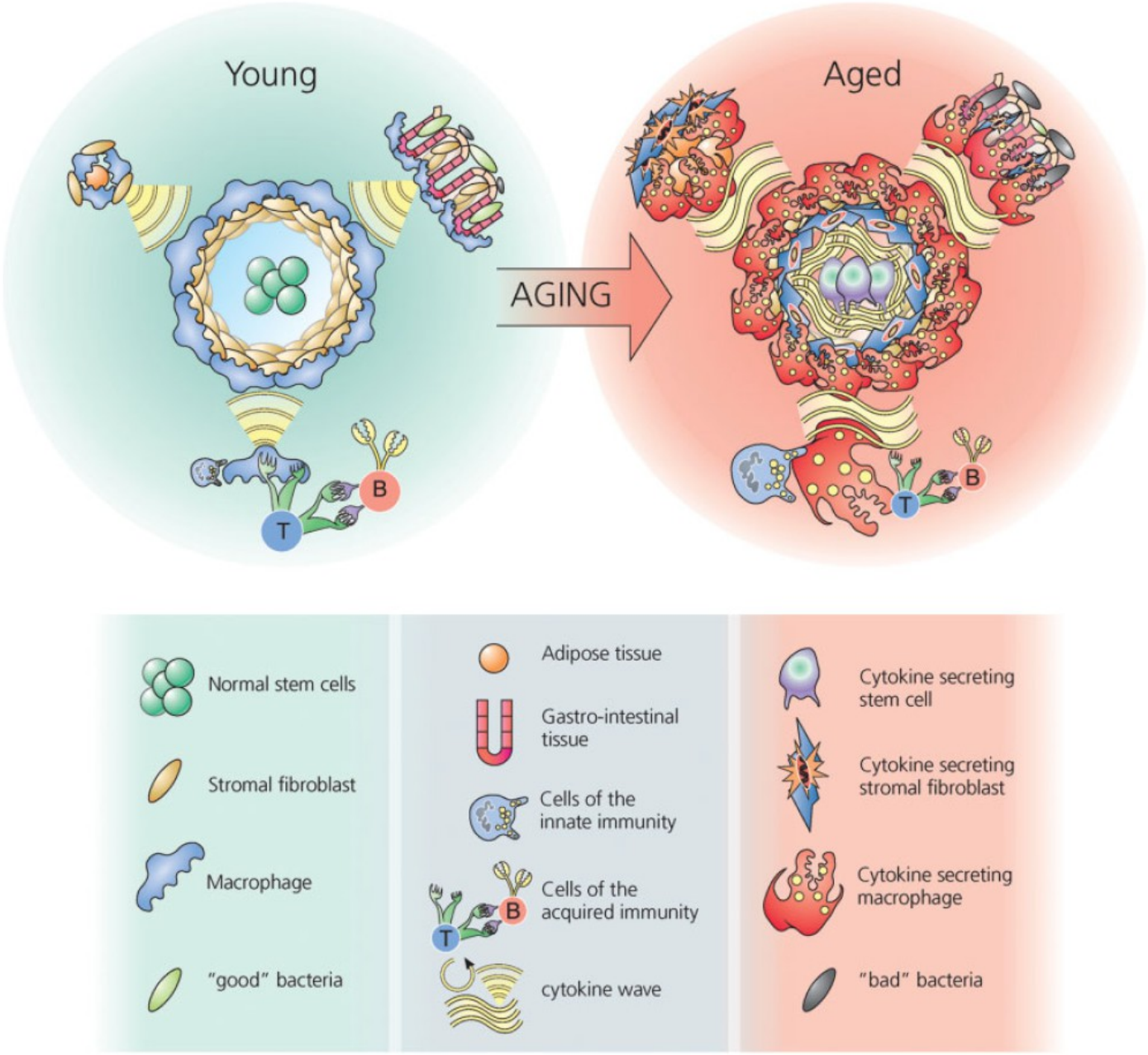
Massimiliano Bonafè[1)2)], Gianluca Storci[1)2)3)] and Claudio Franceschi[1)]*

Bioessays 34: 40–49,© 2011

Aging-driven Inflammation of stem and stromal cells



The multishell cytokine network

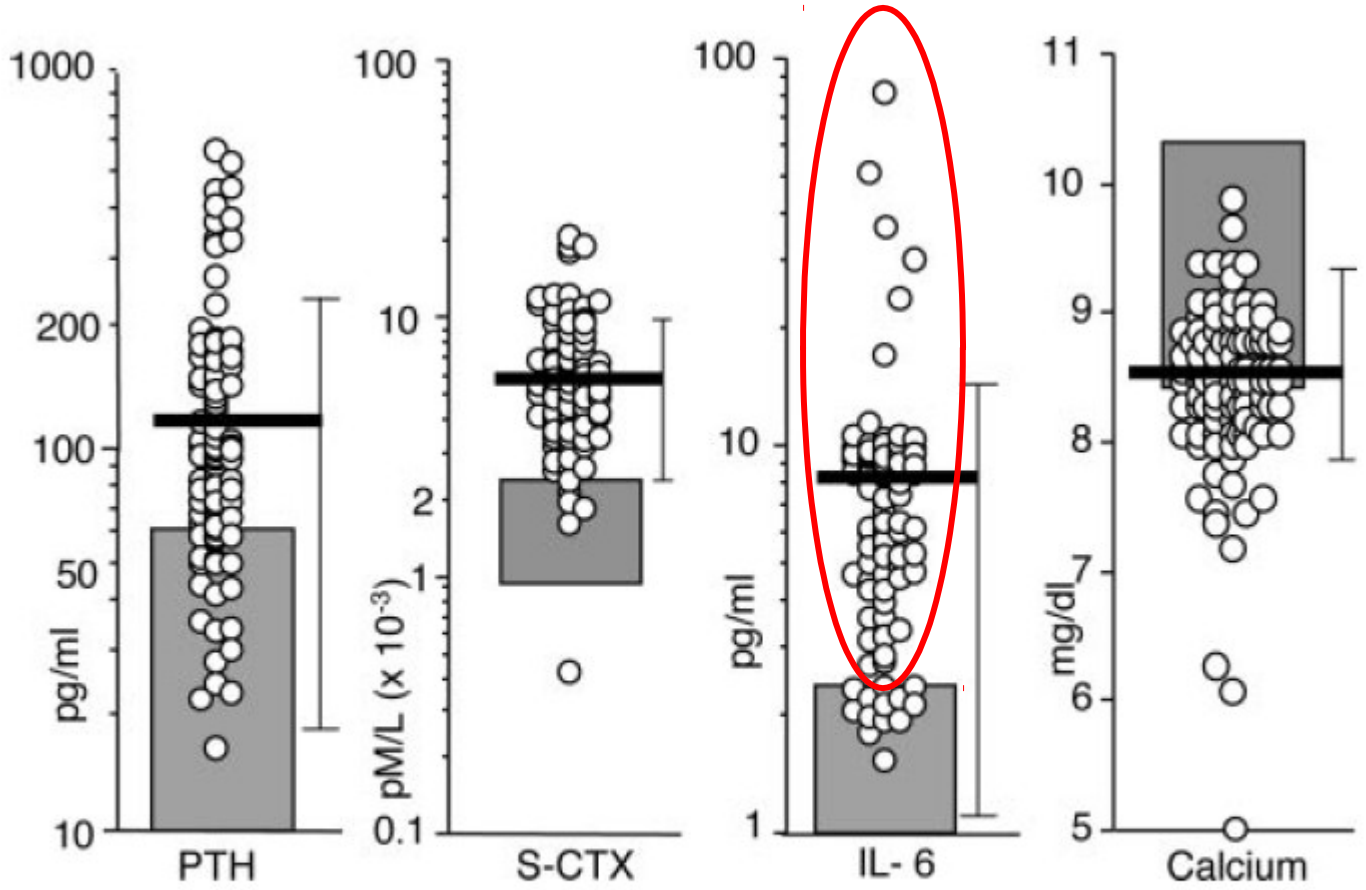


DNA Damage Response
Inflamm-aging and Cancer

1. The role of **inflamm-aging** is particularly important in **cancers of the elderly**
2. **Local** and **Systemic** Inflamm-aging is **a promising target** to stop (prevent) cancer growth and metastasis

CENTENARIANS ARE INFLAMMED

inflammatory markers (*e.g.* IL-6) are elevated in centenarians



Passeri et al. *J Clin Endocrinol Metab* 2003, 88:5109-15

are expressed in log scale.

Centenarians have (slightly) hypofuntioning thyroid

STEFANO MARIOTTI, CLAUDIO FRANCESCHI
ANDREA COSSARIZZA , ALDO PINCHERA

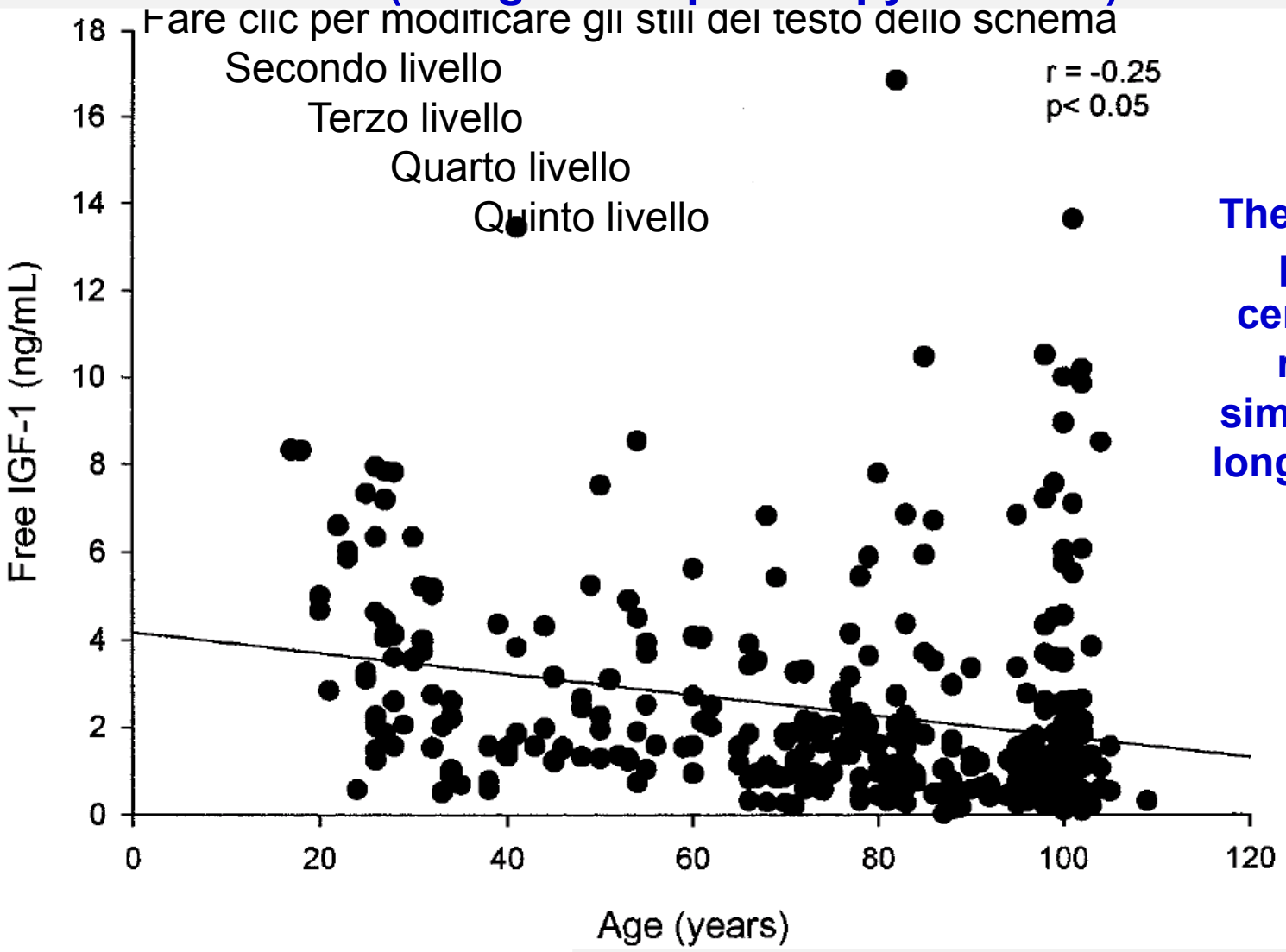
The Aging Thyroid

Endocrine Reviews 1995 16: 686-715

Very recent data from New York, Leiden and Calabria have confirmed these findings

IGF-1 plasma levels are low in centenarians protecting from cancer but favoring sarcopenia

(antagonistic pleiotropy trade off)



The IGF-1/insulin pathway of centenarians is remarkably similar to that of long living animal models

Bonafè et al., J Clin Endocrinol Metab 2003

Serum IGF-1 bioactivity is low
in 100+ and their offspring

	Controls (n=80)	Centenarians' Offspring (n=192)	Centenarians (n=106)
Total IGF-I (nmol/L)	17 (13.6-20.8)	14.4 (11.9-18.2)	9.3 (7.1-12.9)
IGF-I Bioactivity (pmol/L)	161 (134-187)	144 (119-170)	132 (107-157)
Total IGF-II (nmol/L)	114 (89-137)	134 (92-168)	72 (55-117)
Glucose (mmol/L)	4.9 (4.5-5.4)	4.8 (4.3-5.4)	4.6 (4.2-5.1)
Insulin (pmol/L)	74 (51-105)	71 (44-103)	39 (27-70)
HOMA2-B%	128 (98-166)	137 (100-174)	109 (81-152)
HOMA2-S%	62 (44-93)	68 (45-110)	122 (68-174)

IGF-I bioactivity was evaluated **through an innovative IGF-I kinase receptor activation (KIRA) assay** that S. Lamberts, L. Hofland and J. Janssen, University of Rotterdam, recently developed. This assay quantify **phosphorylation of tyrosine residues of the activated IGF-I receptor** after stimulation with human serum in vitro. Unlike IGF-I immunoassays, the IGF-I KIRA assay **takes into account modifying effects of IGFBPs and proteases** on the interaction between bioactive ligands and the IGF-I receptor, and it is possible to measure the overall IGF-I receptor activation in blood.

Vitale et al., AGING 2012

Inflammaging and anti-inflammaging: A systemic perspective on aging and longevity emerged from studies in humans

Claudio Franceschi [a,b,c,e,*], Miriam Capri [a], Daniela Monti [d], Sergio Giunta [e], Fabiola Olivieri [e], Federica Sevini [b], Maria Panagiota Panourgia [b], Laura Invidia [a], Laura Celani [b], Maria Scurti [b], Elisa Cevenini [b], Gastone C. Castellani [b,f], Stefano Salvioli [a,b,c]

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[b] *Centro Interdipartimentale "L. Galvani", University of Bologna, via S. Giacomo 12, 40126 Bologna, Italy*
[c] *ER-GenTech laboratory, via Saragat 1, 44100 Ferrara, Italy*
[d] *Department of Experimental Pathology and Oncology, University of Florence, Viale Morgagni 50, 50134 Florence, Italy*
[e] *I.N.R.C.A., Department of Gerontological Sciences, via Birarelli 8, 60121 Ancona, Italy*
[f] *DIMORFIPA, University of Bologna, Via Tolara di Sopra 50, 40064 Ozzano dell'Emilia, Italy*

Mechanisms of Ageing and Development 128 (2007) 92–105

High levels of circulating anti-inflammatory molecules in 100+:

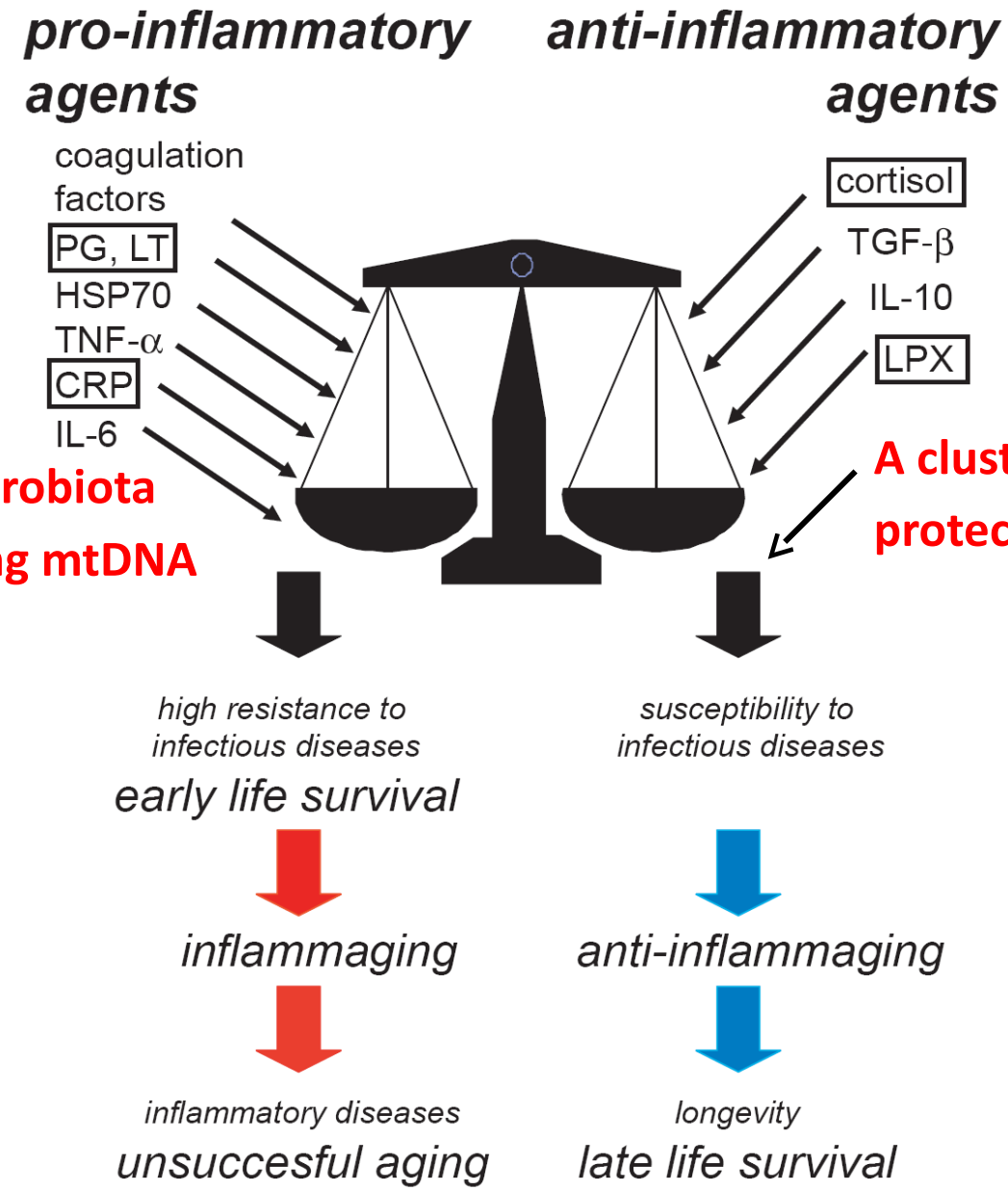
TGFβ

Cortisol

IL-1RA

Adiponectin

LONGEVITY
and the balance between Inflamm-aging & anti-Inflamm-aging



Franceschi et al., MAD 2007

Pro and anti-inflammatory mediators in centenarians, their offspring and offspring of non long-lived parents

	Centenarians, n=156	Centenarians Offspring, n=267	Offspring of non long-lived parents, n=107	p-value
	Median (25th-75th)	Median (25th-75th)	Median (25th-75th)	
IL-6, (pg/ml)	31.5 (18.1-57.5)	16.9 (10.1-35.1)	17.5 (10.7-29.8)	<.0001
IL-10, (pg/ml)	2.4 (1.2-4.6)	2.5 (1.1-5.4)	2.5 (1.2-4.5)	ns
TNF-α, (pg/ml)	9.5 (2.4-37.7)	10.4 (2.8-31.9)	10.5 (3.8-25.0)	ns
TGF-β1, (ng/ml)	3.6 (2.6-6.2)	3.9 (2.6-7.1)	4.0 (2.8-6.9)	ns
Leptin, (ng/ml)	11.2 (4.4-29.8)	14.1 (7.1-30.7)	14.0 (6.4-27.0)	ns
Adiponectin, (μg/ml)	52.0 (27.4-75.7)	33.8 (18.9-56.7)	30.6 (17.2-56.0)	<.0001
Resistin, (ng/ml)	18.2 (12.6-23.8)	9.3 (7.0-12.2)	9.2 (7.1-12.2)	<.0001
hs-CRP, (mg/l)	3.6 (1.3-8.0)	1.4 (0.8-3.1)	1.6 (0.8-3.0)	ns
Serum amiloid – A, (μg/ml)	219.5 (116.2-687.2)	90.3 (44.0-181.5)	82.4 (47.2-164.6)	<.0001
Fibrinogen, (mg/ml)	4.6 (3.1-5.6)	3.2 (2.2-4.9)	3.1 (2.2-4.3)	<.0001
Albumin	3.7 (3.1-4.2)	4.2 (3.3-4.5)	4.2 (3.4-4.4)	ns
IGF-1, (ng/ml)	71.9 (54.6-96.7)	108.9 (91.0-139.6)	138.2 (111.6-160.4)	<.0001
IgG anti-CMV, (IU/ml)	12.3 (7.2-22.0)	6.7 (3.5-11.7)	5.7 (3.3-9.5)	<.0001

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PLOS ONE

Metabolic Signatures of Extreme Longevity in Northern Italian Centenarians Reveal a Complex Remodeling of Lipids, Amino Acids, and Gut Microbiota Metabolism

Sebastiano Collino[1]*, Ivan Montoliu[2], François-Pierre J. Martin[1], Max Scherer[1], Daniela Mari[3,4], Stefano Salvioli[5,6], Laura Bucci[5,6], Rita Ostan[5,6], Daniela Monti[7], Elena Biagi[8], Patrizia Brigidi[8], Claudio Franceschi[5,6], Serge Rezzi[1]

1 Proteomics and Metabonomics, Nestlé Institute of Health Sciences SA, Campus EPFL, Quartier de l'innovation, Lausanne, Switzerland, **2** Applied Mathematics, NESTEC SA, Nestlé Research Center, Lausanne, Switzerland, **3** Department of Medical Sciences, University of Milan, Milan, Italy, **4** Geriatric Unit Ca' Grande Foundation Maggiore Policlinico Hospital, Milan, Italy, **5** Department of Experimental Pathology, University of Bologna, Bologna, Italy, **6** Interdepartmental Centre L. Galvani, University of Bologna, Bologna, Italy, **7** Department of Experimental Pathology and Oncology, University of Florence, Florence, Italy, **8** Department of Pharmaceutical Sciences, University of Bologna, Bologna, Italy

PLOS ONE | www.plosone. March 2013 | Volume 8 | Issue 3 | e56564

Table 1. Demographic characteristics of the recruited age cohorts.

Metabonomics	Centenerians	Elderly-Offspring of centenarians	Elderly-Offspring of non long-lived parents	Young
Serum-Untargeted LC-MS/MS: Gender, *male/female*, Age, years	30/113, 100.9±2 (99–111)	14/32, 68.4±6 (56–81)	19/25, 70.7±6 (59–86)	11/10, 30.±5 (24–40)
Serum-Quantitative LC-MS/MS *Eicosanoids:* Gender, *male/female*, Age, years	2/10, 101±2 (99–104)	11/6, 66.3±6 (59–74)	10/10, 73.1±3 (68–76)	9/9, 31.2±5 (25–40)
Urine-Untargeted ^{1}H-NMR metabonomics: Gender, *male/female*, Age, years	18/74, 100.9±2 (99–111)	91/119, 70.1±6 (55–88)	37/36, 70.3±5 (57–79)	11/10, 30.9±5 (24–40)

Values are presented as mean ±SD with the range in parentheses.
doi:10.1371/journal.pone.0056564.t001

A total of 457 individuals:
N= 143 centenarians
N= 220 offspring of centenarians
N= 73 offspring of non long-lived parents
N= 21 young subjects

Age-Trajectories of Biomarkers

A G E

Metabolomic Biomarkers of Aging and Longevity

- **monotonically increased or decreased with age :**

- **decreased concentrations of Tryptophan (Trp) & lysophospatidylcholines (LPC 18:2, LPC 20:4);**
- **increased levels of PC 32:0 and sphingomyelins (SM 24:1, SM 16:0);**

- **remaining largely unchanged until age 70 and undergoing significant changes in centenarians**

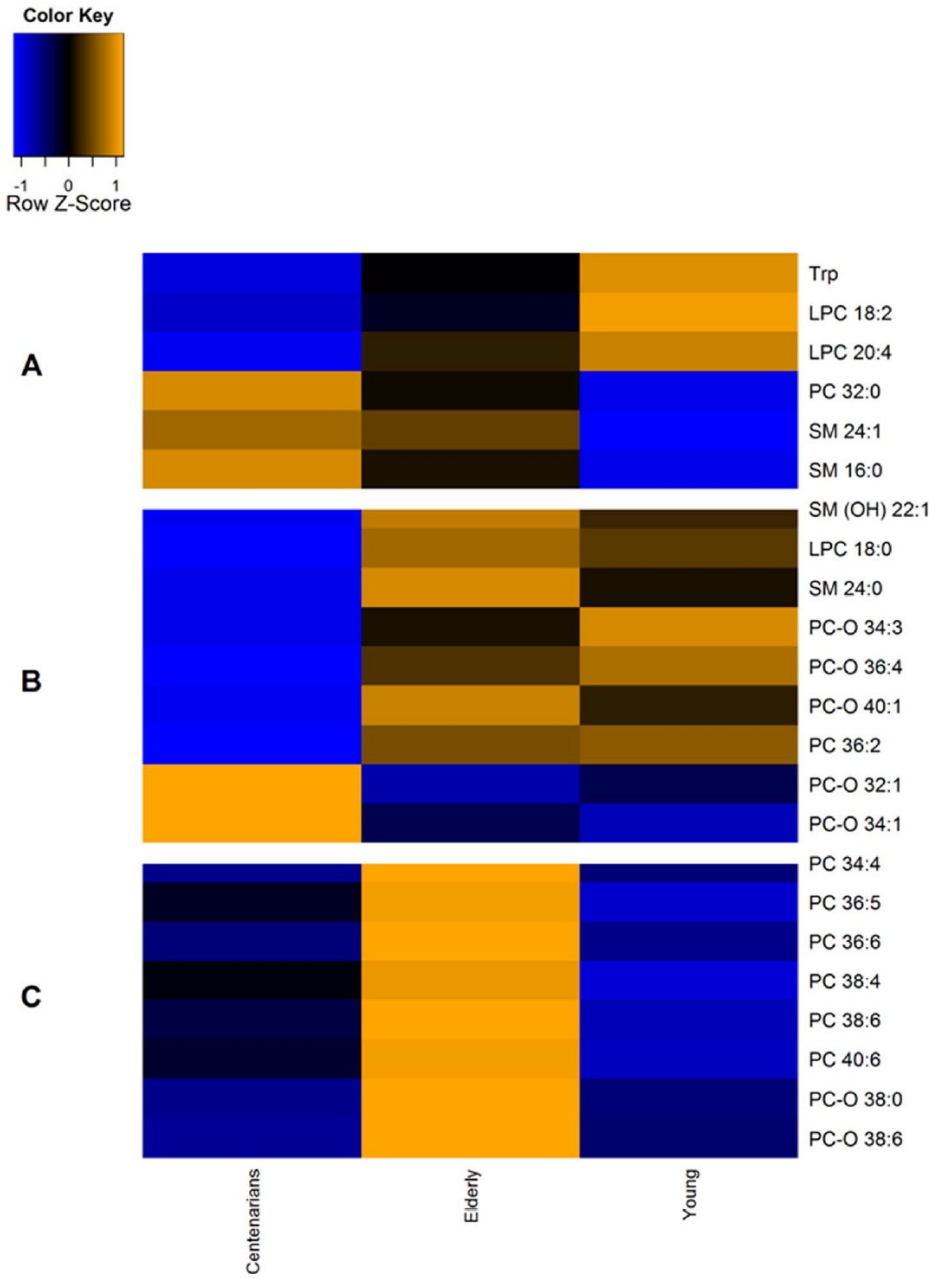
- **decreased concentration in sphingomyelins and specific glycerophospholipids (SM-*OH* 22:1, LPC 18:0, SM 24:0, PC-*O* 34:3, PC-*O* 36:4, PC-*O* 40:1, PC 36:2);**
- **increased concentration in specific glycerophospholipids (PC-*O* 32:1, PC-*O* 34:1)**

- **remarkably similar in young subjects and centenarians**

- **specific glycerophospholipids (PC34:4, PC36:6, PC 36:5, PC 38:4, PC 38:6, PC 40:6, PC-*O* 38:0, PC-*O* 38:6).**

Collino et al., PLoS ONE 2013

Metabolic signature of aging and longevity in serum



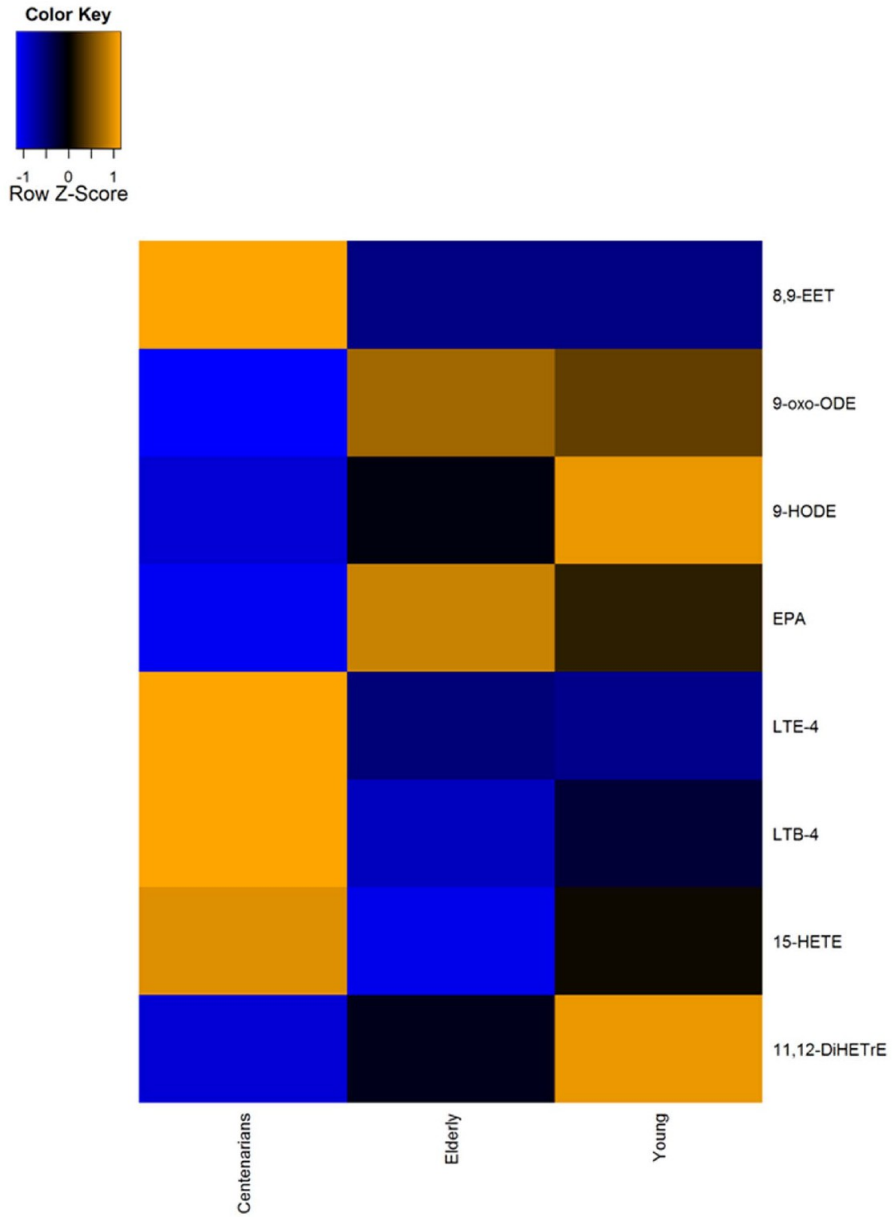
Serum inflammatory biomarkers in centenarians (UPLC-ESI-MS/MS)

Orange : increased vs elderly Blue: decreased vs elderly

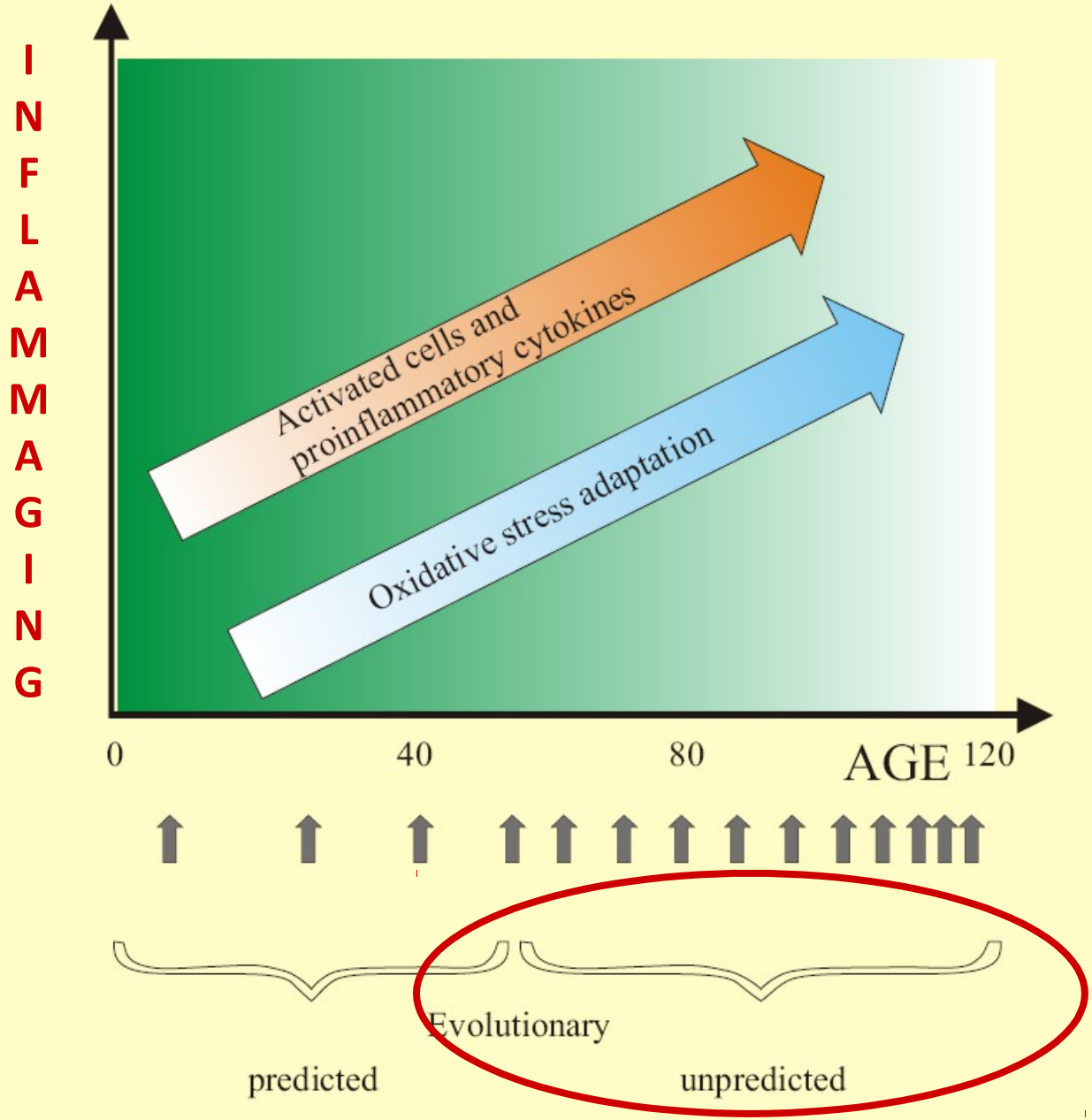
Metabolites [(ng/100 µl serum]	Young	Elderly	Centenarians
LTE4	0.015 ± 0.014	0.013 ± 0.011	0.035 ± 0.031 $^{b(*),c(*)}$
EPA	0.097 ± 0.036	0.123 ± 0.052	0.078 ± 0.026 $^{b(***)}$
15-HpETE	1.512 ± 1.949	1.255 ± 1.245	3.348 ± 2.865 $^{b(*),c(*)}$
11,12-DiHETrE	0.017 ± 0.006	0.017 ± 0.004	0.015 ± 0.006 $^{c(*)}$
9-oxo-ODE	0.042 ± 0.028	0.043 ± 0.039	0.022 ± 0.013 $^{b(***),c(***)}$
9-HODE	0.348 ± 0.223	0.397 ± 0.677	0.204 ± 0.211 $^{c(*)}$
8,9-EpETrE	0.067 ± 0.101	0.074 ± 0.186	0.113 ± 0.107 $^{b(*)}$

Collino et, PLoS ONE 2013

Metabolic signature of aging and longevity in serum as per LC/MS eicosanoids profiling



Inflamm-aging and Oxidative Stress



De Martinis et al., FEBS Lett. 579, 2035-9, 2005

REVIEWS

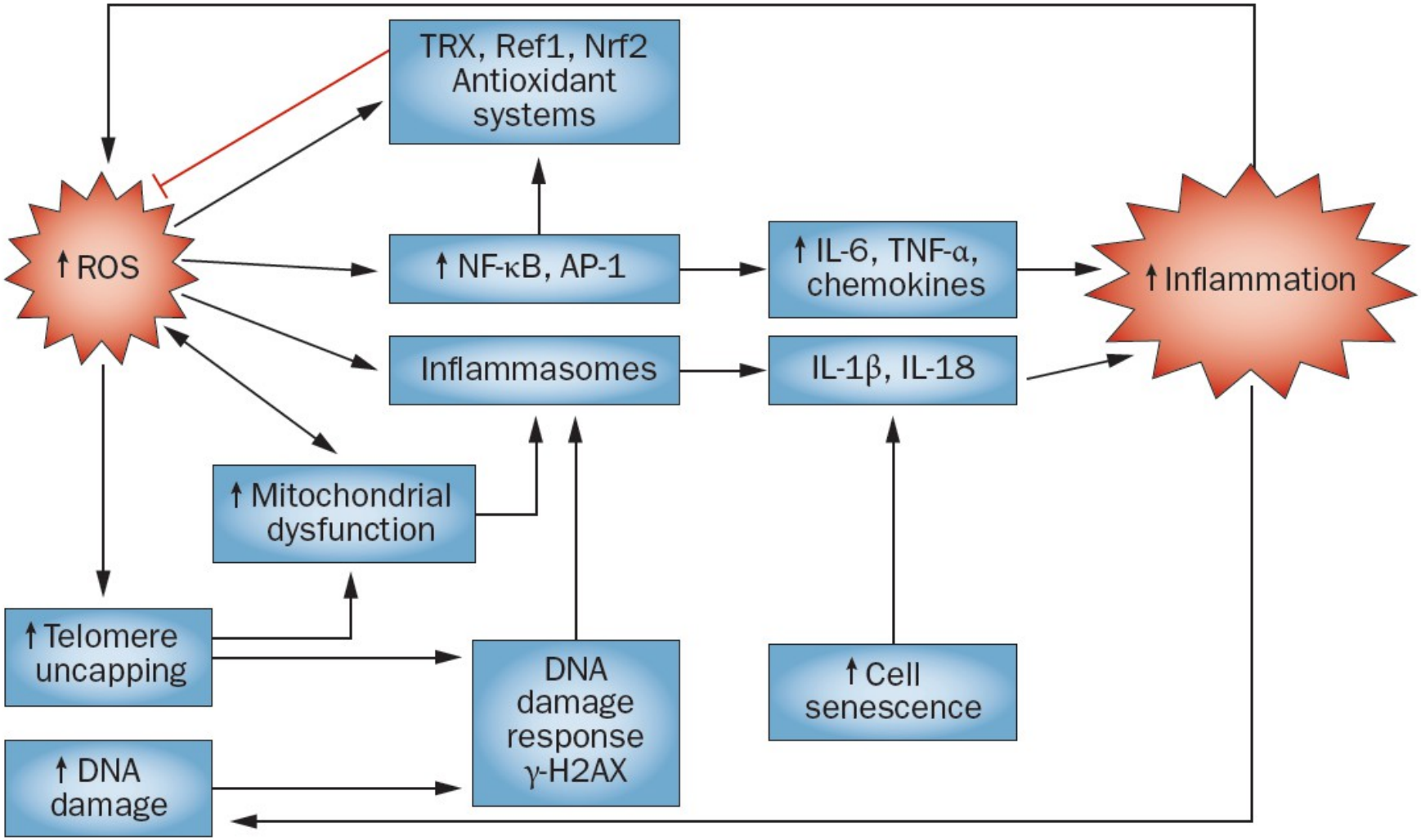
Oxidative stress and the ageing endocrine system

Giovanni Vitale, Stefano Salvioli and Claudio Franceschi

NATURE REVIEWS | ENDOCRINOLOGY

advanced online publication 2013

Oxidative stress & INFLAMM-AGING



Vitale et *al.*, 2013

REVIEW

CURRENT OPINION

Inflamm-ageing

Elisa Cevenini[a,b,*], *Daniela Monti*[c,*], *and Claudio Franceschi*[a,b]

Curr Opin Clin Nutr Metab Care 2012, 15:000–000

DOI:10.1097/MCO.0b013e32835ada13

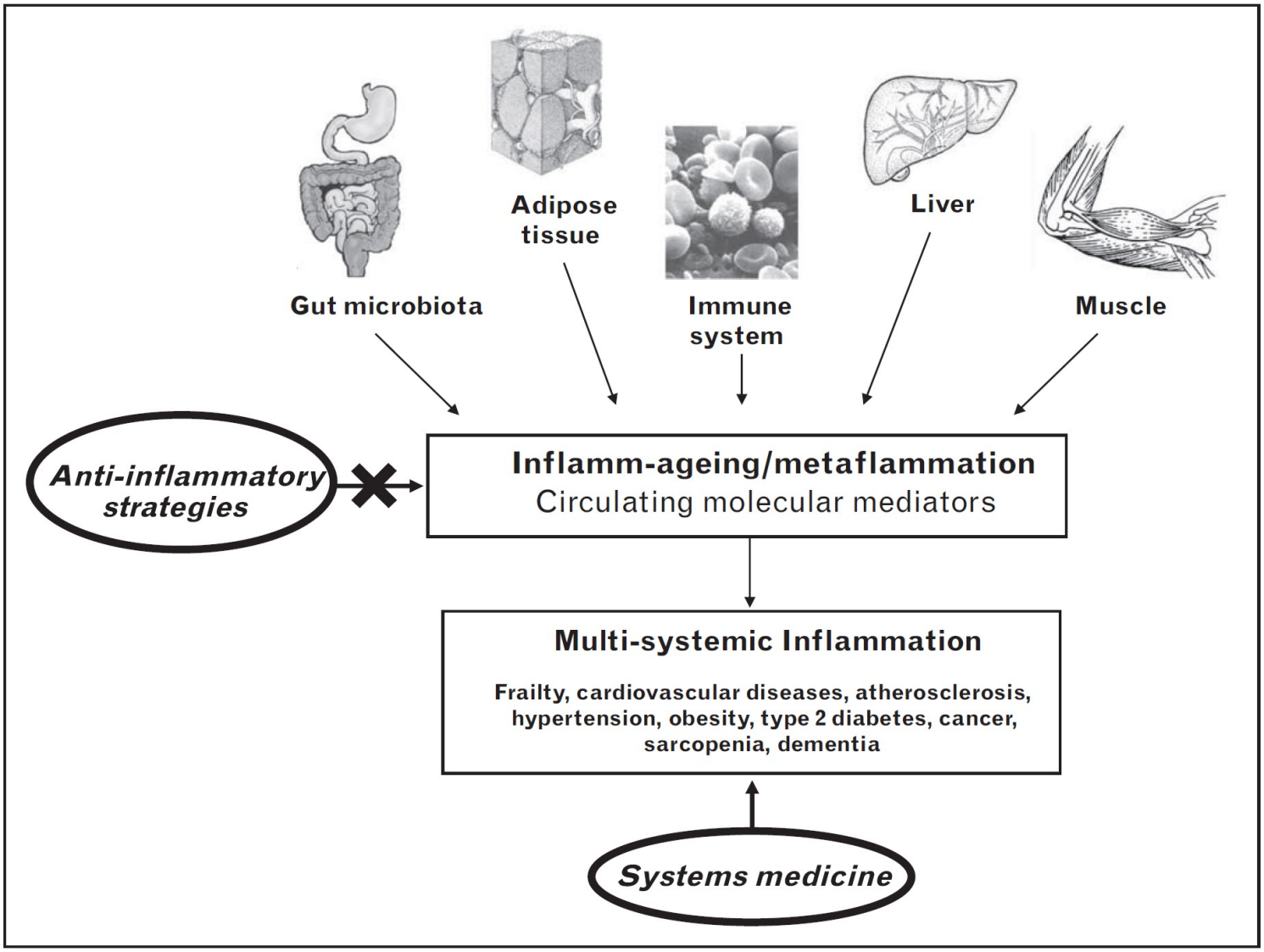
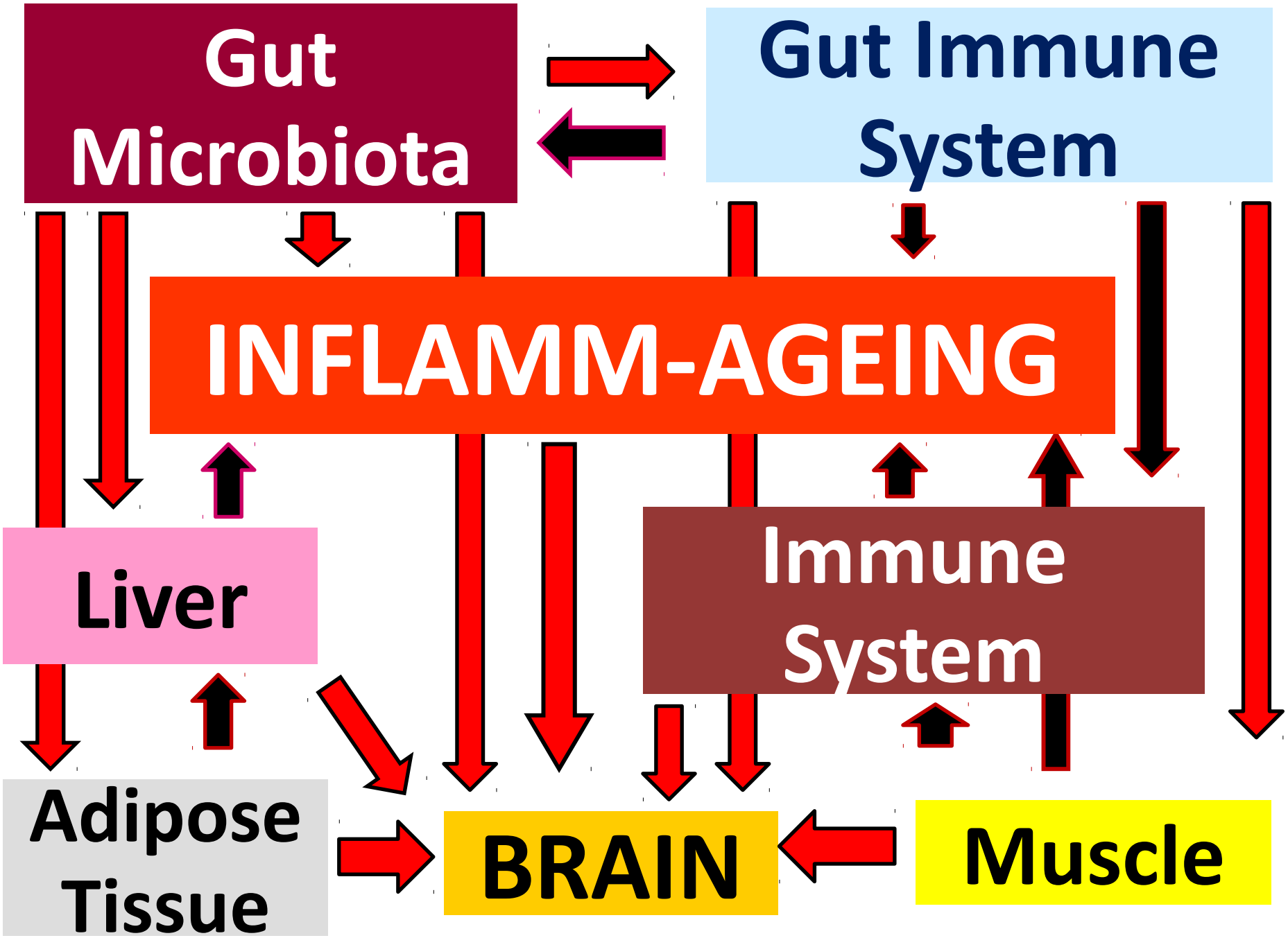


FIGURE 2. Inflamm-ageing and metaflammation. Contribution of some tissues (adipose tissue, muscle), organs (brain, liver), immune system and ecosystem (gut microbiota) to inflamm-ageing and metaflammation onset and progression, thus determining the multisystemic inflammation responsible for the major age-related diseases, that should be faced together in a systems medicine perspective. Anti-inflammatory strategies should be adopted to control both inflamm-ageing and metaflammation.


Gut Microbiota
Gut Immune System
INFLAMM-AGEING
Liver
Immune System
Adipose Tissue
BRAIN
Muscle

Gut Microbiota (faeces) & Metabolomics (serum & urine) in 100+ and their offspring

In collaboration with

Patrizia Brigidi, Marco Candela & Elena Biagi
University of Bologna
Willem de Vos
University of Wageningen
Serge Rezzi & Sebastiano Collino
Nestec, Lausanne

GM bacterial biodiversity

- **10 to 100 trillion**
- **1,800 genera**
- **16,000 phylotypes**
- **their collective genome (microbiome) contains 3.3 million non-redundant microbial genes, ~ 150 times the genes of the human genome**

Each human has a **"metagenome"** (human genome + microbiome) and has to be considered a **"superorganism"**

- ***Firmicutes*** **65%**
- ***Bacteroidetes*** **25%**
- ***Proteobacteria*** **8%**
- ***Actinobacteria*** **5%**
- ***Fusobacteria*** **1%**

Each individual carries about half a million prevalent microbial genes

GM main functions

Protective: against pathogens (barrier effect); competition for available nutrients and space; inhibition of pathogens growth

Trophic: gut epithelial cell survival and proliferation

Cross-talk with the immune system: development and maturation of gut-associated lymphoid system; enhancement of innate immunity and modulation of inflammation through Toll-like-receptor regulated pathways

Metabolic and nutritional: Fermentation of non-digestible dietary residues, salvage of energy as SCFAs and production of vitamins

OPEN ACCESS Freely available online

PLoS one

Through Ageing, and Beyond: Gut Microbiota and Inflammatory Status in Seniors and Centenarians

Elena Biagi[1]*, Lotta Nylund[2,3], Marco Candela[1], Rita Ostan[4], Laura Bucci[4], Elisa Pini[4], Janne Nikkïla[3], Daniela Monti[5], Reetta Satokari[2], Claudio Franceschi[4], Patrizia Brigidi[1], Willem De Vos[3,6]

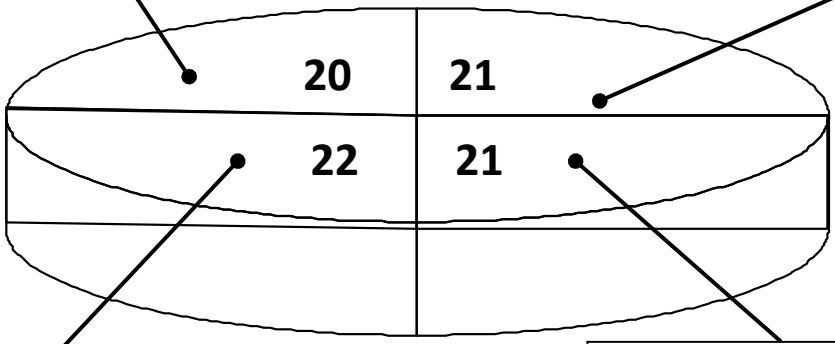
1 Department of Pharmaceutical Sciences, University of Bologna, Bologna, Italy, **2** Functional Foods Forum, University of Turku, Turku, Finland, **3** Division of Microbiology and Epidemiology, Department of Basic Veterinary Medicine, University of Helsinki, Helsinki, Finland, **4** Department of Experimental Pathology and CIG-Interdepartmental Center L. Galvani, University of Bologna, Bologna, Italy, **5** Department of Experimental Pathology and Oncology, University of Florence, Florence, Italy, **6** Laboratory of Microbiology, Wageningen University, Wageningen, The Netherlands

PLoS One 2010

The Gut Microbiota of centenarians

Group Y: Young people
11 men, 9 women
Age 25-40, mean 31

Group C: Centenarians
1 men, 20 women
Age 99-104, mean 100.5

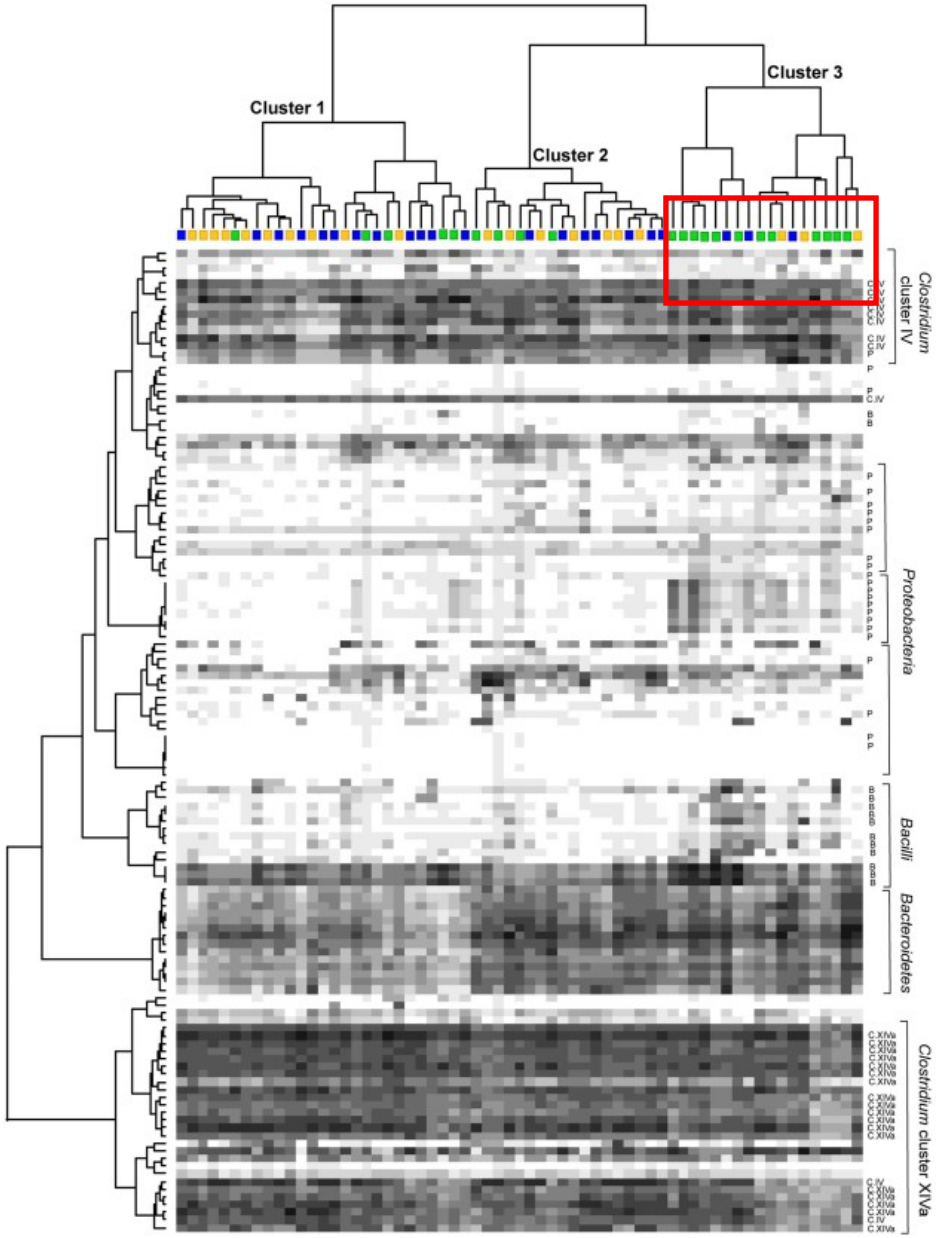


Group K: "control"
11 men, 11 women
Age 63-76, mean 72.7
Unrelated to group C
Mean parental age at death: 59.3

Group F: Offspring of C
11 men, 10 women
Age 59-78, mean 67.5

Biagi et al., 2010

CENTENARIANS HAVE A PECULIAR GM PROFILE



Y

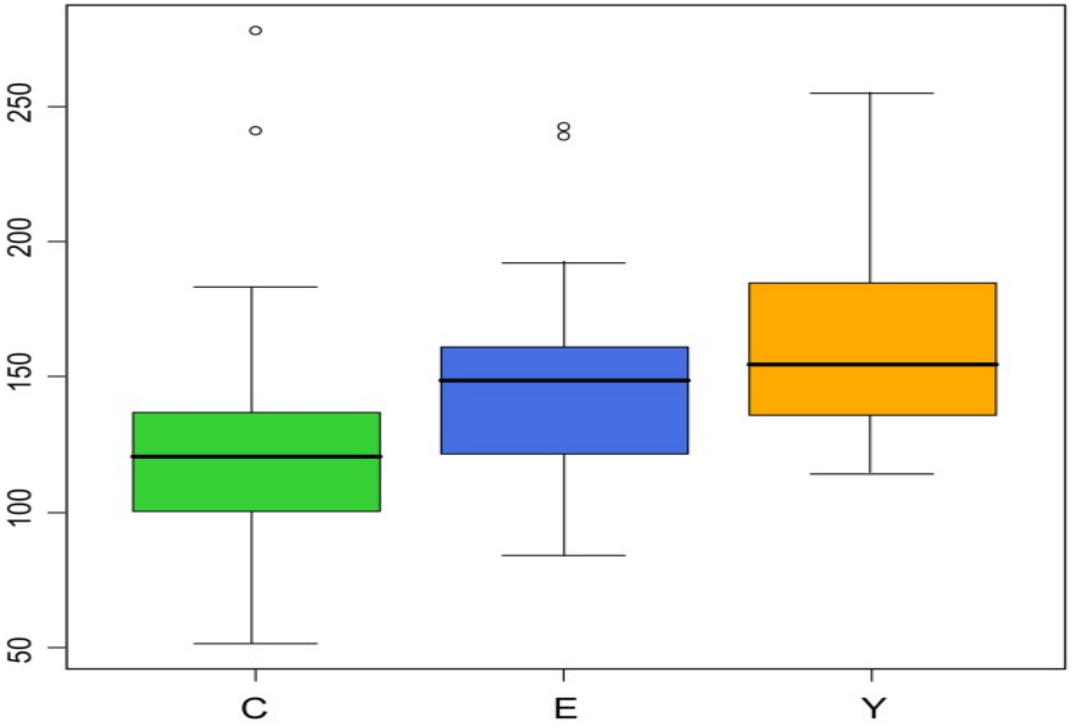
E

C

15 centenarians out of 21

DIVERSITY OF GUT MICROBIOTA: SIMPSON INDEX

The Simpson index is a measure of the diversity of an ecosystem (Simpson, 1949) takes into account both the **number** of taxa present in a sample and their **abundance** in the community; a higher value corresponds to a more diverse community



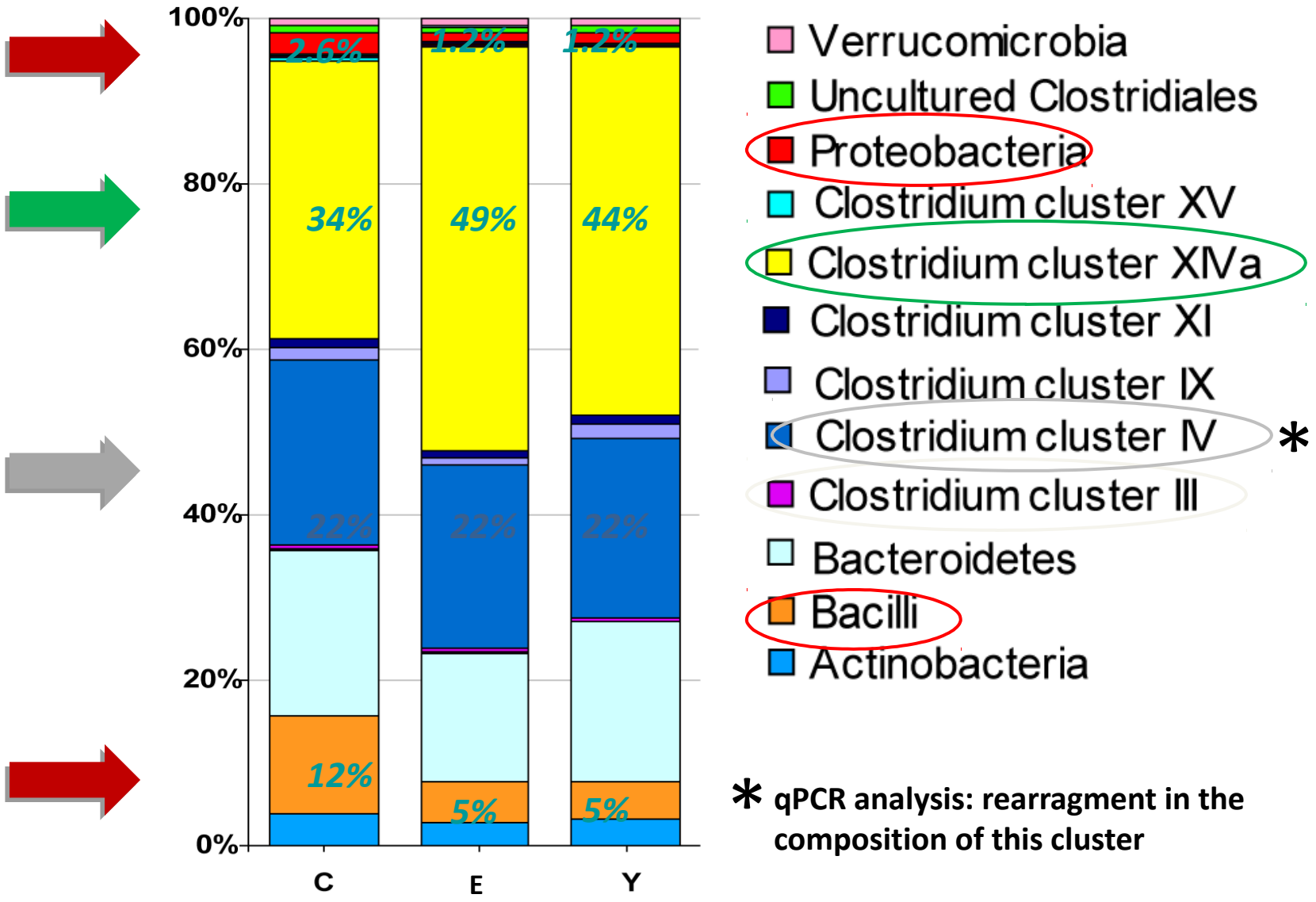
C = 127.0 ± 54.2

E =149.4 ± 40.5 p> 0.02

Y = 162.8 ± 35.1 p> 0.002

Progressive decline of the diversity of the gut ecosystem with the age

MAJOR PHYLOGROUPS WHICH CHANGE WITH AGE



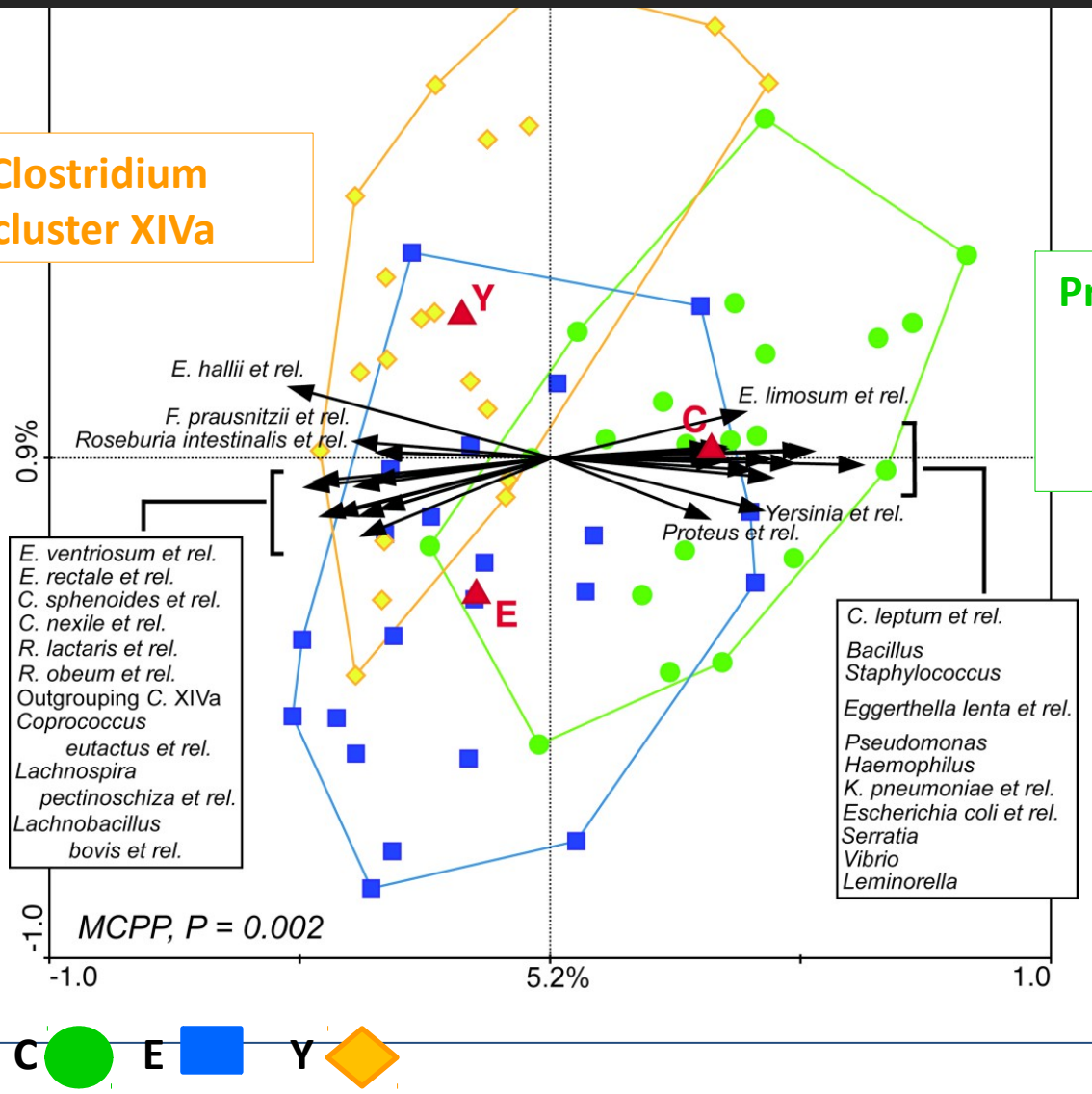
* qPCR analysis: rearragment in the composition of this cluster

MAJOR BACTERIAL GROUPS RESPONSIBLE FOR THE GM DIFFERENCES BETWEEN C, E AND Y

Clostridium cluster XIVa

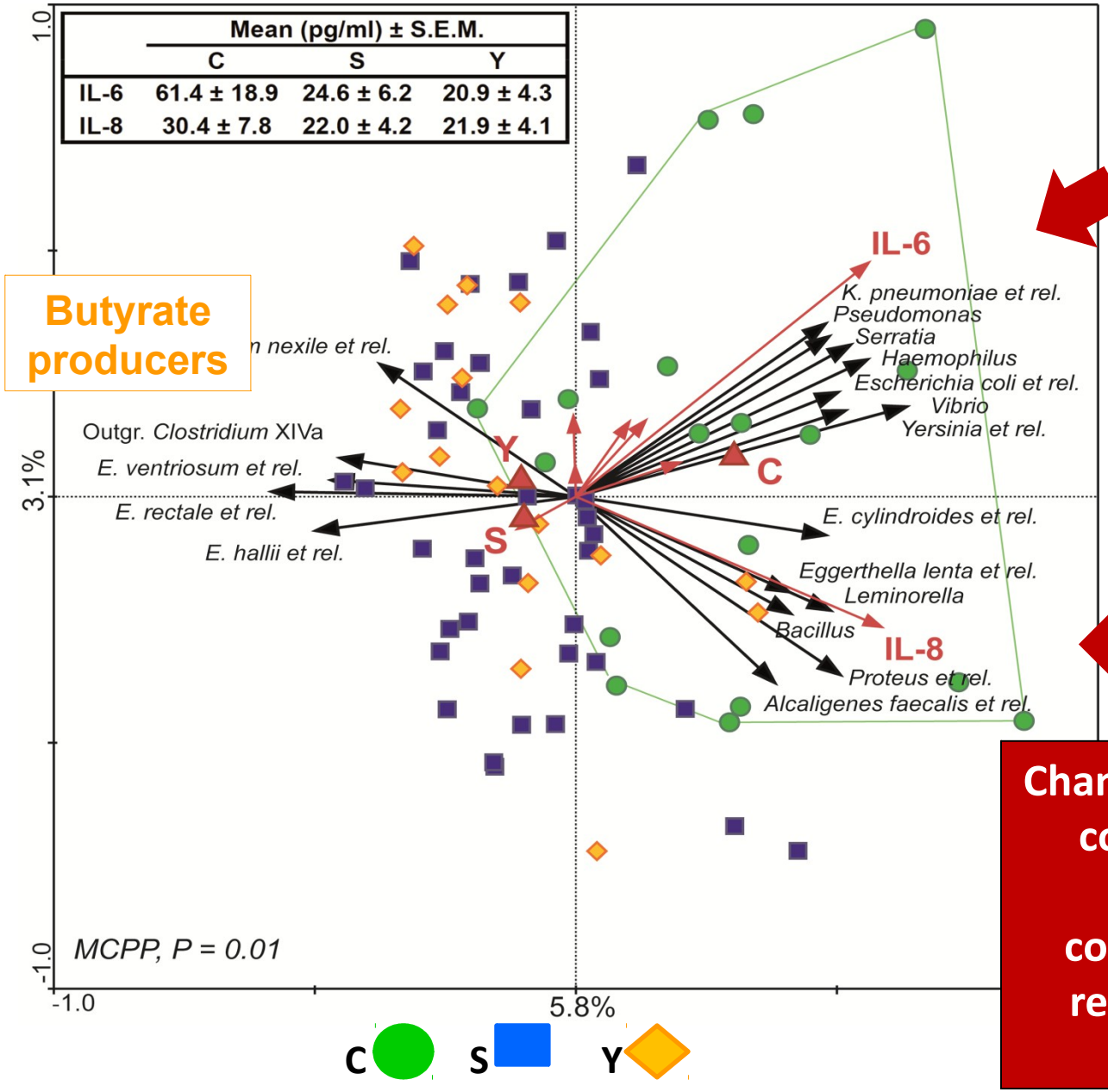
Proteobacteria and other facultative anaerobes

Redundancy Analysis (RDA) indicates that differences in the microbiota fingerprint between C and all the other subjects are greater than the differences between E and Y



CENTENARIAN MICROBIOTA AND INFLAMMATION

	Mean (pg/ml) ± S.E.M.		
	C	S	Y
IL-6	61.4 ± 18.9	24.6 ± 6.2	20.9 ± 4.3
IL-8	30.4 ± 7.8	22.0 ± 4.2	21.9 ± 4.1



Butyrate producers

RDA shows that 8.9% of the total variability of the GM can be related to the pattern of pro-inflammatory cytokines

Pathobionts

Changes in the microbiota composition may be caused by and/or contribute to the age-related inflammatory status

CONCLUSIONS

The fecal microbiota of Centenarians
shows an organization which significantly differs
from the adult-like pattern
(a highly compromised ecosystem)

Decreased biodiversity

↓ *Clostridium* cluster XIVa*

↓ Bifidobacteria

Rearrangement of *Clostridium* cluster IV*

↑ Facultative anaerobes, including Bacilli and Proteobacteria (“pathobionts”)

* **butyrate producer**

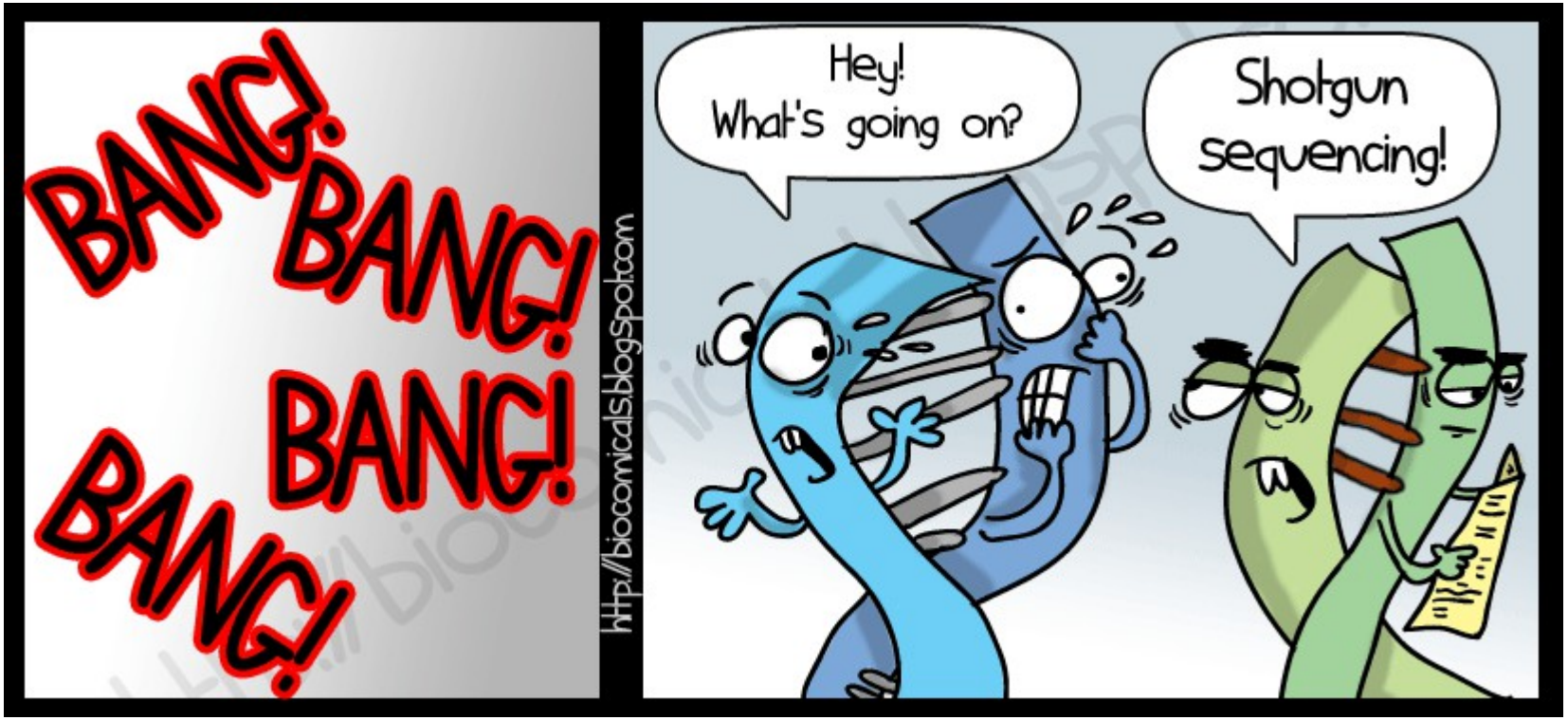
CONCLUSIONS (II)

- GM is **stable** throughout adulthood and its stability may last **longer than expected**
- Changes in GM **do not follow a linear relation with age**
- An **"aged" GM** occurs likely **after age 70-75 yrs**
- GM composition of **parents and offspring shows no significant similarity**
- GM changes in centenarians (*dysbiosis, proliferation of "pathobionts"*) are accompanied by **an increase in pro-inflammatory IL6** and **IL8** in peripheral blood supporting the hypothesis that **GM contributes to inflamm-aging**

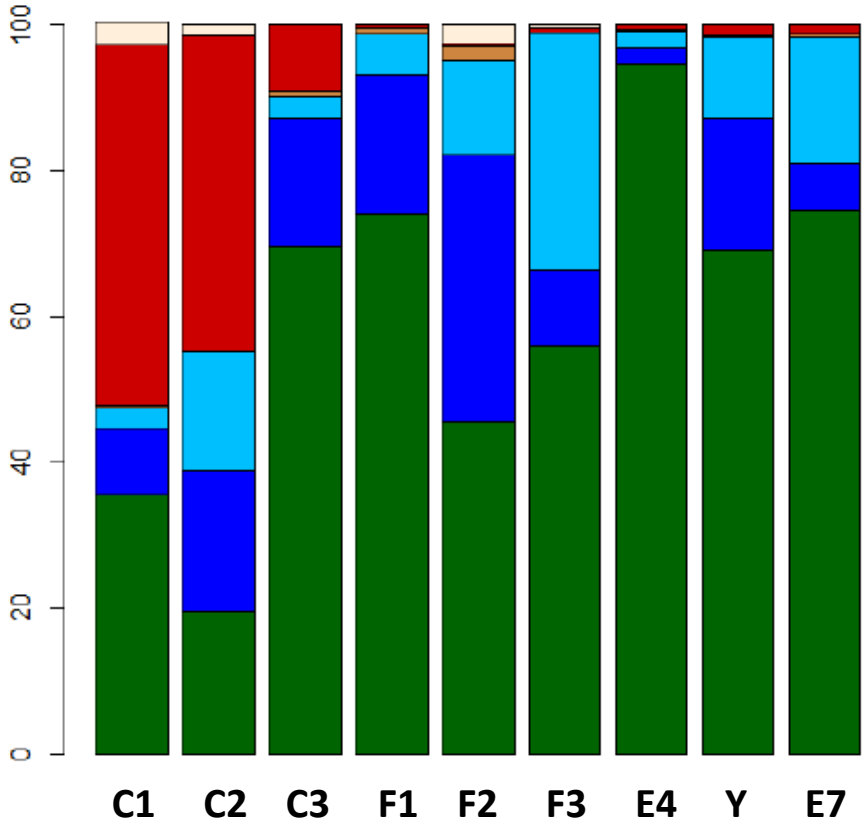
METAGENOMIC ANALYSIS OF CENTENARIANS

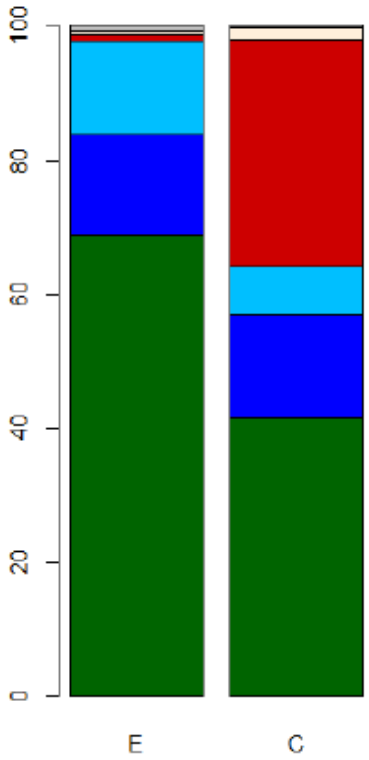
In collaboration with: Paul O´Toole, UCC, Cork

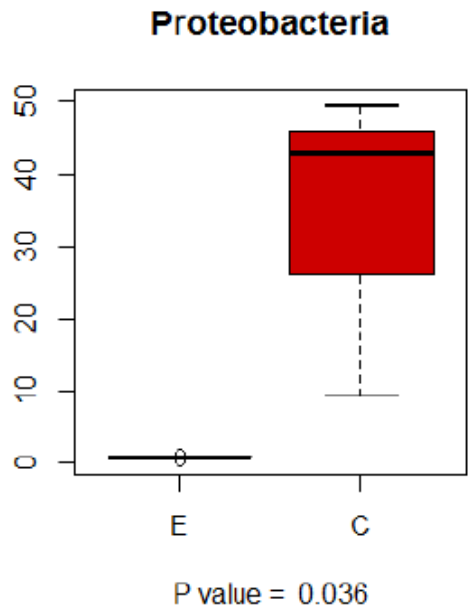
Centenarians 3 samples : C1, C2, C3; average age 100.7 y
Elderly 5 samples : F1, F2 F3 (centenarians' offspring); E4 and E7 average age 66.4 y
Young adult 1 sample: Y1, 38 y

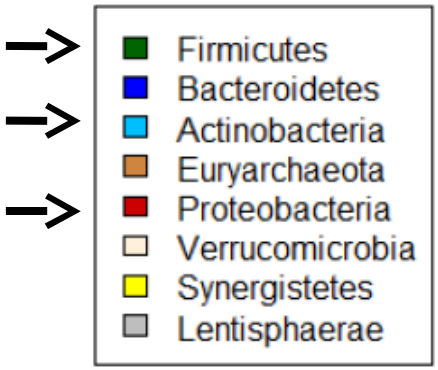


RELATIVE ABUNDANCE OF PHYLA



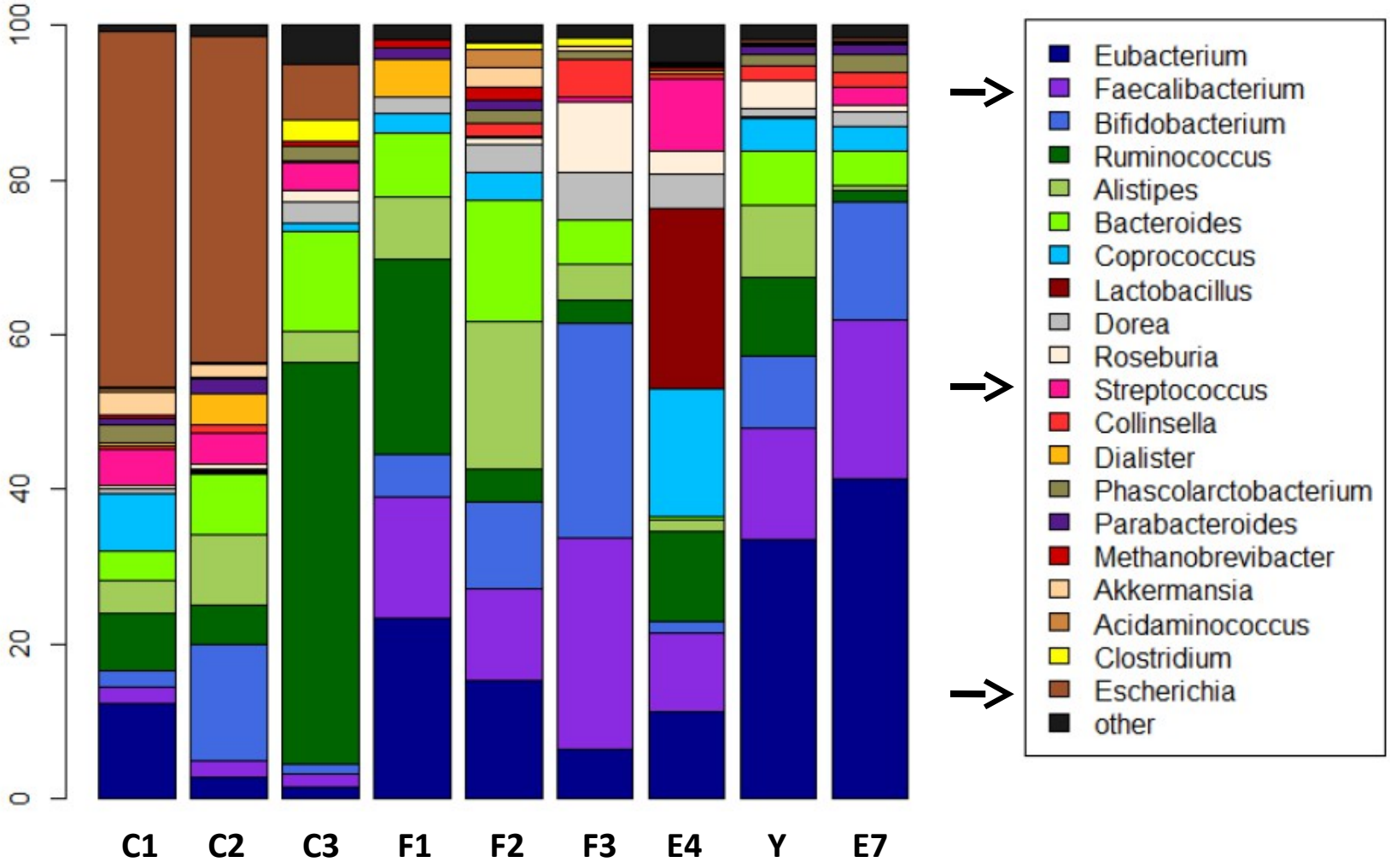




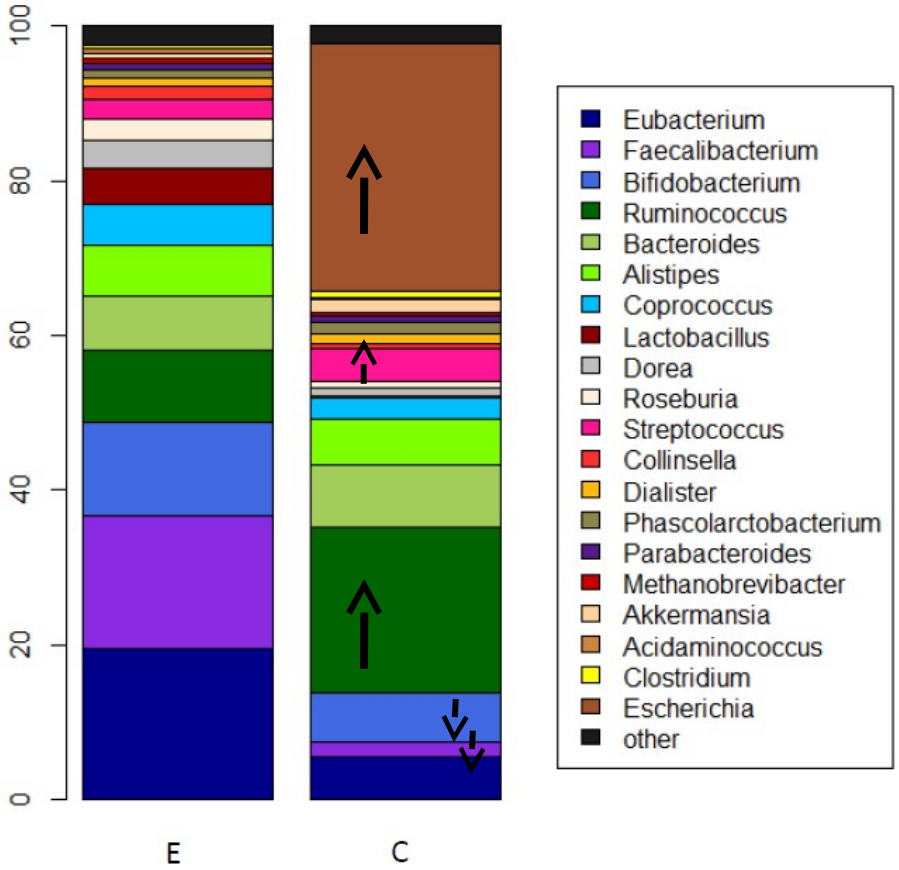


	MeanC	MeanE	MeanY
Proteobacteria	33.86	0.66	1.48
Firmicutes	41.54	68.94	69.07
Bacteroidetes	15.37	14.9	18.22
Verrucomicrobia	1.59	0.63	0
Actinobacteria	7.3	14.16	11.06
Euryarchaeota	0.45	0.7	0.17
Synergistetes	0	0	0
Lentisphaerae	0.01	0	0

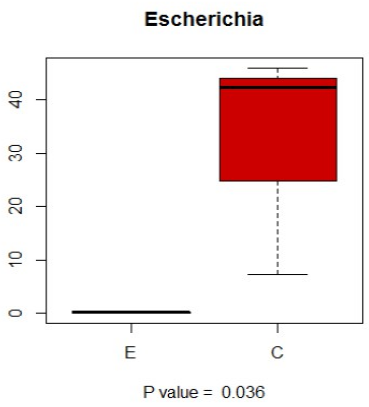
RELATIVE ABUNDANCE OF GENERA

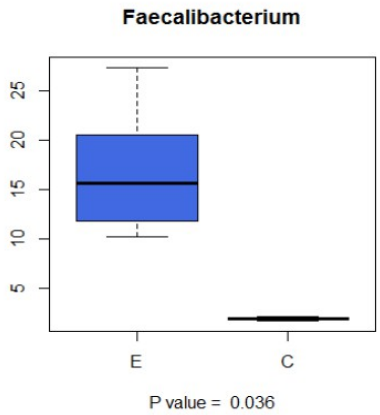


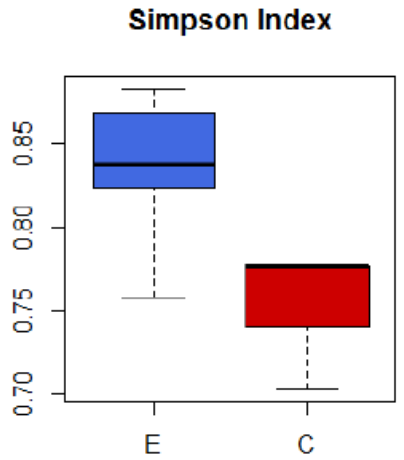
RELATIVE ABUNDANCE OF GENERA



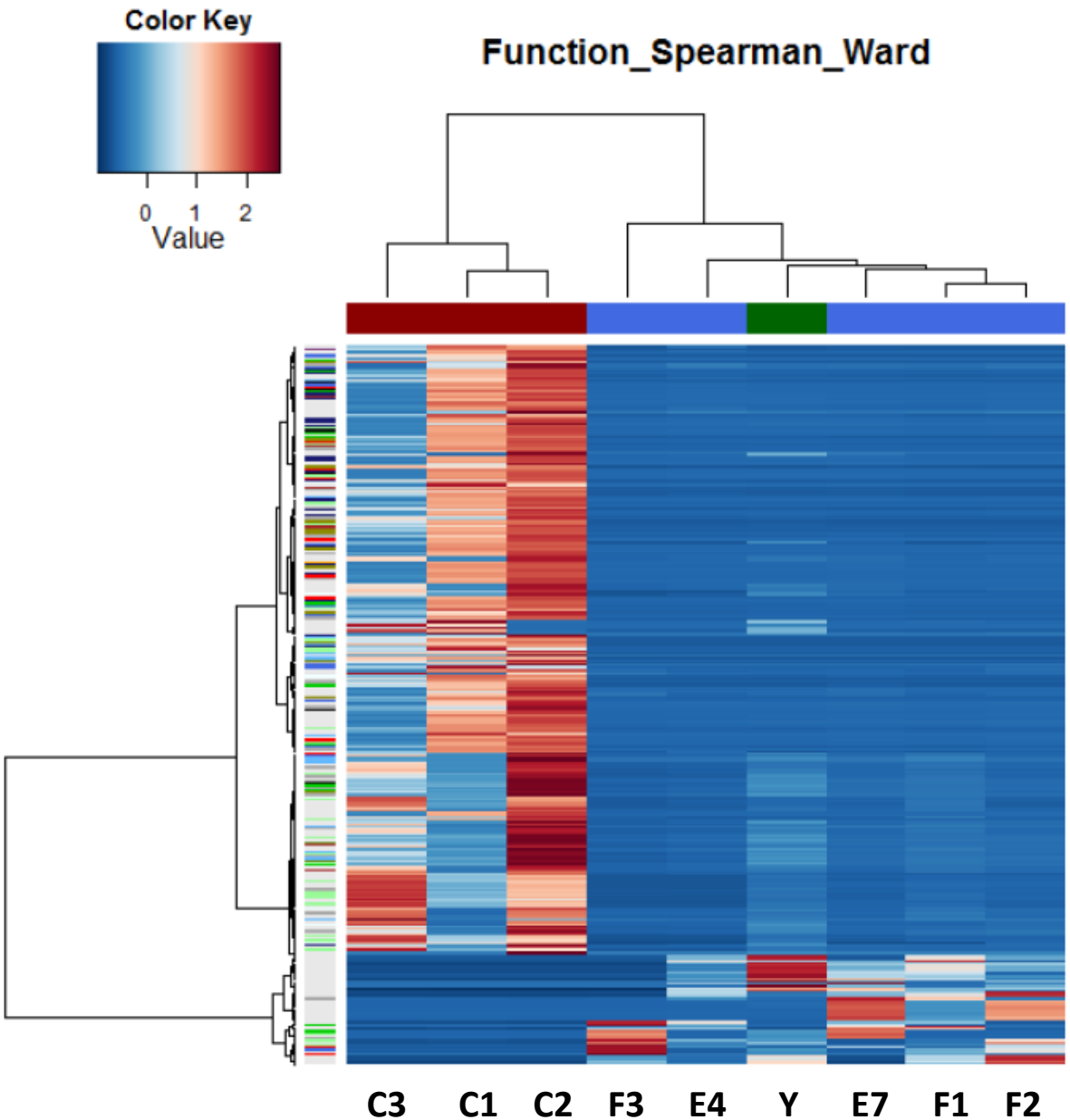
	meanC	meanE	meanY
Eubacterium	5.50151	19.45263	33.49068
Faecalibacterium	1.941863	17.14373	14.47249
Bifidobacterium	6.205453	12.23239	9.30204
Ruminococcus	21.43516	9.205746	10.09336
Alistipes	5.81489	6.701016	9.38046
Bacteroides	8.221277	6.929142	7.0597
Coprococcus	2.89546	5.177708	4.26248
Lactobacillus	0.01644	4.690194	0.04331
Dorea	1.209463	3.619744	1.04502
Roseburia	0.851427	2.774258	3.59276
Streptococcus	4.153	2.540286	0.1996
Collinsella	0.5914	1.818082	1.73463
Dialister	1.39114	1.010174	0
Phascolarctobacterium	1.421033	1.008962	1.6407
Parabacteroides	0.927917	0.825108	1.07121
Methanobrevibacter	0.45226	0.690544	0.16936
Akkermansia	1.585307	0.634002	0
Acidaminococcus	0.1306	0.534966	0
Clostridium	1.050593	0.444998	0.05685
Escherichia	31.81525	0.128928	0.44968
other	2.388563	2.4374	1.93567







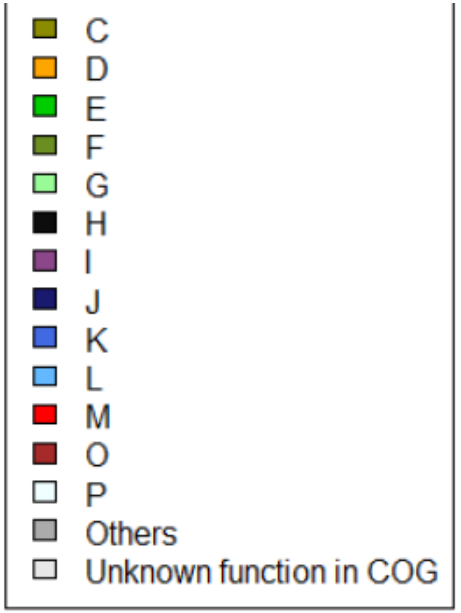
Different present genes classified in COG categories



Different present Genes classified in COG categories

Centenarians		
COG categories	Function name	Gene count
J	Translation, ribosomal structure and biogenesis	22
K	Transcription	12
L	Replication, recombination and repair	14
O	Posttranslational modification, protein turnover, chaperones	7
M	Cell wall/membrane/envelope biogenesis	12
T	Signal transduction mechanisms	2
D	Cell cycle control, cell division, chromosome partitioning	1
V	Defense mechanisms	5
C	Energy production and conversion	12
G	Carbohydrate transport and metabolism	29
E	Amino acid transport and metabolism	13
F	Nucleotide transport and metabolism	3
P	Inorganic ion transport and metabolism	7
H	Coenzyme transport and metabolism	6
I	Lipid transport and metabolism	1
U	Intracellular trafficking, secretion, and vesicular transport	1
R	General function prediction only	4
S	Function unknown	9
others		12

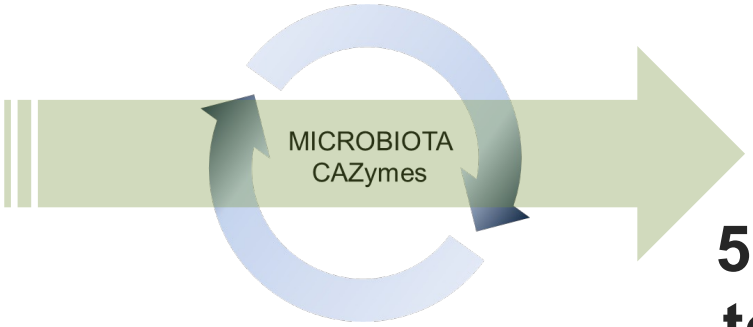
Elderly		
COG categories	Function name	Gene count
M	Cell wall/membrane/envelope biogenesis	2
V	Defense mechanisms	2
G	Carbohydrate transport and metabolism	3
E	Amino acid transport and metabolism	2
R	General function prediction only	1



SCFAs

THE METABOLISM OF DIETARY COMPOUNDS BY GUT MICROORGANISMS IS A KEY PROCESS FOR THE HOST TO MAXIMIZE THE EXTRACTION OF ENERGY FROM DIET

indigestible plant polysaccharides

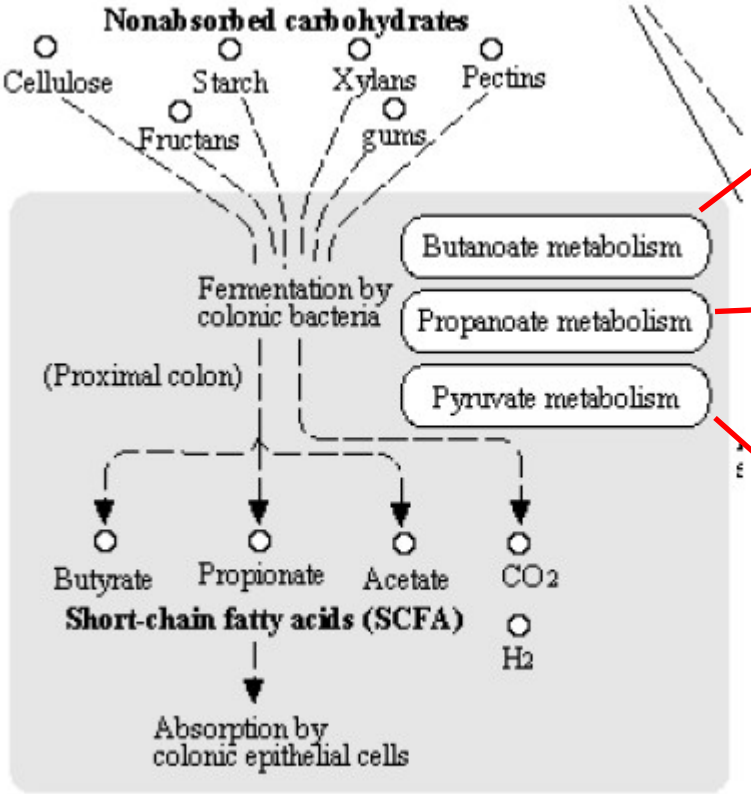


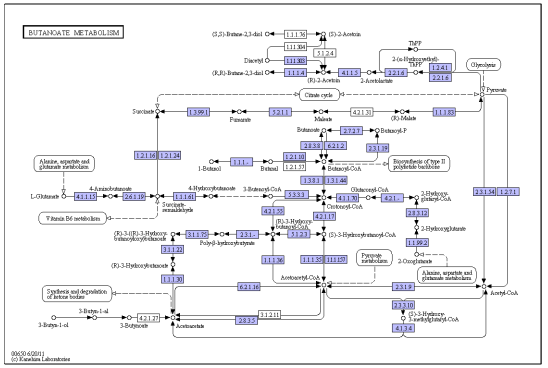
SCFAs

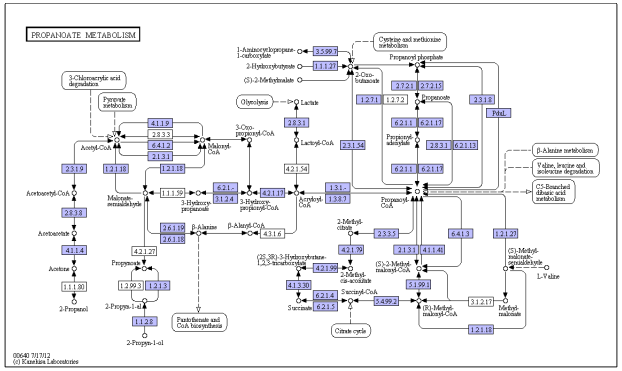
5-15% of the total energy required

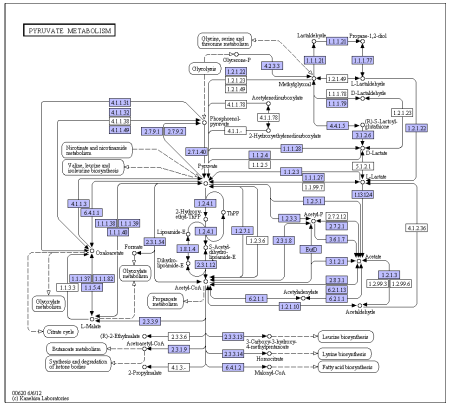
SCFA PATHWAY

- translate the KO IDs to COG IDs, of the core genes involved in Butanoate, Propanoate and Pyruvate metabolisms in KEGG database
- evaluate the presence of these genes in the metagenomes

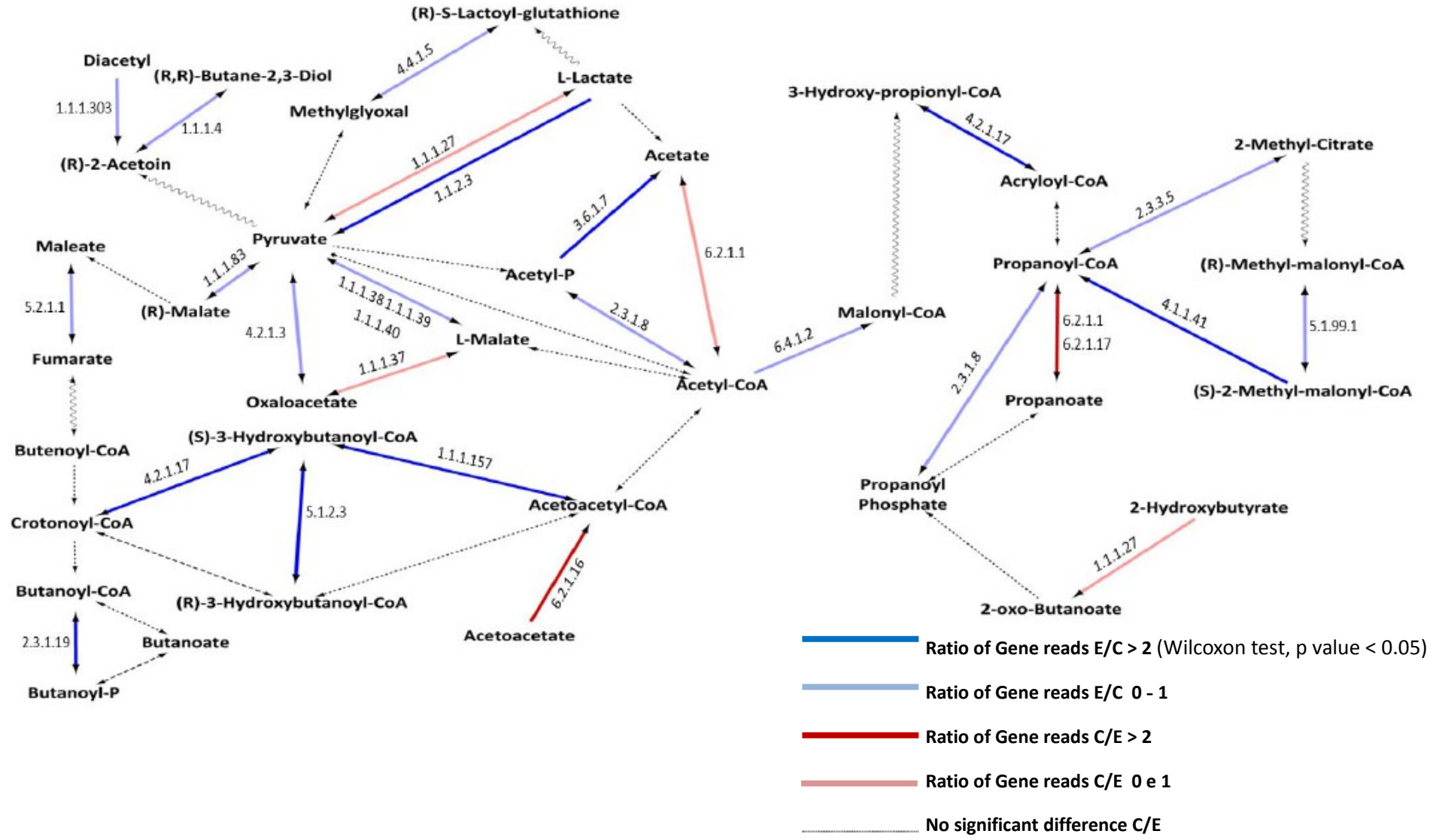








SCFA PATHWAY IN ELDERLY AND CENTENARIANS



Significant decrease of gene sequences involved in SCFA production in centenarians

CONCLUSION

- **Confirming the compromised microbiota in centenarians and the decreased biodiversity**

- **Confirming the increase of pathobiont genera**
  ↑ *Escherichia, Ruminococcus, Streptococcus, (Akkermansia)*

  - **Confirming the decrease of health-promoting genera**

  ↓ *Faecalibacterium, Eubacterium, Roseburia, Bifidobacterium, Lactobacillus*

  - **Decrease of genes involved in health-promoting pathways (SCFAs)**

OPEN ACCESS Freely available online

PLOS ONE

Metabolic Signatures of Extreme Longevity in Northern Italian Centenarians Reveal a Complex Remodeling of Lipids, Amino Acids, and Gut Microbiota Metabolism

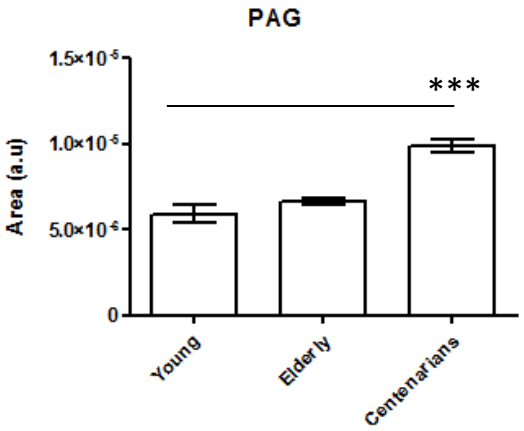
Sebastiano Collino[1]*, Ivan Montoliu[2], François-Pierre J. Martin[1], Max Scherer[1], Daniela Mari[3,4], Stefano Salvioli[5,6], Laura Bucci[5,6], Rita Ostan[5,6], Daniela Monti[7], Elena Biagi[8], Patrizia Brigidi[8], Claudio Franceschi[5,6], Serge Rezzi[1]

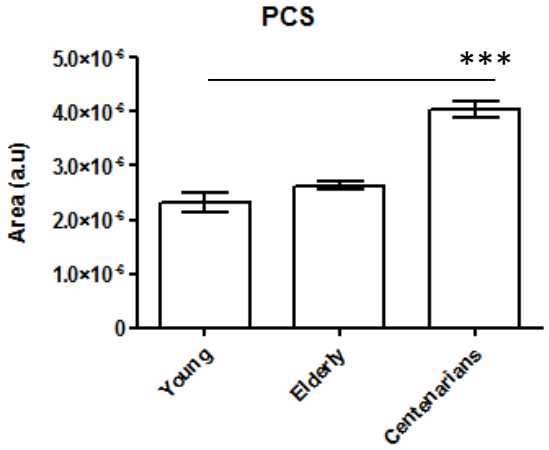
1 Proteomics and Metabonomics, Nestlé Institute of Health Sciences SA, Campus EPFL, Quartier de l'innovation, Lausanne, Switzerland, **2** Applied Mathematics, NESTEC SA, Nestlé Research Center, Lausanne, Switzerland, **3** Department of Medical Sciences, University of Milan, Milan, Italy, **4** Geriatric Unit Ca' Grande Foundation Maggiore Policlinico Hospital, Milan, Italy, **5** Department of Experimental Pathology, University of Bologna, Bologna, Italy, **6** Interdepartmental Centre L. Galvani, University of Bologna, Bologna, Italy, **7** Department of Experimental Pathology and Oncology, University of Florence, Florence, Italy, **8** Department of Pharmaceutical Sciences, University of Bologna, Bologna, Italy

PLOS ONE | www.plosone. March 2013 | Volume 8 | Issue 3 | e56564

URINE METABOLOMIC PROFILING

Significant increased secretion of metabolites related to changes in centenarians'microbiota

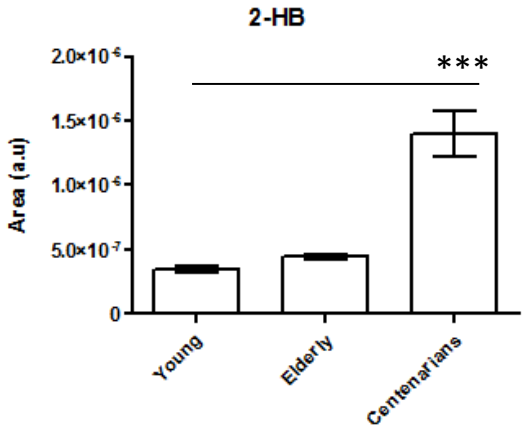




PAG
Phenylacetylglutamine

PCS
p-cresol-sulfate

2HB 2hydroxybenzoate



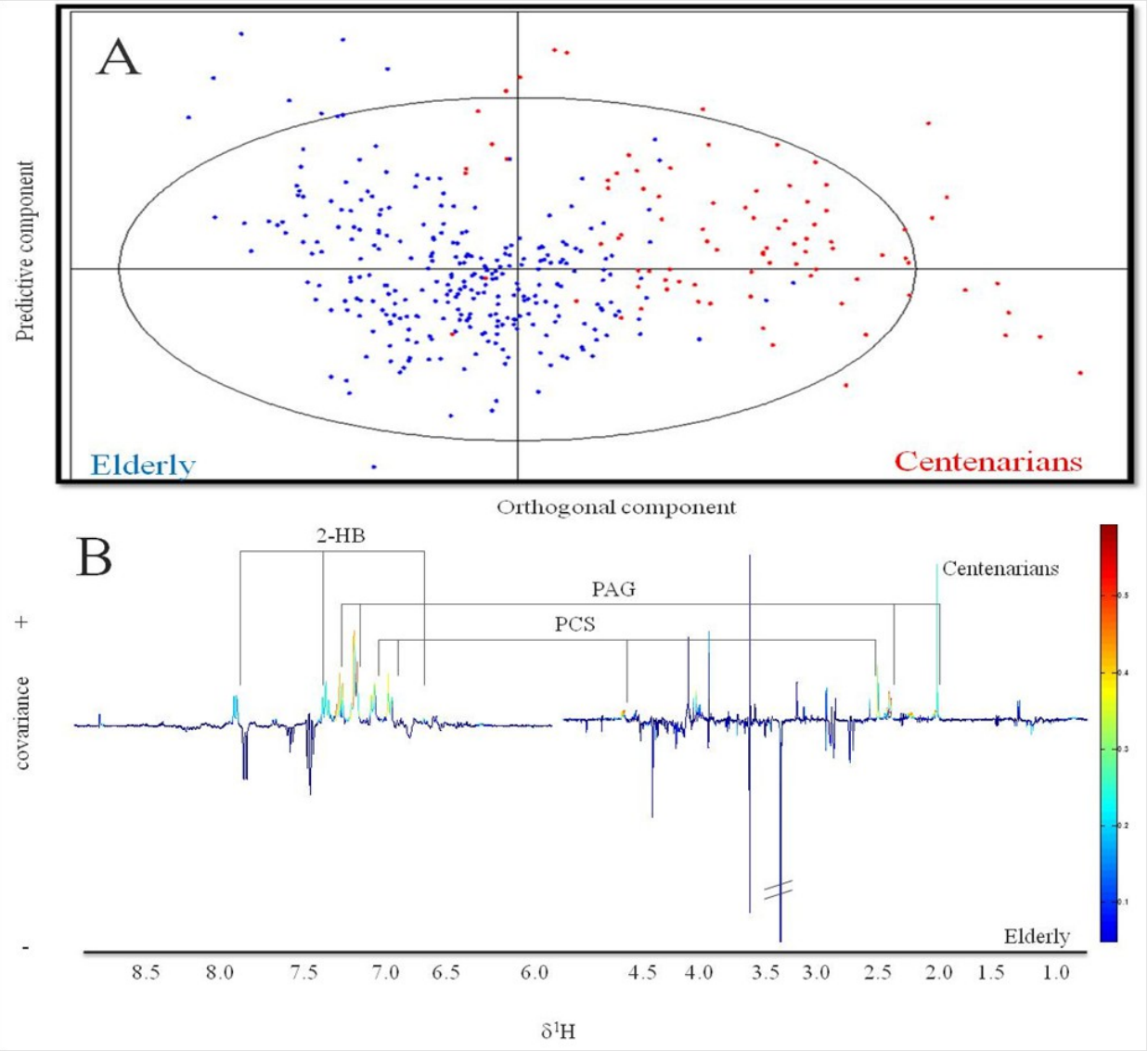
Positive correlation between PAG and PCS and proteobacteria

Catabolism of protein and aromatic AA by proteobacteria

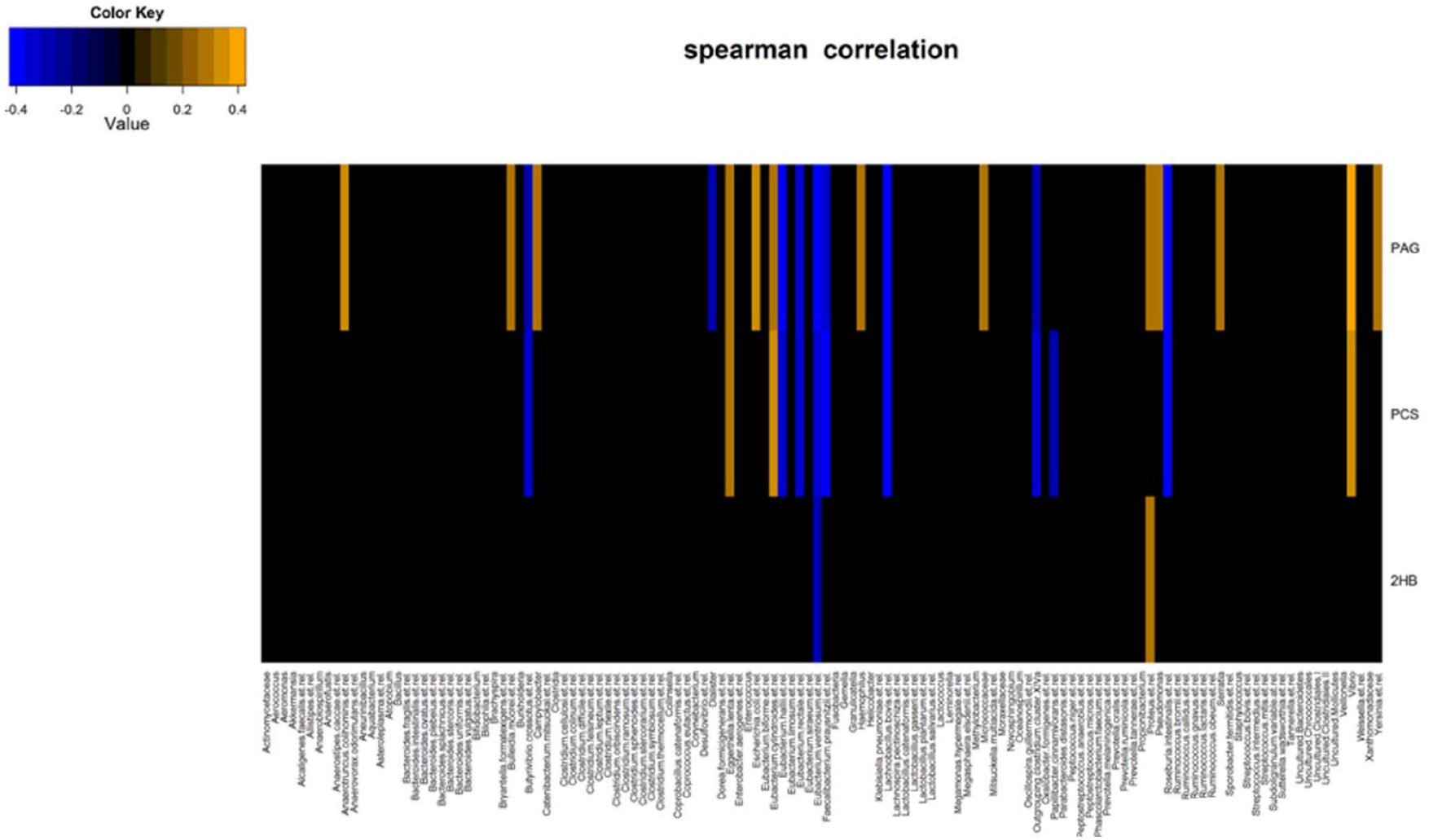
Collino et al., PLoS One, 2013

OPLS-DA score (A) and coefficient plots (B) derived from urinary 1H-NMR spectra from elderly (blue) and centenarians (red).

PAG = phenylacetylglutamine, PCS = p-cresol sulfate, 3-HB = 3-hydroxybenzoate.



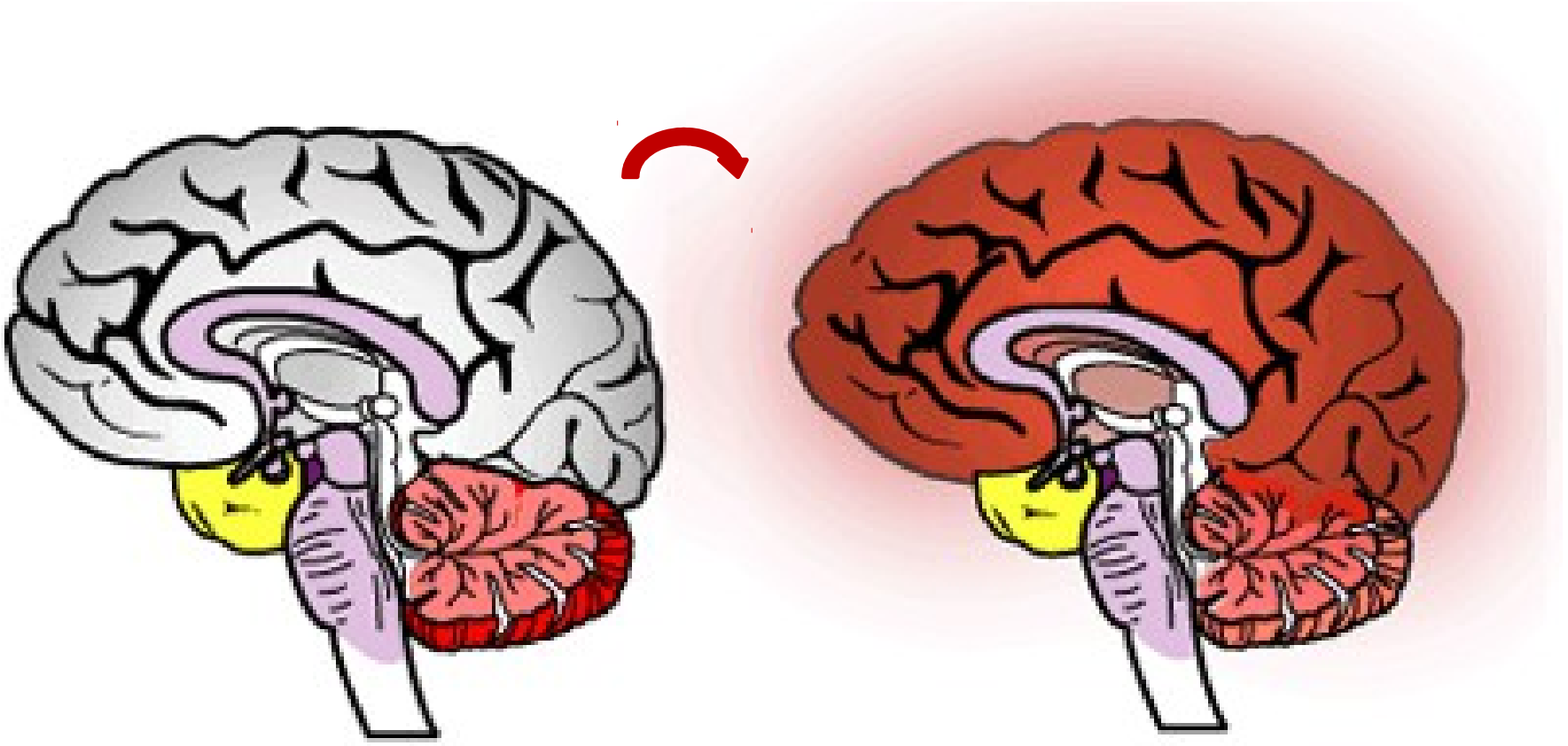
Spearman correlation map of PAG, PCS and HB and order/genus-like bacterial phylogroups



GUT MICROBIOTA-ASSOCIATED DISEASES

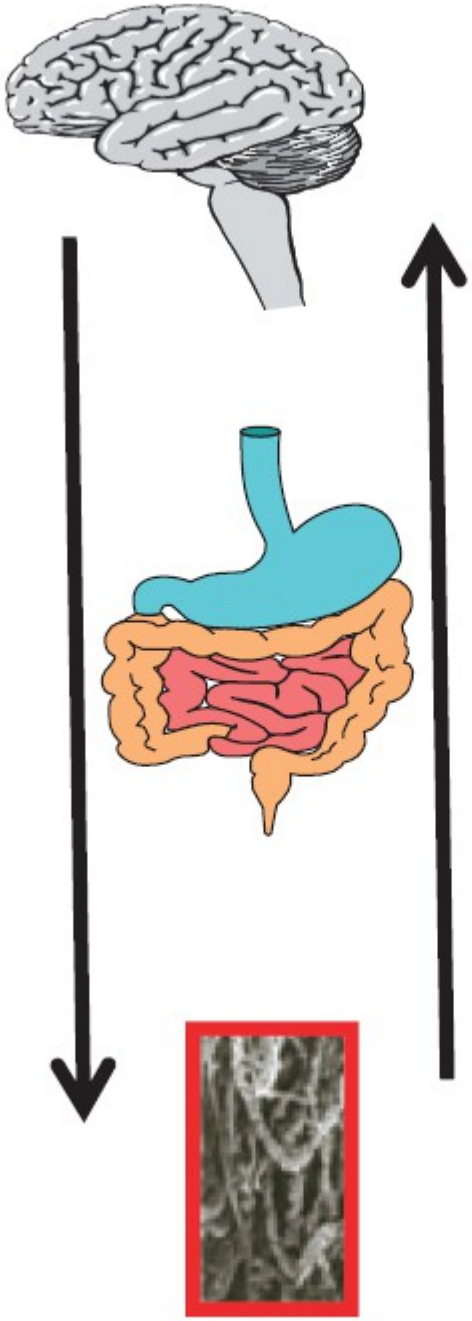
- Inflammatory Bowel Diseases
- Obesity
- Diabetes (type 1 and 2)
- Cardio-Vascular Diseases
- Atopy/Allergy
- Colorectal Cancer
- Gut-Brain Axis (Depression, Autism, MS, AD?, Dementia?)

Where neuro-inflammation come from?



The Gut-Brain Axis

Behavioural perturbation alters gut function, changes the habitat for bacteria and alters the composition of intestinal commensal bacteria



Changes in the microbiota alter gut and brain function

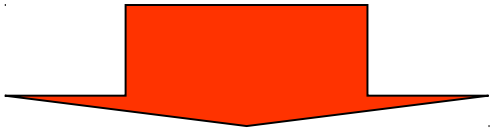
GM & lifespan extension

Ottaviani E, Ventura N, Mandrioli M, Candela M, Franchini A, Franceschi C.

***Gut microbiota as a candidate for lifespan extension: an ecological/evolutionary perspective targeted on living organisms as metaorganisms*.**

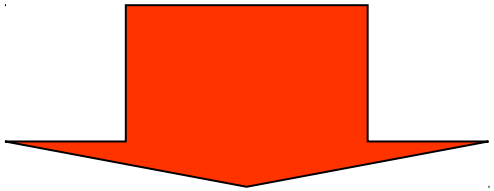
Biogerontology 2011 Dec;12(6):599-609

Appropriate DIET in the elderly



+

Gut microbiota



-

INFLAMM-AGING

SEVENTH FRAMEWORK PROGRAMME

COOPERATION

NU AGE

THEME 2: Food, Agriculture and Fisheries, and Biotechnology FP7-KBBE-2010-4

Proposal full title: **New dietary strategies addressing the specific needs of the elderly population for healthy ageing in Europe**

Proposal Acronym: **NU-AGE**
Coordinator: **Professor Claudio Franceschi**
Starting: **May 1st 2011**

Funding scheme: Large integrating project 9M €

Topic addressed: **KBBE-2010-2-2-02: Diet and prevention of functional decline of the elderly**

NU-AGE Partners

- 1. Alma Mater Studiorum Università di Bologna Research institution
- 2. University of East Anglia Research institution
- 3. Wageningen Universiteit Research institution
- 4. Institut National de la Recherche Agronomique Research institution
- 5. Spread European Safety Geie Stakeholder
- 6. University College Cork, Nat. Univ. of Ireland, Cork Research institution
- 7. Institute of Food Research Research institution
- 8. Szkola Glowna Gospodarstwa Wiejskiego Research institution
- 9. Confederation des Industries Agro-Alimentaires de L'UE Stakeholder
- 10. European Food Information Council Aisbl Stakeholder
- 11. Maa Ja Elintarviketalouden Tutkimuskeskus Research institution
- 12. Ethniko Idryma Erevnon Research institution
- 13. Straticell Screening Technologies SME
- 14. The University of Reading Research institution

NU-AGE Partners

- 15. Karolinska Institutet Research institution
- 16. Valio Enterprise
- 17. Orebro University Research institution
- 18. Lesieur Enterprise
- 19. Villani S.p.A. Enterprise
- 20. Pancrazio S.p.A. SME
- 21. Newsol SME
- 22. Wiesbauer Gourmet Gastro Gmbh Enterprise
- 23. Kanizsa Pékség Zrt. SME
- 24. VIDRERES LLET, S.L. Enterprise
- 25. Zeelandia Enterprise
- 26. MEVGAL Enterprise
- 27. Yoruk Sut Urunleri Hayvancılık Gıda San. Ve Tic. Ltd. Şti. SME
- 28. NEDERLANDSE ORGANISATIE VOOR TOEGEPAST NATUURWETENSCHAPPELIJK ONDERZOE Research institution
- 29. CRNH Auvergne Research institution
- 30. Nestec Enterprise

NU-AGE and Inflamm-aging

BASIC HYPOTHESIS & RATIONALE

Appropriate WHOLE DIET (an *ad hoc* fortified "mediterranean diet") can decrease the level of the chronic, sub-clinical, low grade inflammatory process characteristic of old age we have proposed to call INFLAMM-AGING (Franceschi et al., 2000)

NU-AGE recruited subjebts (65-79 years of age)

A total of **1250 individuals** categorized as not frail or prefrail will be recruited in **5 European countries** (Italy, France, UK, The Netherlands and Poland), and divided into 2 groups:

- will follow **a whole "mediterranean" ad hoc fortified/modified diet**
- will continue its own diet (**control group**)

At **time zero** and **after 12 months** a variety of parameters will be measured (hematological profiles, immunology and inflammation, cognition, genetics, body composition)

NUAGE "OMICS"
before and after dietary intervention

in a subcohort of 125 elderly subjects

- **In depth immunology** (in plasma/serum)
- **Transcriptomics** (PBMC, immune function and inflammation)
- **Oxido-lipidomics** (in plasma)
- **Metabolomics** (in urine)
- **GM by HITChip microarray, metagenomics** (in feces)
- **Epigenetics** (DNA from PBMC, genes involved in inflamm-aging)

NUAGE SYSTEMS BIOLOGY of nutrition and inflamm-aging

Clinical and nutritional data plus “omics” will generate **an integrated massive amount of data** which will analysed by advanced statistics and mathemathical modelling in order to generate **a predictive model for inflammaging-based nutrition in elderly**

DAVID SUTHERLAND/GETTY IMAGES

Could a Mediterranean diet, rich in olive oil, fish and fresh fruit, lead to a healthy microbiome in old age?

MICROBIOME

Cultural differences

Studies of gut bacteria are beginning to untangle how diet affects health in old age — but determining cause and effect is tricky.

BY VIRGINIA HUGHES

Franceschi, O'Toole and two dozen other academic and industry groups are now part of a €9 million project called NU-AGE, which includes 1,250 older individuals from France, Italy, Poland, the Netherlands and the United Kingdom. For one year, half will be given the Mediterranean diet, half will remain on their normal diet, and the NU-AGE researchers will measure how their health changes. O'Toole's team will sequence the participants' gut microbiota before and after the dietary intervention, while other researchers will look at genetic, epigenetic and metabolic signatures in their blood. Each of these biological levels might give insight on how the diet changes the microbiome.

NU-AGE is exactly the kind of large, longitudinal study that scientists the world over are clamouring for.

Inflamma-miRs

In collaboration with

Fabiola Olivieri & Antonio Procopio

Università Politecnica delle Marche

- According to recent studies of Judith Campisi **senescent cells**, which accumulate in tissues and organs with age **have a secretory phenotype (SASP),** can be another important contributor to inflamm-aging, especially in the elderly.
- **MicroRNAs (miRs)** are emerging as modulators of some pathways, such as **NF-kB**, target of rapamycin mammalian complex (**mTOR**), **Sirtuins**, **TGF-β** and **Wnt signalling**, potentially related to inflammation, cellular senescence, and age-related diseases, including cancer.
- Some miRs, like **miR-21, -146a and -126**, targeting mRNAs belonging to NF-kB signaling are classifiable as senescence-associated (*SA-miRs*), **inflammation-associated (*inflamma-miRs*)** and cancer-associated (*onco-miRs*) miRs.

MiR-146a as marker of senescence-associated pro-inflammatory status in cells involved in vascular remodelling

Fabiola Olivieri · Raffaella Lazzarini ·
Rina Recchioni · Fiorella Marcheselli ·
Maria Rita Rippo · Silvia Di Nuzzo ·
Maria Cristina Albertini · Laura Graciotti ·
Lucia Babini · Serena Mariotti · Giorgio Spada ·
Angela Marie Abbatecola · Roberto Antonicelli ·
Claudio Franceschi · Antonio Domenico Procopio

AGE 2012

Mechanisms of Ageing and Development 133 (2012) 675–685

Contents lists available at SciVerse ScienceDirect

Mechanisms of Ageing and Development

journal homepage: www.elsevier.com/locate/mechagedev

Age-related differences in the expression of circulating microRNAs: miR-21 as a new circulating marker of inflammaging

Fabiola Olivieri [a,b,1,*], Liana Spazzafumo [c,1], Gabriele Santini [a], Raffaella Lazzarini [a], Maria Cristina Albertini [g], Maria Rita Rippo [a], Roberta Galeazzi [f], Angela Marie Abbatecola [e], Fiorella Marcheselli [b], Daniela Monti [j], Rita Ostan [h,i], Elisa Cevenini [h,i], Roberto Antonicelli [d], Claudio Franceschi [h,i], Antonio Domenico Procopio [a,b]

[a] *Department of Clinical and Molecular Sciences, Università Politecnica delle Marche, Ancona, Italy*
[b] *Center of Clinical Pathology and Innovative Therapy, INRCA-IRCCS, Ancona, Italy*
[c] *Biostatistical Center, INRCA-IRCCS, Ancona, Italy*
[d] *Cardiology Unit, INRCA-IRCCS, Ancona, Italy*
[e] *Scientific Direction, INRCA-IRCCS, Ancona, Italy*
[f] *Clinical Laboratory & Molecular Diagnostics, INRCA-IRCCS, Ancona, Italy*
[g] *Dipartimento di Scienze Biomolecolari, Sezione di Biochimica e Biologia molecolare, Università degli Studi di Urbino "Carlo Bo", Urbino, Italy*
[h] *Department of Experimental Pathology, "Alma Mater Studiorum" University of Bologna, Bologna, Italy*
[i] *Centro Interdipartimentale Galvani "CIG", "Alma Mater Studiorum", University of Bologna, Bologna, Italy*
[j] *Department of Experimental Pathology and Oncology, University of Florence, Florence, Italy*

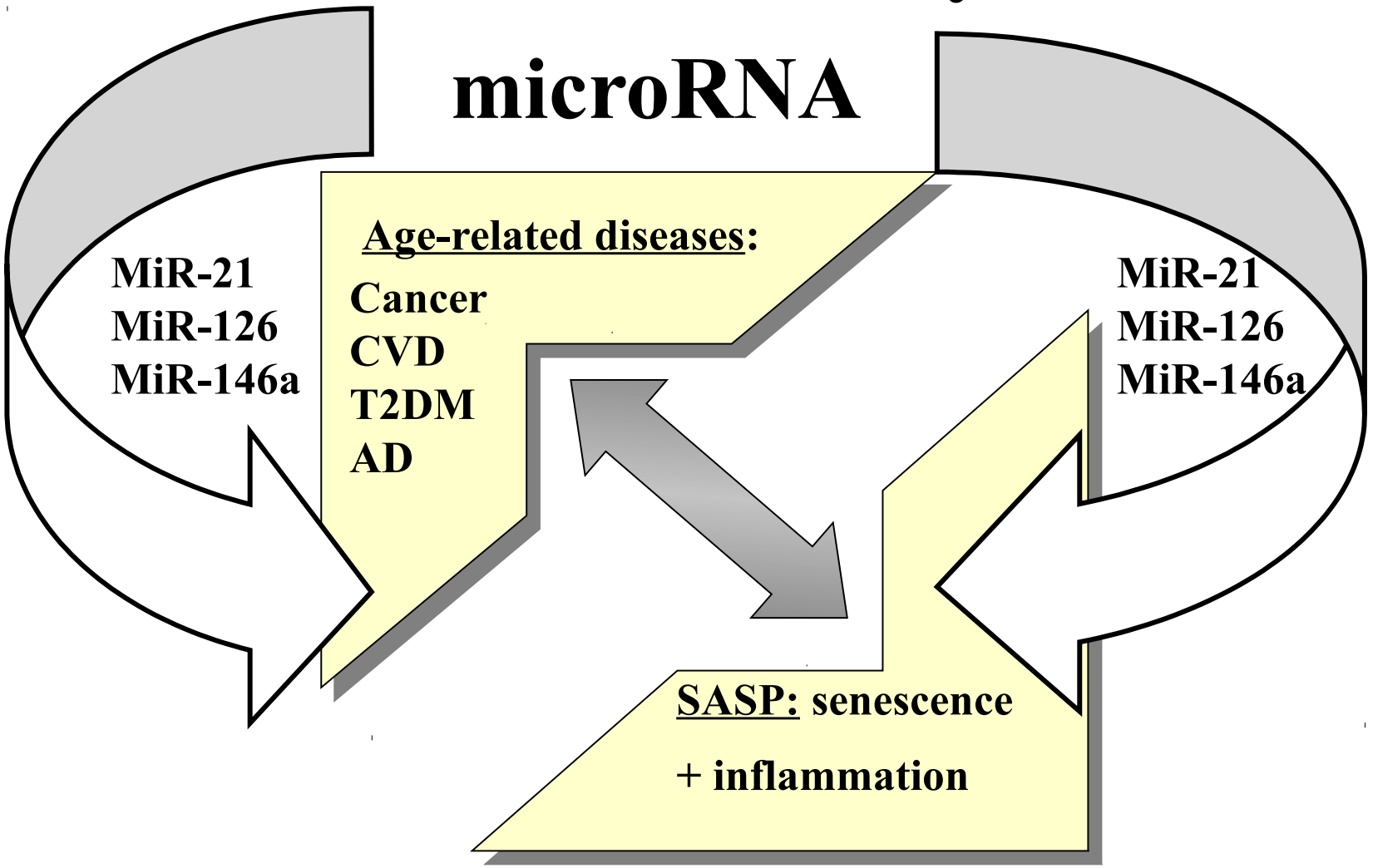
Inflamma-miR

MicroRNAs linking inflamm-aging, cellular senescence and cancer

Olivieri F, Rippo MR, Monsurro V, Salvioli S, Capri M , Procopio AD and Franceschi C

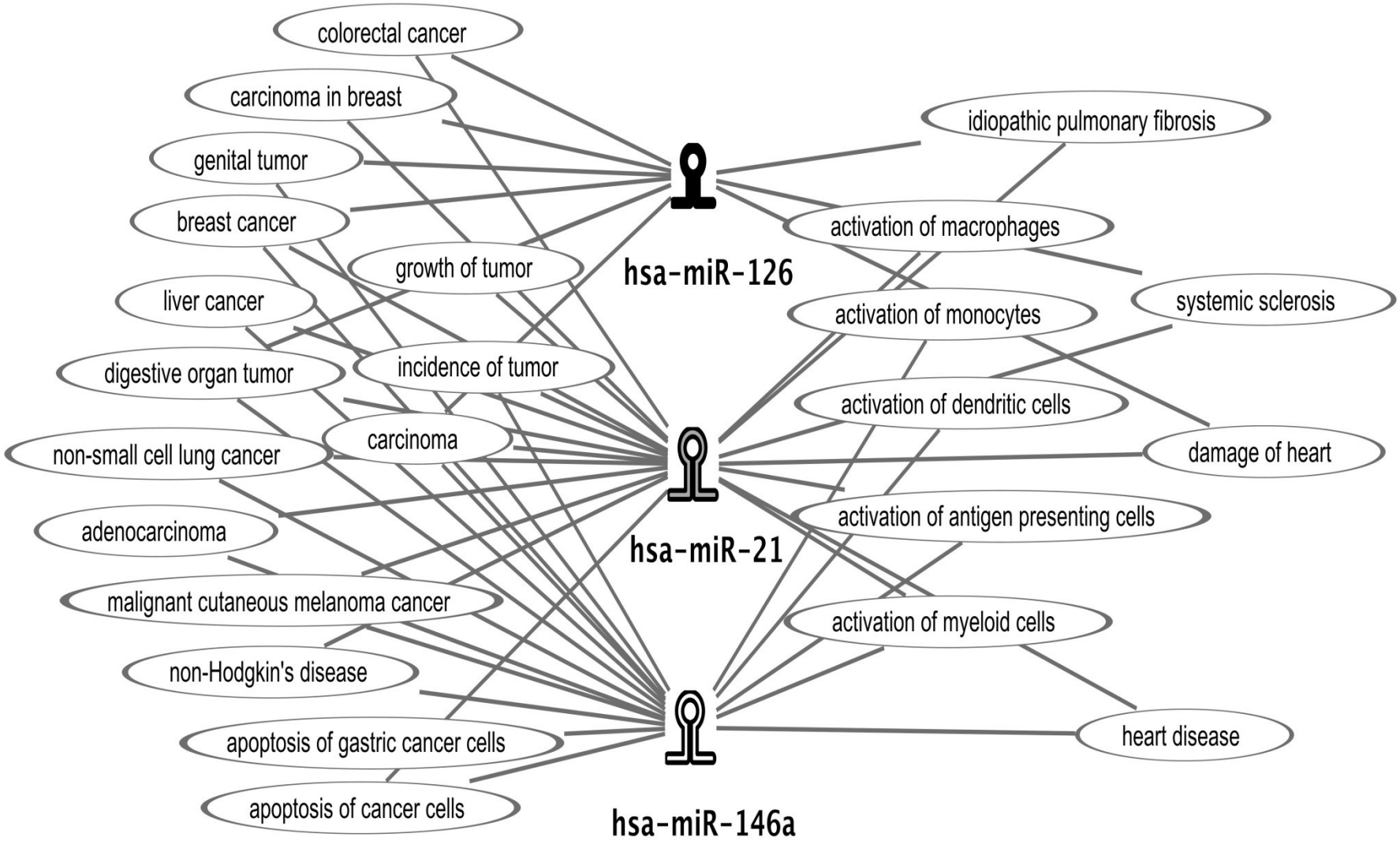
Aging Research Reviews, 2013 in press

Pro-inflammatory microRNA



Inflamma-miRs

Targets and Diseases of Inflamma-miRs



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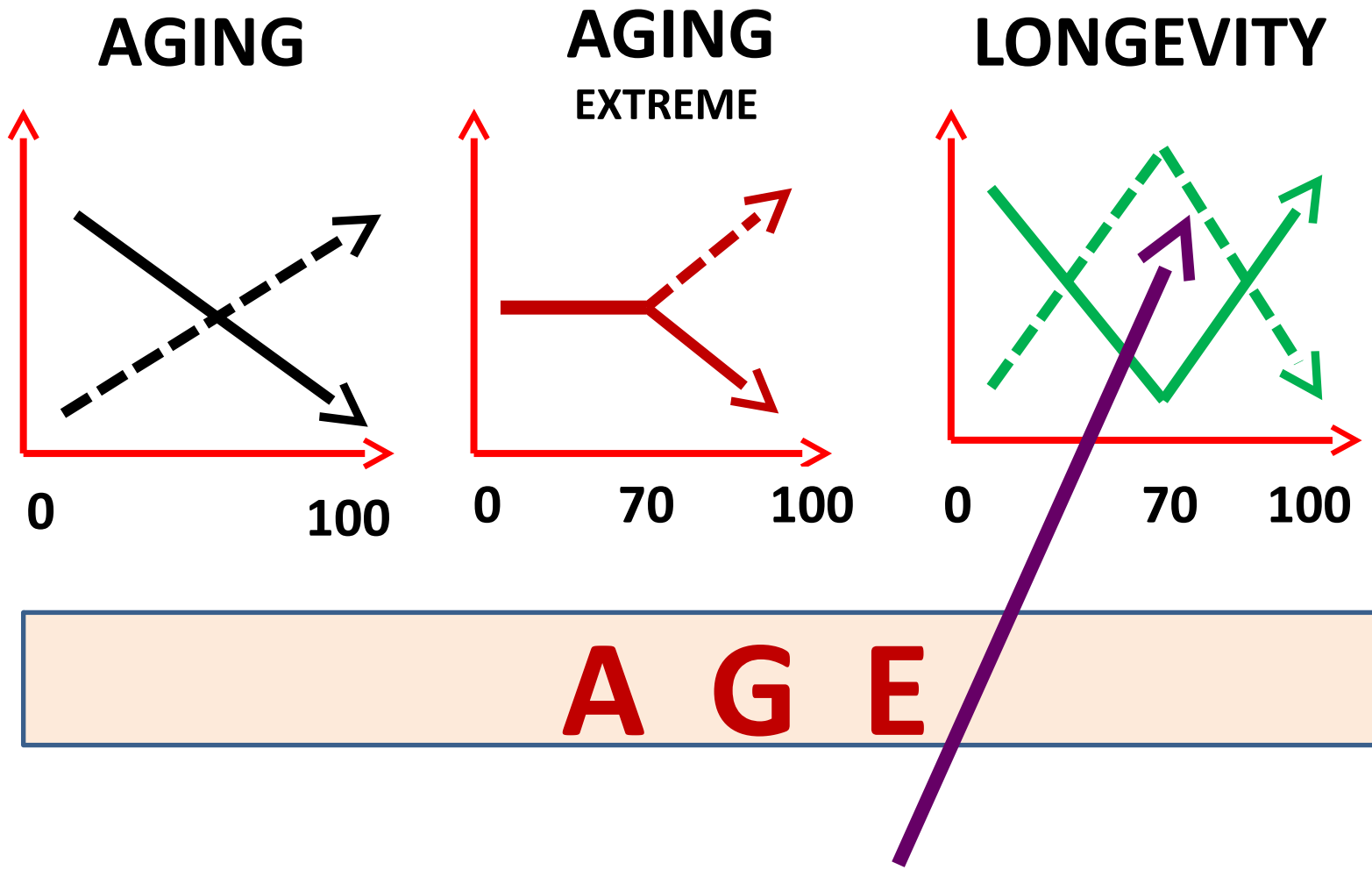
Age-Trajectories of Biomarkers



miR-146a and vascular cell senescence

- Among the **367 profiled miRs in HUVECs**, **miR-146a, -9, -204 and -367** showed the highest **up-regulation in senescent cells**.
- Their predicted target genes belong to nine common pathways, including **Toll Like Receptor signalling (TLR)** that plays a pivotal role in inflammatory response, a key feature of senescence (**inflammaging**).
- MiR-146a was the most up-regulated miR in the validation analysis (>10 fold).
- Significant correlations were observed among **miR-146a** expression and **β-galattosidase** expression, **telomere length** and **telomerase activity**.

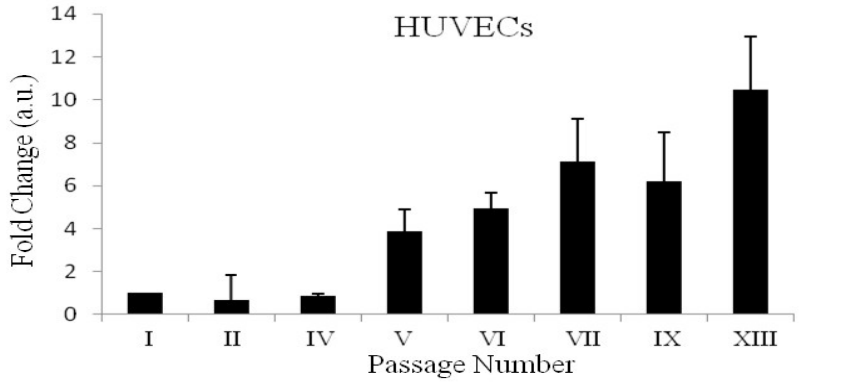
miRNA and vascular cell senescence

In order to identify new markers of **vascular cell senescence** primary cultured endothelial cells, including:

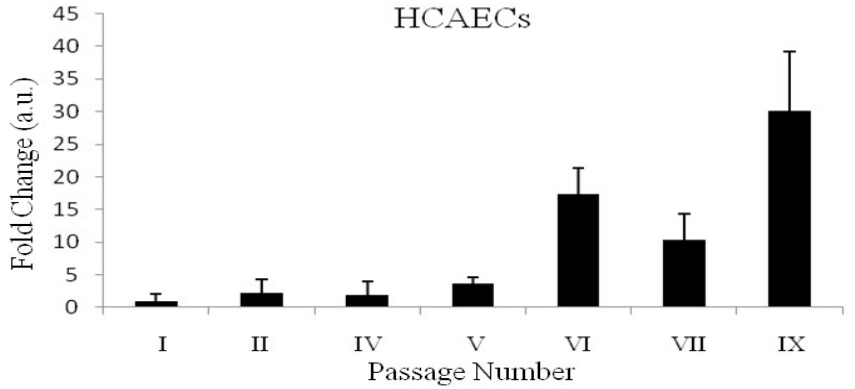
- **H**uman **U**mbilical **V**ein **E**ndothelial **C**ells (**HUVEC**)
- **H**uman **A**ortic **E**ndothelial **C**ells (**HAEC**)
- **H**uman **C**oronary **A**rtery **E**ndothelial **C**ells (**HCAEC**)
- ex vivo **C**irculating **A**ngiogenic **C**ells (**CAC**)

were analysed for microRNA (miR) expression.

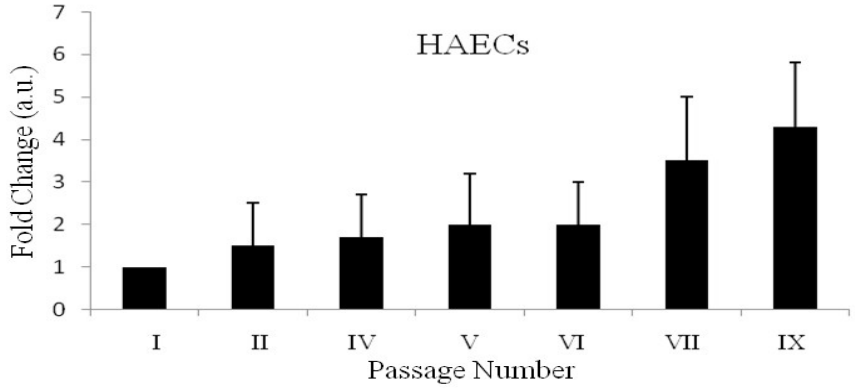
miRNA 146a expression in HUVEC, HCAEC & HAEC during replicative senescence



HUVEC
Human **U**mbilical **V**ein **E**ndothelial **C**ells



HCAEC
Human **C**oronary **A**rtery **E**ndothelial **C**ells



HAEC
Human **A**ortic **E**ndothelial **C**ells

Senescence-associated secretory phenotype (pro-inflammatory) in HUVEC

	II	III	V	IX	XI	XII	XIII
IL-1 α (pg/ml)	0.012±0.005	0.016±0.004	0.019±0.006	0.068±0.007	0.066±0.008	0.241±0.030	0.791±0.083
IL-1 β (pg/ml)	0.035±0.007	0.062±0.007	0.065±0.008	0.069±0.008	0.277±0.014	0.707±0.080	2.669±0.120
IL-2 (pg/ml)	0.036±0.004	0.088±0.009	0.146±0.015	0.231±0.060	0.587±0.042	0.966±0.085	2.966±0.145
IL-6(pg/ml)	3.206±0.135	9.166±0.234	11.135±1.234	15.769±1.345	50.443±4.156	60.137±6.987	80.123±10.565
IL-8 (pg/ml)	3.094±0.178	4.768±0.145	11.575±2.456	17.003±1.756	30.820±3.798	49.016±4.778	100.123±15.678
IL-10 (pg/ml)	0.109±0.008	0.175±0.009	0.163±0.008	0.251±0.012	0.721±0.019	2.6743±0.545	5.636±0.985
IL-12 (pg/ml)	0.006±0.002	0.065±0.005	0.079±0.009	0.071±0.005	0.199±0.010	0.397±0.099	2.175±0.245
INF-γ (pg/ml)	0.113±0.007	0.212±0.010	0.352±0.076	0.376±0.085	0.821±0.086	2.364±0.765	9.591±0.785
TNF-α (pg/ml)	0.007±0.002	0.009±0.001	0.023±0.003	0.045±0.005	0.066±0.007	0.312±0.085	0.791±0.095
MPO (pg/ml)	0.027±0.003	0.037±0.003	0.040±0.006	0.043±0.008	0.039±0.005	0.069±0.019	0.445±0.014

miRNA and vascular cell senescence

- We also included miR-146a expression determination in **CACs** from **37 CHF patients** and **35 healthy controls** (CTR) subjects.
- **A 1,000-fold increased expression of miR-146a was observed in CACs of CHF patients** compared to CTR.
- With these cells also, significant correlations among miR-146a expression, telomere length and telomerase activity were observed.
- Overall, our findings indicate that **miR-146a is a marker of a senescence-associated pro-inflammatory status in vascular remodelling cells.**

miRNA and vascular cell senescence

Overall, our findings indicate that

miR-146a is a marker of a senescence-associated pro-inflammatory status in cells involved in vascular remodelling.

Thank for your
attention

Common pathways shared by miR-146a, miR-204, miR-367 and miR-9

KEGG Pathway name	KEGG Pathway ID
MAPK signaling	hsa04010
Small cell lung cancer	hsa05222
Glycan structures - biosynthesis 1	hsa01030
Axon guidance	hsa04360
Neuroactive ligand-receptor interaction	hsa04080
Cell adhesion molecules (CAMs)	hsa04514
Erb signaling	hsa04012
Ubiquitin mediated proteolysis	hsa04120
Toll-like receptor signaling	hsa04620

REJUVENATION RESEARCH
Volume 10, Number 4, 2007
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DOI: 10.1089/rej.2007.0556

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Terzo livello
Quarto livello
Quinto livello

N-Glycomic Changes in Serum Proteins During Human Aging

Valerie Vanhooren,[1] Liesbeth Desmyter,[1] Xue-En Liu,[1] Maurizio Cardelli,[2] Claudio Franceschi,[3] Antonio Federico,[4] Claude Libert,[1] Wouter Laroy,[1] Sylviane Dewaele,[1] Roland Contreras,[1] and Cuiying Chen[1]

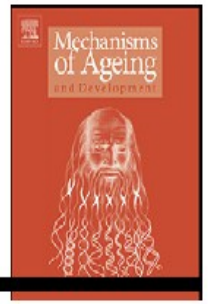
Fare clic per modificare stili del testo dello schema
Secondo livello
Terzo livello
Quarto livello
Quinto livello

Mechanisms of Ageing and Development 130 (2009) 92–97

Contents lists available at ScienceDirect

Mechanisms of Ageing and Development

journal homepage: www.elsevier.com/locate/mechagedev



N-glycan profiles as tools in diagnosis of hepatocellular carcinoma and prediction of healthy human ageing

Vanhooren Valerie[a,b], Liu Xue-En[a,b,e], Franceschi Claudio[c], Gao Chun-Fang[d], Libert Claude[a,b], Contreras Roland[a,b], Chen Cuiying[a,b,*]

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[e] *Department of Microbiology, Peking University Health Science Center, 38 Xue-yuan Road, Haidian District, Beijing 100083, People's Republic of China*

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Quinto livello

Experimental Gerontology 45 (2010) 738–743

Contents lists available at ScienceDirect

Experimental Gerontology

journal homepage: www.elsevier.com/locate/expgero

Serum N-glycan profile shift during human ageing

Valerie Vanhooren [a,b], Sylviane Dewaele [a,b], Claude Libert [a,b], Sebastiaan Engelborghs [c,d,j], Peter Paul De Deyn [c,d,j], Olivier Toussaint [e], Florence Debacq-Chainiaux [e], Michel Poulain [f], Youri Glupczynski [g], Claudio Franceschi [h], Koos Jaspers [k], Ingrid van der Pluijm [i], Jan Hoeijmakers [k], Cuiying Chitty Chen [a,b,*]

[a] *Department for Molecular Biomedical Research, VIB, Technologiepark 927, B-9052 Gent-Zwijnaarde, Belgium*
[b] *Department of Biomedical Molecular Biology, Ghent University, Technologiepark 927, B-9052 Gent-Zwijnaarde, Belgium*
[c] *Reference Center for Biological Markers of Memory Disorders, Laboratory of Neurochemistry and Behavior, University of Antwerp, Universiteitsplein 1, B-2610 Antwerp, Belgium*
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[g] *Cliniques universitaires UCL de Mont-Godinne 5530 Yvoir, Belgium*
[h] *Dept. Experimental Pathology, via S. Giacomo, 12 I-40126 Bologna, Italy*
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An **N-glycan (N-linked oligosaccharide)** is a sugar chain covalently linked to an asparagine residue of a polypeptide chain. The linkage commonly involves a **GlcNAc residue** and the consensus peptide sequence **Asn-X-Ser/Thr.**

Glycans can mediate a wide variety of biological roles by virtue of their mass, shape, charge and other physical properties. It is important to emphasize that unlike protein sequences, which are primary gene products, **glycan chain structures are not encoded directly in the genome** but are secondary gene products.

Glycans are synthesized in many different combinations by a variety of competing and sequentially acting **glycosidases** and **glycosyltransferases** without use of a template

The serum GlycoAgeTest values of a population of healthy Belgian (full lines) and Italian (dotted lines) volunteers of different ages: 20–49 (Belgian $n = 60$, Italian $n = 35$), 50–75 (Belgian $n = 160$, Italian $n = 37$), 76–89 (Belgian $n = 30$, Italian $n = 33$), 90–99 (Belgian $n = 175$, Italian $n = 39$) and ≥ 100 years (only Italian $n = 84$).

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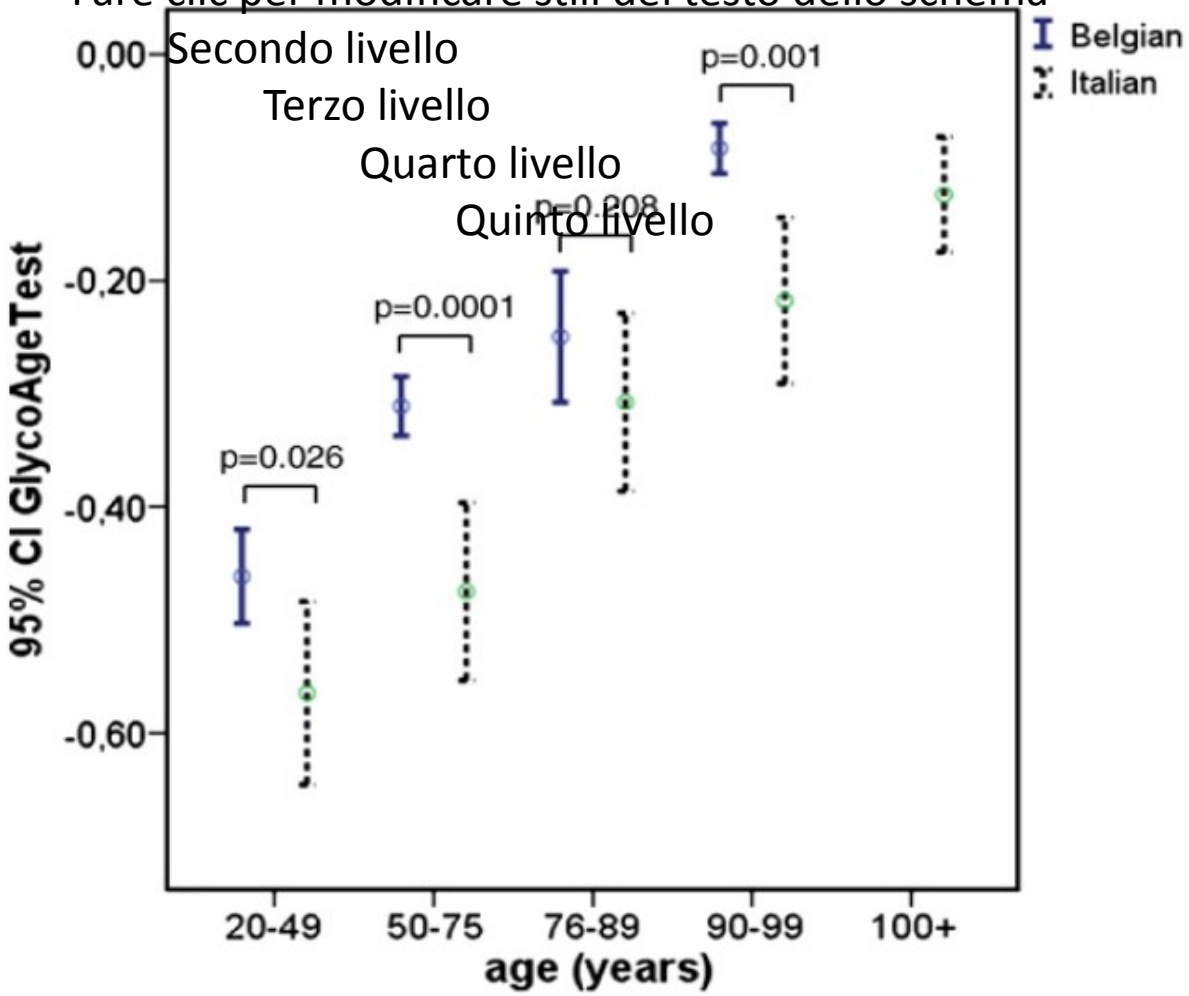
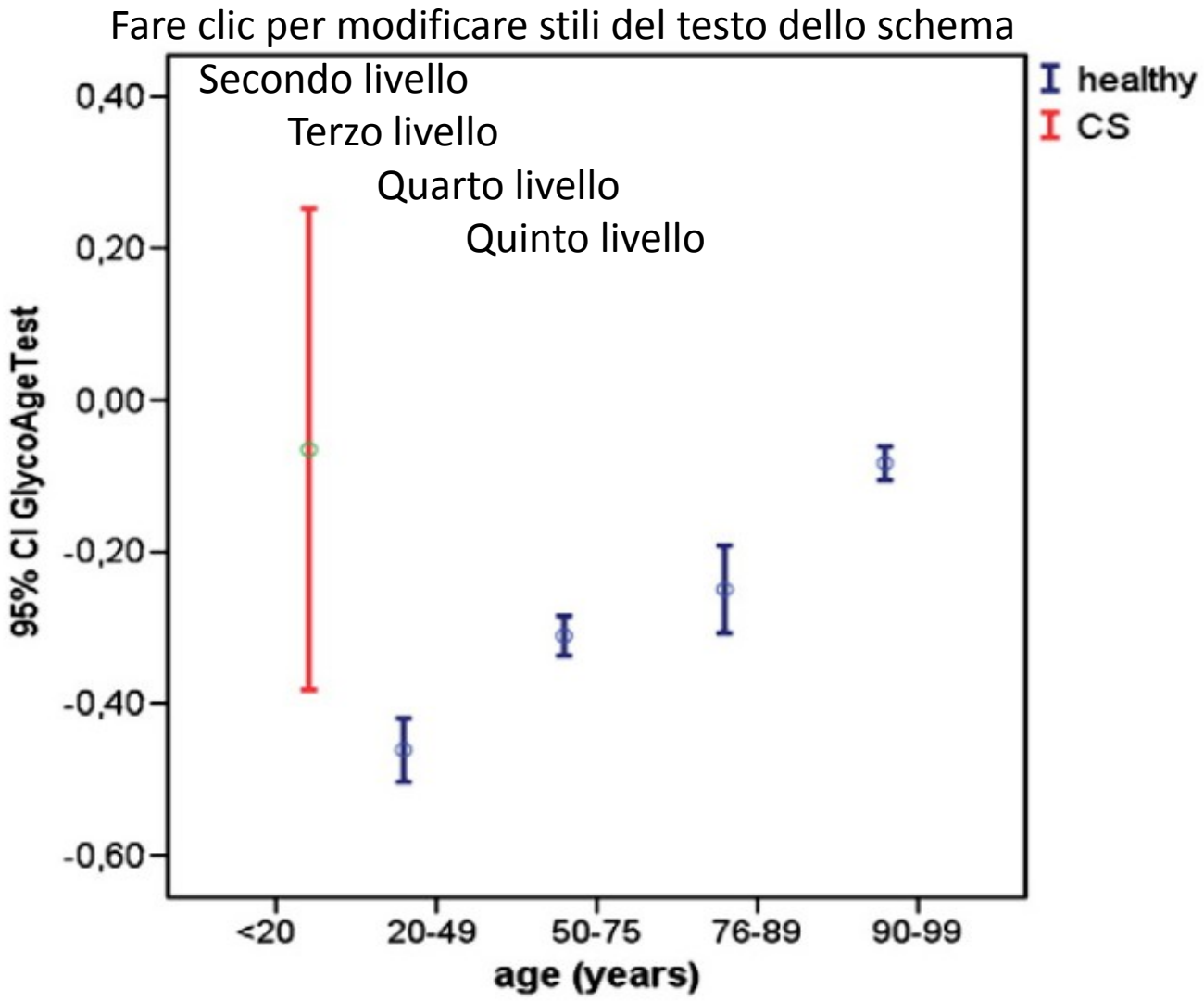
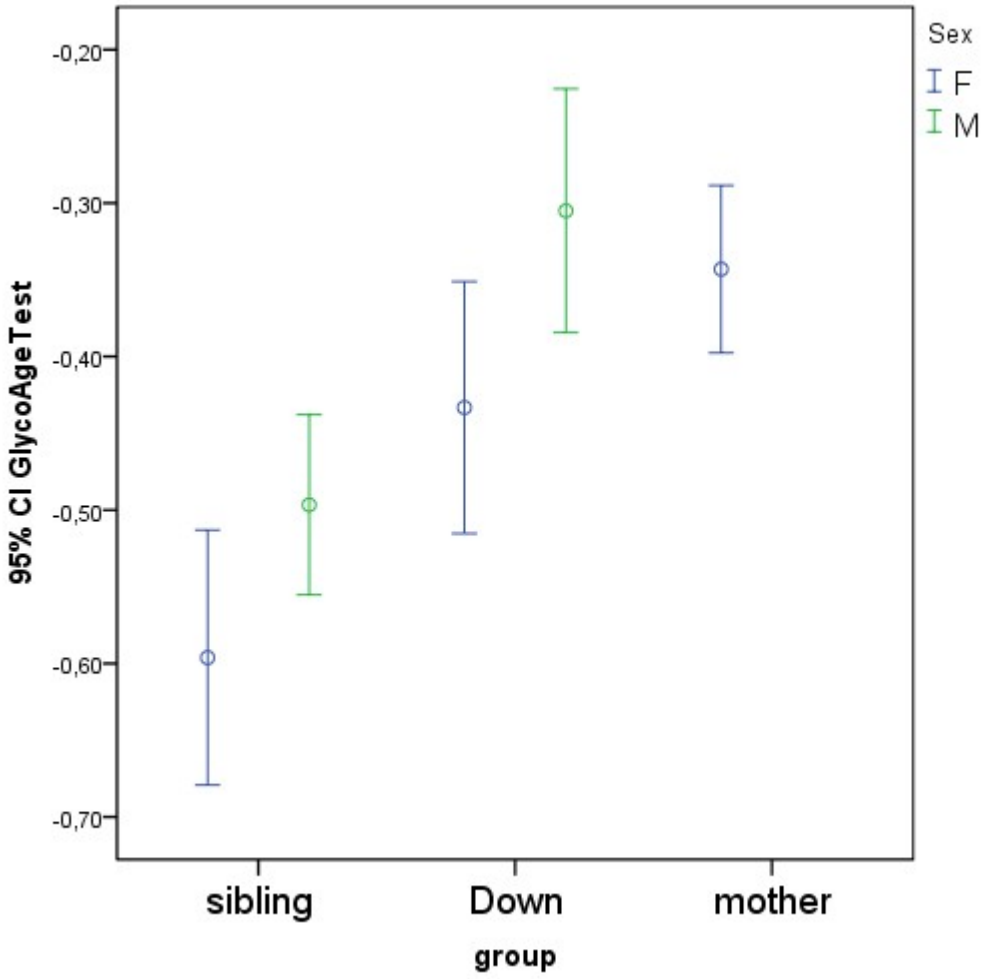


GlycoAge test
in 6 patients with Cockayne syndrome



The GlycoAgeTest value increases faster in Down' Syndrome subjects than in their siblings and is similar to that of their mothers (unpublished results)



n.38 n.52 n.46

mtDNA as an internal source of potent inflammatory molecules

Damage-**A**ssociated **M**olecular **P**atterns (**DAMPs**), are a family of conserved molecules, which can trigger an innate immune response, causing inflammation through the engagement of several TLRs.

Mitochondrial DAMPs (MTDs) can be released by damaged tissues, and are highly present in plasma of patients with systemic inflammatory response syndrome (SIRS).

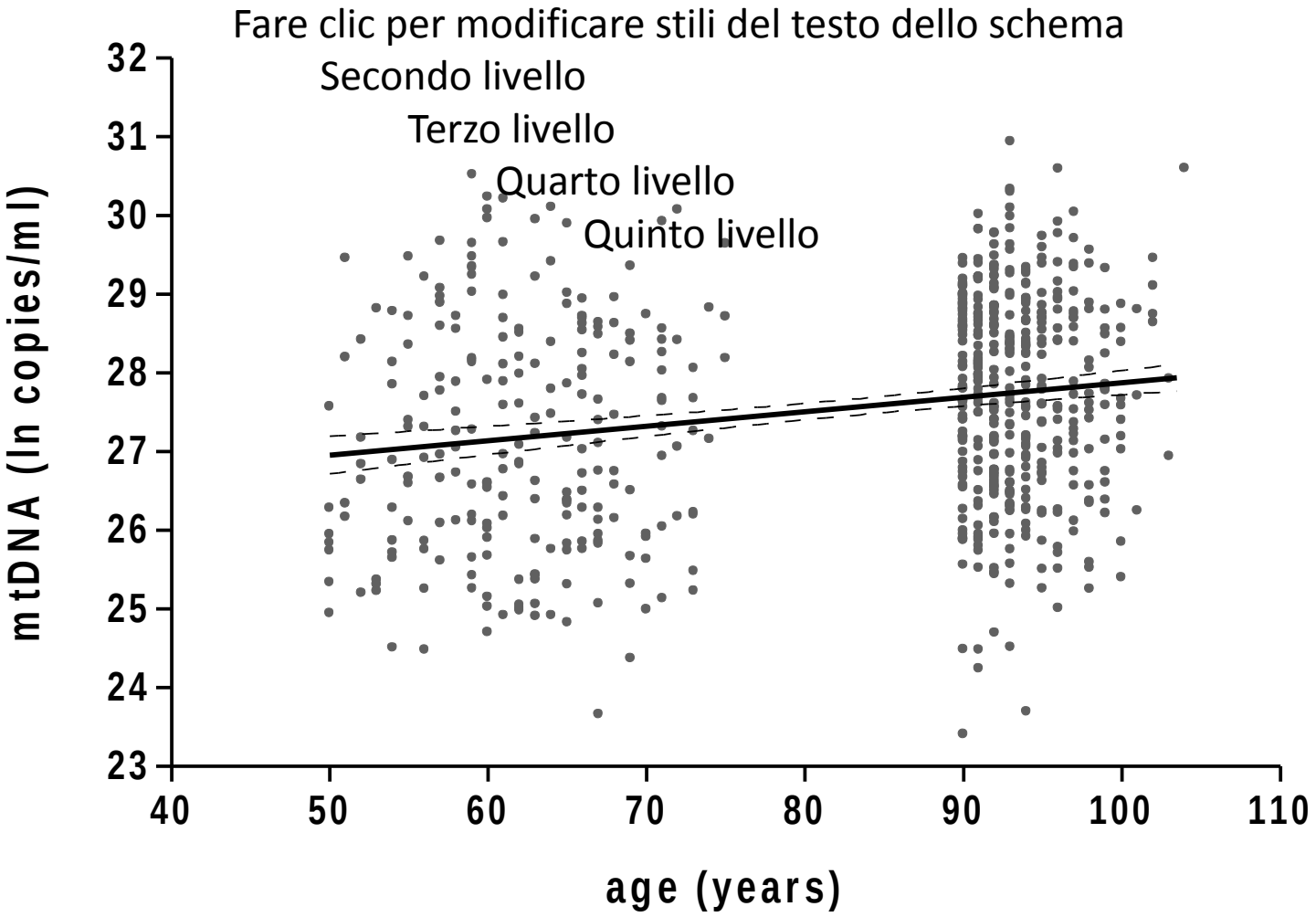
MTDs are represented by **formyl peptides**, that bind the formyl peptide receptor-1 (FPR-1), and **circulating mtDNA**, **that binds TLR-9, whose activation causes a potent inflammatory reaction.**

Circulating mtDNA and inflammation

- To investigate the possible role of extracellular mtDNA in inflammaging, we measured their plasma levels in **232 adults** (50-75 years of age) and in **428 siblings older than 90 years of age (90+),** including 20 centenarians, all geographically and ethnically-matched and belonging to the subjects recruited within the EU Integrated Project **GEHA** (GEnetics of Healthy Ageing, 2004-2010, Coordinator: C. Franceschi) .
- Human longevity clusters in families and **90+ long-living siblings are enriched in longevity genes.**
- The 90+ sibs have been followed for **4 years**

Direct correlation between age and circulating mtDNA content ($p<0.0001$).

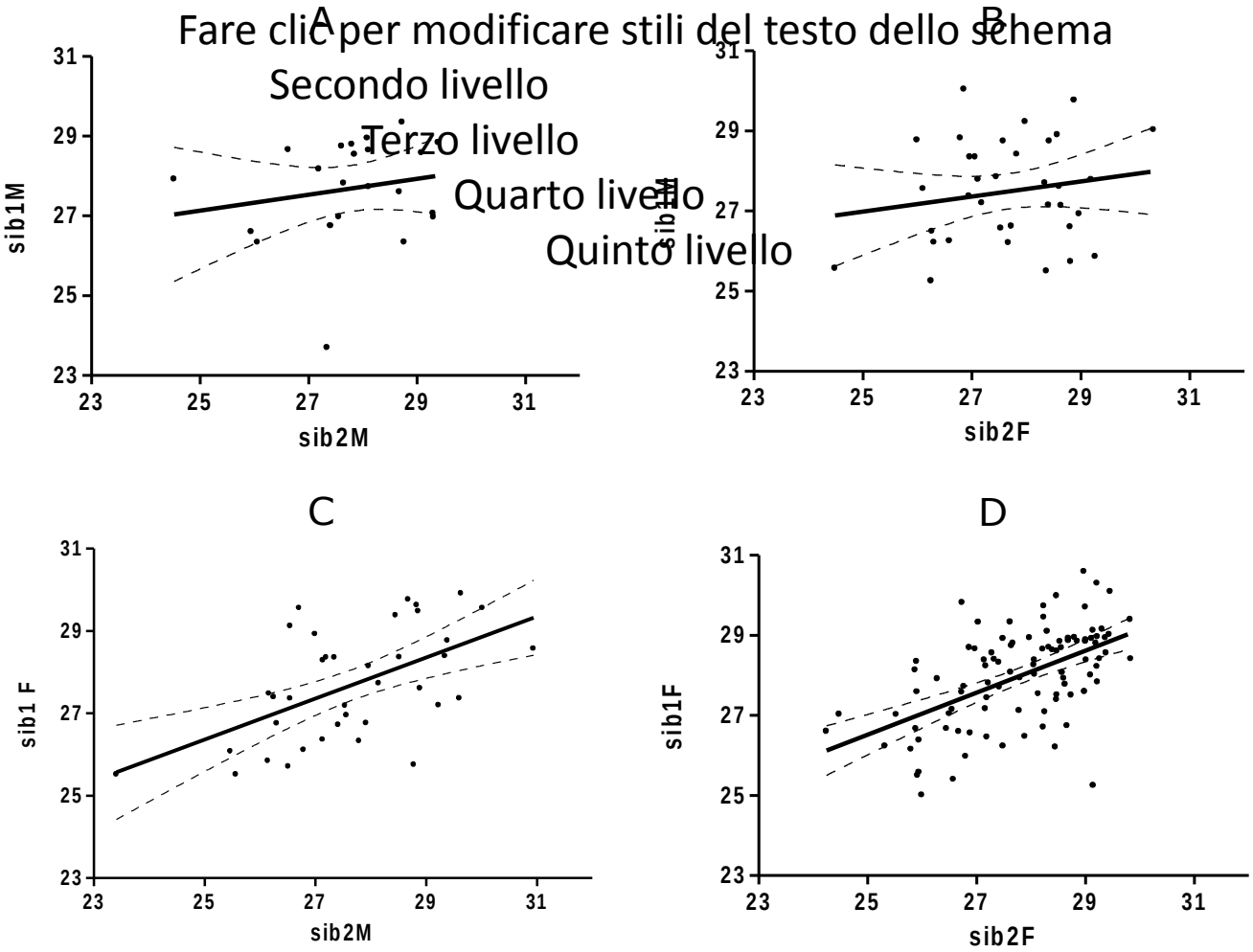
the estimated increase was of 1.0187 ln copies/mL per year.



Linear regression analysis

Correlation in mtDNA content between sib1 and sib2 according to the gender of the couple

A: p=0.4028; B: p=0.3089; **C: p=0.0003; D: p<0.0001**



M=male F=female

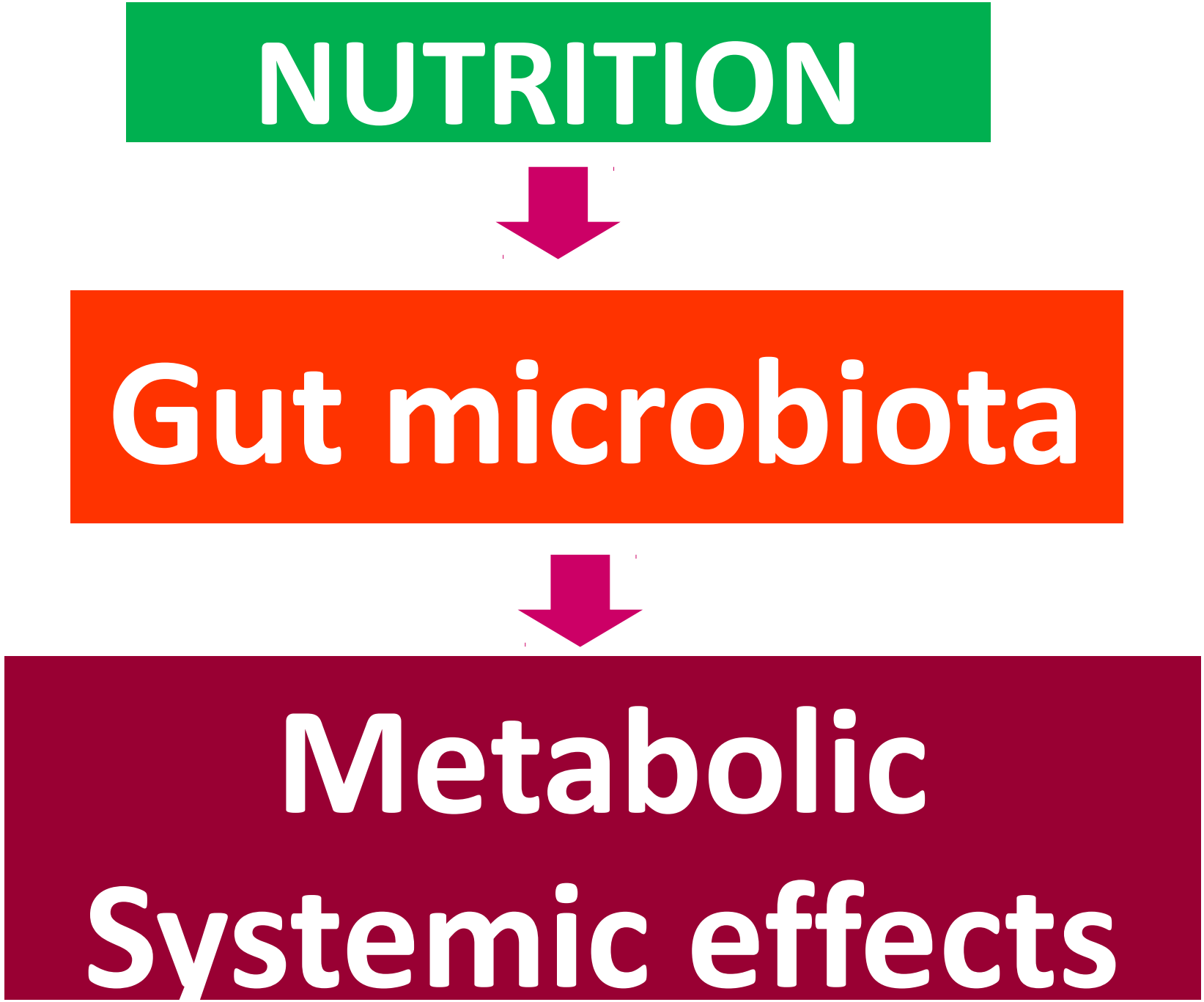
CONCLUSION

- **Circulating mtDNA increases with age**, suggesting that it can contribute to “inflammaging”.

  - The number of copies of circulating mtDNA is significantly correlated between siblings, suggesting that it is **a familial/genetic trait**.

Thanks for your attention

Installation by Claudio Parmigiani


NUTRITION
Gut microbiota
Metabolic
Systemic effects

- Larbi, A. *et al. Aging of the immune system as a prognostic factor for human longevity. Physiol.* ***23, 64–74 (2008).***

Lio, D., F. Licastro, L. Scola, M. Chiappelli, L. M. Grimaldi, A. Crivello, G. Colonna-Romano, G. Candore, C. Franceschi, and C. Caruso. 2003. Interleukin-10 promoter polymorphism in sporadic Alzheimer's disease. Genes Immun. 4: 234–238.

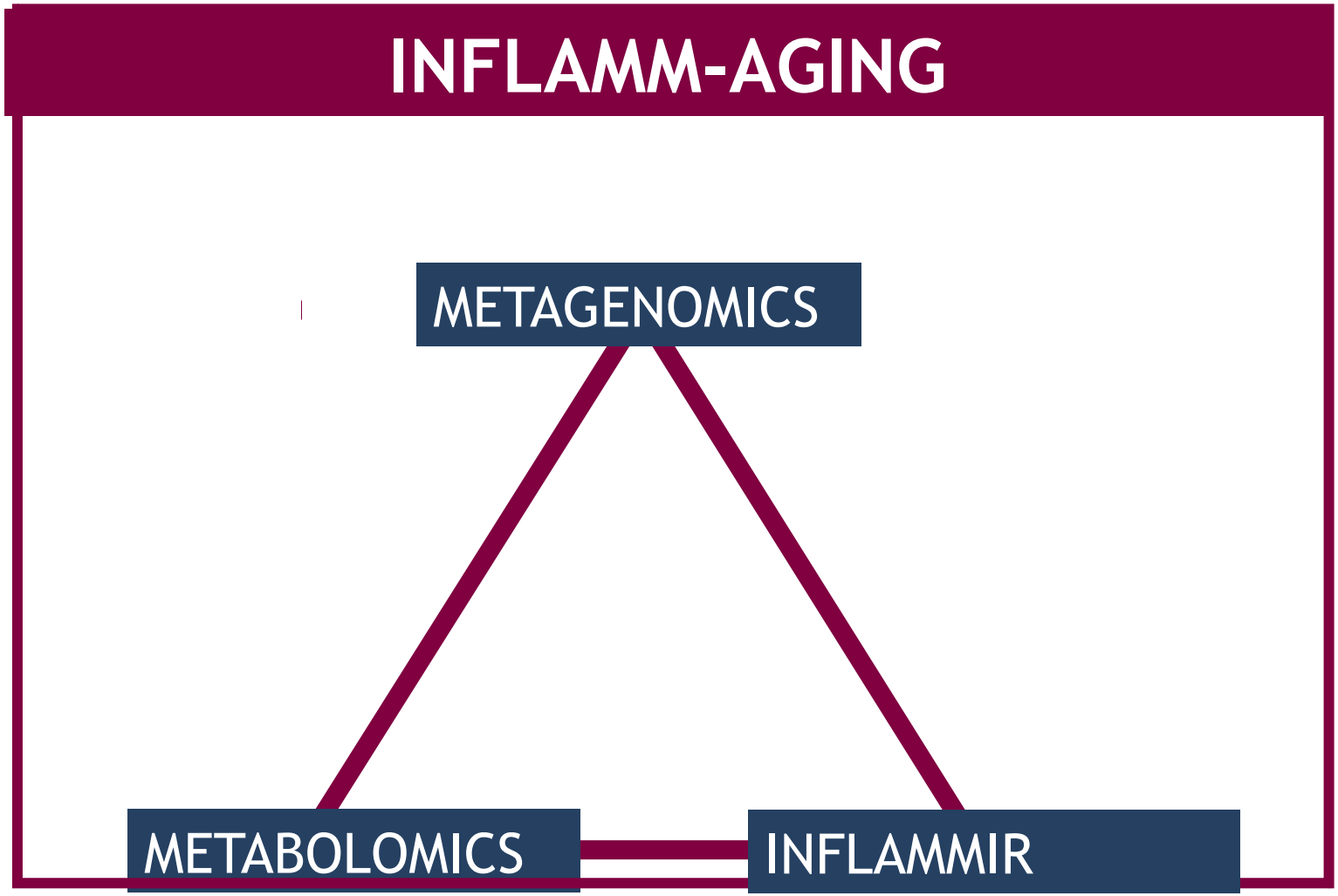
- Caruso, C., D. Lio, L. Cavallone, and C. Franceschi. 2004. Aging, longevity, inflammation, and cancer. Ann. N. Y. Acad. Sci. 1028: 1–13.
- Licastro, F., G. Candore, D. Lio, E. Porcellini, G. Colonna-Romano, C. Franceschi, and C. Caruso. 2005. Innate immunity and inflammation in ageing: a key for understanding age-related diseases. Immun. Ageing 2: 8–22.

- Lio, D., L. Scola, A. Crivello, G. Colonna-Romano, G. Candore, M. Bonafe`, L. Cavallone, C. Franceschi, and C. Caruso. 2002. Gender-specific association between -1082 IL-10 promoter polymorphism and longevity. Genes Immun. 3: 30–33.

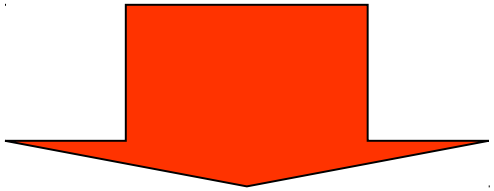
- **Lio, D., F. Licastro, L. Scola, M. Chiappelli, L. M. Grimaldi, A. Crivello, G. Colonna-Romano, G. Candore, C. Franceschi, and C. Caruso. 2003. Interleukin-10 promoter polymorphism in sporadic Alzheimer's disease. Genes Immun. 4: 234–238.**

- **Appropriate nutrition** contributes substancially to healthy aging and longevity
- **Nutrition + physical activity** are the two pillars of any intervention to prevent all major age-related diseases
- **Nutritional re-programming** of the aging process is apparently possible and should be pursued

LOCAL & SYSTEMIC INFLAMMATION

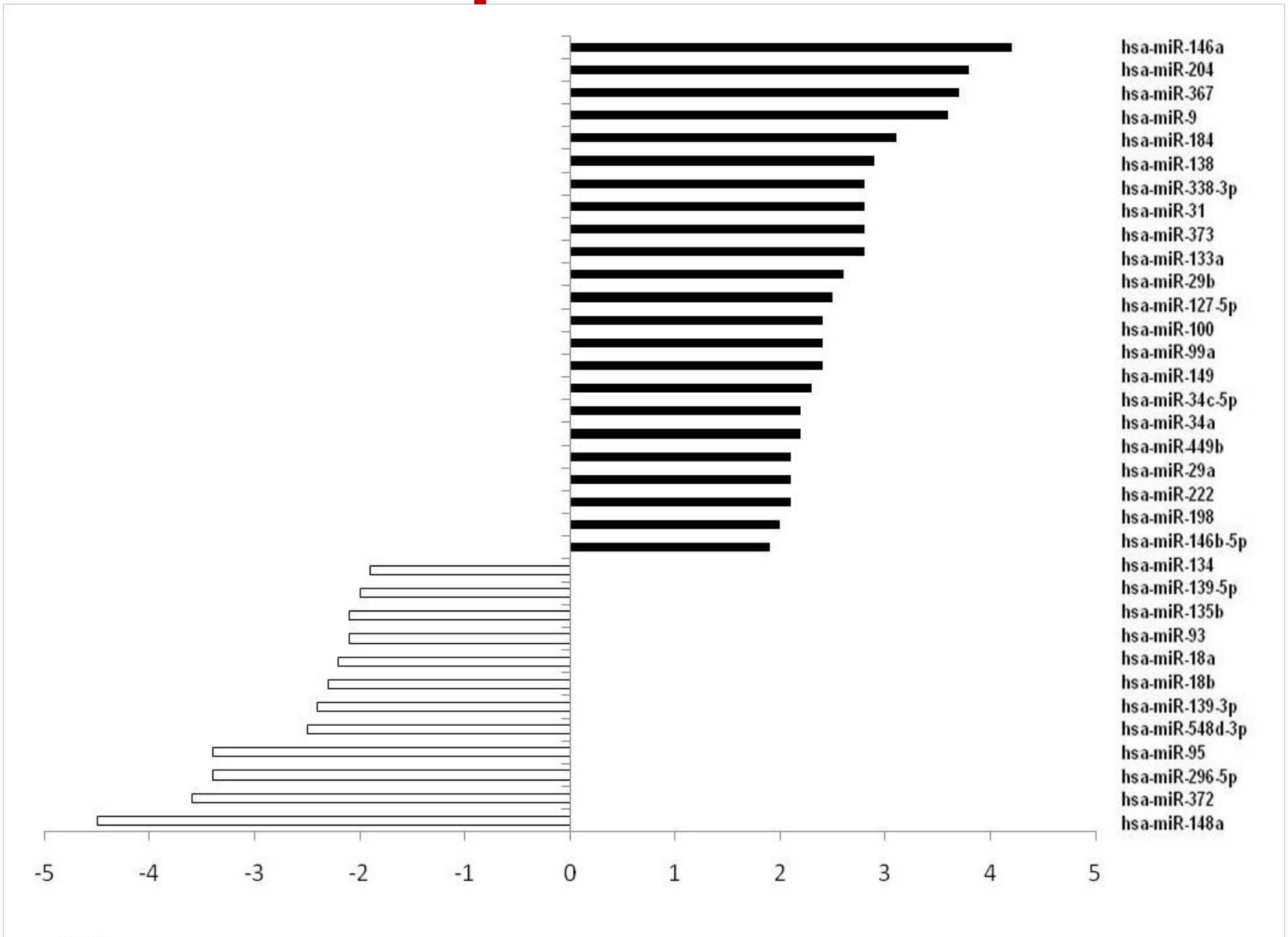


LIFELONG ANTIGENIC LOAD



INFLAMM-AGING

miRNA expression in HUVEC



miRNA expression in HUVEC

hsa-miRs	Fold change (senescent vs. young HUVEC)
hsa-miR-146a	4.25
hsa-miR-204	3.89
hsa-miR-367	3.73
hsa-miR-9	3.68
hsa-miR-184	3.60
hsa-miR-138	3.47
hsa-miR-338-3p	3.39
hsa-miR-31	3.00
hsa-miR-373	2.80
hsa-miR-133a	2.58
hsa-miR-29b	2.53
hsa-miR-127-5p	2.44
hsa-miR-100	2.40
hsa-miR-99a	2.38
hsa-miR-149	2.34
hsa-miR-34c-5p	2.25
hsa-miR-34a	2.25
hsa-miR-449b	2.16
hsa-miR-29a	2.11
hsa-miR-222	2.11
hsa-miR-198	2.07

Available online at www.sciencedirect.com
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Ageing Research Reviews 7 (2008) 83–105
ageing research reviews
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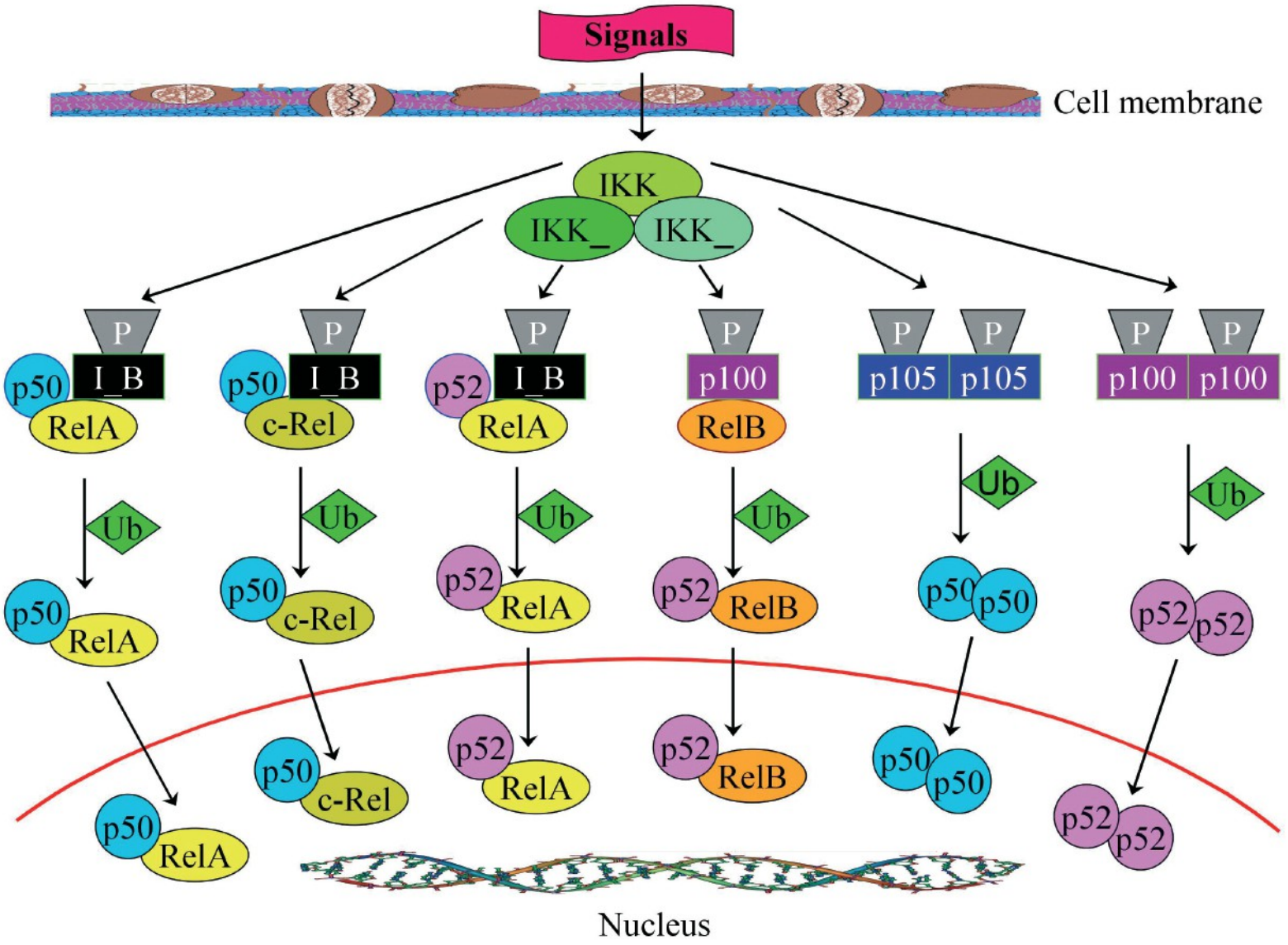
Review

Activation of innate immunity system during aging: NF-kB signaling is the molecular culprit of inflamm-aging

Antero Salminen [a,b,*], Jari Huuskonen [a], Johanna Ojala [a], Anu Kauppinen [a], Kai Kaarniranta [c], Tiina Suuronen [a]

[a] *Department of Neuroscience and Neurology, University of Kuopio, P.O. Box 1627, FIN-70211 Kuopio, Finland*
[b] *Department of Neurology, University Hospital of Kuopio, P.O. Box 1777, FIN-70211 Kuopio, Finland*
[c] *Department of Ophthalmology, University of Kuopio, P.O. Box 1627, 70211 Kuopio, Finland*

NFkB signalling pathway



X-S. Sun and H. Zhang Histol Histopathol (2007) 22: 1387-1398

INTERACTOMICS

the in silico

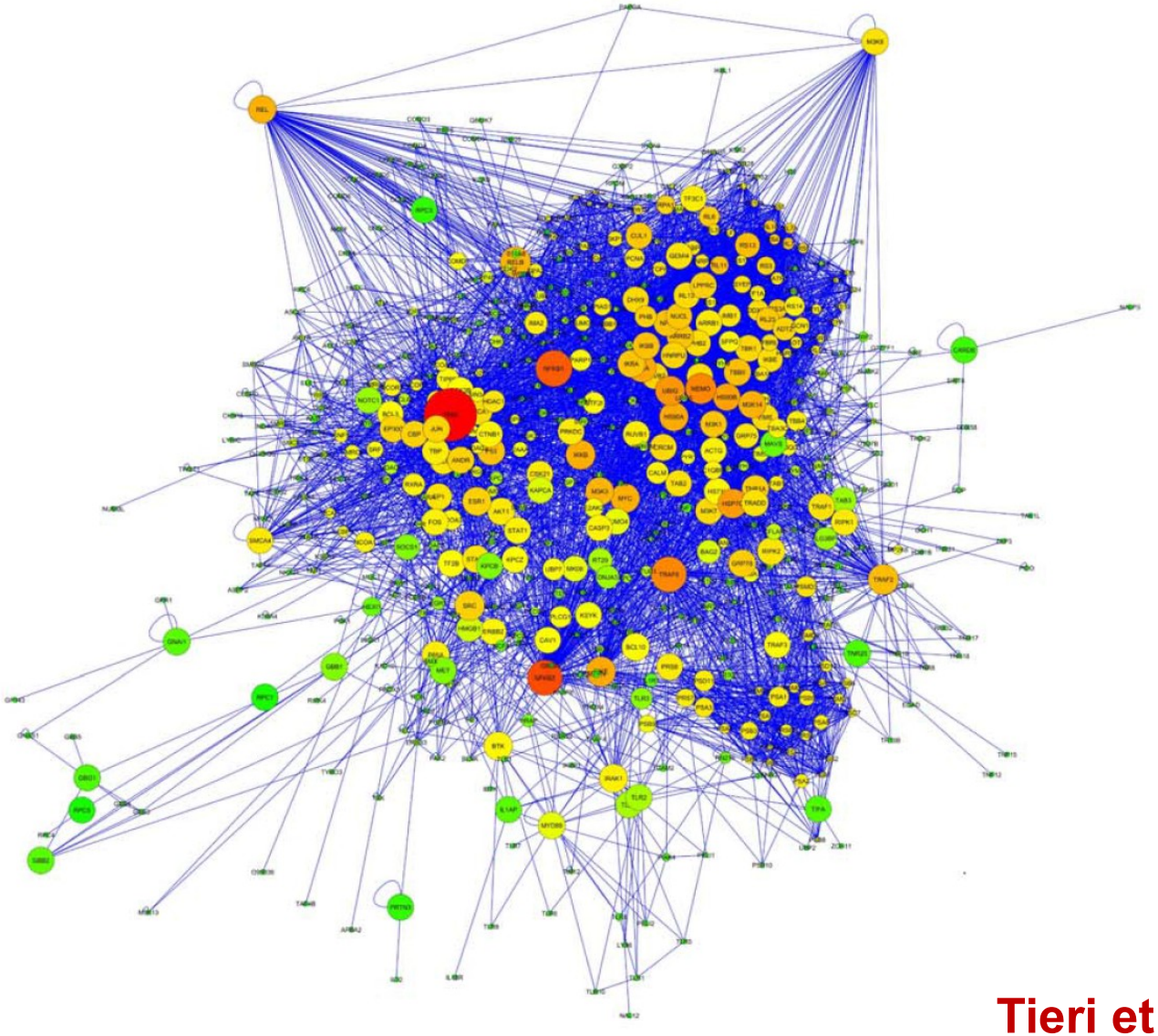
NFKB interactome

the aim is to identify and integrate

ALL THE PROTEINS

that – on the basis of published papers - interact, directly or indirectly, with the proteins activated by the NF-kB transcription factor

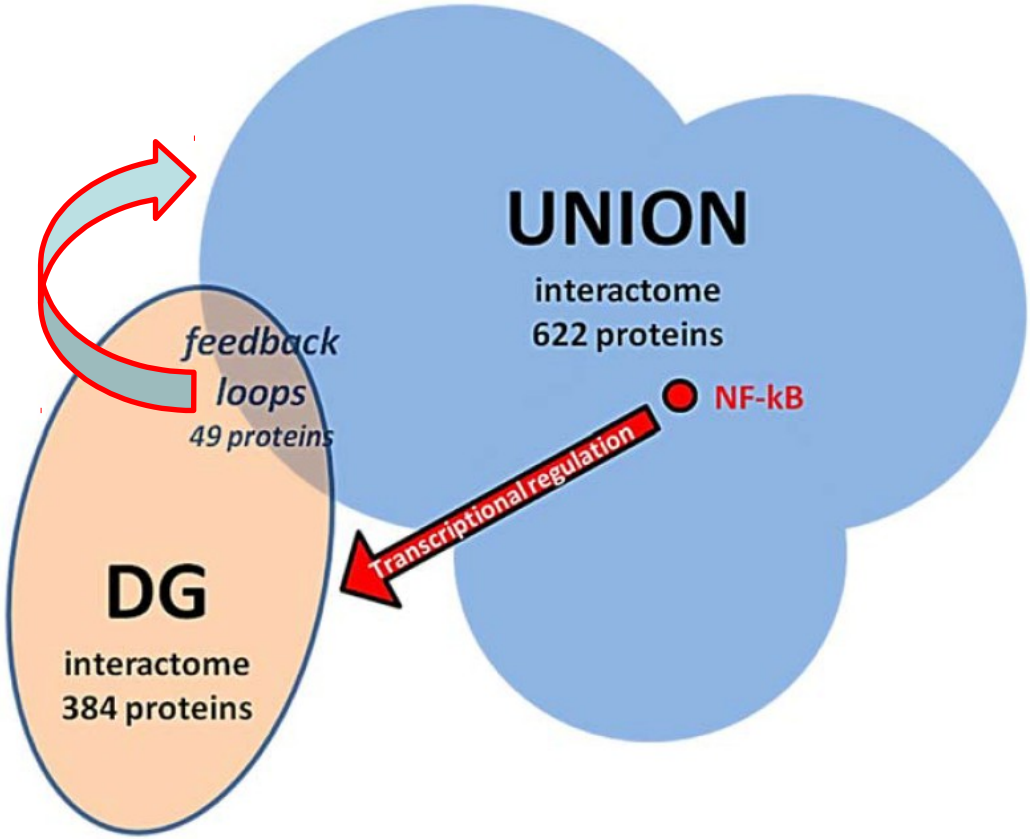
NF-kB INTERACTOME



Tieri et al., 2012

Forty nine proteins are shared by both the UNION and the DG sets, establishing a feedback loop: "*interaction with NF-κB pathway → transcription factor activation → transcriptional regulation → interaction with NF-κB pathway*",

DG = Dowstream Gene dataset

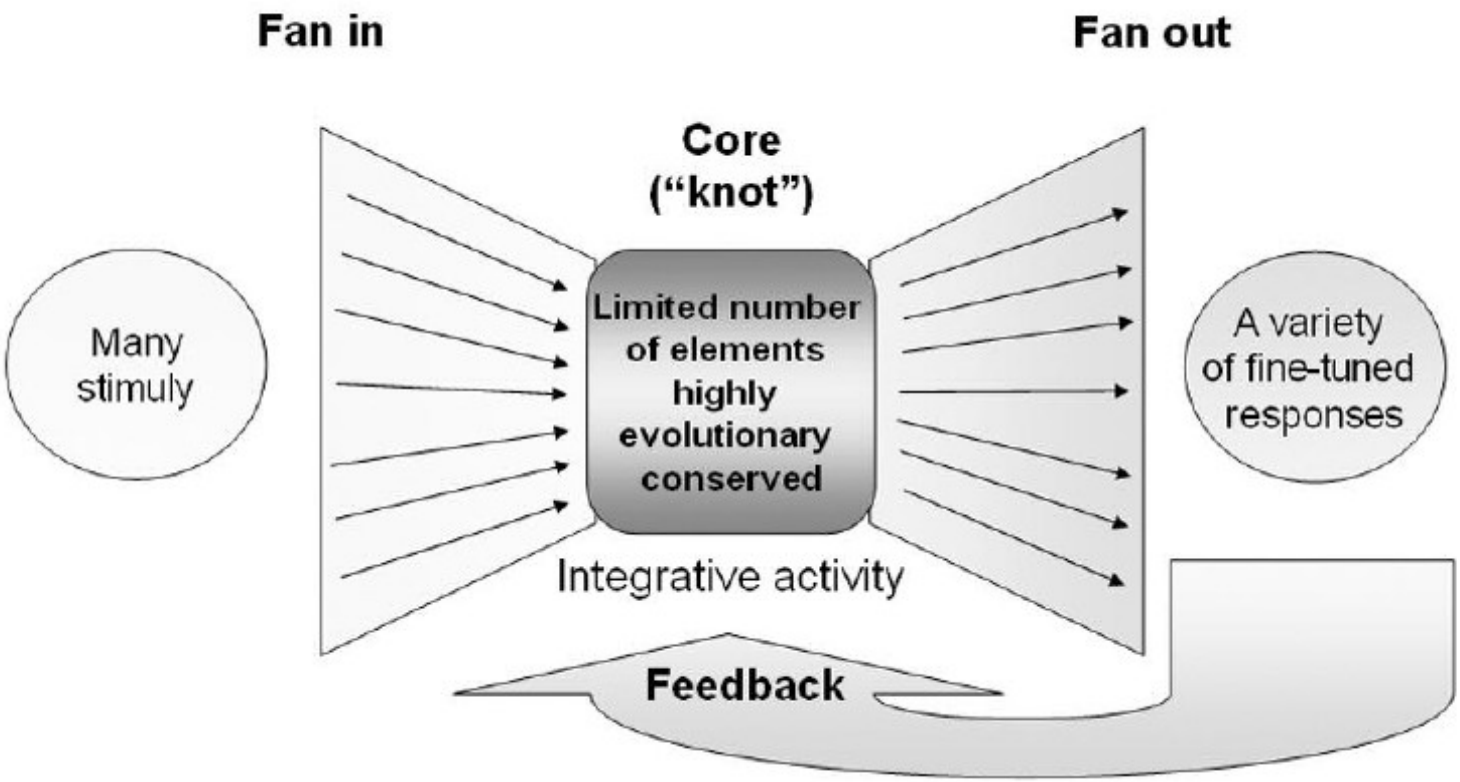


NF-kB interactome has a bow-tie architecture

Tieri et al.,

BOW TIE FUNCTIONAL ARCHITECTURE

OPTIMIZATION ROBUSTNESS EVOLVABILITY



Ottaviani, Capri and Franceschi, *BioEssays* 2008