Alzheimer and DNA methylation

?Hyperhomocysteinemia is associated with LOAD *(risk factor or consequence?)*

(McCaddon et al., 1998; Seshadri et al., 2002)

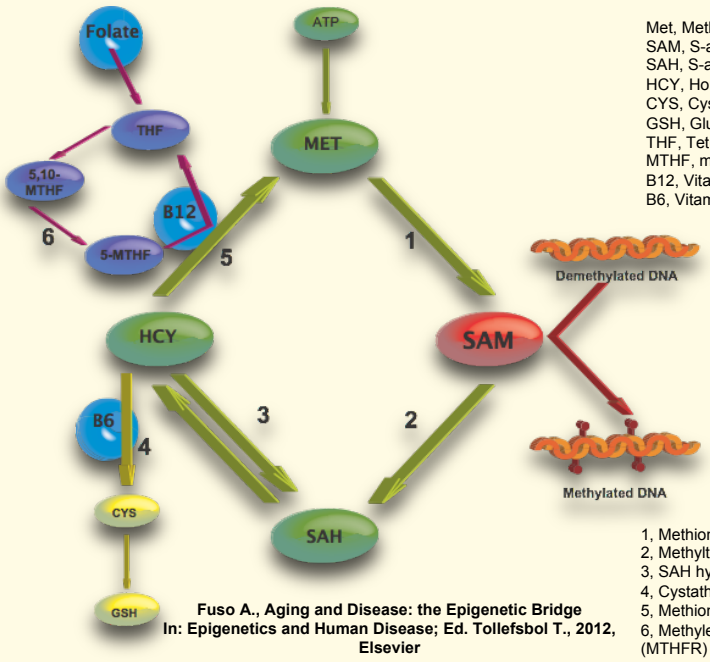
?Impairment of Methylation Potential (MP) in ageing, particularly in LOAD

(Bottiglieri and Hyland, 1994; Morrison et al., 1996)

?Low Folate, vitamin B12 and vitamin B6 in LOAD
(Selhub et al., 2000)

Hyperhomocysteinemia (HHcy) ⋯⋯ **???** ⋯⋯▶ **Alzheimer**

One-carbon metabolism and methylation



Met, Methionine
SAM, S-adenosylmethionine
SAH, S-adenosylhomocysteine
HCY, Homocysteine
CYS, Cystathionine
GSH, Glutathione
THF, Tetrahydrofolate
MTHF, methyltetrahydrofolate
B12, Vitamin B12
B6, Vitamin B6

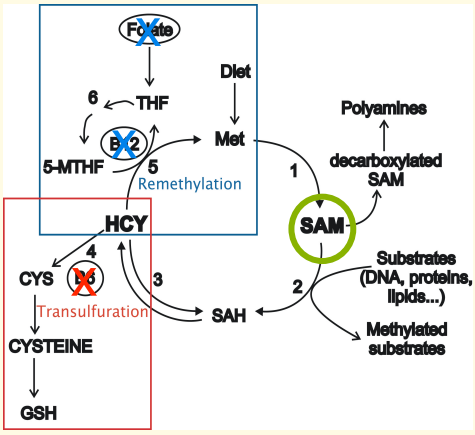
Fuso A., Aging and Disease: the Epigenetic Bridge In: Epigenetics and Human Disease; Ed. Tollefsbol T., 2012, Elsevier

1, Methionine adenosyltransferase (MAT)
2, Methyltransferase(s)
3, SAH hydrolase
4, Cystathionine-β-synthase (CBS)
5, Methionine synthase
6, Methylenetetrahydrofolate reductase (MTHFR)

Alzheimer and DNA methylation: hypothesis

- HCY involved in methylation metabolism
- DNA methylation regulates gene expression

Experimental model



TgCRND8 mice carrying double mutated (Swe/Ind) human APP transgene.

- Rapid Amyloid plaque deposition starting at 2-3 months of age
- Normal endogenous PSEN1 gene
- No deposition of Tau fibrils

	Folate (mg/kg)	Vitamin B12 (mg/kg)	Vitamin B6 (mg/kg)
Control diet (AIN-93M)	1,98	0,025	7
B vitamin deficient diet	0,11	0,0018	0,85

Fuso et al., NBA 2010

C1 metabolites in TgCRND8 mice

		Brain			Blood	
		SAM (nmol/g)	SAH (nmol/g)	SAM/SAH ratio	SAM (μM)	Hcy (μM)
TgCRND8	Ctrl	39.6±1.6	80.0±1.0	0.47±0.08	10.0±3.0	0.41±0.57
	B def	52.0±6.8	190.0±30.0 (p<0.05)	0.32±0.09	11.0±7.8	118.75±65.8 (p<0.05 vs Ctrl)
129Sv	Ctrl	70.0±20.0	90.0±10.0	0.63±0.08	7.0±1.0	2.04±0.56
	B def	28.0±7.0	250.0±70.0 (p<0.05 vs Ctrl)	0.16±0.1 (p<0.01 vs Ctrl)	9.0±5.0	90.68±34.08 (p<0.05 vs Ctrl)

B vitamin deficiency induces hyperhomocysteinemia and brain SAH accumulation

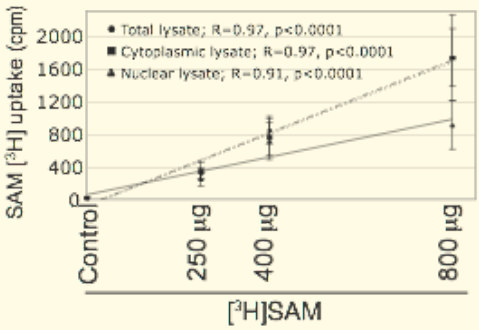
Fuso et al., NBA 2012

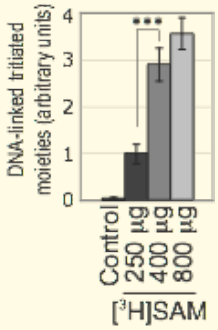
SAM uptake

	Brain	Blood
	SAM (nmol/g)	SAM (μM)
Ctrl	39.6±1.6	10.0±3.0
Ctrl+SAM	175.9±46.4 (p<0.05 vs. Ctrl)	51.66±11.4 (p<0.05 vs Ctrl)
B def	52.0±6.8	11.0±7.8
B def+SAM	127.0±45.6 (p<0.05 vs B def)	60.37±13.7 (p<0.05 vs B def)

Measured by:

- **HPLC in brain and blood**
- **Tritiated SAM in brain lysates**
- **Tritiated CH_3 in brain DNA**

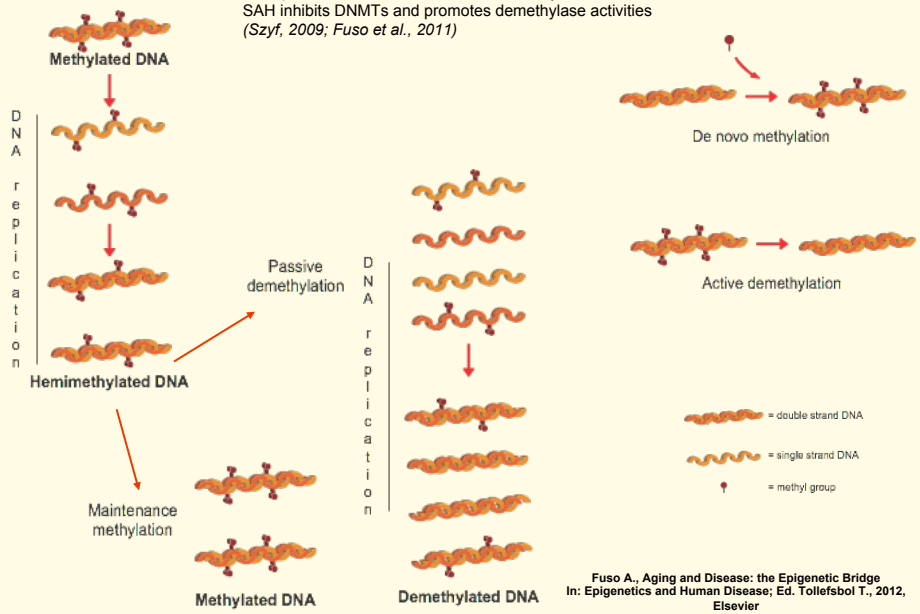




Fuso et al., NBA 2012

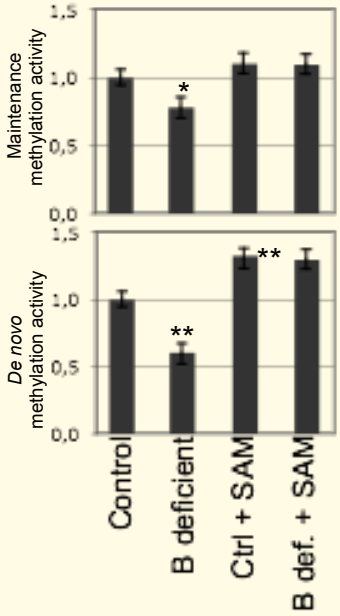
DNA methylation machinery

SAM promotes DNMTs and inhibits demethylase activities
SAH inhibits DNMTs and promotes demethylase activities
(Szyf, 2009; Fuso et al., 2011)

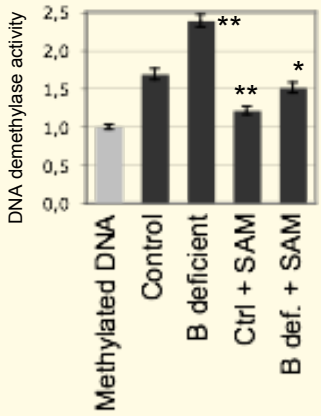


Fuso A., Aging and Disease: the Epigenetic Bridge
In: Epigenetics and Human Disease; Ed. Tollefsbol T., 2012, Elsevier

DNA methylase and demethylase activity in mice brain



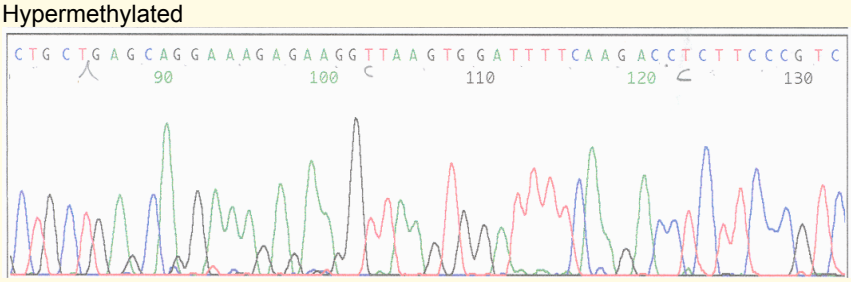
In vitro emimethylated, non methylated and H^3-methylated DNA is mixed to nuclear extracts from TgCRND8 mice brain to asses, respectively, maintenance and de novo methylation and demethylation.



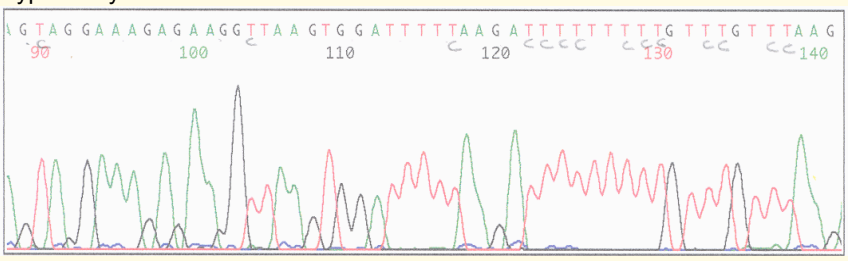
Fuso et al., JNB 2010

Bisulfite assay and sequencing (experimental samples)

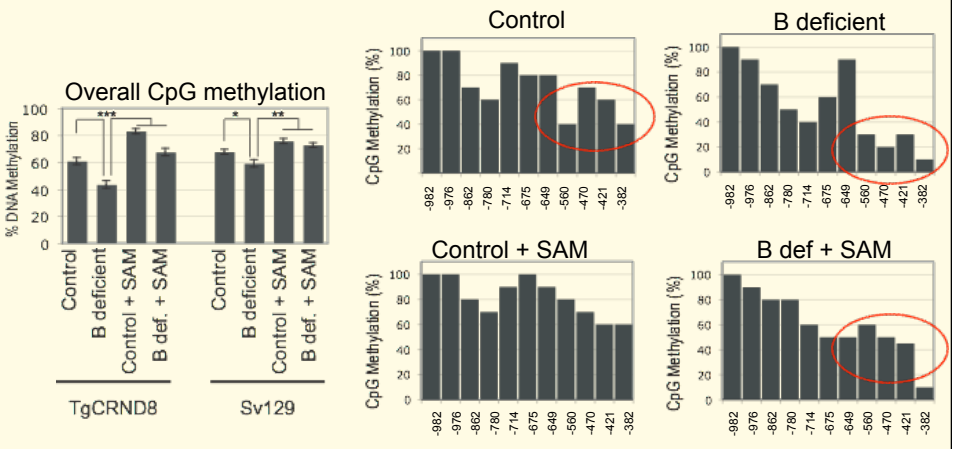
Hypermethylated



Hypomethylated

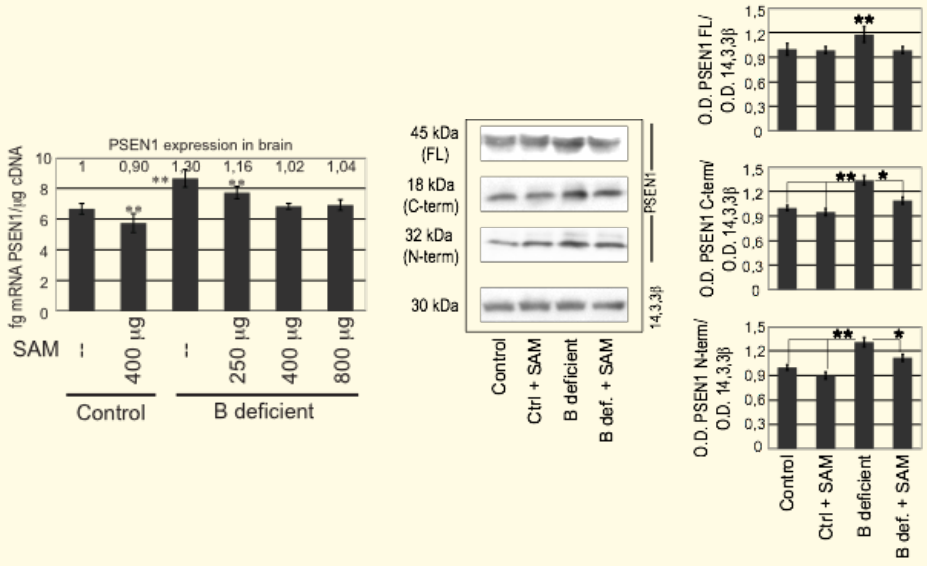


PSEN1 methylation



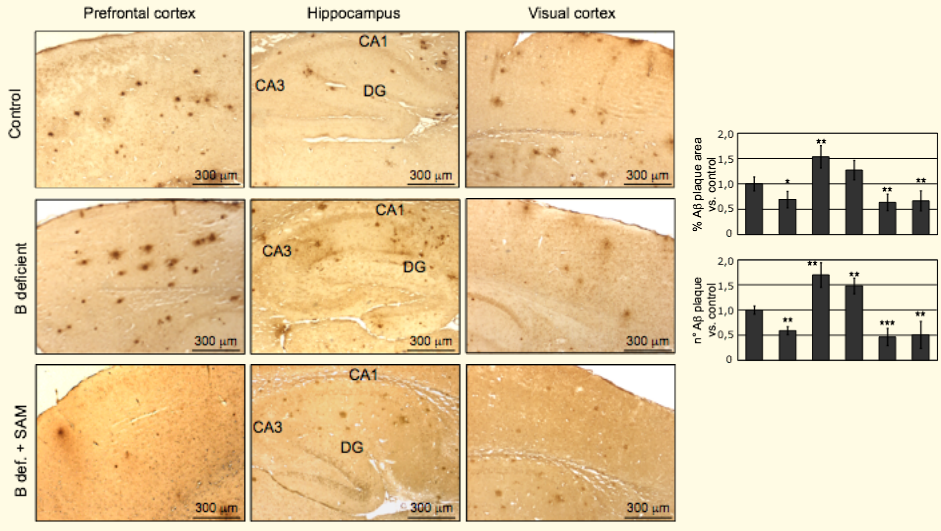
Fuso et al., NBA 2011

PSEN1 expression



Fuso et al., NBA 2012

Amyloid plaques



Fuso et al., NBA 2012

PSEN1 methylation in old (10 mo.) TgCRND8 mice

Figure 1

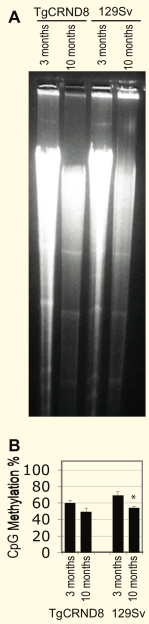
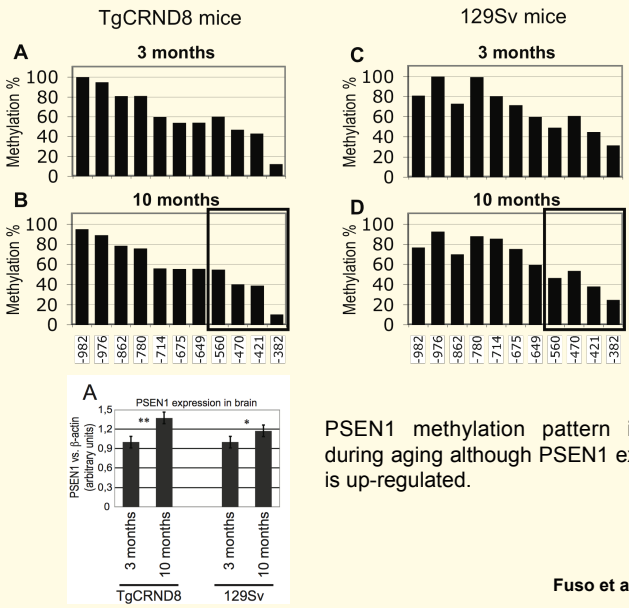


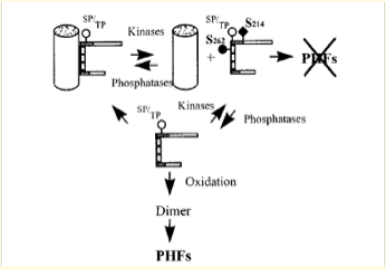
Figure 2

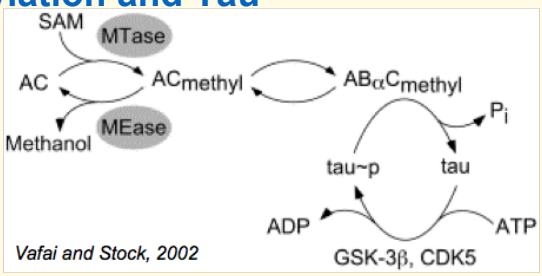


PSEN1 methylation pattern is stable during aging although PSEN1 expression is up-regulated.

Fuso et al., CAR 2011

Methylation and Tau





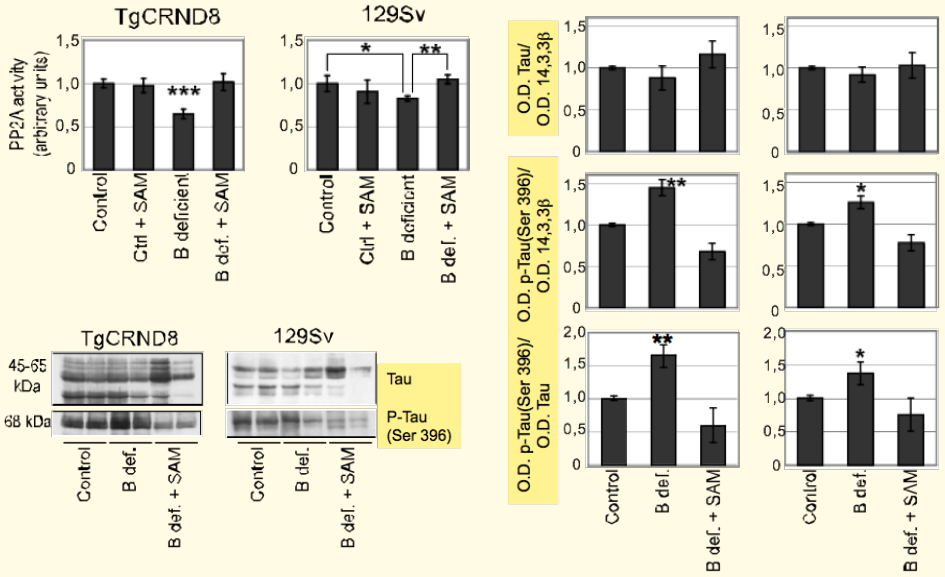
PP2A methylation promotes phosphatase activity and Tau dephosphorylation

•Downregulation of neuronal PPMT and PP2A methylation occur in affected brain regions from AD patients.

•Reduced PP2A methylation promotes the accumulation of both phosphorylated tau and APP isoforms and increased secretion of amyloid-ß peptides.

•S-adenosylmethionine enhance PP2A methylation. This leads to the accumulation of dephosphorylated tau and APP and increased secretion α-secretase-cleaved APP fragments.

Tau phosphorylation in TgCRND8 mice



Nicolia et al., JAD 2010

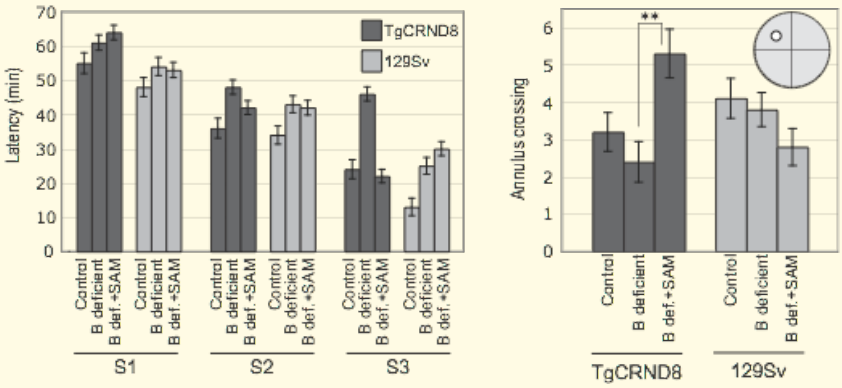
Water-maze test: training

Fuso et al., NBA 2012

Water-maze test: trained mice (SAM supplemented, obviously!!!)

Fuso et al., NBA 2012

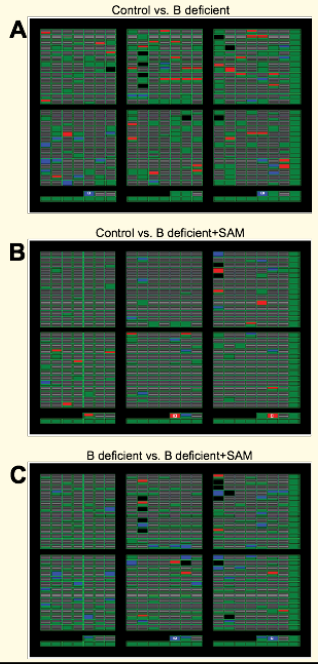
Cognitive improvement with SAM



SAM supplementation improves cognitive performance in the cued task of the water maze test.

Fuso et al., NBA 2012

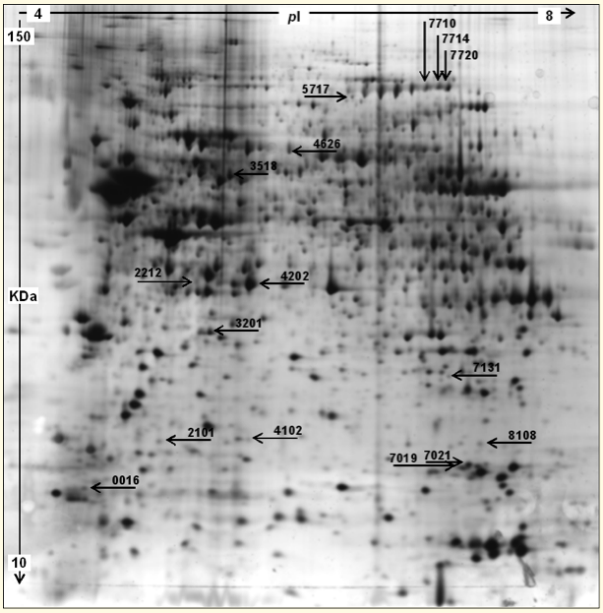
Transcriptomic analysis in mice brain

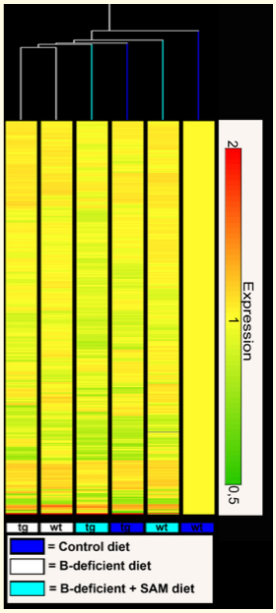


Only 1% of genes on the array are modulated!!!

Fuso et al., NBA 2012

Proteomic analysis in mice brain



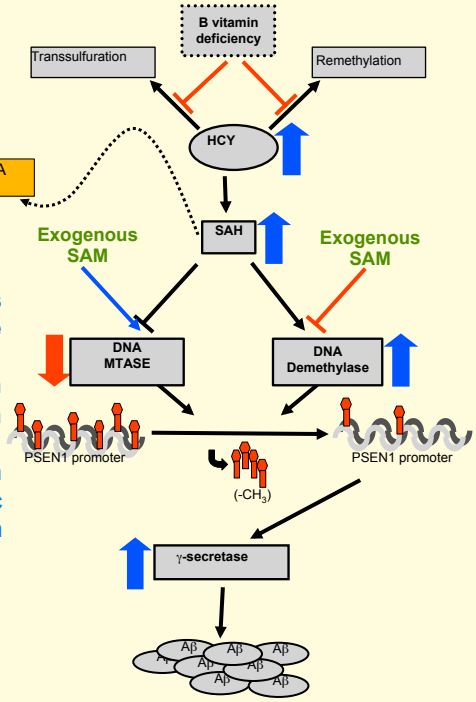


Only 16 proteins are modulated!!!

Borro et al., JAD 2010

C1 metabolism alteration in AD

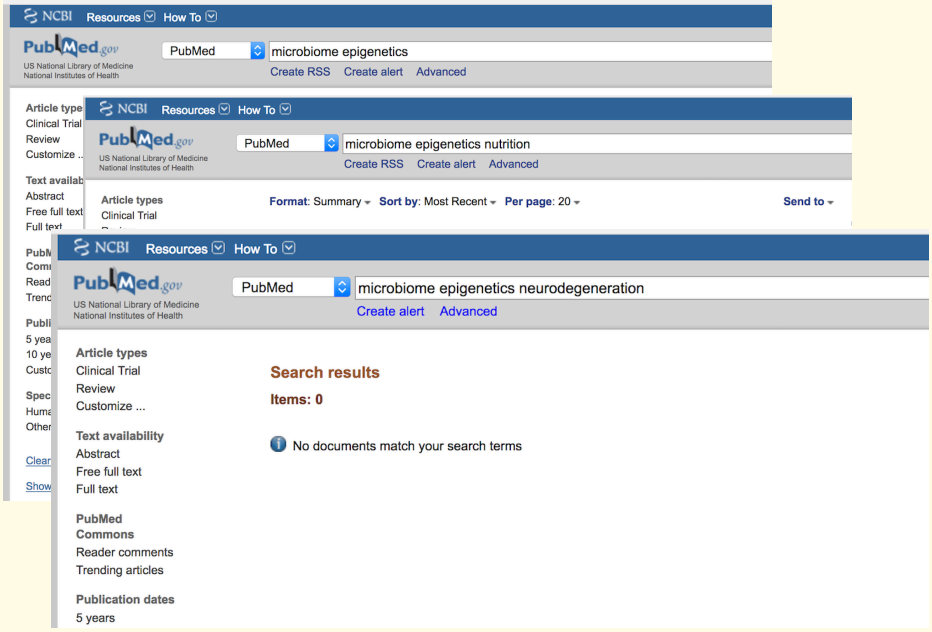
- SAM supplementation restores methyltransferase/demethylase homeostasis. As a consequence, control levels of PSEN1 methylation and expression and Aβ production were restored
- SAM or B vitamin supplementation could be considered as therapeutic or, at least, co-adjuvant agent in LOAD treatment and prevention



NUTRITION, EPIGENETICS AND NEURODEGENERATION…

…AND THE GUT MICROBIOME

Microbiome-epigenetics-neurodegeneration



Microbiota regulates brain development, aging and neurodegeneration

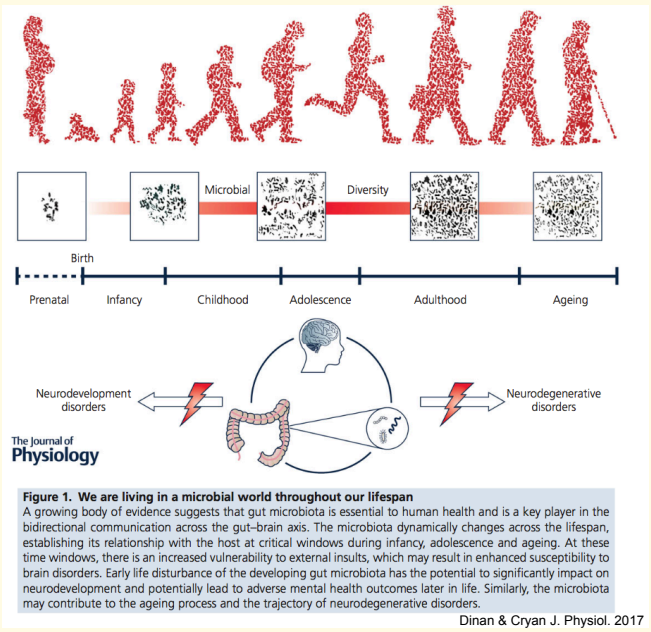
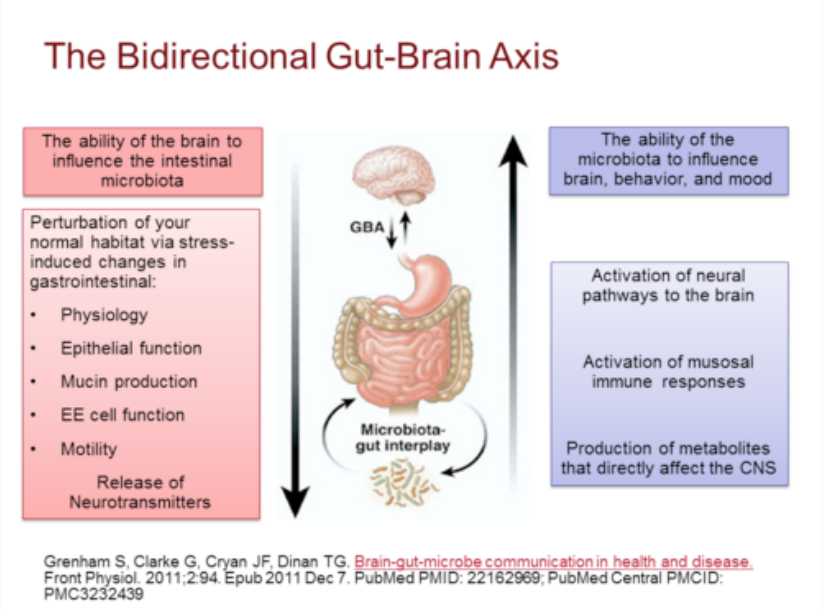


Figure 1. We are living in a microbial world throughout our lifespan
A growing body of evidence suggests that gut microbiota is essential to human health and is a key player in the bidirectional communication across the gut–brain axis. The microbiota dynamically changes across the lifespan, establishing its relationship with the host at critical windows during infancy, adolescence and ageing. At these time windows, there is an increased vulnerability to external insults, which may result in enhanced susceptibility to brain disorders. Early life disturbance of the developing gut microbiota has the potential to significantly impact on neurodevelopment and potentially lead to adverse mental health outcomes later in life. Similarly, the microbiota may contribute to the ageing process and the trajectory of neurodegenerative disorders.

Dinan & Cryan J. Physiol. 2017

The Gut-Brain axis



Grenham S, Clarke G, Cryan JF, Dinan TG. Brain-gut-microbe communication in health and disease. Front Physiol. 2011;2:94. Epub 2011 Dec 7. PubMed PMID: 22162969; PubMed Central PMCID: PMC3232439

The Gut-Brain axis and the epigenetics

- Epigenetic changes are responsible for normal/aberrant development and for disease onset
- Epigenome is a dynamic entity, modulated by particular stimuli.
- Nutrition and diet can influence development and aging. Dietary exposures can have consequences even many years later.
- The gut microbiota is now known to have a crucial role in the development and functionality of innate and adaptive immune responses and in regulating gut motility, intestinal barrier homeostasis, nutrient absorption and fat distribution.
- Impact of enteric microbiota on brain and behavior = the gut–brain axis
- Diet can substantially affect microbiota composition, in a sort of mutual regulation existing between colonizing microbiota and the host organism.
- **Microbiota can influence the epigenetic signature, particularly the DNA methylation, by altering nutrient absorption (Cryan, 2012). Colonization by different microbiota can result in differential gut development and regulate nutrient absorption.**
- **Low-molecular-weight substances of indigenous gut microbiota origin should be considered one of the main endogenous factors actively participating in epigenomic mechanisms that are responsible for the mammalian genome reprograming**

Gut microbiome influences epigenetic marks

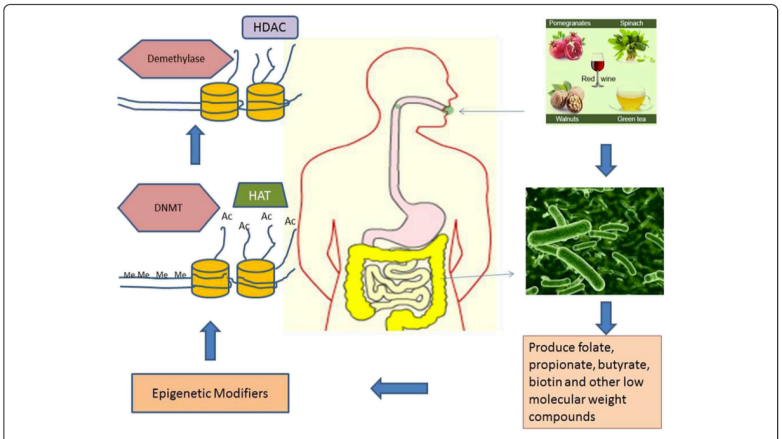


Fig. 2 The molecular interaction of the gut microbiota is greatly influenced by the dietary compounds consumed. The microbes residing in the human gut produce a number of low molecular weight molecules such as butyrate, folate, propionate, and biotin. These compounds either directly bring about epigenetic modifications such as changes in DNA methylation and histone acetylation or indirectly act via activation or inhibition of certain enzymes such as DNMTs, HDACs, or even hTERTs. *Me* DNA methylation, *Ac* histone acetylation

Gut microbiome alterations in disease

Table 1 Alteration of gut microbiome in human diseases

Disease	Microbiome alteration	Reference
Irritable bowel syndrome	Increased ratio of the *Firmicutes* to *Bacteroidetes*	(Rajilić-Stojanović, Biagi et al. 2011) [70]
Crohn's disease	Increased *Clostridium* species, *Ruminococcus torques*, and *E. coli*	(Martinez-Medina, Aldeguer et al. 2006) [53]
Gastric cancer	*H. pylori* induces production of pro-inflammatory cytokines	(Tsuji, Kawai et al. 2003) [88]
Colorectal cancer	Abundance of *Fusobacteria* and *Coriobacteria*	(Castellarin, Warren et al. 2012) [9]
Obesity	Reduced ratio of *Bacteroidetes* to *Firmicutes*	(Ley, Bäckhed et al. 2005) [44]
Type 1 diabetes	Altered gut permeability to mannitol and lactulose	(Kuitunen, Saukkonen et al. 2002) [41]
Atherosclerosis	Metabolism of phospholipids by gut microbiota to trimethylamine-*N*-oxide	(Loscalzo 2011) [48]
Rheumatoid arthritis	Less *Bifidobacteria* and bacteria of the *Bacteroides-Porphyromonas-Prevotella*	(Vaahtovuo, Munukka et al. 2008) [90]
Autism	Higher number of *Clostridium* species known to produce tetanus neurotoxin (TeNT)	(Parracho, Bingham et al. 2005) [64] (Bolte 1998) [4]
Chronic fatigue syndrome	Lower levels of *Bifidobacteria* and small-intestinal bacterial overgrowth	(Logan, Venket Rao et al. 2003) [47]
Alzheimer's disease	Excess ammonia production by gut microbiota	(Samsel and Seneff 2013) [76]

Genes involved in regulation of gut function and maturation (**APC, Sfrp1, DKK1**) and in neurodevelopment (**BDNF, Reelin**), and that are already known to be modulated by DNA methylation (Licchesi 2008, Matrisciano 2011).

Acknowledgements

Sapienza University of Rome

Dept. of Surgery "P. Valdoni"
- Sigfrido Scarpa
- Sara Cavallaro
- Vincenzina Nicolia
- Alessandra Di Napoli

Dept. of Psychology
- Maria Teresa Fiorenza
- Sonia Canterini
- Giampiero Palladino
- Noemi Monti
- Viviana Ciraci

Dept. of Biochemical Sciences
- Maria D'Erme
- Luciana Mosca
- Sara Martire

Dept. of Cellular Biotechnologies and Hematology
- Marco Lucarelli
- Giampiero Ferraguti

Università Cattolica del Sacro Cuore, Rome
Institute of Clinical Biochemistry
- Silvia Persichilli
- Claudia Desiderio

Gnosis s.p.a., Milano
(SAM supplementation)

DEVELAGE
EC 7th Framework program (FP7/2007-2013) grant n° 278486
Pathways common to brain development and ageing: defining strategies for preventive therapy and diagnostics.
- Medizinische Universität Wien, *G. Kovacs (coordinator)*
- Fundació Institut d'Investigació Biomèdica de Bellvitge, *I. Ferrer*
- Amsterdam Medical Center, *E. Aronica*
- Sapienza University of Rome, *S. Scarpa*
- Tierärztliche Hochschule Hannover, *E. Zimmermann*
- University of Montpellier 2, *J.M. Verdier*
- Université Paris Diderot, *H. Adle-Biasette*
- Biolution GmbH, *I. Grunert*

Institute of Biochemistry Food Science and Nutrition
The Hebrew University of Jerusalem
- Aron Troen

This lecture has been possible thanks to the project

REP-EAT

EU funded Horizon 2020 (Marie Skłodowska-Curie grant agreement No 713714)

Join the Epigenetics Society

http://epigeneticssocietyint.com/

Improving the website with resources, databases, protocols.

Monthly newsletter advertising in press and just published abstracts from ES members

Thank you for your attention

Questions welcomed!