



**VALUTAZIONE DELL'ESPRESSIONE DI GENI SELEZIONATI IN UNA
COPPIA DI GEMELLE OMOZIGOTI DISCORDANTI
PER LA MALATTIA DI ALZHEIMER**

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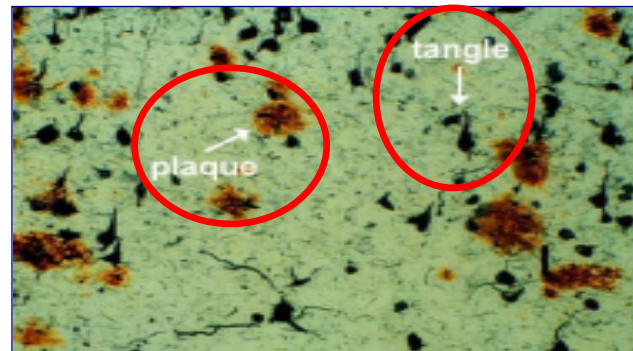
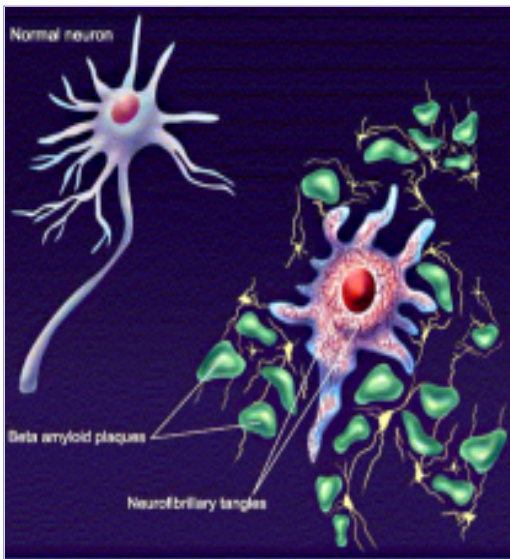
FIRENZE, 3 Dicembre 2010, 55° Congresso SIGG

ALZHEIMER DISEASE

The Hallmarks of AD: Plaques and Tangles

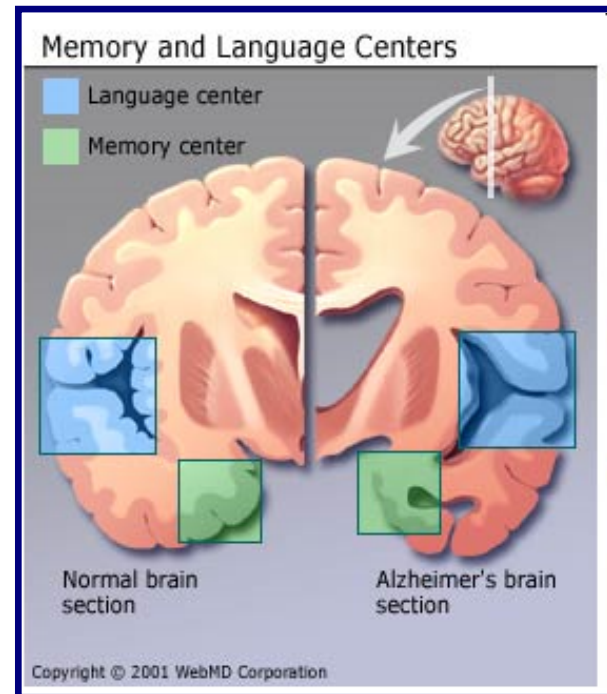
The brains of people with AD have an abundance of two abnormal structures:

- beta-amyloid plaques, which are dense deposits of protein and cellular material that accumulate outside and around nerve cells
- neurofibrillary tangles, which are twisted fibers that build up inside the nerve cell.



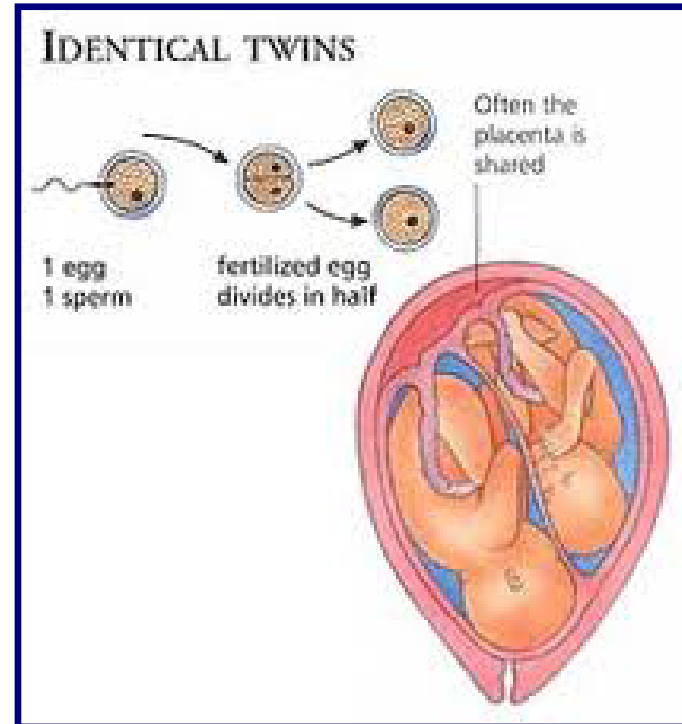
Presentation

- Early-onset AD, affect people aged 30 to 60. Researchers have identified mutations in three genes that cause early-onset AD (APP, PS1 e PS2).
- Late-onset AD is more common. It usually affects people over age 65. Researchers have identified a gene that produces a protein called apolipoprotein E (ApoE).



HOMOZYGOTIC TWIN

- single egg is fertilized to form one zygote (genetically identical)
- rate of about three in every 1000 deliveries worldwide
- The number of epigenetic differences between identical twins increases with age.





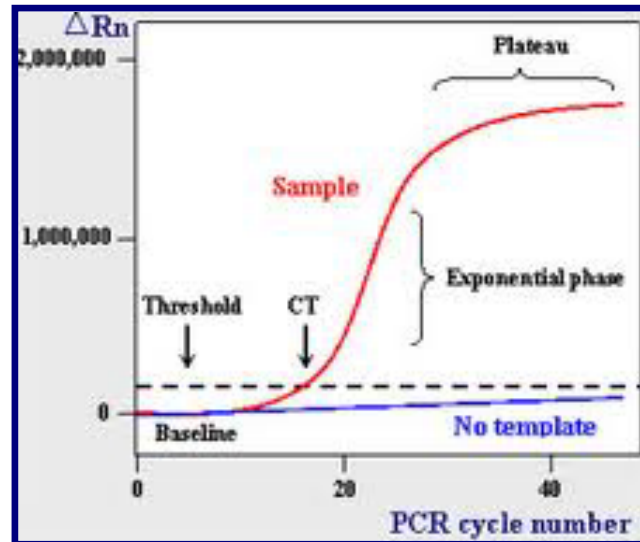
AIM of the STUDY

- 1. Evaluation of changes in the expression of selected genes possibly involved in AD in MZ twins**
- 2. Examination of epigenetic mechanisms to study non-genetic effects on genes expression**

DNA, RNA and protein from peripheral blood cells (PBMC), are peripheral markers which could reflect the pathology within the brain and suggested to be a useful model of epigenetic gene regulation (*Gavin & Sharma, 2009*)

Gene expression quantification by Real Time PCR

- ✓ APP
- ✓ SIRT1
- ✓ APOE
- ✓ BDNF
- ✓ PSEN1
- ✓ PIN1



Gene Expression

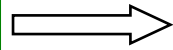
<i>APP</i>	<i>SIRT1</i>	<i>APOE</i>	<i>PSEN1</i>	<i>PIN1</i>	<i>BDNF</i>
Cont AD	Cont AD	Cont AD	Cont AD	Cont AD	Cont AD
1 ↑↑↑	1 ↑↑↑↑	1 0,97	1 0,46	1 41	1 0,55

- Overexpression of **APP** in certain areas of the brain in AD patients (Johnson et al., 1990)
- Induction of **SIRT1** expression levels in various neurotoxic conditions (Kim et al., 2007) may be interpreted as a neuroprotective adaptation response

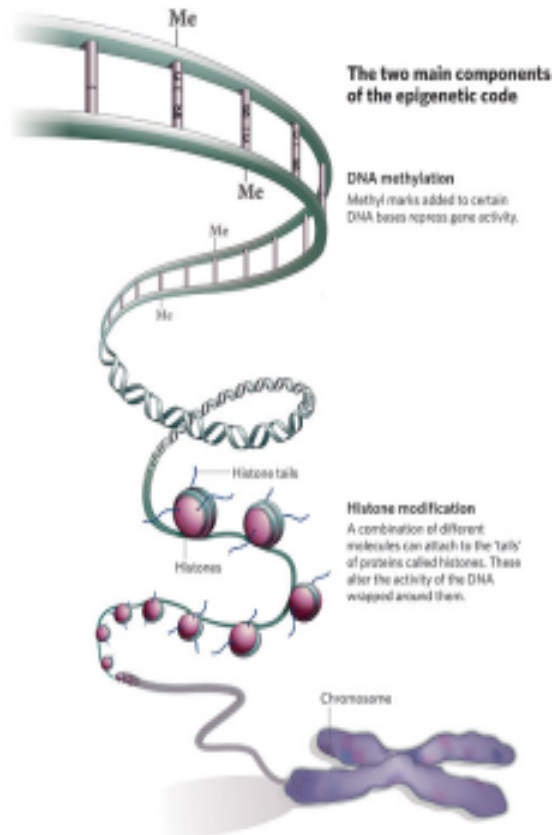
Gene Expression

APP		SIRT1		APOE		PSEN1		PIN1		BDNF	
<i>Cont</i>	<i>AD</i>	<i>Cont</i>	<i>AD</i>	<i>Cont</i>	<i>AD</i>	<i>Cont</i>	<i>AD</i>	<i>Cont</i>	<i>AD</i>	<i>Cont</i>	<i>AD</i>
1	↑↑↑	1	↑↑↑↑	1	0,97	1	0,46	1	41	1	0,55

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Study of the epigenetic mechanisms of gene regulation



Regulation of transcription

Mark ^a	Transcriptionally relevant sites [†]	Transcriptional role [‡]
DNA methylation		
Methylated cytosine (meC)	CpG islands	Repression
Histone PTMs		
Acetylated lysine (Kac)	H3 (9, 14, 18, 56), H4 (5, 8, 13, 16), H2A, H2B	Activation
Phosphorylated serine/threonine (S/Tph)	H3 (3, 10, 28), H2A, H2B	Activation
Methylated arginine (Rme)	H3 (17, 23), H4 (3)	Activation
Methylated lysine (Kme)	H3 (4, 36, 79) H3 (9, 27), H4 (20)	Activation Repression
Ubiquitylated lysine (Kub)	H2B (123 [§] /120 [¶]) H2A (119 [¶])	Activation Repression
Sumoylated lysine (Ksu)	H2B (6/7), H2A (126)	Repression
Isomerized proline (Pisom)	H3 (30-38)	Activation/ repression

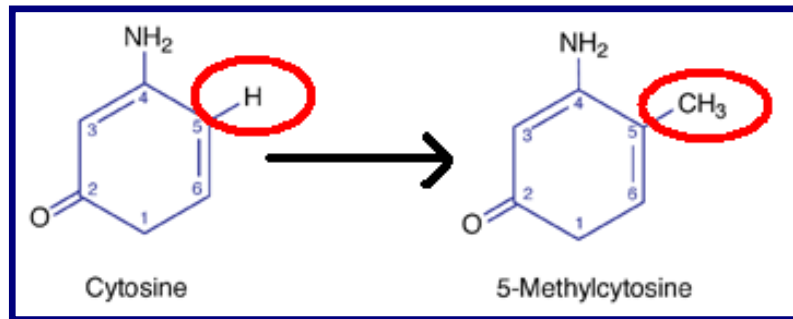
from *Oui et al, 2006*

DNA Methylation

- Established during embryonic development
- Stable through multiple cell divisions (cellular memory)
- Differs between tissues
- Normally associated with gene silencing
- Replacement of the 5' hydrogen atom in Cytosine with the methyl group.
- Hypermethylation in the promoter area of a gene prevent the gene from being expressed.

Regulatory involvement of DNA methylation

- Repeat elements
- Imprinted Genes
- X Chromosome Inactivation



Unmethylated promoter

CGCGCGCGC

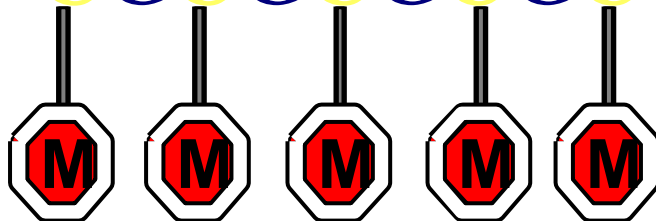
Coding Sequence



Gene
Expression

Methylated promoter

CGCGCGCGC



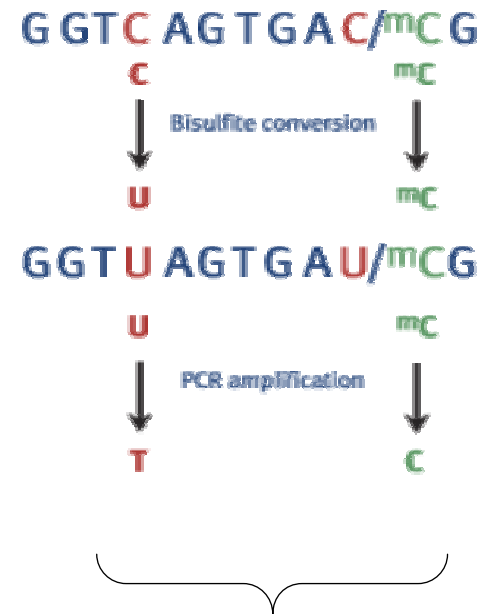
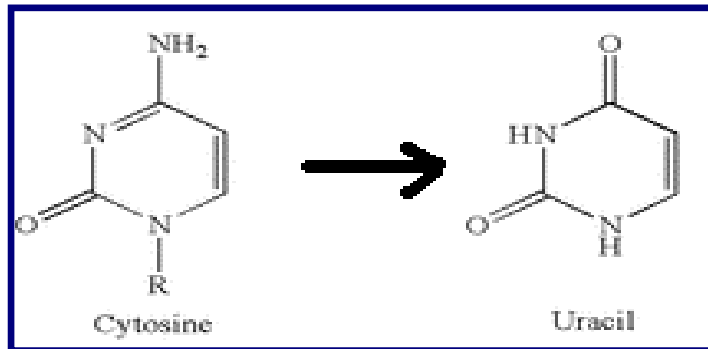
Coding Sequence



Method: Bisulfite conversion of genomic DNA followed by Methylation Specific Primer (MSP) Real Time PCR:

1. Bisulfite Treatment

- Convert unmethylated Cytosine into Uracil.
- Methylated Cytosine unaffected by this process.



2. Real Time Detection

DNA Methylations

<i>APP</i>	<i>SIRT1</i>	<i>APOE</i>	<i>PSEN1</i>	<i>PIN1</i>	<i>BDNF</i>
<i>Cont AD</i>	<i>Cont AD</i>	<i>Cont AD</i>	<i>Cont AD</i>	<i>Cont AD</i>	<i>Cont AD</i>
2,3 5,8	1,1 3,8	100 100	0 0	2,9 0,9	13,1 7,1

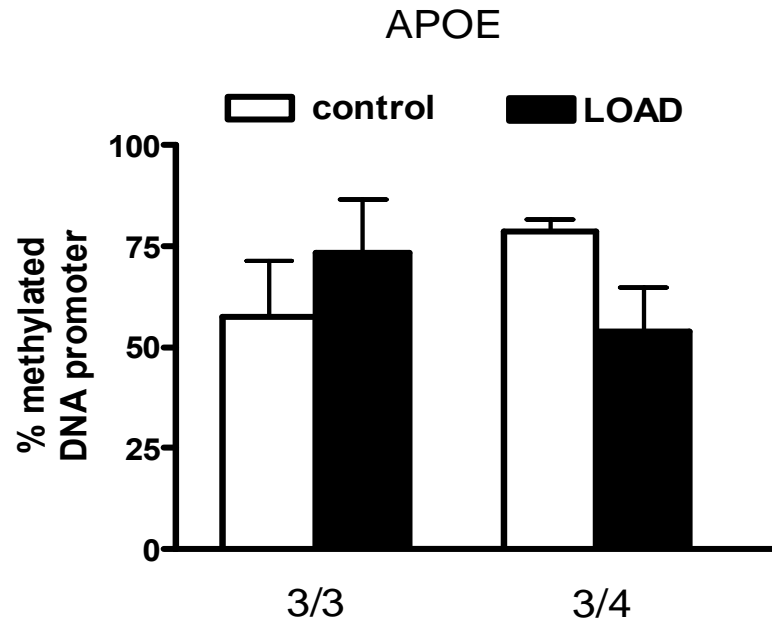
- ↓ **APP** in frontal cortex of AD patients (Toghi et al, 1999).
- ↑ **APOE** in prefrontal cortex and peripheral lymphocytes of AD patients (Wang et al, 2008).

DNA Methylations

<i>APP</i>		<i>SIRT1</i>		<i>APOE</i>		<i>PSEN1</i>		<i>PIN1</i>		<i>BDNF</i>	
<i>Cont</i>	<i>AD</i>	<i>Cont</i>	<i>AD</i>	<i>Cont</i>	<i>AD</i>	<i>Cont</i>	<i>AD</i>	<i>Cont</i>	<i>AD</i>	<i>Cont</i>	<i>AD</i>
2,3	5,8	1,1	3,8	100	100	0	0	2,9	0,9	13,1	7,1

- ↓ **APP** in frontal cortex of AD patients (Toghi et al, 1999).
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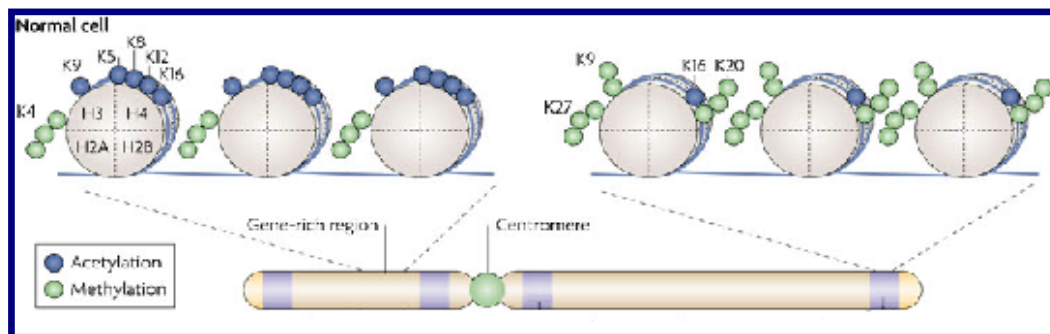
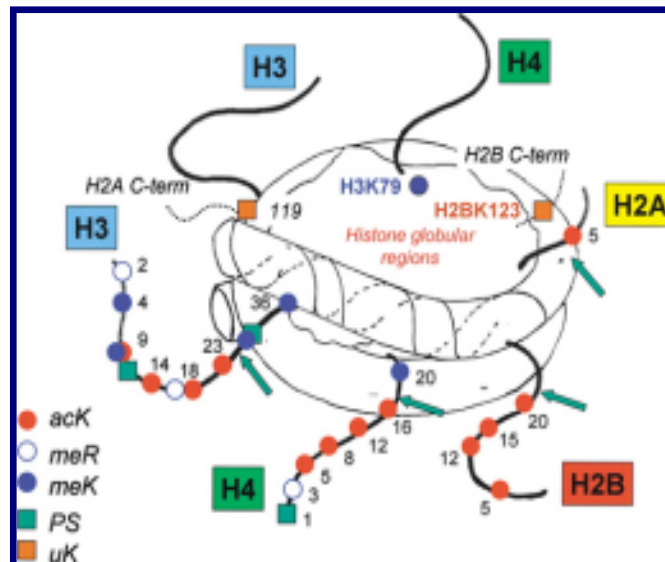
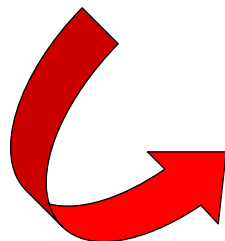
DNA Methylations



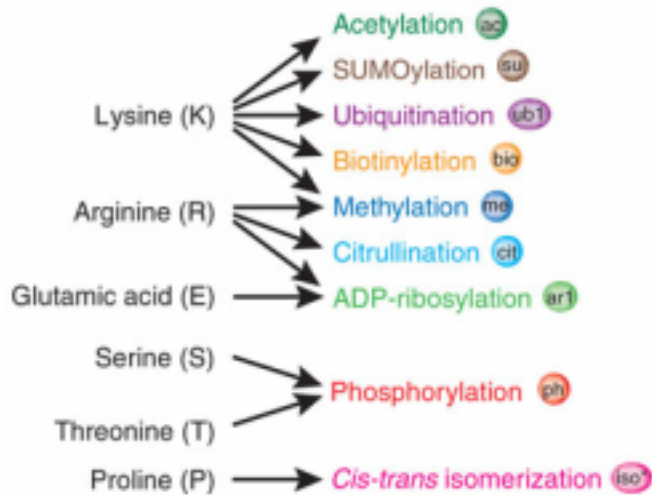


Histone Structure

Nucleosomes are the basic unit of DNA consisting of a segment of DNA wound around a histone protein core.



Known post-translational modifications and the amino acid residues they modify



- Acetylation (K)
- Methylation (K & R)
- Phosphorylation (S & T)
- Ubiquitination (K)
- Others

ACTIVATION

Histone 3 lysine 4 meth (H3K4me3)

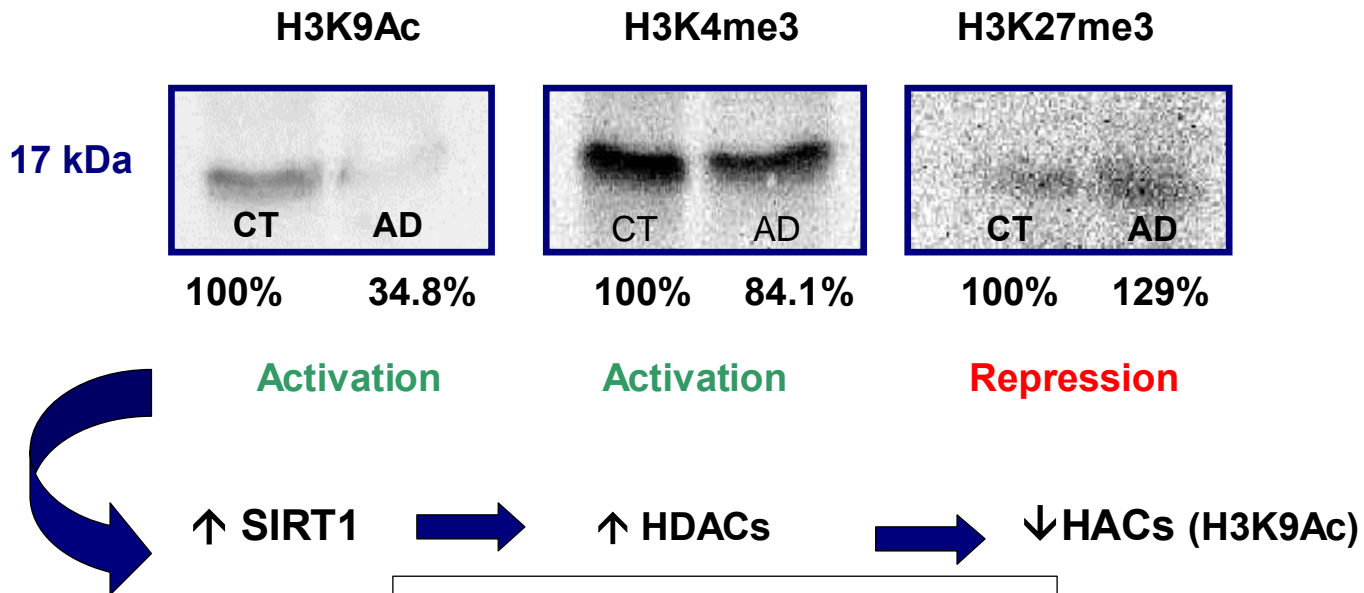
Histone 3 lysine 9 Acet (H3K4Ac)

REPRESSION

Histone 3 lysine 9 meth (H3K9me)

Histone 3 lysine 27 meth (H3K27me3)

Histone modifications by Western Blotting



Narayan and Dragunow
British Journal of Pharmacology, 2010



Conclusions:

We found in MZ twins discordant for AD:

- differences in the expression of selected genes and in some chromatin modifications (i.e. H3K9Ac; H3K4me3).**
- general hypomethylation in the genes promoter studied.**

Our study should provide insights into the molecular causes of differential susceptibility to AD in genetically identical organisms and clarify the importance of epigenetic factors in its mediation.