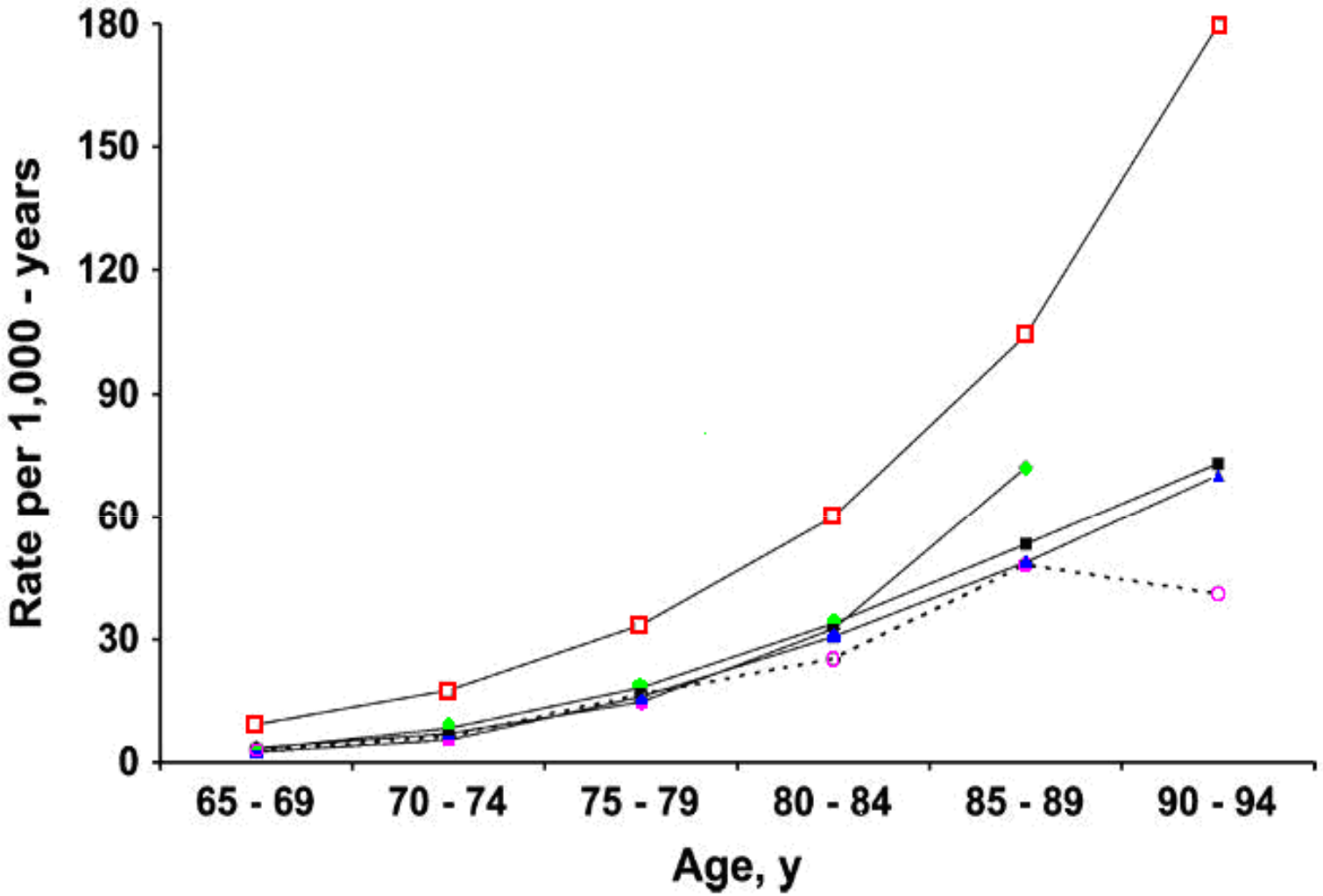
LATENT HERPES VIRUS INFECTION COGNITIVE DETERIORATION AND DEMENTIA

Prof. Federico Licastro, MD

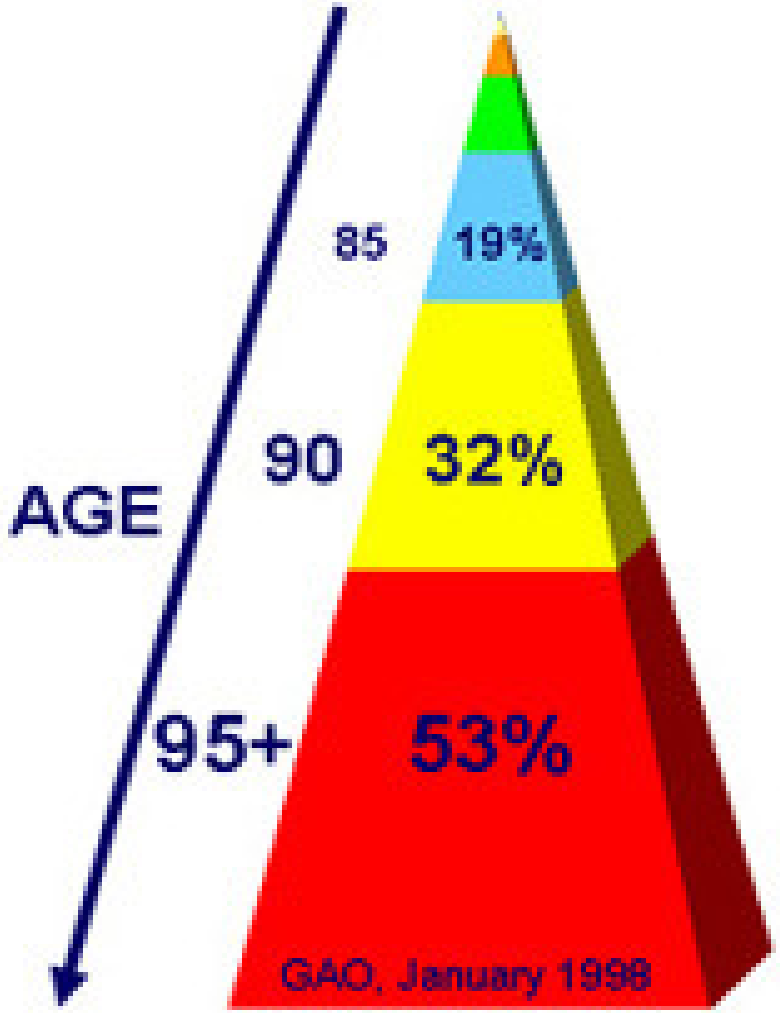
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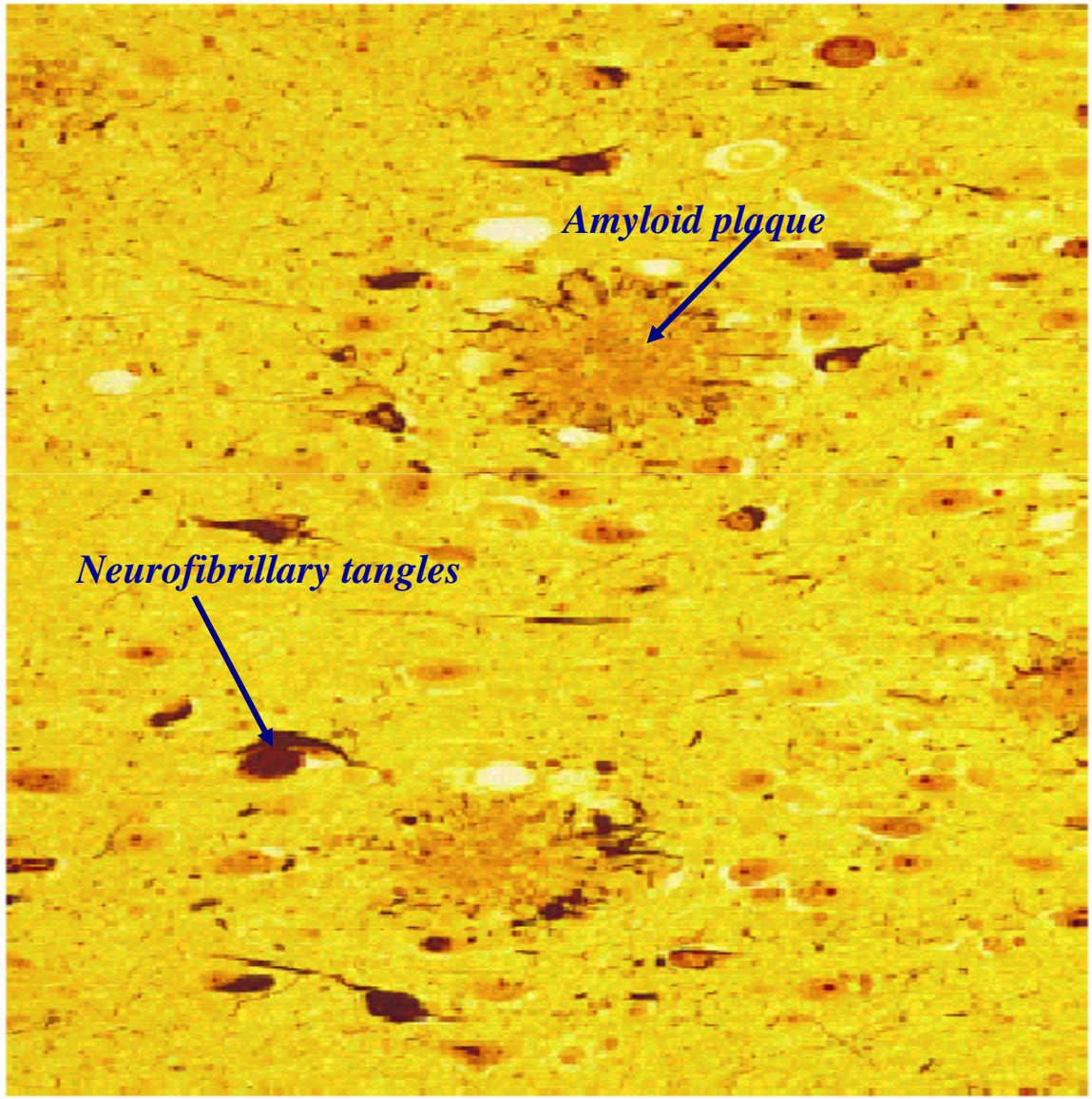
Annual incidence rates of dementia. □, Meta-analysis of European studies. ◆, Meta-analysis of East Asian studies. ■, Meta-analysis of Western and Asian studies. ▲, Meta-analysis of European studies. ○, Brazilian incidence study.

PREVALENCE of AD in the elderly

AGE	%
65	1
70	2
75	5
80	9
85	18
90	32
95+	53



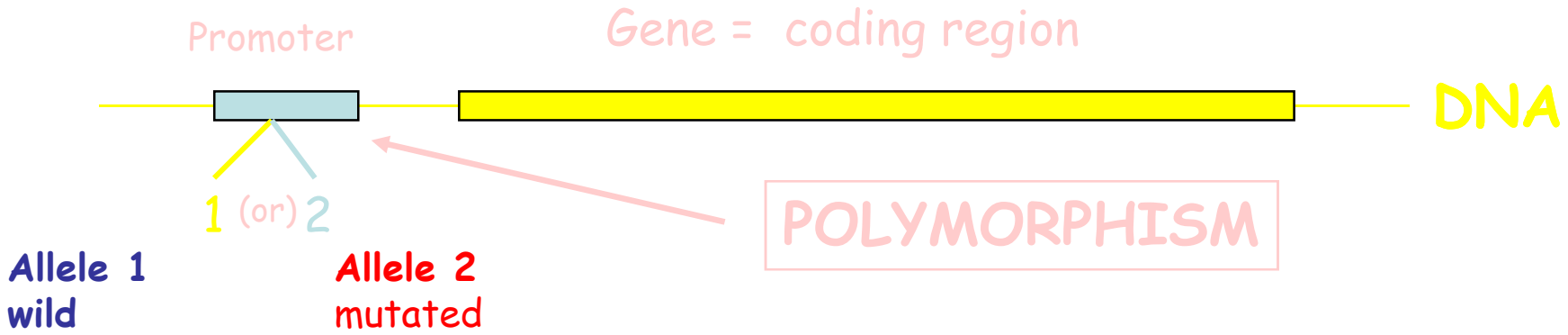
Neuritic amyloid plaques e neurofibrillary tangles.



AD is a multifactorial disease with complex aetiology and pathogenesis

- Several risk factors are involved.
- Dominant genetic transmission accounts for less than 2 % of AD familial cases. Several genes are involved in non familial AD and gene epistasis (gene-gene interaction) may play a pivotal role.
- The interaction of genetic factors with environmental components may play a pivotal role in mechanisms inducing AD neurodegeneration.
- However, environmental factors remain largely unknown.
- The goal is to reveal possible environmental components involved in cognitive deterioration and dementia.

GENE ALLELE POLYMORPHISM AND HUMAN DISEASES



Mutated allele in the promoter region often induces an impaired production of the cognate protein.

Nature Genetics 41, 1094 - 1099 (2009)

nature genetics

Genome-wide association study identifies variants at *CLU* and *CR1* associated with Alzheimer's disease

Jean-Charles Lambert[1–3], Simon Heath[4], Gael Even[1,2], Dominique Campion[5], Kristel Sleegers[6,7], Mikko Hiltunen[8], Onofre Combarros[9], Diana Zelenika[4], Maria J Bullido[10], Béatrice Tavernier[11], Luc Letenneur[12], Karolien Bettens[6,7], Claudine Berr[13], Florence Pasquier[3,14], Nathalie Fiévet[1,2], Pascale Barberger-Gateau[12], Sebastiaan Engelborghs[7,15], Peter De Deyn[7,15], Ignacio Mateo[9], Ana Franck[16], Seppo Helisalmi[8], Elisa Porcellini[17], Olivier Hanon[18], the European Alzheimer's Disease Initiative Investigators[19], Marian M de Pancorbo[20], Corinne Lendon[21], Carole Dufouil[22,23], Céline Jaillard[24], Thierry Leveillard[24], Victoria Alvarez[25], Paolo Bosco[26], Michelangelo Mancuso[27], Francesco Panza[28], Benedetta Nacmias[29], Paola Bossù[30], Paola Piccardi[31], Giorgio Annoni[32], Davide Seripa[33], Daniela Galimberti[34], Didier Hannequin[5], Federico Licastro[17], Hilkka Soininen[8], Karen Ritchie[13], Hélène Blanché[35], Jean-François Dartigues[12], Christophe Tzourio[22,23], Ivo Gut[4], Christine Van Broeckhoven[6,7], Annick Alpérovitch[22,23], Mark Lathrop[4,35] & Philippe Amouyel[1–3,14]

The gene encoding apolipoprotein E (*APOE*) on chromosome 19 is the only confirmed susceptibility locus for late-onset Alzheimer's disease. To identify other risk loci, we conducted a large genome-wide association study of 2,032 individuals from France with Alzheimer's disease (cases) and 5,328 controls. Markers outside APOE with suggestive evidence of association (P < 10–5) were examined in collections from Belgium, Finland, Italy and Spain totaling 3,978 Alzheimer's disease cases and 3,297 controls. Two loci gave replicated evidence of association: one within CLU (also called APOJ), encoding clusterin or apolipoprotein J, on chromosome 8 (rs11136000, OR = 0.86, 95% CI 0.81–0.90, P = 7.5 × 10–9 for combined data) and the other within CR1, encoding the complement component (3b/4b) receptor 1, on chromosome 1 (rs6656401, OR = 1.21, 95% CI 1.14–1.29, P = 3.7 × 10–9 for combined data). Previous biological studies support roles of CLU and CR1 in the clearance of β amyloid (Aβ) peptide, the principal constituent of amyloid plaques, which are one of the major brain lesions of individuals with Alzheimer's disease.

Common variants at *ABCA7*, *MS4A6A/MS4A4E*, *EPHA1*, *CD33* and *CD2AP* are associated with Alzheimer's disease

nature genetics

Hollingworth P, Harold D, Sims R, Gerrish A, Lambert JC, Carrasquillo MM, Abraham R, Hamshere ML, Pahwa JS, Moskvina V, Dowzell K, Jones N, Stretton A, Thomas C, Richards A, Ivanov D, Widdowson C, Chapman J, Lovestone S, Powell J, Proitsi P, Lupton MK, Brayne K, Rubinsztein DC, Gill M, Lawlor B, Lynch A, Brown KS , Passmore PA……Porcellini E, Licastro F….. Riemenschneider M, Morgan K, Younkin SG, Owen MJ, O'Donovan M, Amouyel P and WilliamsJ

We sought to identify new susceptibility loci for Alzheimer's disease through a staged association study (GERAD+) and by testing suggestive loci reported by the Alzheimer's Disease Genetic Consortium (ADGC) in a companion paper. We undertook a combined analysis of four genome-wide association datasets (stage) and identified ten newly associated variants with P ≤ × 10^{-5}. We tested these variants for association in an independent sample (stage 2). Three SNPs at two loci replicated and showed evidence for association in a further sample (stage 3). Meta-analyses of all data provided compelling evidence that ABCA7 (rs3764650, meta P = 4.5 × 10^{-7}; including ADGC data, meta P = 5.0 × $10^{-21)}$ and the MS4A gene cluster (rs60932, meta P =1.8 ×10^{-4}; including ADGC data, meta P = 1.2 × 10^{-6}) are new Alzheimer's disease susceptibility loci. We also found independent evidence for association for three loci reported by the ADGC, which, when combined, showed genome-wide significance: CD2AP (GERAD+, P = 8.0 × 10^{-4}; including ADGC data, meta P = 8.6 × 0–9), CD33 (GERAD+, P = 2.2 × 10^{-4}; including ADGC data, meta P = 1.6 × $^{10-9}$) and EPHA1 (GERAD+, P = 3.4 × 10^{-4}; including ADGC data, meta P = 6.0 × 10^{-10}).

Provisional conclusions

- **Several SNPs in selected genes are highly associated with AD risk.**
- **Each SNP shows a low OR and are of limited informative value.**
- **Most genes have a regulatory function on inflammatory responses.**
- **These genes are linked to AD pathogenesis, however it is unlikely that they are causative of AD.**
- **Interactions among these genes and environmental factors may be relevant to the pathogenesis of the disease.**

Unusual interpretation of GWA data: from genes to environment

Alzheimer's disease gene signature says: beware of brain viral infections.

Porcellini E, Carbone I, Ianni M, Licastro F.Immun Ageing. 2010 Dec 14;7:16.

Gene signature in Alzheimer's disease and environmental factors: the virus chronicle.

Licastro F, Carbone I, Ianni M, Porcellini E.

J Alzheimers Dis. 2011;27(4):809-17.

Virus genomes of latent CMV, HHV-6 and EBV in human peripheral blood leukocytes (PBL) and brain

• Blood samples from the longitudinal investigation "Conselice study on brain aging". **Elderly followed up for 5 years and cognitively tested at the beginning and at the end of the follow up.**

150 brain samples from The Department of Neurosciences and Pathology of the University of California, San Diego, USA.

•DNA samples from PBLs of AD (n=103) and Controls (CTR, n=165).

•DNA samples from mid-frontal cortex of AD (n=85).

•Real-Time PCR Termal Cycler CFX 9600 (BioRad).

EBV and HHV-6 presence in PBL

EBV

	Positive		Negative		APOE ε4 carrier				APOE ε4 noncarrier				
					Positive		Negative		Positive		Negative		
	n	%	n	%	n	%	n	%	n	%	n	%	
CTR	33	31.1	73	68.9	11	55	9	45	22	25.9	63	74.1	$\chi^2 = 6.369$ $p = 0.012$ OR = 3.5 CI = 1.280–9.569
AD	30	45.5	36	54.5	9	52.9	8	47.1	21	42.9	28	57.1	$\chi^2 = 4.11$ $p = 0.472$
	$\chi^2 = 3.595$, $p = 0.050$ OR = 1.843 CI = 0.976–3.480												

Key: CI, confidence interval; OR, odds ratio.

HHV-6

	Positive		Negative		APOE ε4 carrier				APOE ε4 noncarrier				
					Positive		Negative		Positive		Negative		
	n	%	n	%	n	%	n	%	n	%	n	%	
CTR	3	4.4	65	95.6	1	6.2	15	93.8	2	3.8	50	96.2	$\chi^2 = 0.168$ $p = 0.682$
AD	12	23.1	40	76.9	3	30	7	70	9	21.4	33	8.6	$\chi^2 = 0.334$ $p = 0.563$
	$\chi^2 = 9.386$, $p = 0.002$ OR = 6.5 CI = 1.728–24.455)												

Key: CI, confidence interval; OR, odds ratio.

AD progression

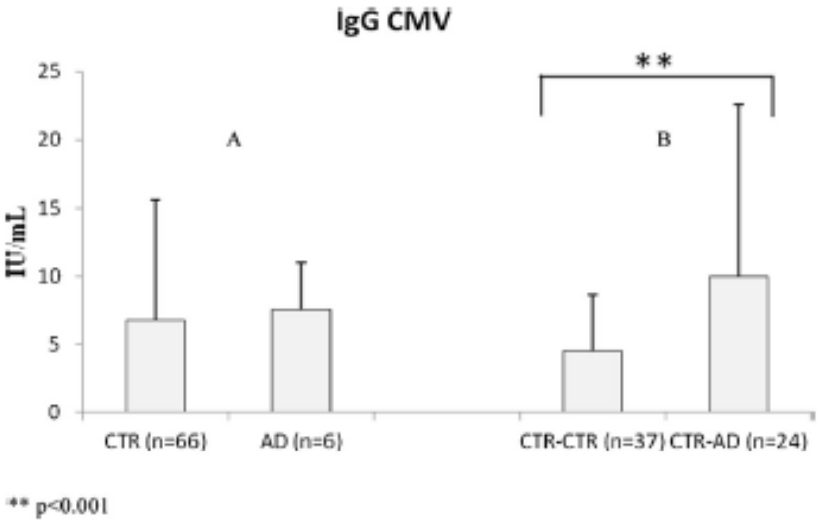
	EBV				HHV-6			
	Positive	%	Negative	%	Positive	%	Negative	%
CTR-CTR	33	31.1	73	68.9	3	4.4	65	95.6
CTR-AD	19	52.8	17	47.2	7	21.9	25	78.1
Total	142				100			
	$p = 0.020$				$p = 0.007$			
	OR = 1.458				OR = 1.224			
	CI = 1.009–2.108				CI = 1.011–1.480			

Key: CTR-AD, controls who developed Alzheimer's disease (AD); CTR-CTR, controls who remained cognitively healthy.

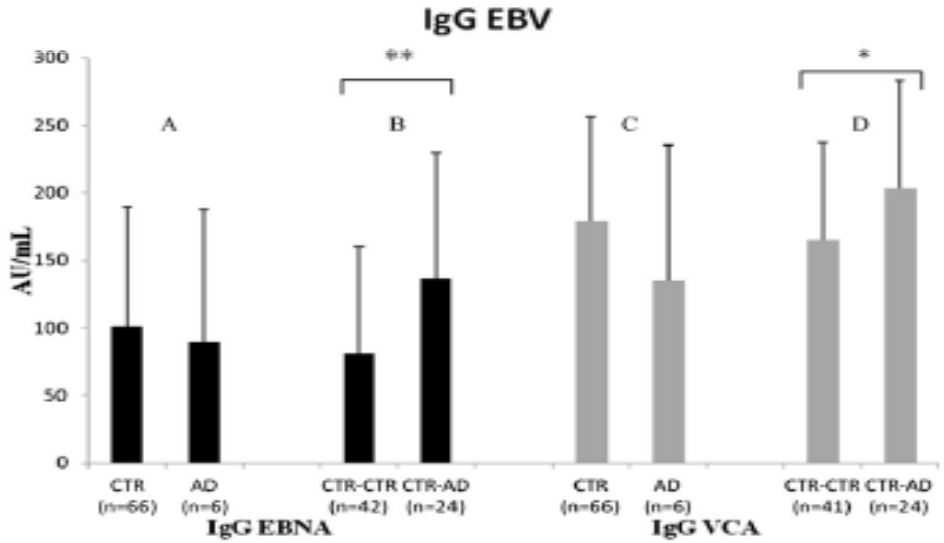
Samples has been divided in CTR-CTR and CTR-AD based on the cognitive performances at the baseline of the study and at the end of the five year follow up:

CTR-CTR = cognitively healthy during the 5 yrs follow up;
CTR-AD = elderly developing clinical AD during the 5 yrs follow up.

Serology: IgG plasma levels in association with AD progression



** p<0.001



* p<0.05

** p<0.001

EBV and HHV-6 presence in frontal cortex samples from AD patients

	EBV						HHV-6					
	Frequency		APOE ε4				Frequency		APOE ε4			
			Positive		Noncarrier				Positive		Noncarrier	
	n	%	n	%	n	%	n	%	n	%	n	%
Positive	5	5.9	5	100	0	0	14	17.3	8	16.7	6	18.2
Negative	80	94.1	47	58.8	33	41.2	67	82.7	40	83.3	27	81.8
			$\chi^2 = 3.371, p = 0.066$						$\chi^2 = 0.031, p = 0.859$			

Conclusions

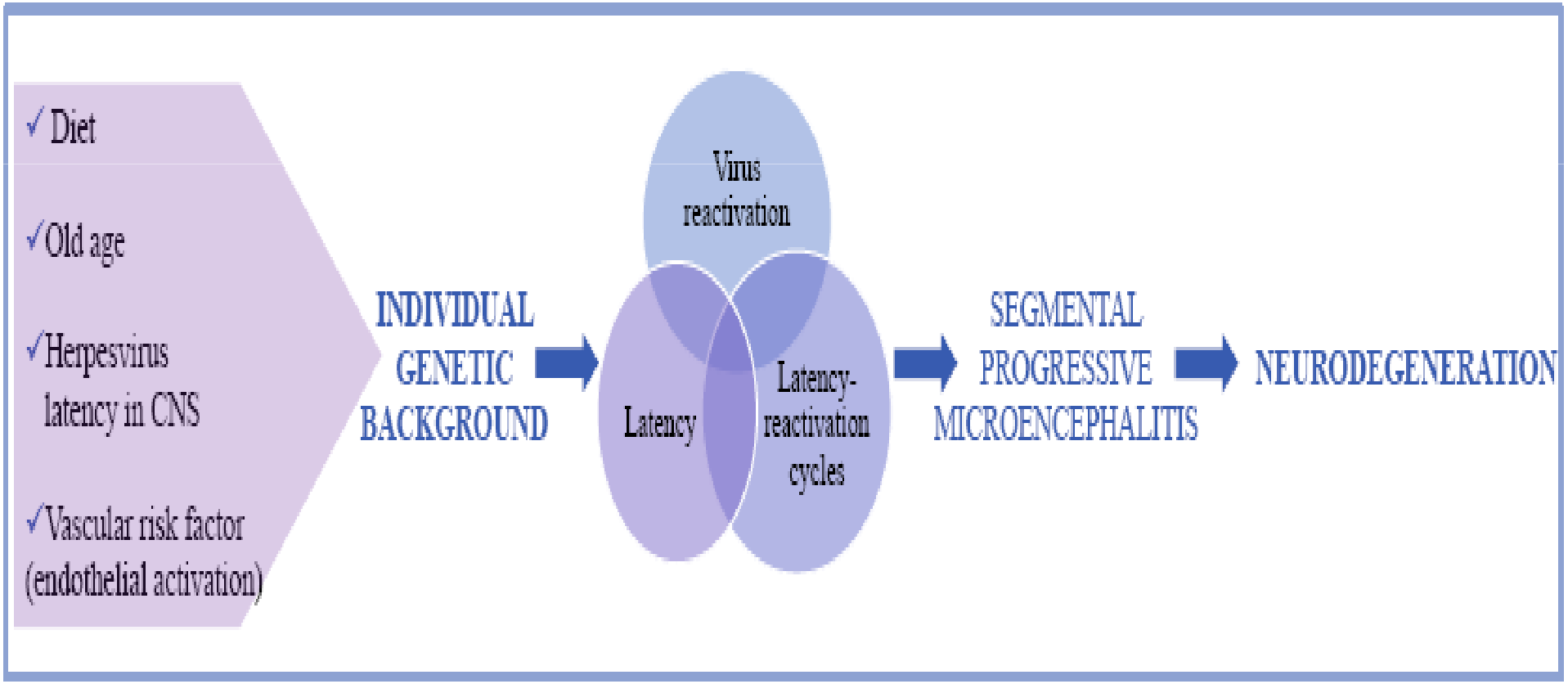
1) Viruses of the herpes family appear to be involved in the clinical progression of age associated cognitive decline to AD.

2) Herpes viruses (EBV and HHV-6) have also been found in brain from AD patients.

3) *Whether these and other viruses may be associated with the etiology of the disease remains an open question.*

4) **These data suggest new therapeutic approaches to control the progression of cognitive deterioration to AD.**

Alzheimer's disease: a syndrome with different etiological factors



Many Thanks

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- Prof Eliezer Masliah, Dept. Neurosciences and Pathology, UCSD, USA.
- Genetic European Research Consortium on AD.