HLA-A*01 IS ASSOCIATED WITH LATE ONSET OF ALZHEIMER'S DISEASE IN ITALIAN PATIENTS

F.R. GUERINI[1], C. TINELLI[2], E. CALABRESE[3], C. AGLIARDI[1], M. ZANZOTTERA[1], A. DE SILVESTRI[2], M. FRANCESCHI[4], L.M.E. GRIMALDI[5], R. NEMNI[3,6] and M. CLERICI[1,6]

[1]Laboratory of Molecular Medicine and Biotechnology, Don C. Gnocchi Foundation IRCCS, Milan; [2]Clinical Epidemiology and Biometric Unit, IRCCS Policlinico S. Matteo Foundation, Pavia; [3]Department of Neurorehabilitation, Don C. Gnocchi Foundation IRCCS, Milan[4]; Department of Neurology, IRCCS Multimedica, Santa Maria, Castellanza, Varese; [5]Department of Neurology, Fondazione Istituto San Raffaele "G. Giglio", Cefalù; [6]Department of Biomedical Sciences and Technology, University of Milan, Italy

Received March 30, 2009 – Accepted August 6, 2009

In this study, the distribution of HLA-A alleles was analyzed in Italian Alzheimer's Disease (AD) patients. Interaction between HLA alleles, APOE genotypes, age of onset, and gender were also analyzed. The results were compared to those obtained in healthy controls (HC). One hundred-seventy-three AD patients and 258 age-and-sex-matched healthy controls were enrolled in the study. AD patients were classified according to age at the onset of disease using quartiles of the distribution. HLA-A genotyping was performed by PCR-SSP; APOE genotyping was performed by RFLP. A correlation between late disease onset and HLA-A*01 was observed. Thus, HLA-A*01, calculated as number of alleles, was significantly more present in patients with age of onset > 74 years than in HC (20% vs 10.5%; p=0.014); the distribution of this allele was skewed also in patients 68.1 -74.0 years of age (16.3%), even if the difference did not reach statistical significance. The relative risk ratio (RRR) of AD onset calculated by a multinomial logistic regression adjusted for sex and presence of APOE-4 confirmed a significant association of HLA-A*01 with AD onset > 74.0 years of age (RRR=2.2; 95%CI: 1.1 - 4.6; p=0.033). A high RRR (2.04) was also present in patients 68.1 - 74 years (p=0.064). Lower age of disease onset did not correlate with HLA-A*01. Data herein suggest that the presence of HLA-A*01 results in delayed AD development, even in patients carrying APOE-4. These results could offer new insights into the etiopathogenesis of Alzheimer's disease.

The major cause of cognitive decline in the elderly is Alzheimer's disease (AD), a clinical syndrome characterised by complex and heterogeneous pathogenic mechanisms that may include infectious, immunologic, and genetic factors. Several genetic determinants are suggested to be involved in modulating the susceptibility to disease (1). Among these factors, autosomal mutations of the gene encoding the amyloid precursor protein, presenilin 1 and 2, were reported to be causative of familial and

Key words: Alzheimer's disease, HLA, APOE, immunology, late onset

Mailing address: Mario Clerici, MD.,
Dept. of Biomedical Sciences and Technology,
University of Milano,
Via Fratelli Cervi 93,
20090, Segrate (Mi), Italy
Tel: ++39 02 40308376 Fax: ++39 02 40308438
e-mail: mario.clerici@unimi.it

0394-6320 (2009)

usually early-onset AD cases (2).

Genetic factors have been also associated with the sporadic or non-familial form of the disease, and an array of data clearly demonstrates that the allele ε4 of the apolipoprotein E (APOE) significantly increases the risk of AD, but is neither necessary nor sufficient for the development of the disease (3). Genetic factors very likely interact with an inflammatory background, as inflammation has repeatedly been suggested to be associated with the neurodegenerative process characteristic of the AD brain. Thus, molecules and proteins that take part in the inflammatory cascade have been involved in the pathogenesis of AD, and over-expression of cytokines and other inflammatory molecules is a common feature of the AD brain pathology (4). The role of inflammation in the pathogenesis of AD is further confirmed by epidemiological studies showing that the long term use of non-steroid anti-inflammatory drugs is associated with a decreased incidence of AD in twin-control studies, and that the incidence of AD is decreased in individuals treated with anti-inflammatory drugs, including COX2 specific inhibitors (5-7).

As is the case of other neurodegenerative conditions, an initiating role for viral infections has been suggested to play an important pathogenetic role. In AD, different hints point to a possible role for herpes simplex virus type 1. Thus clonotypic immunity directed to viruses and/or amyloid peptides might be the initial event in the development of AD (8-10). In this regard, an intriguing potential role for human leukocyte antigen (HLA) genes was postulated. The HLA locus is a highly polymorphic site positioned on chromosome 6, coding for antigens directly involved in immunological recognition. The high polymorphism of this locus is responsible for the sophisticated ability of the immune system to respond to an enormous variety of different peptides. Because immune responses depend on the ability of HLA molecules to preferentially bind peptides, the vigour and degree of an immune response differs according to the presence of different HLA antigens. Therefore, distinct HLA molecules induce specific immune reaction, thus playing a role in modulating susceptibility to diseases (11).

Several studies have suggested a possible association between AD and the expression of particular HLA molecules, and significant results have been reported when AD patients were sub-grouped according to age at onset (12), APOE genotypes (13), gender, and family history (14). AD susceptibility has repeatedly been suggested to be associated with HLA-A*02 (15), even if these results were not confirmed by all investigators (16-17).

In the attempt to further clarify possible interactions between AD and HLA molecules, we studied HLA-A distribution among Italian AD patients and healthy controls (HC), taking into account possible interactions between the presence of HLA molecules, APOE pattern, age at onset, and gender.

MATERIALS AND METHODS

Patients and controls

A total of 173 patients (63 male, 110 female) with a clinical diagnosis of probable AD according to the NINCDS-ADRDA Work Group criteria (18) were recruited for this study from two Memory Clinics. The epidemiologic characterization of the patients, including age and gender ratios, is shown in Table I.

Each patient underwent a complete medical examination that included a detailed medical history, physical examination, neurological and neuro-psychological assessments, and neuro-imaging studies. The degree of cognitive impairment was evaluated by administration of a complete battery of neuropsychological tests that included the Mini-Mental State Examination (MMSE) (19). Mean MMSE score in our patients was 18.1 ± 5.9. AD patients had a normal complete blood count, urine analysis, blood chemistry screen, serum folate, B12 levels, and thyroid function tests. The brain MRI or CT images of these patients showed degrees of cerebral atrophy. AD patients were classified according to gender and age at onset of the disease, using quartiles of the distribution: group 1 (onset before 61 years old), group 2 (61.1 - 68.0 years old), group 3 (68.1 – 74.0 years old), group 4 (onset after 74 years old). Age at onset was defined as the age when memory loss was first noticed by relatives. Twenty of the 173 patients (3 in group 1, 3 in group 2, 4 in group 3 and 10 in group 4) had a familial history of dementia, with no known pathological mutations.

A group of 258 age- [mean age 68.6 (s.d. 10.8 years)] and sex- (94 male, 164 female) matched healthy controls (HC) were enrolled in the study. Healthy controls were selected from the patients' spouses or relatives of staff

members and did not refer the presence of either acute or chronic disease. All HC underwent an MMSE evaluation, the results of which showed a mean MMSE score of 27.3 ± 1.5.

The present study conforms to the principles outlined in the Declaration of Helsinki. The study protocol was approved by the Institutional Review Board of the Fondazione Don Gnocchi, Milano. All the subjects, or their relatives if so required, provided written informed consent before admission to the study.

Genetic analyses

Genomic DNA was isolated from peripheral blood by phenol-chloroform extraction using standard procedures. HLA-A genotyping was performed by standard sequence specific primer polymerase chain reaction (PCR- SSP) method (20), using Histo Type DNA well plates (BAG, Formedic Diagnostici, Milan, Italy); APOE genotyping were performed by RFLP as already described (21).

Statistical analyses

Chi-square analysis was used to exclude any deviation of HLA-A and APOE genotype distribution from Hardy-Weinberg equilibrium, p value was >0.05 both in cases and in controls. Quantitative data are shown as mean and standard deviation (SD). The chi-squared statistics or Fisher's exact test, as appropriate, were applied to 2X2 table to compare specific HLA-A and APOE genotype distributions between controls and patients. Multinomial (polytomous) logistic regression (22) was performed to calculate the HLA-A*01 effect on AD risk in four different groups of age at onset, taking into account also gender and APOE-4 positivity. Results are expressed as relative risk ratio (RRR) using HC as reference group. 95% confidence intervals (95%CI) are also given.

Statistical analyses were performed using the Stata Software 10.0, and the study has a power of 80% to detect a difference in proportions characterized by a Variance of proportions across groups of 0.005 and an average proportion of 0.224.

RESULTS

APOE polymorphisms in early and late onset disease

Since APOE polymorphism is the main genetic determinant involved in the pathogenesis of AD, we performed APOE genotyping in all the individuals enrolled in the study. As expected, the frequency of both APOE-4 and APOE-2 alleles was skewed in AD patients compared to HC. Thus (Table I), the frequency of APOE ε4 allele was higher in AD patients (48.6%) compared to HC (20.9%). Finally, APOE-2 was more frequent in HC (19.0%) compared to AD patients (2.9%); both these differences were highly statistically significant ($p<0.001$ in each case).

*Association of HLA-A*01 with late onset AD*

Possible correlations between HLA genetic polymorphisms and disease onset were analyzed by studying HLA-A genotype distribution in 173 AD patients; results were compared to those obtained in 258 age-and sex-matched healthy controls. A familial history of AD was present in 20/173 cases; since no differences in HLA distributions were observed when familial and sporadic AD patients were compared, the familial cases were evaluated together with all the other patients.

A total of nineteen HLA-A alleles were analysed in all the individuals studied. Results showed that the distribution of HLA-A alleles was not significantly different in AD patients compared to HC, even if a trend toward a higher frequency of HLA-A*01 was seen in AD (13.9%) compared to HC (10.5%) individuals. Interestingly, though, the distribution of HLA-A alleles was skewed when AD patients were divided according to age at onset (Table II). In particular, distribution analysis of HLA-A alleles was significantly different between older patients and HC. Thus, HLA-A*01 calculated as number of allele was significantly more present in group 4 than in HC (20% *vs* 10.5%; p=0.014); the distribution of this allele was skewed also in group 3 (16.3%), even if the difference did not reach statistical significance.

We subsequently calculated the relative risk ratio (RRR) of AD onset by a multinomial logistic regression adjusted for sex and presence of APOE-4 in each group of patients compared to HC. RRR the relative risk ratio for a change (M/F or yes/no) in the corresponding variable and the risk is measured as the risk of the outcome relative to the base outcome i.e. controls.

The results of this analysis showed that, whereas gender was not associated with AD risk of onset, APOE-4 was always associated to AD risk (data not shown); results also confirmed the presence of an association between HLA-A*01 and late age at onset of AD. In particular, a significant association of HLA-A*01 with AD onset > 74 years of age was evidenced by a RRR =2.2 (95%CI: 1.1 - 4.6; p=0.033) (Table III). A high RRR (2.04) was also observed in the quartile group with age at onset of AD between 68.1 and 74.0 years, even if in this case the RRR did not reach statistical significance (p=0.065). Lower age at onset of disease did not correlate with HLA-A*01. Fig.1 shows how the RRR increases when disease onset takes place over 68 years of age.

Moreover, dichotomizing the patients, i.e. group 1 + 2 *vs* 3 + 4, and performing a post-hoc analysis to determine the strength of association with HLA-A*01, revealed that the presence of HLA-A*01 results in a significantly

Table I. *Demographic features, HLA-A*01, APOE-4 and APOE-2 frequency distribution of Alzheimer patients and healthy controls.*

		Mean age at Onset (SD)	HLA-A*01		APOE-4		APOE-2	
			N	%	N	%	N	%
All cases	AD (N=173)	67.2 (9.6)	43	24.9	84	48.6*	5	2.9*
	HC (N=258)	68.6 (10.8)	49	19.0	54	20.9	49	19.0
Men	AD (N=63)	66.35 (9.9)	11	17.5	26	41.3°	3	4.8°
	HC (N=94)	69.7 (9.6)	16	17.0	24	25.5	14	14.9
Women	AD (N=110)	67.6 (9.4)	32	29.1	58	52.7*	2	1.8*
	HC (N=164)	67.9 (11.7)	33	20.1	30	18.3	35	21.3

*HC: Healthy Controls; N: number of subjects; SD: standard deviation; %: percentages observed; *: significant difference between AD and controls $p<0.01$; °: significant difference between AD and controls $p<0.05$*

higher risk of LOAD (RRR 4.15, p=0.001)

HLA-A*01 patterns of hetero/homozygosity were subsequently considered to evaluate whether a dose-dependent influence of A*01 allele could influence age at disease onset. An HLA-A*01/A*01 homozygosity was detected in only 5 patients, four of whom, noticeably, showed late disease onset.

Analysis of other HLA alleles

HLA-A*02 was described to be associated with an increased susceptibility to the development of AD (11-12). Analysis of this allele in our patients showed that, even if a similar frequency of this allele was observed in AD (22.0%) and HC (26.3%) individuals, HLA-A*02 was less present in group 2, 3 and 4 patients (21.1, 18.6 and 21.3%) compared to either HC (26.3%) or group 1 (26.7%). These trends did not reach statistical significance [data reported in 23]. Finally, HLA-A*25 was more present in HC (4.6%) compared to AD (1.7%) patients (Table II).

DISCUSSION

The data herein present an analysis of the pattern of distribution of the frequencies of HLA class I molecules in patients affected by Alzheimer's disease. AD patients were divided into groups based on clinical parameters: in particular, patients were differentiated on the basis of disease onset into four groups, and HLA-A*01 association with age at onset was evaluated in each group adjusting for gender and APOE-4 positivity.

The results indicate that the frequency of HLA-A*01 is skewed such that this molecule is over-represented in AD patients with late disease onset. Thus, the relative risk ratio was higher in patients with age of onset between 68 and 74 years old, and it was even more significant in patients with disease onset after 74 years.

Table II. *HLA-A gene frequency in 173 Alzheimer patients (346 alleles) subdivided into: 45 Group 1 patients (onset <61 years old; 90 alleles), 45 Group 2 patients (onset between 61.1-68.0 years old; 90 alleles), 43 Group 3 patients (onset between 68.1-74.0 years old; 86 alleles) and 40 Group 4 patients (onset >74 years old; 80 alleles) and 258 Healthy Controls (516 alleles)*

	HC N (%)	AD N (%)	Group 1 N (%)	Group 2 N (%)	Group 3 N (%)	Group 4 N (%)
A*01	54 (10.5%)	48 (13.9%)	9 (10%)	9 (10%)	14 (16.3%)	16 (20%)*
A*11	40 (7.8%)	20 (5.8%)	9 (10%)	4 (4.4%)	3 (3.5%)	4 (5%)
A*02	136 (26.4%)	76 (22%)	24 (26.7%)	19 (21.1%)	16 (18.6%)	17 (21.3%)
A*23	13 (2.5%)	6 (1.7%)	2 (2.2%)	1 (1.1%)	3 (3.5%)	0 (0%)
A*24	68 (13.2%)	48 (13.9%)	8 (8.9%)	19 (21.1%)	6 (7%)	15 (18.8%)
A*25	24 (4.7%)	6 (1.7%)	0 (0%)	2 (2.2%)	3 (3.5%)	1 (1.3%)
A*26	24 (4.7%)	25 (7.2%)	6 (6.7%)	6 (6.7%)	6 (7%)	7 (8.8%)
A*29	16 (3.1%)	15 (4.3%)	2 (2.2%)	4 (4.4%)	3 (3.5%)	6 (7.5%)
A*03	49 (9.5%)	37 (10.7%)	10 (11.1%)	10 (11.1%)	11 (12.8%)	6 (7.5%)
A*30	19 (3.7%)	12 (3.5%)	8 (8.9%)	2 (2.2%)	0 (0%)	2 (2.5%)
A*31	12 (2.3%)	8 (2.3%)	1 (1.1%)	2 (2.2%)	5 (5.8%)	0 (0%)
A*32	21 (4.1%)	12 (3.5%)	2 (2.2%)	3 (3.3%)	5 (5.8%)	2 (2.5%)
A*33	16 (3.1%)	8 (2.3%)	1 (1.1%)	2 (2.2%)	4 (4.7%)	1 (1.3%)
A*34	0 (0%)	2 (0.6%)	1 (1.1%)	0 (0%)	0 (0%)	1 (1.3%)
A*36	0 (0%)	1 (0.3%)	0 (0%)	1 (1.1%)	0 (0%)	0 (0%)
A*66	4 (0.8%)	9 (2.6%)	4 (4.4%)	2 (2.2%)	2 (2.3%)	1 (1.3%)
A*68	18 (3.5%)	11 (3.2%)	3 (3.3%)	4 (4.4%)	3 (3.5%)	1 (1.3%)
A*69	2 (0.4%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
A*74	0 (0%)	2 (0.6%)	0 (0%)	0 (0%)	2 (2.3%)	0 (0%)

HC: Healthy Controls; %: percentages observed; N: number of alleles; Significant difference of distribution between Group 4 AD patient and HC p=0.014 OR:2.52 IC(95%): 1.18-5.35

HLA-A*01 allele frequency was neither significantly increased in the entire group of patients nor in those with early disease onset

These results could indicate that HLA-A*01 carriers have an increased risk for LOAD. Nevertheless, the observation that the frequency of HLA-A*01 was comparable in AD patients *in toto* and HC, seems to indicate that this allele does not augment the risk of developing AD, but rather that carriers of HLA-A*01 who are prone to developing AD are allowed a longer symptom-free period of life.

Alzheimer's disease is a complex, multifactorial degenerative pathology. Amongst the genetic factors involved in the pathogenesis of AD, HLA molecules play an important role (24). The majority of published results focuses on HLA-A*02; a uniform consensus on the interaction between HLA-A*02 and AD is nevertheless still missing. Thus, data showing a protective effect, lack thereof, a correlation between HLA-A*02 and earlier disease onset, and the lack of any influence of this HLA allele on age at AD onset have been published (12-17).

Fewer analyses have focused on HLA-A*01. Previous results showed a higher prevalence of HLA-A*01 in DNA extracted from frozen brain tissue of AD patients with late disease onset (25). Those data, nevertheless, did not reach statistical significance, presumably because of the small number of cases examined. HLA-A*01 is an interesting HLA allele and its presence has been correlated with disease susceptibility in other pathologies. In particular,

Table III. *Multinomial logistic regression analysis of HLA-A*01 association with AD age of onset adjusted for sex and APOE4 positivity.*

AD Group	Age of onset	RRR	IC(95%)	p
1	<61.0	1.09	0.48-2.44	0.83
2	61.1-68.0	0.77	0.32-1.84	0.55
3	68.1-74.0	2.04	0.96-4.36	0.06
4	>74	2.22	1.06-4.64	0.03

N: number of subjects; RRR: relative risk ratio; IC: interval of confidence; p= p value

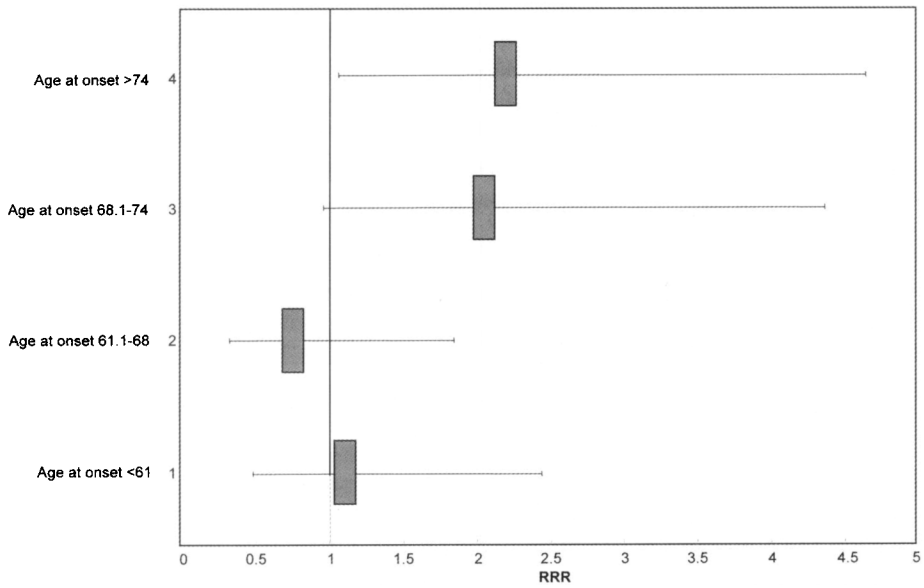

Fig. 1. *Multinomial logistic regression analysis of HLA-A*01 association in different AD age of onset classes compared to HC, adjusted for sex and APOE4 positivity.*

HLA-A*01 was shown to have a protective effect against idiopathic immunoglobulin A nephropathy (26), a disease that can lead to end-stage renal failure, as well as against Henoch Schonlein purpura, the most common vasculitis of childhood (27). This allele is also suggested to correlate with late onset of gluten-sensitive enteropathy (28). Finally, upon Epstein-Barr virus (EBV) infection, HLA-A*01-expressing individuals are more likely to develop symptomatic mononucleosis as well as EBV+ Hodgkin lymphoma (9-10).

In Caucasians, and in particular in Northern Europe, HLA-A*01 is part of an ancestral haplotype that is in strong linkage disequilibrium with other

HLA alleles including HLA-B8, DR3, Cw7 (29-30). This haplotype, is also associated with a number of immune system dysfunctions including alterations of neutrophil chemotaxis and an increased production of the pro-inflammatory cytokine TNFalfa by PBMC (31). Interestingly, though, IL-10 serum concentration is also increased in individuals carrying this ancestral haplotype (31). It could thus be hypothesized that the higher IL-10 production associated with this haplotype could dampen the inflammation that accompanies the development of AD, resulting in a delayed disease onset.

Late disease onset is the dominant modality observed in AD (32). The APOE gene, and in particular its allele ε4, significantly increases the risk of AD, and accounts for approximately 50% of the cases (33). A number of other genetic markers have been reported to be associated with increased susceptibility to late onset AD (34). The relative importance of these factors in the pathogenesis of AD is not clear, and it is not clear whether these factors play a causative role or rather are markers of susceptibility to disease. Our results add to this list the HLA-A*01 allele, a marker that distinguishes itself because it might have a delaying effect of disease development.

In conclusion, our data indicate HLA-A*01 as a possible additive marker prognostic of AD development. We suggest that the presence of HLA-A*01 may result in delayed AD development, even in patients carrying APOE-4. These data merit to be evaluated in a larger group of AD patients to better elucidate the role of HLA-A*01 in AD onset and development. Moreover, it will be interesting to evaluate possible associations between HLA-A*01 and the presence of the other genes shown to be correlated with late onset AD. These results offer new insights in the etiopathogenesis of Alzheimer's disease.

ACKNOWLEDGEMENTS

This research was made possible by grants from Ricerca Finalizzata (Italian Ministry of Health); 2007 Ricerca Corrente (Italian Ministry of Health), Italian Ministry of Health (ISS 2007; Conv. No. 526/ D25), the Istituto Superiore di Sanita' "Programma Nazionale di Ricerca sull' AIDS"; the EMPRO and AVIP EC WP6 Projects; the nGIN EC WP7 Project; the Japan Health Science Foundation; Progetto FIRB RETI, Rete Italiana Chimica Farmaceutica CHEM-PROFARMA-NET [RBPR05NWWC].

REFERENCES

1. Kehoe P, Wavrant-De Vrieze F, Crook R, et al. A full genome scan for late onset Alzheimer's disease. Hum Mol Genet 1999; 8:237-45.
2. Lleó A, Blesa R, Queralt R, et al. Frequency of mutations in the presenilin and amyloid precursor protein genes in early-onset Alzheimer disease in Spain. Arch Neurol 2002; 59:1759-63.
3. Corder EH, Saunders AM, Strittmatter WJ, et al. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. Science 1993; 261:921-3.
4. McGeer PL, Rogers J, McGeer EG. Inflammation, anti-inflammatory agents and Alzheimer disease: the last 12 years. J Alzheimers Dis 2006; 9:271-6.
5. Rogers J, Kirby LC, Hempelman SR, et al. Clinical trial of indomethacin in Alzheimer's disease. Neurology 1993; 43:1609-11.
6. Hauss-Wegrzyniak B, Willard LB, Del Soldato P, Pepeu G, Wenk GL. Peripheral administration of novel anti-inflammatories can attenuate the effects of chronic inflammation within the CNS. Brain Res 1999; 815:36-43.
7. Zandi PP, Breitner JC. Do NSAIDs prevent Alzheimer's disease? And, if so, why? The epidemiological evidence. Neurobiol Aging 2001; 22:811-7.
8. Dobson CB, Wozniak MA, Itzhaki RF. Do infectious agents play a role in dementia? Trends Microbiol 2003; 11:312-7.
9. Diepstra A, Niens M, Vellenga E, et al. Association with HLA class I in Epstein-Barr-virus-positive and with HLA class III in Epstein-Barr-virus-negative Hodgkin's lymphoma. Lancet 2005; 365:2216-24.
10. Niens M, Jarrett RF, Hepkema B, et al. HLA-A*02 is associated with a reduced risk and HLA-A*01 with an increased risk of developing EBV+ Hodgkin lymphoma. Blood 2007; 110:3310-5.
11. Listi F, Candore G, Balistreri CR, et al. Association between the HLA-A2 allele and Alzheimer disease. Rejuvenation Res 2006; 9:99-101.

12. Payami H, Schellenberg GD, Zareparsi S, et al. Evidence for association of HLA-A2 allele with onset age of Alzheimer's disease. Neurology 1997; 49:512-8.
13. Ballerini C, Nacmias B, Rombolà G, Marcon G, Massacesi L, Sorbi S. HLA A2 allele is associated with age at onset of Alzheimer's disease. Ann Neurol 1999; 45:397-400.
14. Harris JM, Cumming AM, Craddock N, St Clair D, Lendon CL. Human leucocyte antigen-A2 increases risk of Alzheimer's disease but does not affect age of onset in a Scottish population. Neurosci Lett 2000; 294:37-40.
15. Zareparsi S, James DM, Kaye JA, Bird TD, Schellenberg GD, Payami H. HLA-A2 homozygosity but not heterozygosity is associated with Alzheimer disease. Neurology 2002; 58:973-5.
16. Araria-Goumidi L, Lambert JC, Cottel D, Amouyel P, Chartier-Harlin MC. No association of the HLA-A2 allele with Alzheimer's disease. Neurosci Lett 2002; 335:75-8.
17. Nourhashémi F, Gillette-Guyonnet S, Fort M, Andrieu S, Abbal M, Albarede JL, Vellas B. Association between the A2 allele of the HLA system and age at onset of Alzheimer's disease. Presse Med 2003; 32:158-61.
18. McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's Disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Service Task Force on Alzheimer's Disease. Neurology 1984; 34: 939-44.
19. Folstein MF, Folstein SE, McHugh PR. „Mini-mental state" A practical method for grading the cognitive state of patients for the clinicians. J Psychiat Res 1975; 12:189-98.
20. Olerup O, Zitterquist H. HLA –DR typing by PCR amplification with sequence-specific primers (PCR-SSP) in 2 hours: an alternative to serological DR typing in clinical practice including donor-recipient matching in cadaveric transplantation. Tissue Antigens 1992; 39:225-35.
21. Hixson JE, Vernier DT. Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with HhaI. J Lipid Res 1990; 31:545-8.
22. Awadzi KD, Classen S, Hall A, Duncan RP, Garvan CW. Predictors of injury among younger and older adults in fatal motor vehicle crashes. Accid Anal Prev 2008; 40:1804-10.
23. Guerini FR, Calabrese E, Agliardi C, et al. Association study of the HLA-A2 allele in Italian Alzheimer disease patients. Neurobiol Aging 2008; doi:10.1016/j.neurobiolaging.2008.02.001
24. Candore G, Balistreri CR, Colonna-Romano G, Lio D, Caruso C. Major histocompatibility complex and sporadic Alzheimer's disease: a critical reappraisal. Exp Gerontol 2004; 39:645-52.
25. Middleton D, Mawhinney H, Curran MD, et al. Frequency of HLA-A and B alleles in early and late-onset Alzheimer's disease. Neurosci Lett 1999; 26: 140-2.
26. Doxiadis IN, de Lange P, de Vries E, Persijn GG, Claas FHJ. Protective and susceptible HLA polymorphisms in IgA nephropathy patients with end-stage renal failure. Tissue Antigens 2001; 57: 344-7.
27. Peru H, Soylemezoglu O, Gonen S, Cetinyurek A, Bakkaloðlu SA, Buyan N, Hasanoglu E. HLA class 1 associations in Henoch Schonlein purpura: increased and decreased frequencies. Clin Rheumatol 2008; 27:5-10.
28. Vogelsang H, Panzer S, Mayr WR, Granditsch G, Fischer GF. Distribution of HLA Class I Alleles Differs in Celiac Disease Patients According to Age of Onset. Dig Dis Sci 2003; 48:611-4.
29. Lio D, Candore G, Colombo A, et al. A genetically determined high setting of TNF-alpha influences immunologic parameters of HLA-B8,DR3 positive subjects: implications for autoimmunity. Hum Immunol 2001; 62:705-13.
30. Fensterle J, Trefzer U, Berger T, Andersen MH, Ugurel S, Becker JC. HLA-B8 association with late-stage melanoma-an immunological lesson? BMC Med 2006; 13:4-5.
31. Candore G, Campagna AM, Cuppari I, Di Carlo D, Mineo C, Caruso C. Genetic control of immune response in carriers of the 8.1 ancestral haplotype: correlation with levels of IgG subclasses: its relevance in the pathogenesis of autoimmune diseases. Ann N Y Acad Sci 2007; 1110:151-8.
32. Lambert JC, Amouyel P. Genetic heterogeneity

of Alzheimer's disease: complexity and advances. Psychoneuroendocrinology 2007; 1(S):62-70.

33. Strittmatter WJ, Weisgraber KH, Huang DY, et al. Binding of human apolipoprotein E to synthetic amyloid beta peptide: isoform-specific effects and implications for late-onset Alzheimer disease. Proc Natl Acad Sci USA 1993; 90:8098-102.
34. Waring SC, Rosenberg RN. Genome-wide association studies in Alzheimer disease. Arch Neurol 2008; 65:329-34.