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On the lack of a clear-cut association between alpha-2-macroglobulin deletion and the risk of Alzheimer disease in Poland

Grażyna Michałowska-Wender[1,2], Alicja Wawrzynek[1], Grzegorz Rossa[3], Wojciech Kozubski[4], Mieczysław Wender[2]

[1]Laboratory of Neurogenetics, Department of Neurology, University of Medical Sciences, Poznań, Poland, [2]Neuroimmunological Unit, Mossakowski Medical Research Centre, Polish Academy of Sciences, Poznań, Poland, [3]Regional Hospital for Neurological and Psychiatric Diseases, Cibórz, Poland, [4]Department of Neurology, University of Medical Sciences, Poznań, Poland

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Abstract

Alzheimer disease (AD) is a complex, multi-factorial disease with the potential involvement of several genes. Alpha-2-macroglobulin (A2M) has been implicated in AD on the basis of its ability to mediate the clearance and degradation of β-amyloid peptide. Nevertheless, it is not clear whether there are racial differences in frequency of polymorphisms of A2M in AD. We examined a group of 50 unrelated patients from Poland (38 women and 12 men), who were diagnosed clinically as probably developing AD (according to the N1NCD3 – ADR PA criteria). The patients were examined by a neurologist and a psychologist and had a CT or MRI scan of the brain. Fifty individuals of matched age, without any signs of dementia, were studied as a control group. DNA was extracted by a routine method from a blood sample. Amplification and genotyping at A2M was performed as described by Blacker et al. (1997). The genotypic distribution in A2M exon 18 in patients with AD and genotype TT in A2M exon 24 was similar to that in the controls. Significant differences were noted only in early onset AD in males and for old onset disease in females. The deletions were found more frequently in AD; however, they were found in only a small proportion of studied patients. These findings indicate that A2M is not the only biological candidate gene for AD determination.

Key words: *Alzheimer disease, alpha-2-macroglobulin, polymorphism.*

Introduction

Alzheimer disease (AD) is a complex multi-factorial disease with the potential involvement of several genes. Alpha-2-macroglobulin (A2M) has been implicated in AD on the basis of its ability to mediate the clearance and degradation of β-amyloid peptide. Nevertheless, it is not clear whether there are racial differences in frequency of polymorphisms of A2M in AD.

Association of A2M deletion polymorphism with sporadic AD was reported in Korea [11] and in southern Italy [23]. Genetic association of A2M with AD was also noted in the Finnish elderly population [16]. Similar results were reported in the USA [5,7,13]. These data seem to support the view about susceptibility for AD, linked to A2M polymorphism.

However, there are also many studies with negative results, including in Poland [21], Hungary [10],

Communicating author:

Grazyna Michalowska-Wender, Neuroimmunological Unit, Mossakowski Medical Research Centre, Polish Academy of Sciences, 49 Przybyszewskiego St., 60-355 Poznań, Poland, phone/fax: +48 61 869 17 91, e-mail: grazynawender@wp.pl

Japan [9], China [20], USA [22], Italy [17], Germany [3], France [8] and Sweden [6].

The discussed differences between various human populations may be explained by the known gene expression variability between different human populations, with the impact on disease susceptibility [14], and by biochemical individuality probably with potential relevance to AD [15].

The above, briefly presented differences regarding whether existing data point to an association between A2M polymorphism and AD justify our interest in this problem.

Material and methods

We examined a group of 50 unrelated patients from Poland (38 women and 12 men), who were diagnosed clinically as probably developing AD (according to the N1NCD3 – ADR PA criteria), including 35 patients with an onset of over 65 years of age (late AD) and 15 with an early AD (onset before the 65th year of age). The patients were examined by the neurologist and psychologist and had a CT or MRI scan of the brain. Fifty individuals of matched age, without any signs of dementia, were studied as control subjects.

DNA was extracted by a routine method from a 1 ml EDTA blood sample. All patients were pseudosporadic ones, without evident familiar association. Amplification and genotyping at A2M was performed as described by Blacker *et al*. [5].

Results

The genotype and allelic distribution were compared between controls and patients with AD. In the control group the genotype WW in A2M exon 18 was detected in 74% of individuals. Heterozygous W/Δ was found in 24%, and ΔΔ was found in only 2% of control group individuals. Allelic distribution in the control group was 86% for W and 14% for Δ.

The genotypic distribution in A2M exon 18 in patients with AD was similar to that in the controls. The genotype WW was detected in 32 cases (64%), while heterozygous W/Δ was detected in 3 cases (6%). A polymorphism (deletion) was uncovered in 4 cases. The allelic distribution was found to be 78% for W and 22% for Δ. The comparisons of genotype and allele frequencies between the early (before 65 years) and late onset (older than 65 years) as well as between male and female patients with AD are presented in Table I. Statistically significant differences were not found.

The genotype TT in A2M exon 24 of the control group was detected in 52%, TC in 30% and CC in 18% instances. In the control group the allelic distribution was as follows: T 67% and C 33%. In AD the TT genotype in A2M exon 24 was found in 19 patients (38%), TC in 19 individuals (38%) and CC in 12 cases (29%), and the allelic distribution was C 43% and T 57%. The genotype and allele frequencies, divided according to gender, in early onset AD are presented in Table II. Significant differences were detected between the subgroup of males in early onset AD and the controls, as well as between the subgroup of females with late onset AD and the respective controls.

Discussion

The molecular basis of AD is of general interest and in recent years has been broadly discussed in

Table I. Genotype and allele frequencies for alpha-2-macroglobulin (A2M) exon 18 in Alzheimer disease and control groups

	Age < 65, *n* = 15			Age > 65, *n* = 35			Control, *n* = 50		
		Female	Male		Female	Male		Female	Male
W/W	12	4	8	20	13	7	37	26	11
W/Δ	3	1	2	11	5	6	12	9	3
Δ/Δ	0	0	0	4	3	1	1	1	0
W	27	9 *1	18 *2	51	31 *3	20 *4	86	61 *1*3	25 *2*4
Δ	3	1	2	19	11	8	14	11	3
ratio W : Δ	9 : 1	9 : 1	9 : 1	2.5 : 1	3 : 1	2 : 1	8 : 1	6 : 1	0.8 : 1

*OR = 0.6162; p = 0.6609 (*1)*
*OR = 0.9259; p = 0.9364 (*2)*
*OR = 1.9677; p = 0.1585 (*3)*
*OR = 3.3333; p = 0.1040 (*4)*

Table II. Genotype and allele frequencies for alpha-2-macroglobulin (A2M) exon 24 in Alzheimer disease and control groups

	Age < 65, *n* = 15			Age > 65, *n* = 35			Control, *n* = 50		
		Female	Male		Female	Male		Female	Male
T/T	5	0	5	14	8	6	26	24	2
T/C	9	4	5	10	5	5	15	6	9
C/C	1	1	0	11	8	3	9	6	3
T	19	4 *1	15 *2	38	21 *3	17 *4	67	54 *1*3	13 *2*4
C	11	2	0	32	21	11	33	18	15
ratio T : C	2 : 1	2 : 1	15 : 0	3.8 : 3.2 1 : 1	1 : 1	1.7 : 1	2 : 1	3 : 1	1 : 1

*OR = 15000; p = 0.6551 (*1)*
*OR = 0.02810; p = 0.0161 (*2)*
*OR = 3.0000; p = 0.0076 (*3)*
*OR = 0.5608; p = 0.2855 (*4)*

very interesting review papers [1,2,18,19]. Among several genetic factors which have been implicated in AD, only a few are thought to be causative for the disease. In the majority of sporadic AD cases, genetic factors act as predisposing agents, without the capacity to induce the disease but able to increase the risk of disease above that found in the general population. They may also interact among themselves to further enhance the probability of inducing the disease (synergic effect) [21].

In our studies we have used the typical approach to evaluate the genetic contribution to the risk of AD by analyzing the frequency distribution of the allelic variants at polymorphic sites of the candidate gene, A2M, in cases of the disease and in controls. Alpha-2-macroglobulin is a serum pan-protease inhibitor, also expressed in the brain, which has been implicated in AD on the basis of its ability to mediate the clearance and degradation of Aβ, the major component of β-amyloid deposits. Alpha-2-macroglobulin is a component of senile plaques. The A2M gene, mapped to chromosome 12p, became a candidate as a disease locus for late-onset AD. Based on the positional information about the candidate gene, Blacker *et al.* [5] focused on a 5 bp deletion/insertion polymorphism of the A2M gene, located in the 5' splice site of exon 18. By using a family-based association test method, they found an association between the deletion allele (A2M-D) and late-onset AD. With a deletion, A2M may be less effective in β-amyloid binding and clearance and in preventing its deposition in senile plaques. Sequencing of the RT-PCR products from the deletion and insertion homozygotes revealed no alteration in sequence from that expected, despite the loss of 5 bp adjacent to exon 18. These molecular findings suggest that A2M deletion might be nonfunctional [6].

In our studied material we have not found any clear-cut differences in the frequency of genotype and allele frequency between AD and controls. Significant differences were noted only in early onset AD in males and for old onset disease in females. The deletions were found more frequently in AD; however, they were found in only a small proportion of studied patients.

There are three biological manifestations of the Aβ-A2M interactions that may be directly relevant to the etiology of AD [13]. First, the interaction between A2M and Aβ prevents Aβ fibril formation and fibril-associated neurotoxicity. Secondly, protease activation of Aβ-A2M complexes or protease activation of A2M followed by Aβ binding can promote the protease-mediated degradation of A2M-bound Aβ. And thirdly, protease activated Aβ-A2M complexes may undergo LPR-mediated endocytosis followed by trafficking of Aβ to lysosomes for degradation. The established late-onset risk factor ApoE ε4 accelerates Aβ deposition, and ApoE is found in a complex with A2M in plasma. Taken together, these findings indicate that A2M is not the only biological candidate gene for AD determination. So far, only the ApoE ε4 allele is known to be a genetic risk factor for the later onset sporadic AD.

Similar negative results in the Polish population were obtained in studies of CR1, PICALM and CLU gene polymorphisms [12]. Therefore the slightly positive results obtained in studies by Bednarska-Makaruk *et al.* [4] are very interesting. The authors con-

cluded that paraoxonase 1 (PON1) gene promoter polymorphism may have a role in AD development. However, the role seems to be only an additional one.

Disclosure

Authors report no conflict of interest.

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