Short report

Possible protective role of the 489C>T P2X7R polymorphism in Alzheimer's disease

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A B S T R A C T

Inflammation is a key factor in the onset and progression of Alzheimer’s disease (AD). The P2X7 receptor (P2X7R) is increasingly recognized as key pro-inflammatory receptor. A recent study has shown that activation of microglia by amyloid β (Aβ) and associated release of IL-1β, requires P2X7R expression. In this study we assessed by RT-PCR in genomic DNA samples, the frequency of two single-nucleotide polymorphisms (SNP) of P2X7R in AD patients compared to age-matched non demented elderly. Our data show that the 489C>T SNP was significantly less frequent in AD patients than in controls (p = 0.01), whereas there was no statistical difference in 1513A>C frequency in either groups. In addition, presence of the 1513C allele and absence of the 489C allele decreased the probability of having AD by about four fold. In conclusion, our data show a strong negative association between the P2X7R 489C>T polymorphism and AD, especially in the presence of the 1513C allele.

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1. Introduction

Alzheimer’s disease (AD) is the leading cause of dementia in the elderly and the number of patients is increasing due to the increment of the average lifespan. There is little doubt that inflammation is a key factor in the initiation and progression of AD (Niranjan, 2013; Zhang et al., 2013). It has in fact been shown that overt neuroinflammation and increased central nervous system IL-1β levels are present only in AD patients and not in healthy elderly subjects, even in the presence of the typical AD plaques (Rao et al., 2011). The P2X7R is a potent activator of the NLRP3 (NOD-like receptor family, pyrin domain containing 3) inflammasome and a strong stimulant of IL-1β release, besides being also involved in antigen presentation, endosome–lysosome fusion and phagocytosis (Di Virgilio, 2013; Rayah et al., 2012). In a transgenic murine model of AD P2X7R is overexpressed by glial cells surrounding Aβ plaques, and is a necessary requirement for Aβ-stimulated IL-1β release in vivo (Parvathenani et al., 2003; Sanz et al., 2009). In addition, P2X7 is up-modulated by microglia isolated from brains of AD patients (Parvathenani et al., 2003; McLarnon et al., 2006).

The P2X7R, a member of the P2X subfamily of purinergic receptors, is a cation-selective ion channel activated by extracellular ATP. When over-activated, P2X7R undergoes a channel-to-pore transition that causes the opening of a non-selective plasma membrane pore permeable to aqueous solutes up to a MW of 900 D. The precise physiological function of P2X7R is as yet unknown, but rather intriguingly this gene is highly polymorphic, with over thirty single-nucleotide polymorphisms (SNPs) described (Backlund et al., 2012; http://www.genecards.org/cgi-bin/carddisp.pl?gene=P2X7&search=97e20c4c94f763e865b40c278d3b2d).

Recently, attention has been focused on two SNPs, 1513A>C (rs3751143) and 489C>T (rs208294), leading the substitution of glutamate for alanine at position 496 (E496A) and histidine for tyrosine at position 155 (H155Y), respectively. The 1513A>C substitution, localized in the carboxyl-terminal cytoplasmic tail of the receptor, is associated in heterozygosis (AC) with a 50% decrease in P2X7R responses, and in homozygosis (CC) to a near complete loss of P2X7R function (Gu et al., 2001). This SNP has been linked with different diseases such as a familial form of chronic lymphocytic leukemia, the follicular variant of papillary thyroid cancer, and increased susceptibility to tuberculosis infections (Sluyter and Stokes, 2011). The 489C>T SNP, localized in P2X7R...
ectodomain, causes a gain of function and has not been associated so far to any disease (Cabrini et al., 2005; Sluyter and Stokes, 2011).

The purpose of this study was to assess the frequency of 1513A–C and 489C–T in AD patients versus age-matched subjects.

2. Methods

2.1. Study participants

The study population comprised 84 Caucasian Late Onset Alzheimer’s disease subjects (age range 65 to 88 years, average age 78 ± 6) diagnosed according to the National Institute of Neurological and Communicative Disorders and Stroke/Alzheimer’s Disease and Related Disorders Association (NINCDS-ADRDA) criteria. Consecutive patients referring to the Day Hospital Services (Ferrara, Italy) were enrolled during a four-year period. Subjects affected by severe congestive heart failure (New York Heart Association classes III–IV), severe liver or kidney disease, severe chronic obstructive pulmonary disease, or cancer were excluded. For neuropsychological assessment, all patients were given a battery of tests as previously described (Zuliani et al., 2007). 148 free-living elderly subjects (control group) (age range 65 to 93 years, average age 73 ± 5.6) were recruited among those referring to the “Ageing Center” of University of Chieti (Italy). Patients with cerebrovascular lesions, mixed or vascular dementia or clinically significant neurologic disease due to conditions other than AD, or major depressive disorder were excluded. All control patients enrolled in the control group were requested to have a Mini Mental State Examination (MMSE) score of 28 or higher. Written informed consent was obtained from participants and/or legally authorized representatives. The study was approved by the local ethics review committee.

2.2. P2X7R polymorphism analysis

Genomic DNA was extracted from blood leukocytes with the salting out method. 1513A–C and 489C–T SNPs were analyzed in genomic DNA samples with the TaqMan MGB probe technique as previously described (Cabrini et al., 2005). 148 free-living elderly subjects (control group) (age range 65 to 93 years, average age 73 ± 5.6) were recruited among those referring to the “Ageing Center” of University of Chieti (Italy). Patients with cerebrovascular lesions, mixed or vascular dementia or clinically significant neurologic disease due to conditions other than AD, or major depressive disorder were excluded. All control patients enrolled in the control group were requested to have a Mini Mental State Examination (MMSE) score of 28 or higher. Written informed consent was obtained from participants and/or legally authorized representatives. The study was approved by the local ethics review committee.

2.3. Statistical analysis

We analyzed P2X7R polymorphisms, alleles, genotypes and haplotypes by Arlequin 3.5.1.2 software (Excoffier et al., 2007). Statistical analysis was performed using StatView software package (SAS Institute Inc., Cary, NC) and GraphPad Prism 6.0 software (Graphpad Software, San Diego, CA, USA). Significance was assumed for p < 0.05.

3. Results and discussion

Baseline characteristics of subjects of both groups are shown in Table 1. The frequency of patients with hypertension, coronary heart disease (CHD) and diabetes is significantly higher in the AD group compared to the control group.

Table 2 shows frequencies of 1513A–C and 489C–T SNPs from non-demented control subjects and AD patients. Both P2X7R polymorphisms were in Hardy–Weinberg equilibrium in both control and AD populations (1513A–C: controls χ²: 0.29, p = 0.59; AD patients χ²: 3.7, p = 0.054; 489C–T controls χ²: 0.24, p = 0.62; AD patients χ²: 0.08, p = 0.78).

The minor allele frequency of 1513A–C and 489C–T polymorphisms was comparable with frequencies found in other published European reports (Cabrini et al., 2005; Sluyter and Stokes, 2011). Curiously, we have observed an increase, although not significant, of the frequency of the 1513A–C and 489C–T polymorphisms and 1513CC and 489TT homozygotes in control subjects compared to values obtained previously in our laboratory by analyzing these polymorphisms and genotypes in young volunteers (Cabrini et al., 2005). This increase could be due to the difference in age between the two groups, similar to results obtained by Zhang et al. (2003).

When the possible relationship between the 1513A–C polymorphism with AD was analyzed, no significant association was detected. Instead, analysis of the 489C–T polymorphism frequency showed an increase in the 489C allele and the 489CC genotype in AD patients compared to controls. Thus, the 489CC genotype was more frequent than 489TT in AD, whereas in elderly non-demented 489TT was more frequent than 489CC.

Analysis of haplotypes revealed a different distribution between controls and AD population. To examine these differences, subjects were subdivided on the basis of the presence/absence of both alleles in combination; because the most common genotype for 1513A–C and 489C–T P2X7R polymorphisms was AA and CT, respectively, the most frequent subgroup, was formed by the absence of the 1513C allele and the presence of the 489C allele; thus, this subgroup was taken as referent for analysis. Odd’s ratio (OR) and 95% confidence interval (CI) were computed to assess the possible association between alleles–genotypes of 1513A–C and 489C–T P2X7R polymorphisms and the risk of having a diagnosis of AD (Table 3). The presence of the 1513C allele and the absence of the 489C allele decreased the probability of having AD by about four fold versus the reference subgroup.

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The presence of the 1513C allele causes a severe decrease in P2X7R-associated responses. This receptor is well known for its pro-inflammatory activity (Bours et al., 2011), thus a reduced function
should confer an “anti-inflammatory” phenotype that recently has been associated to an increase in Aβ phagocytosis in microglia (Ni et al., 2013). On the other hand, the P2X7R ectodomain, in particular the “dolphin nose” region (residues 115–168) appears to be associated with phagocytosis (Wiley and Gu, 2012); the presence at residue 155 of the 489T genotype inside this region may potentiate phagocytosis activity of P2X7R, and subsequent Aβ elimination. Fiala et al. have shown that monocyte and macrophages from AD patients are inefficient in Aβ phagocytosis and clearance, it is not clear if it was the cause and/or a consequence of the disease (Fiala et al., 2005). However, control of Aβ deposition is certainly an important step in the onset and development of the disease and P2X7 could play an important role in this process and may be useful as target for prevention or treatment therapies; actually there are only palliative therapies for symptoms. Further studies will certainly help to solve this issue.

4. Conclusions

In conclusion, this manuscript shows that the 489C>T P2X7 polymorphism alone or in comcomitant with 1513C allele of 1513A>C P2X7R polymorphism are less frequent in Alzheimer’s disease (AD) patients than in age-matched non-demented elderly. This is the first study that associates the 489C>T P2X7R polymorphism with a disease.

Conflict of interest

JSM, SF, RR, FC, GZ declare no conflict of interest. FDV has served as consultant for Biosceptre Ltd, a company involved in the development of P2X7R-targeted drugs.

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Table 3

Relationship between combination of 1513A>C and 489C>T genotypes and risk of receiving the diagnosis of Late Onset Alzheimer’s disease.

| 1513A > C | 489C > T | Control n (%) | AD n (%) | OR (95% C.L.) | p* |
|-----------|---------|---------------|---------|---------------|---|
| Absent C  | Absent C| 19 (12.8)     | 8 (9.5) | 0.62 (0.25–1.66) | 0.38 |
| Absent C  | Present C| 64 (43.2)    | 44 (52.4) | Reference |
| Present C  | Absent C| 29 (19.6)    | 5 (6.0) | 0.25 (0.09–0.71) | 0.007 |
| Present C  | Present C| 26 (18.5)  | 27 (32.1) | 1.08 (0.58–2.0) | 0.87 |

Total 148 84

Bold entries indicate a significant p value.

* Fisher exact test.