ELSEVIER

Brain Research 951 (2002) 77–81

BRAIN RESEARCH

www.elsevier.com/locate/bres

Research report

Olfactory identification and apolipoprotein E ε4 allele in mild cognitive impairment

Qing-Song Wang[a,c], Lin Tian[a], Yong-Lu Huang[b], Song Qin[a], Long-Quan He[c], Jiang-Ning Zhou[a,b,*]

[a]*Laboratory of Neurodegenerative Diseases, School of Life Science, University of Science and Technology of China, Hefei 230027, PR China*
[b]*Anhui Geriatric Institute, the First Affiliated Hospital of Anhui Medical University, Hefei 230001, PR China*
[c]*Department of Neurology, the 105th Hospital, Hefei 230031, PR China*

Accepted 20 May 2002

Abstract

To investigate olfactory identification and apolipoprotein E ε4 allele in patients with mild cognitive impairment (MCI), we used Cross-Cultural Smell Identification Test (CC-SIT) from University of Pennsylvania to assess olfactory identification performance and polymerase chain reaction (PCR) to detect apolipoprotein E ε4 (ApoE ε4) allele in 28 patients with MCI and the 30 age-matched control subjects in present study. The Mann–Whitney U test demonstrated that the MCI group performed significantly worse on CC-SIT than the normal aging group ($P<0.01$). For MCI patients olfaction scores correlated positively with CAMCOG-C ($r=0.61$, $P<0.01$), but not with age, gender or years of education. In normal subjects, the CC-SIT score showed no significant associations with age, gender, years of education, or CAMCOG-C. As the least common allele in Chinese, ε4 was found in 13.3% of controls and in 35.8% of MCI in this study. ApoE ε4 was significantly higher in MCI group than normal group ($\chi^2=4.65$, $P<0.01$). There was a significant effect of allele status on odor identification: subjects with ε4 allele were not able to identify as many odors as the subjects without ε4 allele ($P<0.01$). These results suggested that the decreased olfactory identification in MCI may be a marker for the early diagnosis of Alzheimer's disease, and ApoE genotype may be part of the basis of olfactory identification decline.

Theme: Disorders of the nervous system

Topic: Degenerative disease: Alzheimer's-other

Keywords: Mild cognitive impairment; Olfactory identification; Apolipoprotein E ε4

1. Introduction

The transition from normal cognition to AD is gradual [8,10,11,20]. Affected individuals typically pass through a disease state recognized as mild cognitive impairment (MCI), which refers to non-demented aged persons with mild memory or cognitive impairment that cannot be accounted for by any recognized medical or psychiatric condition [2,9,18,19]. MCI is thought to be the transitional state between normal aging and AD. The rate at which MCI patients convert to AD is substantially greater than that of the general older population [2,20,24]. It is difficult for clinicians to decide which persons are MCI since some memory failure can be seen in normal aging [21]. Moreover, there is also a clinical problem of the non-specificity of neuropsychometric testing for diagnosing MCI. Difficulties on a memory task may be attributable to other cognitive difficulties, in particular attentional, central executive, and comprehension impairment [24]. Lack of awareness of cognitive deficits is common in early stage of Alzheimer's disease and may occur at the mild cognitive impairment stage before the diagnosis of AD is made. Early detection of AD has clinical and potential therapeutic applications in MCI [20,24]. It has, therefore, become the responsibility of clinical investigators to try to characterize the transitional state between normal aging and AD.

*Corresponding author. Tel.: +86-551-360-7778; fax: +86-551-262-4887.
E-mail address: anhuigi@mail.hf.ah.cn (J.-N. Zhou).

0006-8993/02/$ – see front matter
PII: S0006-8993(02)03137-2

The processes of odor identification involve medial temporal lobe structures, which are affected early in the course of AD, and therefore olfactory deficit may be a clinical manifestation of early pathology [1,30,34]. Studies of olfactory tasks have generally shown olfactory identification (discrimination among odors) deficits in AD [6,13]. The ability to identify odors is affected early in the disease, whereas the ability to detect odors is affected later [6,27].

Little is known about olfactory identification test performance in patients with mild cognitive impairment, defined broadly as fitting into the category between being 'normal' and having 'dementia'. In present study we hypothesized that there may be olfactory identification decline with MCI. It has been found that individuals with the ApoE $\varepsilon 4$ genotype are at a higher risk for developing AD than those in general population [16]. Investigating the link between olfactory dysfunction and the presence of this gene may, therefore, also yield valuable information concerning preclinical diagnosis. We hypothesized further that persons with one or two ApoE $\varepsilon 4$ alleles may be at higher risk of olfactory identification decline.

2. Materials and methods

2.1. Recruitment and evaluation of subjects

The subjects for this study were recruited through the Memory Clinic in the First Affiliated Hospital of Anhui Medical University and Hefei Nursing Home using a standardized clinical protocol. Informed consent was obtained for participation in the studies. Criteria for the diagnosis of MCI described by Peterson et al. were as follows [18,19]: (1) memory complaints as documented by the patient or collateral source; (2) normal general cognitive function, as determined by measurements of general intellectual function and screening instruments by Cambridge Cognitive Examination Chinese version (CAMCOG-C) and Mini-Mental State Examination (MMSE) [25,32]; (3) normal activities of daily living, as documented by history and record of independent living; (4) dementia ruled out by DSM criteria for probable or possible AD, and met no NINCDS-ADRDA criteria for AD; (5) objective memory impairment, defined by performance at 1.5 standard deviations below age and education-matched controls on indices of memory functions (by Logical Memory in Wechsler Memory Scale-Revised); (6) age 60 through 89 years; (7) Clinical Dementia Rating scale (CDR) score of 0.5. General physical examinations and neurological examinations were done to all subjects. Laboratory studies were performed, including measures of serum electrolytes, liver and renal function, thyroid function, VDRL, complete blood cell count, chest radiograph, and ECG. Brain imaging (CT or MRI) was performed to all subjects. None of the subjects had a history of a neurological or psychiatric disease.

2.2. Assessment of olfactory identification

All subjects were screened for cognitive, medical, and psychiatric disorders that might interfere with olfactory score and thus preclude their participation [26]. These disorders included head trauma, hypothyroidism, insulin-dependent diabetes mellitus, Cushing's disease, active hepatitis, cirrhosis, chronic renal failure, multiple sclerosis, vitamin B_{12} deficiency, and local respiratory tract factors such as active rhinitis or sinusitis (allergic or infectious), active asthma, and history of nasal polyps or surgery. Potential subjects were also not included if they were currently taking antidepressants, neuroleptics, antihistamines, or anticholinergics. Current smokers were excluded from participation. Olfactory identification performance was assessed using the 12-item Cross-Cultural Smell Identification Test (CC-SIT) from University of Pennsylvania. The CC-SIT is a standardized, four-alternative, forced-choice test of olfactory identification. The stimuli are embedded in 'scratch and sniff' microcapsules fixed and positioned on strips at the bottom of each page. The items of this test are well known in most non-English-speaking cultures, making it a popular item in Europe, South America and Asia [5]. The number of correct odors identified out of the 12 was summed for a total score. All missing items were assigned a value of 0.25, which is the same probability as a random guess.

2.3. APOE genotype

Genomic DNA was extracted from peripheral blood with a Genomix DNA extraction kit according to the manufacturer's protocol. The genotype of each extracted DNA sample was determined by PCR amplification using the primers 5′-TCCAAGAGCTGCAGGCGGCGCA-3′ and 5′-ACAGAATTCGCCCGGCCTGTACACTGCCT-3′. Then, the PCR product was digested by CfoI using previously described methods [33].

2.4. Statistic analysis

We used Statistic 6.0 to analyze the data. Difference in CC-SIT, CAMCOG-C, age, education, and MMSE between controls and MCI were tested using the Mann–Whitney U test. The deference in the frequency of ApoE alleles between controls and MCI was tested by χ^2 analysis. Correlations of years of education, age, and CAMCOG-C vs. CC-SIT were analyzed by Correlation matrices. Difference were considered statistically significant at the $P<0.05$ level.

3. Results

In the present study, no difference was found in age, education and gender distribution between normal aging

group and MCI group (see Table 1). There was a significant decline in the function of orientation ($P<0.01$), praxis ($P<0.01$) and language ($P<0.01$) besides memory impairment ($P<0.01$) in patients with MCI (see Table 1). Though general cognitive function in MCI group is in normal range (the cut-off of CAMCOG-C is 79/80), there was a significant decline in general cognitive function in MCI group ($P<0.01$).

We compared the performance on CC-SIT of the 28 MCI patients and the 30 aged-matched control subjects. The Mann–Whitney *U* test demonstrated that MCI group performed significantly worse on CC-SIT than the normal aging group ($P<0.01$) (see Table 1). For MCI patients olfaction scores correlated positively with CAMCOG-C ($r=0.61$, $P<0.01$), but not with age ($r=-0.39$, $P=0.09$), years of education ($r=0.38$, $P=0.09$). In normal subjects, the CC-SIT score showed no significant associations with age ($r=0.12$, $P=0.54$), years of education ($r=-0.18$, $P=0.35$), or CAMCOG-C ($r=0.03$, $P=0.35$).

As the least common allele in Chinese, $\varepsilon 4$ was found in 13.3% of controls and in 35.8% of MCI in this study. ApoE $\varepsilon 4$ was significantly increased in MCI group vs. Normal group ($\chi^2=4.65$, df=1, $P<0.01$). (see Table 2).

Table 3 illustrated the significant effect of allele status on odor identification and cognitive function: when subjects were grouped according to $\varepsilon 4$ allele, there was a significant decline ($P<0.01$) in CMCOG-C in the group with ApoE $\varepsilon 4$. Moreover, the group with ApoE $\varepsilon 4$ allele was not able to identify as many odors as the group without ApoE $\varepsilon 4$ allele ($P<0.01$).

Table 1
Descriptive information of normal aging group and MCI group

	Normal group ($n=30$)	MCI group ($n=28$)
Age (years)	73.84±5.90	71.90±7.78
Sex, F/M	16/14	15/13
Education (years)	9.00±2.63	8.71±3.78
WMS-R		
LM^{I}	20.60±1.44	13.34±0.87**
LM^{II}	14.85±0.78	5.23±0.81**
Subscales of CAMCOG-C		
Orientation	9.73±0.50	9.40±0.73
Language	26.13±1.68	24.41±2.13**
Memory	23.70±1.25	19.79±1.40**
Attention	6.17±1.12	5.34±1.37*
Praxis	10.43±0.86	9.87±1.29
Calculation	1.97±0.18	1.94±0.21
Abstract thinking	5.40±1.71	5.20±1.38
Perception	6.97±1.35	6.95±1.34
CAMCOG-C	90.2±4.80	82.70±4.39**
MMSE	26.20±1.51	25.53±1.39
CC-SIT	9.27±1.26	7.25±1.41**

Data represent mean±S.D. ($n=20$). * $P<0.05$ compared with normal aging group. ** $P<0.01$ compared with normal aging group.
Abbreviations: MMSE, mini-mental state examination; WMS-R, Wechsler memory scale-revised; LM, Logical Memory; CAMCOG-C, Cambridge cognitive examination Chinese version; and CC-SIT, Cross-Cultural Smell Identification Test.

Table 2
The ApoE genotype and $\varepsilon 4$ frequency in controls and MCI patients

	Normal group ($n=30$)	MCI group ($n=28$)
Genotype		
$\varepsilon 2/2$	1	1
$\varepsilon 2/3$	4	3
$\varepsilon 3/3$	18	6
$\varepsilon 2/4$	2	7
$\varepsilon 3/4$	4	9
$\varepsilon 4/4$	1	2
Frequency (%)		
$\varepsilon 2$	13.3	21.4
$\varepsilon 3$	73.4	42.8
$\varepsilon 4$	13.3	35.8*

* $P<0.05$ compared with control.

4. Discussion

There is high vulnerability of episodic memory in MCI and the most prominent, and perhaps the only specific, age-related cognitive decline in normal aging is a decrease in episodic memory performance [2,28,32]. The present study shows markedly lower scores in CC-SIT of MCI patients, which indicated a significant olfactory identification impairment in MCI. Since the presence of olfactory dysfunction is very well established in prevalent AD patients, some researchers have hypothesized its potential usefulness as a possible biological marker of AD [3,14,17,23]. The decreased olfactory identification in MCI

Table 3
Descriptive information of cognitive performance in the group with and without $\varepsilon 4$ allele

	Group without $\varepsilon 4$ ($n=33$)	Group with $\varepsilon 4$ ($n=25$)
Age (years)	71.67±6.28	70.70±7.36
Sex, F/M	18/15	13/12
Education(years)	8.87±2.51	8.43±3.39
Subscales of CAMCOG-C		
Orientation	9.57±0.51	9.41±0.58
Language	25.93±1.76	23.65±1.97*
Memory	21.99±1.87	21.20±2.19*
Attention	5.86±1.32	5.67±1.13
Praxis	10.39±0.76	9.7±1.41
Calculation	1.95±0.26	1.96±0.13
Abstract thinking	5.30±1.76	5.39±1.30
Perception	7.28±1.23	6.71±1.37
CAMCOG-C	88.27±5.28	83.70±5.23**
CC-SIT	9.11±1.37	7.34±1.43**

Data represent mean±S.D. * $P<0.05$ compared with normal aging group. ** $P<0.01$ compared with normal aging group.
Abbreviations: CAMCOG-C, Cambridge cognitive examination Chinese version; CC-SIT, Cross-Cultural Smell Identification Test.

makes it to be a possible biomarker for the early diagnosis of AD.

Odor detection refers to a person's ability to determine the presence or absence of an odor, while odor identification refers to one's ability to produce and attach a verbal label to an odor or to identify an odor that matches a verbal por nonverbal (picture) label provided by another person. Odor identification is generally found to be impaired in the early stage of AD. The two studies that found no impaired detection recruited patients with mild AD. The change in olfactory detection is a later phenomenon that appears in advanced stages of AD [13,27]. Serby found that olfactory identification performances were nearly 40% lower in mild AD patients than in age, and gender-matched controls [27]. Interestingly, our data show that olfactory identification performances were about 20% lower in MCI patients than in age, and gender-matched controls. This suggested that deficiency in olfactory identification from normal to AD is gradual one. While the entorhinal cortex is not exclusively an olfactory region, it does receive a large direct olfactory input from the olfactory bulb via the lateral olfactory tract, and abnormalities in this region are likely to disrupt olfactory functioning [7,29,34]. Little is known about the neuropathology of MCI and related disorders, because there have been few longitudinal studies that include neuropathological confirmation. An olfaction deficit may be a clinical manifestation of early pathology in MCI.

ApoE $\varepsilon 4$ allele is a well documented risk factor of AD. A higher rate of ApoE $\varepsilon 4$ allele was reported both in people with a positive family history of AD and in sporadic AD [4,18]. It was reported that 80% of people with family AD and 64% of sporadic AD have at least one $\varepsilon 4$ allele, compared to 25–31% of control subjects possessing an $\varepsilon 4$ allele [22,31]. In our present study, 64.2% (18/28) of MCI have at least one $\varepsilon 4$ allele, compared to 23.3% (7/30) of control subjects possessing an $\varepsilon 4$ allele. Murphy and colleagues have demonstrated that ApoE $\varepsilon 4$ allele is associated with odor identification deficits in nondemented older persons [15]. Olfactory dysfunction in the presence of one or more ApoE $\varepsilon 4$ alleles is associated with a high risk of cognitive decline [12]. Our data showed that patients with MCI had much higher frequency of ApoE $\varepsilon 4$ allele. Moreover, there was significant decline in cognitive function in subjects with $\varepsilon 4$ allele versus subjects without $\varepsilon 4$ allele and subjects with $\varepsilon 4$ allele was not able to identify as many odors as the subjects without $\varepsilon 4$ allele. These results indicated that presence of the ApoE $\varepsilon 4$ allele may be considered as a risk factor or a genetic factor for MCI and olfactory identification is associated with ApoE $\varepsilon 4$ allele.

The use of olfactory assessment to distinguish MCI from normal aging has several advantages. The CC-SIT is easy to administer, time- and cost-efficient (it takes about 5–10 min and costs about 13$ for one subject). It is also not greatly affected by education level, while many standard neuropsychological tests are usually affected by education level. A weakness of this approach is that olfactory impairment is not specific for AD type dementia [5,26]. We view olfactory testing as a potentially useful adjunctive technique; its ultimate diagnostic value remains to be cross-validated empirically in future research. Whether, in combination with other tests (such as APOE genotyping) and perhaps neuropsychological testing, the specificity of olfactory dysfunction in MCI may be enhanced, should be shown by follow-up studies.

Acknowledgements

This project was supported by the Chinese National Key Project of Basic Research (G19990054).

References

[1] H. Braak, E. Braak, Neuropathological staging of Alzheimer-related changes, Acta Neuropathol. 82 (1991) 239–259.
[2] P. Celsis, Age-related cognitive decline, mild cognitive impairment or preclinical Alzheimer's disease?, Ann. Med. 32 (2000) 6–14.
[3] L.K. Chiodo, D.R. Royall, C.J. Jaramillo, M.J. Polk, Olfactory function and cognition in independent elders, J Am. Geriatr. Soc. 46 (1998) S71.
[4] E.H. Corder, A.M. Saunders, W.J. Strittmatter, D.E. Schmechel, P.C. Gaskell, G.W. Small, A.D. Roses, J.L. Haines, M.A. Pericak-Vance, Gene dose of Apolipoprotein type 4 allele and risk of Alzheimer's disease in late onset families, Science 261 (1993) 921–923.
[5] R. Doty, A. Marcus, W. Lee, Development of the 12-item Cross-Cultural Smell Identification Test, Laryngoscope 106 (1996) 353–356.
[6] H. Eichenbaum, T.H. Morton, H. Potter, S. Corkins, Selective olfaction deficits in case HM, Brain Res. Bull. 18 (1983) 597–600.
[7] G. Ellison, The *N*-methyl-D-aspartate antagonists phencyclidine, ketamine and dizocilpine as both behavioral and anatomical models of the dementias, Brain Res. Rev. 20 (1995) 250–267.
[8] D.A. Fleischman, D. Gabrieli, Long-term memory in Alzheimer's disease, Curr. Opin. Neurobiol. 9 (1999) 240–244.
[9] C. Flicker, S.H. Ferris, B. Reisberg, Mild cognitive impairment in the elderly: predictor of dementia, Neurology 41 (1991) 1006–1009.
[10] G.B. Frisoni, M.P. Laakso, A. Beloramello, C. Geroldi, A. Bianchetti, H. Soininen, M. Trabucchi, Hippocampal and entorhinal cortex atrophy in frontotemporal dementia and Alzheimer's disease, Neurology 52 (1999) 91–100.
[11] T. Gomez-Isla, J.L. Price, D.M. Mckeel, J.C. Morris, J.H. Growdon, B.T. Hyman, Profound loss of layer entorhinal cortex neurons occurs in very mild Alzheimer's disease, J. Neurosci. 16 (1996) 4491–4500.
[12] A.B. Graves, J.D. Bowen, M.S. Rajaram, W.C. McCormick, S.M. McCurry, G.D. Schellenberg, E.B. Larson, Impaired olfaction as a marker for cognitive decline interaction with ApoE $\varepsilon 4$ status, Neurology 53 (1999) 1480–1487.
[13] E. Koss, J.M. Weiffenbach, J.V. Haxby, R.P. Friedland, Olfactory detection and recognition in Alzheimer's disease, Lancet 1 (1987) 622.
[14] J.A. Mortimer, I. Fortier, E.P. Pepin, D. Gauvreau, Sensory loss as a predictor of dementia and questionable dementia in a community of elderly persons, Neurobiol. Aging 17 (1996) S 79.
[15] C. Murphy, A.W. Bacon, M.W. Bondi, D.P. Salmon, Apolipoprotein

E status is associated with odor identification deficts in nondemented older persons, Ann. N.Y. Acad. Sci. 855 (1998) 744–750.
[16] S. Noguchi, K. Murakami, N. Yamada, Apolipoprotein E polyphism and Alzheimer's disease, Lancet 342 (1993) 737.
[17] S. Nordin, C. Murphy, Impaired sensory and cognitive olfactory function in questionable Alzheimer's disease, Neuropsychology 10 (1996) 113–119.
[18] R.C. Petersen, G.E. Smith, R.J. Ivnik, E.G. Tangales, D.J. Schaid, S.N. Thibodeau, E. Kokmen, S.C. Waring, L.T. Kurland, Apolipoprotein E status as a predictor of the development of Alzheimer's disease in memory impaired individuals, J. Am. Med. Assoc. 273 (1995) 1274–1278.
[19] R.C. Petersen, G.E. Smith, S.C. Waring, R.J. Ivnik, E.G. Tangles, E. Korkme, Mild cognitive impairment clinical characterization and outcome, Arch. Neurol. 56 (1999) 303–308.
[20] R.C. Petersen, Mild cognitive impairment or questionable dementia?, Arch. Neurol. 57 (2000) 643–644.
[21] R.C. Petersen, G. Smith, E. Kokmen, R.J. Ivnik, E.G. Tangalos, Memory function in normal aging, Neurology 42 (1992) 396–401.
[22] J. Poirier, J. Davignon, D. Bouthillier, S. Kogan, P. Bertrand, S. Gauthier, Apolipoprotein E polymorphism and Alzheimer's disease, Lancet 342 (1993) 697–699.
[23] D.L. Rezek, Olfactory deficits as a neurologic sign in dementia of the Alzheimer type, Arch. Neurol. 44 (1987) 1030–1032.
[24] K. Ritchie, J. Touchon, Mild cognitive mild:conceptual basis and current nosological status, Lancet 355 (2000) 225–228.
[25] M. Roth, E. Tym, C.Q. Mountjoy, F.A. Huppert, H. Herdrie, S. Verma, R. Goddard, CAMDEX. A standardised instrument for the diagnosis of mental disorder in the elderly with spcial reference to the early detection of dementia, Br. J. Psychiatry 149 (1986) 698–709.
[26] S.S. Schiffman, Taste and smell in disease, N. Engl. J. Med. 308 (1983) 1275–1279.
[27] M. Serby, P. Larson, D. Kallkstein, The nature and course of olfactory defects in Alzheimer's Disease, Am. J. Psychiatry 148 (1991) 357–360.
[28] S. Small, Y. Stern, M. Tang, R. Mayeux, Selective decline in memory function among healthy elderly, Neurology 52 (1999) 1392–1396.
[29] U. Stabuli, G. Ivy, G. Lynch, Hippocampal denervation causes rapid forgetting of olfactory information in rats, Proc. Natl. Acad. Sci. USA 81 (1984) 5585–5597.
[30] B.R. Talamo, R. Rudel, K.S. Kosik, V.M. Lee, S. Neff, L. Adelman, J.S. Kauer, Pathological changes in olfactory neurons in patients with Alzheimer's disease, Nature 337 (1989) 736–739.
[31] J. Travis, New piece in Alzheimer's puzzle, Science 261 (1993) 828–829.
[32] Q.S. Wang, J.N. Zhou, Retrieval and encoding of episodic memory in normal aging and patients with mild cognitive impairment, Brain Res. 924 (2002) 113–115.
[33] P.R. Wenham, W.H. Price, G. Blundel, Apolopoprotein E genotyping by one-stage PCR, Lancet 337 (1991) 1158–1159.
[34] Y. Xu, C.R. Jack Jr., P.C. O'Brien, E. Kokmen, G.E. Smith, R.J. Ivnik, B.F. Boeve, R.G. Tangalos, R.C. Petersen, Usefulness of MRI measures of entorhinal cortex versus hippocampus in AD, Neurology 54 (2000) 1760–1767.