ApoE gene and familial risk of Alzheimer’s disease as predictors of odour identification in older adults

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Abstract

The study examined odour identification ability in healthy older adults at increased risk for developing Alzheimer’s disease (AD). We recruited a sample (n = 24) of siblings related to probable AD cases and an age-matched control sample (n = 47). All participants were genotyped for the presence of the ApoE ε4 allele. Performance on a simple olfactory task of odour identification was compared according to positive family history of AD and ApoE ε4 status. The sibling group showed an odour identification deficit compared to the control group. Whilst there was no independent influence of ApoE ε4 status on odour identification, there was a significant interaction between positive family history and ApoE ε4 status. Sibling ε4 carriers showed the greatest odour identification deficit and their performance was significantly poorer than both the sibling non-ε4 carrier and control ε4 carrier groups. Odour identification deficits like those reported here are considered to be early cognitive markers of incipient AD. In this respect, these findings support the need to both monitor individuals at increased risk of the disease and introduce olfactory-mediated cognitive tasks into the diagnostic setting.

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1. Introduction

Alzheimer’s disease (AD) is characterised as a relentless neurodegenerative disease; onset is insidious, and no distinct pattern of deficits emerges with its progression, making clinical diagnosis during the early stages of the disease difficult [6]. Known risk factors for developing the disease include age, positive family history, education, gender and genetic predisposition [1,17,33,37].

The strongest known genetic risk factor for sporadic AD is possession of the ε4 allele of the apolipoprotein E (ApoE) gene, located on chromosome 19. The function of ApoE is to transport cholesterol and aid plasma lipoprotein metabolism [22]. Possession of the ε4 allele is associated with reduced age of AD onset and acts in a dose-dependent manner [8,44]. The precise role of ε4 presence in AD is unknown, although strong evidence suggests a pathogenic role where an increased binding ability to beta-amyloid (Aβ) and more intense and extensive Aβ deposits and neurofibrillary tangle density are found in the brains of ε4 homozygotes than ε3 homozygotes [4,39,45]. Moreover, healthy older ε4 carriers are more likely to show early cognitive signs of AD than non-ε4 carriers [3,23,37], which may be a reflection of the underlying neuropathology associated with the allele.

Familial risk of AD is well documented, such that the likelihood of developing the disease increases for individuals with family history of the disease [13,46]. Neuropsychological profiling of this high-risk population has identified possible cognitive markers of incipient AD including more rapid decline in general cognitive function and impaired episodic memory [5,37]. One argument suggests that increased susceptibility in these individuals may be due to an, as yet, unidentified genetic susceptibility to developing AD [31,34].
Olfactory dysfunction is widely reported in AD, and is known to affect the ability to identify, recognise and detect odours [30,36]; see [25] for a review. Indeed, one of the most salient features of preclinical and early stage AD is the reduction in accurate odour identification [10,15,29]. The ability to differentiate olfactory memory performance according to familial risk of AD has been investigated directly in only two studies to date [38,40]. Both found that first-degree relatives (FDRs) showed greater odour identification deficits compared to their control counterparts. The genetic influence of the e4 allele on olfaction has been more widely examined (e.g. [36,47]). Healthy older e4 carriers have shown increased odour detection and odour identification deficits compared to their non-e4 carrying counterparts. More recently, Gilbert and Murphy [14] found that healthy older e4 carriers made more false recognition errors than healthy older non-e4 carriers on an odour recognition memory task. Moreover, the number of false recognition errors committed by healthy older e4 carriers was similar to the number committed by patients with AD, suggesting that this subgroup of healthy older adults was closer to the threshold of incipient AD.

Deficits of this nature may signify the first signs of disease expression in the AD brain as some of the earliest neuropathological changes are known to occur in areas involved in transfer of olfactory information. Degeneration to the transentorhinal and entorhinal regions, particularly the hippocampus and the orbital frontal cortex, and certain association areas of the neocortex [7,11,20,32] disrupts the transfer of olfactory information, possibly giving rise to an odour memory deficit [27].

Given that both possession of the e4 allele and family history of AD have each been identified as risk factors for AD, and that odour identification deficits occur early in the course of AD, the presence of these factors in combination offers potential diagnostic utility as well as theoretical value. The current study, therefore, investigated the proposal that odour identification is impaired in individuals possessing either one or both of these risk factors. For family history of AD, we predicted greater odour identification deficits in relatives of AD patients compared to age-matched control participants. For ApoE e4 presence, we similarly predicted greater odour identification deficits for e4 carriers compared to non-e4 carriers. Finally, an interactive effect of family history of AD and ApoE e4 presence on odour identification deficits was predicted proposing that individuals possessing both risk factors would show greater odour identification deficits compared to those possessing a risk factor in isolation.

2. Method

2.1. Participants

Two participant groups were recruited in this study. A sibling group (n = 24) aged between 59 and 88 years with a mean age of 74 years 1 month, where participants were siblings of probable late-onset AD patients [24] attending the Memory Clinic at Llandough Hospital, Wales, UK. A control group (n = 47) aged between 61 and 87 years with a mean age of 73 years 2 months comprised members of the Community Participant Panel of the School of Psychology, Cardiff University; control participants were negative for family history of dementia. The two participant groups were matched for age, years of education, score on the Mini Mental State Exam (MMSE; [12]) and smoking habits; there were proportionally more females in the control group (0.85) than in the sibling group (0.63; $\chi^2(1) = 4.65, p < 0.05$). Exclusion criteria for all participants included a diagnosis of dementia or primary major affective disorder, head injury, nasal congestion on the day of examination, history of anosmia and a MMSE score of less than 24 [12].

2.2. Procedure

Local research ethical approval was obtained for this study, which is part of a wider research initiative examining neuropsychological profiles in individuals at risk of developing AD. Written informed consent was obtained from all participants. Siblings were tested in their homes and control participants were tested in the School of Psychology, Cardiff University, with the exception of six participants who requested home visits. An information form was completed by the experimenter following a semi-structured interview. This provided demographic information including history of illness and medication, which might affect cognitive functions including olfactory performance, smoking habits and screening of family history for AD. The MMSE was administered to all participants to screen for dementia.

Odour identification performance was assessed using a battery of 10 common odours (Orange, Strawberry, Chocolate, Coffee, Lemon, Fish, Garlic, Bacon, Almond and Banana) and was derived from a task devised by Morgan et al. [26]. Each odour (supplied by Dale Air Deodorising Limited, UK) was presented in a 20 ml brown-tinted glass bottle containing gauze saturated with the relevant themed aroma oil. A 4 × 5 array of 20 colour photographic pictures was provided on a sheet of A4 paper. Ten of the photographs represented images of each of the test odours, for instance, the odour ‘orange’ was represented by a picture of an orange, and 10 of the photographs represented images of each of the distractor odours. Both sets of photographs were matched for ratings of perceived object familiarity and naming accuracy: a pilot study (n = 12) revealed familiarity ratings (1 = not at all familiar, 7 = very familiar) of target pictures (M = 6.50, S.E. = 0.23) and distractor pictures (M = 6.37, S.E. = 0.27) did not differ significantly ($t(22) = -0.36$, ns); picture naming accuracy of both target and distractor pictures was high at 96 and 95%, respectively ($t(12) = -0.36$, ns). Prior to commencing the odour identification task, the participant was required to name each pictured object to ensure consistent nameability across participants. If a participant produced an incorrect name, the experimenter provided the correct response. For
each trial, an odour was presented birhinically for 10 s. The participant was required to match the odour with a picture in the array; responses could be made by either pointing to the picture or responding verbally with the name of the picture. A change of response to an odour was accepted only during the 10 s presentation period of that odour. There was a 45 s rest period between each odour presentation to control for adaptation to the previous odour stimulus [26]. The order of odour presentation was randomised for each participant. As the trial progressed, a participant was free to choose an earlier response.

Genomic DNA for ApoE genotyping was prepared from two saliva samples obtained from participants at time of testing. Two 25 ml saline solutions (Sterets® Normasol®, topical irrigation solution containing 0.9% (w/v) Sodium Chloride Ph. Eur.) were used as mouthwashes to collect DNA from saliva. Each saliva sample was stored in a 50 ml centrifuge tube prior to DNA extraction. The genomic DNA was amplified by the Polymerase Chain Reaction (PCR) using primers A1: 5′-ACAGAATTCCCGCGCCTGGTACAC-3′ and A2: 5′-TAAGCTTGCCAGCTGTCCAAAGGA-3′ [18]. This enabled six possible genotypes to be extracted (ε2/ε2, ε2/ε3, ε3/ε3, ε2/ε4, ε3/ε4 and ε4/ε4).

3. Results

3.1. ApoE distribution

ApoE genotypes were categorised according to ɛ4 carriers (ɛ2/ɛ4 and ɛ4/ɛ4) and ɛ4 absence (ɛ2/ɛ2, ɛ2/ɛ3 and ɛ3/ɛ3). There were 10 ɛ4 carriers and 14 non-ɛ4 carriers in the sibling group, and 33 ɛ4 carriers and 14 non-ɛ4 carriers in the control group. Non-parametric analyses confirmed no difference in the distribution of the ɛ4 allele between sibling and control participants (χ² (1) = 1.00, p = 0.32).

3.2. Odour identification

A 2 × 2 between-subjects analysis of variance (ANOVA) was performed to compare odour identification scores according to both risk group (siblings versus control participants) and ApoE ɛ4 status (ɛ4 carriers versus non-ɛ4 carriers). There was a main effect of risk group reflecting lower odour identification scores for the sibling group, F(1, 67) 10.42, p < 0.01; means = 4.17 (S.D. = 2.44) and 5.09 (S.D. = 2.20) for sibling and control groups, respectively. However, for ApoE ɛ4 status there was no difference in odour identification accuracy between ɛ4 carriers and non-ɛ4 carriers, F < 1; means = 5.17 (S.D. = 2.44) and 5.09 (S.D. = 2.44), respectively. The group × ApoE ɛ4 status interaction was significant, F(1, 67) 6.10, p < 0.05 (see Fig. 1). Further comparisons revealed that the sibling ɛ4 carrier group had the poorest mean odour identification scores, significantly lower than control ɛ4 carriers (t(22) = −4.12, p < 0.01) and control non-ɛ4 carriers (t(44) = −2.33, p < 0.05) but not significantly lower than sibling non-ɛ4 carriers (p > 0.1). Sibling non-ɛ4 carriers were significantly poorer at odour identification than control ɛ4 carriers (t(23) = −2.34, p < 0.05) but not lower than control non-ɛ4 carriers (p > 0.1). Although the figure suggests a difference between control non-ɛ4 carriers and control ɛ4 carriers, this difference did not reach significance (t(48) = −1.914, p > 0.05). In summary, the interaction indicates that the combined effects of sibling status and ɛ4 status put those who have a sibling with AD and who carry the ɛ4 allele at particular disadvantage in this test of olfactory ability. Because there was a significant difference in gender distribution according to risk group and gender is known to influence odour identification [21], the ANOVA was re-run adding sex as a covariate. Gender did not covary with odour identification (F < 1).

4. Discussion

In this study, we have shown the independent and combined effects of family history of AD and ApoE ɛ4 status on an odour identification task. The hypothesis that odour identification accuracy would be more impaired for individuals with a family history of AD was supported; siblings made significantly more errors on the odour identification task compared to control participants. For ApoE ɛ4 status, however, the findings were less clear and there was no support for an independent effect of ɛ4 presence on odour identification. However, participants in possession of both risk factors had the poorest odour identification accuracy, a finding that is
consistent with our prediction. This observation was strengthened by an interaction revealing that sibling ε4 carriers were significantly more impaired at identifying odours than both control ε4 carriers and control non-ε4 carriers. To our knowledge, this is the first time that a combined effect of possessing both these risk factors has been used to identify early signs of cognitive decline. The present data suggest that it may be possible to enhance the accuracy of early detection of AD by considering these risk factors in combination alongside a simple test of odour identification.

Odour identification deficits such as those reported here are recognised as a salient feature of incipient AD, and correspond closely to early neuropathological changes in the AD brain. Impaired odour identification and recognition have been reported in patients with mild cognitive impairment (MCI) and questionable AD patients [9,36] strengthening the argument that detection of olfactory dysfunction has potential value as a preclinical marker of AD. In addition to reduced odour identification, higher olfactory thresholds are typically exhibited in patients with AD and may mediate the nature of olfactory dysfunction in early stage AD (see [10] for review).

Support for a link between odour identification deficits and family history of AD was originally provided by Serby et al. [40] who reported that FDRs were impaired on a task of odour identification. Our results further validate the work by Serby et al. demonstrating that odour identification deficits were more pronounced for siblings of probable AD cases. Although the precise mechanism predisposing relatives to develop AD is unknown, it is suggested that a number of genes combine in their operation, thus increasing relatives’ genetic proclivity to this disease [35]. Until such time as these genetic markers have been identified it would be worthwhile to profile this high-risk group for early disease markers using a task of odour identification, as the present study shows that this is a simple and accurate means of identifying people who are intact on standard measures of performance such as MMSE, yet who show isolated deficits to olfactory memory that could be indicative of future cognitive decline.

The ApoE ε4 allele has emerged as the largest known genetic risk factor for developing AD [44,45]. Past studies have provided some evidence that odour identification accuracy discriminates between healthy older ε4 carriers and healthy older non-ε4 carriers [2,28], and that odour identification deficits in the presence of at least one copy of the ε4 allele suggest high risk of cognitive decline [16]. For the present study, the most powerful evidence in support of this relationship is the main effect of the ε4 allele on reducing odour identification accuracy. Whilst the current study showed no evidence that odour identification performance distinguished healthy older ε4-carriers from non-ε4 carriers, analysis of the interaction between ApoE ε4 presence and familial risk indicated a clear deficit for siblings carrying at least one copy of the ε4 allele. This group demonstrated poorer odour identification performance, and was significantly worse than both the control ε4 carrier and control non-ε4 carrier groups. We suggest that since possession of the ε4 allele alone does not account for the odour identification deficits, siblings possessing at least one copy of the ε4 allele may be closer to the threshold of developing the disease possibly because of interactions with other, presently unidentified, genes hosted by family members.

A further feature of the interaction showed that sibling non-ε4 carriers were significantly poorer at odour identification than control ε4 carriers, suggesting that unlike familial risk, possession of the ε4 allele in isolation is not a sufficient condition to produce odour identification deficits in healthy older adults. Precisely why sibling non-ε4 carriers demonstrate greater odour identification deficits compared to control ε4 carriers is still open to investigation. One possibility is that the aforementioned enriched genetic predisposition in family members may play host to a number of as yet unidentified, and possibly more potent, biological risk factors than the ε4 allele, and as such are encumbering family members with a greater risk for developing AD.

Explaining why control participants with a copy of the ε4 allele were not significantly poorer than control non-ε4 carriers requires some attention particularly since the majority of past research supports a malignant effect of ε4 presence on cognitive function in normal ageing [23,42]. However, an emerging body of literature argues that presence of the ε4 allele is not related to pathological cognitive decline in healthy older adults [19,41,43]. For instance in the Small et al. study [41], healthy older adults underwent a comprehensive neuropsychological assessment and showed no evidence to suggest that the ε4 allele is a risk factor for pathological cognitive decline in normal ageing.

In conclusion, our results demonstrate that siblings of probable AD cases show a clear deficit on a simple test of odour identification despite intact general cognitive status. The role of the ε4 allele in odour identification in normal aging remains unresolved. However, an interaction between positive family history of AD and carrying a copy of the ε4 allele shows that ε4 presence in siblings is a significant modifier of olfactory dysfunction in normal ageing. The implications of this interaction suggest that other, presently unknown, genetic factors play a significant role in the prevalence of olfactory deficits in population samples at familial risk of developing AD. These data further demonstrate that odour identification deficits may reflect early disease expression in individuals at increased risk for developing the disease. In summary, the use of olfactory-mediated cognitive tests within the diagnostic setting may enhance diagnostic confidence, particularly if such tests are measured in individuals at known risk for developing the disease.

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