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Aldehyde dehydrogenase 2 genetic variations may increase susceptibility to Parkinson's disease in Han Chinese population

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ABSTRACT

Genetic variations of *ALDH2*, encoding aldehyde dehydrogenase-2 which regulates aldehyde oxidation in the brain, have been recently suggested to impact on the association of pesticide exposure with Parkinson's disease (PD). However, the link between *ALDH2* polymorphism and PD remains elusive. In the present study, tag-single nucleotide polymorphisms of *ALDH2*, including rs4767944, rs441, and rs671, were extracted and analyzed in a Chinese cohort consisting of 584 PD patients and 582 controls. Results from genotyping analyses showed that rs4767944 ($p = 0.002$), but not rs441 and rs671, were associated with PD. The C allele of rs4767944 served a risk factor toward PD. Further analysis presented a significant association between haplotype frequencies and the risk for PD, primarily driven by the preponderance of the C-T-A ($p = 0.03$) or C-T-G ($p = 0.003$) haplotype of rs4767944, rs441, and rs671 in PD patients. In conclusion, these novel results suggest an association between PD susceptibility and *ALDH2* genetic variations.

1. Introduction

Parkinson's disease (PD) is the second most prevalent neurodegenerative disorder characterized by progressive loss of dopaminergic neuron in substantia nigra, and the formation of Lewy bodies primarily comprised α-synuclein. Although its unequivocal etiology remains elusive, PD is believed to be caused by genetic factors (Nuytemans et al., 2010; Spatola and Wider, 2014), environmental exposures (Kieburtz and Wunderle, 2013), and their interactions (Gao and Hong, 2011; Gao et al., 2012).

Aldehyde dehydrogenase (ALDH) is a group of enzymes responsible for the oxidation of aldehydes to carboxylic acids and detoxification of exogenous and endogenous aldehydes (Marchitti et al., 2008). Among the ALDH superfamily, ALDH2 is one of the most important enzymes for aldehyde oxidation in the brain (Marchitti et al., 2008). Aldehyde metabolites have been suggested to be involved in the pathogenesis of PD. For instance, 4-hydroxynonenal (4-HNE), a common aldehyde product of lipid peroxidation, promotes the formation of α-synuclein oligomers (Nasstrom et al., 2011). Monoamine oxidase metabolizes dopamine to form 3,4-dihydroxyphenylacetaldehyde (DOPAL), an aldehyde metabolite toxic to dopaminergic neurons and associated with neurological pathologies (Marchitti et al., 2007; Mattammal et al., 1995). 4-HNE inhibits mitochondrial biotransformation of DOPAL to 3,4-dihydroxyphenylacetic acid (Florang et al., 2007), which can be excreted as a sulfate conjugate or further metabolized by catechol-O-methyltransferase to form homovanillic acid (Marchitti et al., 2007). ALDH2 plays a critical role in reducing 4-HNE toxicity and in maintaining low levels of DOPAL by catalyzing its metabolism to 3,4-dihydroxyphenylacetic acid (Goldstein et al., 2013; Kong and Kotraiah, 2012; Wey et al., 2012). These lines of evidence highlight the importance of ALDH activity in the detoxification of dopaminergic neurons and suggest that ALDH genetic variation may be associated with PD predisposition.

Recently, Fitzmaurice et al. (2013, 2014) found that ALDH activity could be inhibited by pesticides such as ziram, benomyl, and folpet. Exposure to pesticides is known to be associated with elevated risk for PD (Kamel et al., 2007; Tanner et al., 2011). Further analysis suggested that certain *ALDH2* haplotypes based on 5 variants enhanced the effect of pesticide association with PD. However, no direct association was observed between these haplotypes and PD susceptibility (Fitzmaurice et al., 2014). To the best of our knowledge, the link between each single variant of

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Table 1
PCR primers and RFLP products

Tag-SNPs	Restriction enzyme	Primers	PCR product, bp	RFLP sizes, bp
rs4767944	PsiI	Forward: 5′-CATAGGCACCATACAGAA-3′ Reverse: 5′-AGAGTCCTCGTTCATCAC-3′	392	CC: 392 CT: 392 + 246 + 146 TT: 246 + 146
rs441	HaeIII	Forward: 5′-AGGAGGGTTGCTTGAGTT-3′ Reverse: 5′-TAAATGGGACGGAGAAGG-3′	326	CC: 219 + 107 CT: 326 + 219 + 107 TT: 326
rs671	—	Forward: 5′-GAGCCCAGTCACCCTTTG-3′ Reverse: 5′-ACCAGCAGACCCTCAAGC-3′	149	

Key: PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; tag-SNPs, tag-single nucleotide polymorphisms.

ALDH2 and PD has not been studied. Polymorphisms of *ALDH2* vary by ethnicities, and the rs671 is thought to be exclusively occurred in Asians (Eng et al., 2007). The susceptibility of ALDH2 activity to pesticides indicates a potential interaction with local environmental exposures. Thus, in this study, we aim to determine whether *ALDH2* genetic variations, single or in the form of haplotype, are associated with the risk of PD in a large Chinese cohort by analyzing tag-single nucleotide polymorphisms (tag-SNPs).

2. Materials and methods

2.1. Subjects

A total of 1166 Han Chinese from eastern China were enrolled in this study, including 584 sporadic PD patients (310 males and 274 females) and 582 age and gender-matched controls (294 males and 288 females). The median age of the patients and controls were 64.0 (interquartile range: 58–73) and 62.6 (interquartile range: 53–73) years, respectively. PD cases were diagnosed by 2 movement disorder specialists according to the UK Parkinson's Disease Society Brain Bank Criteria (Hughes et al., 1992). Patients with a family history of PD or with secondary and atypical parkinsonism were excluded. Control subjects were free of neurological disorders according to medical history, physical and laboratory examinations. This study was approved by the Ethics Committee of The Second Affiliated Hospital, Wenzhou Medical University. All participating subjects in the study signed informed written consents.

2.2. Tag-single nucleotide polymorphisms

To broadly cover the variability of *ALDH2*, 3 tag-SNPs, rs4767944 (C/T), rs441 (C/T), and rs671 (A/G), were selected according to implications of the HapMap project (http://www.hapmap.org/) and Haploview v.4.2 (Barrett et al., 2005) using pairwise tagging with the followings parameters: r^2, cutoff $\geq$ 0.8; mean allele frequency, $\geq$0.1 in population Han Chinese individuals from Beijing, China.

2.3. Genotyping

Genomic DNA was extracted from peripheral blood samples as described previously (Zhang X et al., 2014). Primer pairs for these tag-SNPs were synthesized as shown in Table 1. Polymerase chain reactions (PCRs) were carried in a total volume of 25 μL containing 0.1 μg of genomic DNA, 12.5 μL of 2× PCR Mastermix (Tiangen, Beijing, China), and 0.5 μM of each primer and subjected to the following conditions sequentially: initial denaturation at 94 °C for 3 minutes, 35 cycles of 30 seconds at 94 °C, 30 seconds at 58 °C for rs671 (54 °C for rs441 or 52 °C for rs4767944), 1 minute at 72 °C, and a final extension at 72 °C for 5 minutes. The PCR products of

Table 2
Genotype and allele frequencies of the 3 tag-SNPs, rs4767944, rs441, and rs671, in PD patients and controls

tag-SNPs	Genotype, n (%)			*p*	Allele, n (%)		*p*	OR (95% CI)
rs4767944	CC	CT	TT		C	T		
Control	62 (10.7)	301 (51.7)	219 (37.6)		425 (36.5)	739 (63.5)		
PD[a]	92 (15.8)	316 (54.1)	176 (30.1)	0.004*	500 (42.8)	668 (57.2)	0.002*	1.302 (1.102–1.537)
Men control	32 (10.9)	156 (53.1)	106 (36.1)		220 (37.4)	368 (62.6)		
Men PD[b]	53 (17.1)	171 (55.2)	86 (27.7)	0.023*	274 (44.2)	346 (55.8)	0.017*	1.325 (1.052–1.668)
Women control	30 (10.4)	145 (50.3)	113 (39.3)		205 (35.6)	371 (64.4)		
Women PD[b]	39 (14.2)	145 (52.9)	90 (32.8)	0.180	223 (40.7)	325 (59.3)	0.078	1.242 (0.976–1.580)
rs441	CC	CT	TT		C	T		
Control	33 (5.7)	256 (44.0)	293 (50.3)		322 (27.7)	842 (72.3)		
PD[a]	25 (4.3)	278 (47.6)	281 (48.1)	0.323	328 (28.1)	840 (71.9)	0.822	1.021 (0.852–1.224)
Men control	17 (5.8)	120 (40.8)	157 (53.4)		154 (26.2)	434 (73.8)		
Men PD[b]	13 (4.2)	151 (48.7)	146 (47.1)	0.131	177 (28.5)	443 (71.5)	0.358	1.126 (0.874–1.451)
Women control	16 (5.6)	136 (47.2)	136 (47.2)		168 (29.2)	408 (70.8)		
Women PD[b]	12 (4.4)	127 (46.4)	135 (49.3)	0.765	151 (27.6)	397 (72.4)	0.549	0.924 (0.713–1.198)
rs671	AA	AG	GG		A	G		
Control	35 (6.0)	208 (35.7)	339 (58.3)		278 (23.9)	886 (76.1)		
PD[a]	27 (4.6)	236 (40.4)	321 (55.0)	0.193	290 (24.8)	878 (75.2)	0.595	1.053 (0.871–1.272)
Men control	18 (6.1)	101 (34.4)	175 (59.5)		137 (23.3)	451 (76.7)		
Men PD[b]	12 (3.9)	131 (42.3)	167 (53.9)	0.089	155 (25.0)	465 (75.0)	0.490	1.097 (0.843–1.429)
Women control	17 (5.9)	107 (37.2)	164 (56.9)		141 (24.5)	435 (75.5)		
Women PD[b]	15 (5.5)	105 (38.3)	154 (56.2)	0.947	135 (24.6)	413 (75.4)	0.952	1.008 (0.769–1.323)

*$p < 0.05$.
Key: CI, confidence interval; OR, odds ratio; PD, Parkinson's disease; tag-SNPs, tag-single nucleotide polymorphisms.
[a] Compared with total controls.
[b] Compared with controls of the same gender.