Polymorphism of α-estrogen receptor and aryl hydrocarbon receptor genes in dementia patients in Shanghai suburb[1]

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ABSTRACT

AIM: To explore the possible association of different polymorphic forms of human α-estrogen receptor (*ER*-α) and aryl hydrocarbon receptor gene (*Ahr*) with the risk to senile dementia in farmers in Shanghai suburb. **METHODS:** Senile dementia patients (*n*=52) were examined for *ER*-α and *Ahr* gene polymorphism genotyping. Healthy individuals (*n*=125) in the same area were selected as a community control group. Two polymorphic loci, *Pvu* II locus and *Xba* I locus, of human *ER*- *a* gene were investigated by a PCR-RFLP-based procedure. The population frequencies of two polymorphic loci in exon 10 of *Ahr* gene, $G_{1721}A$ ($R_{554}K$) and $G_{1768}A$ ($V_{570}I$) were compared between patients and healthy controls using an allele-specific PCR (AS-PCR) procedure. **RESULTS:** The mutant allele frequencies of *ER*-α gene in the AD group were significantly higher than those in the control group (*P*= 0.023, OR=2.94, 95 % CI 1.13-7.71 for *Pvu* II locus; *P*=0.046, OR=2.28, 95 % CI 1.003-5.17 for *Xba* I locus). The mutant allele frequencies among female AD patients were higher than those in the female controls (*P*=0.016, OR=3.68, 95 % CI 1.22-11.08 for *Pvu* II locus, *P*=0.029, OR=2.95, 95 % CI 1.10-7.94 for *Xba* I locus). The mutant form, neither in the homozygous, nor in the heterozygous form was detected at the locus of *Ahr* $G_{1768}A$ in a normal local population. No significant difference of *Ahr* genotype frequency at locus $G_{1721}A$ was noticed between the patients and healthy individuals. **CONCLUSION**: The distribution of *ER* polymorphisms was significantly different between Chinese and some other ethnic populations. The results suggested that *ER-a* gene polymorphisms might be related to the individual susceptibility to AD, especially in the females. However, it did not support the association of *Ahr* gene polymorphism with higher risk of senile dementia.

INTRODUCTION

Most of human health disorders are modulated by the interaction of individual genetic background and various environmental factors. Alzheimer's disease is a common form of dementia with progressive deterioration of cognitive function. Polymorphisms of several gene loci have been reported to be associated with the etiology of Alzheimer's disease[1,2]. Women who had taken estrogen replacement therapy during their postmenopause usually are at lower risk of dementia[3]. Estrogen modulates the function of the central nervous system via its receptor binding. It is demonstrated that estrogen up-regulates the expression of apolipoprotein E (*ApoE*) gene[4], which is involved in the metabolism

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of β-amyloid related to the development of Alzheimer's disease. Aryl hydrocarbon receptor (Ahr) is a kind of orphan receptor, which mediates most of the toxic effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)[5]. Ahr not only functions in modulating the expression of many xenobiotics metabolism enzymes but also plays an important role in the signal transduction pathways[6]. Coactivators of Ahr can decrease the affinity of estradiol to ER receptor and, in consequence, inhibit the downstream reactions. Ahr may compete with ER and other hormone receptors for binding with their coactivators[7].

There are two polymorphic loci with only 50 bp apart in intron 1 of *ER-a* gene, *Pvu II* locus and *Xba I* locus[8]. These polymorphic loci may influence the expression of *ER-a* gene. Studies in HeLa cells indicated that *xp* haplotype of *ER-a* has higher expression than the *XP* one, but the difference was not significant[9]. In exon 10 of *Ahr* gene there are two polymorphic loci, $G_{1721}A$ ($R_{554}K$) and $G_{1768}A$ ($V_{570}I$), this results in the production of functionally altered proteins. Recently, polymorphism of *ER-a* gene was investigated in several populations. Some of them confirmed the association of polymorphism of *ER-a* gene with Alzheimer's disease[2,10], some did not[9]. To investigate whether the polymorphic alleles of *ER-a* gene and *Ahr* gene are related to senile dementia such as Alzheimer's disease in Chinese population, a group of Alzheimer dementia and non-Alzheimer dementia patients and a group of healthy individuals as controls were genotyped.

MATERIALS AND METHODS

Populations A group of senile dementia patients (n=52, 14 males and 38 females, with average age of (79±14) years, ranging from 53 to 95 years) in a remote rural area in Shanghai suburb were selected and a group of healthy controls (n=125, 62 males and 63 females) in the same area were enrolled in this study. All of them are ethnic Han Chinese (Chinese majority, representing 93 % of the resident population of China). At the time of sampling, non of the individuals in the control group have ever been diagnosed the illness as any kind of cancers, cardiovascular diseases, mental disorders, or any other serious diseases. The medical examinations were performed in the local county mental health center. Within the case group, neurological/psychiatric examination confirmed the diagnosis of 30 cases as sporadic Alzheimer's disease, 22 cases as non-Alzheimer's dementia.

Blood sampling and DNA extraction Blood was taken from each subject by venopuncture. DNA was extracted from the ethylenediaminetetraacetic acid (EDTA) anticoagulated blood with a standard phenol/chloroform procedure.

***ER* genotyping** The *ER-a* gene polymorphism identification was conducted based on the method of Kobayashi *et al*[11]. Products of 1.3 kb long were obtained with a pair of primers (5'-CTG CCA CCC TAT CTG TAT CTT TTC CTA TTC TCC-3' and 5'-TCT TTC TCT GCC ACC CTG GCG TCG ATT ATC TCA-3'). The PCR products were digested with restriction endonucleases *Pvu* II and *Xba* I to identify the *Pvu* II and *Xba* I polymorphism, respectively. Capital letters (*P* or *X*) denoted the absence of the restriction site, and small letters (*p* or *x*) denoted its presence. The restriction endonucleases were purchased from New England Biolabs.

***Ahr* genotyping** Two polymophic loci of *Ahr* gene were genotyped by an allele specific PCR (AS-PCR) procedure described by Zhang *et al*[12]. Primers were designed according to the sequence of exon 10 in the *Ahr* cDNA[13]. The primer sequences for locus $G_{1721}A$ are as follows: AHR5: 5'-ACT CTC TCA ATC CTA GTT CC-3' and AHR3A1: 5'-TTT CAT TCT GCA TGT GTC-3' or AHR3A2: 5'-TTT CAT TCT GCA TGT GTT-3'. The product is of 333 bp and is coamplified with a 493-bp fragment of human actin gene (primers: 5'-GGG CAC GAA GGC TCA TCA TT-3' and 5' -GGC CCC TCC ATC GTC CAC CG-3') as an internal control. To genotype the polymorphism in locus $G_{1768}A$ of *Ahr* gene the following primers were used: AHR5: 5'-ACT CTC TCA ATC CTA GTT CC-3' and AHR3B1: 5'-GTC AAT GTC TCT GAA GTC AAT-3' or AHR3B2: 5'-GTC AAT GTC TCT GAA GTC AAC-3'. The PCR product was 383 bp and a coamplificated 268-bp segment of human β-globin gene (primers: 5'-CAA CTT CAT CCA CGT TCA CC-3' and 5'-GAA GAG CCA AGG ACA GGT AC-3') was taken as internal control. All the primers were synthesized by Gibco-BRL Life Technologies (Grand Island, NY, USA).

Statistical analysis Chi-squared test was used to determine the significance difference in genotype distribution between case and control groups. Odd ratios (OR) and 95 % confidence interval (95 % CI) were calculated to estimate the risk of developing AD on the basis of different genotypes. According to Smart *et al*[14], the *G/G* genotype of $G_{1721}A$ locus in *Ahr* gene

codes for the lower-active form, and the *G/A* and *A/A* genotypes code for the higher-active ones. Then, the risk of combined *G/A* and *A/A* genotypes versus *G/G* genotype was estimated. For *ER-a* gene polymorphism, conventionally, risk of combined mutation homozygous (*PP* or *XX*) and heterozygous genotypes (*Pp* or *Xx*) versus homozygous wild genotypes (*pp* or *xx*) were evaluated.

RESULTS

Polymorphism of *ER-a* gene Healthy individuals (n=125) were genotyped for the *ER-a* gene. The distribution of *ER-a* gene polymorphism in this normal Chinese population and other ethnic populations were compared (Tab 1). Unlike in other reports[2], we did not find the existence of *ppXX* genotype in this Chinese population. No significant difference has been found between Chinese male and Swedish male population for both loci. The significant differences of distribution frequency at *Pvu* II locus between Chinese and Italian females and American females were observed (P=0.004 and P=0.001, respectively). For *Xba* I locus, significant difference has been confirmed between normal Chinese females and Japanese females , Swedish females, and American females (P=0.04, P=0.003, P<0.001, respectively). The distribution of the combined genotypes of two loci in the different ethnic population was also compared. It was shown that there were significant differences of distribution of the combined genotype either between normal Chinese female and Japanese females, and Italian females (P=0.01, P<0.001, respectively) (Tab 2).

No significant difference in *Pvu* II polymorphic genotype distribution between all the senile dementia patients or the non-AD senile dementia subgroup and the control group was observed. The same results were found for the *Xba* I locus (Tab 3). In contrast, it was shown that an over-representation of *P* or *X* alleles was detected among AD patients compared with healthy controls (P=0.023, OR=2.94, 95 % CI 1.13-7.71 for *P* allele and P=0.046, OR=2.28, 95 % CI 1.003-5.17 for *X* allele, respectively). This trend was more distinct in the female individuals (P=0.016, OR=3.68, 95 % CI 1.22-11.08 for *P* allele and P=0.029, OR=2.95, 95 % CI 1.10-7.94 for *X* allele). When focus was laid on the combined genotypes of the two loci, 9 genotypes were obtained (Tab 4), several kinds of genotypes were uncommon in the genotyped individuals because of the linkage disequilibrium between the *Pvu* II and *Xba* I loci. *PPXX* has a significantly more frequentcy in the Alzheimer's disease patients than that in the control group

Tab 1. Comparison of genotype distribution of *ER-*a gene polymorphisms in different ethnic groups.

Populations	*ER*-α *Pvu* II				*ER*-α *Xba* I			
	PP	*Pp*	*pp*	*P*-value	*XX*	*Xx*	*xx*	*P*-value
Normal male in Shanghai, this study (n=62)	10 (16%)	30 (48%)	22 (36%)	1	3 (5%)	20 (33%)	39 (62%)	1
Normal male Swedish[15] (n=90)	20 (22%)	40 (44%)	30 (33%)	0.65	8 (9%)	36 (40%)	46 (51%)	0.31
Normal female in Shanghai, this study (n=63)	6 (10%)	26 (41%)	31 (49%)	1	5 (8%)	12 (19%)	46 (73%)	1
Normal female Japanese[11] (n=318)	57 (18%)	146 (45%)	115 (36%)	0.09	9 (3%)	98 (31%)	211 (66%)	0.04
Normal female Swedish[16] (n=101)	14 (14%)	56 (56%)	31 (31%)	0.06	8 (8%)	45 (45%)	48 (47%)	0.003
Normal female Italian[17] (n=426)	90 (21%)	210 (49 %)	126 (30%)	0.004	84 (20%)	191 (45%)	151 (35%)	1.01×10^{-7}
Normal female American[18] (n=145)	34 (23%)	75 (52%)	36 (25%)	0.001	-	-	-	-

Tab 2. Distribution of combined genotype of *ER-*a gene in different ethnic female populations.

Population	*ER*-α genotype							*P*-value
	PPXX	*PPXx*	*PPxx*	*PpXX*	*PpXx*	*Ppxx*	*ppxx*	
Normal female in Shanghai, this study (n=63)	4 (6%)	2 (3%)	0 (0%)	1 (2%)	10 (16%)	15 (24%)	31 (49%)	1
Normal female Japanese[11] (n=318)	9 (3%)	33 (10%)	15 (5%)	0 (0%)	65 (20%)	81 (26%)	115 (36%)	0.01
Normal female Italian[17] (n=426)	73 (17%)	17 (4%)	0 (0%)	11 (3%)	174 (41%)	25 (6%)	126 (30%)	6.93×10^{-8}

Tab 3. *Pvu* II and *Xba* I polymorphism of *ER-a* gene in the investigated groups[1)].

Groups (*n*, Male/Female)	Genotype			Risk[2)]	
	PP	*Pp*	*pp*	*P*-value	OR (95 % CI)
AD (*n*=30, 6/24)	7 (2/5)	17 (3/14)	6 (1/5)	0.023	2.94 (1.13-7.71)
Non-AD dementia (*n*=22, 8/14)	5 (3/2)	6 (1/5)	11 (4/7)	0.51	0.74 (0.30-1.83)
All dementia (*n*=52, 14/38)	12 (5/7)	23 (4/19)	17 (5/12)	0.23	1.52 (0.77-2.99)
Normal (*n*=125, 62/63)	16 (10/6)	56 (30/26)	53 (22/31)	Ref.[3)]	Ref.
Groups		Genotype		Risk[4)]	
	XX	*Xx*	*xx*	*P-value*	OR (95 % CI)
AD (*n*=29, 6/23)[5)]	6 (3/3)	9 (0/9)	14 (3/11)	0.046	2.28 (1.003-5.17)
Non-AD (*n*=22, 8/14)	1 (1/0)	7 (2/5)	14 (5/9)	0.69	1.21 (0.47-3.13)
All dementia (*n*=51, 14/37)	7 (4/3)	16 (2/14)	28 (8/20)	0.10	1.75 (0.90-3.40)
Normal (*n*=125, 62/63)	8 (3/5)	32 (20/12)	85 (39/46)	Ref.[3)]	Ref.

AD: Alzheimer dementia. 1) *Chi*-square test was used. 2) *P* values and Odds ratios were calculated by comparison of the normal population and dementia groups for *Pvu II PP* or *Pp* versus *pp*. 3) The distribution of genotypes among normal population was in Hardy-Weinberg equilibrium. 4) *P* value and Odds ratios were calculated by comparison of the normal population and dementia groups for *Xba I XX or Xx* versus *xx*. 5) Blood of one of the samples is insufficient to perform genotyping.

Tab 4. Distribution of *ER-*a gene *Pvu* II and *Xba* I combined genotype in the investigated groups.

Subjects		*Pvu* II- *Xba* I combined genotype									
		n	*PPXX*	*PPXx*	*PPxx*	*PpXX*	*PpXx*	*Ppxx*	*ppXX*	*ppXx*	*ppxx*
AD patients (*n*=29)	Male	6	2	0	0	0	0	3	1	0	0
	Female	23	3	2	0	0	7	6	0	0	5
All dementia patients (*n*=51)	Male	14	2	2	1	1	0	3	1	0	4
	Female	37	3	4	0	0	9	9	0	1	11
Controls (*n*=125)	Male	62	1	4	5	2	16	12	0	1	21
	Female	63	4	2	0	1	10	15	0	0	31

AD: Alzheimer dementia

(*P*=0.029, OR=5.00, 95 % CI 1.34-18.62), but this trend was not obvious when only female subjects were concerned (*P*=0.58). This conflict might be caused by the higher frequency of *PPXX* in male patients with Alzheimer's disease (2 of 6 male patients are *PPXX* carriers) and small sample size. Conversely, *ppxx* has a significantly lower frequency in the AD patients than that in control group (*P*=0.012, OR=0.28, 95 % CI 0.10-0.79). Although the 5 *ppxx* genotype carriers in the AD patient group are all women, the genotype frequency is significantly lower than that among female control subjects (*P*=0.022, OR=0.29, 95 % CI 0.10-0.87) (Tab 5).

Polymorphism of *Ahr* gene No mutated alleles of $G_{1768}A$ were found in 125 healthy controls (62 males, 63 females). Further genotyping of this locus in the patients was not performed. Polymorphism of this locus has been reported in the Caucasian population, but was not observed before in Chinese population. It suggests that different genetic background might exist between different ethnic groups or different resident populations in the world.

For $G_{1721}A$ locus, distributions of the different polymorphisms were of high homogeneity both in the senile dementia patient group and the healthy control group.

DISCUSSION

Estrogen exerts its function through receptor-binding. ER-α was thought to be the exclusive

Tab 5. Risk estimation of PPXX and ppxx genotype in AD patients and controls.

Genotype	AD (*n*=29)	Controls (*n*=125)	*P*-value	OR	95 % CI
PPXX	5 (17.24 %)	5 (4.00 %)	0.029	5.00	1.34-18.62
PPXX (in female)	3 (13.04 %)	4 (6.35 %)	0.58	2.21	0.46-10.75
ppxx	5 (17.24 %)	53 (42.40 %)	0.012	0.28	0.10-0.79
ppxx (in female)	5 (21.74 %)	31 (49.21 %)	0.022	0.29	0.10-0.87

AD: Alzheimer dementia

receptor of estrogen before ER-β was discovered in 1996[19]. Two polymorphic loci were found in the intron 1 of *ER*-α gene. There are important regulation sequences such as enhancer and promotor sequences in this intron, suggesting that mutations in this intron may affect the expression and biological function of ER-α[18]. Different *ER*-α genotypes may rather determine individuals' differences in ER-α expression than influence the final effect of ER-α. The estrogen level of women decreased dramatically during the postmenopause. Numerous studies indicated that women who had taken estrogen replacement therapy were at lower risk to develop dementia. In this study, the history of estrogen usage and endogenous estrogen level of the investigated subjects were not interviewed. This may limit the conclusions from the study. We found that subjects who bore *P* or *X* allele were overrepresented in the AD patient group. But in female AD patients, *PPXX* genotype frequency was not higher than that in the healthy females. The discrepant results may be due to limited sample size and the unequal distribution of genders in cases and controls ($P<0.01$). So it remains uncertain whether the *PPXX* genotype is associated with the risk of AD. Meanwhile, the *ppxx* genotype is obviously associated with AD as a "protective" factor according to the statistical data. Very recently, a study accomplished in an Caucasian population in UK suggested that *px* allele of *ER*-α might be a "protective" haplotype to AD[20]. It must be noted that as in the present study, individuals who bore the "protective" genotype represented a very small subgroup. The sample size was not large enough to provide an accurate interpretation after stratification for gender and genotype. As the modulator of several xenobiotic metabolism enzymes, *Ahr* gene may play an important role in the induction and expression of these enzymes. The polymorphism of *Ahr* gene was believed to modify individual's susceptibility to environmental related diseases. In this study, different genotypes at locus *Ahr* $G_{1721}A$ have similar distribution frequencies either in the case group or in the control one. The results of our study provide no evidence for the association of a polymorphism of locus $G_{1721}A$ with the risk of senile dementia in Chinese population studied.

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