MEDICAL SCIENCE MONITOR

META-ANALYSIS

e-ISSN 1643-3750
© Med Sci Monit, 2016; 22: 1924-1935
DOI: 10.12659/MSM.895984

Received: 2015.09.16
Accepted: 2015.11.24
Published: 2016.06.07

Polymorphisms of *CHAT* but not *TFAM* or *VR22* are Associated with Alzheimer Disease Risk

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Manuscript Preparation E
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Source of support: Departmental sources

Background: Alzheimer disease (AD) is a chronic neurodegenerative disease that is one of the most prevalent health problems among seniors. The cause of AD has not yet been elucidated, but many risk factors have been identified that might contribute to the pathogenesis and prognosis of AD. We conducted a meta-analysis of studies involving *CHAT*, *TFAM,* and *VR22* polymorphisms and AD susceptibility to further understand the pathogenesis of AD.

Material/Methods: PubMed/Medline, Embase, Web of Science, the Cochrane Library, and Google Scholar were searched for relevant articles. Rs1880676, rs2177369, rs3810950, and rs868750 of *CHAT*; rs1937 and rs2306604 of *TFAM*; and rs10997691 and rs7070570 of *VR22* are studied in this meta-analysis.

Results: A total of 51 case-control studies with 16 446 cases and 16 057 controls were enrolled. For *CHAT*, rs2177369 (G>A) in whites and rs3810950 (G>A) in Asians were found to be associated with AD susceptibility. No association was detected between rs1880676 and rs868750 and AD risk. For *TFAM* and *VR22*, no significant association was detected in studied single-nucleotide polymorphisms (SNPs).

Conclusions: Rs2177369 and rs3810950 of *CHAT* are associated with AD susceptibility, but rs1880676 and rs868750 are not. Rs1937 and rs2306604 of *TFAM*, and rs10997691 and rs7070570 of *VR22* are not significantly associated with AD risk.

MeSH Keywords: **1-Acylglycerophosphocholine O-Acyltransferase • Alzheimer Disease • Meta-Analysis as Topic • Polymorphism, Single Nucleotide • Genes, Mitochondrial**

Full-text PDF: http://www.medscimonit.com/abstract/index/idArt/895984

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Background

Alzheimer disease (AD) is one of the most prevalent health problems among seniors. It is a chronic neurodegenerative disease characterized by progressive cognition impairment and short-term memory loss, which usually deteriorates with aging. Amyloid plaques and neurofibrillary tangles are identified as 2 hallmarks in the AD process [1].

The amyloid cascade hypothesis is one of the most influential hypotheses regarding AD pathogenesis. It suggests that the initial pathological event in AD is triggered by deposition of amyloid β (Aβ) in the brain, which further leads to the formation of tau-immunoreactive neurofibrillary tangles (NFT), extracellular senile plaques (SP), neuron dysfunction, and neuronal loss [2]. Aβ peptides are cut from amyloid precursor protein (APP) by secretases and aggregate to form oligomers. The malformation of oligomers or the dysfunction of oligomers further break down enzymes, leading to amyloid plaques and neurofibrillary tangles and triggering the process of AD. Tau as a microtubules-associated protein is also suspected to play an important part in the progression of AD, and was found to be the major constituent of neurofibrillary tangles. According to the amyloid cascade hypothesis, formation of the insoluble aggregates of tau is triggered by increased Aβ level via the induced hyperphosphorylation of tau [3]. In contrast, in the tau hypothesis it is the tau protein abnormality that is thought to trigger the disease [4]. Another important hypothesis regarding the pathogenesis of AD is the acetylcholine hypothesis; it is also the basis of most currently available AD drugs. According to this theory, AD is caused by reduced synthesis of the neurotransmitter acetylcholine (ACh) [5], and, by external supplementation of ACh, the symptoms of AD can be reduced.

Aside from cells, the mitochondrial cascade hypothesis indicates that critical changes in mitochondrial function initiate other pathologies characteristic of AD. Accumulation of amyloid-β (Aβ) causes mitochondrial dysfunction in AD, leading to decreased ATP levels and increased ROS generation. It can also enhance mitochondrial dysfunction and apoptosis, and inhibit protein import inside the mitochondria. Mitochondrial DNA mutations and mitochondrial DNA damage are also involved in the pathogenesis of AD. Phosphorylated tau and Aβ can lead to increased mitochondrial fission and neurodegeneration. Aβ and APP impair mitochondrial fusion/fission processes, mitophagy, and mitochondrial movement, and cause abnormal morphology [6].

In addition to the various AD hypotheses, many genes involved in the pathway are suspected to be risk factors of AD, including *APP*, *APOE*, *CASS4*, and *CELF1* [7]. Although the association of AD with some genes has been verified by many studies, the contradictions between different studies make it difficult to form firm conclusions about such associations. Therefore, we performed a meta-analysis of published studies to investigate the correlation between suspected genes and AD susceptibly.

CHAT (choline O-acetyltransferase) gene encodes an enzyme that catalyzes the biosynthesis of ACh. The enzyme is also characteristic of cholinergic neurons, and changes in these neurons may contribute to some AD symptoms. The A allele of *CHAT* c.2384G>A polymorphism was also associated with earlier onset and possibly accelerated progression of AD [8]. *CHAT* was considered as a suspected gene in this meta-analysis.

TFAM (transcription factor A, mitochondrial) gene encodes a key mitochondrial transcription factor that functions in mitochondrial DNA replication and repair. Impaired expression of *TFAM* may influence the function of mitochondria and thus lead to AD.

Supplementary Table 1. Research terms.

AD	Alzheimer Disease[Mesh] OR Alzheimer Disease[tiab] OR Alzheimer Sclerosis[tiab] OR Alzheimer Syndrome[tiab] OR Alzheimer Type Senile Dementia[tiab] OR Alzheimer-Type Dementia[tiab] OR Alzheimer Type Dementia[tiab] OR Alzheimer Type Dementia[tiab] OR Senile Dementia[tiab] OR Primary Senile Degenerative Dementia[tiab] OR Alzheimer Dementia[tiab] OR Alzheimer's Disease[tiab] OR Acute Confusional Senile Dementia[tiab] OR Presenile Dementia[tiab] OR Late Onset Alzheimer Disease[tiab] OR Focal Onset Alzheimer's Disease[tiab] OR Familial Alzheimer Disease[tiab] OR Presenile Alzheimer Dementia[tiab] OR Early Onset Alzheimer Disease[tiab] OR AD
SNP	Polymorphism, Genetic[Mesh] OR Polymorphisms, Genetic[tiab] OR Genetic Polymorphism[tiab] OR Polymorphism[tiab] OR Genetic Polymorphisms[tiab] OR Polymorphism, Single Nucleotide[Mesh] OR Nucleotide Polymorphism, Single[tiab] OR Nucleotide Polymorphisms, Single[tiab] OR Single Nucleotide Polymorphisms[tiab] OR SNPs[tiab] OR Single Nucleotide Polymorphism[tiab] OR Polymorphisms, Single Nucleotide[tiab]
CHAT	CHAT[Mesh] OR CHAT[tiab] OR CHOACTASE[tiab] OR Choline O-Acetyltransferase[tiab] OR Choline Acetylase[tiab] OR Choline Acetyltransferase[tiab] OR rs868750[tiab] OR rs3810950[tiab] OR rs2177369[tiab] OR rs1880676[tiab]
TFAM	TFAM[Mesh] OR TFAM[tiab] TCF6[tiab] OR MTTF1[tiab] OR MTTFA[tiab] OR transcription factor A, mitochondrial[tiab] OR rs1937[tiab] OR rs2306604[tiab]
VR22	CTNNA3[Mesh] OR CTNNA3[tiab] OR VR22[tiab] OR ARVD13[tiab] OR rs10997691[tiab] OR rs7070570[tiab]

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Supplementary Table 2. Main characteristics of studies selected in the meta-analysis.

Gene	SNP	First Author	Year	Country	Ethnicity	Case	Control	Case			Control		
								WW	MW	MM	WW	MW	MM
ChAT	**rs1880676**	Ahn Jo	2006	Korea	Asian	316	264	211	99	6	193	69	2
	G>A	Giedraitis	2009	Sweden	Caucasians	84	384	54	29	1	222	144	18
		Harold	2003	UK	Caucasians	68	85	34	25	9	49	33	3
		Harold	2003	UK	Caucasians	135	135	71	56	8	64	62	9
		Harold	2003	UK	Caucasians	194	209	105	77	12	127	79	3
		Li	2008	Canada	Caucasians	690	681	386	256	48	364	275	42
		Ozturk	2005	USA	Caucasians	1001	705	563	376	62	369	292	44
		Reiman	2007	USA	Caucasians	853	550	478	329	46	303	206	41
	rs2177369	Cook	2014	UK	Caucasians	381	370	158	207	105	162	164	55
	G>A	Cook	2005	UK	Caucasians	202	295	95	124	76	88	85	29
		Cook	2005	UK	Caucasians	202	295	29	85	88	76	124	95
		Cook	2005	UK	Caucasians	179	175	26	79	74	29	83	63
		Piccardi	2007	Italy	Caucasians	158	118	44	75	39	40	57	21
		Scacchi	2008	Italy	Caucasians	442	218	167	200	75	61	117	40
	rs3810950	Ahn Jo	2006	Korea	Asian	316	264	211	99	6	192	70	2
	G>A	Cook	2005	UK	Caucasians	210	315	112	76	22	161	128	26
		Gruenblatt	2008	Austria	Caucasians	120	456	63	45	12	268	164	24
		Harold	2003	UK	Caucasians	131	118	69	51	11	65	47	6
		Kim	2004	Korea	Asian	246	561	171	61	14	419	133	9
		Lee	2012	Korea	Asian	736	1386	505	205	26	1023	342	21
		Mubumbila	2002	Germany & French	Caucasians	122	112	48	32	42	64	34	14
		Ozturk	2005	USA	Caucasians	999	708	562	377	60	363	296	49
		Schwarz	2003	Germany	Caucasians	242	143	139	94	9	83	52	8
		Tang	2008	China	Asian	273	271	190	75	8	179	83	9
	rs868750	Harold	2003	UK	Caucasians	119	116	72	39	8	83	31	2
	G>A	Harold	2003	UK	Caucasians	135	131	88	42	5	95	33	3
		Harold	2003	UK	Caucasians	209	222	129	75	5	130	84	8
		Ozturk	2005	USA	Caucasians	989	706	628	322	39	476	217	13

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Supplementary Table 2 continued. Main characteristics of studies selected in the meta-analysis.

Gene	SNP	First Author	Year	Country	Ethnicity	Case	Control	Case			Control		
								WW	MW	MM	WW	MW	MM
TFAM	**rs1937**	Alvarez	2008	Spain	Caucasians	300	183	277	23	0	158	23	2
	G>C	Belin	2007	Swedish	Caucasians	423	313	339	78	6	251	55	7
		Blomqvist	2005	Scotland	Caucasians	122	152	95	21	6	123	27	2
		Blomqvist	2005	Sweden	Caucasians	204	174	156	43	5	143	30	1
		Gunther	2004	–	Caucasians	372	295	301	67	4	221	71	3
		Zhang	2011	China	Asian	394	390	274	116	4	250	126	14
	rs2306604	Alvarez	2008	Spain	Caucasians	300	183	93	151	56	50	99	34
	A>G	Belin	2007	Swedish	Caucasians	406	318	164	169	73	100	152	66
		Giedraitis	2009	Sweden	Caucasians	85	400	29	41	15	146	200	54
		Gunther	2004	–	Caucasians	353	291	123	163	67	84	136	71
		Zhang	2012	China	Asian	394	390	98	204	92	100	192	98
VR22	**rs10997691**	Busby	2004	UK(I)	Caucasians	133	110	94	35	4	81	24	5
	T>C	Busby	2004	UK(II)	Caucasians	108	104	79	25	4	85	19	0
		Busby	2004	USA(I)	Caucasians	265	448	214	45	6	362	82	4
		Busby	2004	USA(II)	Caucasians	94	90	68	23	3	71	18	1
	rs7070570	Blomqvist	2004	Scotland	Caucasians	119	151	53	54	12	75	66	10
	T>C	Blomqvist	2004	Sweden	Caucasians	534	173	277	214	43	89	73	11
		Busby	2004	UK(I)	Caucasians	145	121	72	63	10	54	58	9
		Busby	2004	UK(II)	Caucasians	107	106	56	41	10	53	41	12
		Busby	2004	USA(I)	Caucasians	266	423	141	110	15	226	172	25
		Busby	2004	USA(II)	Caucasians	422	381	222	169	31	195	159	27
		Cellini	2005	Italy	Caucasians	302	164	168	116	18	94	56	14
		Kuwano	2006	Japan	Asian	348	328	23	155	170	33	122	173

W – wild allele; M – mutation allele.

Another suspected gene in this study is *VR22* (also known as CTNNA3, catenin [cadherin-associated protein], alpha 3). The encoded protein plays a role in cell-cell adhesion. The association between *VR22* and AD was first reported in several linkage studies [9–12]. Further studies also provided evidence of significant interaction between *APOE-4* and *VR22* SNPs [13], indicating that *VR22* or a nearby gene may influence susceptibility to AD.

We conducted a meta-analysis of studies concerning *CHAT*, *TFAM*, and *VR22* polymorphisms and AD susceptibility to further understand the pathogenesis of AD.

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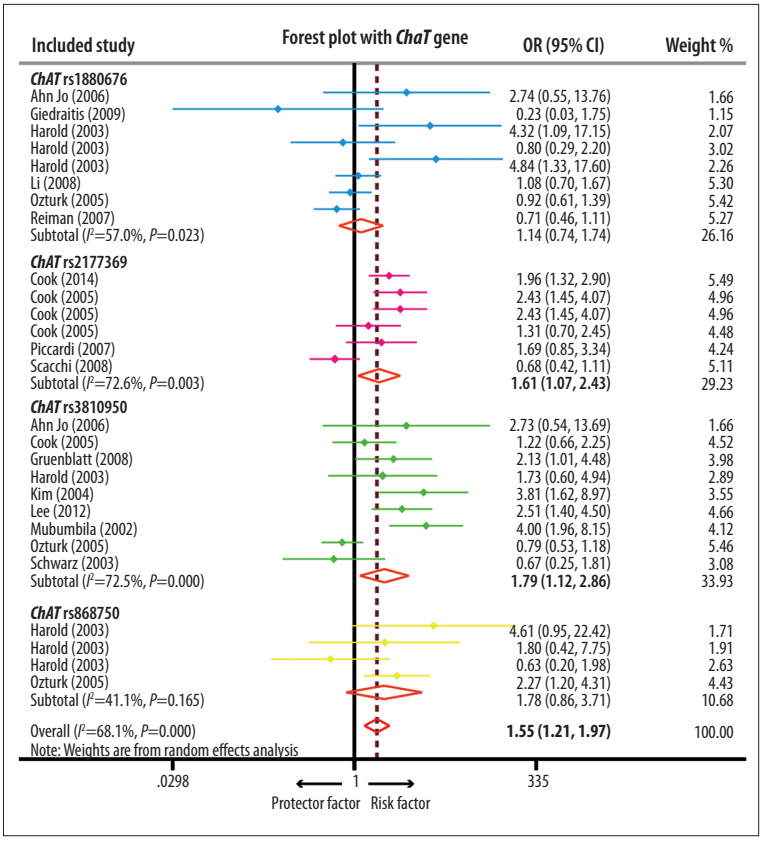

Figure 1. Forest plots showed the relationship of the 4 SNPs – rs1880676, rs2177369, rs3810950, and rs868750 – in *CHAT* gene and the risk of AD. The odds ratio from each study is represented by a square and the confidence interval is indicated by error bars. The subtotal and overall odds ratio is signified by a rhombus.

Material and Methods

Search strategy

In the current study, PubMed/Medline, Embase, Web of Science, the Cochrane Library, and Google Scholar were searched with related terms (details shown in Supplemenatry Table 1). Articles published prior to August 2015 were searched for potential SNP targets. References of retrieved articles were manually checked for other relevant publications.

Study selection and data extraction

The following criteria had to satisfied by eligible studies: (a) case-control studies covering the association between SNPs on *CHAT*, *TFAM*, or *VR22* genes and susceptibility to AD; (b) sufficient requirements for estimating odds ratios (ORs) and their 95% confidence interval (CIs) must have been satisfied; (c) the diagnosis of AD was confirmed by the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR) criteria [14] published by the American Psychiatric Association, or the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) – the Alzheimer's Disease and Related Disorders Association (ADRDA) Alzheimer's Criteria [15]. Studies were excluded if they were: (a) not a case-control study; (b) had insufficient data provided; (c) were cited by a previous meta-analysis of same subject. The name of first author, publication year, country of origin, ethnicities of subjects, studied SNPs and genes, number of subjects, frequencies of allele and genotype, and indication of Hardy-Weinberg equilibrium (HWE) in the controls were documented for each study. Ethnicity was categorized as white or Asian. No study was conducted in African populations. Four SNPs for *CHAT* gene (rs1880676, rs2177369, rs3810950, and rs868750); 2 SNPs for *TFAM* gene (rs1937 and rs2306604); and 2 SNPs for *VR22* gene (rs10997691 and rs7070570) were included in this meta-analysis. Data from retrieved studies were independently extracted by 2 reviewers. In cases of conflicting evaluations, 2 of the authors discussed the issues to reach a consensus; if no agreement could be reached, a third author would decide.

Statistical analysis

The strength of associations between the studied SNPs and susceptibility to AD were assessed by OR corresponding to 95% CI. Four genetic models (the allele, the dominant, the recessive, and the homozygous) were examined. A 2-sided $P<0.05$ in the Z test was considered as statistically significant. Subgroup analyses were performed by ethnicity (Asian/white). Heterogeneities were tested with Cochran's Q-statistic [16] with a $P_h>0.05$ indicating lack of heterogeneity. Mantel-Haenszel (M-H) method for the fixed-effects model [17] was used to calculate the

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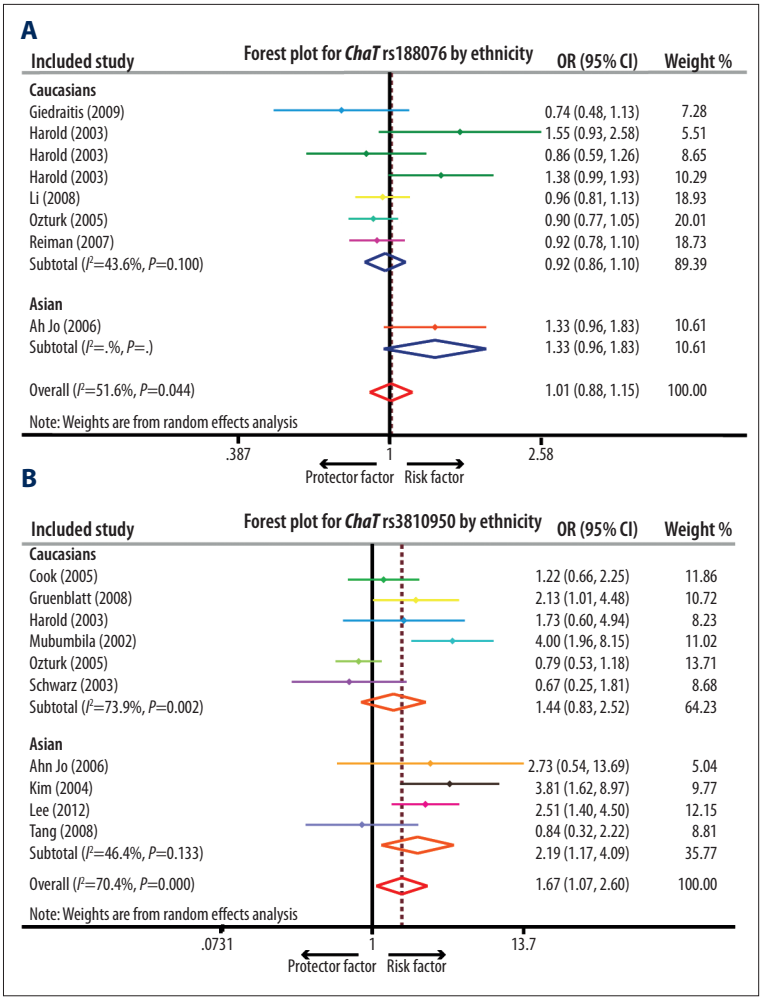

Figure 2. The forest plots of (**A**) *CHAT* rs1880676 and (**B**) *CHAT* rs3810950 by ethnicity. The odds ratio from each study is represented by a square and the confidence interval is indicated by error bars. The subtotal and overall odds ratio is signified by a rhombus.

pooled OR estimate of studies without heterogeneity; otherwise, the DerSimonian and Laird (D-L) method [18] was used for the random-effects model. A funnel plot was used to detect publication bias. The standard error of log (OR) of each study was plotted against its log (OR) in the plot. Possible funnel plot asymmetry was evaluated by Egger's linear regression test on the natural logarithm scale of OR [19]. All statistical analyses were performed with STATA version 12.0 software (Stata Corp, College Station, TX), using 2-sided *P*-values.

Results

Study characteristics

In the search for *CHAT* gene polymorphisms and AD association, we retrieved 26 articles [8,20–44] from PubMed/Medline, Embase, Web of Science, the Cochrane Library, and Google Scholar, with 28 studies related to rs1880676, rs2177369, rs3810950, and rs868750. For *TFAM* gene polymorphisms, 10 articles and 11 studies were enrolled. For *VR22*, 4 articles and 12 studies were enrolled. A total of 51 case-control studies were included in our meta-analysis, with 16 446 cases and 16 057 controls. The details of methodological and characteristics qualities of the eligible studies are compiled in Supplementary Table 2.

CHAT gene polymorphisms correlated with AD risk

Among the studied SNPs, rs2177369 (G>A) and rs3810950 (G>A) were found to be associated with AD susceptibility, but no association was detected between rs1880676 and rs868750 and AD risk (Figures 1, 2A). As shown in Table 1, rs2177369 (G>A) was a risk factor for AD onset (OR=1.61, 95% CI=1.07–2.43, *P*=0.022). For rs3810950 (G>A), a mutation is a risk factor for AD (OR=1.79, 95% CI=1.12–2.86, *P*=0.016, Figure 1). In subgroup analysis by ethnicity, the association was confirmed in Asians (Figure 2B), but not in whites (allele model: OR=1.23, 95%CI=1.01–1.48; homozygous model: OR=2.19, 95%CI=1.17–4.09; recessive model: OR=2.14, 95%CI=1.20–3.84, Table 1).

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Table 1. Meta-analysis of four polymorphisms in *ChAT* gene and AD susceptibility.

Gene	SNP	Genetic model	OR (95% CI)	$P_{odds\ ratio}$	Tau^2	I^2	$P_{hetero-geneity}$	Ethnicity		Publication bias	
								Caucasians	Asians	P_{Begg}	P_{Egger}
ChAT	rs1880676	**A** *vs.* G	1.01 (0.88–1.15)	0.896	0.017	51.6%	0.044	0.97 (0.86–1.11)	1.33 (0.96–1.83)	0.386	0.165
		AA+G**A** *vs.* GG	0.97 (0.85–1.11)	0.687	0.010	30.4%	0.185	0.93 (0.83–1.03)	1.35 (0.95–1.94)	0.536	0.239
		AA *vs.* GG	1.14 (0.74–1.74)	0.551	0.170	57.0%	0.023	1.08 (0.70–1.66)	2.74 (0.55–13.76)	0.536	0.095
		AA *vs.* GG+G**A**	1.16 (0.77–1.75)	0.474	0.151	55.1%	0.029	1.11 (0.73–1.69)	2.54 (0.51–12.67)	0.536	0.104
	rs2177369	**A** *vs.* G	1.13 (0.83–1.54)	0.439	0.133	88.6%	<0.0001	1.13 (0.83–1.54)	–	0.348	0.178
		AA+G**A** *vs.* GG	1.14 (0.76–1.67)	0.531	0.198	82.6%	<0.0001	1.14 (0.76–1.69)	–	0.452	0.220
		AA *vs.* GG	**1.61 (1.07–2.43)**	**0.022**	0.185	72.6%	0.003	**1.61 (1.07–2.43)**	–	1.000	0.831
		AA *vs.* GG+G**A**	**1.53 (1.17–2.00)**	**0.002**	0.063	57.0%	0.040	**1.53 (1.17–2.00)**	–	0.707	0.659
	rs3810950	**A** *vs.* G	**1.23 (1.02–1.48)**	**0.033**	0.060	77.2%	<0.0001	1.18 (0.90–1.55)	**1.23 (1.01–1.48)**	0.592	0.214
		AA+G**A** *vs.* GG	1.16 (0.97–1.38)	0.105	0.042	61.5%	0.008	1.09 (0.85–1.39)	1.20 (0.10–1.44)	0.592	0.292
		AA *vs.* GG	**1.79 (1.12–2.86)**	**0.016**	0.346	72.5%	<0.0001	1.44 (0.83–2.52)	**2.19 (1.17–4.09)**	0.858	0.325
		AA *vs.* GG+G**A**	**1.76 (1.14–2.70)**	**0.010**	0.273	68.5%	0.001	1.45 (0.87–2.41)	**2.14 (1.20–3.84)**	1.000	0.355
	rs868750	**A** *vs.* G	1.21 (0.96–1.52)	0.113	0.027	49.3%	0.116	1.21 (0.96–1.52)	–	0.308	0.689
		AA+G**A** *vs.* GG	1.19 (0.95–1.47)	0.125	0.014	27.5%	0.247	1.19 (0.95–1.47)	–	0.308	0.628
		AA *vs.* GG	1.78 (0.86–3.70)	0.123	0.229	41.1%	0.165	1.78 (0.86–3.71)	–	0.734	0.858
		AA *vs.* GG+G**A**	1.72 (0.87–3.37)	0.117	0.161	33.1%	0.213	1.72 (0.87–3.37)	–	0.734	0.919

OR – odds ratio; CI – confidence intervals; In genetic model, the bold one means mutation allele.

No association observed between SNPs of *TFAM* and *VR22* and AD

A total of 3353 cases and 3089 controls from 11 studies were involved in the meta-analysis concerning rs1937 and rs2306604 of TFAM. No significant association was detected between the 2 SNPs and the risk of AD by the allele, the dominant, the recessive, or the homozygous model (Figure 3, Table 2). In subgroup analysis, 9 of the studies were in whites and only 2 were in Asians. No clear correlation could be identified in the stratification by ethnicity (Figure 4A, 4B).

The association of rs10997691 and rs7070570 polymorphism of *VR22* and AD risk was investigated in 12 studies. No statistically significant correlation with AD was observed in the 4 models (Figure 5, Table 3). Nevertheless, increased or decreased AD susceptibility was not observed in subgroup analysis by ethnicity in the studies of rs7070570 polymorphism (Figure 4C).

Publication bias

Publication biases of the articles were assessed by Begg's funnel plot and Egger's linear regression test on the metadata. The distribution of different studies on the funnel plot of each

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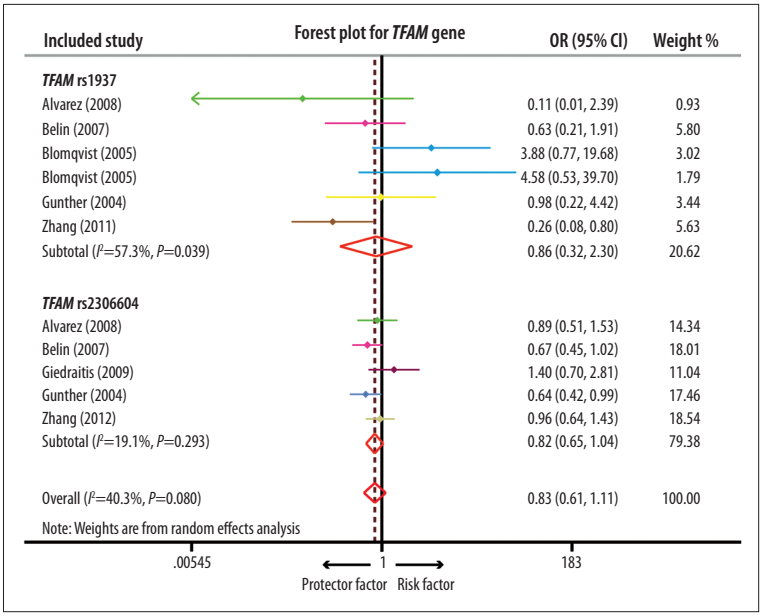

Figure 3. Forest plots showed the association of rs1937 and rs2306604 in *TFAM* gene with the risk of AD. The odds ratio from each study is represented by a square and the confidence interval is indicated by error bars. The subtotal and overall odds ratio is signified by a rhombus.

Table 2. Meta-analysis of two polymorphisms in *TFAM* gene and AD susceptibility.

Gene	SNP	Genetic model	OR (95% CI)	$P_{odds\ ratio}$	Tau^2	I^2	$P_{hetero-geneity}$	Ethnicity		Publication bias	
								Caucasians	Asians	P_{Begg}	P_{Egger}
TFAM	rs1937	**C** *vs.* G	0.90 (0.90–1.17)	0.432	0.066	63.5%	0.018	0.94 (0.68–1.32)	0.76 (0.59–0.99)	1.000	0.395
		CC+G**C** *vs.* GG	0.88 (0.69–1.13)	0.310	0.045	49.2%	0.080	0.91 (0.66–1.26)	0.78 (0.58–1.05)	1.000	0.496
		CC *vs.* GG	0.86 (0.32–2.30)	0.759	0.817	57.3%	0.039	1.20 (0.43–3.34)	0.26 (0.09–0.80)	0.452	0.330
		CC *vs.* GG+G**C**	0.87 (0.33–2.30)	0.783	0.759	55.6%	0.046	1.22 (0.45–3.32)	0.28 (0.09–0.84)	0.452	0.335
	rs2306604	**G** *vs.* A	0.90 (0.79–1.0)	0.084	0.006	28.0%	0.235	0.87 (0.75–1.01)	0.98 (0.80–1.20)	0.462	0.308
		GG+A**G** *vs.* AA	0.85 (0.70–1.02)	0.074	0.010	22.7%	0.270	0.78 (0.65–0.94)	1.04 (0.76–1.44)	0.462	0.387
		GG *vs.* AA	0.82 (0.65–1.04)	0.107	0.014	19.1%	0.293	0.79 (0.59–1.06)	0.96 (0.64–1.43)	0.806	0.192
		GG *vs.* AA+A**G**	0.89 (0.74–1.07)	0.200	0.000	0.0%	0.504	0.89 (0.70–1.11)	0.91 (0.66–1.26)	0.462	0.103

OR – odds ratio; CI – confidence intervals; In genetic model, the bold one means mutation allele.

SNP appeared to be symmetrical, and no statistically significant asymmetry was detected by Egger's test. Hence, no evidence of publication bias for the correlation between the SNPs and AD susceptibility was found (Tables 1–3).

Discussion

We performed a systematic meta-analysis of case-control association studies for susceptibility to AD. We screened 3 candidate genes – *CHAT*, *TFAM*, and *VR22* – and their major polymorphisms. In the end, 51 studies of 16 446 cases and 16 057 controls were involved in the analysis. Our results showed that 2 SNPs of *CHAT* (rs2177369 and rs3810950) were significantly associated with AD susceptibility. We also observed ethnic

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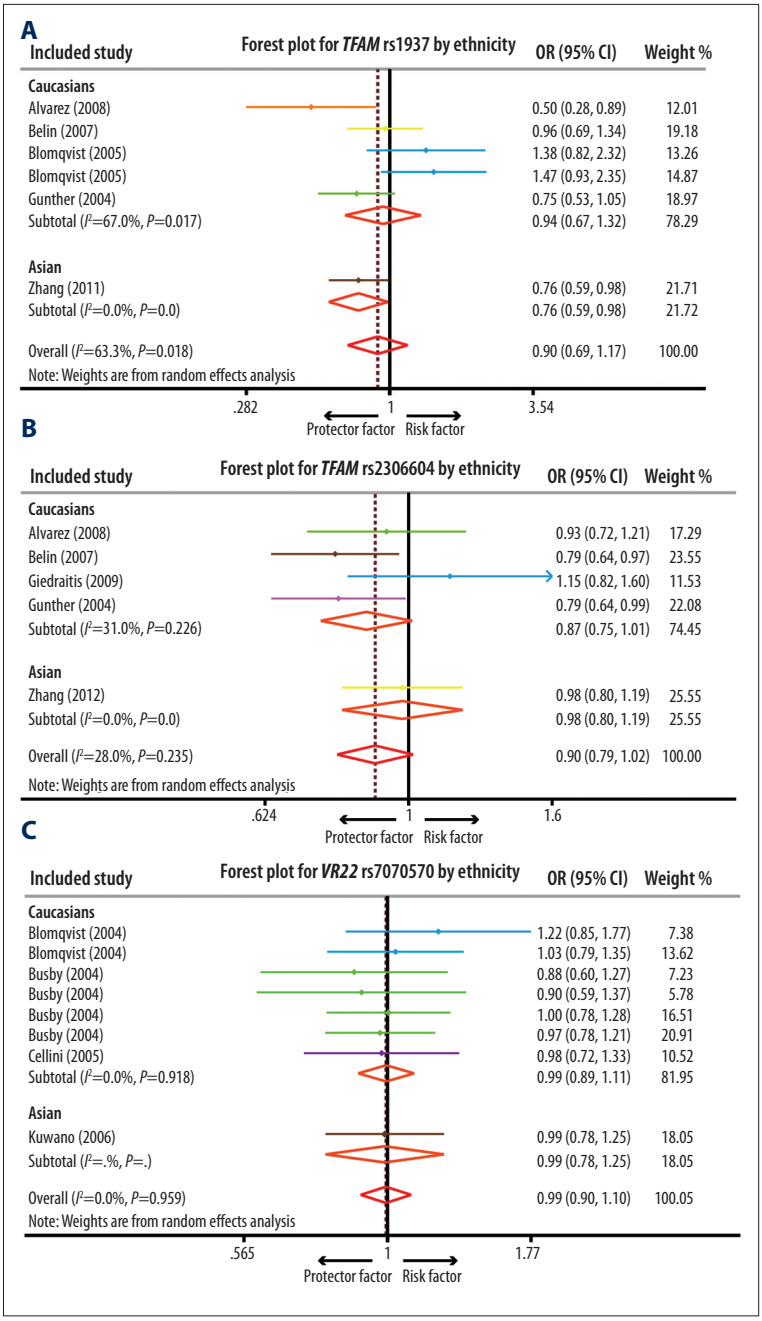

Figure 4. The forest plots of (**A**) *TFAM* rs1937, (**B**) *TFAM* rs2306604, and (**C**) *VR22* rs7070570 by ethnicity. The odds ratio from each study is represented by a square and the confidence interval is indicated by error bars. The subtotal and overall odds ratio is signified by a rhombus.

differences for rs3810950 of *CHAT*, with A allele of rs3810950 in Asians as risk factors for AD, whereas rs1880676 and rs868750 of *CHAT*, rs1937 and rs2306604 of *TFAM*, and rs10997691 and rs7070570 of *VR22* did not contribute to AD risk.

CHAT encodes the enzyme responsible for the biosynthesis of ACh. CHAT protein is a marker used in evaluating the function of basal forebrain cholinergic cells and dementia severity in AD [45,46]. Previous studies indicated that basal forebrain cholinergic neuron abnormalities are present very early in the course of AD, with altered expression of *CHAT* [47,48]. Mutations or polymorphisms of *CHAT* are also suspected to be related to AD and its treatment [49]. In agreement with previous results, we identified 2 SNPs of *CHAT* that contribute to the onset on AD.

TFAM locates in mitochondrial deoxyribonucleic acid (MTDNA) and encodes a key mitochondrial transcription factor that functions in mitochondrial DNA replication and repair. Mutations on *TFAM* can affect the function of mitochondria and contribute to the pathogenesis of AD. In accordance with the mitochondrial cascade hypothesis, the synergistic interactions

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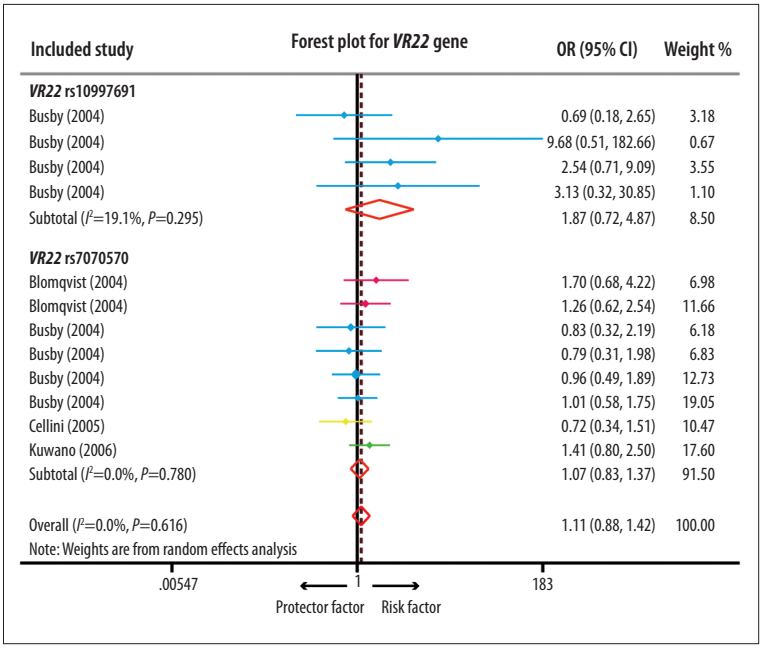

Figure 5. Forest plots showed the association of rs10997691 and rs7070570 in *VR22* gene with the risk of AD. The odds ratio from each study is represented by a square and the confidence interval is indicated by error bars. The subtotal and overall odds ratio is signified by a rhombus.

Table 3. Meta-analysis of two polymorphisms in *VR22* gene and AD susceptibility.

Gene	SNP	Genetic model	OR (95% CI)	$P_{odds\ ratio}$	Tau^2	I^2	$P_{hetero-geneity}$	Ethnicity		Publication bias	
								Caucasians	Asians	P_{Begg}	P_{Egger}
VR22	rs10997691	**C** *vs.* T	1.22 (0.96–1.54)	0.106	0.0000	0.0%	0.436	1.22 (0.96–1.54)	–	0.308	0.211
		CC+TC *vs.* TT	1.18 (0.91–1.54)	0.212	0.0000	0.0%	0.579	1.18 (0.91–1.54)	–	0.308	0.098
		CC *vs.* TT	1.87 (0.72–4.87)	0.200	0.1895	19.1%	0.295	1.87 (0.72–4.87)	–	0.308	0.183
		CC *vs.* TT+TC	1.82 (0.69–4.82)	0.229	0.2212	21.8%	0.280	1.82 (0.69–4.82)	–	0.308	0.203
	rs7070570	**C** *vs.* T	0.99 (0.90–1.10)	0.903	0.0000	0.0%	0.959	1.00 (0.89–1.11)	0.99 (0.78–1.25)	0.902	0.930
		CC+TC *vs.* TT	1.02 (0.89–1.17)	0.802	0.0000	0.0%	0.740	0.99 (0.86–1.14)	1.58 (0.91–2.75)	0.386	0.269
		CC *vs.* TT	1.07 (0.83–1.37)	0.617	0.0000	0.0%	0.780	1.00 (0.75–1.32)	1.41 (0.80–2.50)	0.536	0.710
		CC *vs.* TT+TC	0.94 (0.76–1.14)	0.513	0.0000	0.0%	0.828	1.00 (0.77–1.32)	0.86 (0.63–1.16)	0.711	0.326

OR – odds ratio; CI – confidence intervals; In genetic model, the bold one means mutation allele.

between *TFAM* rs1937 and *APOE4* status have been reported to influence AD risk [50], and rs2306604 A allele of *TFAM* was also found to be a moderate risk factor for AD [22]. However, in the present study, we failed to confirm the results of Belin et al. and Zhang et al. [22,44].

VR22, also known as CTNNA3, plays a role in cell-cell adhesion. *VR22* can bind directly to b-catenin, whereas b-catenin forms a complex with presenilin 1 (PSEN1) [51], mutations of which cause familial cases of early-onset AD [52]. Nonetheless, the 2 SNPs we enrolled in this meta-analysis failed to show significant associations with AD.

The principal results of the present study suggest that *TFAM* and *VR22* gene polymorphisms are not associated with risk of AD. All eligible case-control studies were included in this

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meta-analysis, including the most recent ones. However, there remain certain issues that need to be addressed in interpreting our results. Firstly, most of the subjects covered in our study were white (81.6% in cases and 76.0% in controls), which limits the general application of the results. As we have already observed, the association of AD with some SNPs can only be observed in certain ethnic groups. Further studies with more Asian and African subjects are recommended. Secondly, although it is statistically sufficient, the overall sample size for each SNP is still relatively small. Furthermore, individual genetic factors, the biological characteristics of tumors, and their interaction with the environment may influence cancer susceptibility and carcinogenesis. Because the diagnosis of most of the AD cases enrolled in the studies were based on diagnostic criteria rather than pathological examination, we cannot exclude that some cases might have been misdiagnosed, which further influences the results of this meta-analysis, and further work is required to minimize this effect.

Conclusions

Rs2177369 and rs3810950 of *CHAT* are associated with AD susceptibility, but rs1880676 and rs868750 are not. Rs1937 and rs2306604 of *TFAM* and rs10997691 and rs7070570 of *VR22* are not significantly associated with AD risk.

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