# 科技部補助專題研究計畫報告

## 探討性別差異對醇脫氫酶與乙醛去氫酶酵素之基因多型性，基因調控，與粒線體功能於阿茲海默症的角色

報 告 類 別 ： 成果報告
計 畫 類 別 ： 個別型計畫
計 畫 編 號 ： MOST 108-2629-B-182A-005-
執 行 期 間 ： 108年08月01日至109年10月31日
執 行 單 位 ： 長庚醫療財團法人神經內科

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本研究具有政策應用參考價值：■否 □是，建議提供機關
（勾選「是」者，請列舉建議可提供施政參考之業務主管機關）
本研究具影響公共利益之重大發現：□否 □是

中 華 民 國 110 年 01 月 21 日

中 文 摘 要 ： 阿茲海默症是老年人中最常見的失智症，其罹病率和預後具性別差異。在阿茲海默症病理生理中可能存在控制性別差異的分子途徑。粒線體功能損傷在阿茲海默症早期即已發生。醇脫氫酶（ADH）與乙醛去氫酶（ALDH）對氧化壓力調節及酒精代謝很重要。在台灣，男性飲酒比例較女性高，可能造成兩性因代謝酶變異而致病的差異。ADH / ALDH途徑涉及許多罹患阿茲海默症的風險，包括氧化壓力，高血壓，飲酒習慣和腦血管內皮細胞完整性。因此，本計劃希望透過檢測醇脫氫酶與乙醛去氫酶基因多型性及表現差異，來了解性別對於此基因群與阿茲海默症的關連性與可能機轉。此1年期計畫收案157位阿茲海默症病患和168位年齡和性別匹配的對照受試者，以研究阿茲海默症與ADH / ALDH單計畫分析了核苷酸多態性（SNP）和單套型構建(haplotype)的關聯，包括ADH1C rs2241894，ADH1B rs1229984，ALDH1B1 rs2073478，ALDH2 rs886205，rs4767944，rs4648328和rs671。我們發現在隱性遺傳模型中，女性ADH1C rs2241894 TT基因型與阿茲海默症呈負相關（OR = 0.25，95%CI 0.09-0.75，p <0.0001）。在ALDH2的四個SNP之間具連鎖不平衡。我們未發現ALDH2單套型與阿茲海默症有關。阿茲海默症病患中血漿ADH1C濃度高於對照組。我們還發現了阿茲海默症與rs2241894基因型對血漿ADH1C濃度的顯著相互作用（p = 0.04）。這種相互作用的作用歸因於阿茲海默症和血漿ADH1C濃度之間的關聯（β估計值= 366，95%CI 92.7～639.4，p = 0.009）。 ADH1C rs2241894的遺傳分佈顯示出強烈的種族異質性，其中T等位基因為次要等位基因(minor allele)，在我們的研究中佔28.5%，在東亞地區佔23.6%，而在全球人群中它卻是主要等位基因(major allele)。這項研究揭示了ADH1C rs2241894 TT基因型與女性阿茲海默症之間存在可能的保護關聯性。未來需要進行更多的樣本研究來確認此發現。

中 文 關 鍵 詞 ： 阿茲海默症 性別 粒線體 乙醇

英 文 摘 要 ： Alzheimer’s disease (AD) is the commonest type of dementia among older people. There are substantial evidence of sex differences in AD prevalence, disease course, and prognosis. There are molecular pathways governing sex differences in AD pathophysiology. Mitochondrial dysfunction is an early feature of AD. Alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) play central role of oxidative stress. In Taiwan, men are more likely being alcoholics than women. Alcohol-gene interaction in diseases has been found between sexes. ADH/ALDH pathway was involved in a number of risks of AD, including oxidative stress, hypertension, alcohol habit, and cerebral vascular endothelial cell integrity. This 1-year project enrolled 157 AD and 168 age- and sex-matched control subjects to examine the association of AD with ADH/ALDH single nucleotide polymorphisms (SNPs) and haplotype construction, including ADH1C rs2241894, ADH1B rs1229984, ALDH1B1 rs2073478, ALDH2 rs886205, rs4767944,

rs4648328, and rs671. This study observed that ADH1C rs2241894 TT genotype was negatively associated with AD in a recessive genetic model (OR=0.25, 95% CI 0.09–0.75, p<0.0001) in women. A strong linkage disequilibrium was observed among the four examined SNPs of ALDH2. No haplotype was related to AD. The plasma ADH1C level in AD was higher than that in control. We also found a significant interaction effect of AD–rs2241894 genotype on plasma ADH1C level (p=0.04). This interaction effect was attributable to the association between AD and plasma ADH1C level ($\beta$ estimate =366, 95% CI 92.7~639.4, p=0.009). The genetic distribution of ADH1C rs2241894 showed strong ethnic heterogeneity, in which the T allele was the minor allele accounting for 28.5% in our study and 23.6% in East Asians, while it was a major allele in global populations. In summary, this study revealed a suggestive association between ADH1C rs2241894 and female AD. Further large sample size case-control studies are needed before rs2241894 can be interpreted as a protective genetic factor of AD in women.

英文關鍵詞：Alzheimer's disease, sex, mitochondria, alcohol

# 科技部補助專題研究計畫成果報告

**（□期中進度報告/■期末報告）**

探討性別差異對醇脫氫酶與乙醛去氫酶酵素之基因多型性，基因調控，與粒線體功能於阿茲海默症的角色

計畫類別：■個別型計畫　□整合型計畫

計畫編號：MOST108-2629-B-182A-005-

執行期間： 108 年 08 月 01 日至 109 年 10 月 31 日

執行機構及系所：長庚醫療財團法人

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本計畫除繳交成果報告外，另含下列出國報告，共 ___ 份：

□執行國際合作與移地研究心得報告

□出席國際學術會議心得報告

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1. 公開方式：

   非列管計畫亦不具下列情形，立即公開查詢

2.「本研究」是否已有嚴重損及公共利益之發現：■否 □是

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中　華　民　國　110 年 01 月 18 日

# 目錄

# 中文摘要

關鍵詞：阿茲海默症 性別 粒線體 乙醇

阿茲海默症是老年人中最常見的失智症，其罹病率和預後具性別差異。在阿茲海默症病理生理中可能存在控制性別差異的分子途徑。粒線體功能損傷在阿茲海默症早期即已發生。醇脫氫酶（ADH）與乙醛去氫酶（ALDH）對氧化壓力調節及酒精代謝很重要。在台灣，男性飲酒比例較女性高，可能造成兩性因代謝酶變異而致病的差異。ADH / ALDH 途徑涉及許多罹患阿茲海默症的風險，包括氧化壓力，高血壓，飲酒習慣和腦血管內皮細胞完整性。因此，本計劃希望透過檢測醇脫氫酶與乙醛去氫酶基因多型性及表現差異，來了解性別對於此基因群與阿茲海默症的關連性與可能機轉。此 1 年期計畫收案 157 位阿茲海默症病患和 168 位年齡和性別匹配的對照受試者，以研究阿茲海默症與 ADH / ALDH 單計畫分析了核苷酸多態性（SNP）和單套型構建(haplotype)的關聯，包括 ADH1C rs2241894，ADH1B rs1229984，ALDH1B1 rs2073478，ALDH2 rs886205 ，rs4767944，rs4648328 和 rs671。我們發現在隱性遺傳模型中，女性 *ADH1C* rs2241894 TT 基因型與阿茲海默症呈負相關（OR = 0.25，95%CI 0.09-0.75，p <0.0001）。在 *ALDH2* 的四個 SNP 之間具連鎖不平衡。我們未發現 *ALDH2* 單套型與阿茲海默症有關。阿茲海默症病患中血漿 ADH1C 濃度高於對照組。我們還發現了阿茲海默症與 rs2241894 基因型對血漿 ADH1C 濃度的顯著相互作用（p = 0.04）。這種相互作用的作用歸因於阿茲海默症和血漿 ADH1C 濃度之間的關聯（$\beta$ 估計值= 366，95%CI 92.7～639.4，p = 0.009）。 *ADH1C* rs2241894 的遺傳分佈顯示出強烈的種族異質性，其中 T 等位基因為次要等位基因(minor allele)，在我們的研究中佔 28.5%，在東亞地區佔 23.6%，而在全球人群中它卻是主要等位基因(major allele)。這項研究揭示了 *ADH1C* rs2241894 TT 基因型與女性阿茲海默症之間存在可能的保護關聯性。未來需要進行更多的樣本研究來確認此發現。

**Abstract**
Key words: Alzheimer’s disease, sex, mitochondria, alcohol

Alzheimer’s disease (AD) is the commonest type of dementia among older people. There are substantial evidence of sex differences in AD prevalence, disease course, and prognosis. There are molecular pathways governing sex differences in AD pathophysiology. Mitochondrial dysfunction is an early feature of AD. Alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) play central role of oxidative stress. In Taiwan, men are more likely being alcoholics than women. Alcohol-gene interaction in diseases has been found between sexes. ADH/ALDH pathway was involved in a number of risks of AD, including oxidative stress, hypertension, alcohol habit, and cerebral vascular endothelial cell integrity. This 1-year project enrolled 157 AD and 168 age- and sex-matched control subjects to examine the association of AD with *ADH/ALDH* single nucleotide polymorphisms (SNPs) and haplotype construction, including *ADH1C* rs2241894, *ADH1B* rs1229984, *ALDH1B1* rs2073478, *ALDH2* rs886205, rs4767944, rs4648328, and rs671. This study observed that *ADH1C* rs2241894 TT genotype was negatively associated with AD in a recessive genetic model (OR=0.25, 95% CI 0.09–0.75, $p<0.0001$) in women. A strong linkage disequilibrium was observed among the four examined SNPs of *ALDH2*. No haplotype was related to AD. The plasma ADH1C level in AD was higher than that in control. We also found a significant interaction effect of AD–rs2241894 genotype on plasma ADH1C level ($p=0.04$). This interaction effect was attributable to the association between AD and plasma ADH1C level (β estimate =366, 95% CI 92.7~639.4, $p=0.009$). The genetic distribution of *ADH1C* rs2241894 showed strong ethnic heterogeneity, in which the T allele was the minor allele accounting for 28.5% in our study and 23.6% in East Asians, while it was a major allele in global populations. In summary, this study revealed a suggestive association between *ADH1C* rs2241894 and female AD. Further large sample size case-control studies are needed before rs2241894 can be interpreted as a protective genetic factor of AD in women.

## 1. Background

Alzheimer's disease (AD) is the commonest type of dementia among older people (QUERFURTH AND LAFERLA 2010; SCHELTENS *et al.* 2016). There are substantial evidence of sex differences in AD prevalence, clinical manifestation, disease course, and prognosis (ROCCA *et al.* 2014; MAZURE AND SWENDSEN 2016; BACIGALUPO *et al.* 2018). Although having low education partly contributes to higher incidence of dementia in women than in men, non-modifiable biological factors, such as sexual dimorphism in brain structure and function, also contributes to sex differences in AD phenotypes (ROCCA *et al.* 2014; FILON *et al.* 2016). Sex difference in genetic instability has been suggested as the observation that effects of *Apolipoprotein E* (*APOE*) variants on the AD risks are more pronounced in women (ALTMANN *et al.* 2014). *APOE* ε4 allele increases the AD risk by 3-fold higher in people carrying one ε4 allele and 12-fold higher in those with two (CORDER *et al.* 1993). A recent study utilizing global gene expression profiles found four other genes expressed differently between sexes in AD (*glutamate metabotropic receptor 2, estrogen-related receptor beta, kinesin family member 26B, and aspartoacylase*) (SUN *et al.* 2019). Therefore, there are molecular pathways governing sex differences in AD pathology. Although genes cannot be modified, factors that interact with sex related variants may be intervened to prevent AD.

Mitochondrial dysfunction is an early feature of AD. Accumulation of β-amyloid peptide (Aβ, β-amyloid plaques) plays an important role in AD pathogenesis. Accumulation of Aβ causes neuronal death via a number of mechanisms including oxidative stress, neuroinflammation, excitotoxicity, and apoptosis and directly affects mitochondrial respiratory enzyme activity and triggers mitochondrial membrane permeability (PARIHAR AND HEMNANI 2004; HANSSON PETERSEN *et al.* 2008; ABRAMOV *et al.* 2009). Mitochondria are vulnerable to oxidative stress and are major sources of intracellular reactive oxygen species (ROS) (MOREIRA *et al.* 2007). Studies suggest that $A\beta_{1\text{-}42}$ inhibits cytochrome c oxidase (COX) activity at an early stage of AD (KISH *et al.* 1992; PARKER AND PARKS 1995; CARDOSO *et al.* 2004; CROUCH *et al.* 2005).

Mitochondrial Aβ-binding alcohol dehydrogenase (ABAD, EC 1.1.1.178) , HGNC: *HSD17B10*, GRCh38: X:53,431,257-53,434,375) has been found to be up-regulated in AD neurons (MORSY AND TRIPPIER 2018). Aβ interacts with ABAD to induce a conformational change, which prohibits ABAD binding to nicotinamide adenine dinucleotide (NAD+) and prevents its role in the oxidation of substrates and consequently causes changes in mitochondrial membrane permeability (LUSTBADER *et al.* 2004). In addition to Aβ toxicity, removal of toxic aldehydes in cells also plays a central role of the other two protein families, alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) (BOSRON *et al.* 1980; JACKSON *et al.* 2011). ADH families (EC 1.1.1.1) are a group of dehydrogenase enzymes that facilitates the interconversion between alcohols and aldehydes or ketones with the reduction of NAD+ to NADH during biosynthesis of

various metabolites (BOSRON *et al.* 1980). ALDHs families (EC 1.2.1.3) are a group of enzymes that catalyse the oxidation of aldehydes. Mitochondria ALDH convert acetaldehyde to acetate as the rate-limiting step in liver mitochondria (JACKSON *et al.* 2011; CHEN *et al.* 2014). These genes participate in a wide variety of biological processes involved in the physiological and pathological effects from exogenously and endogenously generated aldehydes. Both high ADH activity and low ALDH activity cause an excess of acetaldehyde and results in oxidative stress. In the clinical significance, ADH genetic variants were associated with alcohol dependence (ENG *et al.* 2007; CHIANG *et al.* 2016) and coronary artery disease (WANG *et al.* 2014), while ALDH2 deficiency causes acetaldehyde accumulation and mitochondria dysfunction and increase risks of AD (KAMINO *et al.* 2000; GRUNBLATT AND RIEDERER 2016), Parkinson's disease (PD) (GRUNBLATT AND RIEDERER 2016), hypertension (KATO *et al.* 2011), ischemic stroke (CHEN *et al.* 2014), and cerebral vascular endothelial cell integrity (SOLITO *et al.* 2013).

**Sex difference in alcohol exposure, alcohol bio-distribution, and status of central nervous system diseases**

A recent prospective cohort study examining alcohol consumption and risk of dementia in a 23 year follow-up suggested that alcohol consumption is a risk of dementia for both sexes (SABIA *et al.* 2018). In Taiwan, men are more likely being alcoholics. Our hospital-based studies showed the rate of alcohol consumption was 0% in women vs. 26% in men in normal controls and 1.5% in women vs. 46% in men in stroke patients (CHEN *et al.* 2006b; CHEN *et al.* 2009a). The impact of alcohol is greater in men then in women due to the difference in their alcohol habit. In addition, the toxic effect of alcohol can be influenced by gene; for example, men carrying *APOE* ε2ε3 tended to have more stroke than those with ε3ε3, when they have alcohol exposure (CHEN *et al.* 2009a). Another example for alcohol-gene interaction is class III ADH (glutathione-dependent ADH). Women develop higher blood alcohol levels than men in spite of an equal alcohol intake due to a smaller gastric metabolism in women by lesser activity of class III ADH in females (BARAONA *et al.* 2001). Therefore, sex difference in the effects of *ADH* and *ALDH* genes on AD should be tested to illuminate the genetic roles on AD for a personalized management (SULTATOS *et al.* 2004).

**Regulatory effect of *ADH* and *ALDH* genes**

To date, the majority of the identified genetic variants of AD reside in noncoding regions with unclear functions. The functional genetic variants associated with AD and the related quantitative traits are largely unknown. Expression quantitative trait loci (eQTL) are defined as loci that harbor sequence variants which regulate gene expression in specific cell or tissue types (TURPEINEN *et al.* 2015). Expression differences result in phenotypic variation among individuals. eQTLs therefore can serve as major determinants for the function of certain genes, in this proposal, *ADH* and *ALDH* genes, that regulate the expression of causal genes.

There are no reports concerning transcriptomic patterns of the various *ADH* and *ALDH* mRNAs in the AD brain tissues to date, whereas protein alterations were demonstrated in distribution of the mitochondrial ALDH2 in glia cells of cerebral cortex and hippocampus of AD patients (PICKLO *et al.* 2001). ALDH2 protein activity was also shown to significantly increase in the putamen of AD patients (MICHEL *et al.* 2010). In the temporal cortex of AD, activated astrocytes expressing both ALDH1A1 and glial fibrillary acidic protein were more prominent than those in controls (SERRANO-POZO *et al.* 2013).

ADH exists in multiple forms as a dimer and is encoded by at least seven different genes. There are five classes of ADH, and the hepatic form (class 1) is the primarily ones in human cells. Class 1 consists of α, β, and γ subunits that are encoded by the genes *ADH1A* (HGNC symbol, Cytogenetic location: 4q23, Genomic coordinates (GRCh38): 4:99276365-99291027), *ADH1B* (GRCh38: 4:99306386-99321441), and *ADH1C* (4:99336491-99353044) (SULTATOS *et al.* 2004). The well-studied genetic variants for *ADH* genes in Asian included rs2241894 (*ADH1C*, chr4:99344976, c.453A>T, p.Thr151=) and rs1229984 (*ADH1B*, chr4:99318162, Missense Variantc.143A>G, p.His48Arg) (ENG *et al.* 2007).

ALDH2 is one of the 19 ALDH isozymes in human cells that are essential for the metabolism and detoxification of a wide range of endogenous and exogenous aldehyde substrates (ENG *et al.* 2007). ALDH2 is most efficient in metabolizing aldehydes and is the rate-limiting enzyme in the ethanol metabolism, oxidizing acetaldehyde to acetic acid both in the liver and brain (CHEN *et al.* 2014). The well-studied genetic variants for *ALDH* genes in Asian included rs2073478 (*ALDH1B1*: chr9:38396068, Missense Variant, c.320G>A, p.Arg107His) (JACKSON *et al.* 2013), rs886205 (*ALDH2*, chr12:111766623, 2KB Upstream Variant, A>G), rs671 (*ALDH2*, chr12:111803962, Missense Variant, c.1510G>A, p.Glu504Lys) (ZHAO AND WANG 2015), rs4648328 (*ALDH2*, chr12:111784984, Intron Variant, C>T), and rs4767944 (*ALDH2*, chr12:111771537, Intron Variant, C>G,T).

## 2. Objective and specific aims

Given that *ADH/ALDH* pathway is involved in a number of risks of AD, including oxidative stress, hypertension, alcohol habit, and cerebral vascular endothelial cell integrity, this 1-year project aimed to discover genetic functional loci for aiding AD diagnosis and understanding mechanisms in both sexes. This proposal also aimed to discover the effect of the modifiable factor, alcohol consumption, on the associations of *ADH* and *ALDH* genes with AD association. SNPs and haplotype construction were used for association tests. Plasma levels of ADH and ALDH2 were measured in normal controls and AD patients. Sex difference in alcohol consumption and gene-alcohol interaction were tested and adjusted.

## 3.研究方法

Patients were recruited from Chang Gung Memorial Hospital (CGMH). A patient or her/his legally acceptable representative provided written informed consent to participate. If the patient was incapable of giving informed consent, the legally acceptable representative consented on behalf of the patient.

*Patient and control subject recruitment*

This study was designed as a two-step process. First, we enrolled 157 AD patients and 168 age- and sex-matched control subjects in a pilot study. AD diagnosis was made according to the 2011 diagnostic criteria of the National Institute on Aging-Alzheimer's Association workgroups (NIAAA) (McKhann *et al.* 2011). Participants of the control group were recruited randomly utilizing the following inclusion criteria: (1) gender- and age-matched subjects providing informed consent, (2) subjects who came to CGMH for a health exam or treatment for diseases other than neurodegenerative diseases or cerebrovascular diseases, (3) subjects ascertained from community, and (4) no medical history of neurodegenerative or cerebrovascular diseases, no overt medical diseases, such as renal failure, myocardial infarction, or cancer. Second, the number of AD patients was expanded to 339. A total of 2504 healthy participants selected from the Taiwan Biobank were included in the extension study. The Taiwan Biobank is a prospective population-based study that enrolled healthy seniors with extensive baseline phenotypic measurements, genomic data, and stored biological samples. The criteria for selecting the control groups from the Taiwan Biobank were the age range 50–70 years, no history of stroke or dementia, and self-reporting as being of Taiwanese Han Chinese ancestry (Chen *et al.* 2016). Details on the Taiwan Biobank can be found on its official website (https://taiwanview.twbiobank.org.tw/index).

*Definition of alcohol consumption:*

Our prior studies discovered that alcohol drinking ≥210 g/week is a risk factor of stroke in the Taiwan population (Chen *et al.* 2006b; Chen *et al.* 2009a). Therefore, this proposal applies the same cutoff point for alcohol consumption to study the inter-correlation of gene, alcohol, and dementia risks. For the investigation of the alcohol effect on the genetic expression and effect, participants who have alcohol consumption in the prior year before enrollment is defined as current drinker. We classify participants who used to have alcohol and report no alcohol consumption over the previous year as former drinkers.

*Sample collection and genomic DNA preparation*

Genomic DNA was extracted from 10-ml peripheral blood samples using the QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA, USA) as follows: 20 μL of QIAGEN Protease (or protease K) was mixed with 200 μL of buffy coat via a 15-s vortex step. The mixture was then incubated at

56 °C for 10 min after adding 200 μL of Buffer AL. Next, 200 μL of 96–100 % ethanol was added, followed by a 15-s vortex and transfer to a QIAamp Mini spin column. The silica membrane was washed via centrifugation with Buffers AW1 and AW2. Genomic DNA was eluted with Buffer AE, and the quantity and quality were determined using a Nanodrop (Thermo Fisher Scientific, Waltham, MA, USA) and Qubit 2.0 fluorometer (Life Technologies, Carlsbad, CA, USA).

*Genotyping and haplotype construction for cases and control*

Based on a previously reported association with alcohol dependence (EDENBERG AND FOROUD 2013), this study analyzed seven SNPs, namely, *ADH1C* rs2241894, *ADH1B* rs1229984, *ALDH1B1* rs2073478, *ALDH2* rs886205, *ALDH2* rs4767944, *ALDH2* rs4648328, and *ALHD2* rs671. Genomic DNA was extracted from peripheral leukocytes using the Stratagene DNA extraction kit (La Jolla, CA, USA). SNP polymorphisms were genotyped using TaqMan® Assays in the ABI Prism 7900HT Sequence Detection System (catalogue #4317596, Applied Biosystems, Foster City, CA, USA) (SCHLEINITZ *et al.* 2011). Plasma ADH1C level was determined using human ADH1C ELISA kit (catalogue #MBS2889930, MyBioSource, San Diego, CA, USA) and monitored spectrophotometrically at 450 nm on a multifunctional microplate reader (Tecan infinite 200) by following the manufacturer's instructions. Levels of ADH1C were determined from a standard curve. Patterns of linkage disequilibrium (LD) were evaluated using Haploview v4, and haplotypes were reconstructed using PHASE 2.0 (BARRETT *et al.* 2005) based on the LD results. Haplotypes with a frequency <1% were excluded from the association analysis. In participants from the Taiwan Biobank, SNP genotypes were obtained from the data derived from the custom Taiwan Biobank chips and run on the Axiom Genome-Wide Array Plate System (Affymetrix, Santa Clara, CA, USA).

*Statistical analysis*

Pearson's $\chi 2$-test or t-test was used to compare the demographic data and the distributions of genotypes of AD and control. Two-tailed P-values were derived from the $\chi 2$-test or Fisher's exact test. Association analyses were performed stratified by sex. Hardy–Weinberg equilibrium was performed via $\chi 2$-test for all SNPs at a significance level of 0.05. Multivariable logistic regression was used to analyze the phenotype-genotype associations of AD with *ADH* and *ALDH* alleles under dominant, recessive, and additive genetic models. The covariables included age, years of education, HTN, DM, and alcohol use. Since considering Bonferroni correction, the significance level was set to 0.007 in pilot study and 0.01 in extension study. The permutation testing was performed when the P-value was under Bonferroni correction. Analysis of interaction effect (CHEN *et al.* 2009b) was performed to evaluate how carrying APOE ε4 influence the *ADH1C* rs2241894 to AD susceptibility. All the data analyses were performed using SAS software version 9.1.3 (SAS Institute, Cary, NC, USA). Association of the interaction effect between AD and rs2241894 genotypes on the plasma ADH1C level was tested by the general linear models (GLM) with

adjustment for age, sex, DM, HTN, and alcohol. We also perform analysis of interaction effect of AD-rs2241894 genotype in ADH1C level.

*Power estimation*

We evaluated the ability to detect an association between an SNP and AD via a power calculation implemented in QUANTO version 1.0 (GAUDERMAN 2002). When Minor allele frequency (MAF) >0.2 under a recessive genetic model at a significance level of 5%, we observed that the power to identify an association was greater than 0.8 when the per-allele genetic effect was greater than 3.5 and 2.0 in the pilot case-control study and in the extension study, respectively.

## 4. 結果與討論

*Demography of the 1-year case-control study*

A total of 157 AD patients and 168 controls were included. The years of education were higher in the female AD patients than in the controls (Table 1). The age and sex between the AD patients and the controls were matched in this dataset. The proportion of APOE ε4 carriers was higher in the AD patients than in the controls. The proportion of DM was higher in the female patients with AD than in the controls. There were no differences in age, HTN frequency, and the proportion of alcohol use. As the proportion of alcohol use was remarkably different between sexes, the analyses were stratified by sex.

Table 1. Background demographic distribution and frequency of the genotype in the pilot study

| | Males (n =147) | | | Females (n = 178) | | |
|---|---|---|---|---|---|---|
| | AD (n =73) | Controls (n =74) | *p*-Value | AD (n = 84) | Controls (n =94) | *p*-Value |
| Age (years) | 69.4 ± 9.0 | 67.1 ± 5.3 | 0.06 | 65.4 ± 5.9 | 67.0 ± 6.3 | 0.08 |
| Education (yrs) | 8.4 ± 4.1 | 9.3 ± 4.7 | 0.31 | 7.4 ± 4.5 | 5.6 ± 4.7 | 0.01 |
| Hypertension(%) | 55.40% | 52.51% | 0.69 | 45.2% | 44.7% | 0.94 |
| DM (%) | 20.30% | 21.10% | 0.9 | 36.9% | 20.2% | 0.01 |
| Alcohol use (%) | 17.60% | 16.90% | 0.92 | 1.2% | 1.1% | 0.94 |
| APOE ε4 carrier | 30.1% | 12.3% | 0.02 | 43.4% | 21.7% | 0.01 |
| *ADH1B* rs1229984 TT/TC/CC | 49.4/39.5/11.1 | 56.2/41.1/2.7 | 0.14 | 60.7/35.7/3.6 | 53.2/42.6/4.3 | 0.6 |
| *ADH1C* rs2241894 CC/CT/TT | 43.8/42.5/13.7 | 52.1/38.4/9.6 | 0.55 | 54.8/44.0/1.2 | 46.8/43.6/9.6 | 0.05 |
| *ALDH1B1* rs2073478 GG/GT/TT | 47.9/36.6/15.5 | 52.8/31.9/15.3 | 0.82 | 41.0/51.8/7.2 | 48.4/36.6/15.1 | 0.07 |
| *ALDH2* rs886205 GG/GA/AA | 77.0/21.6/1.4 | 78.1/20.5/1.4 | 0.99 | 82.1/17.9/0.0 | 74.5/22.3/3.2 | 0.18 |
| rs4767944 TT/TC/CC | 48.6/41.9/9.5 | 41.1/49.3/9.6 | 0.63 | 51.8/41.0/7.2 | 47.8/38.0/14.1 | 0.34 |
| rs4648328 CC/CT/ TT | 67.1/28.8/4.1 | 57.5/38.4/4.1 | 0.46 | 68.7/26.5/4.8 | 64.9/31.9/3.2 | 0.66 |
| rs671 GG/GA/AA | 46.6/35.6/17.8 | 46.6/42.5/11.0 | 0.44 | 47.6/41.7/10.7 | 41.5/50.0/8.5 | 0.53 |

Abbreviations: n=number, AD= Alzheimer disease, ADH= alcohol dehydrogenase, ALDH= aldehyde dehydrogenase

Data are expressed as percentage or mean ± SE. Comparisons between AD cases and controls were analyzed using the $\chi 2$ test or t-test where appropriate.

*Genotype frequency and association analysis of the 1-year case-control study*

All seven SNPs were in Hardy–Weinberg equilibrium at a significance level of 0.05. The frequencies of each genotype in the AD and control subjects are listed in Table 1. The proportion of *ADH1C* rs2241894 TT genotype (minor allele T) was significantly lower in the female patients with AD than in the female controls. In the female group, *ADH1C* rs2241894 was significantly associated with AD in the recessive genetic model (OR=0.25, 95% CI 0.09–0.75, $p<0.0001$). APOE ε4 carriers had no interactive effect between AD and *ADH1C* rs2241894. This study did not find an association between AD and the other six SNPs in the female groups and any candidate SNPs in the male groups.

*Haploview analysis of ALDH2 SNPs*

In the pilot case-control study, a haplotype block of *ALDH2* was further constructed by rs886205, rs4767944, rs4648328, and rs671 using Haploview (4.2), where there was one block with strong LD. In the haplotype analyses, there was no association between the haplotype and AD susceptibility.

*Plasma ADH1C level*

We examined the plasma level of ADH1C and ALDH2. AD had higher ADH1C level in comparison to control group (n=78, n=72, 781 ± 383, 665 ± 242, respectively) ($p=0.03$). After adjusted by age, sex, HTN, DM, and alcohol, we found a significant interaction effect of AD–rs2241894 genotype on plasma ADH1C level ($p=0.04$). This interaction effect was attributable to the association between AD and plasma ADH1C level (β estimate =366, 95% CI 92.7~639.4, $p=0.009$). There was no association between SNPs and plasma levels for ADH1C and ALDH2.

**Discussion**

Our study demonstrated a suggestive association between AD and *ADH1C* rs2241894 genotypes in a recessive fashion. To the best of our knowledge, this is the first study to propose *ADH1C* rs2241894 genotypes as a protective factor of AD in the Taiwanese female population. This study did not find associations between AD and *ADH1B* (rs1229984), *ALDH1B1* (rs2073478), and *ALDH2* (rs886205, rs4767944, rs4648328, and rs671), indicating that *ADH1B*, *ALDH1B1*, and *ALDH2* played no role in the relationship between alcohol and AD.

The genetic distribution of *ADH1C* rs2241894 showed strong ethnic heterogeneity, in which the T allele was the minor allele accounting for 28.5% in our study, 23.6% in East Asians, and 40% in South Asians, while it was a major allele in Americans (83.0%), Europeans (76.5%), and the global populations (52.8%) (HUANG *et al.* 2020). *ADH1C* rs2241894 (A > G, synonymous variant Thr151, exon 5) is a synonymous variant. Moreover, we did not find functional SNPs that have LD with rs2241894 on SNPsnap (https://data.broadinstitute.org/mpg/snpsnap/). To the best of our knowledge, there is no report showing an association between AD and rs2241894 or nearby SNPs.

In Asia, men are prone to alcohol drinking in contrast to women (MILLWOOD *et al.* 2019), in which we have demonstrated that the rate of alcohol consumption was 0% in women versus 26% in men (CHEN *et al.* 2006a; CHEN *et al.* 2009b). In addition, the toxic effect of alcohol can be influenced by genes; for example, men carrying APOE ε2ε3 have a greater tendency to suffer from strokes than those with ε3ε3 when they have alcohol exposure (CHEN *et al.* 2009b). In alcoholic pharmacokenetics, women have increased bioavailability and a faster clearance rate (MUMENTHALER *et al.* 1999). Women develop higher blood alcohol levels than men in spite of an equal alcohol intake due to a smaller gastric metabolism in women due to the lesser activity of class III ADH in females (BARAONA *et al.* 2001). Therefore, sex differences in the effects of alcohol metabolism on AD should be tested to illuminate the genetic roles of AD in personalized management (SULTATOS *et al.* 2004).

There are some limitations to our study. First, alcohol intake was much lower in Asian females than in males; therefore, the sample size was small, especially for those with alcohol use. Second, the frequencies of alcohol-metabolizing genes differ among ethnicities (HUANG *et al.* 2020). Besides, the sizes of the examined samples are small and have limited power to detect genetic association of minor/modest effect with AD. The result should be interpreted with caution and further studies with larger sample size were indicated for further confirmation of the results herein.

**Summary**

This study revealed a suggestive association between the genetic variant of *ADH1C* rs2241894 and female AD in Taiwanese population. Carrying the *ADH1C* rs2241894 TT genotype may be a protective factor for elderly female Taiwanese individuals.

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附件二

# 科技部補助專題研究計畫成果報告自評表

請就研究內容與原計畫相符程度、達成預期目標情況、研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）、是否適合在學術期刊發表或申請專利、主要發現（簡要敘述成果是否有嚴重損及公共利益之發現）或其他有關價值等，作一綜合評估。

1. 請就研究內容與原計畫相符程度、達成預期目標情況作一綜合評估

■ 達成目標

□ 未達成目標（請說明，以 100 字為限）

□ 實驗失敗

□ 因故實驗中斷

□ 其他原因

說明：

2. 研究成果在學術期刊發表或申請專利等情形：

論文：■已發表 □未發表之文稿 □撰寫中 □無

專利：□已獲得 □申請中 □無

技轉：□已技轉 □洽談中 □無

其他：（以 100 字為限）

3. 請依學術成就、技術創新、社會影響等方面，評估研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性），如已有嚴重損及公共利益之發現，請簡述可能損及之相關程度（以 500 字為限）

*ADHIC* rs2241894 的遺傳分佈顯示出強烈的種族異質性，其中 T 等位基因為次要等位基因(minor allele)，在我們的研究中佔 28.5%，在東亞地區佔 23.6%，而在全球人群中它卻是主要等位基因(major allele)。這項研究揭示了 *ADHIC* rs2241894 TT 基因型與女性阿茲海默症之間存在可能的保護關聯性。未來需要進行更多的樣本研究來確認此發現。

# 108年度專題研究計畫成果彙整表

| 計畫主持人：陳怡君 | 計畫編號：108-2629-B-182A-005- |
|---|---|
| 計畫名稱：探討性別差異對醇脫氫酶與乙醛去氫酶酵素之基因多型性，基因調控，與粒線體功能於阿茲海默症的角色 | |

| 成果項目 | | | 量化 | 單位 | 質化<br>（說明：各成果項目請附佐證資料或細項說明，如期刊名稱、年份、卷期、起訖頁數、證號...等） |
|---|---|---|---|---|---|
| 國內 | 學術性論文 | 期刊論文 | 0 | 篇 | |
| | | 研討會論文 | 0 | | |
| | | 專書 | 0 | 本 | |
| | | 專書論文 | 0 | 章 | |
| | | 技術報告 | 0 | 篇 | |
| | | 其他 | 0 | 篇 | |
| 國外 | 學術性論文 | 期刊論文 | 5 | 篇 | 1.Wei-Min Ho, Yah-Yuan Wu, Yi-Chun Chen (2020, Dec). Genetic Variants behind Cardiovascular Diseases and Dementia. Genes, Genes 2020, 11(12), 1514. (SCI, 54/178,GENETICS & HEREDITY). 本人為通訊作者.<br>2.Yu-Hua Huang, Kuo-Hsuan Chang, Yun-Shien Lee, Chiung-Mei Chen, Yi-Chun Chen (2020, Feb). Association of alcohol dehydrogenase and aldehyde dehydrogenase Polymorphism with Spontaneous Deep Intracerebral Haemorrhage in the Taiwan population. Scientific Reports, 10(1), 3641-3648. (SCI, 15/69, MULTIDISCIPLINARY SCIENCES - SCIE). 本人為通訊作者.<br>3.Yah-Yuan Wu, Wen-Chuin Hsu, Yu-Hua Huang, Wei-Min Ho, Yi-Chun Chen (2019, Nov). Memory complaint is a surrogate for memory decline in the middle-aged: A register-based study. J.Clin.Med, 8(11), 1900. (SCI, 15/160,MEDICINE, GENERAL & INTERNAL). 本人為通訊作者.<br>4.Yi-Chun Chen, Wen-Hai Chou, Chiu-Ping Fang, Tung-Hsia Liu, Hsiao-Hui Tsou, Yun Wang, Yu-Li Liu (2019, Oct). Serum level and activity of butylcholinesterase: A biomarker for post-stroke dementia. J.Clin.Med, 8(11), 1778. (SCI, 15/160,MEDICINE, GENERAL & INTERNAL). 本人為第一作者. |

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|---|---|---|---|---|---|
| | | | | | 5.Yah-Yuan Wu, Yun-Shien Lee, Yu-Li Liu, Wen-Chuin Hsu, Wei-Min Ho, Yu-Hua Huang, Shih-Jen Tsai, Po-Hsiu Kuo, Yi-Chun Chen (2021, Jan). Association study of alcohol dehydrogenase and aldehyde dehydrogenase polymorphism with Alzheimerdisease in the Taiwanese population.. Frontiers in Neuroscience, Manuscript ID: 625885. (Accepted). (SCI, 97/272,NEUROSCIENCES). 本人為通訊作者. |
| | | 研討會論文 | 0 | | |
| | | 專書 | 0 | 本 | |
| | | 專書論文 | 0 | 章 | |
| | | 技術報告 | 0 | 篇 | |
| | | 其他 | 0 | 篇 | |
| 參與計畫人力 | 本國籍 | 大專生 | 0 | 人次 | |
| | | 碩士生 | 0 | | |
| | | 博士生 | 0 | | |
| | | 博士級研究人員 | 0 | | |
| | | 專任人員 | 0 | | |
| | 非本國籍 | 大專生 | 0 | | |
| | | 碩士生 | 0 | | |
| | | 博士生 | 0 | | |
| | | 博士級研究人員 | 0 | | |
| | | 專任人員 | 0 | | |
| 其他成果<br>（無法以量化表達之成果如辦理學術活動、獲得獎項、重要國際合作、研究成果國際影響力及其他協助產業技術發展之具體效益事項等，請以文字敘述填列。） | | | | | |