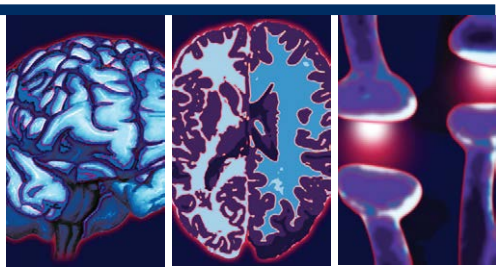

Effect of Aldehyde Dehydrogenase 2 Gene Polymorphism on Executive Function in Opiate User

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Abstract

Objective: The use of opiate substances, such as heroin and methadone, has been proven to cause executive function damage. The extent of damage may be mediated by the activity of enzyme aldehyde dehydrogenase 2 (ALDH2), which plays an important role in neurotransmitter dopamine metabolism. The single nucleotide polymorphism Glu487Lys distributes mostly in Asia and codes for ALDH2 with greatly reduced enzyme activity. The current study aims to explore the effect of ALDH2 on executive function in opiate users.

Methods: A total of 94 opiate users were recruited and 58 patients finished the neuropsychological assessment and blood genotyping.

Results: After co-variating the influence of age and the years of education, we found that participants with ALDH2 A allele performed worse on the Category Complete index of the Modified Wisconsin Card Sorting Test ($F = 5.34$, $p = 0.02$), suggesting an impaired planning ability in this group. We also found that ALDH2 A carriers went through the Trail 2 in Color Trails Test faster ($F = 8.21$, $p = 0.01$), in part suggesting better set-shifting abilities; however, impulsiveness and lack of planning associated with this study group may also explain the faster performances.

Conclusions: The role that ALDH2 plays in the pathology of cognitive impairment in opiate users may lead new focus in studies of the pathophysiology in opiate usage and its consequences on cognitive function.

Keywords

Aldehyde dehydrogenase, Cognitive dysfunction, Dopamine, Executive function, Heroin, Methadone, Opiate

Introduction

Substance use especially the use of heroin is an important health problem. For users, heroin is the most mentally dependent and physically harmful substance among illegal drugs [1]. Heroin or methadone using may damage one's executive functions such as, cognitive flexibility, cognitive inhibition, sustained attention, strategic planning, and decision-

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making [2]. Certain genotypes of key enzymes that catalyze the metabolism of these substances may underlie the extent of damage that these substances may cause in the users. Specifically, aldehyde dehydrogenase 2 (ALDH2) catalyzes the metabolism of opiates, by modulating levels of metabolites, and of the neurotransmitters involved in the process, dopamine is the most critical [3].

The ALDH2 gene leads to the transcription of the enzyme ALDH2, and depending on the single nucleotide polymorphism Glu487Lys, the level of activity in the ALDH2 enzymes may vary [4,5]. According to the homotetrametic enzyme hypothesis, the inactive allele (ALDH2 A) is the main phenotype gene, instead of the active allele (ALDH2 G) [6]. From the perspective of ALDH2 A allele regional distribution in East Asia [7], related studies become more prevalent to medical practices in Asian countries.

In dopamine metabolism, dopamine and related catecholamines were first turned into toxic aldehydes 3,4-dihydroxyphenyl-acetaldehyde (DOPAL) and 3,4-dihydroxyphenylglycolaldehyde (DOPEGAL) in reactions catalyzed by monoamine oxidase A (MAOA). These aldehydes then turned into carboxylic acids 3,4-dihydroxyphenyl-acetic acid (DOPAC) and 3,4-dihydroxymandelic acid (DOMA), respectively via the catalyzing properties of ALDH2 [8]. If the activity of ALDH2 is limited, the toxic aldehydes generated in this metabolism may accumulate, causing neural death [9,10].

One of the dopamine pathways in the brain originates from substantia nigra and ventral tegmental area, and projects to cortical regions like mesocortical system [11]. Dopamine could influence one's prefrontal cortex function through the mesocortical pathway or the nigrostriatal pathway via the striatothalamocortical loops [12]. Prefrontal lobe related cognitive functions were collectively included under the umbrella term *executive functions* [13]. Chung had divided executive functions into five main constructions-planning, verbal fluency, inhibition, and set-shifting [14].

Heroin bind to μ-opioid receptors in substantia nigra and ventral tegmental area, triggering dopamine release [15,16] and the subsequent projection along its pathway [11]. In this way, the use of heroin could influence executive functions [17]. Despite causing less damage, methadone induces dopamine reactions similar to those triggered by heroin [18]. The combination of low ALDH2 catalytic efficiency and elevated dopamine concentration in the ventral tegmental area propagated by opiate intake could lead to the accumulation of neuro-toxic aldehydes, byproducts in dopamine metabolism. These aldehydes accumulate and harm dopaminergic neurons in ventral tegmental area, causing further neural deceases [19]. Considering the dopamine projection along the frontostriatal pathway, an injury in the ventral tegmental area involves dopaminergic dysfunction in the prefrontal cortex [20], ultimately leading to executive dysfunction in the given individual. Previous studies have investigated the role of dopamine system in cognitive functions of opiate users [21]. However, to the best of our knowledge, no studies have investigated the role of ALDH2 in the executive function of opiate users.

Past studies on ALDH2 have focused on its relation to general cognitive function in patients with Alzheimer's disease (AD), Parkinson's disease (PD), and substance use disorders. Some studies proposed that ALDH2 A allele may be a risk factor of AD [22-24], but others disagreed [25-28]. Of the studies listed above, only Kim et al. and Shin et al. evaluated the general cognitive function of AD patients, but both of them failed to find a relation between general cognitive function and ALDH2 [25,26]. Previous studies on ALDH2 and PD have focused on catechol-aldehyde hypothesis, which suggested that ALDH2 inactivity may mediate the extent of damage to the dopamine system due to increased levels of toxic aldehydes [13]. Further studies have supported this hypothesis by associating ALDH2 with the chemicals related to parkinsonism, such as pesticides [29], fungicide [30], and rotenone model [31]. The study conducted by Zhao et al. reported similar results, supporting the neuro-toxic theory [32], but another study failed to find the same relation between ALDH2 and PD development [33]. Furthermore, Yu et al. analyzed the role of specific ALDH2 genotypes on cognitive functions in PD and found that ALDH2 A allele carriers performed worse on cognitive function tests and had higher dementia rate [34], compared to those without the allele.

On a separate note, in a study of alcohol use and ALDH2, individuals who carried ALDH2 A allele, interestingly, tended to consume less alcohol; nonetheless, no significant impairment in general cognitive functions or memory were noted in these participants [35]. In addition, previous study on ALDH2 has investigated its

relation with opiate use. The allele frequency of the ALDH2 A, that studies found, was higher in opiate users, and these ALDH2 A carriers showed more novelty seeking and harm avoidance personality [36].

Although previous studies have explored general cognitive function in patients with AD, PD, and substance users, a study probing the role of ALDH2 genotype in cognitive function in opiate users has yet to be performed. Opiate usage could cause dopaminergic system damage, and the extent of damage may be mediated by the efficiency of ALDH2 to catalyze toxic aldehydes.

The present study hypothesized that the increased level of toxic aldehydes may damage the dopaminergic system, thereby leading to vulnerability in developing executive dysfunctions on top of the cognitive impairment associated with opiate use. Thus, our study aims to clarify the relation between ALDH2 genotypes and executive function in opiate users.

Materials and Methods

■ Participants

The participants were recruited from the methadone clinic. The inclusion criteria were: (1) outpatients attending methadone clinic due to heroin use (2) intact sensation and perception to follow test directions and tested stimuli (3) literacy and ability to communicate in Chinese. The exclusion criteria were: (1) comorbidities, including other psychiatric disorders diagnosed under the Chinese version of Mini-International Neuropsychiatric Interview [37] (2) auditory or visual impairment (3) insufficient proficiency in the Chinese language, either in listening, speaking, or reading.

Ninety-four participants were contacted at the beginning of our study, and 58 of them completed the study. Of the 36 patients who dropped out of the study, some did not complete the screening test and others failed to provide blood sample. Therefore, the study analysis included results from a total of 58 participants.

■ Standard protocol approvals, registrations, and patient consent

All the participants were well informed about the procedures and purpose of the current study before signing a written informed consent. The current study had been approved by the Institutional Review Board. Participants completed the interviews, blood drawings, and neuropsychological tests in succession. All procedures were performed in accordance with the approved guidelines.

■ Neuropsychological assessment

All participants underwent psychometric testing by neuropsychologists experienced in the following tests. The Modified Wisconsin Card Sorting (M-WCST) [38] was given to evaluate planning [39]. Examiners provided cards with figures of varying shapes, colors, and numbers, and the participants were asked to group each card into a category. The examiners showed the cards, one by one, and gave only a confirmative or negative feedback, corresponding to the accuracy of participant's responses. To complete the task successfully, participants needed to initiate and maintain a certain rule within a category then switch to a new rule in between categories, without being directly told the sorting rule in effect. The categories completed and the different errors made were recorded. For the Verbal Fluency Test (VFT) [40], participants were asked to generate as many words as possible, in a given semantic category under a time limit. The Color Trails Test (CTT) [41] mainly measured set-shifting and processing speed, and the results comprised several scores. Trail 1 test purely evaluated processing speed, while the Trail 2 test evaluated set-shifting in addition to the processing speed. To get an estimation of the set-shifting function, the Interference Index was calculated based on the Trail 1 and Trail 2 scores, yielding the formula: (Trail 1 – Trail 2) / Trail 1 [42]. The assessments utilized by the present study are reliable, valid, and sensitive to prefrontal cortex impairment.

■ Blood samples and Genotyping

Twenty milliliters of venous blood was drawn from each participant, and DNA extracted from the sample. The genotyping of ALDH2 gene was conducted via polymerase chain reaction, using protocols proposed by Chen [43] and Dandre [44]. The laboratory technician who performed the genotyping and read the genotypes was blinded from the patients' clinical data.

■ Statistical analysis

Without knowing participants' performances or clinical characteristics, they were divided into ALDH2 GG and AG/AA groups based on the homotetrameric hypothesis by Crabb [6]. Considering the effect of patient age and years of education on test performance, we co-variated age and years of education, compared

executive function test scores from different gene groups via ANCOVA in parametric variables (M-WCST and CTT) and via rank ANCOVA [45] in non-parametric variables (VFT). All levels of significance in tests were set to 0.05.

Results

A total of 94 participants were recruited in our study and 58 participants completed all study protocols and thus remained in the current study. There were no significant differences in the sex, age, and the years of education between participants who completed and those who dropped out of the study **(Table 1)**. Out of the remaining participants, 24 had ALDH2 GG, 27 ALDH2 AG, and 7 ALDH2 AA genotypes. According to Ting et al., the ALDH2 A allele incidence rate in Taiwan was 0.29 [46], but the incidence rate in our study participants was 0.35; statistical analysis revealed that the incidence rate of ALDH2 A alleles found in our study groups was representative of the incidence rate in the population, according to Hardy-Weinberg equilibrium. Nonetheless, it might be a point of future studies to verify that the observed increase in incidence rate of ALDH2 A alleles in opiate users were merely by chance.

We divided participants into a group with ALDH2 GG and a group with ALDH2 AG/AA, based on the homotetrameric hypothesis by Crabb [6]. In the demographic variables, there were no significant differences in the sex, years of education, methadone dosage, and years of heroin use, between the study groups. However, the mean age of ALDH2 GG group was lower than that of AG/AA group (t = 2.16, p = 0.03) **(Table 1)**.

Considering that executive functions may be influenced by patient age and years of education, we co-variated these two variables before comparing the performances in executive function between the ALDH2 AG/AA and GG groups. The results showed that, gene group had a significant effect on performances in Category Complete of M-WCST (F = 5.34, p = 0.02) and Trail 2 in CTT (F = 8.21, p = 0.01) **(Table 2)**.

Discussion

The primary purpose of current study was to explore various subdomains under executive functions in heroin and related substance users with different ALDH2 genotypes, by comparing their performance in planning, verbal fluency, and set-shifting tests. In the current study, after co-variating the influence of patient age and years of education, we found that participants with ALDH2 GG performed better in the Category Complete index of the M-WCST than those with the ALDH2 A allele, suggesting a deficit in abstract concept formation in these

Table 1: Demographic and clinical characteristics of the study groups and dropout group.

	ALDH2 GG		ALDH2 AG/AA		Statistic	*p* value	Dropout		Statistic (+)	*p* value
	Mean	SD	Mean	SD			Mean	SD		
N	24	-	34	-	-		36	-	-	-
Gender (male%)[a]	71%	-	88%	-	χ^2 = 2.77	0.10	89%	-	χ^2=1.02	0.31
Age (years)[b]	41.43	6.19	45.26	6.79	t = 2.16	0.03*	45.72	7.08	t =1.36	0.18
Education (years)[c]	9.83	1.90	10.12	2.10	U=349.50	0.60	8.94	2.78	U=785.50	0.07
Methadone (mg/day)[b]	40.63	20.56	44.65	25.90	t = 0.53	0.60	-	-	-	-
Past heroin use (years)[c]	11.31	7.60	14.26	7.71	U=314.50	0.14	-	-	-	-

Note: Abbreviations: SD, standard deviation. [a]Chi-square test. [b]t test. [c]Mann-Whitney U test. [†]Compare with the whole study group.

Table 2: Executive function in the study groups.

	ALDH2 GG		ALDH2 AG/AA		Statistic (+)	*p* value
	Mean	SD	Mean	SD		
Modified Wisconsin Card Sorting Test						
Category Complete[a]	5.90	1.09	5.15	1.42	F = 5.34	0.02*
Perseverative Error[a†]	2.71	2.80	3.59	3.22	F = 2.29	0.14
Nonperseverative Error[a†]	4.67	3.23	6.12	3.57	F = 3.99	0.05
Verbal Fluencty Test[b]	33.21	8.63	34.45	8.22	F = 0.16	0.82
Color Trails Test						
Trail 1[a†]	41.70	16.02	40.38	14.12	F = 2.20	0.14
Trail 2[a†]	94.70	23.80	85.53	38.50	F = 8.21	0.01*
Interference Score[a†]	1.48	0.85	1.14	0.57	F = 1.84	0.18

ALDH2 A carriers. To go through the Category Complete assessment successfully, an individual needed to initiate, maintain, and terminate categorization based on an abstract concept [47]. Although statistical significance was not reached, the trend of lower performances by ALDH2 A carrier was present in other indices, such as more preservative and non-preservative errors, in the M-WCST. Therefore, the present study suggests relative vulnerability in ALDH2A allele carriers to progress to impairment in learning, rule following, and decision making, with opiate use.

In analyzing set-shifting among the study groups, we found that the ALDH2 A allele carrier performed significantly better in the Trail 2 but insignificantly in the Trail 1 and the Interference Index in the CTT. Considering that CTT evaluated different functions simultaneously, the success ALDH2 A allele carriers had may reflect the insensitivity of the assessment instead of a true measure of set-shifting functions.

Although one way to explain this success might be faster processing speed and set-shifting, we propose otherwise. First, results from assessment of ALDH2 A patients' processing speed (Trail 1) and set-shifting (Interference Index) did not reach a significant difference between the study groups. Secondly, previous literatures have not shown that damaged dopaminergic system could lead to improved- or even intact- set-shifting skills. More studies would therefore be needed to conclude a protective role of ALDH2 A allele in the abilities of set-shifting. Another proposal that might explain ALDH2 A patients' significantly higher score on Trial 2 raises the point of impulsivity associated with this patient group. CTT is a timed assessment; the time required before tasks execution were recorded. Therefore, participants who went through the tests faster would have been scored higher, given the same percentage of response accuracy. If the tasks that the participants were asked to do were easy enough to answer right away, immediate responses naturally led to better performances. In our opinion, the better performance on CTT in ALDH2 A carriers in the current study may be attributed to impulsiveness and lack of planning. However, its detailed mechanism remains unclear. More researches with more sensitive set-shifting tests are needed to confirm this hypothesis.

Previous studies of ALDH2 and cognitive function focused on the genotype's relation with highly prevalent neurodegenerative diseases, like AD and PD. Although etiologies of AD and PD are different from that of opiate use in our study, these diseases share common clinical characteristics of cognitive function decline and toxic aldehydes- induced damage to the dopaminergic system that underlies the pathology. Many studies have reported relations between ALDH2 and the incidence of AD [48,49] and PD [32,33], but the results remained controversial. Out of the studies that conducted cognitive function tests, only Kim et al. reported that cognitive function were not related to ALDH2 A allele in AD [25]. Yu et al., on the other hand, reported that ALDH2 A carrier performed worse in cognitive functions compared to patients without the ALDH2 A allele [34].

We also arranged some cognitive function tests in the current study, specifically to evaluate executive functions. Intrigued by the pathophysiology of toxic aldehydes in dopamine metabolism, we picked opiate users as our study groups because an altered dopamine system activity underlies the pathological lesion.

In the course of exploring the effects of ALDH2, there were several breakthroughs in the current study. First, we extended the patient base to opiate users, whose pathology are different from AD and PD, yet are nonetheless characterized by dopaminergic system involvement. Second, we selected executive function tests specific to prefrontal cortex, which are more sensitive to cognitive decline, so that the ALDH2 effect can be seen more distinctly. Lastly, we expanded the patient age range of ALDH2 related studies to those in a younger generation (43 in average).

Nevertheless, there were some limitations to our study. First, we did not recruit normal controls in our studies, which restricted the generalizability of results. Second, the sample size the study ended up with was relatively small, which may be due to the substance users' trait- dropped out easily; future studies that target opiate users as the study groups might devise a study-adherence plan to minimize the percentage of study drop-outs. Last, even though we had arranged several tests covering subdomains under the executive function, such as planning, verbal fluency, set-shifting, and processing speed, in the scope of construction of executive function, there remains uncovered territories that have yet to be evaluated by the present study, such as working memory. Further research will be needed to explore this issue.

To the best of our knowledge, there has not been a study conducted on the effect of ALDH2 genotype on executive function in opiate users. Hence, the subject became the purpose of the current study. In brief, our study was based on (1) the influence of ALDH2 genotype on aldehydes metabolism (2) the process of aldehyde formation in dopamine metabolism (3) dopamine projection pathway in the prefrontal cortex, related to executive function (4) effects of opiate use on the dopaminergic system. We analyzed the relation between ALDH2 genotypes and executive functions in opiate users and found that participants with ALDH2 A exhibited impaired planning abilities when compared to those with ALDH2 GG genotype, and this finding suggests that carriers of ALDH2 A allele may experience relative vulnerability in their planning skills with opiate use, providing more evidence that opiate usage may harm cognitive functions. These results still need more meticulous and longitudinal studies to verify and confirm.

Author Contributions

Po-Chih Liu and Rwei-Ling Yu conceptualized the study and contributed to the execution, critique, and revision of the data analysis and manuscript. Po-See Chen, Ru-Band Lu, Chun-Hsiang Tan, Shao-Ching Tu contributed to the data acquisition and critique of the manuscript.

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References

1. Nutt D, King LA, Saulsbury W, *et al.* Development of a rational scale to assess the harm of drugs of potential misuse. *Lancet* 369(9566), 1047-1053 (2007).
2. Ersche KD, Sahakian BJ. The neuropsychology of amphetamine and opiate dependence: implications for treatment. *Neuropsychol. Rev* 17(3), 317-336 (2007).
3. Wang TY, Lee SY, Chen SL, *et al.* The aldehyde dehydrogenase 2 gene is associated with heroin dependence. *Drug. Alcohol. Depend* 120(1-3), 220-224 (2012).
4. Li D, Zhao H, Gelernter J. Strong protective effect of the aldehyde dehydrogenase gene (ALDH2) 504lys (*2) allele against alcoholism and alcohol-induced medical diseases in Asians. *Hum. Genet* 131(5), 725-737 (2012).
5. Yoshida A, Hsu LC, Yasunami M. Genetics of human alcohol-metabolizing enzymes. *Prog. Nucleic. Acid. Res Mol. Biol* 40, 255-287 (1991).
6. Crabb DW, Edenberg HJ, Bosron WF, *et al.* Genotypes for aldehyde dehydrogenase deficiency and alcohol sensitivity: The inactive ALDH2 (2) allele is dominant. *J. Clin. Invest* 83(1), 314 (1989).
7. Li H, Borinskaya S, Yoshimura K, *et al.* Refined geographic distribution of the oriental ALDH2*504Lys (nee 487Lys) variant. *Ann. Hum. Genet* 73(Pt 3), 335-345 (2009).
8. Grünblatt E, Riederer P. Aldehyde dehydrogenase (ALDH) in Alzheimer's and Parkinson's disease. *J. Neural. Transm* 123 (2), 83-90 (2016).
9. Burke WJ, Li SW, Chung HD, *et al.* Neurotoxicity of MAO metabolites of catecholamine neurotransmitters: role in neurodegenerative diseases. *Neurotoxicology* 25(1-2), 101-115 (2004).
10. Marchitti SA, Brocker C, Stagos D, *et al.* Non-P450 aldehyde oxidizing enzymes: the aldehyde dehydrogenase superfamily. *Expert .Opin. Drug. Metab. Toxicol* 4(6), 697-720 (2008).
11. Halliday GM, Leverenz JB, Schneider JS, *et al.* The neurobiological basis of cognitive impairment in Parkinson's disease. *Mov. Disord* 29(5), 634-650 (2014).
12. Klostermann EC, Braskie MN, Landau SM, *et al.* Dopamine and frontostriatal networks in cognitive aging. *Neurobiol. Aging* 33(3), 623. e615-624 (2012).
13. Goldstein DS, Sullivan P, Holmes C, *et al.* Determinants of buildup of the toxic dopamine metabolite DOPAL in Parkinson's disease. J Neurochem 126(5), 591-603 (2013).
14. Chung HJ, Weyandt LL, Swentosky A. The Physiology of Executive Functioning. In: Goldstein S, Naglieri JA, editors, Handbook of Executive Functioning: Springer Science & Business Media (2014).
15. Di Chiara G, Imperato A. Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. *Proc .Natl. Acad .Sci* 85(14), 5274-5278 (1988).
16. Spanagel R, Herz A, Shippenberg TS. The effects of opioid peptides on dopamine release in the nucleus accumbens: an in vivo microdialysis study. *J. Neurochem* 55(5), 1734-1740 (1990).
17. Severtson SG, Hedden SL, Martins SS, *et al.* Patterns of cognitive impairments among heroin and cocaine users: The association with self-reported learning disabilities and infectious disease. *J. Learn. disabil* 45(2), 139-150 (2012).
18. Kreek MJ, Levran O, Reed B, *et al.* Opiate addiction and cocaine addiction: underlying molecular neurobiology and genetics. *J. Clin .Invest* 122(10), 3387-3393 (2012).
19. Horvath MC, Kovacs GG, Kovari V, *et al.* Heroin abuse is characterized by discrete mesolimbic dopamine and opioid abnormalities and exaggerated nuclear receptor-related 1 transcriptional decline with age. *J. Neurosci.* 27(49), 13371-13375 (2007).
20. Rogers TD, Dickson PE, Heck DH, *et al.* Connecting the dots of the cerebro-cerebellar role in cognitive function: neuronal pathways for cerebellar modulation of dopamine release in the prefrontal cortex. *Synapse* 65(11), 1204-1212 (2011).
21. Liang CS, Ho PS, Yen CH, *et al.* Reduced striatal dopamine transporter density associated with working memory deficits in opioid-dependent male subjects: a SPECT study. *Addict. Biol* 21(1), 196-204 (2016).
22. Kamino K, Nagasaka K, Imagawa M, *et al.* Deficiency in mitochondrial aldehyde dehydrogenase increases the risk for late-onset Alzheimer's disease in the Japanese popu-

lation. *Biochem. Biophys. Res. Commun* 273(1), 192-196 (2000).

23. Ohta S, Ohsawa I, Kamino K, *et al.* Mitochondrial ALDH2 deficiency as an oxidative stress. Mitochondrial Pathogenesis: From Genes and Apoptosis to Aging and Disease 1011, 36-44 (2004).
24. Wang B, Wang J, Zhou S, *et al.* The association of mitochondrial aldehyde dehydrogenase gene (ALDH2) polymorphism with susceptibility to late-onset Alzheimer's disease in Chinese. *J. Neurol. Sci* 268(1-2), 172-175 (2008).
25. Kim JM, Stewart R, Shin IS, *et al.* Assessment of association between mitochondrial aldehyde dehydrogenase polymorphism and Alzheimer's disease in an older Korean population. *Neurobiol .Aging* 25(3), 295-301 (2004).
26. Shin IS, Stewart R, Kim JM, *et al.* Mitochondrial aldehyde dehydrogenase polymorphism is not associated with incidence of Alzheimer's disease. *Int .J .Geriatric .Psychiatry* 20(11), 1075-1080 (2005).
27. Zhou S, Huriletemuer, Wang J, *et al.* Absence of association on aldehyde dehydrogenase 2 (ALDH2) polymorphism with Mongolian Alzheimer patients. *Neurosci. Lett* 468(3), 312-315 (2010).
28. Komatsu M, Shibata N, Ohnuma T, *et al.* Polymorphisms in the aldehyde dehydrogenase 2 and dopamine β hydroxylase genes are not associated with Alzheimer's disease. *J. Neural. Transm* 121(4), 427-432 (2014).
29. Fitzmaurice AG, Rhodes SL, Cockburn M, *et al.* Aldehyde dehydrogenase variation enhances effect of pesticides associated with Parkinson disease. *Neurol* 82(5), 419-426(2014).
30. Casida JE, Ford B, Jinsmaa Y, *et al.* Benomyl, Aldehyde Dehydrogenase, DOPAL, and the Catecholaldehyde Hypothesis for the Pathogenesis of Parkinson's Disease. *Chem. Res. Toxicol* 27(8), 1359-1361 (2014).
31. Chiu CC, Yeh TH, Lai SC, *et al.* Neuroprotective effects of aldehyde dehydrogenase 2 activation in rotenone-induced cellular and animal models of parkinsonism. *Exp. Neurol* 263, 244-253 (2015).
32. Zhao CC, Cai HB, Wang H, *et al.* Role of ADH2 and ALDH2 gene polymorphisms in the development of Parkinson's disease in a Chinese population. *Genet. Mol. Res* 15(3) (2016).
33. Zhang X, Ye YL, Wang YN, *et al.* Aldehyde dehydrogenase 2 genetic variations may increase susceptibility to Parkinson's disease in Han Chinese population. *Neurobiol. Aging* 36(9), 2660.e2669-2613 (2015).
34. Yu RL, Tan CH, Lu YC, *et al.* Aldehyde dehydrogenase 2 is associated with cognitive functions in patients with Parkinson's disease. *Sci. Rep* 6, 30424 (2016).
35. Au Yeung S, Jiang C, Cheng K, *et al.* Evaluation of moderate alcohol use and cognitive function among men using a Mendelian randomization design in the Guangzhou biobank cohort study. *Am. J. Epidemiol* 175(10), 1021-1028 (2012).
36. Wang TY, Lee SY, Chen SL, *et al.* Association between DRD2, 5-HTTLPR, and ALDH2 genes and specific personality traits in alcohol- and opiate-dependent patients. *Behav. Brain. Res* 250, 285-292 (2013).
37. Sheehan DV, Lecrubier Y, Sheehan KH, *et al.* The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *J. Clin .Psychiatry* 59(20), 22-33 (1998).
38. Nelson HE. A modified card sorting test sensitive to frontal lobe defects. *Cortex* 12(4), 313-324 (1976).
39. Demakis GJ. A meta-analytic review of the sensitivity of the Wisconsin Card Sorting Test to frontal and lateralized frontal brain damage. *Neuropsychology* 17(2), 255-264 (2003).
40. Hua M-S, Chang S-H, Chen S-T. Factor structure and age effects with an aphasia test battery in normal Taiwanese adults. *Neuropsychology* 11(1), 156 (1997).
41. D'Elia L, Satz P, Uchiyama C, *et al.* Color Trails Test; Odessa. FL Professional manual, psychological assessment resources (1996).
42. Golden CJ. A Manual for the Clinical and Experimental Use of the Stroop Color and Word Test (1978).
43. Chen CC, Lu RB, Chen YC, *et al.* Interaction between the functional polymorphisms of the alcohol-metabolism genes in protection against alcoholism. *Am. J. Hum. Genet* 65(3), 795-807 (1999).
44. Dandre F, Cassaigne A, Iron A. The frequency of the mitochondrial aldehyde dehydrogenase I2 (atypical) allele in Caucasian, Oriental and African black populations determined by the restriction profile of PCR-amplified DNA. *Mol. Cell. Probes* 9(3), 189-193 (1995).
45. Quade D. Rank Analysis of Covariance. *J. Am. Statistical .Association* 62(320), 1187-1200 (1967).
46. Ting TT, Huang SY, Chen KH, *et al.* Effects of genetic variants of ADH1B and ALDH2 and social network on continued alcohol drinking among young adolescents in Taiwan. *Drug. Alcohol.Depend* 147, 38-45 (2015).
47. Welsh MC, Pennington BF. Assessing frontal-lobe functioning in children views from developmental-psychology. *Developmental. Neuropsychology* 4(3), 199-230 (1988).
48. Hao PP, Chen YG, Wang JL, *et al.* Meta-analysis of aldehyde dehydrogenase 2 gene polymorphism and Alzheimer's disease in East Asians. *Can. J. Neurol .Sci* 38(3), 500-506 (2011).
49. Zhao Y, Wang C. Glu504Lys single nucleotide polymorphism of aldehyde dehydrogenase 2 gene and the risk of human diseases. *Biomed. Res. Int* 174050 (2015).