

Evidence-Based Genetics and Identification of Key Human Alzheimer's Disease Alleles with Co-morbidities

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Abstract

Advancements in biomedical research have contributed to increasing the life expectancy of humans, but we now observe an increase in age-related diseases such as Alzheimer's disease. Genome-Wide Association Studies (GWAS) and linkage studies have identified human genes associated with Alzheimer's disease (referred to as *AD* genes). A previous study by Vahdati in 2017 has revealed the human *AD* genes and counterparts in model species [1]. Thus, we further investigate the co-morbidity genes and alleles. Using ontology analysis combined with cluster analysis, the study identified functional pathways enriched among the human *AD* genes, including 179 genes out of 695 human *AD* genes (26%) that were associated with one or more of the four neurological diseases including Amyotrophic lateral sclerosis, Multiple sclerosis, Parkinson's disease, and Schizophrenia [1]. More importantly, the results indicate co-morbidities with Late-Onset Alzheimer's Disease (LOAD) and other neurological conditions, implying the complexity of the phenotypes in the human AD. The co-morbidity genes may account for mixed symptoms for human AD as well as age-related risks of infections. Of them, the three genes are well conserved (Angiotensin I Converting Enzyme gene, *ACE*; Methylenetetrahydrofolate Reductase gene, *MTHFR*; and tumor necrosis factor gene, *TNF*). In this study, we confirmed the comorbidity of the three genes associated with AD. We further identified the comorbidity of two alleles in the *MTHFR* gene, *C677T* and *A222V*, significantly associated with Alzheimer's disease. This study provides an example of evidence-based analysis that is cost-effective and may be an effective approach to develop cure-alls for multiple diseases.

Keywords

Alzheimer's disease, Beta-amyloid plaques

Abbreviations

AD: Alzheimer's Disease; **ACE:** Angiotensin I Converting Enzyme Gene; **ALS:** Amyotrophic Lateral Sclerosis; **EOAD:** Early-Onset Alzheimer's Disease; **GWAS:** Genome-Wide Association Studies; **LOAD:** Late-Onset Alzheimer's Disease; **MS:** Multiple Sclerosis; **MTHFR:** Methylenetetrahydrofolate Reductase Gene; **PD:** Parkinson's Disease

Introduction

Alzheimer's Disease (AD) has been recognized as a multifactorial disease that affects 5.8 million people in America [2]. Despite the controversy, the

earliest stage of AD includes an excess production of beta-amyloid plaques [3]. Genetics is an essential component of the onset of AD [4]. AD can be classified as either Early-Onset Alzheimer's Disease (EOAD) or Late-Onset Alzheimer's Disease (LOAD). EOAD symptoms, by definition, are manifested before 65 and usually involve the accumulation of beta-amyloid plaques. This form is rare and accounts for less than 1% of cases, but is usually more aggressive and has a shorter survival time. In contrast, most of the AD cases fall into the category of LOAD, in which symptoms are manifested at the age 65 and older [5]. LOAD shows complex genetic patterns, do not follow Mendelian inheritance patterns and do not show full penetrance, which likely interacts with environmental, lifestyle, and epigenetic factors. The genetic factors contribute to the predisposition of the disease with multiple genes playing a role. Although the most well-characterized LOAD gene is the apolipoprotein E (APOE) gene, the genome-wide association studies and other linkage analysis identified more than 100 genes [1, 6], which remain to be investigated.

Using meta-analysis, the data from Genome-Wide Association Studies (GWAS) were compiled to extract and identify single nucleotide polymorphisms that are associated with Alzheimer's disease [7-9]. Both the Alzgene database [10] and the National Institute of Aging Genetics of Alzheimer's Disease Data storage site (niagads.org) were referenced. This study further performed a meta-analysis of the three genes that show co-morbidities with other neurological diseases. We narrowed down our search by accounting for mechanisms and inhibitor drugs. The drugs may have a significant impact on the symptoms of AD, which may provide an effective intervention for treating the symptoms of AD. We found significant correlations to justify further study, including the model systems such as the semi-automated system developed using the nematode *C. elegans* [11].

Methods

Meta-analysis was performed as described previously [1]. To compare the previous data, publications from January 1, 2013 to April 15, 2017 were investigated from the PubMed database and Scopus to evaluate the genes that are associated with Alzheimer's disease. Searches were done for English peer-reviewed articles, with their dates and p-values organized into an Excel spreadsheet. The p-values were evaluated to determine the validity of the studies. The study was determined valid if the p-value corresponds to a 95% confidence interval.

Articles pertaining to the 3 AD genes of interest (*ACE*, *MTHFR*, and *TNF*) were searched using Scopus and PubMed. General keywords were used first and then more keywords were added to help narrow down results. Scopus was accessed by going to the website (scopus.com). PubMed was accessed by going to the website (ncbi.nlm.nih.gov). When searching for specific genes, we used the keyword, either *TNF*, *MTHFR*, or *ACE*. For articles pertaining to the *ACE* gene, angiotensin-converting enzyme had to be entered fully in the box. Entering ACE in the search could yield false positives in

PubMed, where the database includes articles that involve the Spanish Fundación ACE foundation which were excluded from the analysis.

Keywords including Alzheimer's, GWAS, Aging and Dementia, were systematically used to search the literature relevant to each gene (*ACE*, *MTHFR* and *TNF*). We tested the gene name plus all the combinations of the keywords. The keywords used were indicated in results section. Typically, three keywords (Alzheimer's, GWAS and each gene) are sufficient to narrow down the literature that was below a total of 10 or less for each gene. PubMed and Scopus yielded the highest number of articles that demonstrated significant associations with *ACE* and *AD*. Further searches were done primarily to focus on the mechanisms of *ACE* inhibiting drugs. The drug names were identified by using the keyword, *ACE* inhibitor using PubMed, Scopus and UpToDate (UpToDate.com).

Results

We conducted a systematic review using PubMed and Scopus between 2013 and 2017 (methods). The result was summarized in table 1 and figure 1. The two searches provided a total of 751 publications (Table 1). The analysis was done through a current literature search on GWAS involving *ACE*, *TNF*, and *MTHFR*. This included the review of 751 publications total. Of the 751 publications, 569 articles were from PubMed and 182 articles were from Scopus.

Table 1: A summary of the search results from PubMed and Scopus.

Initial search: 751 publications total			
PubMed: 569 publications (keywords; No of literatures)		Scopus: 182 publications (keywords; No of literatures)	
<i>ACE</i> + <i>GWAS</i>	102	<i>ACE</i> + <i>GWAS</i>	41
<i>TNF</i> + <i>GWAS</i>	337	<i>TNF</i> + <i>GWAS</i>	98
<i>MTHFR</i> + <i>GWAS</i>	130	<i>MTHFR</i> + <i>GWAS</i>	43

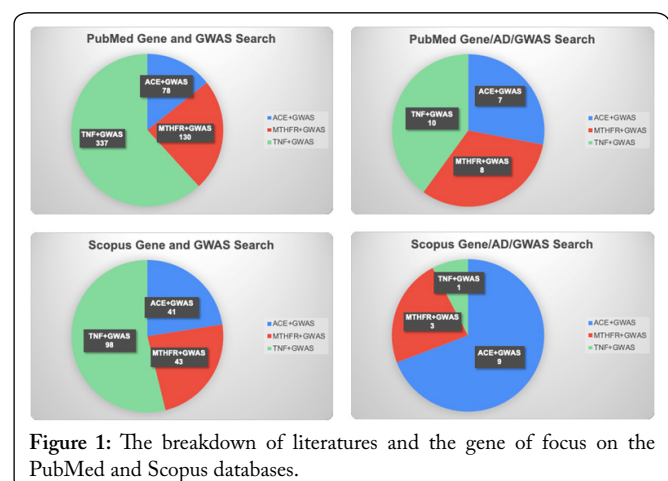


Figure 1: The breakdown of literatures and the gene of focus on the PubMed and Scopus databases.

Importantly, *ACE*, *TNF*, and *MTHFR* were found to be risk factor genes in other neurological disorders, including ALS (Amyotrophic Lateral Sclerosis), MS (Multiple Sclerosis), PD (Parkinson's Disease), and Schizophrenia [1].

Table 2 confirms that our previous study is the first report of the three *AD* genes associated with Alzheimer's disease (Table 2). We further searched for the alleles of the *ACE*, *TNF*, and *MTHFR* genes that are associated with Alzheimer's disease.

Table 2: Publications involving GWAS studies with a focus on *ACE* genes and AD.

PMID	Date of Publication	Gene	P-value
29777097	5/12/2018	<i>ACE</i>	5×10^{-8}
28650998	6/26/2017	<i>ACE</i>	0.358*
28553317	5/12/2017	<i>ACE, TNF, MTHFR</i>	7.73×10^{-13}
26159191	9/1/2015	<i>ACE</i>	N/A
23857120	6/19/2014	<i>ACE ATP5H/KCTD2</i>	5×10^{-6}
22917148	8/23/2012	<i>ACE, APOE, PICALM</i>	N/A
20413850	2010	<i>ACE</i>	N/A
19501718	2009	<i>ACE, CHRN2, GAB2</i>	N/A

(* - not significant)

MTHFR

The search on PubMed for GWAS studies on *MTHFR* provided a total of 130 publications. Narrowing down the search by adding Alzheimer's to the key terms filtered this total down to a total of 8 publications. Of these 8 publications, five indicated a significant correlation between the *MTHFR* gene and AD (Table 3 and Figure 1). Further analysis of *MTHFR* identified two alleles, C677T and A222V with statistical significance (Table 3). Search for *MTHFR* on Scopus led to a total of 43 publications. It is not known about the functions of the *MTHFR* alleles. Searching for studies on PD and *MTHFR* genes returned a total of 50 publications on PubMed (summarized in Table 4). Limiting the same search parameters to only GWAS studies led to a total of 5 publications. Of those 5 publications, one study demonstrated a significant association with susceptibility to PD for individuals in Asian and European populations with the functional SNP, rs1801133. A search conducted on studies focusing on ALS and *MTHFR* yielded 9 studies. Of those 9 studies, 2 were GWAS studies (Table 5).

Table 3: Results pertaining to GWAS studies involving the *MTHFR* gene.

PMID	Date of Publication	Gene	P-value
28553317	5/12/2017	<i>MTHFR</i>	N/A
27713094	9/18/2016	<i>MTHFR C677T</i>	N/A
23916622	8/3/2013	<i>MTHFR C677T</i>	0.004
23754956	6/9/2013	<i>MTHFR A222V</i>	9.5×10^{-53}
20413850	4/20/2010	<i>MTHFR</i>	N/A
18258338	10/30/2009	<i>MTHFR C677T</i>	0.031
18830724	10/2/2008	<i>MTHFR</i>	0.03
27031476	3/22/2006	<i>MTHFR C677T</i>	2.9×10^{-5}

All publications on GWAS studies focusing on *MTHFR* in context of AD are included. Three of these publications did not yield any p-values.

ACE

The search including both *ACE* and GWAS yielded 102 publications. Adding the keyword of Alzheimer's identified a total result of 7 publications focusing on *ACE* on PubMed (Figure 1). Only 4 of these articles demonstrated a significant relationship between *ACE* genes and the cognitive symptoms of AD (Supplementary Table 1). The articles provide an association between AD and the genes, which do not provide information about specific alleles. Thus, no specific allele was found. Similarly, of the 182 publications identified by Scopus, the additional keyword, *ACE*, narrowed down the results to 8 publications (out of 182). Three of these publications showed significant p-values, resulting in no alleles in the gene.

Another search conducted for Parkinson's and *ACE* was performed on PubMed, leading to a total of 75 publications (Table 4). Adding GWAS as a parameter yielded only 1 study and no clear alleles. Searches for studies on *ACE* in the context of ALS and dementia resulted in a total of 13 studies, with only 1 of those being a GWAS study (Table 5).

Table 4: Results pertaining to GWAS studies involving PD and the *ACE*, *MTHFR* or *TNF* genes.

PMID	Date of Publication	Gene	P-value
25143327	8/20/2014	<i>ACE</i>	N/A
28927418	9/19/2017	<i>MTHFR</i>	N/A
24694231	2/1/2014	<i>MTHFR</i>	N/A
27713094	7/1/2017	<i>MTHFR</i>	N/A
23916622	11/15/2013	<i>MTHFR</i>	0.0149, 0.0178
28553317	5/12/2017	<i>MTHFR, TNF</i>	N/A
23380027	9/1/2013	<i>TNF</i>	N/A
27487029	8/3/2016	<i>TNF</i>	0.006

Table 5: Results pertaining to GWAS studies involving ALS and the *ACE*, *MTHFR*, or *TNF* genes.

PMID	Date of Publication	Gene	P-value
28553317	5/12/2017	<i>ACE, MTHFR, TNF</i>	N/A
27713094	7/1/2017	<i>MTHFR</i>	N/A
27487029	8/3/2016	<i>TNF</i>	N/A

TNF

PubMed yielded a total of 337 publications involving GWAS studies and *TNF* genes. A search for *TNF* genes in the background of AD and GWAS resulted in a total of 6 publications. Only 3 of the 6 publications indicated a significant relationship between the influence of *TNF* and the symptoms of AD (Supplementary Table 2). No alleles were found. Similarly, a keyword, *TNF*, on Scopus yielded a total of 98 publications, which do not provide information about specific alleles. A search on PubMed for studies involving PD and *TNF* genes yielded 209 publications. Limiting the search to GWAS studies involving PD and *TNF* genes led to a result

of 4 publications, one study demonstrated a relationship between *PD* and *TNF* genes with a p-value of 0.006, but none of the studies was able to elucidate any specific alleles. Searching for studies involving *TNF* and ALS resulted in 173 studies, with only 2 of them being GWAS studies (Table 5).

Drug

A drug search was conducted to determine the most affordable drugs in the categories of *ACE* inhibitors and *TNF*-alpha blockers. We did not search for *MTHFR* as drugs are not well known. The search indicated lisinopril to be the most cost-effective *ACE* inhibitor (Table 6). A search on PubMed identified 5 publications focused on lisinopril interactions with AD. Scopus presented a larger volume of publications directed towards both *ACE* inhibitors and AD. The search on Scopus showed 25 studies about lisinopril, but only 3 of them showed significant correlations between Lisinopril use and improved cognitive function in AD patients. Lastly, another drug search identified renflexis, inflectra, and cimzia as three of the most cost-effective *TNF*-alpha blockers (Table 6).

Table 6: A list of drugs identified.

Drug Name	Standard Dose	Cost
Prinivil (lisinopril/ ACE inhibitor)	5-10 mg once daily 20-40 mg for maintenance	\$7 for 30 tablets of 20 mg
Zestril (lisinopril/ ACE inhibitor)	5-10 mg once daily 20-40 mg for maintenance	\$7 for 30 tablets of 20 mg
Renflexis (<i>TNF</i> blocker)	3-5 mg per kg (of patient weight) every 8 weeks	\$2,245 for 4 vials of 100 mg
Inflectra (<i>TNF</i> blocker)	3-5 mg per kg (of patient weight) every 8 weeks	\$2,817 for 4 vials of 100 mg
Cimzia (<i>TNF</i> blocker)	200 mg every 2 weeks	\$4,289 for 200 mg

*- 5 drugs were identified as the most affordable option for *ACE* inhibitors and *TNF*-alpha blockers. The standard dose, retrieved from Medscape, is included for reference.

Discussion

Recent biomedical research has identified a wide variety of the genetic and non-genetic interventions that can increase the life expectancy of humans. With increasing life spans, age-related diseases, including AD have been a major concern. Over the past decade, only several genes are known to be associated with AD. Three of those AD genes (*ACE*, *MTHFR*, and *TNF*) are associated with multiple neurological disorders [1], the result of which also suggest three major ontology pathways but not limited to, lipoprotein metabolism, hemostasis and extracellular organizations. The common GWAS hits for AD are also hits in other diseases is evidence of complex gene interactions. It insinuates that the three target genes may be cure-alls for multiple diseases. In this study, we further extended the finding of co-morbidities of the genes to commonly seen health problems in the middle age. *ACE* is known to be involved in hypertension and cardiovascular disease, which are the leading causes of human mortality [12] and the involvement of *ACE* in AD has been confirmed in other studies [13]. *ACE* is a part of the Renin system that is involved

in controlling blood pressure and fluid and electrolyte balance in the body [14] (Supplementary Figure 1). *ACE* inhibitors are commonly used drugs for the treatment of hypertension, which may also be effective for treating AD and other neurological disorders. *MTHFR* codes for the enzyme that is the rate-limiting step in the methyl cycle that couples with methionine and homocysteine metabolism. *TNF* codes for a cytokine that has a role in the activation of acute phase inflammation. Thus, *TNF* is involved in the response to infection, while *ACE* is involved in the renin system, in which *ACE2* is a binding site of virus infection, such as a new coronavirus COVID-19 [15, 16]. The genes are associated with infection, which is a major cause of human mortality (e.g., diabetes, hypertension and cardiovascular disease) (Coronavirus Disease 2019, cdc.org). Lastly, our result showed that the finding is the first evidence for all three genes.

The purpose of this study was to analyze the number of current studies that have focused on the three AD genes: *ACE*, *MTHFR*, and *TNF*. The two databases we used were PubMed and Scopus. Our search on PubMed showed a relatively even distribution in the number of articles for the three AD genes after more parameters were added to filter the results. Scopus, on the other hand, showed a more prominent number of publications on GWAS studies targeting *ACE* and *AD*. Despite this, the overall number of publications in our literature search shows that the three gene alterations have not been studied rigorously concerning AD. This creates room for further studies on *ACE*, *MTHFR*, and *TNF*.

The publications for *ACE*, *MTHFR*, and *TNF* showed overall similar numbers of significant findings based on p-values. However, *ACE* had a larger number of studies, many of which focused on the specific mechanisms of *ACE* inhibitors. Compared to *ACE*, the other genes do not have widely available or cost-effective drugs for further research. *TNF* blockers, such as etanercept and infliximab, can cost several thousand dollars [14]. Lisinopril is the most cost-effective drug that has been studied in the context of the three genes of interest. Although this may be a valuable aspect to take into consideration, further studies including functional analysis of the gene effect on the toxicity of beta-amyloid and other AD proteins remains to be performed. The recent discovery of muscarinic receptor genes as crucial for REM sleep [17] and that on the importance of night sleep for removing brain debris [18]. This and earlier studies may miss the genes involved in the mechanism of sleep and others, while non-coding RNAs [19] are also identified, including miR142 [1, 8, 13]. The study of the areas will require a further analysis designed to study the mechanisms in the future. Computational and artificial intelligence studies will open a window to the analysis of the overwhelming amount of data that will be beneficial for this type of studies.

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Conflict of Interest Statement

No conflict of interest noted.

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