Re-thinking Alzheimer's disease therapeutic targets using gene-based tests

Man Ki Kwok a, Shi Lin a, C. Mary Schooling a,b,*

a School of Public Health, Li Ka Shing Faculty of Medicine, The University of Hong Kong, 1/F, Patrick Manson Building (North Wing), 7 Sassoon Road, Hong Kong, China
b City University of New York, Graduate School of Public Health and Health Policy, New York, United States

A R T I C L E   I N F O
Article history:
Received 6 June 2018
Received in revised form 11 September 2018
Accepted 1 October 2018
Available online 9 October 2018

Keywords:
Alzheimer's disease
Genetics
Genetic drug targets
Gene-based test

A B S T R A C T
Background: Alzheimer’s disease (AD) is a devastating condition with no known effective drug treatments. Existing drugs only alleviate symptoms. Given repeated expensive drug failures, we assessed systematically whether approved and investigational AD drugs are targeting products of genes strongly associated with AD and whether these genes are targeted by existing drugs for other indications which could be re-purposed.

Methods: We identified genes strongly associated with late-onset AD from the loci of genetic variants associated with AD at genome-wide-significance and from a gene-based test applied to the most extensively genotyped late-onset AD case-control (n = 17,008)-control (n = 37,154) study, the International Genomics of Alzheimer's Project. We used three gene-to-drug cross-references, Kyoto Encyclopedia of Genes and Genomes, Drugbank and Drug Repurposing Hub, to identify genetically validated targets of AD drugs and any existing drugs or nutraceuticals targeting products of the genes strongly associated with late-onset AD.

Findings: A total of 67 autosomal genes (forming 9 gene clusters) were identified as strongly associated with late-onset AD, 28 from the loci of single genetic variants, 51 from the gene-based test and 12 by both methods. Existing approved or investigational AD drugs did not target products of any of these 67 genes. Drugs for other indications targeted 11 of these genes, including immunosuppressive disease-modifying anti-rheumatic drugs targeting PTK2B gene products.

Interpretation: Approved and investigational AD drugs are not targeting products of genes strongly associated with late-onset AD. However, other drugs targeting products of these genes exist and could perhaps be re-purposing to combat late-onset AD after further scrutiny.

© 2018 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

List of abbreviations

| Abbreviation | Description |
|--------------|-------------|
| AD           | Alzheimer’s disease |
| FDA          | Food and Drug Administration |
| GATES        | Gene-based association test with an extended Simes procedure |
| GEO          | Gene Expression Omnibus |
| GWAS         | genome-wide association study |
| HGNC         | HUGO Gene Nomenclature Committee |
| IGAP         | International Genomics of Alzheimer’s Project |
| KEGG         | Kyoto Encyclopedia of Genes and Genomes |
| OMIM         | Online Mendelian Inheritance in Man |
| SNP          | single nucleotide polymorphism |

* Corresponding author at: School of Public Health, Li Ka Shing Faculty of Medicine, The University of Hong Kong, 1/F, Patrick Manson Building (North Wing), 7 Sassoon Road, Hong Kong, China.
E-mail address: cms1@hku.hk (C.M. Schooling).

1. Introduction

Alzheimer's disease (AD) has remained incurable since its first discovery in 1906 [1]. An estimated one-third of AD cases may be associated with lifestyle or related attributes, education and other health conditions (hypertension, obesity, diabetes, depression) [2], although the exact interventions require clarification [3]. Mendelian randomization studies using single nucleotide polymorphisms (SNPs) as instruments suggest that apolipoprotein E, systolic blood pressure, smoking and vitamin D are inversely associated with AD [4–6]. Effective therapies to prevent and treat AD were called for globally at the G8 dementia summit in 2013 [7], because of the escalating societal costs if AD remains untreated [8]. Effective drug treatments for AD have proved elusive.

Current understanding of AD is based on factors identified from brain autopsies, which in 1976 implicated acetylcholine, “the
cholinergic hypothesis” [9,10], and subsequently implicated beta-amyloid (Aβ) in extracellular plaques in 1984 and tau proteins in neurofibrillary tangles in 1986 [11]. Drug development based on these hypotheses has yielded two types of drugs for AD approved by the U.S. Food and Drug Administration (FDA), including cholinesterase inhibitors, N-methyl-D-aspartate receptor antagonists, and the combination of these inhibitors. These drugs relieve symptoms rather than delaying progression. No new AD drugs have been approved by the U.S. FDA since 2003, despite over 400 trials from 2002 to 2012 mainly targeting Aβ [12]. These difficulties may be due to lack of pre-trial biomarker screening to identify high risk patients as selection criteria [13], or of subjectively rated cognitive and functional outcomes rather than validated biomarkers [14]. However, failure of anti-Aβ drugs has raised questions about the causal role of amyloid protein in AD and the relevance of the amyloid protein hypothesis to AD drug development [15,16]. In vitro studies suggest Aβ and its precursor amyloid protein precursor are damage response proteins [17] so amyloid plaque may be a consequence rather than a cause [18]. More importantly, both Aβ and tau protein may not only be neurotoxins, but also be relevant to normal physiological functions [15].

In response to issues with drug development, genetic validation of drug targets is becoming increasing popular, and has recently explained the failure of several cardiovascular drugs, such as darapladib and varespladib, where functionally relevant genetic variants were found relevant to the drug but not to the disease [19,20]. However, overall a small number of drug identified from SNP-based genome-wide association studies (GWAS) have raised the concerns that GWAS may fail to identify causal SNPs by excluding SNPs with greater effect but low frequency [21]. To identify new targets for the most common form of AD, i.e., late (65 + years)-onset AD agnostically, we considered genetic variation in naturally occurring functional units, i.e., genes, as an initial step. A previous gene-based study of people of European ancestry in the International Genomics of Alzheimer’s Project (IGAP) found 13 genes known from genetic loci of SNPs identified in GWAS, 3 genes in close proximity to 2 known genes and 2 new genes (TP53INP1, IGHV1-67) [22]. Another gene-based study of AD in a trans-ethnic GWAS of people of European, African-Americans, Japanese and Israeli-Arabs ancestry replicated 7 known genes and identified 1 new gene (TPBG) [23]. However, these studies did not compare late-onset AD gene products with targets of AD drugs. To our knowledge, only one previous study has compared AD genetic loci with targets of approved AD drugs [21]. Here, we build on this previous work by firstly identifying genes associated with specifically late-onset AD from a gene-based test and from the loci of SNPs found in previous GWAS, secondly assessing the overlap between these late-onset AD gene products and targets of currently approved or investigational AD drugs, and thirdly identifying any other existing drugs or nutraceuticals targeting products of these late-onset AD genes that could potentially be re-purposed to prevent or treat AD. Given genes from GWAS have been shown to be more likely to be drug targets than random genes for complex traits [24] or heritable diseases [25], our findings of any discrepancy between products of these AD genes and drug targets would indicate opportunities for using AD genes for drug discovery and repurposing. Conversely, genes without even a nominal association with late-onset AD might be screened out and de-prioritized as indicators of potential targets of intervention.

2. Materials and methods

2.1. Genes associated with AD

We obtained genes strongly associated with late-onset AD using two approaches. First, we ascertained AD genes from all loci of single nucleotide polymorphisms (SNPs) associated with late-onset AD at genome wide significance in a recent review of GWAS [26] (i.e., SNP-based GWAS). In addition, we did not use the Online
Mendelian Inheritance in Man (OMIM) because no additional genes associated with late-onset AD arising through genome-wide Bonferroni corrected significance could be identified. Second, we ascertained AD genes as genes associated with AD at Bonferroni corrected significance using a gene-based test applied to the largest, most densely genotyped (7,055,881 SNPs genotyped or imputed in autosomal chromosomes using the 1000 Genomes Catalog case (n = 17,008) control (n = 37,154) study of AD, stage 1 discovery sample from the iGAP, in people of European descent (mean age 71.4 years) adjusted for age and sex and corrected for population stratification [27]. Among all these late-onset AD genes, we then obtained gene clusters based on two or more genes that encode for similar proteins within the same gene family classified by the HUGO Gene Nomenclature Committee (HGNC) [28].

2.2. Therapies targeting identified genes

To assess whether existing AD drugs are targeting products of late-onset AD genes and whether genes strongly associated with AD are being fully exploited, two researchers independently identified the genes targeted by AD drugs using three gene-to-drug cross-references, Kyoto Encyclopedia of Genes and Genomes (KEGG) [29], Drugbank [30], and Drug Repurposing Hub [31] as of August 30, 2018. For existing AD drugs, in KEGG, we searched each drug on “KEGG Drug” and identified the targets given in the “Target pathway”. In Drugbank, we searched for each drug by specifying “Drugs”, and identified the targets based on “Targets: Gene Name”. In Drug Repurposing Hub, we searched each drug by specifying “Name:” and identified the targets based on the “Target”. To identify treatments targeting AD genes, in KEGG, we searched each gene on “KEGG Disease” and identified the treatments for that particular gene as listed in “Gene” given in the “Drug target”. In Drugbank, we searched for each gene by specifying “Targets”, and identified the treatments based on “Drug relations” including drugs labeled as “approved” with known pharmacological action. In Drug Repurposing Hub, we searched each gene by specifying “Target:” and identified the treatments based on the “Name” (including drugs labeled as “Launched” phase). We considered all existing or investigational AD drugs. The list of investigational drugs was based on drugs being tested in recruiting or active Phase III or IV trials registered in ClinicalTrials.gov or listed in AlzForum (http://www.alzforum.org/therapeutics) as of January 28, 2018, supplemented by drugs in Phase III trials listed in Cummings J, et al. [32], excluding diagnostic drugs and drugs for sequelae of AD, such as agitation or sleep disorders.

2.3. Statistical analysis

We obtained an overall P-value for the association of each autosomal gene with late-onset AD by combining P-values for the association of all the SNPs within each gene using GATES accounted for linkage disequilibrium between loci [33]. GATES has the advantage of not requiring permutations or simulations, and maintains a Type I error (false positive) rate regardless of gene size or linkage disequilibrium patterns among SNPs. We only considered genes reaching genome-wide Bonferroni corrected significance (nominal P-value < 0.000002, i.e., 0.5/25000 genes) such that genes identified from this gene-based test are selected on the same basis as genes identified from SNP-based GWAS [26]. We performed a hypergeometric test to obtain the probability of the identified genes being targeted by AD drug or drugs for other indications, assuming 5% of the human genomes (same as the significance level of one-tailed Fisher test) are targeted by AD drugs or drugs for other indications (n = 1250 genes) and the remaining genes are not targeted. This analysis of publicly available data does not require ethical approval.

3. Results

Table 1 shows that 28 genes strongly associated with AD were identified from the SNP-based GWAS [26] and 51 from the gene-based test, with 12 genes (APOE, CLU, BIN1, CR1, PICALM, MS4A6A, EPFA1, SORL1, ABCA7, PTK2B, CD33, CD2AP) identified by both approaches. A total of 16 genes (SLC2A4-RIN3, CELFI, CASS4, TRIP4, ZCWPW1, HLA-DRB5-HLA-DRB1, FERMT2, NME8, INPP5D, TREM2, TREM1L, MEF2C, ACE, APP, PDL3, DSG2) were only identified from the SNP-based GWAS, and 39 genes (including 2 pseudogenes: APOC1P1, CEACAM22P) were only identified from the gene-based test. Most of these 67 genes (n = 33) were on chromosome 19, where all 4 genes with the smallest P-value (APOC1, APOE, NECTIN2, TOMM40) are located. When considering these 67 genes together, 9 gene clusters were identified including apolipoproteins (APOC1, APOE, APOC2, APOC4, APOC4-APOC2), carboxyribonuclease antigen related cell adhesion molecule (CEACAM16, CEACAM19), cluster of differentiation molecule (ACE, CD33, CD2AP), complement system (CR1, CR1L), ephrins (EPHA1, EPHA1-AS1), histocompatibility complex (HLA-DQAI, HLA-DRB5-HLA-DRB1), membrane spanning 4-domains (MS4A6E, MS4A4A, MS4A6A, MS4A4E, MS4A4D), microRNAs (mir6843, mir6503, mir4531) and V-set domain containing (TREM2, TREML2). As such, in addition to identifying the same 12 genes and 7 gene clusters as previous SNP-based GWAS, this gene-based test newly identified 39 genes (of which 4 genes were in strong linkage disequilibrium with previously known genes) and 2 gene clusters (CEACAM, MIR) (formed from 5 genes).

The current approved or investigational AD drugs did not target products of any of these 67 genes strongly associated with AD (Table 2). However, drugs for other indications targeted products of 11 of the 67 late-onset AD genes, with P-value = 0.0001 which indicates our results unlikely occurred by chance. These other drugs targeting products of these 11 genes (ACE, APPE, CD33, CLU, EPFA1, HHECGF, HLA-DQAI, HLA-DRB1, MS4A2, PTK2B) including Gemtuzumab ozogamicin which targets CD33 gene products, Omalizumab which targets MS42 gene products and Baricitinib/Fostamatinib/Leflunomide which targets PTK2B gene products (Table 3).

4. Discussion

Among the 67 genes found strongly associated with late-onset AD, this gene-based study replicated 12 genes and 7 gene clusters found by previous SNP-based GWAS and identified 39 new genes (of which 4 genes were in strong linkage disequilibrium with previously known genes) and 2 new gene clusters (formed from 5 genes). However, we did not replicate 3 genes (TP53NPI1, IGHV1-67, TPBG) found by previous gene-based studies, possibly because the study of people of European ancestry in iGAP used Fisher’s combination test [22], which is prone to type I error [33], and the trans-ethnic GWAS using the same GATES method may have identified population-specific genes [23]. Moreover, our study suggests existing AD drugs (cholinesterase inhibitors or N-methyl-d-aspartate receptor antagonists) may not be targeting products of these late-onset AD genes, consistent with the previous comparison of AD genetic loci with approved AD drugs [21]. We also found that existing investigational AD drugs currently in Phase III trials (anti-Aβ agents, anti-tau agents, other neurotransmitters agonist/antagonists and insulin sensitizers) did not appear to be targeting products of late-onset AD drugs. We cannot rule out the possibility that incomplete knowledge of AD genes and/or drug targets could generate such null findings. Finally, we found products of 11 AD genes are targets of existing drugs for other indications, which could possibly be considered for further scrutiny of directionality of disease genetics and drug actions within an integrated biological networks before re-purposing to mitigate or cure late-onset AD.
Our study suggests a ‘mismatch’ between approved or investigational AD drugs and the genes strongly related to late-onset AD. Cholinesterase inhibitors are not the only neurotransmitter related to AD [11] and may have missed the window of intervention in late-onset AD because of the accumulated loss of neurological functions [13]. N-methyl-D-aspartate receptor antagonists aim to oppose the effects of the excitatory neurotransmitter glutamate [34], however the causal role of glutamate in AD is yet to be elucidated [35]. The investigational drugs are mainly anti-Aβ and anti-tau agents, which are not known to target products of genes associated with late-onset AD, but are largely based on causal associations with familial AD. Aβ is the product of the APP gene which is primarily associated with early-onset AD [36] except for a rare genetic variant [37]. The latest failures of these drug classes in late-onset AD [38,39], have raised questions as to whether Aβ generation [42], and off-target pathways.
Table 2
Genes with products targeteda by approved or investigational Alzheimer's disease drugsb.

| Type               | Drug               | Target Gene | Gene | P-value | Class                          |
|--------------------|--------------------|-------------|------|---------|--------------------------------|
| Approved drugs     | Donepezil          | ACHÉ        | 0.65 | Acetylcholinesterase inhibitor |
|                    |                    | HTR2A       | 0.03 |         |                                |
|                    | Rivastigmine       | ACHÉ        | 0.65 | Acetylcholinesterase inhibitor |
|                    |                    | BCHE        | 0.73 |         |                                |
|                    | Galantamine        | ACHÉ        | 0.65 | Acetylcholinesterase inhibitor |
|                    |                    | CHRNA1-ACHÉ | 0.22 |         |                                |
|                    |                    | CHRNB1-B4   | 0.77 |         |                                |
|                    |                    | CHRND       | 0.89 |         |                                |
|                    |                    | CHRNE       | 0.0003 |      |                                |
|                    |                    | CHRNA4      | 0.58 |         |                                |
|                    |                    | BCHE        | 0.73 |         |                                |
|                    | Memantine          | CHRNA7      | 0.72 | N-methyl-D-aspartate receptor antagonist |
|                    |                    | CYP2E1      | 0.94 |         |                                |
|                    |                    | DAB2       | 0.23 |         |                                |
|                    |                    | GRIN1       | 0.97 |         |                                |
|                    |                    | GRIN2A/2B   | 0.50 |         |                                |
|                    |                    | GRIN3A      | 0.85 |         |                                |
|                    |                    | HTR3A       | 0.24 |         |                                |
| Investigational drugs | Aducanumab        | Nil         | –   | Anti-amyloid |                                |
|                    | ALZT-0P1a/b        | --          | –   | Anti-amyloid |                                |
|                    |                    | ASCL1       | 0.28 |         |                                |
|                    |                    | BCL2        | 0.56 | Anti-inflammation | |
|                    |                    | CFTR        | 0.98 |         |                                |
|                    |                    | FABP2       | 0.99 |         |                                |
|                    |                    | KCNMA1      | 0.59 |         |                                |
|                    |                    | S100P       | 0.22 |         |                                |
|                    |                    | SLC15A1/A8  | 0.40 |         |                                |
|                    |                    | PLAT        | 0.57 |         |                                |
|                    |                    | PPARGA      | 0.97 |         |                                |
|                    |                    | PPARG       | 0.98 |         |                                |
|                    |                    | PTGS1/2     | 0.43 |         |                                |
|                    |                    | THBD        | 0.44 |         |                                |
|                    | Azeliragon         | AGER        | 0.13 | Anti-amyloid |                                |
|                    |                    | --          | –   | Anti-inflammation |                                |
|                    | CNP520             | Nil         | –   | Anti-amyloid |                                |
|                    | Crenezumab         | Nil         | –   | Anti-amyloid |                                |
|                    | Elenbecestat       | Nil         | –   | Anti-amyloid |                                |
|                    | Gantenerumab       | Nil         | –   | Anti-amyloid |                                |
|                    | JNJ-54861911       | Nil         | –   | Anti-amyloid |                                |
|                    | Pioglitazone       | INS         | 0.97 | Insulin sensitizer |                                |
|                    |                    | PPARRA      | 0.97 | Anti-inflammation |                                |
|                    |                    | PPARG       | 0.72 |         |                                |
|                    |                    | TRPM3       | 0.27 |         |                                |
|                    |                    | MAOB        | –   |         |                                |
|                    | Intepirdine        | Nil         | –   | Other neurotransmitters (Selective serotonin 5-HT, receptor antagonist) | |
|                    | Solanezumab        | Nil         | –   | Anti-amyloid |                                |
|                    | TRx0237            | Nil         | –   | Anti-tau |                                |
|                    | Verubecestat       | BACE1       | –   | Anti-amyloid |                                |
|                    | Guanfacine         | ADRB2A-2C   | 0.16 | Other neurotransmitters (Attention Deficit Hyperactivity Disorder drug) | |
|                    |                    | CYP1A1      | 0.38 |         |                                |
|                    |                    | HCN1/4      | 0.62 |         |                                |
|                    | Insulin            | INSR        | 0.83 | Intranasal insulin |                                |
|                    | (Humulin®RU-10)    | IGF1R       | 0.78 |         |                                |
|                    |                    | RB1         | 0.26 |         |                                |
|                    |                    | CTSD        | 0.11 |         |                                |
|                    |                    | IDE         | 0.45 |         |                                |
|                    |                    | PCSK1/2     | 0.41 |         |                                |
|                    |                    | CPK         | 0.09 |         |                                |
|                    |                    | NOV         | 0.30 |         |                                |
|                    |                    | LRP2        | 0.40 |         |                                |
|                    |                    | IGBP7       | 0.70 |         |                                |
|                    |                    | SYTL4       | –   |         |                                |
|                    | Lanabecestat       | Nil         | –   | Anti-amyloid |                                |
|                    | Sodium oligomannurrate | Nil    | –   | Anti-amyloid (Oligosaccharide) | |
|                    | Docosahexaenoic acid | PPARRA     | 0.97 | Other (Unsaturated fatty acid synthesis) | |
|                    |                    | PPARG       | 0.72 |         |                                |
|                    |                    | RXRA        | 0.25 |         |                                |
|                    |                    | RXRB        | 0.86 |         |                                |
|                    |                    | RXRG        | 0.13 |         |                                |
|                    |                    | SREBF1      | 0.58 |         |                                |
|                    | Tricaprilin        | Nil         | –   | Other (triacylglycerols) |                                |
|                    | Lithium Carbonate  | GSK3B       | 0.98 | Other (alkali metal compounds) | |
|                    |                    | IMPA1/2     | 0.04 |         |                                |
|                    |                    | GRA3        | –   |         |                                |

(continued on next page)
that induce adverse effects might negate the possible benefits of Aβ accumulation. Notably, the possibility of citation bias in favour of the beta-amyloid hypothesis for AD has been raised [43].

We identified 67 genes strongly associated with late-onset AD (12 genes from both approaches, 16 from SNP-based GWAS and 39 from the gene-based test). The 12 genes identified by both approaches relate to lipid metabolism or transport (e.g. APOE, SORL1, ABCA7), synaptic function (BIN1, PICALM, MA4A6A, PTK2B), immune response (CR1, CD33, CD2AP) or cell proliferation or apoptosis (CLU, EPHA1) [44]. With replication by the gene-based test of findings from previous SNP-based GWAS, products of these 12 genes could possibly be potential drug targets. Conversely, 16 genes inferred from previous SNP-based GWAS as associated with late-onset AD were not associated with AD on the gene-based test, perhaps indicating that these SNPs' might not indicate causal genes [27]. As such, considering genetic variants in functional units, i.e., genes, as here may be informative, in addition to considering single genetic variants. For genes with variants representing loss-of-function, drugs that activate (agonists) proteins in human cells would be needed, whereas drugs that inhibit (antagonists) would be needed for variants representing gain-of-function [25]. Understanding AD pathogenesis and drug actions are necessary for translating our genetic validations into identification of therapeutic targets.

Other identified genes, if substantiated by future studies with clearly defined disease genetics and drug actions, could provide new, promising directions for late-onset AD therapeutic investigation. For example, NECTIN2 relates to cell-to-cell spreading of the herpes simplex virus and pseudorabies virus, when herpes simplex virus type 1 is thought to play a role in AD [45]. A recent drug trial targeting the virus (VALZ-PILOT) has been launched, although it is not known to target products of the 67 identified late-onset AD genes [46]. Further, the 9 gene clusters identified by the gene-based test and/or SNP-based GWAS could be potentially relevant genetic loci implicated in late-onset AD. The APO gene cluster (lipoprotein metabolism) consistently identified by both approaches may play a causal role. Particularly the APOE gene, as substantiated

| Type | Drug | Target Gene | Gene P-value | Class |
|------|------|-------------|--------------|-------|
| Resveratrol | NQO2 | 0.97 | Anti-inflammation (herpes simplex virus) |
| | CSN2GA1 | 0.29 | |
| | PITG1/2 | 0.43 | |
| | ALOX5/15 | 0.06 | |
| | AHR | 0.75 | |
| | P4A28 | 0.99 | |
| | ITGAS | 0.44 | |
| | ITGB3 | 0.51 | |
| | APP | 0.08 | |
| | ESRI | 0.13 | |
| | MITRRI/18 | 0.20 | |
| | CLEC4A | 0.93 | |
| | NR112/3 | 0.16 | |
| | SLC2A1 | 0.77 | |
| | SCN1A | 0.91 | |
| | CBR1 | 0.67 | |
| | PPARG | 0.97 | |
| | AKT1 | 0.26 | |
| | RHYP | 0.64 | |
| | YAS | 0.91 | |
| | APOA1 | 0.27 | |
| | BACE1 | 0.25 | |
| | SCN5A | 0.82 | |
| | BTR | 0.71 | |
| | TTR | 0.75 | |
| | XDH | 0.56 | |
| | MOA | -- | |
| Acetyl-L-Carnitine | Nil | -- | Other (fatty acyls) |
| Angiotensin II receptor blocker + calcium channel blocker | AGTR1 | 0.97 | Anti-hypertensives |
| | CACNA1B/1C | 0.83 | |
| | CACNA1D/1S | 0.03 | |
| | CACNA2D1/D3 | 0.98 | |
| | CACNB1/B2 | 0.05 | |
| | C1 | 0.35 | |
| | SPPD | 0.96 | |
| Choline alfoscerate | GM2A | 0.63 | Other (glycerophosphocholines) |
| | SLC6A2 | 0.06 | Other (possibly improving blood flow) |
| | PLA2G2A | 0.90 | |
| | GLRA1 | 0.57 | |
| | GABRA1 | 0.45 | |
| | GABBR2 | 0.81 | |
| | GABBC2 | 0.96 | |
| Octohydroaminoacridine Succinate | Nil | -- | Acetylcholinesterase inhibitor |

a Source: Kyoto Encyclopedia of Genes and Genomes (KEGG) (http://www.genome.jp/kegg/), Drugbank (https://www.drugbank.ca/) and Drug Repurposing Hub (https://clue.io/repurposing) as of August 30, 2018.

b Source: ClinicalTrial.gov (https://clinicaltrials.gov/), Cummings J, et al. (2016) Alzheimer’s & Dementia: Translational Research & Clinical Interventions (http://www.trci.alzdem.com/article/S2352-8737(16)30019-1.pdf) and AlzForum (http://www.alzforum.org/therapeutics). The list of investigational drugs was based on drugs being tested in recruiting or active Phase III or IV trials registered in ClinicalTrial.gov or listed in AlzForum as of January 28, 2018, supplemented by drugs in Phase III trials listed in Cummings J, et al. (2016), excluding diagnostic drugs and drugs for sequelae of AD, such as for agitation or sleep disorders.
Table 3

Genes strongly associated with late-onset Alzheimer’s disease with products targeted by existing drugs\(^a\).

| Drug                  | Target Gene | Gene P-value | Class                           |
|-----------------------|-------------|--------------|---------------------------------|
| Afatinib              | HBE GF      | 1.83 × 10\(^{-6}\) | Anti-cancer EGFR inhibitors     |
| Brigatinib            | HLA-DRB1    | 8.77 × 10\(^{-5}\) | Anti-cancer immunomodulator     |
| Cetuximab             | EPHA1       | 7.57 × 10\(^{-10}\) | Anti-thyroid cancer EGFR, RET tyrosine, VEGFR kinase inhibitors |
| Erlotinib             | CLU         | 5.91 × 10\(^{-16}\) | Metal compounds                 |
| Gefitinib             | FTK2B       | 3.66 × 10\(^{-7}\) | Anti-Rheumatoid JAK inhibitor   |
| Lapatinib             | MS4A2       | 4.47 × 10\(^{-9}\) | Anti-IGF for severe allergic asthma, Anti-hypertensive ACE inhibitors |
| Necitumumab           |             |              |                                 |
| Neratinib             |             |              |                                 |
| Olmutinib             |             |              |                                 |
| Osimertinib           |             |              |                                 |
| Panitumumab           |             |              |                                 |
| Glatiramer acetate    | HLA-DQA1    | 9.36 × 10\(^{-7}\) | Immunomodulator for multiple sclerosis |
| Apolizumab            | HLA-DBR1    | 8.77 × 10\(^{-5}\) | Anti-cancer immunomodulator     |
| Vandetanib            | EPHA1       | 7.57 × 10\(^{-10}\) | Anti-thyroid cancer EGFR, RET tyrosine, VEGFR kinase inhibitors |
| Copper                | CLU         | 5.91 × 10\(^{-16}\) | Metal compounds                 |
| Zinc                  |             |              |                                 |
| Zinc acetate          |             |              |                                 |
| Zinc chloride         |             |              |                                 |
| Baricitinib           | PTK2B       | 3.66 × 10\(^{-7}\) | Anti-Rheumatoid JAK inhibitor   |
| Leflunomide           |             |              |                                 |
| Omalizumab            | MS4A2       | 4.47 × 10\(^{-9}\) | Anti-IGF for severe allergic asthma, Anti-hypertensive ACE inhibitors |
| Alacepril             | ACE         | 0.01         | Metal compounds                 |
| Benazepril            |             |              |                                 |
| Benazeprilat          |             |              |                                 |
| Captopril             |             |              |                                 |
| Ceronapril            |             |              |                                 |
| Cilazapril            |             |              |                                 |
| Delapril              |             |              |                                 |
| Deserpidine           |             |              |                                 |
| Enalapril             |             |              |                                 |
| Enalaprilat           |             |              |                                 |
| Fosinopril            |             |              |                                 |
| Fosinoprilat          |             |              |                                 |
| Gemopatrilat          |             |              |                                 |
| Imidapril             |             |              |                                 |
| Indolapril hydrochloride |         |              |                                 |
| Liensapril            |             |              |                                 |
| Lisinopril            |             |              |                                 |
| Moxepin               |             |              |                                 |
| Pentopril             |             |              |                                 |
| Perindopril           |             |              |                                 |
| Pivopril              |             |              |                                 |
| Quinapril             |             |              |                                 |
| Quinaprilat           |             |              |                                 |
| Ramipril              |             |              |                                 |
| Rescinnamine          |             |              |                                 |
| Spirapril             |             |              |                                 |
| Spiraprilat           |             |              |                                 |
| Temocapril            |             |              |                                 |
| Trandolapril          |             |              |                                 |
| Zofenopril            |             |              |                                 |
| Zofenoprilat arginine |             |              |                                 |
| Copper                | APOE        | 0            | Metal compounds                 |
| Zinc                  |             |              |                                 |
| Zinc acetate          |             |              |                                 |
| Zinc chloride         |             |              |                                 |
| Gemtuzumab ogzomicin  | CD33        | 8.09 × 10\(^{-7}\) | Treatment for acute myeloid leukemia |
| Curcumin              | APP         | 0.08         | Nutraceuticals extracted from tumeric |

\(^a\) Source: Kyoto Encyclopedia of Genes and Genomes (KEGG) (http://www.genome.jp/kegg/), DrugBank (https://www.drugbank.ca/) and Drug Repurposing Hub (https://clue.io/repurposing) as of August 30, 2018.

by a Mendelian randomization study on apolipoprotein E [4], which has long been considered as a drug target [47]. Recently, APOE ε4 alleles have been shown to promote a gain of toxic effects by independently promoting Aβ42 and tau protein production in human neurons [41]. Taken together with the null findings from randomized controlled trials of statin on cognitive functions [48] and Mendelian randomization studies of low-density lipoprotein cholesterol [49] and triglyceride [50] on late-onset AD, better understanding on the functionality of both gene and gene clusters would add more nuanced mechanistic insights on lipid metabolism e.g. apolipoprotein E as a cholesterol transport protein may be more relevant than cholesterol synthesizing proteins.

Importantly, this study identified products of 11 late-onset AD genes that are currently targeted by other therapies [51–53] that could possibly be further investigated to clarify the disease genetics and drug actions before repurposing for late-onset AD. Gemtuzumab ozogamicin, which targets CD33 gene products, is an approved treatment for acute myeloid leukemia and has been considered for repurposing in AD [54]. Omalizumab, which targets MS4A2 gene products and another gene (FCER1A), is a subcutaneous injectable controlling moderate-to-severe allergic asthma; Leflunomide, which targets PTK2B gene products in addition to 2 other genes (AHKR, PTK2B), is an orally-administered immunosuppressive disease-modifying anti-rheumatic drug. These existing drugs already have phase II trial results such that pharmacodynamics, pharmacokinetics, toxicity, preclinical effects in vitro and in vivo and phase I safety and dosage results are known, which reduces the risk, time and resources needed for possible drug re-purposing after more scrutiny of their relevance to AD.

Our findings provide an initial step in translating AD genetics to therapeutic targets and should be enriched using network medicine by characterizing any drug actions within biological networks. Given many diseases including late-onset AD may be related to functional disruption of multiple genes rather than single genes and these genes may cluster and interact as disease modules, network medicine may help integrate regulatory networks, RNA networks, protein-protein interactions and metabolic pathways to delineate the complex link from disease genes to drug targets [55]. Moreover, disease proximity and similarity between two different drugs might indicate shared mechanisms between two diseases with implications for drug re-purposing [56]. Drugs may target specific but not entire disease modules [56] and may partly inhibit or activate interactions and functions [57], thereby AD drug targets are not necessarily the products of AD genes. In addition, only a fraction of proteins (gene products) can be manipulated by small molecule drugs [57]. Clarifying the direction of drug actions as well as the drug effects within integrated biological networks would help identify gene products as druggable targets. As such, building and integrating drug-drug relationships, gene-protein interactions, and disease-disease networks may provide a framework for further identifying drug targets [58]. Further, the use of computational drug-target interactions may help predict druggability by integrating genomics, pharmacological properties, biochemical interactions supplemented with similarities between drugs and target proteins [59]. Although the network-based analytical techniques are evolving and the interactome and drug-protein interactions remain to be fully understood, to date, network-based analysis using GWAS data has contributed in prioritizing disease genes by mapping candidate genes within the protein–protein interaction network [60] supplemented by further searches of sub-networks [61]. Specifically for AD, Talwar et al. (2014) found 6 gene clusters related to 7 proteins encoded by genes EGFR, ACTB, CDC2, IRAK1, APOE, ABCA1, AMPH with EGFR and ACTB as key genes [62]. Browne et al. (2015) found 32 prioritized AD genes with
PSEN1 and TRAF1 as key genes [63]. Both studies identified AD genes primarily related to neurogenesis and its regulation based on functional annotation using gene-ontology [62,63]. Hu et al. (2017) found 3 main modules related to neuronal pathways and metabolism, cell growth or survival and neuroendocrine, and immune response using pathway crosstalk analysis [64]. Given these previous studies only considered AD genes as a whole, our systematically identified late-onset AD genes could be utilized for late-onset AD-related gene cluster identification and functional annotation. Mostafavi et al. (2018) found INPPL1 and PLXNB1 related to late-onset AD using RNA sequencing from a cohort of 478 older adults with brain autopsy [65]. While these previous network-based studies do not replicate in their most relevant genes, two of these genes (ABCA1, INPPL1) are paralog of genes identified in our study (ABCA7, INPPD5). Alternatively, comparison of gene expression profiles and drug-induced RNA expression profiles could provide further insights to connect genes to existing drugs [66,67]. Issa et al. (2016) found several approved or experimental drugs (such as Rasagiline, Interferon, Calcium, Dovitinib, Somatotropin Re-combinant) related to AD [68]. Vargas et al. (2018) found 6 approved drugs (Cefuroxime, Cyproterone, Dydrogesterone, Metrizamide, Trimethadione, Vorinostat) negatively associated with AD [69]. Both studies used disease-related gene expression from the Gene Expression Omnibus (GEO) database, but applied to different drug-induced expression profiles, which might account for the differences in the identified drugs [68,69]. The late-onset AD genes and existing drugs for other indications identified in this study would allow more refined expression of comparison profiles in future studies for drug repurposing, of which timely evidence is needed considering this study identified that current approved and investigational AD drugs may not be targeting late-onset AD genes.

This systematic and agnostic identification of genes for late-onset AD provides directions for re-thinking AD treatments. Nevertheless, several limitations are noted. First, the genes identified here from IGAP relate to late-onset AD. As such, they may not be relevant to early-onset AD, i.e., AD occurring before the age of 65 years, which may explain the non-identification of the 3 genes (APP, PSEN1, PSEN2) associated with early-onset AD [36]. Although a rare variant of APP might be related to late-onset AD as well, but is likely context-specific as it has been found in Nordic countries whereas it is very rare in the United States [37]. Second, the genes identified might not be a complete set and did not include genes on the sex chromosomes. Replication of the gene-based test in a different population or using a future GWAS with a larger sample size may reveal additional genetic targets. The recent trans-ethnic GWAS for AD using a gene-based test with the same GATES method identified 8 late-onset AD genes, of which nearly all genes (CR1, BIN1, PTK2B, CLU, PICALM, and ABCA7) were also identified in our study. The only new gene (TPBG) that study identified was neither found in the previous GWAS of European ancestry [26] nor reached genome-wide significance in their European ancestry-specific result [23], indicating the relevance of certain genes may be population-specific. Nevertheless, consistency in genes identified in both trans-ethnic GWAS and our study with a larger sample size and greater power without excluding SNPs with P-value < 1 × 10^{-6} lends credence to the gene-based association approach. Third, this study aims to identify potential genetically validated drug targets rather than map out the full genetic functionality or pathophysiology of these targets, because repurposing therapies acting on genetically validated targets may be the swiftest way of developing effective new treatments. We acknowledge that the current gene-based analysis might not provide comprehensive therapeutic effects and AD pathogenesis. Comparing disease gene-related and drug-induced gene expression profiles may provide further insights [66]. Fourth, our identified drug targets depend on the validity and completeness of KEGG, Drugbank and Drug Repurposing Hub. While the Drug Repurposing Hub incorporates extensive in-house drug target data, more publicly available data from pharmaceutical companies would make the search more comprehensive especially for investigational drugs [70]. Moreover, these curated cross-references are constantly being made more comprehensive to reflect new discoveries, so it is possible that new targets will be found in the future. Fifth, some valid targets of existing drugs might not reach genome wide significance. For example the gene related to statins (HMGCR) [71] is only nominally (P-value = .004) associated with ischemic heart disease [72]. Sixth, the gene-based test considers SNPs within or near the genes using the same amount of SNPs as the SNP-based GWAS, but not intergenic regions [33]. Our findings can be supplemented by future studies identifying SNPs outside of genes associated with late-onset AD to provide a comprehensive AD genome. Seventh, this study considers genes reported based on index SNPs to allow comparison with previous AD GWAS [26]. However, SNP-based GWAS identifies genomic regions (loci) which may correspond to multiple genes that do not necessarily correspond to the genes implicated in late-onset AD [73]. Consideration of multiple genes within the loci given in a recent review of GWAS of late-onset AD [73] identified 4 more genes (HLA-DQA1, M54A4A, M54A6E, M54A2) using both approaches. Our gene based test identified 35 genes not found by previous SNP-based GWAS. Finally, case-control studies of older people are inevitably open to selection bias, meaning that genes lethal for other diseases may appear relevant to late-onset diseases [74]. However, the genes we identified are not associated with major diseases that may result in death before the onset of AD, such as ischemic heart disease [72] or stroke [75].

In conclusion, our study provides no evidence that approved and investigational AD drugs are targeting products of genes strongly associated with late-onset AD, which might explain the lack of efficacy to date. Genetic validation of potential AD drugs might help to identify the most promising drugs to try to combat AD, conversely a gene-based test may also provide an initial screening tool to identify drugs that are unlikely to be successful. Other drugs targeting products of late-onset AD genes do already exist, but the mechanisms of disease genetics and drug actions need further clarification before proposing drug targets to combat late-onset AD.

Acknowledgements

Data have been contributed by the IGAP investigators and have been downloaded from http://web.pasteurlille.fr/en/recherche/u744/igap/igap_download.php. The authors thank Mr. Stanley Chan for independently searching for the gene targets of existing drugs.

Funding

This work receives no funding.

Declaration of interests

All authors declared no support from any organisation for the submitted work; no financial relationships with any organisations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work.

Author contributions

KMK conceptualized ideas, performed the literature review, conducted data analysis, interpreted findings and drafted the manuscript. LSL provided advice on data analysis and critically reviewed
the manuscript. CMS conceptualized ideas, directed analytic strategy, interpreted findings, revised drafts of the manuscript critically and supervised the study from conception to completion. KMK and CMS had full access to all of the data (including statistical reports and tables) in the study and can take responsibility for the integrity of the data and the accuracy of the data analysis. CMS is the guarantor.

References

[1] Hippisley C, Neudorffer G. The discovery of Alzheimer's disease. Dialogues Clin Neurosci 2003;5(2):109–13.
[2] Norton S, Matthews FE, Barnes DE, Yaffe K, Brayne C. Potential for primary prevention of Alzheimer's disease: an analysis of population-based data. Lancet Neurol 2014;13(8):788–94.
[3] Kane RL, Butler M, Fink HA, et al. Interventions to prevent age-related cognitive decline, mild cognitive impairment, and clinical Alzheimer's-type dementia [Internet]. https://www.ncbi.nlm.nih.gov/pubmed/26693784; 2017. (accessed Jun 1, 2018).
[4] Rasmussen KL, Tybjerg-Hansen AN, Nordestgaard BG, Frielle-Schmidt R. Plasma apo-lipoprotein E levels and risk of dementia: A Mendelian randomization study of 106,562 individuals. Alzheimers Dement 2018;14(1):71–80.
[5] Ostergaard SD, Mukherjee S, Sharp SJ, et al. Associations between Potentially Modifiable Risk Factors and Alzheimer Disease: A Mendelian Randomization Study. PLoS Med 2015;12(6):e1001841.
[6] Moklyk RE, Ross S, Morris JA, Manousakis D, Forgetta V, Richards JB. Genetically decreased vitamin D and risk of Alzheimer disease. Neurology 2016;87(24):2567–74.
[7] Cumming R, Allen S, Foy B, et al. Drug development in Alzheimer's disease: the path to 2025. Alzheimers Res Ther 2016;8:39.
[8] Vradianburg G. A pivotal moment in Alzheimer's disease and dementia: how global unity of purpose and action can beat the disease by 2025. Expert Rev Neurother 2018;18(11):173–82.
[9] Bartus RT, Dean 3rd RL, Beer B, Lipps AS. The cholinergetic hypothesis of geriatric memory dysfunction. Science 1982;217(458):408–14.
[10] Davies P, Maloney AJ. Selective loss of central cholinergic neurons in Alzheimer's disease. Lancet 1976;2(8000):1403.
[11] Hardy J. A hundred years of Alzheimer's disease research. Neuro 2006;52(1):3–13.
[12] Cummings JL, Morstorf T, Zhong K. Alzheimer's disease drug-development pipeline: few candidates, frequent failures. Alzheimers Res Ther 2014;6(4).
[13] Schelten S, Blennow K, Breitner MM, et al. Alzheimer's disease. Lancet 2016;388(10043):505–17.
[14] Becker RE, Greig NH, Giacobini E. Why do so many drugs for Alzheimer's disease fail in development? Time for new methods and new practices? J Alzheimers Dis 2008;15(2):23–32.
[15] Mullanke K, Williams M. Alzheimer's therapeutics: continued clinical failures question the validity of the amyloid hypothesis—but what lies beyond? Biochem Pharmacol 2013;85(3):285–90.
[16] Honig LS, Vellas B, Woodward M, et al. Trial of Solanezumab for Mild Dementia Due to Alzheimer's Disease to N Engl J Med 2018;378(4):321–30.
[17] Hardy J. The amyloid hypothesis for Alzheimer's disease: a critical reappraisal. J Neurosci Res 2009;87(1):23–34.
[18] Castellani RJ, Smith MA. Compounding artefacts with uncertainty, and an amyloid cascade hypothesis that is ‘too big to fail’. J Pathol 2011;224(2):147–52.
[19] Talmud PJ, Holmes MV. Deconvulsing the Casual Role of sPLA2s and PLA2 in Coro-nary Disease. Mol Cell Proteom Thromb Vasc Dis 2015;24(11):2281–9.
[20] Gregory JM, Freitag DF, Suredran P, et al. Genetic invalidation of PLA2 as a therapeut-ic target: Large-scale study of five functional PLA2-lowering alleles. Eur J Prev Cardiol 2017;24(3):492–504.
[21] Cao C, Moult J, GWAS and drug targets. BMC Genomics 2014;15(Suppl. 4):S5.
[22] Esscit-Price V, Bellenguez C, Wang LS, et al. Gene-wide analysis detects two new susceptibility genes for Alzheimer's disease. PLoS One 2014;9(6):e94661.
[23] Jun GR, Chung J, Mez J, et al. Transethnic genome-wide scan identifies novel Alzheimer's disease loci. Alzheimers Dement 2017;13:727–38.
[24] Sasseau P, Agarwal P, Barnes MR, et al. Use of genome-wide association studies for drug repositioning. Nat Biotechnol 2012;30(4):317–20.
[25] Zeng Z, Zhang HY. Rational drug repositioning by medical genetcs. Nat Biotechnol 2013;31(11):1080–2.
[26] Naj AC, Schellenberg GD. Alzheimer's Disease Genetics Consortium. Genomic vari-ants, genes, and pathways of Alzheimer's disease: an overview. Am J Med Genet B Neuropsychiatr Genet 2017;174(1):1–26.
[27] Lambert JC, Ibrahim-Verbaas CA, Harold D, et al. Meta-analysis of 74,046 individuals for late-onset Alzheimer’s disease: A Mendelian randomisation study. BMJ 2017;357:j1648.
[28] Mostafavi S, Gaiteri C, Sullivan SE, et al. A molecular network of the aging human brain provides insights into the pathology and cognitive decline of Alzheimer's disease-gene candidates. BMC Genomics 2015;16(Suppl. 9):S2.
[29] Talwar P, Silla Y, Grover S, et al. Genomic convergence and network analysis ap-Proach to identify candidate genes in Alzheimer's disease. BMC Genomics 2014;15:68.
[30] Gatteres M, Aqwa B, Krogher R, et al. Apolipoprotein E: a potential therapeutic target for human disease. Nat Rev Genet 2011;12(1):56–68.
[31] Guey E, Menche J, Vidal M, Barabasi AL. Network-assisted analysis to prioritize GWAS results: principles, methods and perspectives. Hum Mol Genet 2014;23(12):125–38.
[32] Shahi P, Silla Y, Grover S, et al. Genomic convergence and network analysis approach to identify candidate genes in Alzheimer’s disease. BMC Med Genet 2014;15:195.
[33] Brownie F, Wang H, Zheng H. A computational framework for the prioritization of disease-gene candidates. BMC Genomics 2015;16(Suppl. 9):S2.
[34] Ouyang Y, Xia S, Zhang GY, et al. Mining microRNA-mRNA expression data for diagnostic miRNA targets in breast cancer. PLoS One 2015;10(3):1–8.
[35] Mostafavi S, Gaiteri C, Sullivan SE, et al. A molecular network of the aging human brain provides insights into the pathology and cognitive decline of Alzheimer's disease. Nat Neurosci 2018;21(6):811–9.
[36] Soeh HC, Chau CK, Chia WT, et al. A basis of genome-wide association data highlights candidates for drug repositioning in psychiatry. Nat Neurosci 2017;20(10):1342–9.
[67] Donertas HM, Fuentesalba Valenzuela M, Partridge L, Thornton JM. Gene expression-based drug repurposing to target aging. Aging Cell 2018;e12819 https://www.ncbi.nlm.nih.gov/pubmed/?term=29959820.

[68] Issa NT, Kruger J, Wathieu H, Raja R, Byers SW, Dakshanamurthy S. DrugGenEx-Net: a novel computational platform for systems pharmacology and gene expression-based drug repurposing. BMC Bioinformatics 2016;17(1):202.

[69] Vargas DM, De Bastiani MA, Zimmer ER, Klarm F. Alzheimer’s disease master regulators analysis: search for potential molecular targets and drug repositioning candidates. Alzheimers Res Ther 2018;10(1):59.

[70] Nelson MK, Tipney H, Painter JL, et al. The support of human genetic evidence for approved drug indications. Nat Genet 2015;47(8):856–60.

[71] Collins R, Reith C, Emberson J, et al. Interpretation of the evidence for the efficacy and safety of statin therapy. Lancet 2016;388(10059):2532–61.

[72] Schooling CM, Huang JV, Zhao JV, Kwok MK, Au Yeung SL, Lin SL. Disconnect between genes associated with ischemic heart disease and targets of ischemic heart disease treatments. EBioMedicine 2018;28:311–5.

[73] Efthymiou AG, Goate AM. Late onset Alzheimer’s disease genetics implicates microglial pathways in disease risk. Mol Neurodegener 2017;12(1):43.

[74] Anderson CD, Nalls MA, Biffi A, et al. The effect of survival bias on case-control genetic association studies of highly lethal diseases. Circ Cardiovasc Genet 2011;4(2):188–96.

[75] Malik R, Chauhan G, Traylor M, et al. Multiancestry genome-wide association study of 520,000 subjects identifies 32 loci associated with stroke and stroke subtypes. Nat Genet 2018;50(4):524–37.