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Research paper

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

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ABSTRACT

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 (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.

List of abbreviations

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

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

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Research in context

Evidence before this study

We searched PubMed for articles on Alzheimer's disease (AD) published between January 1, 1960 and August 28, 2018 using the terms: ("Alzheimer's disease" AND genetics AND GWAS) to identify AD genes; and ("Alzheimer's disease" AND "drug therapy") to identify AD treatments. We screened articles by title and abstract to locate full-text articles relevant to the study aims, supplemented by reference lists of the relevant articles. We considered articles that examined the associations of genetic variants or genes with late-onset AD, or evaluated existing or investigational AD drugs. AD drug development has encountered limited efficacy of existing drugs and repeated expensive failures of investigational drugs. Genetic validation of drug targets may provide the impetus to rethink effective drug treatments for late-onset AD. Genetic variants related to late-onset AD have been extensively identified from genome-wide association studies (GWAS); however, late-onset AD genes, rather than loci, are less studied. Only two gene-based studies of late-onset AD have been published. One study in people of European ancestry found two new genes and one trans-ethnic study found another new gene. However, none of these gene-based studies compared with AD drug targets. Only one study compared genetic loci from previous GWAS with targets of approved AD drugs and found no overlaps. No studies have considered whether products of late-onset AD genes are targets of numerous on-going investigational AD drugs or whether any late-onset AD gene products are targeted by drugs for other indications.

Added value of this study

We revisited the late-onset AD genes of European ancestry using a new gene-based association analysis and compared them with known late-onset AD genes indicated by loci from previous GWAS. We then compared all these late-onset AD gene products with targets of currently approved or investigational AD drugs and identified drugs for other indications targeting any late-onset AD gene products. This gene-based association analysis of late-onset AD replicated 12 genes found by previous GWAS and additionally identified 39 genes. We did not replicate 3 genes found by the two previous gene-based studies, perhaps partly due to population-specific relevance of particular genes. Moreover, this study found no evidence that approved AD drugs or AD drugs in Phase III trials are targeting the products of genes strongly associated with late-onset AD, consistent with a previous study comparing AD genetic loci with approved AD drugs, although false negative findings due to incomplete knowledge remains possible. Among all 67 late-onset AD genes from this gene-based study and previous GWAS, 11 genes are targets of other existing drugs, which could possibly be further scrutinized for appropriate alignment of disease genetics and drug actions before re-purposing.

Implications of all the available evidence

This study forms an initial step in translating genetic evidence to AD drug development. It also highlights the importance of genetic validation of investigational AD drugs as an initial screening tool to identify promising late-onset AD drug.

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 use 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 reaching 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 ClinicalTrial.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. (2016) [32], excluding diagnostic drugs and drugs for sequelae of AD, such as for 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<.000002, i.e., .05/25000 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, EPHA1, SORL1, ABCA7, PTK2B, CD33, CD2AP)* identified by both approaches. A total of 16 genes (*SLC24A4-RIN3*, *CELF1*, *CASS4*, *TRIP4*, *ZCWPW1*, *HLA-DRB5-HLA-DRB1*, *FERMT2*, *NME8*, *INPP5D*, *TREM2*, *TREML2*, *MEF2C*, *ACE*, *APP*, *PLD3*, *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*), carcinoembryonic 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-DQA1*, *HLA-DRB5-HLA-DRB1*), membrane spanning 4-domains (*MS4A6E, MS4A4A, MS4A6A, MS4A4E, MS4A2*), 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 = .0001 which indicates our results unlikely occurred by chance. These other drugs targeting products of these 11 genes *(ACE, APP, APOE, CD33, CLU, EPHA1, HBEGF, HLA-DQA1, HLA-DRB1, MS4A2, PTK2B)* including Gemtuzumab ozogamicin which targets *CD33* gene products, Omalizumab which targets *MS4A2* 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 (*TP53INP1, 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 1 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 genes. 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.

Table 1
Genes associated with late-onset Alzheimer's disease[a] identified from gene-based association test and/or single nucleotide polymorphism (SNP)-based genome-wide association studies (GWAS).

Chromosome	Gene-based test + SNP-based GWAS			Gene-based test[b]			SNP-based GWAS		
	Position	Gene	*P*-value	Position	Gene	*P*-value	Position	Gene	*P*-value
1	207,669,472	*CR1*	2.25×10^{-13}	207,818,457	*CR1L*	3.01×10^{-08}			
2	*127,805,598*	*BIN1*	2.86×10^{-14}				*234,068,476*	*INPP5D*	1.21×10^{-03}
5				139,712,427	*HBEGF*	1.83×10^{-06}	*88,223,420*	*MEF2C*	0.01
6	*47,445,524*	*CD2AP*	1.76×10^{-06}	32,605,182	*HLA-DQA1*	9.36×10^{-07}	*32,578,530*	*HLA-DRB5-HLA-DRB1*	8.77×10^{-05}
							41,129,252	*TREM2*	5.09×10^{-03}
							41,154,650	*TREML2*	9.98×10^{-03}
7	*143,088,204*	*EPHA1*	7.57×10^{-10}	143,104,905	*EPHA1-AS1*	1.92×10^{-09}	*100,004,446*	*ZCWPW1*	8.56×10^{-05}
							37,841,534	*NME8*	3.70×10^{-04}
8	*27,454,433*	*CLU*	5.91×10^{-16}	27,468,117	*MIR6843*	6.39×10^{-16}			
	27,183,080	*PTK2B*	3.66×10^{-07}						
11	*85,668,213*	*PICALM*	9.31×10^{-11}	60,102,354	*MS4A6E*	9.71×10^{-12}	*47,557,871*	*CELF1*	2.90×10^{-05}
	59,939,970	*MS4A6A*	6.24×10^{-10}	60,048,013	*MS4A4A*	1.04×10^{-10}			
	121,322,911	*SORL1*	5.25×10^{-09}	59,968,725	*MS4A4E*	1.59×10^{-09}			
				59,976,543	*MIR6503*	1.76×10^{-09}			
				59,856,136	*MS4A2*	4.47×10^{-09}			
14							*92,926,952*	*SLC24A4-RIN3*	6.71×10^{-06}
							53,400,629	*FERMT2*	3.50×10^{-04}
15							*64,725,490*	*TRIP4*	4.79×10^{-05}
17							*61,538,148*	*ACE*	0.01
18							*29,088,958*	*DSG2*	0.98
19	*45,409,657*	*APOE*	0	45,417,811	*APOC1*	0	*40,877,595*	*PLD3*	0.90
	1,040,101	*ABCA7*	2.12×10^{-07}	45,430,059	*APOC1P1*	0			
	51,728,334	*CD33*	8.09×10^{-07}	45,349,392	*NECTIN2*	0			
				45,394,476	*TOMM40*	0			
				45,312,315	*BCAM*	7.50×10^{-68}			
				45,281,125	*CBLC*	3.09×10^{-42}			
				45,251,977	*BCL3*	9.04×10^{-42}			
				45,653,007	*NKPD1*	5.26×10^{-20}			
				45,458,480	*CLPTM1*	5.75×10^{-20}			
				45,596,430	*PPP1R37*	8.07×10^{-20}			
				45,666,185	*TRAPPC6A*	2.78×10^{-18}			
				45,682,002	*BLOC1S3*	2.78×10^{-17}			
				45,504,706	*RELB*	5.31×10^{-17}			
				45,449,238	*APOC2*	6.67×10^{-16}			
				45,542,297	*CLASRP*	1.13×10^{-15}			
				45,445,494	*APOC4*	2.91×10^{-15}			
				45,445,494	*APOC4-APOC2*	3.38×10^{-15}			
				45,715,634	*EXOC3L2*	9.26×10^{-14}			
				45,147,097	*PVR*	2.53×10^{-12}			
				45,588,586	*LOC105372419*	9.04×10^{-12}			
				45,583,162	*GEMIN7*	1.17×10^{-11}			
				45,202,420	*CEACAM16*	3.97×10^{-11}			
				45,174,723	*CEACAM19*	1.58×10^{-09}			
				45,156,955	*MIR4531*	3.97×10^{-09}			
				45,116,939	*IGSF23*	4.25×10^{-08}			
				1,076,632	*ARHGAP45*	8.21×10^{-08}			
				45,041,044	*CEACAM22P*	9.34×10^{-08}			
				46,213,886	*FBXO46*	1.23×10^{-06}			
				46,236,508	*BHMG1*	1.30×10^{-06}			
20							*55,018,260*	*CASS4*	3.60×10^{-05}
21							*27,269,932*	*APP*	0.08

Abbreviations: GWAS: genome wide association studies; SNPs: single nucleotide polymorphisms.
[a] All gene *P*-values were based on gene-based association test.
[b] Among the 39 genes newly identified from the gene-based test, 4 genes were in strong linkage disequilibrium with previously known genes (previous gene vs. new gene with r2: *CLU* vs. *MIR6843* (0.875); *MS4A6A* vs. *MS4A2* (0.844); *MS4A6A* vs. *MIR6503* (0.844) and *ABCA7* vs. *ARHGAP45* (0.967).

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 and tau proteins are biomarkers [15] or underlying causal targets [18]. The exact reasons for such an exceptionally high failure rate remains elusive, but several explanations have been proposed including: anti-Aβ trials have not reported changes in cerebrospinal fluid Aβ [40], results from animal models may not be comparable to human trials [41], clearance of existing Aβ protein may be as important as reduction in Aβ generation [42], and off-target pathways

Table 2
Genes with products targeted[a] by approved or investigational Alzheimer's disease drugs[b].

Type	Drug	Target Gene	Gene *P*-value	Class
Approved drugs	Donepezil	*ACHE*	0.65	Acetylcholinesterase inhibitor
		HTR2A	0.03	
	Rivastigmine	*ACHE*	0.65	Acetylcholinesterase inhibitor
		BCHE	0.73	
	Galantamine	*ACHE*	0.65	Acetylcholinesterase inhibitor
		CHRNA1-A8;A10	0.22	
		CHRNB1-B4	0.77	
		CHRND	0.89	
		CHRNE	0.0003	
		CHRNG	0.58	
		BCHE	0.73	
	Memantine	*CHRNA7*	0.72	*N*-methyl-D-aspartate receptor antagonist
		CYP2E1	0.94	
		DRD2	0.23	
		GRIN1	0.97	
		GRIN2A/2B	0.50	
		GRIN3A	0.85	
		HTR3A	0.24	
Investigational drugs	Aducanumab	Nil	–	Anti-amyloid
	ALZT-OP1a/b	*ASIC1*	0.28	Anti-amyloid
		BCL2	0.56	Anti-inflammation
		CFTR	0.98	
		FABP2	0.99	
		KCNMA1	0.59	
		S100P	0.22	
		SLC15A1/A8	0.40	
		PLAT	0.57	
		PPARA	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
		PPARA	0.97	Anti-inflammation
		PPARD	0.50	
		PPARG	0.72	
		TRPM3	0.27	
		MAOB	--	
	Intepirdine	Nil	–	Other neurotransmitters (Selective serotonin 5-HT_6 receptor antagonist)
	Solanezumab	Nil	–	Anti-amyloid
	TRx0237	Nil	–	Anti-tau
	Verubecestat	*BACE1*	–	Anti-amyloid
	Guanfacine	*ADRA2A-2C*	0.16	Other neurotransmitters (Attention Deficit Hyperactivity Disorder drug)
		CYP3A5	0.38	
		HCN1/4	0.62	
	Insulin (Humulin®RU-10)	*INSR*	0.83	Intranasal insulin
		IGF1R	0.78	
		RB1	0.26	
		CTSD	0.11	
		IDE	0.45	
		PCSK1/2	0.41	
		CPE	0.09	
		NOV	0.30	
		LRP2	0.40	
		IGFBP7	0.70	
		SYTL4	--	
	Lanabecestat	Nil	–	Anti-amyloid
	Sodium oligomannurarate	Nil	–	Anti-amyloid (Oligosaccharide)
	Docosahexaenoic acid	*PPARA*	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	
		GRIA3	--	

(continued on next page)

Table 2 (*continued*)

Type	Drug	Target Gene	Gene *P*-value	Class
	Resveratrol	*NQO2*	0.97	Anti-inflammation (herpes simplex virus)
		CSNK2A1	0.29	
		PTGS1/2	0.43	
		ALOX5/15	0.06	
		AHR	0.75	
		PI4K2B	0.99	
		ITGA5	0.44	
		ITGB3	0.51	
		APP	0.08	
		ESR1	0.13	
		MTNR1A/1B	0.20	
		CLEC14A	0.93	
		NR1I2/3	0.16	
		SLC2A1	0.77	
		SNCA	0.91	
		CBR1	0.67	
		PPARA	0.97	
		PPARG	0.72	
		AKT1	0.26	
		KHSRP	0.64	
		YARS	0.91	
		APOA1	0.27	
		BACE1	0.25	
		SCN5A	0.82	
		SIRT1	0.71	
		TXNRD1/D2	0.75	
		XDH	0.56	
		MAOA	--	
	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	
		CA1	0.35	
		SMPD1	0.96	
	Choline alfoscerate	*GM2A*	0.63	Other (glycerophosphocholines)
	Ginkgo biloba	*SLC6A2*	0.06	Other (possibly improving blood flow)
		PLA2G2A	0.90	
		GLRA1	0.57	
		GABRA1	0.45	
		GABRB2	0.81	
		GABRG2	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.

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

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	*HBEGF*	1.83×10^{-06}	Anti-cancer EGFR inhibitors
Brigatinib			
Cetuximab			
Erlotinib			
Gefitinib			
Lapatinib			
Necitumumab			
Neratinib			
Olmutinib			
Osimertinib			
Panitumumab			
Glatiramer acetate	*HLA-DQA1*	9.36×10^{-07}	Immunomodulator for multiple sclerosis
Apolizumab	*HLA-DRB1*	8.77×10^{-05}	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^{-07}	Anti-Rheumatoid JAK inhibitor
Leflunomide			
Omalizumab	*MS4A2*	4.47×10^{-09}	Anti-IgE for severe allergic asthma
Alacepril	*ACE*	0.01	Anti-hypertensive ACE inhibitors
Benazepril			
Benazeprilat			
Captopril			
Ceronapril			
Cilazapril			
Delapril			
Deserpidine			
Enalapril			
Enalaprilat			
Fosinopril			
Fosinoprilat			
Gemopatrilat			
Imidapril			
Indolapril hydrochloride			
Libenzapril			
Lisinopril			
Moexipril			
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 ozogamicin	*CD33*	8.09×10^{-07}	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 Aβ 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 (*AHR*, *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 links 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 subnetworks [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, Interferons, Calcium, Dovitinib, Somatropin Recombinant) 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 comparison of expression 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$<1 \times 10^{-5}$ 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*, *MS4A4A*, *MS4A6E*, *MS4A2*) 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.

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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.

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