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Towards precision medicine in Alzheimer's disease: deciphering genetic data to establish informative biomarkers

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Abstract

Introduction—Developing biomarker tools for identification of individuals at high-risk for late-onset Alzheimer's disease (LOAD) is important for prognosis and early treatment. This review focuses on genetic factors and their potential role for precision medicine in LOAD.

Areas covered—*APOE*e4 is the strongest genetic risk factor for non-Mendelian LOAD, and the *APOE*-linkage disequilibrium (LD) region has produced the most significant association signal in multi-center genome-wide-association-studies (GWAS). Consideration of extended haplotypes in the *APOE*-LD region and specifically, non-coding variants in putative enhancer elements, such as the *TOMM40*-polyT, in-addition to the coding variants that comprise the *APOE*-genotypes, may be useful for predicting subjects at high-risk of developing LOAD and estimating age-of-onset of early disease-stage symptoms. A genetic-biomarker based on *APOE–TOMM40*-polyT haplotypes, and age is currently applied in a clinical trial for prevention/delay of LOAD onset. Additionally, we discuss LOAD-GWAS discoveries and the development of new genetic risk scores based on LOAD-GWAS findings other than the *APOE*-LD region.

Expert Commentary—Deciphering the precise causal genetic-variants within LOAD-GWAS regions will advance the development of genetic-biomarkers to complement and refine the *APOE*-LD region based prediction model. Collectively, the genetic-biomarkers will be translational for early diagnosis and enrichment of clinical trials with subjects at high-risk.

Keywords

APOE; genetic-based LOAD prediction models; genetic biomarkers; Late-Onset Alzheimer's disease (LOAD); precision medicine; *TOMM40* poly-T

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1. Introduction

An ultimate goal for precision medicine is to develop and identify personalized disease treatment and prevention approaches considering individual variability in genetics, environmental and lifestyle factors. Gaining better insight into the biological pathways and molecular basis underlying disease etiology will advance the potential role of precision medicine. For many human complex diseases (*i.e.* don't exhibit Mendelian inheritance pattern) of aging, including late-onset Alzheimer's disease (LOAD), it is clear that family history is associated with increased risk; however, understanding the precise genetic variants, target genes and specific biological pathways contributing to disease risk has greatly lagged the discovery of disease-associated Single Nucleotide Polymorphisms (SNPs) from genome-wide association studies (GWAS).

The two strongest risk factors for the most common form of Alzheimer's disease, non-Mendelian LOAD, are age [1] followed by *APOE* genotype[2]. Recently, epidemiological, biological and clinical studies have pointed to sex and gender differences in cognitive decline and the progression of LOAD [3, 4, 5]. Increasingly, it is clear that the risk of LOAD and the associated genetic loci must be interpreted in a framework of age-dependent risk and sex-specific risk of the disease.

In this paper, we focus on genetic factors and their potential role for precision medicine in LOAD. Developing biomarkers for precise identification of subjects at high-risk for LOAD is important for prognosis and early intervention. Here, we review the discovery of genetic markers of LOAD risk that provide a greater level of precision and accuracy for the early prediction of LOAD and mild cognitive impairment (MCI) due to AD and the impact of these markers on precision diagnosis for LOAD. These genetic markers can be assayed using DNA from a simple blood draw and used for prediction of LOAD-risk either alone or in conjunction with neuroimaging, cerebrospinal fluid (CSF), clinical, neuropsychological, and metabolomics biomarkers. As *APOE* is the strongest genetic risk factor for non-Mendelian LOAD, we primarily describe a comprehensive characterization of genetic variation in the *APOE* linkage disequilibrium (LD) region of the human genome and in particular within the neighboring *TOMM40* gene. We suggest that consideration of extended haplotypes in this region of the genome and specifically non-coding variants in putative enhancer elements, in addition to the coding variants that comprise the *APOE* genotype, may be useful for predicting who is at risk of developing LOAD and estimating age of onset of early disease-stage symptoms. Supporting this premise are a number of clinical studies that have examined episodic memory and brain imaging changes associated with the manifestation of early-stage LOAD. In addition, we include a discussion of the development of new genetic risk score models based on LOAD-GWAS findings other than the *APOE* region. We conclude with highlighting the application of genetic risk prediction tools for stratification and enrichment of clinical trials for prevention and/or delay the onset of LOAD with individuals at high-risk.

2. *APOE* – the first and most firmly established genetic risk locus for LOAD

Over 20 years ago, genetic linkage analysis of pedigrees with familial Alzheimer's disease identified a disease-associated region on chromosome 19[6]. Over the ensuing years, it has been demonstrated that the e4 allele of *APOE* gene, within the chromosome 19 linkage region, is the strongest and most highly replicated genetic risk factor for LOAD, is associated with lower age of clinical disease onset, and with development of amyloid plaques, a pathological hallmark of AD[7, 8, 9, 10]. The *APOE* alleles are defined by two coding SNPs (rs429358 and rs7412) that are located in exon 4 of the *APOE* gene and present in humans three forms, e2, e3 and e4. The protein coding consequences of these SNPs are to change two amino acids in the protein: Cys130Arg (rs429358) and Arg176Cys (rs7412). Age of onset (AOO) Kaplan-Meier (KM) curves for the development of LOAD stratified by *APOE* genotype are shown in Figure 1. The *APOE* e3/3 genotype is often considered the reference or "neutral" genotype. *APOE* e4 alleles contribute a dose-dependent increase in risk and a shift of the KM curves to earlier ages of disease onset. *APOE* e2 containing genotypes are associated with later ages of LOAD onset compared with genotypes that carry only e3 or e4 *APOE* alleles.

There is a body of work that explores how *APOE* isoforms differentially affect a number of processes that lead to development of AD-related pathologies[2, 11, 12]. While these examples directly change the nature of the encoded proteins, it is also possible that regulatory variants within the *APOE* LD region that affect gene expression or splicing may be directly related to the pathophysiology of LOAD [13, 14, 15]. Common variants in genomic regulatory elements that affect gene expression, transcript splicing, or epigenomic modification may also modify the phenotypic expression of LOAD (*e.g.* severity and AOO).

3. LOAD Genetics post *APOE* discovery

3.1 The GWAS era

GWAS have produced an extensive list of genetic variants associated with LOAD[16, 17, 18]. However, the translation of these data into biochemical targets for drugs development has not followed. Moreover, these studies have not provided significantly better prediction of risk or age of onset of LOAD than does *APOE* genotype alone [19]. The GWAS studies have provided valuable information on biological pathways involved in the etiology of AD, including cholesterol metabolism, immune response, neuroinflammation and endocytosis[20, 21].

3.2. Phylogenetic Analysis to identify *TOMM40* poly-T as a risk locus for LOAD

GWAS of LOAD produced genetic variants with relatively low odds ratios (typically 1.1 – 1.3) that have been followed up by examining proximal genes, conducting computational analysis of enriched biological pathways or whole-genome/whole exome sequencing to identify rare variants. In contrast, as a consequence of the high LD present in the *APOE-TOMM40* region which has an extremely high association with LOAD, the identification of genetic variants in this region that modify LOAD age of onset or risk requires a different approach that allows multiple variants within an LD region to contribute to the association

with the phenotype. Such general strategy constitutes of three major steps, a search using bioinformatics tools to find and annotate highly polymorphic structural variants such as simple sequence repeats (SSRs) and homopolymers followed by phase sequencing of the region that contains these variants and phylogenetic analyses of the sequence data[22]. An important aspect of this general strategy is to fully characterize the defined genomic region and consider single variants as well as long-range haplotypes and interactions between variants. The strengths of this general approach are that sequencing captures broadly defined structural variants[23] (including insertion/deletions, inversions, homopolymers and other repeated sequences) in addition to SNPs and that haplotype phase is known with certainty. This methodology is likely to be particularly applicable to the genetics of phenotypes that result from *cis*-acting alleles, each with relatively small effect size and collectively adding to a larger effect size. From a mechanistic standpoint, this strategy is suitable for identification of variants that impact gene regulation or genomic structure or tag other variants that affect regulatory functions[24]. Phylogenetic approaches are used to interrogate regions of interest previously highlighted by genetic association studies (e.g. linkage analysis or GWAS) and are particularly relevant for regions that exhibit high LD. A recent review by Templeton presents the rationale, limitations and illustrative examples for using phylogenetic methods for genotype/phenotype associations[22]. Detailed examples of specific methods are covered in several publications[25, 26, 27]. For regions of weaker LD, methods based on analysis of phylogenetic network analysis are needed [28] since recombination will limit the applicability of phylogenetic analysis.

Roses *et al.* employed phased sequencing coupled with phylogenetic analysis to identify variants associated with LOAD-risk within the high LD region at chromosome 19 proximal to *APOE,* a genomic region that has repeatedly shown the most significant, by a wide margin, LOAD-GWA signal [29]. Briefly, the *TOMM40-APOE* region of both chromosomes from all subjects was cloned and sequenced, so that the phase of all variants was precisely known, not inferred, and all types of variants were included in the analysis [30]. The sequences, including all distinguishing variants, were then used to construct a phylogenetic, or more appropriately a genealogical, tree. A number of methods, including Bayesian and maximum parsimony, were used to estimate the possible genealogies for the *TOMM40-APOE* region. Combinations of variants that separate clades of the tree were identified and tested for association with the phenotype of interest. The *TOMM40-APOE* genomic region is a fairly unique case in that it is a region of very strong LD, is populated with relatively abundant polymorphisms, and contains large effect-size, causal alleles in *APOE* [29]. This study resulted in the identification of the rs10524523 (‘523) locus, a highly polymorphic poly-T repeat tract positioned in intron 6 of the *TOMM40* gene[29] (hereafter *TOMM40* poly-T) and subsequent work demonstrated that this *TOMM40* poly-T improve the precision of age-of-onset (AOO) prediction for LOAD [31].

The use of haplotype-based methods, including clustering based on evolutionarily-informed haplotypes, is currently limited by the size of a region of the genome that can be sequenced at a reasonable cost. In the discovery of *TOMM40* Poly-T, Roses et al. overcame this challenge by conducting phased sequencing by cloning long-range PCR products and sequencing multiple clones per individual using the traditional Sanger method [29]. However, this approach is very expensive to carry out on a genome wide scale. Newer next

generation sequencing technologies have made it possible to sequence entire human genomes; for example the 1000 Genomes Project has sequenced and analyzed over 1000 individual human genomes and made the data publicly available. While access to this data enables analysis of rare and structural variants in phenotype association studies, the phase of the polymorphisms on each of the two chromosomes must still be statistically inferred, *i.e.* the content of variants unique to each of the two homologous chromosomes, or diplotype, is usually not directly observed.

4. Clinical and biological implications of the role of *TOMM40* poly-T in LOAD

Three allele groups were defined for the *TOMM40* poly-T polymorphism, based on the modes of the distributions of the number of 'T'-residues: 'Short' (S, T≤19), 'Long' (L, 20≤T≤29) and 'Very Long' (VL, T≥30). The haplotypic relationship between the *TOMM40* poly-T polymorphism and *APOE* SNPs in different populations [32, 33] and the association between specific *TOMM40* poly-T – *APOE* haplotypes and age of onset of LOAD has been described in several publications[31, 34]. The Kaplan-Meier (KM) curves illustrated in Figure 2 represent a retrospective stratification by *TOMM40* poly-T genotype of the age of LOAD onset. These KM curves were constructed using genotypic and AOO data for Caucasian individuals followed longitudinally at the Joseph & Kathleen Bryan Alzheimer's Disease Research Center at Duke University [32, 33]. Subjects were tested over time with a battery of neuropsychological tests to monitor cognitive changes and to diagnose onset of cognitive impairment and probable AD dementia. The L/L curve in Figure 2 corresponds closely to *APOE* e4/4 since 98% of all *APOE* e4 alleles are linked to *TOMM40* poly-T 'L' in Caucasians. In this particular cohort, 50% of the L/L subjects were diagnosed with probable AD by age 76. By the age of 78 years, 50% of subjects in this cohort with the L/VL genotype developed cognitive impairment whereas, in general, L/S subjects developed cognitive impairment at later ages (50% had clinical symptoms by age 81). L/S and L/VL genotypes are also *APOE* e3/4; therefore, stratification by *TOMM40* poly-T genotype gave two, separable age-of-onset (AOO) curves for *APOE* e3/4 subjects and provided more granularity to the age of disease onset distributions. Similarly, instead of one curve for *APOE* e3 homozygotes, three distinct curves, for S/S, S/VL and VL/VL, were resolved thus creating three different AOO risk groups. As with *APOE* e3, *APOE* e2 may be linked to an S or a VL allele. Carriage of at least one allele of *APOE* e2 appears to result in later AOO[35], regardless of the *TOMM40* poly-T allele in the haplotype, although AOO may be modified by *APOE* e4 (or an L allele) in the genotype. In conclusion, the combination of the *TOMM40* poly-T – *APOE* haplotypes provides a more precise estimation of LOAD AOO compared to AOO estimations based on *APOE* status solely.

The *TOMM40* poly-T polymorphism has also been associated with performance in the recall of words from the primacy portion of a long word list, a cognitive deficiency noted in the early manifestation of AD, in cognitively healthy individuals in midlife[36]. An association was also reported between the S allele and improved memory and executive function into very old age in *APOE* e3/3 cognitively healthy elderly people[37]. Furthermore, the *TOMM40* poly-T locus was also associated with gray matter volume in regions of the brain affected early in the development of AD in *APOE* e3/3, cognitively normal, middle-age people [36]. A recent study based on data from the Religious Orders Study and Rush

Memory and Aging Project reported an association of *APOE* e3/3-*TOMM40* poly-T haplotypes with cognitive decline in community based older persons[38]. The large sample size (N=1170) and long-term follow up (up to 21 years) were critical factors in demonstrating that there was an *APOE*-independent effect of the *TOMM40* poly-T locus on cognitive decline by limiting the analysis cohort to *APOE* e3/3 individuals thus removing the confounding effects of *APOE* e4 or e2 alleles. The results showed that subjects with *TOMM40* poly-T-S/S had faster decline in global cognition than subjects with *TOMM40* poly-T-S/VL or VL/VL (p=0.002). The same association was observed for episodic memory (p=0.0004) and semantic memory (p=0.003), but not for working memory, processing speed or visuospatial ability (all p>.05). Collectively, these studies provided genetic evidence for the contribution of *TOMM40* poly-T locus to LOAD and related endophenotypes. However, other studies reported conflicting data and did not replicate the reported association of *TOMM40* poly-T with LOAD risk and AOO [39, 40, 41].

The biochemical mechanism by which the *TOMM40* poly-T variant affects AD pathophysiology and biochemistry is not clear, however, it is different from the effects of *APOE* status. The *TOMM40* gene encodes the Tom40 protein, dimers of which create the pore in the outer mitochondrial membrane through which almost all proteins enter mitochondria. The protein is essential for mitochondrial biogenesis and function [42]. Structural DNA variations, especially those in intronic or intergenic regions like the *TOMM40* poly-T variant, most likely exert their effects on phenotype by altering gene transcription efficiency, the timing of transcription, transcript stability, transcript splicing, or possibly by changing patterns of epigenomic modification[43, 44, 45, 46, 47, 48] [49]. There is support for the idea that short structural variants, rather than or in addition to SNPs, may be responsible for many human complex traits[50, 51, 52]. It has been demonstrated that *TOMM40* poly-T affects the expression levels of *APOE* and *TOMM40* mRNAs in the temporal and occipital cortexes of LOAD patients and normal controls[32], and the effect of the *TOMM40* poly-T variation on transcription regulation was recapitulated in a cell-based luciferase reporting system[32] [53]. Gene expression studies suggest that the number of protein import channels per mitochondrion may be regulated by *TOMM40* poly-T variants. Bekris *et al.* described a complex transcriptional regulatory region for *TOMM40* and *APOE* expression that extends throughout both genes and is influenced by multiple polymorphisms including the *TOMM40* poly-T locus[54]. It has been suggested that a relatively modest change in mRNA expression may produce pathology that accumulate over time and is expressed clinically in later age. Changes in levels of the Tom40 protein have also been detected in the brains of Parkinson's disease patients, and in a mouse model of Parkinson's disease[55], suggesting that expression of the Tom40 protein may have a broader impact on a spectrum of neurological diseases in aging[56].

5. Developing LOAD prediction models

Utilization of any biomarker is dependent not only on its performance characteristics (NPV, PPV) but also by the intended application (*e.g.* clinical trial enrichment, diagnostic use in a clinical setting companion diagnostic to a therapeutic) often referred to as "*fit for purpose*". In addition to the test performance (specificity, sensitivity), features like availability of specialized reagents, instruments and/or qualified personnel, willingness of subjects to be

tested, invasiveness, cost/reimbursement and consideration of the clinical utility in the practice of medicine contribute to the use of any given test. The usage of a biomarker to enrich a clinical trial in normal subjects at risk for conversion to disease symptoms needs to consider how that test would be employed in the practice of medicine in a global environment.

Combinations of biomarkers could conceivably be used to further refine selection of subjects for clinical trials, balancing cost and invasiveness of the assay with the improvement in accuracy of conversion prediction in a pre-specified time frame. There is strong precedent for the combination of *APOE*, CSF, imaging biomarkers and neurocognitive measures to improve predictive accuracy [57], and recent work using the Biomarkers for Older Controls at Risk for Dementia (BIOCARD) cohort [58, 59], with 20 years of longitudinal follow up has provided promising results [60]. Specifically, for prediction of the onset of MCI in a five-year time frame, the combination of six measures provided the best performance: Two memory and thinking tests (the Digit Symbol and Paired Associates Immediate Recall tests), levels of CSF amyloid beta and p-tau, two measures of MRI brain scan – one to assess the thickness of the right entorhinal cortex and another to measure the volume of the hippocampus. Accuracy of prediction was reported as: area under the receiver operating characteristic curve (AUC)=0.89, sensitivity = 0.85, specificity = 0.70 [60].

Genetics-based risk biomarkers rely on genomic DNA readily collected from a blood draw, using robust, widely available inexpensive DNA testing of an analyte that is unaffected by environmental, disease state or other conditions. A two-stage process is also possible for specific settings, where the genetic biomarkers would be used to initially screen subjects for a prevention trial using an inexpensive blood test followed by CSF and/or imaging biomarkers for further screening of individuals.

5.1 Prediction of LOAD risk based on GWAS findings

Recently, two studies used genotype data from the International Genomics of Alzheimer's Project (IGAP) to investigate the accuracy of LOAD prediction models based on risk alleles identified in GWAS. Escott-Price *et al.* conducted a polygenic score analysis to test whether the alleles identified to associate with LOAD in one sample set were significantly enriched in the cases relative to the controls in an independent sample, and found a significant enrichment for a polygenic component in LOAD [61]. They further showed that best prediction accuracy, AUC = 78.2%, was achieved by a logistic regression model with *APOE*, the polygenic score, sex and age as predictors. Another study constructed a genetic risk score (GRS) using top 19 GWAS SNPs and evaluate its capacity to improve prediction of LOAD-risk in prospective cohorts. The GRS was associated with a 17% increase in risk of developing LOAD. This study showed a small improvement in LOAD risk prediction when adding the GRS to age, sex, *APOE*ε4 and education [62]. Overall, these studies demonstrated that the LOAD-GWAS based genetic predictors have utility alongside other models based on traditional LOAD predictors, however, their inclusion provided only a minor improvement in LOAD-risk prediction.

5.2 Prediction of LOAD risk based on *TOMM40* poly-T – *APOE* haplotypes and age

A simple, robust genetics-based biomarker risk algorithm (GBRA) utilizing a combination of *APOE* genotype, *TOMM40* poly-T genotypes and age has been developed as a prognostic tool for assessing LOAD age of onset in asymptomatic people [31, 63]. Figure 3 summarizes the principles of the LOAD-GBRA algorithm. The positive predictive values (PPV) and negative predictive values (NPV) of the GBRA are in the range of 70–80%. The relatively high odds ratio (approximately 3–5) comparing the GBRA to predictive models based on *APOE* and age alone support the value of the GBRA in risk prediction for MCI due to LOAD. In addition, the GBRA "high" and "low" LOAD-risk categorizations correlated well with pathological CSF biomarker levels, Positron Emission Tomography (PET) amyloid burden and neurocognitive scores. For example, for the data from the Alzheimer's Disease Neuroimaging initiative (ADNI), subjects predicted to be high risk by the GBRA showed a significantly ($p < 0.0001$) lower mean level of CSF Aβ1-42 (157.15±3.78) in comparison to subjects predicted to be low risk (195.59±4.67); subjects predicted to be high risk by the GBRA showed a significantly ($p < 0.0001$) higher (worse) Standard Uptake Value ratio (SUVR) measurement of amyloid burden (mean SUVR 1.90±0.06, n=40) in comparison to subjects predicted to be low risk (mean SUVR 1.52±0.07, n=30)[63].

Receiver operating curves and comparative analysis of area under the curve (AUC) showed that the combinations of *APOE* genotype, *TOMM40* poly-T genotype and age outperformed age in sensitivity and specificity for risk prediction for MCI due to LOAD. For a well-characterized cohort of individuals with LOAD, MCI and cognitively normal controls followed at the Duke Bryan Alzheimer's Disease Research, the GBRA outperformed age and either genotype (*APOE* or *TOMM40*) in terms of sensitivity and specificity[63]. For the replication ADNI cohort, the performance of age combined with either or both genotypes was equivalent. For both cohorts, the combination of age and *APOE* genotype provided slightly better performance than age and *TOMM40* poly-T genotype, although the difference was not statistically significant[63]. It was surprising that the AUC for the GBRA did not show a statistically-significant improvement for the ADNI cohort when compared with the combination of *APOE* genotype and age considering that age interaction terms with genotype were highly statistically-significant ($p<0.01$)[63]. Testing in additional replication cohorts from longitudinal observational studies with more precise estimation of age of onset of MCI/AD will be needed to more clearly define the performance of the GBRA.

6. Application of the *TOMM40* poly-T – *APOE* haplotypes in Clinical Trial

The development of genetic biomarkers for precise identification of subjects at high-risk to develop LOAD will permit preclinical intervention and improve prognosis. Another important application for genetic risk prediction tools is for enrichment and stratification of clinical trials for individuals with high-risk. This approach is particularly relevant for prevention and/or delay the onset trails. The ultimate proof of the value of including *TOMM40* poly-T in addition to *APOE* variants for risk prediction (GBRA summarizes in Figure 3) will come from the TOMMORROW clinical trial, a pharmacogenetically-enriched, double-blind, delay-of-disease-onset clinical trial of cognitively normal subjects aged 65–83, inclusive, classified as having high or low risk for development of cognitive symptoms over

the course of a 5-year study (TOMMORROW trial; ClinicalTrials.gov Identifier=NCT01931566, [34]). In this study, high risk subjects are randomized to active therapy (low-dose pioglitazone) or placebo; low risk subjects are randomized to placebo only. Stratification for risk of developing MCI due to AD during the study, prior to randomization is accomplished with the GBRA at the beginning of the study (when neuropsychological testing verifies normal cognition). The GBRA will be qualified for use as a prognostic biomarker at the end of the phase 3 trial when the performance characteristics and Receiver Operating Curve (ROC) curves can be calculated from the trial data which will provide a large (n>3,000), prospectively sampled cohort. Once qualified, the biomarker can be used as a companion pharmacogenetics test for a therapeutic to delay the onset of LOAD. While the GBRA was developed as a binary predictive algorithm for a delay-of-disease-onset clinical trial of cognitively normal subjects to high and low risk groups, the algorithm could also be adapted to a continuous scale based on likelihood of conversion within a pre-specified time frame.

7. Conclusion

The *APOE* status was associated with LOAD age-of-onset and stratification by *TOMM40* poly-T genotype provides a more precise estimation of LOAD AOO compared to AOO estimations based on *APOE* status solely. A genetics-based biomarker risk algorithm for LOAD based on the *APOE–TOMM40* poly-T haplotypes, and age was constructed and has been applied in the TOMMORROW study, a clinical trial for prevention and/or delay the onset of LOAD, for enrichment with individuals at high-risk. The utility of the *APOE–TOMM40* poly-T based genetic biomarker will be validate at the end of phase 3 of the TOMMORROW clinical trial. GWAS have identified over 20 genomic regions associated with LOAD in addition to the *APOE* LD region. New genetic risk scores based on LOAD-GWAS have been developed and demonstrated utility alongside other models based on traditional LOAD predictors, however, their inclusion provided only a minor improvement in LOAD-risk prediction compare to models based only on traditional LOAD predictors – age, sex, *APOE*e4. A better understanding of the genetic factors underpinning the etiology of LOAD may provide new informative genetic variants for integration in genetic based disease-risk models that will improve the precision of LOAD-risk prediction.

8. Expert Commentary

Lack of effective therapies for Alzheimer’s stems from poor understanding of the disease’s etiology. Multiple etiologies and age-related prodromal processes contribute to the pathophysiology of LOAD, which highlights the significance of precision medicine in LOAD. Genetics plays an important role in the risk to develop LOAD, nonetheless, deciphering the genetic factors underpinning the etiology LOAD has been a challenge. This challenge attributes, at least in part, to the facts that LOAD is a heterogeneous, multifactorial disease, and the constellation of the genetic determinants might be personalized and distinct among individuals. Large multi-center GWA studies have found associations between over 20 genomic loci and LOAD. However, the precise target genes, the defined causal genetic variants and their molecular mechanisms of action through which they exert their pathogenic effects remain largely unknown.

The large majority of the LOAD-GWAS risk SNPs are in non-coding intragenic or intergenic regulatory regions of the genome. Furthermore, changes in gene expression in LOAD vs. healthy control were described in brain tissues and previous studies reported the *cis*-associations of tagging SNPs with expression of nearby LOAD-risk genes, providing a strong scientific premise for the proposed study. These evidences led us to hypothesize that changes in expression profiles of critical disease genes is an important molecular mechanism underlying disease etiology and that causal variants modulate expression of these disease genes, via transcriptional and post-transcriptional regulatory events, and by that contribute to LOAD susceptibility.

Short Structural Variants (SSVs), variants other than SNPs, include short deletions, short insertions, indels, homopolymer stretches and short tandem repeats (STR). SSVs were not included in GWAS, including for LOAD, and expression traits (eQTL) association studies (eGWA). In the post-GWA era this understudied class of genetic variation is increasingly thought to play an important role in complex disease such as LOAD and it has been suggested that SSVs have regulatory functions in gene transcription and splicing. Therefore, we advocate that investigations of SSVs within the LOAD associated genomic regions will move the field forward towards identification of the functional and causal genetic variations. Moreover, with the exception of the *APOE* status that consists of two SNPs and the combination of *APOE* with *TOMM40* poly-T, haplotypes have been underrepresented in genetic studies of LOAD. The example of the *APOE* LD region demonstrates that extended haplotypes are likely to be more informative than a single variant. Collectively, we suggest that consideration of a broad spectrum of genetic variants and of extended haplotypes is crucial for the elucidation of causal genetic risk-factors and their functional effects that contribute to the risk of developing LOAD.

Uncovering the genetic etiologies of LOAD is the fundamental milestone in order to achieve and practice precision medicine in LOAD. The prospectively identified genome-wide causal variants will be translational for the construction of refined integrative genetic biomarkers to complement and improve other LOAD-risk models based on traditional LOAD predictors.

Complex diseases including LOAD involve the interaction of numerous biological pathways and interactions between genetic, metabolic and environmental factors. Understanding these interactions is critical both to understand the pathophysiology of the disease and to identify potential targets for interventions. In this review, we showed that a region of the genome that has an extremely strong genetic association with LOAD also has two relevant pathways cholesterol metabolism and mitochondrial transport that map back to two genes, *APOE* and *TOMM40* that are in high LD. Integrative disease modeling (IDM) is an approach will link genetic, molecular, neurophysiological and neuroimaging data across multiple physiological levels in order to more clearly elucidate the biological networks that are affected by the causal genetic variants[64, 65, 66, 67, 68, 69]. Integrative disease modeling and systems-biology approaches for understanding the interaction of complex genetic and metabolic factors and networks are increasingly being employed for LOAD research specifically [70, 71, 72, 73]. Moreover, as LOAD develops over the course of decades, IDM will be essential to understand the time course of the development of disease at the levels of the genome, epigenome, transcriptome, microRNome, proteome/peptidome, metabolome/lipidome,

microbiome, lifestyle, and environmental factors that are involved in complex cellular networks.

9. Five-year view

New emerging genomic technologies and cutting-edge biological model systems will advance the identification and validation of the precise LOAD-causal variants and LOAD-risk haplotypes, and the understanding of their molecular mechanisms of action through which they exert their pathogenic effects. Subsequent studies using innovative models will establish functional genotype-phenotype relationships of the identified LOAD-risk genetic variants and will evaluate their direct functional activity and their causal links to molecular and cellular phenotypes. This knowledge will give greater insight into molecular targets contributing to the etiology of LOAD. The variants and haplotypes with direct effects will have a strong translational impact as they will be utilized in combination with *APOE* LD region to develop new genetic LOAD-risk prediction algorithms, *i.e.* integrative genetic biomarkers, that will demonstrate an improved precision of LOAD-risk prediction.

The highly informative integrative genetic biomarkers will be implemented for early diagnosis, and for enrichment of clinical trials with subjects at high risk. In depth understanding of genetic risk factors will also advance the identification of actionable targets for development of novel therapies for LOAD. Ultimately, integrative genetic biomarkers will inform regarding personalized treatment approaches.

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Key issues

- *APOE* is the strongest genetic risk factor for non-Mendelian LOAD. *APOE* e4 allele contributes a dose-dependent increase in risk and is associated with earlier age-of-onset.
- A highly polymorphic, poly-T in intron 6 of the *TOMM40* gene that is adjacent to *APOE*, hereafter *TOMM40* poly-T, is associated with age-of-onset of LOAD and with cognitive performance in the elderly.
- It has been suggested that the *TOMM40* poly-T acts as a regional transcription regulator of the *TOMM40* and *APOE* genes, that may provide the underpinning molecular mechanism for the reported associations with LOAD and related endophenotypes.
- Large multi-center GWA studies have found associations between over 20 genomic loci and LOAD. The GWAS discoveries have provided valuable information on biological pathways involved in the etiology of LOAD. However, the precise target gene and causal genetic variants have yet to be uncovered.
- A genetics-based biomarker risk algorithm for LOAD based on the *APOE–TOMM40* poly-T haplotypes, and age was developed and is being used for enrichment with individuals at high-risk in clinical trials for prevention and/or delay the onset of LOAD, particularly the TOMMORROW study.
- New genetic risk scores based on LOAD-GWAS findings have also been developed by different groups, however, up-to-date provided only a minor improvement in LOAD-risk prediction compared to the traditional LOAD predictors.
- LOAD develops over the course of decades. Integrative disease modeling and systems-biology approaches will be essential to understand the time course of the development of disease at the levels of the genome, epigenome, transcriptome, microRNome, proteome/peptidome, metabolome/lipidome, microbiome, lifestyle, and environmental factors that are involved in complex cellular networks. These approaches will help focus LOAD research on potential therapeutic approaches to apply at the earliest stages of disease pathophysiology.

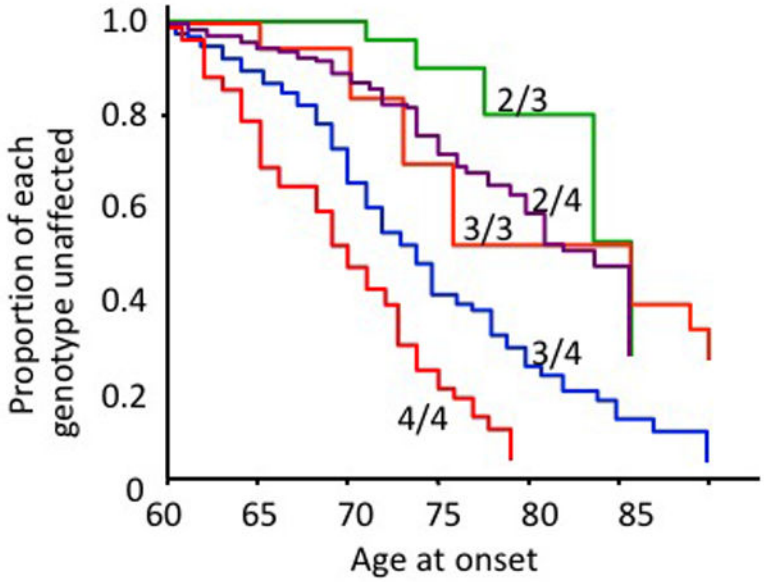


Figure 1. Alzheimer's disease age of onset curves by *APOE* genotypes

This figure is adapted from Corder *et al.* [7]. Kaplan-Meyer survival curves, where the Y axis shows the proportion of individuals unaffected by LOAD who have stratified by the 5 most common *APOE* genotypes. The X axis is the age of onset of LOAD for cases and age when last examined for unaffected individuals. N=234 subjects: 95 diagnosed with LOAD, 139 cognitively normal. N for each genotype: 2/3: 16; 3/3:77; 2/4:5; 3/4:113; 4/4: 23.

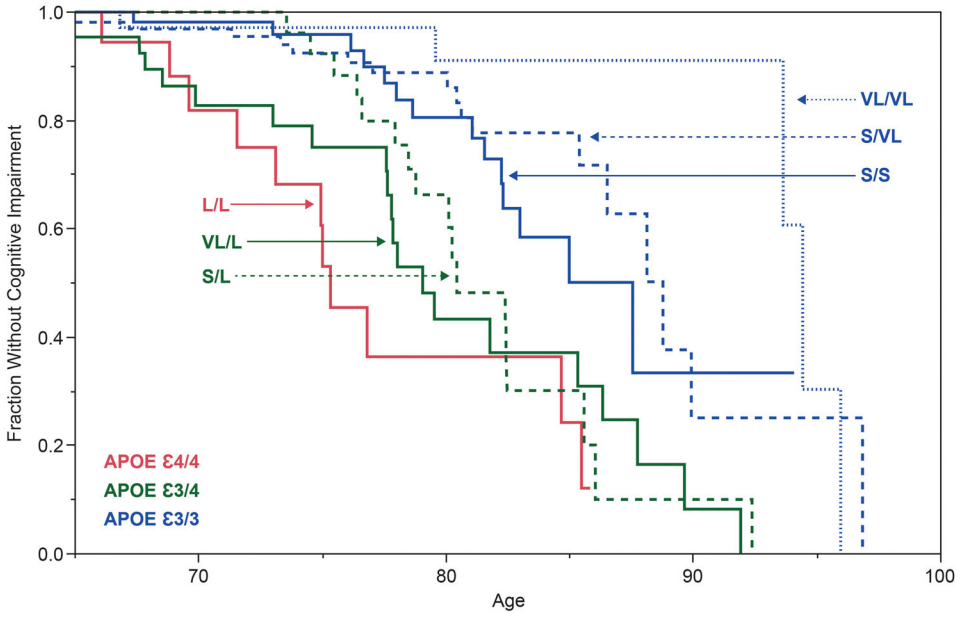
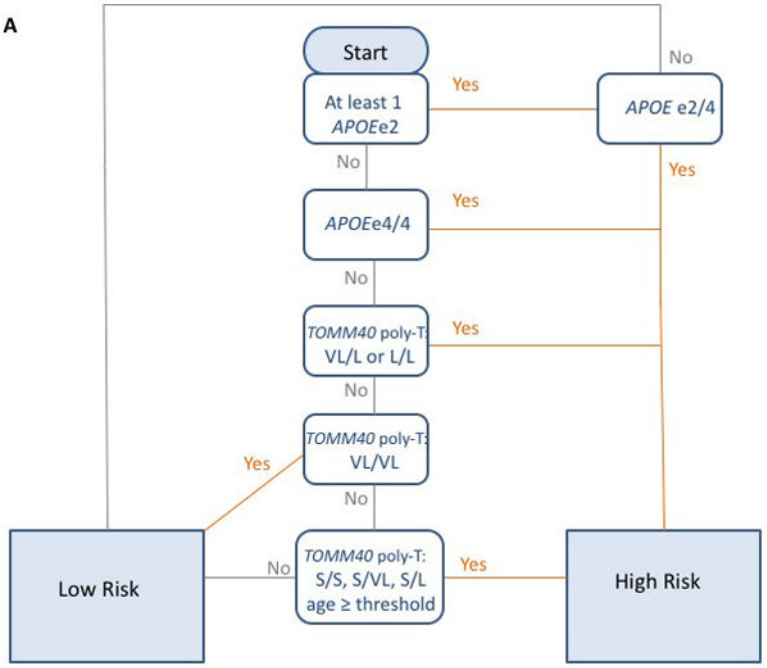


Figure 2. Alzheimer's disease age of onset curves by *TOMM40* poly-T and *APOE* haplotypes

The figure is adapted from Roses *et al.*[34]. Kaplan-Meyer survival curves, where the Y axis shows the percent survival without cognitive impairment, and the X axis represents age. Data was obtained from the Duke Bryan ADRC cohort N=438 subjects: 106 diagnosed with dementia, 332 cognitively normal. N for each genotype: L,L:23; VL,L:54; S,L:72; S,S:100; S,VL:138; VL,VL:51. *TOMM40* genotypes and the corresponding *APOE* genotypes are indicated on the figure. The red line corresponds to *APOE* ε4/4; the two green lines correspond to *APOE* e3/4, and the three blue lines correspond to *APOE* e3/3.

A



B

APOE Genotype	Risk
e2/2	Low risk
e2/3	Low risk
e3/3	Assigned by *TOMM40* polyT/age
e3/4	Assigned by *TOMM40* polyT/age
e2/4	High risk
e4/4	High risk

TOMM40 poly-T	Risk for the Study
L/L	High risk
VL/L	High risk
S/L	≥74 years old = High risk
S/S	≥77 years old = High risk
S/VL	≥76 years old = High risk
VL/VL	Low risk

Figure 3. Genetics-based biomarker risk algorithm (GBRA)

This figure is adapted from Lutz *et al.* [63] Flowchart (A) and Tables (B) for the process for the generation of the risk assessment for MCI due to LOAD using the GBRA. Risk of high or low is assigned based on *APOE* genotype, *TOMM40* poly-T genotype and current age. The GBRA was developed for assessment of asymptomatic individuals and currently being used for the TOMMORROW clinical trial.