Inferring the molecular mechanisms of noncoding Alzheimer’s disease-associated genetic variants

Alexandre Amlie-Wolf*,a,b, Mitchell Tang*,b, Jessica Wayb, Beth Dombroski,b, Ming Jiangb, Nicholas Vrettosc, Yi-Fan Choub, Yi Zhaoa, Nicholas Vrettosc, Elisabeth E. Mlynarski,b, Yuk Yee Leungb, Christopher D. Browna,b,d, Li-San Wang*,a,b,c,d, Gerard D. Schellenberg*,a,b,c,d

aGenomics and Computational Biology Graduate Group, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA
bPenn Neurodegeneration Genomics Center, Department of Pathology and Laboratory Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA
cDepartment of Pathology and Laboratory Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA
dDepartment of Genetics. Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA

Abstract

More than 20 genetic variants associated with late-onset Alzheimer’s disease (LOAD) have been identified by genome-wide association studies (GWAS). However, these variants are tag markers for nearby genetic variants in linkage disequilibrium (LD) and may not be themselves functional. Moreover, most of the significant variants identified by the International Genomics of Alzheimer’s Project (IGAP) meta-analysis are in non-protein-coding regions, implicating gene regulatory mechanisms as underlying the association signal. There are several previously identified coding variant associations with AD, notably the strong APOE signal, but all the novel signals identified in the IGAP GWAS were noncoding.

We set out to characterize the causal variants, regulatory mechanisms, tissue contexts, and target genes underlying noncoding LOAD-associated genetic signals. To do so, we applied our INFERNO method to the IGAP GWAS data, annotating all LD-expanded potentially causal variants at each locus with tissue-specific regulatory activity. Bayesian co-localization analysis of GWAS summary statistics and eQTL data was performed to identify tissue-specific target genes.

INFERNO identified enhancer dysregulation in all 19 tag regions analyzed, significant enrichments of enhancer overlaps in the immune-related blood category, and co-localized eQTL signals overlapping enhancers from the matching tissue class in ten regions (ABCA7, BIN1, CASS4, CD2AP, CD33, CELF1, CLU, EPHA1, FERMT2, ZCWPW1). In several cases, we identified dysregulation of long noncoding RNA (lncRNA) transcripts and applied the lncRNA...
target identification algorithm from INFERNO to characterize their downstream biological effects. We also validated the allele-specific effects of several variants on enhancer function using luciferase expression assays.

Integrating functional genomics with GWAS signals yielded insights into the regulatory mechanisms, tissue contexts, genes, and biological processes affected by noncoding genetic variation associated with LOAD risk.

**Keywords**

Alzheimer’s Disease; Bioinformatics; Genetics; Genomics; Long noncoding RNA

**Introduction**

Alzheimer’s disease (AD) is the most common cause of dementia in the United States [1], but no effective therapies for treatment or prevention exist. Late-onset Alzheimer’s disease (LOAD), defined by age-at-onset after 60 years, is the most common form of AD. Heritability estimates for LOAD stand at 60–80%, implicating genetics as an important factor in disease development [2]. While the APOE locus shows the strongest association [3], LOAD is complex and polygenic [4], and genome-wide association studies (GWAS) have successfully associated over 20 other genetic variants with LOAD [5,6]. Recent studies have implicated a number of different biological processes in LOAD susceptibility such as microglial-mediated innate immunity [7–9].

The majority of top GWAS variants reside in noncoding regions of the genome outside of protein-coding sequences [10]. Any variant in linkage disequilibrium (LD) with a top GWAS variant could be responsible for the association signal, and GWAS data alone lacks the granularity to identify these causal variants. In addition, noncoding variants presumably affect gene regulatory elements, and the affected target genes are often not the closest ones [11]. Thus, functional annotation is needed to reveal the causal variants, regulatory mechanisms, tissue context, and target genes underlying GWAS signals.

Enhancers, which modulate the expression of a target gene independently of orientation and distance, are one of the most common regulatory elements in the noncoding genome [12–14]. Several consortia have generated large-scale functional genomics datasets to characterize regulatory activity in the noncoding genome across different tissue contexts [15–19]. Previous studies used these data to identify noncoding genetic variants with regulatory potential for Alzheimer’s disease [20–22], diabetes [23,24] and schizophrenia [25], but such studies often assume that the relevant tissue context is known a priori.

We hypothesize that noncoding LOAD GWAS signals modulate disease risk by perturbing genomic elements that regulate genes involved in pathogenesis. To explore this hypothesis, we applied our bioinformatics pipeline, INFERNO (INFERring the molecular mechanisms of NOncoding genetic variants) [26] to LOAD GWAS data from the International Genomics of Alzheimer’s Project (IGAP) [6]. INFERNO characterizes noncoding GWAS signals by integrating information across diverse functional genomics data sources to identify causal
noncoding variants and the regulatory mechanisms, tissue contexts, and target genes they affect (Figure 1a). INFERNO identified several putatively causal genetic variants in ten GWAS regions and uncovered strong functional evidence of their effects on immune- and brain-related regulatory mechanisms. Using luciferase reporter assays, we validated the enhancer activity and allelic differences of causal variants in three regions prioritized by relevant tissue context, strength of annotation support, and prior literature.

Materials and Methods

INFERNO analysis of IGAP top hits

INFERNO (details of the algorithm and data sources are described in [26]) was used to analyze 19 top variants from Phase 1 of the IGAP study, excluding the locus near DSG2 (tagged by rs8093731) which did not replicate in Phase 2 and the HLA-DRB5 locus (rs9271192) which is difficult to analyze due to the dense LD structure in the major histocompatibility (MHC) region caused by population-specific selective pressure [27]. INFERNO was run using $P$ value expansion within one order of magnitude and 500 kilobases (kb) of each tagging variant, and the European population from the 1,000 Genomes Project [28] Phase 1 Release v3 (2010/11/23) was used for LD pruning and expansion. All rsID annotations are from dbSNP build 137. For both pruning and expansion, a threshold of $r^2 \geq 0.7$ was used to define LD blocks. All downstream analyses including lncRNA correlation and pathway analysis were performed as defined in [26]: correlation values of each lncRNA with every other expressed gene in the transcriptome were calculated using GTEx RNA-sequencing data and the lncRNA-gene pairs meeting thresholds of 0.5 on the absolute value of both the Spearman and Pearson correlations were considered as targets. WebGestalt was used for all pathway analyses [29–31]. Supplementary Note 1 highlights a few possible ways to prioritize the causal variants for further mechanistic studies.

Luciferase validation

From patient DNA with each allele of interest, insert sequences including the enhancers (800–1,739 base pairs (bp) in length, Supplementary Table 1) overlapping each prioritized variant were PCR-amplified using the Phusion DNA polymerase (New England Biolabs) and primers (Integrated DNA Technologies) with a HindIII overhang (Supplementary Figure 1). The HindIII high fidelity enzyme (New England Biolabs) was used to digest the PCR product along with the vector plasmid containing a minimal promoter and the luciferase reporter gene, pGL4.23[Luc2/minP] (Promega). Enhancer constructs and plasmids were then ligated using the T4 DNA Ligase (New England Biolabs). These ligated vectors were used to transform One Shot TOP10 Competent cells (Invitrogen), and colonies containing the full enhancer sequences along with either allele of the prioritized variants were identified. These clones were amplified using the EndoFree Plasmid Maxi Kit (Qiagen) to generate high levels of DNA suitable for mammalian cell line transfection. Two different vectors were generated this way for each prioritized variant: one with the minor allele of the prioritized variant and one with the major allele. Additionally, we generated vectors containing a minimal promoter with no enhancer inserted and another negative control vector with a minimal promoter and a ~1kb random genomic heterochromatin insert. 300ng of each vector was mixed with one-tenth the amount of a Renilla expressing vector, allowing us to
normalize Luciferase expression for transfection efficiency. This mixture was transfected into separate aliquots of K562 (ATCC) cells using the Lonza Nucleofector Device with Kit V. A mock sample was run through the same transfection procedure with no DNA to account for background luminescence. The Promega Dual-Glo system was used to measure Luciferase and Renilla expression after 24 hours of incubation for the cells to express the luciferase vector. Background-subtracted Luciferase luminescence levels were divided by the corresponding background-subtracted Renilla luminescence, and all ratios were normalized to the average of the minimal promoter condition for quantitative analysis. A total of n = 5 biological replicate experiments were carried out, each including 4 technical replicates per condition. Statistical analysis was performed using a linear mixed model treating experimental days as random effects and alleles as fixed effect using the lmerTest package [32] in R v3.4.4 [33]. P values for the comparisons between conditions were obtained by analysis of variance (ANOVA) using Satterthwaite’s approximation for degrees of freedom.

eGWAS data processing and sampling analysis

For the Zou et al. 2012 eGWAS, eQTL data tables using HapMap2-imputed genotypes were downloaded from the National Institute on Aging Genetics of Alzheimer’s Disease Data Storage Site at the University of Pennsylvania [34]. Custom awk scripts were used to investigate INFERNO-prioritized variants, matching by rsID and/or by hg18 genome position.

Results

Expansion and annotation of IGAP loci

To identify genetic variants with regulatory potential for LOAD, we used INFERNO to analyze the 19 genome-wide significant loci from Phase I of IGAP (Figure 1a, Table 1) [6]. The region tagged by each top variant is referred to by the name of the nearest gene by convention, although these genes are not necessarily causal for the association signals. For each top variant, we identified all variants within 500kb that had a P value within an order of magnitude and the same minor allele effect direction (i.e. variants whose minor alleles are associated with increased AD risk if the top variant is a risk variant, or decreased AD risk if the top variant is protective), identifying a total of 496 variants (Table 1). These variants come from various haplotypes that may underlie the observed association signal at each locus, so to define a comprehensive set of potentially causal variants, we pruned the p-value expanded sets by LD into 52 independent variants tagging each potentially causal haplotype. Then, we re-expanded these by LD, yielding 1,044 unique potentially causal variants (Table 1) used for all subsequent analyses. These variants were primarily in introns and intergenic regions, with only 17 in mRNA exons (Figure 1b-c).

Next, we sought to compute the number of overlaps with different functional genomics data, before statistically quantifying the enrichments of variants overlapping FANTOM5 enhancers, Roadmap enhancers, or both within INFERNO tissue categories (Figure 2b). We overlapped these variants with enhancers defined by bidirectional enhancer RNA (eRNA) transcription in 112 tissues and cell types from the FANTOM5 consortium [17] and by the
ChromHMM epigenomic state-based method [35] in 127 tissues and cell types from the Roadmap Epigenomics Project [15,36,37]. This identified 39 variants overlapping FANTOM5 enhancers in 9 tag regions (Table 1). The FANTOM5 data source with the most enhancer-overlapping variants was monocyte cells, with 25 overlapping variants, whereas the brain harbored only 6 variants (Supplementary Figure 2a). For the Roadmap data, variants were overlapped with a total of 15 ChromHMM states including 3 types of enhancer states (enhancers, genic enhancers, and bivalent enhancers). 659 variants representing all 19 tag regions were found to overlap a ChromHMM-defined enhancer state in at least one tissue (Table 1). Like the FANTOM5 results, primary monocytes from peripheral blood had the most overlapping variants (149 unique variants, Supplementary Figure 2), but 146 unique variants overlapped enhancers in at least one of the brain-related Roadmap datasets.

We also used INFERNO to identify variants overlapping tissue-specific small noncoding RNA (sncRNA) loci from our DASHR database [38,39] as well as those disrupting microRNA (miRNA) seed sites within the 3’ untranslated region (UTR) of miRNA targets predicted by TargetScan [40] since studies have implicated sncRNA dysregulation in LOAD pathogenesis [41]. The DASHR overlap analysis found only one sncRNA overlap, where the variant rs4543938 in the CELF1 region overlapped the piRNA piR-56133. Analysis of miRNA seed disruption found that 46 variants across 5 tag regions (CELF1, FERMT2, INPP5D, MS4A6A, and ZCWPW1) overlapped binding sites for 40 miRNAs in 11 target genes (Supplementary Table 2).

We also used INFERNO to find variants affecting transcription factor binding sites (TFBSs) as identified by HOMER [42]. This identified 451 variants representing all 19 tag regions that either increased or decreased TFBS strength (measured by the change in the positional weight matrix, ΔPWM) for 191 unique transcription factors (Supplementary Figure 3). The majority of these overlaps had negative ΔPWM values, reflecting TFBSs disruptions.

**Integrative analysis of enhancer enrichment patterns**

Using INFERNO’s tissue categorization approach [26] that groups each functional genomics dataset into one of 32 high-level tissue categories, we identified 36 variants from nine tag regions (the CAS54, CELF1, EPHA1, FERMT2, MS4A6A, NME8, PICALM, PTK2B, and SLC24A4/RIN3 regions) that overlapped concordant FANTOM5 and Roadmap ChromHMM enhancers in a tissue category (Figure 2a). All of these regions harbored at least one variant with concordant support in the blood category, supporting the hypothesis of immune mechanisms underlying LOAD genetic signals [7–9]. The CELF1 region was the only one to harbor variants with concordant overlaps of brain enhancers, supporting the approach of performing functional annotation in all available tissues rather than focusing on a single predetermined tissue context. However, we point out that this region contains concordant enhancer overlaps in 9 tissue contexts (Figure 2a), so this regulatory mechanism may exert effects in several tissues.

Many variants overlapped FANTOM5 enhancers in the blood category, which included all the immune-related cell lines such as monocytes and macrophages in addition to whole blood (Supplementary Figure 4a). All 22 of the tissue categories sampled by the Roadmap Epigenomics consortium contained ChromHMM-defined enhancer-related states that...
harbored at least one variant in the expanded set (Supplementary Figure 4b). Again, many variants overlapped Roadmap enhancers in the blood category.

INFERNO includes a method to statistically quantify the enrichments of variants overlapping FANTOM5 enhancers, Roadmap enhancers, or both, in each tissue category. This revealed significant enrichments of variants overlapping both FANTOM5 and concordant enhancers in the blood category and a significant enrichment of Roadmap enhancers in the brain category (Figure 2b) as well as in 13 other tissue contexts including connective tissue, which contains fibroblasts, nervous, and stem cells. Some of these tissue categories had even stronger enrichments than brain or blood, supporting our hypothesis generating approach of performing functional annotation and enrichment analysis in a wide range of tissue contexts. One possible scenario explaining these results is that AD-associated genetic variation could also affect regulatory mechanisms that are active in earlier life and in non-brain regions.

Co-localization analysis with GTEx eQTLs

To identify target genes affected by dysregulated enhancers, INFERNO uses expression quantitative trait loci (eQTLs) – variants whose alleles are correlated with differing levels of a target gene – from the Genotype-Tissue Expression (GTEx) project [16] across 44 tissues. Of the 1,044 potentially causal variants, 750 were significant eQTLs in at least one tissue (corrected q-value ≤ 0.05 on empirical p-values from Matrix eQTL [43]). However, due to dense LD structures in many of our significant regions, this direct overlap approach may yield false positive variants in LD with the truly causal eQTL variant. To address this issue, INFERNO incorporates a Bayesian statistical model (COLOC [44]) to identify co-localized GWAS/eQTL signals with shared causal variants, quantified as the posterior probability \( P(H_4) \). The COLOC method also computes the probability of any individual variant being the shared causal variant, quantified as their Approximate Bayes Factors (ABFs).

We applied COLOC to compare GWAS signals across all 19 tag regions with tissue-specific eQTL signals for 884 unique genes in 44 tissues (median number of genes within each region = 34) for 25,435 tests of GWAS – tissue-specific eQTL co-localization (Supplementary Figure 5a, Methods). We identified 153 co-localized GWAS/eQTL signals \( P(H_4) \geq 0.5 \) representing strong support for a shared causal signal [26]) representing 16 tag regions, 37 tissues and 71 target genes (Supplementary Figure 5b). These co-localization signals were found in 16 tissue categories, with the majority of signals in the brain category. For 32 of these 153 GWAS/eQTL signals, COLOC identified individual variants with ABF \( \geq 0.5 \), but in the majority of cases COLOC was not able to prioritize a single causal variant. This is likely caused by dense LD structures where GWAS and eQTL signals are dispersed across all variants in the LD block (Supplementary Figure 5c). Thus, for each co-localized GWAS/eQTL signal we sampled the highest ABF variants until their sum was 0.5 or greater (Supplementary Figure 5d, [26]). Across the 153 co-localized signals, this yielded 1,291 unique variant–tissue–target gene relationships accounted for by 286 unique variants, 182 of which were in the LD-expanded set.
Comparison of enhancer overlaps with eQTL co-localization signals

We next used the INFERNO tissue categorizations to stratify variants in the ABF-expanded sets by whether they affected a TFBS, overlapped any enhancer, and whether the enhancer came from the concordant tissue category matching the eQTL (Figure 2c). To identify the variants with the strongest INFERNO support, we began by filtering the ABF-expanded variant sets to include only those variants overlapping concordant enhancers, and took two complementary approaches for further variant prioritization: requiring that the variant also overlapped a TFBS (TFBS prioritization) and/or requiring that the variant was the only one in the ABF-expanded set (e.g. ABF ≥0.5; ABF prioritization). TFBS prioritization identified 20 unique variants involved in 43 unique variant–tissue–gene sets across 8 tag regions (Figure 2d, top row) including 15 in the brain or blood categories. ABF prioritization identified 6 unique variants involved in 14 variant–tissue–gene sets across 5 tag regions (Figure 2d, left column), including 2 variants which also had TFBS overlaps. Together, these two approaches identified potentially causal variants in 10 tag regions (Table 2, Supplementary Tables 3-4). We further prioritized four of these signals for experimental validation based on prior literature, strength of annotation support, and relevant tissue contexts: EPHA1, CD33, BIN1, and CD2AP.

EPHA1 region functional variant upregulates lncRNA affecting the JAK2 signaling axis

The strongest signal by both annotation and ABF evidence was in the EPHA1 region, where the variant rs11765305:C>G (chr7.hg19:g.143111112C>G, R² = 1 with tag variant rs11771145) had an ABF of 0.999 underlying an eQTL for the EPHA1-AS1 antisense long non-coding RNA (lncRNA) in whole blood (P(H₄) = 0.516). This variant also colocalized with whole blood eQTLs for the TAS2R60 taste receptor gene (P(H₄) = 0.516, ABF = 1.00) and the TAS2R62P taste receptor gene (P(H₄) = 0.537, ABF = 0.714) (Supplementary Table 3). rs11765305 overlapped FANTOM5 and Roadmap enhancers in the blood category, including white blood cells in the myeloid lineage such as monocytes and macrophages (Figure 3a), and creates a stronger binding site for CEBPB (ΔPWM score = 1.53), an enhancer-binding transcription factor that is associated with immune-related gene regulation [45]. This increase in TF binding is consistent with the positive effect of the rs11765305 minor allele on EPHA1-AS1 expression observed in GTEx (β = 1.25, where a β greater than 1 reflects an increase in gene expression).

To compare enhancer activity between the major and minor alleles of rs11765305, we performed luciferase assays in K562 leukemia cells, which are from the same myeloid cell lineage as monocytes. Although the major allele had no significant luciferase expression compared to controls, the minor allele had significantly higher expression compared to both controls and the major allele (Figure 3b). These results confirm the predicted monocyte enhancer activity in this region and are consistent with the mechanism that the minor allele of rs11765305 creates a stronger CEBPB TFBS, increasing the activity of an enhancer regulating EPHA1-AS1, TAS2R60, and TAS2R62P.

We next set out to computationally identify the downstream effects of EPHA1-AS1, as lncRNAs can modulate gene expression through recruitment of regulatory proteins or binding to target transcripts [46]. INFERNO uses GTEx RNA-seq data to identify genes
whose expression is correlated with that of a lncRNA using a threshold of 0.5 on both Pearson and Spearman correlations across 44 tissues [26]. Note that correlation between the expression of genes does not imply causal regulation. We introduced this computational approach as part of INFERNO for identifying gene hubs for functional enrichment analyses. Experimental approaches (such as knocking down the lncRNAs in the relevant tissues/cell types) could provide mechanistic validation to identify the downstream effects of the identified lncRNAs in followup studies.

For *EPHA1-AS1*, this correlation approach yielded one gene, *JAK2* (Pearson $r^2 = 0.517$, Spearman $r^2 = 0.582$) (Figure 3c). *JAK2* is part of the *JAK2/STAT3* signaling axis, whose disturbance by amyloid-β leads to memory impairment [47]. The tag variant in this region is protective and rs11765305 has the same effect direction, so INFERNO prioritized a mechanism whereby the protective minor allele of rs11765305 increases *EPHA1-AS1* expression which in turn increases the activity of the *JAK2/STAT3* signaling axis, implying that *JAK2/STAT3* activation may protect against LOAD.

To investigate these signals in an eQTL dataset specific to AD, we analyzed the Mayo eGWAS dataset comparing AD patients (n=202) to subjects with non-AD neurodegenerative diseases (n=197) [48] (Methods). This study used expression microarrays and genotyping to perform eQTL mapping in cerebellum and temporal cortex on AD cases, non-AD cases, and the combined patient population. In this study, rs11765305 was not genotyped or imputed, so we investigated the tag variant rs11771145 as a proxy which is in perfect LD. This variant had two significant eQTL signals in the cerebellum combined condition (q-value = 0.02254) and the temporal cortex non-AD patient condition (q-value = 0.02215), both for the gene *ZYX*. One limitation of the expression microarray approach is that the expression of long noncoding RNAs such as *EPHA1-AS1* were not quantified, so this dataset cannot recapitulate these INFERNO results. However, *ZYX* was the most strongly correlated gene with *EPHA1-AS1* by Spearman correlation ($r = 0.6095$), suggesting that these eQTL signals may reflect other downstream effects of *EPHA1-AS1*. *ZYX* encodes the zyxin protein which is part of the actin cytoskeleton, can be induced by amyloid-β, and may interact with *SIRT1*, an important protein in the aging process [49,50]. The eGWAS data could not recapitulate the *JAK2* signal because it is on a different chromosome and the eGWAS analysis only tested cis-eQTLs within 200kb of any gene of interest.

**Functional validation of blood regulation of CD33**

In the *CD33* region, COLOC identified co-localized GWAS/eQTLs for *CD33* itself in whole blood (P(H4) = 0.955) and for *AC018755.1* (P(H4) = 0.683) in brain hypothalamus. In both cases, rs12459419:C>T (chr19.hg19:g.51728477C>T) was prioritized by concordant enhancer and motif overlap. However, the tag variant rs3865444:C>A (chr19.hg19:g.51727962C>A) had a higher ABF in both cases (0.491 and 0.489, respectively) and is in perfect LD (R² = 1) with rs12459419. rs3865444 overlaps Roadmap enhancers in 6 cell lines including primary monocytes and primary T regulatory cells from peripheral blood. In contrast, rs12459419 only overlapped Roadmap enhancers from 3 cell lines including primary T regulatory cells from peripheral blood and fetal brain but lacked the monocyte enhancer overlap (Supplementary Table 3). Additionally, rs3865444 has been
extensively studied, with previous work showing that the protective minor allele (A) decreases the levels of CD33 protein \(^{[51]}\), decreases CD33 mRNA expression consistent with the direction of the GTEx eQTL effect \((\beta = 0.352)\) \(^{[52]}\), and reduces cell surface expression of CD33 in monocytes \(^{[53]}\).

Based on the prior literature, the strong ABF signal, perfect LD with rs12459419, and the monocyte enhancer overlap, we analyzed rs3865444 in our luciferase assays. This found significant increases for the major allele and significant decreases for the minor allele relative to the controls, as well as a striking decrease in enhancer activity of the minor allele relative to the major allele (Figure 4a). This was consistent with prior reports and the GTEx eQTL direction for this variant \((\beta = 0.352)\). We also investigated rs3865444 in the eGWAS dataset but found no significant eQTL signals for that variant or any of the 5 other variants in the LD-expanded set in the CD33 region. This is consistent with the putative monocyte mechanism affected in this region, which we would not expect to detect in bulk brain tissue samples as they only include a small fraction of monocytes \(^{[54]}\).

**Functional validation of lymphocyte regulation of BIN1**

In the BIN1 region, INFERNO identified a co-localized GWAS/eQTL for BIN1 in EBV-transformed lymphocytes \((P(H_4) = 0.652)\) with the variant rs4663105:A>C (chr2.hg19:g.127891427A>C) prioritized by ABF \((\text{ABF} = 0.777)\). This variant overlaps Roadmap enhancers in primary monocyte cells and placenta but does not overlap any TFBSs and is in strong LD with the tag variant rs6733839 \((R^2 = 0.8968)\). rs4663105 has been previously associated with LOAD risk, and an insertion in that region was associated with increased BIN1 expression \(^{[55]}\). This previous study found no difference in luciferase activity between the two alleles of rs4663105 in SKNSH-SY5Y and HEK cells. However, their construct only spanned 60bp around the variant, whereas the monocyte enhancer is 800bp. Therefore, we cloned the full Roadmap enhancer region (Supplementary Table 1) for luciferase assays in K562 cells, which are more relevant to the functional annotations in this region. This found significantly increased enhancer activity for both alleles of rs4663105 relative to the control vectors, and a slight but significant decrease of the minor allele relative to the major allele \((p = 0.0328, \text{Figure 4b})\), consistent with the direction of the GTEx eQTL \((\beta = 0.496)\). We investigated these signals in the Mayo eGWAS datasets, but none of the variants in the BIN1 region were genotyped or imputed in any samples.

**CD2AP region variants modulate lncRNA with widespread brain regulatory effects**

Finally, in the CD2AP region, INFERNO prioritized several co-localized signals including RP11–385F7.1 in brain cerebellar hemisphere and cerebellum \((P(H_4) = 0.904 \text{ and } 0.923, \text{respectively})\) and an eQTL for CD2AP in fibroblasts \((P(H_4) = 0.801)\). TFBS prioritization implicated rs9367279:A>G (chr6.hg19:g.47448336A>G), which overlaps Roadmap enhancers in 33 tissues/cell lines from 13 tissue categories and disrupts a CArG-box binding site \((\Delta \text{PWM} = −1.38)\) for the MADS-box family of transcription factors, which includes the enhancer-related factors SRF and MEF2A \(^{[56,57]}\). CD2AP encodes a scaffolding molecule that regulates the actin cytoskeleton and is involved in endocytic processes \(^{[58]}\), while RP11–385F7.1 is a lncRNA near the promoter for the CD2AP gene.
We performed luciferase assays, but both alleles of rs9367279 had significantly decreased enhancer activity relative to the controls, and there was no strong difference between the two alleles, suggesting that this enhancer may not be active in K562 cells (Figure 4c, p = 0.1892). RPI1–385F7.1 was strongly correlated with 64 transcripts (Figure 4d, Supplementary Table 5), and we performed pathway analysis of these targets using the WebGestalt tool [29,30] to interpret the increased number of targets relative to the single target of EPHA1-AS1, but found no enrichments after controlling for false discovery rate. The gene with the strongest Pearson correlation was PPI1R16A (Pearson $r^2 = 0.641$, Spearman $r^2 = 0.593$) and the gene with the strongest Spearman correlation was COQ4 (Pearson $r^2 = 0.608$, Spearman $r^2 = 0.660$). PPI1R16A, also known as MYPT3, directs the protein phosphatase PP1c to its targets and is involved in actin binding and G-protein coupled receptor pathways [59]. COQ4 is part of the coenzyme Q biosynthesis pathway, an antioxidant that may modify LOAD-associated oxidative damage [60]. The eQTL effects of rs9367279 on RPI1–385F7.1 are weak and inconsistent between the two brain regions ($\beta = 0.969$ in cerebellar hemisphere and 1.194 in cerebellum), suggesting that rs9367279 contributes to fine-scale regulation of RPI1–385F7.1 in brain, although it has a relatively strong repressive effect on CD2AP in fibroblasts ($\beta = 0.505$). We also investigated this variant in the Mayo eGWAS data, but it was only tested for association with CD2AP and not any lncRNAs, and it was not a significant eQTL for CD2AP in any condition.

Discussion

Our application of INFERNO to LOAD GWAS data prioritized perturbations of tissue-specific regulatory mechanisms in 10 IGAP tag regions (Table 2, Supplementary Table 4). In the EPHA1, CD2AP, CELF1, and CASS4 regions, the target genes of the co-localized GWAS/eQTL signals included lncRNAs, so identifying affected enhancers and target genes may be only the first step towards understanding genetic effects on regulatory networks contributing to disease pathogenesis. The tissue classification approach implemented in INFERNO also enabled the unbiased investigation of the relevant tissue contexts affected by each genetic signal. Limiting our analysis to only brain datasets would have missed the blood-category signals that we detected. These immunity-related signals are in line with other recent work highlighting neuroinflammation as a crucial component of LOAD pathogenesis and etiology [7–9]. Our validation experiments used K562 as an immune-related cell line to test that the enhancers predicted by FANTOM5 and Roadmap were indeed active and that there was differential activity between the alleles of our prioritized variants. This cell line does not model specific aspects of AD pathology, but these experiments were aimed at validating the enhancer mechanisms prioritized by INFERNO, and followup studies will elucidate the effects of these regulatory mechanisms in AD-specific contexts.

In our primary INFERNO analysis, we considered GTEx eQTLs that come from healthy, non-diseased tissues, rather than eQTLs from AD patients. We believe that this is a desirable approach for our goal of characterizing the molecular mechanisms underlying AD GWAS signals, which measure genetic susceptibility conferred by inherited (i.e. germline) genetic variation. These susceptibility signals reflect the life-time risk of developing AD, which is present from conception and conferred well before the neurodegenerative disease process.
begins. The molecular mechanisms underlying these associations might exert their effects beginning in the normal state and continuing throughout the life-time. On the other hand, AD eQTL data measures genetic variants associated with AD-associated differential gene expression which could be either up- or down-stream of the actual pathologic processes and susceptibility mechanisms. Also, the onset of AD results in neurodegeneration and the loss of specific cell types which may drive expression changes, but bulk tissue datasets may not account for these proportional changes. Thus, we used GTEx data to generate hypotheses about regulatory mechanisms underlying genetic susceptibility and then incorporated the eGWAS dataset to explore if these mechanisms were recapitulated in the disease state. Consistent with the majority of our signals reflecting immune-related processes and the lack of lncRNA profiling by microarray, the majority of our signals were not detected in the eGWAS dataset, supporting our approach of using the GTEx data to generate hypotheses.

INFERNO did not identify regulatory mechanisms in all 19 of the IGAP regions, and this may be driven by several aspects of this analysis. We are limited by the sample sizes and sets of tissues that were assayed by the FANTOM5, Roadmap, and GTEx consortia and the number of datasets that went into each tissue category, with some categories being much more sparsely sampled than others [26], especially the brain tissues which have some of the smallest GTEx sample sizes [16]. Another issue with this bulk tissue-based approach is that tissue samples are a mixture of a large variety of different cell types such as neurons, astrocytes, and microglia in brain [61], and so the eQTL and enhancer signals that INFERNO analyzes may be mainly driven by the most prevalent cell type in each tissue, potentially missing signals involving rarer cell types. Emerging single cell technologies aimed at analyzing homogeneous cell populations will help to address this gap, but we are not currently aware of any data sources measuring enhancer or eQTL signals in single brain cells.

Our application of INFERNO to IGAP GWAS data for late-onset Alzheimer’s Disease yielded insights into the regulatory mechanisms affected by noncoding LOAD-associated genetic variants. Experimental validation supported our computationally predicted regulatory effects, suggesting that our approach is able to prioritize truly causal regulatory mechanisms at GWAS loci for post-GWAS experiments. Incorporating more functional genomics data as it is generated in concert with more refined validation experiments using a broader range of cell types and molecular techniques will yield insights into a range of phenotypes.

Supplementary Material

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References

1. Association A (2015) 2015 Alzheimer’s disease facts and figures. Alzheimer’s Dement. 11, 332–384. [PubMed: 25984581]
2. Gatz M, Pedersen NL, Berg S, Johansson B, Johansson K, Mortimer J a, Posner SF, Viitanen M, Winblad B, Ahlbom A (1997) Heritability for Alzheimer’s disease: the study of dementia in Swedish twins. Journals Gerontol. Ser. A, Biol. Sci. Med. Sci. 52, M117–M125.
3. Corder EH, Saunders a M, Strittmatter WJ, Schmechel DE, Gaskell PC, Small GW, Roses a D, Haines JL, Pericak-Vance M a (1993) Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer’s disease in late onset families. Science (80-. ). 261, 921–923.
4. Escott-Price V, Sims R, Bannister C, Harold D, Vronskaia M, Majounie E, Badarinarayan N, Morgan K, Passmore A, Holmes C, Powell J, Brayne C, Gill M, Mead S, Goate A, Cruchaga C, Lambert JC, Van Duijn C, Maier W, Ramirez A, Holmans P, Jones L, Hardy J, Seshadri S, Schellenberg GD, Amouyel P, Williams J (2015) Common polygenic variation enhances risk prediction for Alzheimer’s disease. Brain. 138, 3673–3684. [PubMed: 26490334]
5. Naj AC, Jun G, Beecham GW, Wang L-S, Vardarajan BN, Buros J, Gallins PJ, Buxbaum JD, Jarvik GP, Crane PK, Larson EB, Bird TD, Boeve BF, Graff-Radford NR, De Jager PL, Evans D, Schneider J a, Curran-Miller MM, Ertekin-Taner N, Younkin SG, Cruchaga C, Kauwe JSK, Nowotny P, Kramer P, Hardy J, Huentelman MJ, Myers AJ, Parmigiani G, Demirci FY, Baldwin CT, Green RC, Rogaeve E, St George-Hyslop P, Arnold SE, Barber R, Beach T, Bigio EH, Bowen JD, Boxer A, Burke JR, Cairns NJ, Carlson CS, Carney RM, Carroll SL, Chui HC, Clark DG, Corneveaux J, Cotman CW, Cummings JL, DeCarli C, DeKosky ST, Dementia IC, Dick M, Dickson DW, Ellis WG, Faber KM, Fallon KB, Farlow MR, Ferris S, Frolich M, Galasko DR, Galligioni M, Gearing M, Geschwind DH, Ghetti B, Gilbert JR, Gilman S, Giordani B, Glass JD, Growdon JH, Hamilton RL, Harrell LE, Head E, Honig LS, Hulette CM, Hyman BT, Jicha G a, Jin L-W, Johnson N, Karlawish J, Karydas A, Kaye J a, Kim R, Koo EH, Kowall NW, Lah J-J, Levey AI, Lieberman AP, Lopez OL, Mack WJ, Marson DC, Martinuik F, Mash DC, Masliah E, McCormick WC, McCurry SM, McDougal AN, McKee AC, Mesulam M, Miller BL, Miller C a, Miller JW, Parisi JE, Perl DP, Peskind E, Petersen RC, Poon WW, Quinn JF, Rajbhandary R a, Raskind M, Reisberg B, Ringman JM, Roberson ED, Rosenberg RN, Sano M, Schneider LS, Seeley W, Shelanski ML, Slifer Ma, Smith CD, Sonnen Ja, Spina S, Stern Ra, Tanzi RE, Trojanowski JQ, Troncoso JC, Van Deerlin VM, Vinters H V, Vonsattel JP, Wintraub S, Welsh-Bohmer K a, Williamson J, Wolter RL, Cantwell LB, Dombroski B a, Beecky D, Lunetta KL, Martin ER, Kamboh MI, Sainan AJ, Reiman EM, Bennett D a, Morris JC, Montine TJ, Goate AM, Blacker D, Tsuang DW, Hakonarson H, Kukull W a, Foroud TM, Haines JL, Mayeux R, Pericak-Vance Ma, Farrer L a, Schellenberg GD (2011) Common variants at MS4A4/MS4A6E, CD2AP, CD33 and EPHA1 are associated with late-onset Alzheimer’s disease. Nat. Genet. 43, 436–431. [PubMed: 21460841]

6. Lambert JC, Ibrahim-Verbaas C a, Harold D, Naj a C, Sims R, Bellenguez C, DeStefano a L, Bis JC, Beecham GW, Gómez-Arrabal B, Russo G, Thorton-Wells T a, Jones N, Smith a V, Chouraki V, Thomas C, Ikram M a, Zelenika D, Vardarajan BN, Kamatani Y, Lin CF, Gerrish A, Schmidt H, Kunkle B, Dunstan ML, Ruiz A, Bihoreau MT, Choi SH, Reitz C, Pasquier F, Cruchaga C, Craig D, Amin N, Bert C, Lopez OL, De Jager PL, Deramecourt V, Johnston J a, Evans D, Lovestone S, Letenneur L, Morón FJ, Rubinsztein DC, Eiriksdóttir G, Sleeegers K, Goate a M, Fievet N,
Huentelman MW, Gill M, Brown K, Kamboh MI, Keller L, Barberger-Gateau P, McGuiness B, Larson EB, Green R, Myers A J, Dufouil C, Todd S, Wallon D, Love S, Rogaeva E, Gallacher J, St George-Hyslop P, Clarimon J, Lleo A, Bayer A, Tsuang DW, Yu L, Tsolaki M, Bosüi P, Spalletta G, Proitsi P, Collinge J, Sorbi S, Sanchez-Garcia F, Fox NC, Hardy J, Deniz Naranjo MC, Bosco P, Clarke R, Brayne C, Galimberti D, Mancuso M, Matthews F, Moebus S, Meacci P, Del Zompo M, Maier W, Hampel H, Pilotto A, Bullido M, Panza F, Caffarra P, Naçiias B, Gilbert JR, Mayhaus M, Lannefelt L, Hakonarson H, Pichler D, Carrasquillo MM, Ingelsson E, Beekly D, Alvarez V, Zou F, Valladares O, Younkin SG, Coto E, Hamilton-Nelson KL, Gu W, Razquin C, Pastor P, Mateo I, Owen MJ, Faber KM, Jonsson PV, Combarros O, O’Donovan MC, Cantwell LB, Soininen H, Blacker D, Mead S, Mosley TH, Bennett D a, Harris TB, Fratiglioni L, Holmes C, deBruijn RF, Dartigues JF, Mayeux R, Tzourio C, Hofman A, Nöthen MM, Graff C, Psaty BM, Jones L, Haines JL, Holmes P a, Lathrop M, Pericak-Vance M a, Launer LJ, van Duijn CM, Van Broeckhoven C, Moskvina V, Seshadri S, Williams J, Schellenberg GD, Amouyel P (2013) Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer’s disease. Nat. Genet. 45, 1452–8. [PubMed: 24162737]

7. Heneka MT, Golenbock DT, Latz E (2015) Innate immunity in Alzheimer’s disease. Nat. Rev. Immunol. 16, 229–236.

8. Heppner FL, Ramshooff RM, Becher B (2015) Immune attack: the role of inflammation in Alzheimer disease. Nat. Rev. Neurosci. 16, 358–372. [PubMed: 25991443]

9. Sims R, van der Lee SJ, Naj AC, Bellenguez C, Badarinarayan N, Jakobsdottir J, Kunkle BW, Boland A, Raybould R, Bis JC, Martin ER, Grenier-Boley B, Hellmann-Heimbach S, Chouraki V, Kuzma AB, Sleeegers K, Vronskaya M, Ruiz A, Graham RR, Olasso R, Hoffmann P, Grove ML, Vardarajan BN, Hiltunen M, Nöthen MM, White CC, Hamilton-Nelson KL, Epelbaum J, Maier W, Choi S H, Beecham GW, Dulary C, Hermos S, Smith A V, Funk CC, Derbois C, Forstner AJ, Ahmad S, Li H, Bacz D, Harold D, Satizabal CL, Valladares O, Squassina A, Thomas R, Brody JA, Qu L, Sánchez-Juan P, Morgan T, Wolters FJ, Zhao Y, Garcia FS, Denning N, Fornage M, Malamoun J, Naranjo MCD, Majounie E, Mosley TH, Dombroski B, Wallon D, Lupton MK, Dupuis J, Whitehead F, Fratiglioni L, Medway C, van der Lee SJ, Nolthen MM, Graff C, Psaty BM, Jones L, Haines JL, Holmes P a, Lathrop M, Pericak-Vance M a, Launer LJ, van Duijn CM, Van Broeckhoven C, Moskvina V, Seshadri S, Williams J, Schellenberg GD, Amouyel P (2013) Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer’s disease. Nat. Genet. 45, 1452–8. [PubMed: 24162737]
Roberson ED, Rogaeva E, Rosen HJ, Rosenberg RN, Sager MA, Saykin AJ, Schneider JA, Schneider LS, Seeley WW, Smith AG, Sonnen JA, Spina S, Stern RA, Swerdlow RH, Tanzi RE, Thornton-Wellis TA, Trojanowski JQ, Troncoso JC, Van Deerlin VM, Van Eldik LJ, Vinters HV, Vonsattel JP, Weintraub S, Welsh-Boehmer KA, Wilhelmsen KC, Williamson J, Wingo TS, Wolter RL, Wright CB, Yu C-E, Yu L, Garzia F, Golamouly F, Septier G, Engelborghs S, Vandenbergh R, De Deyn PP, Fernandez CM, Benito YA, Thonborg H, Forsell C, Lilis L, Kinhult-Stählbom A, Kilander L, Brundin R, Concari L, Helisalmi S, Koivisto AM, Haapasalo A, Dermecourt V, Fievet N, Hanon O, Dufouil C, Brice A, Ritchie K, Dubois B, Keene CD, Tschanz J, Fitzpatrick AL, Kukwa NA, Norton M, Aspelund T, Larson EB, Munger R, Rotter JI, Lipton RB, Bullido MJ, Hofman A, Montine TJ, Coto E, Boerwinkle E, Petersen RC, Alvarez V, Rivadeneira F, Reiman EM, Gallo M, O’Donnell CJ, Reisch JS, Bruni AC, Royall DR, Dichgans M, Sano M, Galimberti D, St George-Hyslop P, Scarpini E, Tsuang DW, Mancuso M, Bonuccelli U, Winslow AR, Daniele A, Wu C-K, Peters O, Nacmias B, Riemenschneider M, Heun R, Brayne C, Rubinszttein DC, Bras J, Guerreiro R, Al-Chalabi A, Shaw CE, Collinge J, Mann D, Tsolaki M, Clarimon J, Sussams R, Lovestone S, O’Donovan MC, Owen MJ, Behrens TW, Mead S, Goate AM, Uitterlinden AG, Holmes C, Cruchaga C, Ingelsson E, Bennett DA, Powell J, Golde TE, Graff C, de Jager PL, Morgan K, Ertekin-Taner N, Combarros O, Passey BM, Passmore P, Younkin SG, Berr CC, Gudnason V, Rujescu D, Dickson DW, Dartiges J-F, DeStefano AL, Ortega-Cubero S, Hakonarson H, Campion D, Boada M, Kauwe JK, Farrer LA, Van Broeckhoven C, Ikram MA, Jones L, Haines JL, Tzourio C, Launer LJ, Escott-Price V, Mayeux R, Deleuze J-F, Amin N, Holmans PA, Pericak-Vance MA, Amouyel P, van Duijn CM, Ramirez A, Wang L-S, Lambert J-C, Seshadri S, Williams J, Schellenberg GD (2017) Rare coding variants in PLCG2, ABI3, and TREM2 implicate microglial-mediated innate immunity in Alzheimer’s disease. Nat. Genet. 49, 10. Maurano MT, Humbert R, Rynes E, Thurman RE, Haugen E, Wang H, Reynolds AP, Sandstrom R, Qu H, Brody J, Shafer A, Neri F, Lee K, Kutyavin T, Stehling-Sun S, Johnson AK, Canfield TK, Giste E, Diegel M, Bates D, Hansen RS, Neph S, Sabo PJ, Heimfeld S, Raubitschek A, Ziegler S, Cutsapas C, Sotoodehnia N, Glass I, Sunyaev SR, Stamatoyannopoulos JA (2012) Systematic Localization of Common Disease-Associated Variation in Regulatory DNA. Science (80-. ). 337, 1190. 11. Corradin O, Scacheri PC (2014) Enhancer variants: evaluating functions in common disease. Genome Med. 6, 85. [PubMed: 25473424] 12. Bulger M, Groudine M (2010) Enhancers: The abundance and function of regulatory sequences beyond promoters. Dev. Biol. 339, 250–257. [PubMed: 20025863] 13. Wittkopp PJ, Kalay G (2012) Cis-regulatory elements: molecular mechanisms and evolutionary processes underlying divergence. Nat. Rev. Genet. 13, 59–69. 14. Ong C-T, Corces VG (2011) Enhancer function: new insights into the regulation of tissue-specific gene expression. Nat. Rev. Genet. 12, 283–93. [PubMed: 21358745] 15. Consortium RE, Kundaje A, Meuleman W, Ernst J, Bilkeny M, Yen A, Heravi-Moussavi A, Kheradpour P, Zhang Z, Wang J, Ziller MJ, Amin V, Whaitaker JW, Schultz MD, Ward LD, Sarkar A, Quon G, Sandstrom RS, Eaton ML, Wu Y-C, Pfennig AR, Wang X, Claussnitzer M, Liu Y, Coarfa C, Harris RA, Shores N, Epstein CB, Gjoneska E, Leung D, Xie W, Hawkins RD, Lister R, Hong C, Gascard P, Mungall AJ, Moore R, Chuae E, Tam A, Canfield TK, Hansen RS, Kaur R, Sabo PJ, Bansal MS, Carles A, Dixon JR, Farh K-H, Feizi S, Karlic R, Kim A-R, Kulkarni A, Li D, Lowdon R, Elliott G, Mercer TR, Nep S, Onuchic V, Polak P, Rajagopal N, Ray P, Sallari RC, Siebenthal KT, Sinnott-Armstrong N a, Stevens M, Thurman RE, Wu J, Zhang B, Zhou X, Beaudet AE, Boyer L a, De Jager PL, Farnham PJ, Fisher SJ, Haussler D, Jones SJM, Li W, Marra M a, McManus MT, Sunyaev S, Thomson J a, Tlsty TD, Tsai L-H, Wang W, Waterland R a, Zhang MQ, Chadwick LH, Bernstein BE, Costello JF, Ecker JR, Hirst M, Meissner A, Milosavljevic A, Ren B, Stamatoyannopoulos J a, Wang T, Kellis M, Kundaje A, Meuleman W, Ernst J, Bilkeny M, Yen A, Heravi-Moussavi A, Kheradpour P, Zhang Z, Wang J, Ziller MJ, Amin V, Whaitaker JW, Schultz MD, Ward LD, Sarkar A, Quon G, Sandstrom RS, Eaton ML, Wu Y-C, Pfennig AR, Wang X, Claussnitzer Yaping Liu M, Coarfa C, Alan Harris R, Shores N, Epstein CB, Gjoneska E, Leung D, Xie W, David Hawkins R, Lister R, Hong C, Gascard P, Mungall AJ, Moore R, Chuae E, Tam A, Canfield TK, Scott Hansen R, Kaul R, Sabo PJ, Bansal MS, Carles A, Dixon JR, Farh K-H, Feizi S, Karlic R, Kim A-R, Kulkarni A, Li D, Lowdon R, Elliott G, Mercer TR, Nep S, Onuchic V, Polak P, Rajagopal N, Ray P, Sallari RC, Siebenthal KT, Sinnott-Armstrong N a, 16. Amlie-Wolf et al. Page 14 J Alzheimers Dis. Author manuscript; available in PMC 2020 June 25.
16. Ardlie KG, Deluca DS, Segre AV, Sullivan TJ, Young TR, Gelfand ET, Trowbridge CA, Maller JB, Mukame E, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Neri F, Nagarajan RP, Ner...
KR, Lacerda de Sousa B, Beal K, Pignatelli M, Flicek P, Lian J, Kahveci T, Lee D, James Kent W, Ramalho Santos M, Herrero J, Notredame C, Johnson A, Vong S, Lee K, Bates D, Neri F, Diegel M, Canfield T, Sabo PJ, Wilken MS, Reh T a., Giste E, Shafer A, Kutayvin T, Haugen E, Dunn D, Reynolds AP, Neph S, Humbert R, Scott Hansen R, De Bruijn M, Selleri L, Rudensky A, Josefowicz S, Samstein R, Eichler EE, Orkin SH, Levasseur D, Papayannopoulou T, Chang K-H, Skoulitchi A, Gosh S, Distete C, Treuting P, Wang Y, Weiss MJ, Blobel G a., Cao X, Zhong S, Wang T, Good PJ, Lowdon RF, Adams LB, Zhou X-Q, Pazin MJ, Feingold E a., Wold B, Taylor J, Mortazavi A, Weissman SM, Stamatoyannopoulos J a., Snyder MP, Guigo R, Gingeras TR, Gilbert DM, Hardison RC, Beer M a., Ren B (2014) A comparative encyclopedia of DNA elements in the mouse genome. Nature 515, 355–364. [PubMed: 25409824]

19. Consortium TF, Pmi R, Dgt C, Forrest ARR, Kawaji H, Rehli M, Kenneth Baillie J, de Hoon MJL, Haberle V, Lavassan TM, Kulakovskiy I V., Lizio M, Itoh M, Andersson R, Mungall CJ, Meehan TF, Schmeier S, Bertin N, Jørgensen M, Dimont E, Arner E, Schmid C, Schaefer U, Medvedeva Y a., Plessy C, Vitezic M, Severin J, Semple C a., Ishizu Y, Young RS, Francescato M, Alan A, Albanese D, Altschuler GM, Arakawa T, Archer J a. C, Arner P, Babina M, Rennie S, Balwierz PJ, Beckhouse AG, Pradhan-Bhatt S, Blake J a., Blumenthal A, Bodega B, Bonetti A, Briggs J, Brombacher F, Maxwell Burroughs A, Califano A, Cannistraci C V., Carbayo D, Chen Y, Chierici M, Ciani Y, Clevers HC, Dalla E, Davis C a., Detmar M, Diehl AD, Dohi T, Drabløs F, Edge ASB, Edinger M, Ellsworth K, Endoh M, Enomoto H, Fagioli N, Fairbairn L, Fang H, Farach-Carson MC, Faulkner GI, Favorov A V., Fisher ME, Frith MC, Fujita R, Fukuda S, Furlanello C, Furuno M, Furuwasa J, Geijtenbeek TB, Gibson AP, Gingeras T, Goldowitz D, Gough J, Guler R, Gustincich S, Ha TJ, Hamaguchi M, Harra M, Harbers M, Hasegawa A, Hashimoto Y, Hashimoto T, Herlyn M, Hichens KJ, Ho Sui SJ, Hofmann OM, Hook I, Iori F, Hunoldickli L, Iida K, Ikawa T, Jankovic BR, Jia H, Joshi A, Jochum G, Kazekowski B, Kaita K, Kaida K, Kallio A, Kajiyama K, Kato S, Kawaguchi S, Kawamori T, Kawamura YI, Kawashima T, Kempfle JS, Kenna TJ, Kere J, Khachigian LM, Kitamura T, Knopf K, Kojima M, Kojima S, Kondo N, Koseki H, Koyasu S, Kramritz S, Kubasrai K, Kwon AT, Kuro K, Lee W, Lemaransson A, Li K, Lilje B, Lipovich L, Mackay-sim A, Manabe R, March B, Mathelier A, Meynert A, Mizzuno Y, de Lima Morea D a., Morikawa H, Morimoto K, Moroz K, Motakis E, Motofi H, Mummery CL, Murata M, Nagao-Sato S, Nakach Y, Nakahara F, Nakamura T, Nakamura Y, Nakazato K, van Nimwegen E, Ninomiya N, Nishiyori H, Noma S, Nozaki T, Ogishima S, Okada H, Ohkura N, Ohmii H, Ohsako H, Ohshima M, Okada-Hatakeyama M, Okazaki Y, Orlando V, Ovchinnikov D a., Pain A, Passier R, Pearson M, Persson P, Piazza P, Prendergast JGD, Rackham OJL, Ramilowski J a., Rashid M, Rassek T, Rizzu P, Ronconor M, Roy S, Rye MB, Saijyo E, Sajantila A, Saka A, Sakaguchi S, Sakai M, Sato H, Sato H, Savvi S, Saxena A, Schneider C, Schultes E a., Schulze-Tanzil GG, Schwemmann A, Stengst T, Sheng G, Shimojo Y, Shin JW, Simon C, Sugiyama D, Suyama T, Suzuki M, Suzuki N, Suvboka RK, t’Hoen P a. C, Tagami M, Takahashi N, Takai J, Tanaka H, Tatsukawa H, Temudo Z, Thompson M, Toyoda Y, Toyoda T, Valen E, van de Wetering M, van den Berg LM, Verardo R, Vijayan D, Vonrotsve IE, Wasserman WW, Watanabe S, Wells C a., Winteringham LN, Wolvetang E, Wood EJ, Yamaguchi T, Yamamoto M, Yaneda M, Yonekura Y, Yoshida S, Zabierowski SE, Zhang NG, Zhao X, Zucchielli S, Summers KM, Suzuki H, Daub CO, Kawaji H, Heutink P, Hide W, Freeman TC, Lenhard B, Bajic VB, Taylor MS, Makeev VJ, Sandelin A, Hume D a., Carninci P, Hayashizaki Y (2014) A promoter-level mammalian expression atlas. Nature 507, 462–470. [PubMed: 24670764]

20. Gjoneska E, Penning AR, Mathys H, Quon G, Kundaje A, Tsai L-H, Kellis M (2015) Conserved epigenomic signals in mice and humans reveal immune basis of Alzheimer’s disease. Nature 518, 365–369. [PubMed: 25693568]

21. Gagliano SA, Poquelle JG, Hardy J, Knight J, Barnes MR, Retyen M, Weale ME (2016) Genomics implicates adaptive and innate immunity in Alzheimers’s and Parkinson’s diseases. Ann. Clin. Transl. Neurol. 3, 924–933. [PubMed: 28097204]

22. Tansey KE, Cameron D, Hill MJ (2018) Genetic risk for Alzheimer’s disease is concentrated in specific macrophage and microglial transcriptional networks. Genome Med. 10, 14. [PubMed: 29482603]

23. Gaulton KJ, Ferreira T, Lee Y, Raimondo A, Magi R, Reschen ME, Mahajan A, Locke A, William Rayner N, Robertson N, Scott RA, Prokopenko I, Scott LJ, Green T, Sparso T, Thuillier D, Yengo
L, Grallert H, Wahl S, Frånberg M, Strawbridge RJ, Kestler H, Chheda H, Gustafsson S, Steinthorsdottir V, Thorleifsson G, Qi L, Karsen LC, van Leeuwen EM, Willems SM, Li M, Chen H, Fuchsberger C, Kwan P, Ma C, Linderman M, Lu Y, Thomsen SK, Rundle JK, Beer NL, van de Bunt M, Chheda H, Kang HM, Voight BF, Abecasis GR, Almgren P, Baldassarre D, Balkau B, Benediktsson R, Blüher M, Boeke H, Bonnycastle LL, Bottinger EP, Burtt NP, Carey J, Charpentier G, Chines PS, Cornelis MC, Couper DJ, Crenshaw AT, van Dam RM, Doney ASF, Dorkhan M, Edkins S, Eriksson JG, Esko T, Eury E, Fadista J, Fanoniplas P, Fox C, Franks PW, Gertov K, Gieger C, Gigante B, Gottesman O, Grant GB, Grarup N, Groves CJ, Hassinen M, Have CT, Herder C, Holmen OL, Hreidarsson AB, Humphries SE, Hunter DJ, Jackson AU, Jonsson A, Jorgensen ME, Jorgensen T, Kao W-ML, Kerrison ND, Kinnunen L, Klopp N, Kong A, Kovacs P, Kraft P, Kravic J, Langford C, Leander K, Liang L, Lichtner P, Lindgren CM, Lindholm E, Linneberg A, Liu C-T, Lobbens S, Luan J, Lyssenko V, Männistö S, MeLeod O, Meyer J, Mihailov E, Mühleisen TW, Müller-Nurasyid M, Navarro C, Nöthen MM, Oskolkov NN, Owen KR, Peltonen L, Perry JR, Platou CGP, Roden M, Rudferder D, Rybin D, van der Schouw YT, Semmlad B, Sigurdsson G, Stančáková A, Steinbach G, Storm P, Strauch K, Stringham HM, Sun Q, Thorand B, Tikkanen E, Tonjes A, Trakal J, Tremoli E, Tuomi T, Wennauer R, Wiltshire S, Wood AR, Zeggini E, Dunham I, Birney E, Pasquali L, Ferrer J, Loos RJF, Dupuis J, Florez JC, Boerwinkle E, Pankow JS, van Duijn C, Sijbrands E, Meigs JB, Hu FB, Thorsteinsdottir U, Stefansson K, Laak TA, Rauramaa R, Stumvoll M, Pedersen NL, Lind L, Keinanen-Kiukaanniemi SM, Korpi-Hyövätä E, Saaristo TE, Salteivo J, Kuusisto J, Laakso M, Metspalu A, Erbel R, Jöcke K-H, Moebus S, Ripatti S, Salomaa V, Ingelsson E, Boehm BO, Bergman RN, Collins FS, Mohlke KL, Koistinen H, Tuomilehto J, Hveem K, Njolstad I, Deloukas P, Donnelly PJ, Frayling TM, Hattersley AT, de Faire U, Hamsten A, Illig T, Peters A, Cauchi S, Sladek R, Froguel P, Hansen T, Pedersen O, Morris AD, Palmer CN, Kathiresan S, Melander O, Nilsson PM, Groves CJ, Langenberg GD, Wareham NJ, O’Callaghan CA, Gloyon AL, Altshuler D, Boehnke M, Teslovich TM, McCarthy MI, Morris AP (2015) Genetic fine mapping and genomic annotation defines causal mechanisms at type 2 diabetes susceptibility loci. Nat. Genet. 47. 24. Claussnitzer M, Dankel SN, Kim K-H, Quon G, Meuleman W, Haugen C, Glunk V, Sousa IS, Beaudry JL, Puvivrandar V, Abecasis GR, Bentley DR, Chakravarti A, Clark AG, Donnelly P, Eichler EE, Flicek P, Gabriel SB, Gibbs RA, Green ED, Hurles ME, Knoppers BM, Korbel JO, Lander ES, Lee C, Lehrach H, Mardis ER, Martl GT, McVean GA, Nickerson DA, Schmidt JP, Sherry ST, Wang J, Wilson RK, Gibbs RA, Boerwinkle E, Doddapaneni H, Han Y, Korchina V, Kovac C, Lee S, Muzny D, Reid JG, Zhu M, Wang J, Chang Y, Feng Q, Fang X, Guo X, Jin M, Jiang H, Jin X, Lan T, Li G, Li J, Li Y, Liu S, Liu X, Lu Y, Ma X, Tang M, Wang B, Wang G, Wu H, Wu R, Xu X, Yin Y, Zhang D, Zhang W, Zhao J, Zhao M, Zheng X, Lander ES, Altshuler DM, Gabriel SB, Gupta N, Gharani N, Toji LH, Gerry NP, Resch AM, Flicek P, Barker J, Clarke L, Gil L, Hunt SE, Kelman G, Kulesha E, Leinonen R, McLaren WM, RadhaKrishnan R, Roa A, Smirnov D, Smith RE, Streeter I, Thorngren A, Töneva I, Vaughan B, Zheng-Bradley X, Bentley DR, Grocock R, Humphray S, James T, Kingsbury Z, Lehrach H, Sudbrak R, Albrecht MW, Amstislavskiy VS, Borodina TA, Lienhard M, Mertes F, Sultan M, Timmermann B, Yasso M, Mardis ER, Wilson RK, Fulton L, Fulton R, Sherry ST, Ananiev V, J Alzheimers Dis. Author manuscript; available in PMC 2020 June 25.
Desalle R, Narechania A, Wilson Sayres MA, Garrison EP, Handsaker RE, Kashin S, McCarroll SA, Rodriguez-Flores JL, Flicek P, Clarke L, Zheng-Bradley X, Erlrich Y, Gymrek M, Frederick Willems T, Bustamante CD, Mendez FL, David Poznik G, Underhill PA, Lee C, Cervera E, Malhotra A, Romanovitch M, Zhang C, Abecasis GR, Coin L, Shao H, Mittelman D, Tyler-Smith C, Ayub Q, Banerjee R, Cerezo M, Chen Y, Fitzgerald TW, Louzada S, Massaia A, McCarthy S, Ritchie GR, Xue Y, Yang F, Gibbs RA, Kovar C, Kalra D, Hale W, Muzny D, Reid JG, Wang J, Dan X, Guo X, Li G, Li Y, Ye C, Zheng X, Altshuler DM, Flicek P, Clarke L, Zheng-Bradley X, Bentley DR, Cox A, Humphray S, Kahn S, Sudbrak R, Albrecht MW, Lienhard M, Larson D, Craig DW, Izzat T, Kurdoglu AA, Sherry ST, Xiao C, Haussler D, Abecasis GR, Meidan GA, Durbin RM, Balasubramaniam S, Keane TM, McCarthy S, Stalker J, Chakravarti A, Knoppers BM, Abecasis GR, Barnes KC, Beiswanger C, Burchard EG, Bustamante CD, Cai H, Cao H, Durbin RM, Gerry NP, Gharani N, Gibbs RA, Gignoux CR, Grave S, Henn B, Jones D, Jorde L, Kaye JS, Keinan A, Kent A, Kerasidou A, Li Y, Mathias R, McVean GA, Moreno-Estrada A, Ossorio PN, Parker M, Resch AM, Rotimi CN, Royal CD, Sandoval K, Su Y, Sudbrak R, Tian Z, Tishkoff S, Toji LH, Tyler-Smith C, Via M, Wang Y, Yang H, Yang L, Zhu J, Bodmer W, Bedoya G, Ruiz-Linares A, Cai Z, Gao Y, Chu J, Peltonen L, Garcia-Montero A, Orfoa A, Dutil J, Martinez-Cruzado JC, Oleksyk TK, Barnes KC, Mathias RA, Hennis A, Watson H, McKenzie C, Qadri F, LaRocque R, Sabeti PC, Zhu J, Deng X, Sabeti PC, Asgoun D, Folari O, Hapi C, Omnoniwa O, Stremlau M, Tariyal R, Jallow M, Sisay Joof F, Corrah T, Rockett K, Kwiatkowski D, Koerner J, Tinh Hiênh T, Dunstan S, Huy Hang N, Fonnie R, Garry R, Kanchele M, Moses L, Sabeti PC, Sch liefelin J, Grant DS, Gallo C, Poletti G, Saleheen D, Rashed A, Brooks LD, Felsenfeld AL, McEwen JE, Vayidevich V, Green ED, Duncanson A, Dunn M, Schloss JA, Wang J, Yang H, Auton A, Brooks LD, Durbin RM, Garrison EP, Min Kang H, Korbel JO, Marchini JL, McCarthy S, McVean GA, Abecasis GR (2015) A global reference for human genetic variation. Nature 526, 68–74. [PubMed: 26432245]

29. Zhang B, Kirov S, Snoddy J (2005) WebGestalt: an integrated system for exploring gene sets in various biological contexts. Nucleic Acids Res. 33, W741–8. [PubMed: 15980575]

30. Wang J, Duncan D, Shi Z, Zhang B (2013) WEB-based GEne SeT AnaLysis Toolkit (WebGestalt): update 2013. Nucleic Acids Res. 41, W77–83. [PubMed: 23703215]

31. Wang J, Vasaikar S, Shi Z, Greer M, Zhang B (2017) WebGestalt 2017: A more comprehensive, powerful, flexible and interactive gene set enrichment analysis toolkit. Nucleic Acids Res. 45, W130–W137. [PubMed: 28472511]

32. Kuznetsova A, Brockhoff PB, Christensen RHB (2017) ImerTest Package: Tests in Linear Mixed Effects Models. J. Stat. Softw 82,.

33. R Core Team (2014) R: A Language and Environment for Statistical Computing.

34. Kuzma A, Valladares O, Cweibl R, Greenfest-Allen E, Childress DM, Malamon J, Gangadharan P, Zhao Y, Qu L, Leung YY, Naj AC, Stoeckert CJ, Schellenberg GD, Wang L-S (2016) NIAAGDS: The NIA Genetics of Alzheimer’s Disease Data Storage Site. Alzheimer’s Dement. 12, 1200–1203.

35. Ernst J, Kellis M (2012) ChromHMM: automating chromatin-state discovery and characterization. Nat. Methods 9, 215–6. [PubMed: 22955616]

36. Bernstein BE, Birney E, Dunham I, Green ED, Gunter C, Snyder M (2012) An integrated encyclopedia of DNA elements in the human genome. Nature 489, 57–74. [PubMed: 22955616]

37. Bernstein BE, Birney E, Dunham I, Green ED, Gunter C, Snyder M, Bernstein BE (2011) Mapping and analysis of chromatin state dynamics in nine human cell types. Nature 473, 43–49. [PubMed: 21441907]

38. Bernstein BE, Birney E, Dunham I, Green ED, Gunter C, Snyder M, Bernstein BE (2012) An integrated encyclopedia of DNA elements in the human genome. Nature 489, 57–74. [PubMed: 22955616]

39. Leung YY, Kuksa PP, Amlie-Wolf A, Valladares O, Unger LH, Kannan S, Gregory BD, Wang L-S (2016) DASHR: Database of Small Human noncoding RNAs. Nucleic Acids Res. 44,.

40. Kuksa PP, Amlie-Wolf A, Katanic Z, Valladares O, Wang L-S, Leung YY (2018) DASHR 2.0: integrated database of human small non-coding RNA genes and mature products. Bioinformatics 0–0.

41. Agarwal V, Bell GW, Nam JW, Bartel DP (2015) Predicting effective microRNA target sites in mammalian mRNAs. Elife 4, 1–38.

42. Femminella GD, Ferrara N, Rengo G (2015) The emerging role of microRNAs in Alzheimer’s disease. Front. Physiol. 6, 1–5. [PubMed: 25688210]
42. Heinz S, Benner C, Spann N, Bertolino E, Lin YC, Laslo P, Cheng JX, Murre C, Singh H, Glass CK (2010) Simple Combinations of Lineage-Determining Transcription Factors Prime cis-Regulatory Elements Required for Macrophage and B Cell Identities. Mol. Cell 38, 576–589. [PubMed: 20513432]

43. Shabalin AA (2012) Matrix eQTL: Ultra fast eQTL analysis via large matrix operations. Bioinformatics 28, 1353–1358. [PubMed: 22492648]

44. Giambartolomei C, Vukcevic D, Schadt EE, Franke L, Hingorani AD, Wallace C, Plagnol V (2014) Bayesian Test for Colocalisation between Pairs of Genetic Association Studies Using Summary Statistics. PLoS Genet. 10.,

45. Bulger M, Groudnine M (2011) Functional and mechanistic diversity of distal transcription enhancers. Cell 144, 327–39. [PubMed: 21295696]

46. Engreitz JM, Ollikainen N, Guttmann M (2016) Long non-coding RNAs: spatial amplifiers that control nuclear structure and gene expression. Nat. Rev. Mol. Cell Biol.

47. Chiba T, Yamada M, Sasabe J, Terashita K, Shimoda M, Matsuoka M, Aiso S (2009) Amyloid-beta causes memory impairment by disturbing the JAK2/STAT3 axis in hippocampal neurons. Mol. Psychiatry 14, 206–222. [PubMed: 18813209]

48. Zou F, Chai HS, Younkin CS, Allen M, Crook J, Pankratz VS, Cartier MM, Nair AA, Asselin CN, Mullin K, Hooli B, Choi SH, Hyman BT, Tanzi RE (2013) Alzheimer’s disease risk gene CD33 inhibits microglial uptake of amyloid beta. Neuron 78, 631–643. [PubMed: 23623698]

49. Fujita Y, Yamaguchi A, Hata K, Endo M, Yamaguchi N, Yamashita T (2009) Zyxin is a novel interacting partner for SIRT1. BMC Cell Biol. 10, 6. [PubMed: 19173742]

50. Lanni C, Necchi D, Pinto A, Buoso E, Buizza L, Memo M, Uberti D, Govoni S, Racchi M (2013) Zyxin is a novel target for beta-amyloid peptide: Characterization of its role in Alzheimer’s pathogenesis. J. Neurochem. 125, 790–799. [PubMed: 23330981]

51. Griciuc A, Serrano-Pozo A, Parrado AR, Lesinski AN, Asselin CN, Mullin K, Hooli B, Choi SH, Hyman BT, Tanzi RE (2013) Alzheimer’s disease risk gene CD33 inhibits microglial uptake of amyloid beta. Neuron 78, 631–643. [PubMed: 23623698]

52. Malik M, Simpson JR, Parikh I, Wilfred BR, Fardo DW, Nelson PT, Estus S (2013) CD33 Alzheimer’s disease locus: altered monocyte function and amyloid biology. Nat. Neurosci. 16, 848–850. [PubMed: 23708142]

53. Lawson LJ, Perry VH, Dri P, Gordon S (1990) Heterogeneity in the distribution and morphology of microglia in the normal adult mouse brain. Neuroscience 39, 151–170. [PubMed: 2089275]

54. Chapuis J, Hansmann F, Gistelinck M, Mounier A, Van Cauwenberge C, Kolen K V , Geller F, Sottejeau Y, Harold D, Dourlen P, Grenier-Boley B, Kamatani Y, Lepeine B, Demiautte F, Zelenika D, Zommer N, Hamdane M, Bellenguez C, Martin MM, Tavitiyan F, Lebon P, Delevaque C, Misakian D, O’Donovan M, Owen M, Hipple J, Mercken K, Reid M, Batscheff M, Dreyfus G, Jorgensen K, Campion D, Octave J-N, Berr C, Lathrop M, Moechars D, Dermaut B, Lambert J-C (2013) Increased expression of BIN1 mediates Alzheimer genetic risk by modulating tau pathology. Mol. Psychiatry 18, 1225–1234. [PubMed: 23999914]

55. Sullivan AL, Benner C, Heinz S, Huang W, Xie L, Miano JM, Glass CK (2011) Serum Response Factor Utilizes Distinct Promoter-and Enhancer-Based Mechanisms To Regulate Cytoskeletal Gene Expression in Macrophages. Mol. Cell. Biol. 31, 861–875. [PubMed: 21135125]

56. Potthoff MJ, Olson EN (2007) MEF2: a central regulator of diverse developmental programs. Development 134, 4131–4140. [PubMed: 17959722]
58. Lynch DK, Winata SC, Lyons RJ, Hughes WE, Lehrbach GM, Wasinger V, Corthals G, Cordwell S, Daly RJ (2003) A cortactin-CD2-associated protein (CD2AP) complex provides a novel link between epidermal growth factor receptor endocytosis and the actin cytoskeleton. J. Biol. Chem. 278, 21805–21813. [PubMed: 12672817]

59. Yong J, Tan I, Lim L, Leung T (2006) Phosphorylation of myosin phosphatase targeting subunit 3 (MYPT3) and regulation of protein phosphatase 1 by protein kinase A. J. Biol. Chem. 281, 31202–31211. [PubMed: 16920702]

60. Wadsworth TL, Bishop J a, Pappu AS, Woltjer RL, Quinn JF (2008) Evaluation of coenzyme Q as an antioxidant strategy for Alzheimer’s disease. J. Alzheimer’s Dis. 14, 225–34. [PubMed: 18560133]

61. Zhang Y, Chen K, Sloan S a, Bennett ML, Scholze AR, O’Keeffe S, Phatnani HP, Guarnieri P, Caneda C, Ruderisch N, Deng S, Liddelow S a, Zhang C, Daneman R, Maniatis T, Barres B a, Wu JQ (2014) An RNA-Sequencing Transcriptome and Splicing Database of Glia, Neurons, and Vascular Cells of the Cerebral Cortex. J. Neurosci. 34, 11929–47. [PubMed: 25186741]
Figure 1: LD expansion and functional annotation of top IGAP hits.

a) Flowchart of analysis approach. b) Genomic localization of all variants in P-value- and LD-expanded set. c) Genomic partition proportions split by tag regions.
**Figure 2: Integrative analysis of annotations for IGAP top hits.**

a) Integrative tissue context analysis of enhancer overlaps from FANTOM5 and Roadmap datasets. b) Results of LD-collapsed bootstrapping for enhancer annotation overlap enrichments c) Distributions of variant probability of underlying highly colocalized signals stratified by annotation overlap. d) Barplots of numbers of variant – eQTL comparisons across tag regions stratified by motif overlap, enhancer support, and concordant support in a relevant tissue class.
Figure 3: Functional variant in *EPHA1* region upregulates *EPHA1-AS1* IncRNA which regulates the *JAK2* signaling axis.
a) Genome browser view of the region around rs11765305 (in red box) including relevant FANTOM5 and Roadmap enhancer annotations. b) Luciferase assay results for rs11765305 in K562 cells. Luciferase expression is normalized against Renilla expression in the same well. Negative control is randomly sampled heterochromatin insert. A linear mixed model was applied to 5 biological replicates per condition, each with 4 technical replicates per experimental day. c) Scatterplot of Pearson and Spearman correlations between expression of *EPHA1-AS1* and all other genes in the genome across all GTEx tissues.
Figure 4: Luciferase and lncRNA analysis in the BIN1, CD33, and CD2AP regions.
a) Luciferase validation in the CD33 region. b) Luciferase validation in the BIN1 region. c) Luciferase validation in the CD2AP region. For all luciferase analyses, a linear mixed model was applied to 5 biological replicates per condition, each with 4 technical replicates per experimental day. d) Scatterplot of Pearson and Spearman correlations between expression of RP11–385F7.1 (CD2AP region) and all other genes in the genome across all GTEx tissues.
| Tag variant | Nearest Gene | $P$ value expanded | # LD pruned | # LD re-expanded | # FANTOM5 overlaps | # Roadmap ChromHMM enhancer overlaps | # HOMER TFBS overlaps |
|-------------|--------------|------------------|------------|-----------------|-------------------|-------------------------------------|---------------------|
| rs4147929:A>G | ABCA7        | 7                | 2          | 9               | 0                 | 8                                   | 6                   |
| rs35349669:C>T | BIN1        | 2                | 1          | 3               | 0                 | 3                                   | 1                   |
| rs7274581:T>C | CASS4        | 20               | 2          | 23              | 4                 | 19                                  | 10                  |
| rs10948363:A>G | CD2AP            | 80               | 5          | 83              | 0                 | 45                                  | 30                  |
| rs3865444:C>A | CD33        | 3                | 1          | 6               | 0                 | 3                                   | 4                   |
| rs10838725:T>C | CELF1        | 92               | 7          | 264             | 13                | 147                                 | 110                 |
| rs28834970:T>C | CLU          | 10               | 1          | 11              | 0                 | 11                                  | 7                   |
| rs6656401:A>G | CR1         | 20               | 1          | 22              | 0                 | 12                                  | 9                   |
| rs11771145:G>A | EPHA1        | 9                | 3          | 9               | 2                 | 16                                  | 2                   |
| rs17125944:T>C | FERMT2      | 32               | 7          | 92              | 2                 | 79                                  | 43                  |
| rs6733839:C>T | INPP5D      | 65               | 2          | 114             | 0                 | 66                                  | 54                  |
| rs190982:G>A | MEF2C       | 2                | 1          | 3               | 0                 | 2                                   | 1                   |
| rs983392:G>A | MS4A6A      | 80               | 3          | 173             | 5                 | 91                                  | 75                  |
| rs1476679:C>T | TXNDC3 / NME8 | 44             | 5          | 96              | 4                 | 44                                  | 47                  |
| rs10792832:A>G | PICALM     | 2                | 1          | 18              | 2                 | 16                                  | 11                  |
| rs9331896:C>T | PTK2B      | 7                | 2          | 14              | 3                 | 14                                  | 8                   |
| rs10498333:G>T | SLC24A4   | 5                | 3          | 5               | 4                 | 4                                   | 4                   |
| rs11218343:T>C | SORL1     | 1                | 1          | 1               | 0                 | 1                                   | 1                   |
| rs2718058:A>G | ZCWPW1     | 15               | 4          | 98              | 0                 | 78                                  | 28                  |
| **Total**    |             | **496**          | **52**     | **1,044**       | **39**          | **659**                               | **451**              |
Table 2.
Summary of colocalization results in 4 top prioritized tag regions.

| Tag Region | Top affected mechanism(s) and evidence | Direction of effect | Experimental validation performed? |
|------------|---------------------------------------|---------------------|-----------------------------------|
| EPHA1      | Whole blood eQTL for EPHA1-AS1 supported by high ABF and increased CEBP TFBS variant rs11765305 | Protective haplotype has strong increase in EPHA1-AS1 expression | Yes, enhancer activity and significant allelic difference |
| CD33       | Whole blood eQTL for CD33 with high ABF tag variant rs3865444 | Protective tag variant decreases CD33 expression | Yes, strong enhancer activity and significant allelic difference |
| BIN1       | Lymphocyte eQTLs for BIN1, high ABF variant rs4663195 | Risk haplotype decreases BIN1 expression | Yes, moderate enhancer activity with significant allelic difference |
| CD2AP      | Cerebellar eQTL for RP11–385F7.1 and fibroblast eQTL for CD2AP with TF disrupting variant rs9367279 | Risk haplotype with moderate, inconsistent effects on lncRNA expression in brain, decrease in CD2AP expression | Yes, no K562 enhancer activity or allelic difference |