Transfer learning-trained convolutional neural networks identify novel MRI biomarkers of Alzheimer’s disease progression

Yi Li1 | Annat Haber1 | Christoph Preuss2 | Cai John1 | Asli Uyar1 | Hongtian Stanley Yang2 | Benjamin A. Logsdon3 | Vivek Philip2 | R. Krishna Murthy Karuturi1 | Gregory W. Carter1,2 | The Alzheimer’s Disease Neuroimaging Initiative*

1 The Jackson Laboratory, Farmington, Connecticut, USA  
2 The Jackson Laboratory, Bar Harbor, Maine, USA  
3 Sage Bionetworks, Seattle, Washington, USA

Correspondence  
Gregory W. Carter, The Jackson Laboratory, 600 Main St., Bar Harbor, ME 04609, USA.  
E-mail: Gregory.Carter@jax.org

Present address  
Cai John, The University of Tennessee, Knoxville, Tennessee, USA

Abstract

Introduction: Genome-wide association studies (GWAS) for late onset Alzheimer’s disease (AD) may miss genetic variants relevant for delineating disease stages when using clinically defined case/control as a phenotype due to its loose definition and heterogeneity.

Methods: We use a transfer learning technique to train three-dimensional convolutional neural network (CNN) models based on structural magnetic resonance imaging (MRI) from the screening stage in the Alzheimer’s Disease Neuroimaging Initiative consortium to derive image features that reflect AD progression.

Results: CNN-derived image phenotypes are significantly associated with fasting metabolites related to early lipid metabolic changes as well as insulin resistance and with genetic variants mapped to candidate genes enriched for amyloid beta degradation, tau phosphorylation, calcium ion binding-dependent synaptic loss, APP-regulated inflammation response, and insulin resistance.

Discussion: This is the first attempt to show that non-invasive MRI biomarkers are linked to AD progression characteristics, reinforcing their use in early AD diagnosis and monitoring.

KEYWORDS  
Alzheimer’s disease, convolutional neural networks, deep learning, disease progression, imaging phenotypes, machine learning, magnetic resonance imaging; transfer learning
1 | BACKGROUND

Alzheimer’s disease (AD) is a progressive neurodegenerative disorder that slowly degrades memory and cognitive functions. It is neuropathologically defined by intracellular neurofibrillary tangles and aggregated amyloid beta (Aβ) plaques, both of which can currently be estimated accurately only post mortem. The phenotype in current case/control genome-wide association studies (GWAS) for late onset AD (LOAD) are based largely on clinical assessments, in which mild cognitive impairment (MCI) and AD are determined by designed memory and cognitive tests and clinical observations. These criteria fail to reflect early AD hallmark characteristics such as Aβ plaques and neurofibrillary tangles and highlight advanced AD, leaving the MCI category widely heterogeneous and poorly understood. Consequently, current GWAS for LOAD usually exclude MCI and therefore may miss critical genetic variants associated with early AD characteristics and progression.

Non-invasive brain imaging modalities such as magnetic resonance imaging (MRI) and positron emission tomography (PET) are promising tools for monitoring AD progression and its diagnosis. Imaging provides precise quantitative phenotypes, and numerous methods have been proposed for analyzing neuropathology with MRI. However, the high dimensionality of these phenotypes makes it challenging to extract concise and interpretable information. Summary measures for pre-defined regions of interest (ROI) are suboptimal for predicting the onset and progression of AD because they are derived independently of AD status.

In this article, we make use of deep convolutional neural networks (CNN) to simultaneously extract relevant features and classify patients using (structural) MRI data from the Alzheimer’s Disease Neuroimaging Initiative (ADNI) consortium. Deep CNN have become the state-of-the-art methods for image classification due to their ability to form translation invariant hierarchical image features. To mitigate the scarcity of images in the ADNI dataset, and accommodate the high number of model parameters that need to be learned, we adopt a transfer learning technique. This technique uses an independent data-trained 3D CNN model that is then fine-tuned using our dataset of 1381 images. This greatly augments our image dataset and ensures the learned CNN model is more robust to overfitting. Because our baseline MRI images were taken 3 years prior to clinical labeling and provide holistic snapshots of brain states, our CNN-derived image features reflect earlier and more specific AD characteristics than the memory and cognitive performance features used for assessing AD and MCI. This is supported by the significant associations we find between the CNN-derived phenotypes and early AD-related metabolites and genes (Figure 1). To our knowledge, this is the first attempt to link non-invasive MRI biomarkers with AD progression characteristics.

2 | METHODS

2.1 | MRI and clinically labeled data from the ADNI consortium

Data for this study were obtained from the ADNI database (adni.loni.usc.edu). ADNI is a longitudinal study in which initial imaging is followed by annual re-imaging. MRI images taken at the initial stage are later categorized into four major classes based on their follow-up status: control, AD, stable MCI subjects who maintain the same disease status throughout the follow-up period, and progressive MCI subjects who convert from MCI to AD sometime during the follow-up period. MCI were counted as stable MCI only if they were followed-up for at least 3 years in this study. The conversion and follow-up timelines for the 526 MCI patients are shown in Table S1B in supporting information.

We downloaded 817 screening images from the ADNI-1 cohort, 104 ADNI-GO new participants, and 624 ADNI-2 new participants. Because AD patients rarely convert back, we included 162 Year 1...
and 95 Year 2 images from ADNI-1 patients who were diagnosed as AD at screening, 155 of which also had MRIs at screening, totaling 1802 images (Table S1A). The adding of Year 1/2 AD images to screening images was expected to help CNN more accurately recognize progression-related image features. However, there were no duplicate subjects in the downstream metabolite and GWAS analysis. Some subjects in ADNI-1 have two MRI scans from the same session; we kept the one in the "Scaled_2" directory as recommended by ADNI MRI core team. We filtered out Year 1 to 2 AD MRIs with the rank of 4 or -1 (based on downloaded MRIMPRANK.csv) and ADNI-GO/2 MRIs with the quality of 4 or none (based on downloaded MAYOADIRL_MRI_IMAGEQC_12_08_15.csv). When there was more than one MRI scan from the same session after filtering, we kept the one with the highest quality or the latest timepoint when qualities were equal.

FreeSurfer\textsuperscript{18,19} software (-autorecon1 option) was applied to correct motions, normalize image intensities, and strip bone tissue, followed by manual checking of sagittal slice 101 of each MRI image, ensuring that the mean intensity of the white matter was around 110 and skull was stripped correctly (FreeSurfer suggestions). Inappropriate skull stripping was rescued by running mri_watershed with different watershed thresholds. MRIs with incorrect mean intensity of the white matter or inappropriate skull stripping after reslicing were excluded. To investigate whether co-registration is necessary when applying CNN to brain image analysis, we used the Talairach transformation calculated in FreeSurfer (-autorecon1) to obtain MNI305 atlas-registered MRIs.

2.2 CNN and feature formation

CNN is a type of supervised multiple-layer neural network that adopts learnable convolutional kernels to detect hierarchical image features. Because the same kernel slides over the whole image, the detected image features are translation invariant.\textsuperscript{20} To reinforce this, input images are often augmented during CNN training via transformations such as multiple scaling and cropping. A loss value at the last layer of a CNN is computed in the forward pass and iteratively minimized by back-propagating the loss to all hidden layers to update their weights based on the stochastic gradient descent rule.\textsuperscript{21}

The pre-trained 3D CNN model used for the transfer learning adopted ResNext101 network structure, which consists of 101 layers, and was trained using 300,000 Kinetics video clips.\textsuperscript{22} Only the parameters in the last few layers of ResNext101 were fine-tuned during the training stage of our dataset. We added nodes to the second-to-last layer in ResNext101 structure to accommodate covariates (Figure S1 in supporting information). Our preliminary classification results showed that progressive MCI was frequently predicted as AD by the CNN model, suggesting that the CNN classification possibly reclassified patients with pending diagnosis. We therefore trained our CNN models with the target classes of controls, stable MCI, and broad AD (AD and progressive MCI).

To maximize the chance of obtaining an accurate CNN model, we generated 10-fold sample splits. The three classes of subjects were evenly divided into 10 folds in a class-wise fashion; for each sample split, one fold was used as an independent test set, the remaining nine folds were randomly split into training and validation sets with the ratio of 9:1. One CNN model was learned on the training and validation sets of each of the 10 sample splits, and the one with the highest classification accuracy on the (independent) test set was selected as the best model for downstream analyses.

The second-to-last layer of our CNN was the only layer that provided input for the class probabilities at the last layer, and therefore contained the features that are the most predictive of the classification. This layer yielded 2048 image features in the adopted ResNext101 structure (Figure S1). Covariates entered CNN at this layer and their effects were passed forward to compute the loss at the last layer, which was propagated backward to all the hidden layers including the second-to-last layer. Hence, when there are covariates in our CNN model, the extracted image features are covariate adjusted. To reduce the
number of image features for downstream analyses, we applied principal component analysis (PCA) to the 2048 image features, used the broken stick model to estimate the number of PCs needed, followed by a L1-norm regularized regression model (Lasso) to select the most informative PCs for distinguishing stable and progressive MCIs. These PCs are hereafter referred to as CNN-derived image phenotypes. After CNN was trained, covariates were not needed to obtain the image phenotypes, but needed for disease status predictions.

We generated two sets of CNN-derived phenotypes. For the first, we trained a CNN model with age at screening, sex, education level, MRI field strength indicator (1.5T or 3T), and ethnicity as covariates. For the second set, we included APOE genotype as an additional covariate, along with four APOE-correlated cognitive scores at screening (hereafter Augmented CNN model).

To evaluate the performance of our CNN-derived image phenotypes, we correlated them with metabolites and genetic variants. We also compared them with conventional image summary measures, cognitive scores, and clinical labels. See supporting information for additional details.

### 2.3 AIBL MRIs as a validation dataset

We used MR images from the Australian Imaging, Biomarker & Lifestyle Flagship Study of Ageing (AIBL) to evaluate the performance of the trained CNNs. AIBL, designed similarly to ADNI, is a longitudinal study that follows-up participants every 18 months until 6 years from screening. All subjects were assumed to be White, a subset of whose MRIs were provided at the ADNI website. We selected 207 subjects who had one MRI scan at baseline and either remained as controls/MCI/AD for at least 3 years or converted to AD during the 6-year follow-up. There were only 10 stable MCI and 11 progressive MCI among 207 subjects. The MRIs, 60 of which were of 1.5T magnetic strength and 147 of 3T, went through the same pre-processing step as ADNI MRIs. Participants’ characteristics were described in Dang et al. and Ellis et al. Age, sex, APOE ε4 genotype, and MMSE are summarized in Table 1 and used here as covariates. Education level and cognitive scores of ADAS and FAQ, not available to our access, were summarized in Table 1 and used here as covariates. Education level and cognitive scores of ADAS and FAQ, not available to our access, were

### Table 1

| No. of APOE ε4 copies | AIBL data | No. of APOE ε4 copies | AIBL data |
|-----------------------|-----------|-----------------------|-----------|
|                        | No. of subjects | Age | Male/female | 0 | 1 | 2 | MMSE |
| Control               | 373 | 74.3 ± 6 | 182/191 | 274 | 91 | 9 | 76 | 30 | 1 | 29.1 ± 1.1 |
| AD                    | 251 | 74.8 ± 8 | 134/117 | 84 | 114 | 53 | 74 | 73.2 ± 8 | 29/45 | 23 | 37 | 14 | 20.3 ± 5.6 |
| sMCI                  | 424 | 73.1 ± 8 | 255/169 | 246 | 141 | 37 | 10 | 77.2 ± 7 | 8/2 | 5 | 4 | 1 | 28.0 ± 1.5 |
| pMCI                  | 230 | 73.9 ± 7 | 134/96 | 77 | 114 | 39 | 1 | 74.9 ± 6 | 7/4 | 1 | 6 | 4 | 26.3 ± 1.7 |
| P-Value1              | 0.047 | 0.26 | 5.72 × 10⁻³⁰ | 0.28 | 0.26 | 4.51 × 10⁻¹⁰ | 2.93 × 10⁻¹⁶ |
| P-Value2              | 0.72  | 0.64 | 3.21 × 10⁻⁰⁹ | 0.25 | 0.43 | 0.033 | 0.18 |

Notes: Age is presented in a mean ± standard deviation format.
ADNI, sMCI, and pMCI were estimated until 3 years from screening (for CNN training); AIBL, sMCI, and pMCI were estimated until 6 years from baseline (for CNN evaluation).
P-value1: P value of comparing AD and controls.
P-value2: P value of comparing sMCI and pMCI.
Abbreviations: AD, Alzheimer’s disease; ADNI, Alzheimer’s Disease Neuroimaging Initiative; AIBL, Australian Imaging, Biomarker & Lifestyle Flagship Study of Ageing; APOE, apolipoprotein E; MMSE, Mini-Mental State Examination; pMCI, progressive MCI; sMCI, stable MCI.

### 3 RESULTS

#### 3.1 Deep 3D CNN models classify transition from MCI to AD accurately

The confusion matrices for the best CNN models on unregistered MRIs are shown in Table 2. The Augmented CNN yielded prediction accuracy of 0.992 for broad AD, 0.986 for controls, and between 0.911 (1-year follow-up) to 0.801 (the final visit) for progressive MCI. In comparison, the Image CNN achieved prediction accuracy of 0.913 for broad AD, 0.906 for controls, and between 0.822 (1-year follow-up) to 0.69 (the final visit) for progressive MCI. The Image CNN had lower power to differentiate stable MCI from healthy controls. Both models had lower error rate of predicting stable MCI as broad AD with longer follow-up period (0.409 to 0.192 for Augmented CNN, 0.376 to 0.2 for Image CNN), implying that some of the stable MCI that were predicted by CNN as broad AD converted to AD when tracked for longer than 3 years. We also trained the two CNN models using co-registered MRIs. As shown in Figure 2A and B, CNN performance on non-registered and registered MRIs was not significantly different, verifying that CNN is able to learn translation-invariant image features. However, Image CNN with the same structure had a bigger performance difference between training and test samples on registered MRI than on non-registered MRI (Figure 2C), implying that Image CNN might be somewhat overfitting for
TABLE 2  Confusion matrix for CNN predictions

| Clinical label | Image CNN (trained without APOE genotype and cognitive score as covariates) | Augmented CNN (trained with APOE genotype and cognitive score as covariates) |
|---------------|--------------------------------------------------------------------------------|--------------------------------------------------------------------------------|
|               | Control  | Stable MCI | Broad AD | Control  | Stable MCI | Broad AD |
| 3Y_ctrl (373) | 0.895 (334) | 0.0107 (4) | 0.0938 (35) | 0.96 (358) | 0.0268 (10) | 0.0134 (5) |
| AD (482)      | 0.0851 (41) | 0.00207 (1) | 0.913 (400) | 0.0 (0) | 0.0083 (4) | 0.992 (478) |
| 1Y_sMCI (425) | 0.442 (188) | 0.181 (77) | 0.376 (160) | 0.231 (98) | 0.36 (153) | 0.409 (174) |
| 2Y_sMCI (336) | 0.518 (174) | 0.226 (76) | 0.256 (86) | 0.277 (93) | 0.435 (146) | 0.289 (97) |
| 3Y_sMCI (296) | 0.551 (163) | 0.24 (71) | 0.209 (62) | 0.297 (88) | 0.476 (141) | 0.226 (67) |
| 4Y_sMCI (278) | 0.558 (155) | 0.241 (67) | 0.201 (56) | 0.317 (88) | 0.478 (133) | 0.205 (57) |
| 5Y_sMCI (271) | 0.561 (152) | 0.232 (63) | 0.207 (56) | 0.325 (88) | 0.48 (130) | 0.196 (53) |
| final_sMCI (255) | 0.557 (142) | 0.243 (62) | 0.2 (51) | 0.318 (81) | 0.49 (125) | 0.192 (49) |
| 1Y_pMCI (101) | 0.178 (18) | 0 (0) | 0.822 (83) | 0.0198 (2) | 0.0693 (7) | 0.911 (92) |
| 2Y_pMCI (190) | 0.195 (37) | 0.00526 (1) | 0.8 (152) | 0.0368 (7) | 0.0737 (14) | 0.889 (169) |
| 3Y_pMCI (230) | 0.209 (48) | 0.0087 (2) | 0.783 (180) | 0.0522 (12) | 0.0826 (19) | 0.865 (199) |
| 4Y_pMCI (248) | 0.226 (56) | 0.0403 (10) | 0.734 (182) | 0.0484 (12) | 0.109 (27) | 0.843 (209) |
| 5Y_pMCI (255) | 0.231 (59) | 0.0549 (14) | 0.714 (182) | 0.0471 (12) | 0.118 (30) | 0.835 (213) |
| final_pMCI (271) | 0.255 (69) | 0.0554 (15) | 0.69 (187) | 0.0701 (19) | 0.129 (35) | 0.801 (217) |

Notes: Number of samples is given in parentheses. The fraction at each entry stands for the ratio of the number of CNN predictions belonging to the column category to the number of samples belonging to the row (clinical) category. That pMCI or AD were predicted as broad AD, and control/sMCI were predicted as non broad AD can be viewed as correct predictions in a broad sense.

Abbreviations: AD, Alzheimer’s disease; ADNI, Alzheimer’s Disease Neuroimaging Initiative; APOE, apolipoprotein E; CNN, convolutional neural network; pMCI, progressive MCI; sMCI, stable MCI.

registered MRI. This is probably because the transferred pre-trained CNN was trained on non-registered images. Hence, all the downstream analyses are based on non-registered MRIs.

### 3.2 Trained CNNs applied to AIBL MRIs

CNN analysis was also informative in our AIBL validation dataset, especially for Image CNN as the relevant cognitive data were not available for Augmented CNN. Area under the curve (AUC) of distinguishing controls from AD was 0.78 for Image CNN and 0.76 for Augmented CNN. AUC of differentiating stable and progressive MCI were lower—0.61 and 0.6, respectively (Table S3 in supporting information)—probably due to the smaller sample size (21 subjects total). The Augmented CNN performed worse than the Image CNN, probably because ADAS, FAQ and CDR-SOB were not available. Potential reasons for the reduced AUC compared to ADNI analysis include (1) MRI acquisitions in ADNI and AIBL used slightly different protocols and (2) some overfitting in the trained CNN in spite of the transfer learning, as the trained CNN was fitted to a North American population whereas AIBL data were drawn from Australia.

### 3.3 Image phenotypes derived from 3D CNNs

Using Lasso, we selected principal components 1, 4, and 9 as informative phenotypes from Image CNN model (hereafter imageCNN.PC1, imageCNN.PC4, imageCNN.PC9), explaining 0.257, 0.035, 0.019 of the variance of the 2048 CNN-derived image features, respectively. Only PC 2 (hereafter augmentedCNN.PC2) was selected from Augmented CNN model, explaining 0.064 of the variance of the 2048 image features. Only imageCNN.PC4 and augmentedCNN.PC2 had a high correlation coefficient of 0.72; all other pairwise correlations of PCs were below 0.3.

### 3.4 Image phenotypes are associated with early AD-related metabolites

Seven metabolites were found to be significantly (P < 0.05/55 = 0.0009) associated with the four CNN-derived phenotypes in the ADNI-1 participants (Table S4 in supporting information), including two phosphatidylcholines (PC) metabolites (PC ae C44:4 associated with imageCNN.PC1, PC aa C32:3 with augmentedCNN.PC2), and three sphingomyelins (SM) metabolites (SM C16:1, SM C18:0, and SM C20:2, with augmentedCNN.PC2). These PC or SM metabolites were previously found to be significantly associated with cerebrospinal fluid (CSF) Aβ 1-42 and/or CSF tau in ADNI-1 cohort, either directly or indirectly. Significant branched-chain amino acids included histidine (with imageCNN.PC9) and isoleucine (with augmentedCNN.PC2), both of which have been previously implicated in insulin resistance. We need to caution the reader that the interpretation may be overstated as the metabolite association was performed only in ADNI-1 participants while image-derived phenotypes were obtained from ADNI-1/GO/2 participants.
FIGURE 2  Average AUC (area under the receiver operating characteristic curve) of predicting stable and progressive mild cognitive impairment (MCI) among 10 sample splits for six follow-up periods, comparing registered and non-registered images. A, Image CNN model on test samples (with error bar). B, Augmented CNN model on test samples (with error bar). C, Comparison of average AUC between training and test samples. CNN, convolutional neural network; pMCI, progressive mild cognitive impairment; sMCI, stable mild cognitive impairment
3.5 | GWAS using CNN-derived image phenotypes

The results for meta GWAS on imputed SNPs (see supporting information) using the four CNN-derived image phenotypes are shown in Table 3 and Table S5 in supporting information. QQ plots for all phenotypes show no obvious inflation of large P-values (>1 × 10^{-4}), with λGC between 0.993 and 1.01, indicating that our association analyses have accounted for population substructure well (Figure S2 in supporting information).

Using imageCNN.PC1 as a phenotype, we obtained genome-wide significant P-values (<5 × 10^{-8}) for genetic variants at the APOE/TOMM40 locus (Figure S2A). However, its QQ plot shows no upward deviation from the diagonal line when single nucleotide polymorphisms (SNPs) at the APOE/TOMM40 locus are excluded (Figure S2B), suggesting that imageCNN.PC1 is not significantly associated with any genetic variants outside APOE given the current sample size. In contrast, using imageCNN.PC4, imageCNN.PC9, and augmentedCNN.PC2 as phenotypes revealed no significant variants at the APOE/TOMM40 locus (P<1 x 10^{-5}). Their QQ plots show moderate excess of low P-values even when SNPs at the APOE/TOMM40 locus are excluded (Figure S2C-2E), suggesting that these phenotypes are significantly associated with variants outside APOE given the current sample size.

GWAS based on imageCNN.PC4 and imageCNN.PC9 identified 116 and 41 significant (P<1 x 10^{-3}) SNPs, respectively (Figure 3A), which were mapped to 17 protein-coding genes (within ±15 kb) according to FUMA. Identified SLC24A4 (rs12588868, P=9.07 × 10^{-6}) is a known AD gene; two genes, CACNA1C (rs11062078, P=3.14 × 10^{-6}) and DYSF (rs34707417, P=6.38 × 10^{-6}), were significantly enriched in the Accelerating Medicines Partnership-Alzheimer’s Disease (AMP-AD) gene co-expression submodules (Figure S3 in supporting information).

The Gene Ontology (GO) annotations for the enriched submodules include regulation of action potential and calcium-mediated signaling for CACNA1C and regulation of endocytosis for DYSF (Table S7 in supporting information). DYSF has been reported to be significantly associated with a metabolite of histidine, which has been implicated in insulin resistance and p-tau. Furthermore, imageCNN.PC9 is significantly associated with the largest number of lipid metabolites (three sphingomyelin and one phosphatidylcholines; Table S4), which have been previously found significantly associated with CSF Aβ and/or CSF tau. Furthermore, imageCNN.PC9 is significantly associated with a metabolite of histidine, which has been implicated in insulin resistance and p-tau. Their most significant SNPs map to a role in synaptic plasticity. NCAM2 mediates synaptic adhesion, and Aβ-dependent disruption of NCAM2 functions in the AD hippocampus to synapse loss. BRSK1 is the eQTL target gene of significant rs4294948 in the AMP-AD RNA-Seq data, and mediates phosphorylation of tau. Therefore, we believe that Image CNN detected image patterns that are related to calcium ion binding, Aβ-mediated synaptic loss, and tau phosphorylation.

Augmented CNN, CDH13 negatively regulates axon growth and LMF1 is required for maturation and transport of active lipoprotein lipase (LPL). Previous studies have established that LPL is a novel Aβ-binding protein promoting cellular uptake and subsequent degradation of Aβ. ENSA is an inhibitor of protein phosphatase 2A (PP2A) that regulates tau phosphorylation directly. ADcy3 loss-of-function variants increase the risk of obesity and type 2 diabetes. ZC3H12A (a.k.a. MCPIP1), detected by both CNN models, is an APP-regulated inflammation responsive in NT2 cells. All together, we believe that augmentedCNN.PC2 represented both early (Aβ and tau related) and late (insulin resistance/diabetes and inflammation response) AD characteristics.

4 | DISCUSSION

Augmented CNN model achieves higher prediction accuracy than Image CNN model in the ADNI cohort. The high accuracy achieved by both models, as well as the four cognitive scores and APOE genotype, for distinguishing stable and progressive MCI (Table S2 in supporting information) implies that image, cognitive performance, and genetics have complementary roles in disease status prediction.

ImageCNN.PC1 is the only CNN-derived phenotype to identify genome-wide significant SNPs at the APOE locus because principal components are uncorrelated with one another by definition and Augmented CNN-derived image features were APOE-adjusted. This phenotype also had the highest power (AUC = 0.784) to predict the clinical conversion of MCI to AD among all the phenotypes we considered (Table S2), and the highest Pearson correlation with cognitive scores (0.55 with ADAS and 0.52 with CDR-SOB). However, its QQ plot shows no excess of low P-values outside of the APOE locus. We therefore believe that imageCNN.PC1 represents image features that are mainly redundant with APOE genotype and cognitive performance.

The other three CNN-derived phenotypes show low correlations with cognitive scores (<0.1) and have relatively low power to predict the clinical conversion of MCI to AD (Table S2). This is probably due to the conversion assessment being largely based on cognitive performance in the first place. However, these phenotypes are associated with early-stage markers of disease. For example, augmentedCNN.PC2 is significantly associated with the largest number of lipid metabolites (three sphingomyelin and one phosphatidylcholines; Table S4), which have been previously found significantly associated with CSF Aβ1-42 and/or CSF tau. Furthermore, imageCNN.PC9 is significantly associated with a metabolite of histidine, which has been implicated in insulin resistance and p-tau. Their most significant SNPs map to...
### TABLE 3  Genome-wide association results based on the two sets of CNN-derived phenotypes

| Lead SNP     | Image phenotype | Chr | Position (hg19) | A1   | A2   | AF1 (A2) | GMMAT score (A1) | GMMAT standard error | P-value | SNP type   | eQTL genes in AMP-AD | Nearby genes (±15 KB) | AMP-AD logFC | Other associated phenotypes [43] |
|--------------|----------------|-----|-----------------|------|------|----------|------------------|----------------------|---------|------------|----------------------|----------------------|--------------|--------------------------------|
| rs11558606   | PC9            | 1   | 230814668       | A    | G    | 0.072   | 5.243            | 1.153                | 5.41×10⁻⁶ | missense   | COG2*                |                      | 0.096        | edu                          |
| rs6672949    | PC4            | 1   | 37985911        | C    | T    | 0.361   | -12.860          | 2.673                | 1.51×10⁻⁶ | intergenic | ZC3H12A, MEAF6*, SNIP1 |                      | -0.222       | NA                          |
| rs34707417   | PC9            | 2   | 71708830        | T    | G    | 0.236   | 8.605            | 1.906                | 6.38×10⁻⁶ | intronic   | DYFS                 |                      | NA           | NA                          |
| rs12361440   | PC9            | 11  | 74396631        | G    | A    | 0.151   | -12.19           | 2.543                | 1.63×10⁻⁶ | intergenic | POLD3, CHRDL2       |                      | NA           | NA                          |
| rs11062078   | PC4            | 12  | 2135487         | C    | T    | 0.26    | -11.17           | 2.397                | 3.14×10⁻⁶ | intergenic | CACNA1C, DCP1B       |                      | -0.089       | edu, high                   |
| rs35047      | PC9            | 12  | 31163186        | G    | T    | 0.136   | 11.516           | 2.531                | 5.36×10⁻⁶ | intergenic | DDX11                |                      | 0.466        | NA                          |
| rs1258868    | PC9            | 14  | 92909309        | T    | C    | 0.499   | 16.114           | 3.631                | 9.07×10⁻⁶ | intergenic | SLC24A4              |                      | NA           | edu                          |
| rs429498     | PC4            | 19  | 56455746        | G    | A    | 0.441   | -12.830          | 2.664                | 1.46×10⁻⁶ | intergenic | BRSK1                |                      | NA           | NA                          |
| rs8115712    | PC4            | 20  | 4746960         | G    | A    | 0.328   | 11.438           | 2.535                | 6.43×10⁻⁶ | intergenic | RASSF2               |                      | NA           | NA                          |
| rs8116731    | PC9            | 20  | 16718784        | A    | G    | 0.114   | -11.820          | 2.271                | 1.96×10⁻⁷ | intronic   | SNRPB2*, OTOR       |                      | -0.132       | NA                          |
| rs117100735  | PC4            | 20  | 18041336        | G    | T    | 0.063   | 4.461            | 1.005                | 8.97×10⁻⁶ | intergenic | OVOL2                |                      | NA           | NA                          |
| rs35278766   | PC4            | 21  | 22815262        | C    | T    | 0.07    | 6.290            | 1.374                | 4.68×10⁻⁶ | intronic   | NCAM2*               |                      | 0.299        | edu, high, cog             |
| rs3163186    | PC9            | 12  | 31163186        | G    | T    | 0.136   | 11.516           | 2.531                | 5.36×10⁻⁶ | intergenic | DDX11                |                      | 0.466        | NA                          |
| rs1258868    | PC9            | 14  | 92909309        | T    | C    | 0.499   | 16.114           | 3.631                | 9.07×10⁻⁶ | intergenic | SLC24A4              |                      | NA           | edu                          |
| rs429498     | PC4            | 19  | 56455746        | G    | A    | 0.441   | -12.830          | 2.664                | 1.46×10⁻⁶ | intergenic | BRSK1                |                      | NA           | NA                          |
| rs8115712    | PC4            | 20  | 4746960         | G    | A    | 0.328   | 11.438           | 2.535                | 6.43×10⁻⁶ | intergenic | RASSF2               |                      | NA           | NA                          |
| rs8116731    | PC9            | 20  | 16718784        | A    | G    | 0.114   | -11.820          | 2.271                | 1.96×10⁻⁷ | intronic   | SNRPB2*, OTOR       |                      | -0.132       | NA                          |
| rs117100735  | PC4            | 20  | 18041336        | G    | T    | 0.063   | 4.461            | 1.005                | 8.97×10⁻⁶ | intergenic | OVOL2                |                      | NA           | NA                          |
| rs35278766   | PC4            | 21  | 22815262        | C    | T    | 0.07    | 6.290            | 1.374                | 4.68×10⁻⁶ | intronic   | NCAM2*               |                      | 0.299        | edu, high, cog             |

*Allele frequency.

1. Log fold change of transcript abundance for AD cases versus controls in AMP-AD RNA-Seq data.
2. cog, cognitive test performance; edu, educational attainment; high, highest math achievement; math, self-reported math ability; PC, principal component.
3. These two SNPs are in high LD and have P-values < 1×10⁻⁵ in GWAS from the two CNN models.
4. Gene with differential expression in AMP-AD RNA-Seq data.

Abbreviations: AMP-AD, Accelerating Medicines Partnership-Alzheimer's Disease; CNN, convolutional neural network; SNP, single nucleotide polymorphism.
protein-coding genes that are enriched for diverse AD stages, ranging from early Aβ, tau phosphorylation, and calcium ion binding-related synaptic loss to late energy hypo-utilization and inflammation response. Moreover, our CNN-derived phenotypes compare favorably to other AD-related phenotypes (cognitive scores, image summary measures, and clinical labels) in terms of metabolite association and GWAS findings (Table S4 and S6 in supporting information). Although the CNN-derived image phenotypes could be explained to some degree by a linear combination of image summary measures from ROIs—with the highest explaining R2 of 0.358 for imageCNN.PC1 (Figure S4A in supporting information), followed by the explaining R2 of 0.133 for augmentedCNN.PC2 (Figure S4B) the majority of the image phenotypes were unexplained by the ROIs, showing that the CNN-derived image phenotypes provide novel MRI biomarkers.

These findings suggest that our CNN-derived image phenotypes reflect AD progression better than other common phenotypes and refine the genetic associations to key subprocesses for LOAD. Three reasons may explain this. First, the transfer learning technique greatly augments the ADNI image data, making the learned CNN models more robust to overfitting. Second, unlike our image phenotypes, case/control LOAD GWAS often exclude MCI due to their uncertain disease status and could misdiagnose healthy controls that develop AD later, yielding a less precise and less specific phenotype. Third, the categorical clinical labels are more prone to errors due to the use of thresholds than the continuous CNN-derived image phenotypes.

Although we have applied FreeSurfer to the downloaded pre-processed MRI images to correct motion and normalize image intensities, we acknowledge that some confounding effects may not have been accounted for, due to the different MRI acquisition parameters adopted at different sites. This could also explain the lower prediction power in the independent AIBL MRIs.

One direction for future studies is to explore CNN training strategies that can better tolerate inaccurate target labels; another one is to identify the regions in the original MRI images that drive the CNN classification.

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**AUTHOR CONTRIBUTIONS**

YL and GWC designed the project. YL and CJ modified the 3D-CNN structure. YL performed the CNN training, image-derived phenotype construction, and downstream analysis. CP annotated the associated variants and genes, AH carried out the AMP-AD gene expression submodule enrichment analysis, AU and HSY performed RNA-Seq data analysis, KK and VP advised on data analysis. YL and GWC drafted the manuscript. YL, AH, and GWC revised the manuscript. GWC obtained the funding and was responsible for the study supervision. All authors read and approved the final manuscript.

**DATA SHARING**

Summary statistics from the GWAS based on the CNN-derived image phenotypes will be made available for download upon publication from https://www.synapse.org/#!Synapse:syn21069604. The Python codes for CNN fine-tuning and prediction are also available there. However, the ADNI MRI images, which are controlled data, are not allowed to accompany the Python codes.

**URLs**

- ADNI: http://www.adni-info.org
- ADNI database: http://adni.loni.usc.edu/
- AIBL: https://aibl.csiro.au/adni/index.html
- AIBL hosted at ADNI: http://adni.loni.usc.edu/study-design/collaborative-%20studies/aibl/
- AMP-AD: https://adknowledgeportal.synapse.org
- FUMA: http://fuma.ctglab.nl/

**CONFLICTS OF INTEREST**

The authors have no conflicts of interest to disclose.

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