scAGG: Sample-level embedding and classification of Alzheimer's disease from single-nucleus data

T. Verlaan, G. Bouland, A. Mahfouz, M.J.T. Reinders

Abstract

Identifying key cell types and genes in Alzheimer's Disease (AD) is crucial for understanding its pathogenesis and discovering therapeutic targets. Single cell RNA sequencing technology (scRNAseq) has provided unprecedented opportunities to study the molecular mechanisms that underlie AD at the cellular level. In this study, we address the problem of sample-level classification of AD using scRNAseq data, where we predict the disease status of entire samples from the gene expression profiles of their cells, which are not necessarily all affected by the disease. We introduce scAGG, a sample-level classification model which uses a sample-level pooling mechanism to aggregate single-cell embeddings, and show that it can accurately classify AD individuals and healthy controls. We then investigate the latent space learnt by the model and find that the model learns an ordering of the cells corresponding to disease severity. Genes associated with this ordering are enriched in AD-linked pathways, including cytokine signalling, apoptosis, and metal ion response. We also evaluate two attention-based models that perform on par with scAGG, but entropy analysis of their attention scores reveals limited interpretability value. As scRNAseq is increasingly applied to large cohorts, our approach provides a way to link individual phenotypes to single-cell measurements. Our cell- and sample-level severity scores may enable identification of AD-associated cell subtypes, paving the way for targeted drug development and personalized treatment strategies in AD.
A Python implementation of scAGG is available at `http://github.com/timoverlaan/scAGG`

Introduction

Recent advances in RNA sequencing technology allow measurement of gene expression at the individual cell level, through single-cell RNA sequencing (scRNAseq), which has revealed the heterogeneous landscape of cell types and allowed researchers to build cell atlases of various healthy tissues.[1,2] It has also been used to study the cell-type-specific effects of diseases. In the context of Alzheimer's disease (AD), one of the first single-cell studies identified a subtype of microglia (DAM) that has the potential to restrict neurodegeneration.[3] However, analysis of such scRNAseq data introduces a new fundamental problem, because in bulk RNAseq, where the RNA expression of an entire piece of tissue is measured, the metadata of a sample contains information about the disease status of the individual and can be directly related to the measured gene expression of the sample. This approach has, for example, allowed the identification of three distinct AD subtypes that exhibit different combinations of dysregulated pathways.[4] But in scRNAseq, the disease metadata is still at the sample-level, whereas the measured gene expression is now at the cell-level. We refer to this as the *sample-level classification problem in single-cell data*.

Several methods have been proposed to predict the disease status of single-cell data,[5–8] but they focus on the task of cell-level classification. That is, they label each cell individually, according to the disease status of the sample they belong to. However, it has been shown that different types of cells are affected differently in the context of Alzheimer's disease, and that not all cells are equally affected.[9–11] So, if we label the individual cells according to the disease state of the sample they belong to, this may actually be incorrect at the cell-level. This is especially the case if a high proportion of cells is unaffected in terms of gene expression, which is not known a priori.

We argue that the task of sample-level classification should be considered, where the sample is classified as a whole, based on the gene expression of all cells. This way, we do not explicitly enforce the wrong label upon unaffected cells in a diseased sample. The main challenge here is the variability in number of cells between samples, and the permutation invariant nature of the input data. That is, a solution should be able to handle samples of different sizes, and the order of the cells should not matter. To our knowledge, only a few methods directly consider the task of sample-level classification. However, to do so, they either rely on heavy down-sampling to get a fixed number of cells per sample,[12,13] or use a generative approach to learn sample-level embeddings that are not specifically optimised for classification.[14,15]

In this work, we propose scAGG, a machine learning model that performs the task of sample-level classification, that does not rely on down-sampling and directly uses the cell-level data through application of a pooling mechanism. This three-part architecture embeds individual cells, aggregates them into a sample-level embedding through a sample pooling mechanism, and classifies the sample according to disease class, all trained in an end-to-end fashion. We investigate several parametrizations of this architecture, including attention-based modules which allow for interpretability of the model, and we compare the performance of these models to several baseline approaches.

Materials and Methods

ROSMAP Dataset processing

Single-nucleus gene expression data from 465 samples (patients) of the ROSMAP[16] project was processed similar to the standard SCANPY[17] approach for single-cell data: We discard genes detected in fewer than 200 cells and remove cells with less than 200 genes detected or more than 5% mitochondrial gene expression. Next, we normalise each cell to add up to $10,000$ transcripts (CP10k), and log-transform (log1p). We also remove a distinct cluster of 10 outlier samples, which were dominated by a specific cell type (RELN and CHD7 expressing excitatory neurons). Finally, we select only the $1,000$ most highly variable genes (HVGs), using the standard procedure in Seurat.[18] The resulting dataset contains 339 samples, with a total of $2,037,478$ cells.

From the complete set of individuals, we select the 78 most extreme cases of AD and the 61 most healthy controls, leaving 200 intermediate donors. This was done following the approach proposed in,[19] where the Braak stage and CERAD scores, neuropathological marker and cognitive test scores respectively, are used as additional constraints to select only the most severe AD cases and healthiest controls as follows:

$$\begin{cases} AD: & cogdx = 4,\ braaksc \geq 4,\ ceradsc \leq 2 \\ CT: & cogdx = 1,\ braaksc \leq 3,\ ceradsc \geq 3 \\ OTHER: & \text{all the other samples.} \end{cases}$$

Baselines

We used the following three baseline models to compare the quantitative performance of scAGG:

- **Cell-type proportion classifier:** Each sample is represented by its abundance of each of the annotated cell types, normalised to $10k$ cells per sample. The resulting features (cell type proportions) are then standardised over all samples. The resulting sample representations are classified using a Lasso regression model with regularisation strength $\alpha = 0.03$.

- **Pseudobulk classifier:** Raw gene expressions of each sample are aggregated into a single vector per sample by adding the counts per gene in all cells. Resulting representations are normalised to sum to $10k$ counts per sample and log-transformed. The classifier is an MLP with two hidden layers (hidden dims 512 and 256).

- **Cell-level classifier:** Sample-level labels are projected onto each underlying cell, assuming that all cells in a sample are affected by the disease. The classification model is a Lasso regression with $\alpha = 0.3$. To compare these cell-level predictions with the sample-level predictions, we aggregate the results by considering the proportion of cells that were classified as AD in each sample, and pick the optimal decision boundary using the train set.

scAGG: Sample-level embedding and classification architecture

We propose scAGG, a three-part architecture that consists of (1) a cell-level encoder, (2) a sample pooling mechanism, and (3) a sample-level classifier. These three modules can be trained in an end-to-end fashion on the sample-level disease classification task. We first describe the abstract modules, after which we define the specific parametrizations we evaluated in this work.

We define the *cell encoder* module as a learnable mapping from the input space $\mathcal{X} \in \mathbb{R}^{n \times m}$, to a latent space $\mathcal{Z} \in \mathbb{R}^{n \times h}$, where n denotes the number of cells, m the number of features (genes) and h the latent dimensionality.

The *pooling mechanism* is defined to take a set of cell embeddings $Z \subset \mathcal{Z}$ belonging to a single sample (patient) and return a sample-level embedding $s \in \mathbb{R}^{1 \times h'}$. Note that the latent dimensionalities of cell- and sample-level embeddings are not necessarily the same.

Finally, we define a *classifier* module that predicts the disease state $\hat{Y} \in \mathbb{R}^{N \times 2}$ for each sample-level embedding s. In all variations of the model we explore, this classifier part of the architecture is the same.

scAGG: Base parametrization

This model chooses the simplest parametrizations for all three modules of the architecture. The encoder is a two-layer neural network, with a hidden dimension of $h = 128$, interspersed by a ReLU:

$$Enc_{NN}(x) = W_2 \cdot \mathrm{ReLU}(W_1 \cdot x + b_1) + b_2 \tag{1}$$

The pooling layer is a global mean pooling layer P_{mean}, which averages the cell-level embeddings to a single graph-level embedding:

$$P_{mean}(z) = \frac{1}{n} \sum_{i=1}^{n} z_i = s \tag{2}$$

The classifier is a linear layer, followed by a softmax activation function.

scAGG+AP: Attention-based pooling

To allow a sample pooling mechanism to prioritize specific cells when aggregating them, we incorporate an attention mechanism. We adapt the self-attention mechanism to our sample-level pooling task, using a CLS token $T_{CLS} \in \mathbb{R}^h$ as was used, for example, in BERT.[20] This is a learnable token that is appended to the set of cell embeddings for each individual sample $\hat{z} = [z \,||\, T_{CLS}]$, used to represent the entire sample and calculate the attention scores for each cell. Using the CLS token, we can use the self-attention mechanism without any modifications. To increase model capacity, we use four attention heads, which have separate weights, and the resulting embeddings are averaged to obtain the final sample-level embedding.

scAGG+GAT: Graph attention cell encoder

To incorporate attention into the cell encoder part of the model, rather than the sample pooling layer, we incorporate a Graph Attention Network (GAT).[21] Here, each sample is represented as one kNN-graph, where each node represents a cell, and edges are added for each cell to its $k = 30$ nearest neighbours, according to Euclidean distance in the gene expression space.

Given a graph G, and the function $N_k(i)$ that denotes the set of the k-nearest neighbours of a node i, a GAT layer calculates an embedding for each node i as follows:

$$h_i = \sigma\left(\sum_{j \in N_k(i)} \alpha_{i \to j} W x_j\right) \tag{3}$$

Here, x_j is the feature vector of neighbour j, and W is a learnable linear transformation. The attention $\alpha_{i \to j}$ that is paid to j when calculating the embedding of i is calculated following the definition of GATv2, where different weights are learnt for i and j, to allow for "dynamic" attention:[22]

$$\alpha_{i \to j} = softmax_j\left(a \cdot \text{LeakyReLU}\left(W'\left[x_i || x_j\right]\right)\right) \tag{4}$$

where a and W' are learnable transformations, and the $||$ operator denotes vector concatenation. Note that, unlike most GAT implementations, we do not include self-loops: $i \notin N_k(j)$, for better interpretability of attention scores. We use 8 attention heads and concatenate the resulting embeddings before passing them to the classifier.

The classifier and pooling layer are the same as in the base parametrisation, although we also consider a combination of the GAT encoder with the attention-based pooling in our experiments (scAGG+GAT+AP).

Model Training & Evaluation

All models were trained and evaluated using a 5-fold cross-validation, stratified by disease class, where the same splits are used between models to allow for a fair comparison. We used a categorical cross-entropy loss function and the model weights were optimised using Adam[23] ($lr = 0.001$, $wd = 0.0005$ and $dropout_rate = 0.5$).

Mini-batches for graph-based model were constructed using neighbour sampling,[24] where eight seed nodes are sampled from the different training graphs, in a weighted fashion to correct for class imbalance and difference in cell counts between samples. For each seed node, 15 random neighbours are added to the batch graph and for each neighbour, 15 additional (2-hop) neighbours are added to the batch graph. As a result, each batch has at most $8 \times 15 \times 15 = 1800$ nodes. Usually in neighbour sampling, all nodes are used once as seed nodes every epoch, but in our case, we define an epoch as a training iteration over only 200 of such batches, to allow for early stopping, because otherwise the model would overfit within a single epoch.

For the models that do not rely on a graph structure, we found it worked equally well to train on batches generated in the same way, so we use the same batching technique for all models for consistency, and discard the graph structure after sampling for the non-graph models.

Attention Analysis

To identify the cells prioritised by the attention mechanism, we calculated the received attention of each cell i, by summing over attention it received from all neighbours $j \in N(i)$. The score is then corrected for the degree of the cell, using the square root of the degree, so that high-degree cells are penalised less harshly. Finally, we scale the overall distribution to sum to 1 again by multiplying by the average degree of the sample k:

$$att_{recv}(i) = \sum_{i \in N(i)} \alpha_{j \to i} \sqrt{\frac{\deg_{\mu k}}{\deg_i}}$$

To investigate the selectivity of the attention mechanism, we analysed the entropy of the outgoing attention weights of each cell:

$$H(i) = -\sum_{j}^{n} \alpha_{i \to j} \log(\alpha_{i \to j})$$

Note that the direction of the attention here is opposite to the received attention, because selectivity of the attention mechanism occurs in the outgoing attention, whereas the received attention is more indicative of the importance of a cell in the prediction task.

The attention entropy distributions are then compared with a uniform distribution, which is obtained by setting all the attention weights in a graph to $1/n$, where n is the number of neighbours of the cell, and then applying the above formula again.

Results

Dataset

We used a single-nucleus RNAseq dataset, ROSMAP,[16] of patients with Alzheimer's disease and healthy controls of various demographics and stages of AD, providing a comprehensive view of the disease and its progression. After processing 339 donors ($2,037,478$ cells) remained, for which the $1,000$ most highly variable genes were selected (see Methods). Following the approach proposed in (Wang 2022)[19] we identified the most extreme cases of AD (78) and the most healthy controls (61), leaving 200 intermediate donors (see Methods). We use only the extreme cases for training and performance evaluation, so the model learns to separate the most extreme ends of the disease spectrum. In subsequent analyses, we also included intermediate cases.

Pooling of cell embeddings for sample-level classification of extreme AD cases

To address the task of classifying cell-level data with sample-level labels, we propose scAGG, a three-part architecture that consists of (1) a cell-level encoder, (2) a sample pooling mechanism, and (3) a sample-level classifier (Fig 1). In its base form, which we refer to as scAGG, the cell encoder and sample classifier are both simple neural networks, and the sample pooling mechanism is

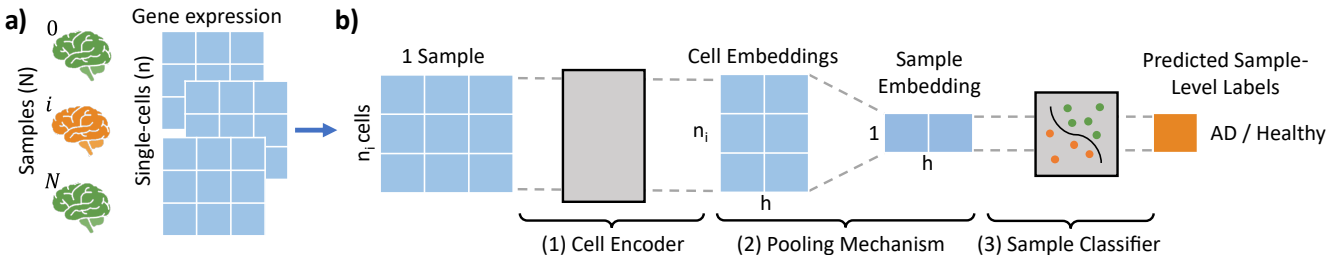

Figure 1: Illustration of the proposed scAGG model: (a) For N samples (brain donors) we have a total of n cells, (b) The cell encoder module (1) produces n cell embeddings that are aggregated by the pooling mechanism (2) into a single embedding per sample. The resulting N sample embeddings are then classified according to disease status using a classifier module (3).

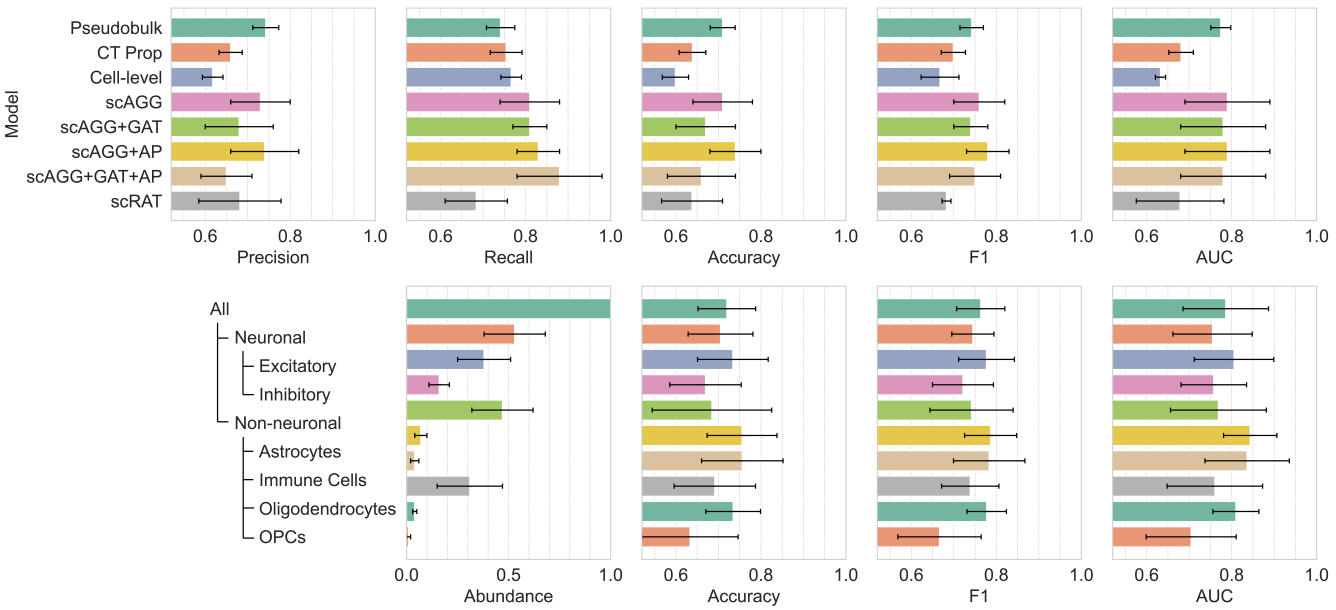

Figure 2: Quantitative performance analysis: a) Baseline models and variations of the proposed scAGG architectures are compared for the 1000 most highly variable genes; b) Performance of scAGG for several (groups of) cell types. Error bars represent the standard deviation over 5 folds of cross-validation, except for the last panel showing relative cell type abundance, where the error bars denote the standard deviation over all samples.

a global mean pooling function that aggregates cell-level embeddings into a sample-level embedding. This sample-level embedding is then used to classify the sample as a whole (see Methods).

To quantitatively assess the performance of scAGG, we consider the task of classifying only the most extreme cases of AD and CT, as these are expected to be the most separable cases on the disease continuum. We compare our performance with three baselines: a cell-type proportion classifier (CT Prop), a pseudobulk model, and a cell-level classifier model that does not rely on pooling together the cell embeddings (see Methods).

We find that scAGG outperforms the CT Prop and Cell-level baselines on all metrics (Fig 2), whereas it performs on par with the pseudobulk model, with a slightly higher F1 and AUC, although not statistically significant. However, we note that this pseudobulk approach discards any opportunity to uncover the cellular heterogeneity captured by the single-cell data. We also observed a high standard deviation between the different folds of cross-validation for scAGG, indicating the sensitivity to the way the data was split. The performance of the other baselines comes closest to the performance of scAGG on the recall metric, which we attribute to the baseline models predicting the AD class more frequently, since this is the majority class. We also compared with scRAT,[12] representing the state of the art, as run with default parameters, and show that scAGG outperforms it.

When we increase the number of genes selected in the dataset from $1,000$ to $5,000$ (Supplementary Fig 1), we find that the performance of scAGG does not improve further. Only on the recall metric we observe a small increase, but since the precision drops, this does not improve the F1 or AUC scores and indicates the model is overfitting due to the higher number of features. However, the performance of the pseudobulk baseline model does improve, but the AUC of scAGG is still on par.

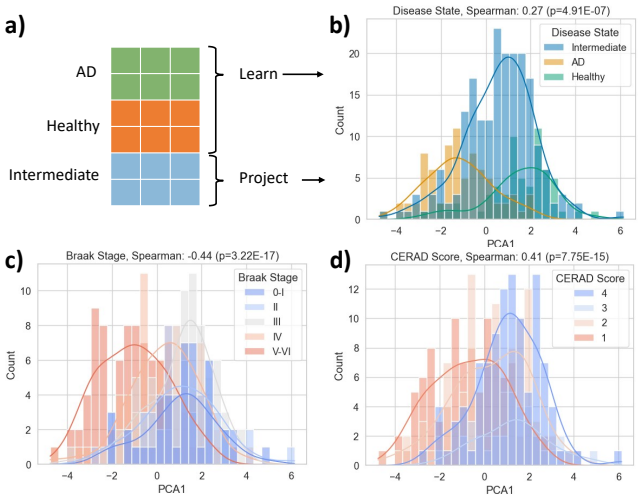

Figure 3: Visualization of sample-level embeddings, showing the distribution of classes along the first principal component, coloured by: (b) disease status of extreme cases, including intermediate cases projected onto latent space (as illustrated in panel a), (c&d) Braak stage and CERAD scores of all samples, including intermediates.

Sample-level embeddings show association between AD classifiability and pathological stage

After training the proposed architecture (Fig 1), we obtain a single embedding for each individual that aggregates all single-cell data of this sample, directly optimised for the classification task. To illustrate how the embeddings of the samples clearly separate disease states, we show the distribution of disease classes along the first principal component of the sample-level embedding space.

Using the model that was trained on the extreme cases only, we projected the held-out intermediate samples onto this spectrum (Fig 3a). These intermediate samples are mapped between the disease extremes (Fig 3b), illustrating that the sample embeddings learnt by our model capture a healthy-to-AD disease trajectory, while only trained on a binary classification task. Moreover, we show that this ordering aligns with the disease severity of the samples, as quantified by the Braak stage and CERAD score, a pathological marker and a clinical score respectively (Fig 3c+d). This indicates that a sample's position on this learnt spectrum can be interpreted as a measure of disease severity.

Cell type-specific performance differences

The aforementioned approaches all rely on the use of all cells in a sample to make a prediction, but these cells belong to different cell types, which may not all be equally informative for the prediction of AD. Therefore, we investigated the classification performance of several major cell types by training and evaluating the model only on cells of that type. We find that there is little difference in performance when considering all neuronal vs. all non-neuronal cell types, which in have similar relative abundances in total. Once we consider individual cell types, we find that, for neuronal cell types, excitatory neurons are more accurately classified, but this could be due to them being more frequently present in the dataset. For non-neuronal cells, we find that astrocytes, immune cells (including microglial cells), and OPCs can be classified exceptionally well considering their far lower abundance compared to oligodendrocytes, which make up the majority of the non-neuronal cells, while being far less accurately classified (Fig 2b).

In general, this indicates that some cell types are more predictive of AD than others, which motivates the use of a model that can prioritise specific cells when making a prediction.

Attention-based sample pooling mechanism does not outperform naive global pooling

Having shown that some cell types are more predictive of AD than others, we propose to incorporate an attention mechanism[25] that allows the model to prioritise specific cells when making a prediction, to make better predictions with only the most informative cells. First, we consider an attention-based sample pooling mechanism (scAGG+AP). Instead of using a simple global sample pooling that averages all cell embeddings, we use an attention mechanism that learns a scoring mechanism per cell that determines its weight in the global embedding (see Methods).

We find that the attention-based sample pooling mechanism does not improve the performance of the model (Fig 2). Only when increasing the number of HVGs to $5,000$ do we see a slight improvement in performance compared to the naive sample pooling mechanism in terms of accuracy, but the F1 and AUC are similar (Supplementary Fig 1). This indicates that the attention-based sample pooling mechanism overfits to the majority class (AD), improving the total accuracy, but not when corrected for class imbalance.

Graph-attention based cell encoder module does not improve performance

The global application of attention as presented above scales quadratically with respect to the number of cells and can become computationally expensive, since the scRNAseq samples may consist of $10,000$s of cells. In addition, it embeds each cell independently and does not consider the relationships between cells, while it has been shown that incorporating information from similar cells can be valuable.[5,6,8]

To address this, we investigated another approach in which we employ a Graph Attention Network (GAT).[21] In this approach, each sample is represented by a cell graph, and the GAT learns to incorporate information from neighbouring cells into the cell embeddings. An attention mechanism is incorporated that learns weights for the neighbours of a cell, which can later be interpreted (see Methods).

We evaluated the quantitative performance of the GAT-based encoder module combined with both the global

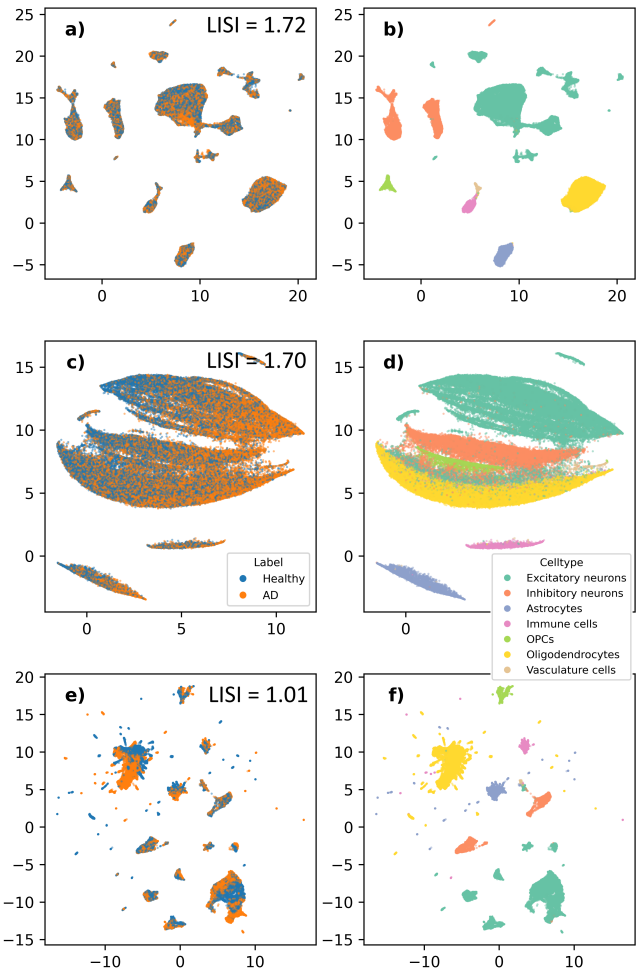

Figure 4: 2D UMAP visualization of the embeddings at the single-cell level, coloured by disease status (left column), and cell type (right column), showing the differences in representations from the input space (a & d), the baseline model (b & e), and the GAT model (c & f). The cell embeddings learned by the attention-based pooling model is very similar to the baseline model and therefore omitted.

mean pooling approach (scAGG+GAT) and the attention-based sample pooling (scAGG+GAT+AP). We find that the GAT-based encoder does not improve the performance of scAGG (Fig 2). On most metrics, the performance is either slightly lower, or on par with scAGG. Only the recall metric is higher for the GAT-based encoder with attention-based sample pooling, which we attribute to the model overfitting to the majority class.

Cell-level embeddings show separability between disease classes

In addition to considering the sample-level embeddings, we can also consider the cell-level embeddings before they are pooled together into a sample-level embedding. We compared a UMAP[26] reduction of the input space with the cell embeddings produced by the baseline model and the GAT model (Fig 4).

Starting with the input space, we see that there is a clear separation between the different cell types (Fig 4a), but little apparent separation between the disease classes (Fig 4d). When we compare this with the embeddings produced by the baseline model, we observe that each cell type has lost most of the underlying structure and was reduced to a single arc-like shape per cell type (Fig 4b). However, we also see that these arcs align with the AD spectrum as all arcs have an AD-end and a healthy-end (Fig 4e), allowing the model to distinguish between them.

Considering the GAT embeddings, we find that they appear to be noisier than the input space, but that more of the structure within and between cell types is retained, compared to the baseline embeddings (Fig 4c). In terms of separability between disease classes, when comparing the base scAGG and scAGG+GAT embeddings, it is hard to draw a conclusion from just this visual inspection. Therefore, we compared the LISI scores for both types of embeddings and input space, which quantify the number classes that occur in a cell's neighbourhood. For perfect separation, we expect $LISI = 1$ and for a homogeneous mixture we expect $LISI = 2$. For the input space, we find a mean LISI score of 1.72, compared to scores of 1.70 and 1.01 for the scAGG and scAGG+GAT models, respectively. This shows that the cell-level embeddings learnt by scAGG are more mixed in terms of disease class, corresponding to the arc-shaped structure that gradually moves across the AD-to-healthy spectrum. For the scAGG+GAT embeddings, we attribute the much lower LISI score to the smoothing that the GAT does over a neighbourhood, making similar cells (from the same sample) even more similar.

Cell-level embedded space associated with several ion response pathways

The UMAP embeddings of the cell-level embeddings learnt by scAGG (Fig 4b) align with a healthy-to-AD spectrum. This is even more pronounced when we consider the first principal component of cell-level embeddings for a single cell type, such as astrocytes (Fig 5a). Here we see that the first PC of the cell embedding seems to order cells along a healthy-to-AD spectrum. To characterise the gene expression patterns that are associated with this spectrum, we calculated the Spearman correlation between the gene expression of each cell and the corresponding value of the first PC. Most genes show a correlation close to zero, but some genes show a strong correlation (Fig 5b).

We then use the absolute values of these correlation scores to rank genes and perform a gene set enrichment analysis (GSEA) for each cell type separately (Fig 5c). We find several significantly enriched gene sets in both the `KEGG (2021) Human Pathways` and `GO Biological Process (2023)` databases for 4 of the 7 major cell types. In particular, we find pathways related to metal ion signalling, regulation of cell growth, and programmed cell death. Furthermore, we find that there is substantial overlap between the enriched gene sets of the different cell types, with 8 out of the 13 gene sets being significantly enriched in at least two cell types.

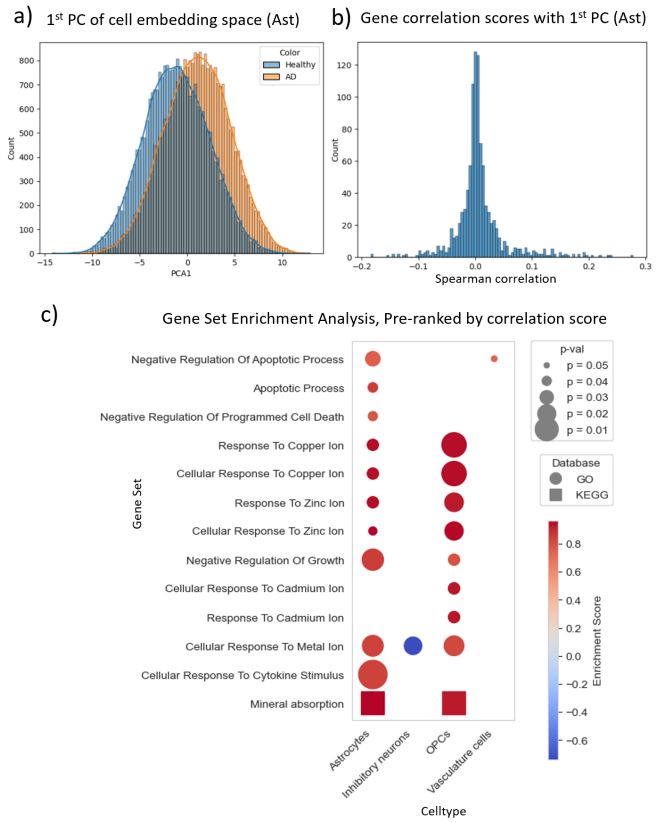

Figure 5: Analysis of the learned cell embedding space of base scAGG. (a) The first principal component all astrocytes, coloured by disease class. (b) Distribution of the correlation scores for each gene, obtain using the spearman correlation between the gene's expression in each cell, and the corresponding cell's 1st PC value. (c) Gene set enrichment analysis per cell type, using the obtained correlation scores to pre-rank the genes.

Entropy analysis of attention scores underscores the limited value in interpretation of attention scores

We found that the attention-based models do not outperform simpler parametrisations of scAGG, raising the question whether the interpretation of the attention scores is meaningful from a biological point of view. The distribution of received attention scores per cell type shows the same average across major cell types, with differences in spread (Fig 6a) that largely correlate with cell type abundance (Fig 6b).

The entropy in the paid attention scores, measuring the spread of the attention distribution, closely matches the uniform attention distribution that characterises the maximum possible entropy, in both layers of the scAGG+GAT model (Fig 6c). In the second layer, the distributions are nearly identical (Fig 6d). From this we conclude that the model spreads attention evenly rather than selectively, which explains why attention-based models do not outperform simpler alternatives.

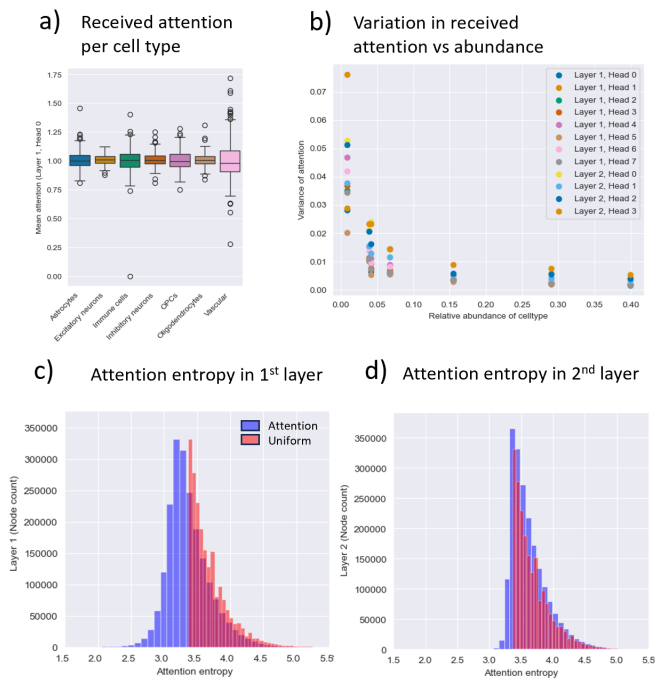

Figure 6: Analysis of the attention scores. (a) Comparison of distributions of received attention per major cell type. (b) Variation observed in the received attention per cell type, compared with the relative abundance of the cell type. (c+d) Attention entropy in the 1st head of the 1st and 2nd GAT layer respectively. Only the first head of each layer is shown, as the distributions are highly similar between different heads in the same layer. Blue denotes the distribution of entropy in the attention given by each cell, and red is the uniform distribution, illustrating what the entropy would look like if all neighbours were paid the same amount of attention. A single neighbour receiving all the attention would result into an entropy of 0 for this cell. Uniform attention is at least $log(k = 30) = 3.5$, since our graphs are built with $k = 30$.

Discussion

We introduced scAGG, a three-part architecture for the purpose of sample-level classification of single-nucleus data, and showed that using a simple parametrisation of this architecture using neural networks already performs well. Through analysis of the learnt sample-level embeddings, we find that the latent space aligns well with two pathological markers of AD, the Braak stage and CERAD score. This indicates that the learnt space spans a spectrum of disease severity, which can be used in future work to identify cell subtypes associated with the disease and potential pathological targets.

Through evaluation of classification performance on isolated cell types, we found that the gene expression patterns of inhibitory neurons and oligodendrocytes were the least predictive of AD, despite their high relative abundance. However, this does not mean that they are not involved in the disease, because we still get reasonable classification performance from just these cell types. This is corroborated by findings of earlier work, that show all major cell types in the brain are transcriptionally affected

by the disease, albeit in different ways.[9–11,27,28]

Genes whose expression was correlated with the learnt AD severity scores were found to be significantly enriched in several AD-associated terms. These terms included the regulation of apoptosis and programmed cell death, which is known to be triggered by Aβ and tau deposition in AD.[29,30] We also found enrichment in cytokine signalling, where it is known that immune cells such as astrocytes and microglia in the brain release inflammatory proteins (cytokines) as part of an immune response to the presence of Aβ plaques and tau tangles.[31,32] Moreover, we found enrichment in multiple terms related to metal-ion responses, with copper, zinc, and cadmium in particular. Existing literature has already established the involvement of metal ions in AD,[33] where copper and zinc dyshomeostasis has been shown to lead to the accumulation of plaques of Aβ plaques,[34–37] and the exposure to even moderate amounts of heavy metals, and in particular cadmium, has been shown to aggravate AD progression.[38–40]

We also considered two attention-based parametrisations of scAGG but found that the performance did not increase as we had expected it to, based on the earlier success of attention mechanisms in similar applications. We hypothesise that, in the case of scAGG+GAT, this is because the attention mechanism can only differentiate between highly similar neighbours of a cell, which are generally not more informative. This warrants the use of deeper GNNs, but these will be difficult to train with the available data. With more data available to train, graph attention could improve the performance. In the case of scAGG+AP, we hypothesise that the limited improvements are due to the high relative abundance of cell types for which we have shown that they can be classified well individually.

Our approach for obtaining cell- and donor-level AD severity scores opens new avenues for understanding complex diseases on multiple scales. By comparing donor-level phenotypes with the cell-level severity scores, we can now identify cases of within-individual heterogeneity, such as the presence of healthy cells in diseased individuals. This could reveal protective mechanisms at the cellular level that may inspire new therapeutic strategies. We also envision applications of the uncovered between-individual heterogeneity, such as the identification of high-resolution disease trajectories and the ability to stratify patients based on cell-type-specific effects, where different cell populations may be primarily affected. As single-cell RNA sequencing is being applied to increasingly large cohorts, our method provides a systematic way to bridge the gap between individual phenotypes and cellular mechanisms, potentially accelerating the development of cell-type-specific or even personalised therapeutic interventions.

Competing interests

No competing interest is declared.

Author contributions statement

All authors worked on conceiving the method and experiments, T.V. performed the experiments and wrote the manuscript, which was reviewed by G.B., A.M. and M.R.

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