**SUMMARY:**

**1. Introduction & Objectives**  
Alzheimer’s disease is a progressive, irreversible neurodegenerative disorder that impairs cognitive functions over time. Our study had two main goals:

* **Biomarker evaluation:** Determine whether brain volume measures extracted from MRI scans can reliably classify three diagnostic groups—Cognitively Normal (CN), Mild Cognitive Impairment (MCI) and Alzheimer’s Disease (AD).
* **Longitudinal assessment:** Analyze how these volumetric measures evolve across four timepoints to understand their trajectories and assess whether they can predict diagnostic transitions.

**2. Dataset & Preprocessing**  
We leveraged data from the Alzheimer’s Disease Neuroimaging Initiative (ADNI), which provides both MRI scans and accompanying clinical information.

* From raw images we quantitatively extracted dozens of volumetric features, compiled into an Excel dataset.
* To reduce noise and control variability, we applied standardization and rigorous quality checks to each feature.

**3. Feature Engineering & Selection**  
To isolate the most informative variables, we adopted a three-step approach:

1. **Literature-driven pre-selection:** We identified brain regions most frequently implicated in Alzheimer’s, based on prior studies.
2. **Recursive Feature Elimination (RFE):** An iterative algorithm that ranks feature importance and discards the least relevant until an optimal subset remains.
3. **Bilateral aggregation:** We combined left- and right-hemisphere measures for each region into single scores, reducing dimensionality while preserving global structural information.

**4. Longitudinal Analysis Across Four Timepoints**  
We first tackled the temporal aspect by applying mixed-effects models to account for repeated measures within subjects:

* **Linear and Multinomial Mixed-Effects Models** were used to model trajectories over time.
* We encountered high collinearity among features, numerical convergence issues, and minimal within-subject variability (78 % of patients did not change diagnostic category).

**5. Cross-Sectional Analysis: Multinomial Regression & Class Balancing**  
Next, we examined the atemporal, cross-sectional relationships by fitting multinomial logistic regression models to predict diagnostic group membership at a single timepoint. Because our classes were imbalanced, we compared three balancing techniques:

* **Class Weights:** Assigning weights inversely proportional to class frequencies.
* **SMOTE (Synthetic Minority Oversampling Technique):** Generating synthetic observations for minority classes.
* **K-Medoids Undersampling:** Reducing the majority class by selecting representative medoids.

**Key findings:** SMOTE and K-Medoids both enhanced the robustness of volume–diagnosis associations, yielding more significant coefficients than Class Weights alone. Clinically, most results aligned with existing literature—except for the thalamus, which showed a paradoxical pattern (larger volume correlating with AD risk), possibly reflecting neuroinflammatory processes.

**6.Unsupervised Clustering: Hierarchical Clustering (Ward’s Method)**  
Given that AD cases showed markedly different volumetric profiles while CN and MCI were largely overlapping, we applied an unsupervised clustering approach to confirm this separation:

* **Ward’s hierarchical clustering** on the standardized feature set naturally partitioned the subjects into two clusters—one almost exclusively containing AD patients and the other combining CN and MCI cases.
* This result underscores the strong structural distinction of AD compared to the minimal volumetric differences between CN and MCI.

**7. Integrating Genetic Data**  
Finally, we explored on a smaller dataset whether adding genetic variants would boost classification performance:

* **Baseline model (volumetric features only):** 58.1 % accuracy
* **Enhanced model (SNPs + APOE genotype + volumetric features):** 67.4 % accuracy

This underscores the synergistic value of combining imaging and genetic data for clinical prediction.

**8. Conclusions & Future Directions**

* **Longitudinal approaches** may require alternative strategies—like clustering—when standard mixed models face convergence or discriminative challenges.
* **Balancing techniques** such as SMOTE and K-Medoids are crucial for uncovering meaningful associations in cross-sectional models.
* **Multimodal integration** of imaging and genetics significantly improves predictive performance, paving the way for more robust, personalized diagnostic tools.

**METHODS:**

**Recursive Feature Elimination (RFE)**  
RFE is an iterative feature‐selection method. It starts by training a model (e.g., a classifier or regression) on all features, ranks each feature by importance, removes the least important ones, and repeats until only the desired number of “optimal” features remain. This reduces dimensionality while retaining the most informative volumetric measures for diagnosis.

**Linear Mixed-Effects Models (LMMs)**  
LMMs extend standard linear models to handle repeated‐measures data by including both fixed effects (the average effect of predictors across all subjects) and random effects (subject-specific deviations, such as each patient’s individual baseline). This lets us model how brain volumes change over time while accounting for within-subject correlation.

**Multinomial Logistic Regression**  
This generalizes binary logistic regression to outcomes with three or more nominal categories (here: CN, MCI, and AD). The model estimates log-odds for each category relative to a reference group, showing how one-unit changes in volumetric measures affect the probability of belonging to each diagnostic class.

**SMOTE (Synthetic Minority Oversampling Technique)**  
SMOTE addresses class imbalance by generating synthetic examples for minority classes. For each minority‐class sample, it creates new points by interpolating between that sample and its nearest neighbors in feature space—balancing class frequencies without simply duplicating existing observations.

**K-Medoids Undersampling**  
In contrast to SMOTE, K-Medoids reduces the majority class by selecting representative “medoids” (cluster centers). Observations closest to each medoid are retained, and the rest are discarded, yielding a more balanced dataset without introducing artificial data.

**Hierarchical Clustering (Ward’s Method)**  
An agglomerative clustering approach that merges pairs of clusters to minimize the increase in within-cluster variance at each step. Applied to standardized volumetric measures, it revealed two clear clusters—AD versus non-AD (CN + MCI)—serving as an exploratory check on structural separability.

**Bilateral Aggregation**  
To simplify the feature set while preserving overall structural information, we combined left- and right-hemisphere volumes (e.g., by summing or averaging). This maintains regional variability but reduces the number of predictors.

**Feature Standardization**  
Before any modeling, we transformed each volumetric measure into a z-score (subtracting the mean and dividing by the standard deviation). This ensures that features on different scales (e.g., hippocampus vs. ventricles) contribute comparably to the models and promotes numerical stability.