|  |  |  |  |
| --- | --- | --- | --- |
| **Total number of participants** | | | |
| NDC | AD | MCI | VaD |
| 117 | 117 | 12 | 12 |
|  |  | |  |

|  |  |  |  |
| --- | --- | --- | --- |
| **Participants selected for training cohort** | | | |
|  | | NDC | AD |
| Microarray |  | 92 | 92 |
| **Final number of participants after quality control** | | | |
|  | | NDC | AD |
| Microarray |  | 90 | 90 |

**Participants selected for validation cohort**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **NDC** | **AD** | **MCI** | **VAD** |
| **qPCR** | 25 | 25 | 12 | 12 |

Final test cohort:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | NDC | AD | MCI | VaD |
| **qPCR** | 25 | 25 | 12 | 12 |

Total of six genes (CNOT8, DDIT4, SESN1, MAP2K1, UCP3, ABCA9) were tested

Sensitivity, specificity, accuracy, ROC evaluated

Test of final genes with test cohort:

-Total of six genes (CNOT8, DDIT4, SESN1, MAP2K1, UCP3, ABCA9) tested in test cohort

-Sensitivity, specificity, accuracy, ROC evaluated

**Verification of microarray**

|  |  |  |  |
| --- | --- | --- | --- |
|  | NDC | AD |  |
| qPCR | 30 | 30 |  |

Models:

1. All expression data (all samples)

2. Expression and staging

3. Expression and gender

4. Expression and ethnic

Feature reduction:

- 299 probes remain after t-test (FC ≥ 2, *p* < 0.05)

- 172 gene with annotation in NCBI

- 50 genes with highest z-score

- 12 gene set was selected (CNOT8, DDIT4, SESN1, MAP2K1, SPOCD1, C5AR1, CAMP, HAPLN2, FBRSL1, UCP3, IQSEC3, ABCA9) with highest sensitivity and specificity

- Six genes (CNOT8, DDIT4, SESN1, MAP2K1, UCP3, ABCA9 that did not influence several confounders (staging, gender, ethnic)

Model optimisation:

PLSDA, HCA

Pathway analysis (IPA)

Gene Ontology (DAVID)

**Figure S1.** Workflow of the present study.