Blood-based Detection of Early-stage Alzheimer's Using Multiomics and Machine Learning

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BACKGROUND

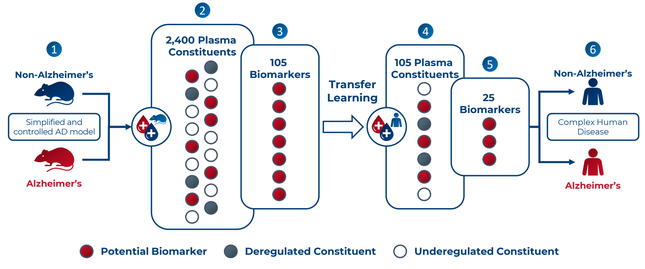
- Currently, the diagnosis of Alzheimer's disease (AD) is largely based on clinical symptoms, including cognitive testing, with a significant number of patients diagnosed when their disease has already advanced.
- A non-invasive blood test capable of detecting patients suffering from Alzheimer's disease, at a prodromal or even pre-symptomatic stage, could reduce the number of dementias through effective clinical management.
- Alzheimer's disease progresses non-linearly in the brain (Bateman et al. (2012), N Engl J Med) resulting in specific blood deregulations at each stage of progression. Identifying these blood signals requires access to the brain information at the molecular level at the time of the blood draw to be sure of the stage of progression. This is not possible in humans without a biopsy.

OBJECTIVE

- The objective of this study was to assess the performance of our multiomics blood test in retrospectively collected plasma samples using Machine Learning to combine metabolomic and proteomic biomarkers, pre-identified in a brand-new rat model.

METHODS

1. We developed a non-transgenic animal model capable of successfully reproducing the continuum of Alzheimer's disease progression at the brain level (Audrain et al. (2018), Cereb Cortex). We then sampled the blood of the animals at key stages of the pathology: we confirmed the molecular stage of the disease by post-mortem cerebral biochemical analysis.
2. The plasma of rats was analyzed by global mass spectrometry: for each sample 2,400 blood constituents were quantified (proteins, metabolites, lipids).
3. We identified by Artificial Intelligence (pipeline of state-of-the-art Machine Learning algorithms: *random forest, support vector machines, artificial neural networks, gradient boosting, etc*) the 105 most informative biomarkers in the simplified and controlled rat model.
4. Then we analyzed the behavior of these biomarkers in humans. The plasma of 232 individuals was analyzed by global mass spectrometry: for each sample the 105 pre-identified biomarkers were quantified (proteins, metabolites).
5. Using AI techniques similar to those previously applied in rats, we were able to identify the 25 first-in-class biomarkers in humans among these 105.
6. Using these 25 biomarkers we developed a neural network (multilayer perceptron) to diagnose the complex human disease with a high level of accuracy.



RESULTS

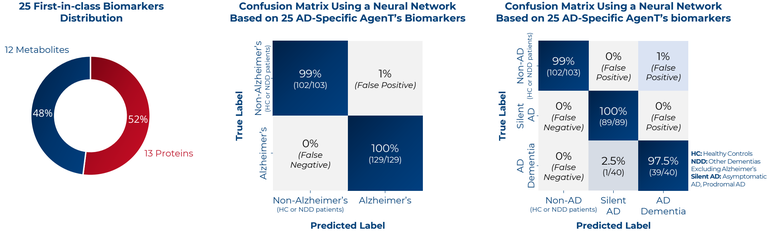
Table 1. Baseline Demographics And Disease Characteristics From Subjects (3 Independent Cohorts)

		Non-Alzheimer's		Alzheimer's		
		Healthy Controls (n=50)	Other Dementias (n=53)	Asymptomatic AD (n=34 DS)	Prodromal AD (n=10 DS & 45 SF)	Dementia Phase (n=10 DS & 30 SF)
Median ± SD	**Age**	66.0±10.6	71.0±8.5	40.0±8.8	48.5±3.8[DS] | 71.0±7.3[SF]	49.5±5.6[DS] | 70.5±7.3[SF]
	MMSE Scores[SF]	28.0±1.5	**23.0±6.2**	NA	27.0±1.4[SF]	**15.0.0±5.0[SF]**
	CAMCOG Score[DS]	NA	NA	75.0±18.6	63.0±21.2[DS]	63.0±14.3[DS]
	CSF pTau[181]/Aβ42 Ratio	0.03±0.01	0.04±0.03	0.02±0.08[DS]	**0.30±0.27[DS] |0.15±0.10[SF]**	**0.34±0.11[DS]|0.15±0.10[SF]**

*Values **in bold** are significantly different from the Healthy Controls group.*

- We validated the biomarkers identified in rats on 232 human plasma samples (1 sample = 1 individual) from 3 independent cohorts: two with the sporadic form of Alzheimer's and one with Down Syndrome individuals. All adults with Down Syndrome show the neuropathological changes of Alzheimer disease by the age of 40. This association is due to overexpression of amyloid precursor protein, encoded by APP, as a result of the location of this gene on chromosome 21 (Fortea et al. (2020), Lancet).
- The control group consists of healthy individuals, without cognitive impairment, and other dementias excluding Alzheimer's. The Alzheimer's group consists of patients sampled in asymptomatic, prodromal and dementia phases. The prodromal AD patients have been followed up to 13 years until their conversion to dementia.

Figure 1. Neural Network Performances Based on 25 First-in-class Biomarkers



- The combination of proteins and metabolites increases the performance of the test by considering different biological pathways.
- Using a neural network based on 25 biomarkers, the multiomics blood test demonstrated 100% sensitivity and 99% specificity for Alzheimer's vs Non-Alzheimer's (5-folds cross-validation). 231/232 samples were correctly classified. MMSE score, age, gender or APOE4 genotype were not use by the algorithm.
- In a second step, the neural network identified Silent AD (Asymptomatic and Prodromal) against AD Dementia by 100% and 97.5% respectively (5-folds cross-validation). 128/129 samples were correctly classified.

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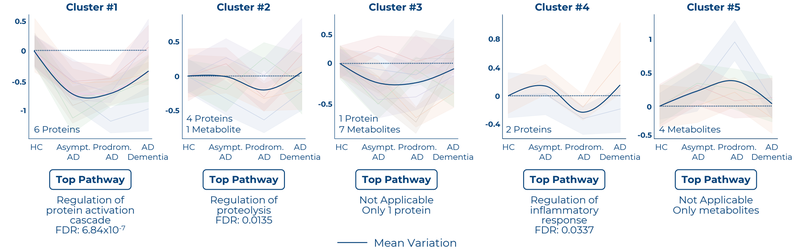
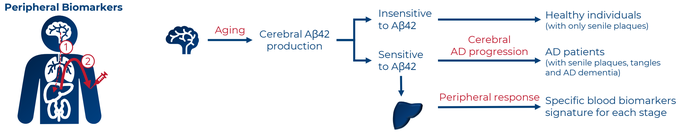
Figure 2. Biomarkers Trajectories Throughout The Alzheimer's Progression



- By clustering the 25 biomarkers according to the Euclidean distance between them, we characterized 5 trajectories describing the global behavior of our biomarkers. All the trajectories are non-linear and non-monotonic. It confirms the hypothesis of an evolutive progression of the plasma biomarkers concentration throughout the Alzheimer's progression.

Figure 3. Peripheral Biomarkers Consider The Sensitivity of Each Individual to Aβ42 Toxicity



- None of the 25 biomarkers are produced by the brain: they are produced or regulated by peripheral organs. This is a major advantage. Using these peripheral biomarkers signals, our blood test can detect Alzheimer's as soon as the amyloid pathway is engaged while being very specific. Indeed, the combination of these biomarkers considers the inherent sensitivity of the subject to amyloid toxicity, thereby reducing the false positives.

CONCLUSIONS

- In our retrospective and multi-center study, we developed a diagnostic algorithm with a high sensitivity (100%) and a high specificity (99%) to detect Alzheimer's disease from the asymptomatic phase.
- Given the biological heterogeneity of Alzheimer's disease and its evolution over time, a multiomics approach coupling proteins and metabolites is necessary for effective early detection of the disease.
- Using peripheral biomarkers increases the sensitivity and specificity of early detection.

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