# Blood-based Biomarkers in Alzheimer's Disease and Related Dementias


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# Overview

- Part I
  - Blood-based biomarker background
- Part II
  - NCRAD Biomarker Assay Lab
- Part III
  - Use of plasma biomarkers in historical study

# Blood-based Biomarkers

# AT(N) Framework for AD

- NIA-AA Research Framework defines AD by pathological processes documented by postmortem examination or by presence of *in vivo* biomarkers
  - AT(N) classification system
    - A = β amyloid deposition
    - T = Pathologic tau
    - N= Neurodegeneration
  - Assessing subjects for AT(N)
    - Neuropathology
    - Imaging
    - Fluid based biomarkers

Risk of short-term cognitive decline based on the biomarker profile and cognitive stage

| | | Syndromal Cognitive Stage | | |
|---|---|---|---|---|
| Biomarker Profile | | **Cognitively unimpaired** | **MCI** | **dementia** |
| | $A^- T^- (N)^-$ | normal AD biomarkers, cognitively unimpaired | normal AD biomarkers with MCI | normal AD biomarkers with dementia |
| | $A^+ T^- (N)^-$ | Preclinical Alzheimer's pathologic change | Alzheimer's pathologic change with MCI | Alzheimer's pathologic change with dementia |
| | $A^+ T^- (N)^+$ | Alzheimer's and concomitant suspected non Alzheimer's pathologic change, cognitively unimpaired | Alzheimer's and concomitant suspected non Alzheimer's pathologic change with MCI | Alzheimer's and concomitant suspected non Alzheimer's pathologic change with dementia |
| | $A^+ T^+ (N)^-$ | Preclinical Alzheimer's disease | Alzheimer's disease with MCI (Prodromal AD) | Alzheimer's disease with dementia |
| | $A^+ T^+ (N)^+$ | | | |

Non-Alzheimer's continuum profiles are not included in table because the risk associated with different combinations of T+(N)-, T+(N)+, T-(N)+ among A- individuals has not been established

Jack et al., 2018

rate of short term clinical progression expected to be low

rate of short term clinical progression expected to be high

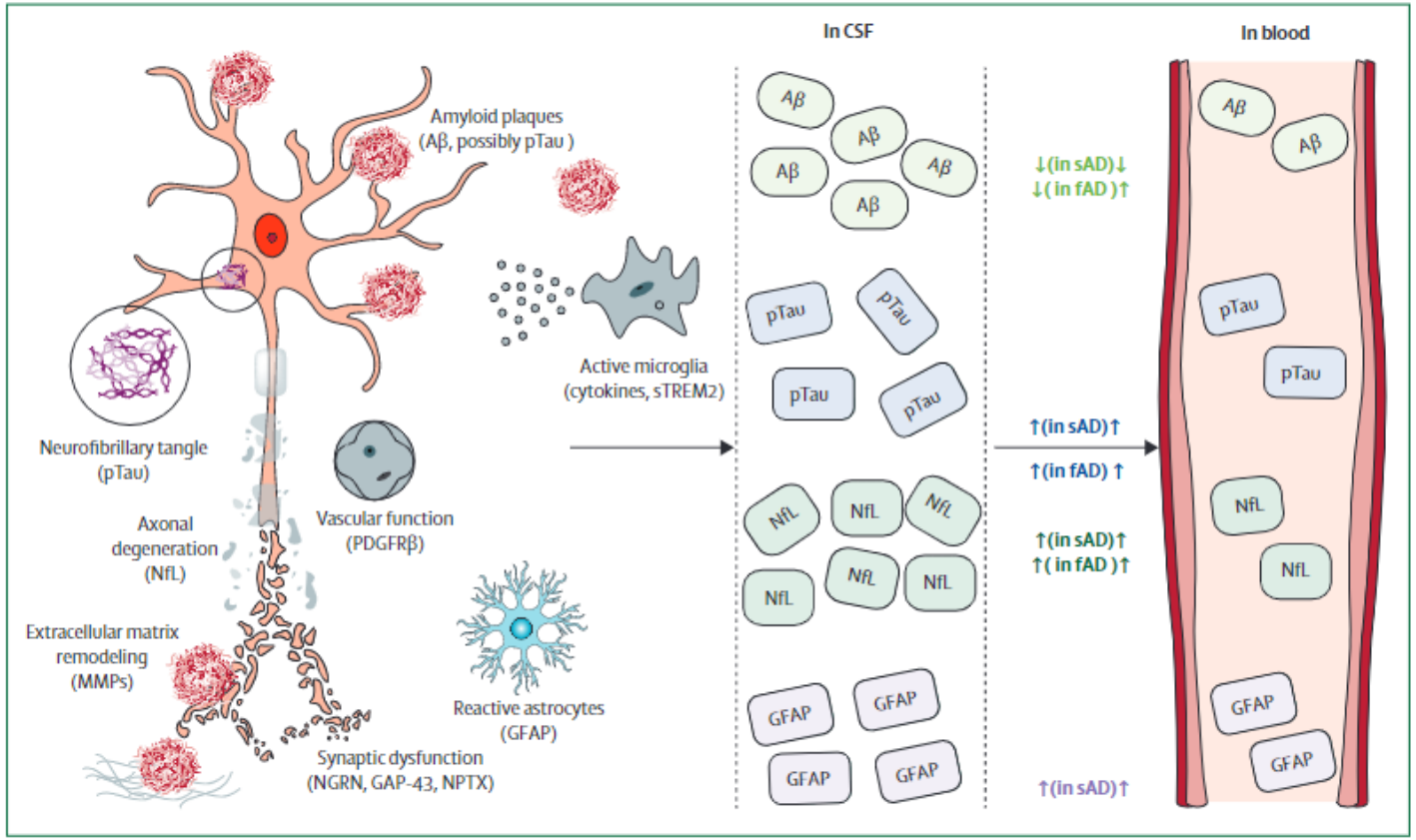
# Application of Blood-based AD Biomarkers to NIA Research Framework

Blood-Based Biomarkers

β Amyloid Deposition (A) → **Amyloid beta peptide ratio (Aβ42/40)**

Pathologic Tau (T) → **Phosphorylated Tau (P-tau)**

Neurodegeneration (N) → **Neurofilament Light Chain (NfL)**




Teunissen et al., 2022

# 2023 Draft Update to the Framework for AD

- Things have changed since the 2018 NIA-AA AT(N) Research Framework development
  - Drugs targeting core pathology have been approved for treatment
  - Plasma biomarkers have been studied and are more reliable
  - Studies have shown not all fluid biomarkers are equivalent for intended uses
- Framework update intended to be a resource reflecting current science
  - Not specific clinical practice guideline

| Biomarker category | CSF or plasma analytes | Imaging |
|---|---|---|
| **Core Biomarkers** | | |
| **Core 1** | | |
| **A** (Aβ proteinopathy) | Aβ42 | Amyloid PET |
| **T1**: (phosphorylated and secreted AD tau) | p-tau 217, p-tau 181, p-tau 231 | |
| **Core 2** | | |
| **T2** (AD tau proteinopathy) | pT205, MTBR-243, non-phosphorylated tau fragments | Tau PET |
| **Biomarkers of non-specific processes involved in AD pathophysiology** | | |
| **N** (injury, dysfunction, or degeneration of neuropil) | NfL | Anatomic MR or CT, FDG PET |
| **I** (inflammation) Astrocytic activation | GFAP | |
| **Biomarkers of non-AD co-pathology** | | |
| **V** vascular brain injury | | Anatomic infarction, WMH |
| **S** α-synuclein | αSyn-SAA* | |

Alzheimer's Association workgroup , 2023

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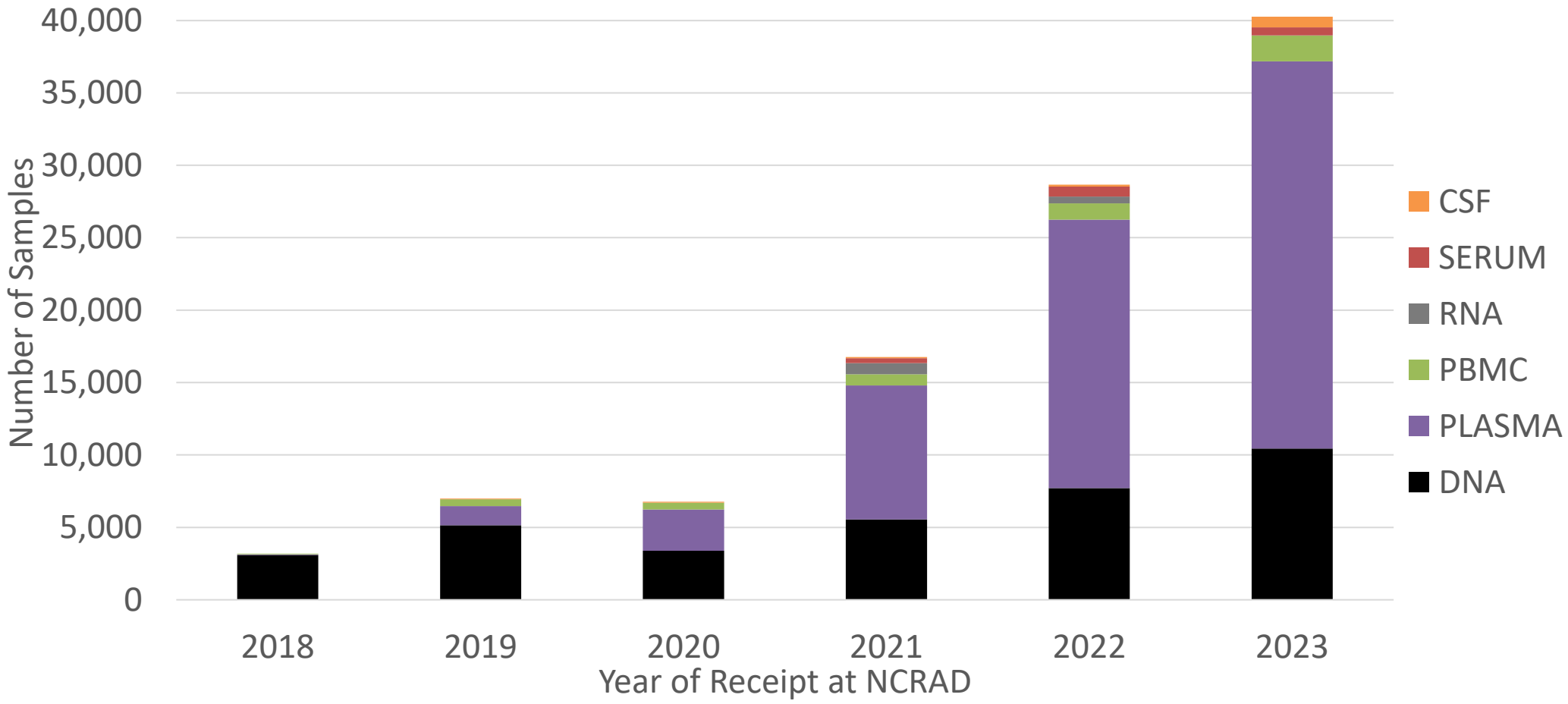
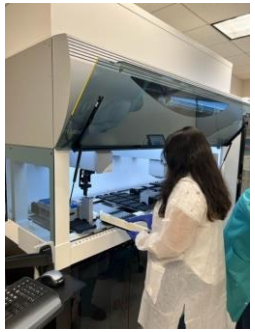
# Biomarker Use in Research

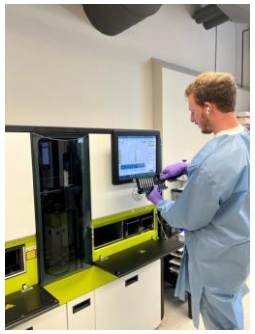
Plasma collection and banking at NCRAD in recent years as plasma biomarkers have been increasingly incorporated into research studies



# NCRAD Biomarker Assay Lab

# NCRAD Biomarker Assay Lab (BAL)





- Leadership and Personnel
  - Scientific Director- Jeff Dage, PhD
    - Internationally recognized leader in the biomarker field with expertise in the development of novel biomarkers
    - Provides overall scientific leadership of the BAL
    - NCRAD Co-investigator
  - Laboratory Director- Kristen Russ, PhD
    - Expertise in a wide range of assay platforms
    - Provides leadership day to day for operation of the BAL
    - NCRAD Co-investigator
  - Laboratory Coordinator- Clairisa Stayton
    - BAL specific project coordinator to interface with researchers and allow for blinding of the lab to sample specific information
  - Laboratory Staff
    - Research Assistant and 3 laboratory technicians
      - Trained in operation of state-of-the-art instrumentation and sample processing/handling SOPs

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# NCRAD Biomarker Assay Lab

- Equipment
  - Automated liquid handling
    - Tecan Fluent (2)
      - Decreases variability during assay processes
  - Immunoassay platforms
    - Quanterix HD-X
      - For use with Quanterix Simoa HD-X kits
        - Excellent sensitivity and low variability for AD biomarkers
    - Fujirebio Lumipulse G1200
      - For use with plasma or CSF


# NCRAD Biomarker Assay Lab

| Assay Kits | Biofluid | Platform | Biomarkers | Qualification Status |
|---|---|---|---|---|
| NF-Light Advantage Kit | Plasma | Quanterix Simoa HD-X | NfL | Complete |
| Neurology 2-Plex B (N2PB) | Plasma | Quanterix Simoa HD-X | NfL, GFAP | Complete |
| Neurology 4-Plex E (N4PE) | Plasma | Quanterix Simoa HD-X | NfL, GFAP, Abeta 40, Abeta 42 | Complete |
| pTau 181 v2.1 Advantage Kit | Plasma | Quanterix Simoa HD-X | pTau 181 | Complete |
| pTau 217 Alzpath | Plasma | Quanterix Simoa HD-X | pTau 217 | Complete |
| Lumipulse G β-Amyloid 1-42 Kit | Plasma | Fujirebio Lumipulse | Abeta 42 | Complete |
| Lumipulse G β-Amyloid 1-40 Kit | Plasma | Fujirebio Lumipulse | Abeta 40 | Complete |
| Lumipulse G pTau 181 Kit | Plasma | Fujirebio Lumipulse | pTau 181 | Complete |
| Lumipulse G pTau217 Kit | Plasma | Fujirebio Lumipulse | pTau 217 | Upcoming |
| Lumipulse G β-Amyloid 1-42 Kit | CSF | Fujirebio Lumipulse | Abeta 42 | Upcoming |
| Lumipulse G β-Amyloid 1-40 Kit | CSF | Fujirebio Lumipulse | Abeta 40 | Upcoming |
| Lumipulse G pTau 217 Kit | CSF | Fujirebio Lumipulse | pTau 217 | Upcoming |

* Funds available for diverse studies interested in collection and banking of plasma

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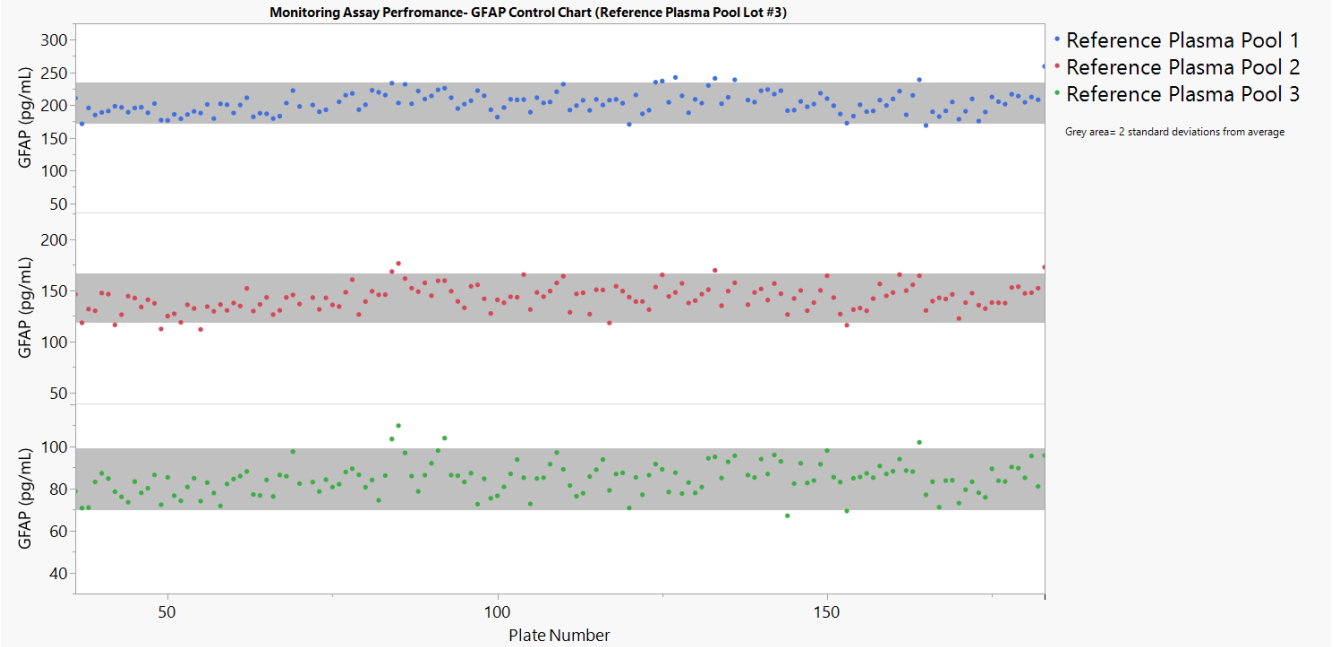
# NCRAD Biomarker Assay Lab

**Bridging and control measures are key to consistency of data across time**

- Control measures
  - Identify assay performance outside established quality guidelines
  - Confirms validity of assay results
- Bridging
  - Allows comparison of data between studies
  - Necessary to fully harmonize laboratories

# NCRAD Biomarker Assay Lab

- Assay monitoring within studies and over time
  - Reference pools
    - BAL produces plasma reference pool controls from our local plasma collections
    - High, medium, and low controls
    - Allows monitoring of assay performance within the batch and between batches
  - Kit QC low and high samples
    - Provided by manufacturer of kits
    - Allows BAL to determine that the assay is working properly from plate to plate
- Bridging
  - Utilizes BAL local collection of plasma
  - 31 samples per bridging plate
  - Controls for variation in lot and instrument performance



# NCRAD Biomarker Assay Lab

**NCRAD cross-laboratory harmonization efforts**

- Harmonization is possible but has become more challenging with the widespread deployment of new assays across many research biomarker laboratories
- Quality assurance system is an important factor every lab will need to properly harmonize
- NCRAD is leading a cross-laboratory harmonization effort
  - Initial organization stages have begun
    - Local collection of samples is underway

# Fluid-based biomarkers: Value of current technology in historical samples

Indianapolis-Ibadan Dementia Project

# Indianapolis-Ibadan Dementia Project (IIDP)

- Prospective population based comparative epidemiological study of the prevalence, incidence rates, and risk factors for Alzheimer's Disease and other age associated dementias
- Study ran from 1991-2011
- Enrolled Participants (age >65 years)
  - African Americans living in Indianapolis
  - Yoruba living in Ibadan, Nigeria

https://iidpportal.medicine.iu.edu/

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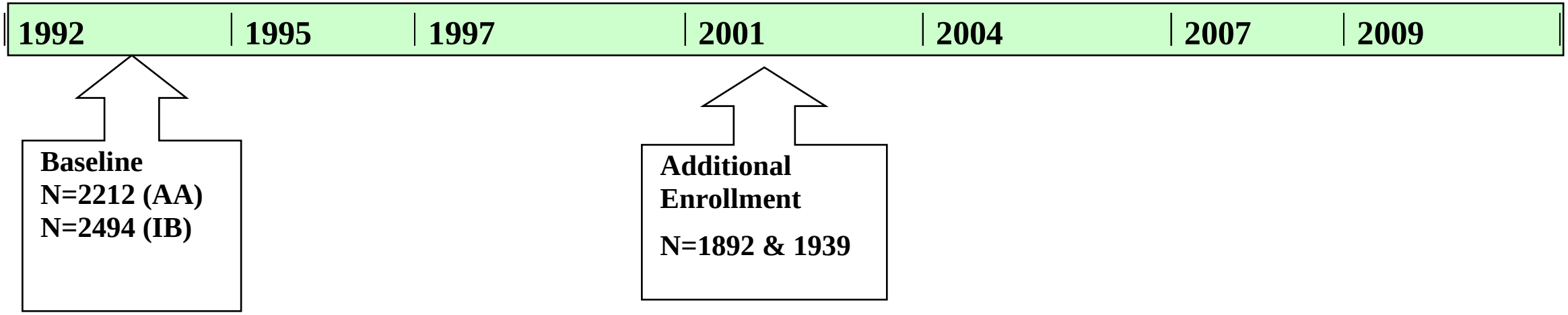
# IIDP Study Timeline




- Total numbers of participants: 4104 African Americans; 4433 Yoruba
- Up to 7 longitudinal evaluations over 19 years
- Two-phase sampling was used at each evaluation wave

https://iidpportal.medicine.iu.edu/

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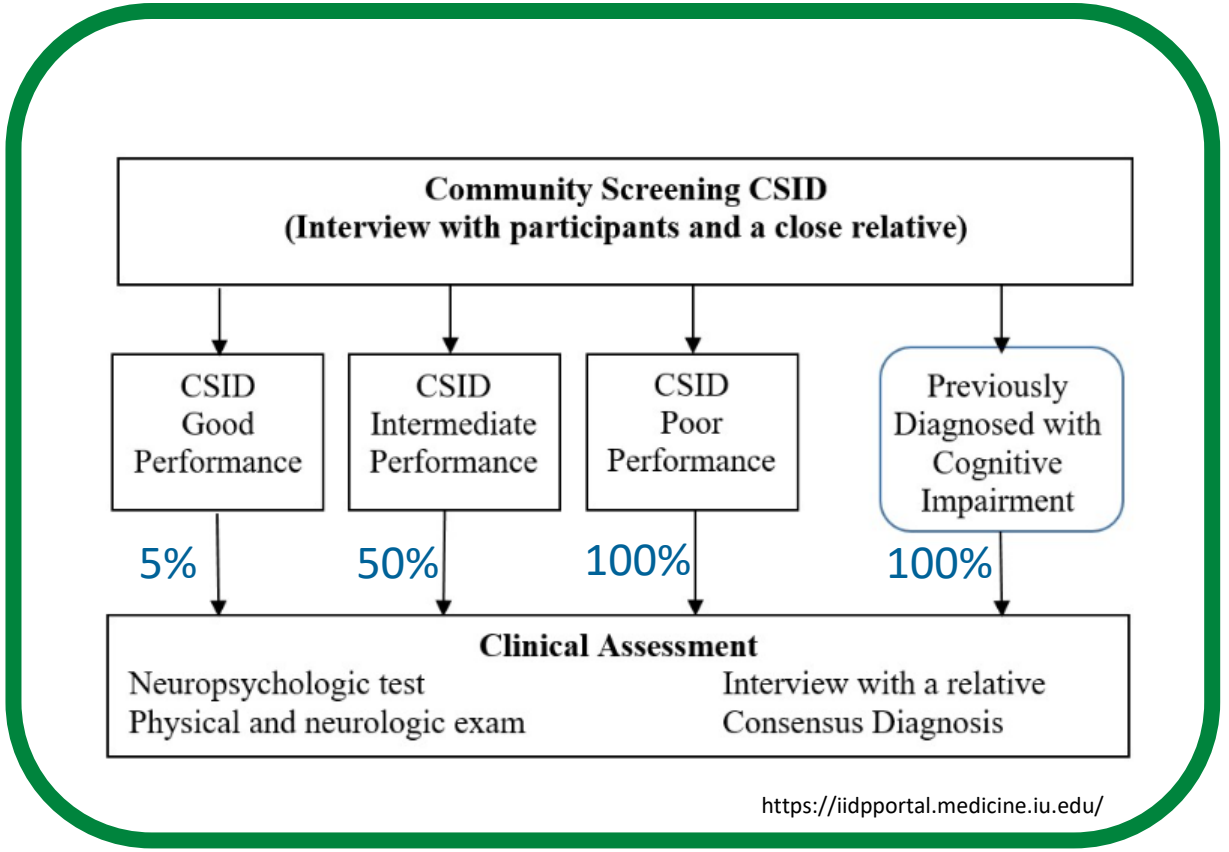
# IIDP Study Design

| Time line | 1992 | 1995 | 1997 | 2001* | 2004 | 2007 | 2009 |
|---|---|---|---|---|---|---|---|
| **Stage 1: Screening** | $n_1$=2212 $n_2$=2486 | $n_1$=1667 $n_2$=2113 | $n_1$=1253 $n_2$=1227 | $n_1$=2628 $n_2$=2806 | $n_1$=1821 $n_2$=2180 | $n_1$=1245 $n_2$=1701 | $n_1$=958 $n_2$=1273 |
| **Interview with participants** | | | | | | | |
| Demographic | x | x | x | x | x | x | x |
| Cognitive Assessment | x | x | x | x | x | x | x |
| Depressive Symptoms | | | | | | x | x |
| Neurological tests | | | x | x | x | x | x |
| Lifestyle | x | x | x | x | x | x | x |
| Medical History | x | x | x | x | x | x | x |
| Medications | | | | x | x | x | x |
| Vital Measurements | | | x | x | x | x | x |
| Engagement Questionnaire | | | x | x | x | x | x |
| **Interview with relatives** | | | | | | | |
| Changes in daily activity | x | x | x | x | x | x | x |
| Activity of Daily Living | x | x | x | x | x | x | x |
| Change in personality | x | x | x | x | x | x | x |
| General Health | x | x | x | x | x | x | x |
| **Stage 2: Clinical Evaluation** | | | | | | | |
| Neuropsychological test | x | x | x | x | x | x | x |
| Geriatric Depression Scale (0-30) | x | x | x | x | x | x | x |
| Functional Assessment (CHIF) | x | x | x | x | x | x | x |
| Physical Examination | x | x | x | x | x | x | x |
| Neurological Exam | x | x | x | x | x | x | x |
| Consensus diagnosis | x | x | x | x | x | x | x |
| **Blood Samples** | | | | x | | | |
| DNA samples are stored at the National Centralized Repository for Alzheimer Disease (NCRAD) | | | | | | | |
| GWAS, Whole Genome Sequencing (ADGC) | | | | | | | |
| Plasma levels of cholesterol, triglyceride, HDL, LDL, 8-isoprostane, CRP, PAI-1, E-selectin, homocysteine, Folate, and vitamin B12[12] | | | | | | | |




Cross-sectional blood collection in 2001!

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# IIDP Findings

- Key Findings
  - AD most common type of dementia at both sites
  - Association between age and incident AD at both sites
  - Inter-site difference with respect to sex and risk of incident AD
  - Indianapolis cohort had a higher BMI and higher prevalence of diabetes and smoking
  - APOE effect differences between sites

**Table 5.** Age-Specific Annual Incidence Rate of Dementia and Alzheimer Disease, Adjusted for Mortality*

| | | Yoruba | African Americans |
|---|---|---|---|
| | Age Group, y | Rate, % (95% Confidence Interval)† | Rate, % (95% Confidence Interval)† |
| Dementia | 65-74 | 0.45 (0.30-0.60) | 1.74 (0.15-3.32) |
| | 75-84 | 1.69 (1.29-2.10) | 4.29 (2.52-6.06) |
| | ≥85 | 5.71 (4.19-7.22) | 9.12 (5.97-12.28) |
| | Age-standardized overall rate | 1.35 (1.13-1.56) | 3.24 (2.11-4.38) |
| Alzheimer disease | 65-74 | 0.38 (0.24-0.52) | 1.38 (0-2.99) |
| | 75-84 | 1.41 (1.04-1.77) | 3.29 (1.56-5.01) |
| | ≥85 | 5.02 (3.62-6.42) | 7.07 (4.54-9.61) |
| | Age-standardized overall rate | 1.15 (0.96-1.35) | 2.52 (1.40-3.64) |

*The overall rates (standardized for age) were derived using the age distributions of African Americans in Indianapolis, Ind, according to the 1990 US Census Bureau data.
†The 95% confidence intervals are narrower in Yoruba than in African Americans' incidence rate because the incidence rate in Yoruba was lower.

Hendrie HC, et al., 2001
Ogunniyi, et al. 2006
Hendrie, et al. 2014

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# IIDP Findings

- Blood collected in 2001 wave was processed to plasma
  - Utilized to investigate assessed cholesterol, HDL, LDL, PAI-1, E-selectin, 8-isoprostane, homocysteine, folate, Vitamin B12, and C-reactive protein in IIDP participants
  - Findings include differences between bio-measures for cardiovascular disease risk
    - Lower lipid levels in Ibadan
    - Higher oxidative stress in Ibadan

Deeg et al. 2008

# Current Biomarker Analysis of Historical Samples

# IIDP Fluid-based Biomarker Study

- Plasma samples were stored from the original 2001 collection at NCRAD
  - Samples were stored at -80C for ~22 years
- Plasma was analyzed in the NCRAD BAL
  - NfL, GFAP, Aβ40, Aβ42, and Ptau181
    - Quanterix Simoa HD-X

| | Study Demographics | | | |
|---|---|---|---|---|
| | **Full Ibadan Study** | **Ibadan Biomarker Subset** | **Full Indianapolis Study** | **Indianapolis Biomarker Subset** |
| **N** | 4425 | 1093 | 4105 | 1017 |
| **Sex** | F: 2913; M: 1512 | F: 733; M: 360 | F: 2667; M: 1438 | F: 683; M: 334 |
| **Age at Baseline, mean ± sd** | 73.9 ± 7.1 (n=4425) | 73.4 ± 5.7 (n= 1093) | 75.8 ± 6.6 (n=4105) | 75.6 ± 5.9 (n=1017) |
| **Age at 2001 Screening, mean ± sd** | 76.8 ± 5.8 (n=2806) | 76.4 ± 5.4 (n= 1093) | 78.2 ± 5.8 (n=2648) | 77.7 ± 5.4 (n=1016) |
| **Age at Blood Draw, mean ± sd** | 77.8 ± 5.4 (n=1234) | 77.8 ± 5.4 (n=999) | 78.1 ± 5.5 (n=1512) | 78.2 ± 5.5 (n=831) |
| **Age at Diagnosis, mean ± sd** | N/A | 81.5 ± 5.6 (n=1093) | N/A | 82.6 ± 5.5 (n=1017) |
| **APoE, n (%)** | E2/E2 29 (1.3%)<br>E2/E3 310 (13.7%)<br>E2/E4 100 (4.4%)<br>E3/E3 1051 (46.4%)<br>E3/E4 664 (29.3%)<br>E4/E4 109 (4.8%) | E2/E2 13 (1.3%)<br>E2/E3 129 (12.8%)<br>E2/E4 42 (4.2%)<br>E3/E3 455 (45.0%)<br>E3/E4 324 (32.1%)<br>E4/E4 46 (4.6%) | E2/E2 20 (1.0%)<br>E2/E3 301 (15.1%)<br>E2/E4 93 (4.7%)<br>E3/E3 950 (47.6%)<br>E3/E4 539 (27.0%)<br>E4/E4 92 (4.6%) | E2/E2 5 (0.5%)<br>E2/E3 153 (15.4%)<br>E2/E4 50 (5.0%)<br>E3/E3 478 (48.1%)<br>E3/E4 273 (27.5%)<br>E4/E4 35 (3.5%) |
| **School (Y/N), n (%)** | 623 (14.1%) | 151 (13.8%) | N/A | N/A |
| **Years of Education, mean ± sd** | N/A | N/A | 10.4 ± 3.1 | 11.0 ± 2.9 |

# IIDP Fluid-based Biomarker Study

- Preliminary Analyses
  - Multiple linear regression models for poor cognition
    - Analyses adjusted for sex, age at diagnosis, years of education, APOE 4 carrier status
      - Indianapolis: Age, years of education, and APOE 4 carrier status were significant in all models
      - Ibadan: Sex and age at diagnosis were significant in all models.
    - Poor cognition
      - In these models, poor cognition is defined as participants diagnosed as cognitively impaired, dementia, or screened as poor at their last study visit

Poor cognition numbers by site

Ibadan

| | *CI* | *D* | *N* | *NCI* | *good* | *interm* | *poor* | *Total* |
|---|---|---|---|---|---|---|---|---|
| *Normal cognition* | 0 | 0 | 82 | 5 | 633 | 59 | 0 | 779 |
| | 0.00 | 0.00 | 10.53 | 0.64 | 81.26 | 7.57 | 0.00 | |
| *Poor cognition* | 79 | 80 | 0 | 0 | 0 | 0 | 72 | 231 |
| | 34.20 | 34.63 | 0.00 | 0.00 | 0.00 | 0.00 | 31.17 | |
| *Total* | 79 | 80 | 82 | 5 | 633 | 59 | 72 | 1010 |

Indianapolis

| | *CI* | *D* | *N* | *NCI* | *good* | *interm* | *poor* | *Total* |
|---|---|---|---|---|---|---|---|---|
| *Normal cognition* | 0 | 0 | 123 | 0 | 577 | 40 | 0 | 740 |
| | 0.00 | 0.00 | 16.62 | 0.00 | 77.97 | 5.41 | 0.00 | |
| *Poor cognition* | 110 | 101 | 0 | 0 | 0 | 0 | 43 | 254 |
| | 43.31 | 39.76 | 0.00 | 0.00 | 0.00 | 0.00 | 16.93 | |
| *Total* | 110 | 101 | 123 | 0 | 577 | 40 | 43 | 994 |

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# IIDP Fluid-based Biomarker Study

Preliminary analyses investigating poor cognition and biomarker interaction

- Site differences in the effect of NfL, GFAP, Ptau181 on poor cognition
- Suggests interactions between AT(N) biomarkers and the 2001 bio-measures that vary by site in models for poor cognition

# IIDP Fluid-based Biomarker Study Limitations

## Study Design

- Participants diagnosed as having dementia were no longer followed
- Variability in compliance for fasting
  - Indianapolis: 1/3 participants fasted
  - Ibadan: All fasted
- Collection of education information

## Samples

- Extended time in storage (>20 year storage)

## Assays

- Use of Ptau181
  - Ptau217 was not commercially available at the time of analysis
  - Ptau217 analysis is a future direction

## Biostatistics

- Very preliminary data
  - Need to determine how best to look at the 2001 labs that require fasted blood

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# IIDP Fluid-based Biomarker Study

- Conclusions
  - Analysis of historical samples with current techniques can provide a wealth of information
    - Limitations of the study and the samples must be critically evaluated
  - AD biomarkers vary by site
    - NfL, GFAP, and Ptau181 in IIDP preliminary studies show differences in looking at poor cognition as an outcome
    - It is critical for research in the AD field to expand beyond Caucasian participants
- Future directions
  - Analysis of AD biomarker data and IIDP existing data will continue
  - Utilization of residual plasma to analyze Ptau217

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