The ALFA Study and nested Sub-studies

Synopsis

The ALFA (ALzheimer and FAmilies) study[1] is a large-scale research initiative aimed at understanding the **early pathophysiological changes and risk factors associated with preclinical Alzheimer's disease (AD)**. The ALFA parent cohort comprises nearly **3,000 cognitively unimpaired individuals**, mostly first-degree descendants of AD patients, aged 45–75, thoroughly characterized through sociodemographic, clinical, blood biomarker, lifestyle, genetic, and cognitive assessments. ALFA is enriched for genetic risk of developing cognitive impairment, particularly a higher prevalence of the *APOE-ε4* allele, is found. ALFA is a highly valuable study to pursue AD prevention research.

Nested within ALFA are two major sub-studies: ALFA-MRI and ALFA+. The **ALFA-MRI study** (n ≈ 1,600) expanded phenotyping via advanced magnetic resonance imaging (MRI), olfactory testing, and biological aging markers such as telomere length. The **ALFA+ study** (n ≈ 420), a longitudinal cohort study, includes, aside from all the variables collected in ALFA, in-depth phenotyping with fluid (both cerebrospinal and plasma) and imaging biomarkers (MRI and several modalities of positron emission tomography), and repeated assessments over time (every 3 years). A significant portion of cognitively unimpaired participants **(35-40%) already exhibit preclinical AD biomarker profiles** (e.g., A+T-, A+T+). These efforts are pivotal in modelling AD's preclinical stages, identifying early biomarkers, and facilitating preventive clinical trials.

[1] The ALFA Study was established in 2013 by the Pasqual Maragall Foundation's research centre, the Barcelonaβeta Brain Research Centre (BBRC), and supported by "la Caixa Foundation".

The ALFA Study

The setup of preventive strategies requires the **understanding, from a molecular perspective, of how risk factors generate the risk** and the **identification of individuals with an increased risk of developing cognitive impairment** in the near future that are suitable to be recruited as asymptomatic subjects in prevention studies and trials. With this in mind, and aiming at increasing our knowledge of the pathophysiology and pathogenic factors emerging at early preclinical Alzheimer's disease (AD) stages, the Barcelonaβeta Brain Research Centre (BBRC), the research centre of the Pasqual Maragall Foundation, started the **ALFA (for ALzheimer and FAmilies) study**, supported by "la Caixa Foundation.

The **ALFA study** (also referred to as ALFA parent cohort) was set-up for the prospective follow-up of a cohort of cognitively unimpaired individuals that were recruited between 2013 and 2014. ALFA is composed of 2,743 cognitively unimpaired participants, most of them first-degree descendants of AD patients, aged between 45 and 75 years, who were thoroughly characterised from a **sociodemographic, clinical, lifestyle, genetic and cognitive** point of view. Other variables of interest obtained during the baseline visit were those lifestyles and cardiovascular risk factors that had been previously suggested as modifiable risk factors that may increase or decrease the risk of cognitive impairment and dementia such as cardiovascular and endocrine-metabolic co-morbidities, the participants' level of physical activity and their smoking habits. In addition, participants' ***APOE*** (*Apolipoprotein E*) haplotype also has been determined as well as a whole **GWAS** is also available (Illumina Infinium Neuro Consortium [NeuroChip] Array). Finally, a subset of ~600 participants that were selected based on their *APOE* genotype (preferentially including *APOE-ε4* allele carriers), underwent cerebral magnetic resonance imaging (MRI, example). In addition, a number of **plasma biomarkers** have been more recently determined from samples that were obtained in the ALFA study visit. These include Aβ42, Aβ40, pTau181, pTau231, NfL and GFAP.

The family history of AD of ALFA participants was recorded during baseline visit. In particular, we registered who, their mother and/or father, had been diagnosed with cognitive impairment. In this regard, 86.3% of the study participants had at least one of their parents that had suffered AD. When considering a more strict family history encoding, it is remarkable that **47.4% of the ALFA study participants had at least one of their parents that had been diagnosed with AD before the age of 75**. Family studies have shown that having a parental history of AD represents a risk factor for sporadic AD and the biggest genetic susceptibility factor is the *APOE-ε4* allele. In agreement with this, a **higher frequency of the *APOE-ε4* allele was found in ALFA parent cohort participants than in the general population (38% and 14%, respectively; *P* <.001).** In brief, of 2,714 ALFA members whose genotype could be determined, 9 were *APOE-ε2/ε2* homozygotes, 171 were *APOE-ε2/e3* heterozygotes, 60 were *APOE-ε2/ε4* heterozygotes, 1,589 were *APOE-ε3/ε3* homozygotes, 796 were *APOE-ε3/ε4* heterozygotes and, finally, 89 were *APOE-ε4/ε4* homozygotes.

Therefore, we have **established a research platform that is enriched in genetic risk factors for AD**. As a consequence, the proportion of individuals presenting altered biomarkers, neuroimaging changes and eventually the development of cognitive decline is also expected to be higher, which is being evaluated in longitudinal assessments. In summary, the ALFA parent cohort represents a valuable infrastructure of middle-aged participants representing the **whole spectrum of risk that will leverage with different studies and trials to prevent AD**.

The main characteristics of ALFA study's participants are: mean age 56.4 years, 63.2% females, 34,3% *APOE-ε4* carriers, mean years of education 13.4.

The ALFA-MRI Study

As a nested study to ALFA, the BBRC established the cross-sectional ALFA-MRI study, with the main objective of expanding the clinical, lifestyle, cognitive and brain characterisation of a subset of cognitively unimpaired ALFA study participants. Around **1,600 participants with no contraindications to MRI** that were selected based on their AD risk profile were recruited from 2016 to 2019.

On top of a review of the clinical and cognitive status, participants underwent a **high-resolution MRI acquisition protocol** in our centre's Philips Ingenia CX 3T including several sequences (**T1, T2, FLAIR, DWI, IR and resting state fMRI**). In addition, the study visits also included an **odour identification test, a blood extraction** to determine basic biochemical variables and be kept for future analyses (e.g. plasma samples to determine AD-related biomarkers). Finally, the **telomere length** (TL), as a proxy of biological age, has been also determined in ALFA-MRI study participants, which allows us to assess whether TL may be generating an age-related structural and functional vulnerability and mediating the effect of ageing on AD pathology.

The main characteristics of ALFA-MRI study's participants are: mean age 60.1 years, 61.2% females, 38,1% *APOE-ε4* carriers, mean years of education 13.5.

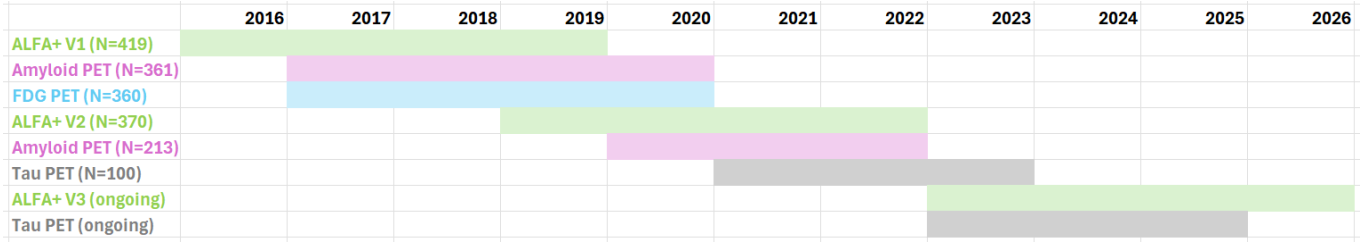
The ALFA+ Study

Also nested to the ALFA parent cohort, the BBRC established the **longitudinal, long-term ALFA+ study** in which a more detailed phenotyping is performed. ALFA+ is a prospective and observational cohort study for the early identification of biomarkers (both fluid and neuroimaging) of AD in **~420 cognitively unimpaired individuals**. Participants with no contraindications to MRI or lumbar puncture (LP) were invited based on their risk profile (mainly *APOE* and family history status). The aim of the study is to describe the **biological processes and identify factors that may precede the clinical phase of AD**. Likewise, thanks to the extensive characterisation of its participants, ALFA+ aims to analyse the **association between the biological, structural, functional and neurocognitive brain markers that characterize the preclinical phase of the disease and its natural history**. The baseline visit (V1) of the ALFA+ cohort study took place between 2016 and 2019 and follow-up visits take place every 3 years: The first follow-up visit (V2) started in 2019 and finished by the end of 2022, and V3 started by 2023 and is currently underway (expected to finalise by 2026).

Each ALFA+ cohort study visit is organised in **three core sessions**: Session 1 (S1) includes a **clinical, cognitive, nursing and lifestyle** characterisation of study participants as well as a **high-resolution MRI acquisition protocol** in our centre's Philips Ingenia CX 3TMRI session (including T1, T2, FLAIR, DWI, IR and resting state fMRI sequences). In S2, participants undergo a **LP to obtain cerebrospinal fluid (CSF)** for their biomarker characterisation and obtaining several CSF aliquots that can be used for future biomarker studies. Various types of biological samples are also collected in S1 (non-fasting conditions) and S2 (fasting conditions) to determine biochemical variables and be kept for future analyses (e.g. plasma samples to determine AD-related biomarkers). Finally, S3 entails a further, more experimental and sensitive **cognitive**

testing session and another MRI scan entailing the acquisition of more experimental sequences (T1, ASL, Spectro, Swip, QFLOW_CSF and multi-b).

In addition to the ALFA+ cohort study core sessions, participants have also been invited to undergo **^{18}F-Flutemetamol (amyloid) and ^{18}F-Fludeoxyglucose Positron Emission Tomography (PET)** in the context of the ALFA+ V1 visit. Similarly, a subset of 213 participants have, in the context of the ALFA+ V2 visit, undergone **longitudinal ^{18}F-Flutemetamol PET**. Finally, a subset of 100 participants have undergone **RO-948 (tau) PET** in the context of the study's V2 and are currently undergoing **longitudinal RO-948 PET.** Please see figure 1 below:



With regard to the **fluid** and **neuroimaging** **biomarker characterization of ALFA+ participants at V1**, 342 have both full CSF (with 25 biomarkers measured [e.g. Aβ42, p-tau and t-tau, Aβ40, GFAP, YKL-40, sTREM2, IL6, NfL, neurogranin, S100 and α-synuclein and various other forms of p-tau in different platforms]) and PET (amyloid and FDG) data, 58 have only CSF data and, finally, 19 have only PET data. As expected due to the selection strategy, **~35%** of (cognitively unimpaired) study participants are already in the **earliest preclinical stages of AD.** Specifically, based on CSF core AD biomarkers (from 396 participants with available CSF biomarkers):

- A-T- 63,1%
- A+T- 26,3%
- A+T+ 7,6%
- A-T+ 3,0%

In addition, we have also expanded the characterization of ALFA+ study participants with regard to recently developed **blood-based biomarkers**. The following blood biomarkers, have been determined in plasma samples (baseline) of the 419 individuals included in the study: Aβ42, Aβ40, GFAP, NfL, APOE-ε4 and various forms of p-tau using a variety of techniques and platforms (a total of 15 blood-based biomarkers).

At present, the **longitudinal determinations (samples from V2) of the following CSF biomarkers** (Aβ42, p-tau and t-tau, Aβ40, GFAP, YKL-40, sTREM2, IL6, NfL, neurogranin, S100 and α-synuclein) have also been performed. **~40%** of (cognitively unimpaired) study participants are already in the **earliest preclinical stages of AD** Specific results are as follows (from 281 participants with available CSF biomarkers):

- A-T- 56,2%
- A+T- 26,0%
- A+T+ 13,5%
- A-T+ 4,3%

Finally, we have also expanded the characterization of ALFA+ study participants with regard to recently developed **blood-based biomarkers in V2**. The following blood biomarkers have been determined in plasma samples (longitudinal): Aβ42, Aβ40, GFAP, NfL, APOE-ε4 and various forms of p-tau using a variety of techniques and platforms (a total of 15 blood-based biomarkers).

In brief, the ALFA+ study will serve to **untangle the natural history of the disease and to model the preclinical stages in order to develop successful trials.**

The main characteristics of ALFA-V1 participants are: mean age 61.1 years, 60.6% females, 55,1% *APOE-ε4* carriers, mean years of education 13.6.

The main characteristics of ALFA-V1 participants are: mean age 64.2 years, 60.1% females, 55.6% *APOE-ε4* carriers, mean years of education 13.7.

The **timelines** of the ALFA, ALFA-MRI and ALFA+ studies as well as a **detailed list of variables** can be found in the following pages of this document.

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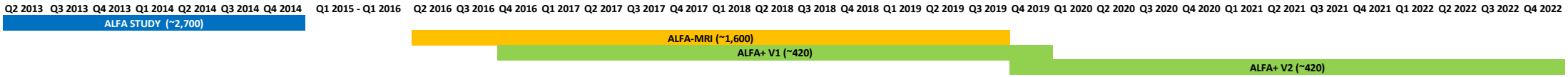
The ALFA Study and nested Sub-studies - TIMELINES



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The ALFA Study and nested Sub-studies – DETAILED LIST OF VARIABLES

STUDY/SUB-STUDY	N
Alfa Parent Cohort	~2,700
Alfa-MRI	~1,600
Alfa+	~420

SOCIODEMOGRAPHIC

VARIABLE	TYPE	STUDY
Gender	Transversal	■ ■ ■
Age at inclusion	Transversal	■ ■ ■
Place of Birth	Transversal	■ ■ ■
Years of education	Longitudinal	■ ■ ■
Level of education	Longitudinal	■ ■ ■
Ethnicity	Transversal	■ ■ ■
Civil status	Longitudinal	■ ■ ■
Employment status	Longitudinal	■ ■ ■
Cohabitation status	Longitudinal	■ ■ ■
Caregiver status	Longitudinal	■ ■ ■
Professional Activity	Longitudinal	■ ■ ■
Socioeconomic status	Longitudinal	■
Laterality/handedness	Transversal	■
Bilingualism / poliglotism	Transversal	■

FAMILY HISTORY

VARIABLE		TYPE	STUDY
Parents	Date of birth	Transversal	■ ■ ■
	History of dementia	Longitudinal	■ ■ ■
	Age of parents at onset of scognitive impairment and diagnosis	Longitudinal	■ ■ ■
	Death (date and cause)	Longitudinal	■ ■ ■
	Anamnesis	Longitudinal	■ ■ ■
Siblings	Number of siblings	Transversal	■ ■ ■
Other	Other family members with cognitive decline	Longitudinal	■ ■ ■
Offspring	Number of children	Longitudinal	■ ■ ■

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CLINICAL

VARIABLE		TYPE	STUDY
Anamnesis	Psychomotor development	Longitudinal	■ ■ ■
	Infancy diseases	Longitudinal	■ ■ ■
	Cardiovascular disease	Longitudinal	■ ■ ■
	Endocrine/metabolic diseases	Longitudinal	■ ■ ■
	Digestive tract diseases	Longitudinal	■ ■ ■
	Psychiatric diseases	Longitudinal	■ ■ ■
	Neurological diseases	Longitudinal	■ ■ ■
	Respiratory diseases	Longitudinal	■ ■ ■
	Hematological diseases	Longitudinal	■ ■ ■
	Immunological diseases	Longitudinal	■ ■ ■
	Ophtalmological diseases	Longitudinal	■ ■ ■
	Reproductive ans renal system diseases	Longitudinal	■ ■ ■
	Infectious diseases	Longitudinal	■ ■ ■
	Neoplastic diseases	Longitudinal	■ ■ ■
	Muscle skeletal diseases	Longitudinal	■ ■ ■
	Other diseases	Longitudinal	■ ■ ■
	Climacteric / Reproductive history	Longitudinal	■ ■ ■
	Medication	Longitudinal	■ ■ ■
Anxiety/depression	GADS	Transversal	■
	HADS	Longitudinal	■ ■
	STAI	Longitudinal	■ ■
	PSS Perceived Stress Scale	Longitudinal	■
	BRS Brief Resilience Scale	Longitudinal	■
	Stressful Life Events (SNAC)	Longitudinal	■ ■
	Surgery procedures	Longitudinal	■ ■ ■
	Practice of contact sports	Longitudinal	■ ■ ■
Anthropometric measures	Weight	Longitudinal	■ ■ ■
	Height	Longitudinal	■ ■ ■
	Hip circumference	Longitudinal	■ ■ ■
	Waist circumference	Longitudinal	■ ■ ■
	Blood pressure	Longitudinal	■ ■ ■
	Heart rate	Longitudinal	■ ■ ■
	Hemogram	Longitudinal	■ ■ ■
	Biochemistry	Longitudinal	■ ■
Substance use	Tobacco	Longitudinal	■ ■ ■
	Alcohol	Longitudinal	■ ■ ■
	Drugs	Longitudinal	■ ■ ■

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COGNITIVE

VARIABLE		TYPE	STUDY
SCD	Subjective Cognitive decline (SCD) Interview	Longitudinal	■ ■ ■
	SCD-Questionnaire (MyCog/TheirCog)	Longitudinal	■ ■ ■
	Cognitive Function Instrument	Longitudinal	■
Cognitive Reserve	CRC	Longitudinal	■ ■
Functional	Clinical Dementia Rating (CDR)	Longitudinal	■ ■
IQ estimate	Test de acentuación de palabras TAP	Longitudinal	■
Screening	Mini–Mental State Examination (MMSE)	Longitudinal	■ ■ ■
Attention working memory	WAIS-IV: Digit Span	Longitudinal	■ ■
	WMS-IV: Symbol Span	Longitudinal	■
Attention	TMT-A	Longitudinal	■
Episodic memory	Free and Cued Selective Reminding Test FCSRT	Longitudinal	■ ■
	Memory Binding Test (MBT)	Longitudinal	■ ■
	WMS-IV Logical Memory subtest	Longitudinal	■
	NIH-toolbox Picture Sequence Memory test	Longitudinal	■
Executive	TMT-B	Longitudinal	■
	Five digits test	Longitudinal	■
	WAIS-IV Coding	Longitudinal	■ ■ ■
	WAIS-IV Matrix reasoning	Longitudinal	■ ■
	NIH-toolbox Flanker inhibiton test	Longitudinal	■
Language	Animal Fluency	Longitudinal	■ ■
Visual processing	WAIS-IV Visual Puzzles	Longitudinal	■ ■ ■
	Judgment of line orientation from RBANS	Longitudinal	■
Personality	Eysenck Personality Questionnaire (EPQ)	Transversal	■

LIFESTYLE HABITS

VARIABLE		TYPE	STUDY
Physical activity	MINNESOTA PHYSICAL ACTIVITY QUESTIONNAIRE	Longitudinal	■ ■
Quality of life	SF-12	Longitudinal	■
Sleep	Pittsburgh Sleep Quality Index	Longitudinal	■
	ESEMED	Transversal	■
	SQQ Sleep Quality Questionaire	Transversal	■
	ISI Insomnia Severity Index	Transversal	■
	ESS Epworth Sleepiness Scale	Transversal	■
	REM	Transversal	■
	RLSYND Restless leg syndrom questionaire	Transversal	■
	SLPDO	Transversal	■
Diet	Adherence to mediterranean diet	Longitudinal	■ ■ ■
	Exposure to pollutants	Transversal	■
	Exposure to green and blue spaces	Transversal	■
Cognitive activities	Leisure Activities Questionnaire (LAQ)	Longitudinal	■
	Spiritual activities	Transversal	■

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GENETICS

VARIABLE	TYPE	STUDY
APOE genotype	Transversal	■ ■ ■
GWAS (Illumina Infinium Neuro Consortium (NeuroChip) Array)	Transversal	■ ■ ■
Telomere length	Transversal	■ ■

MRI

VARIABLE	TYPE	STUDY
Clinincal sequences (T1, T2, FLAIR)	Longitudinal	■ ■ ■
fMRI sequences	Longitudinal	■ ■ ■
Other sequences (ASL, Qflow, SWIP…)	Longitudinal	■

Note: for ALFA, ~600 MRIs available (not for the whole sample)

NEURORADIOLOGIST

VARIABLE	TYPE	STUDY
Incidental Findings	Longitudinal	■ ■ ■
Fazekas scale	Longitudinal	■ ■ ■
Global cortical atrophy scale	Longitudinal	■ ■ ■
Left medial temporal atrophy scale	Longitudinal	■ ■ ■
Right medial temporal atrophy scale	Longitudinal	■ ■ ■
Koedam parietal atrophy scale	Longitudinal	■ ■ ■
Changes in basal ganglia according to the Wahlund scale	Longitudinal	■ ■ ■

Note: for ALFA, ~600 MRIs available (not for the whole sample)

PET

VARIABLE	TYPE	STUDY
Amyloid (Flutemetamol) Whole Cerebellum SUVr	Longitudinal	■
Amyloid (Flutemetamol) SUVr_GreyCerebellum	Longitudinal	■
Amyloid (Flutemetamol) SUVr_WholeCerebellumBrainStem	Longitudinal	■
Amyloid (Flutemetamol) Pons SUVr	Longitudinal	■
Amyloid (Flutemetamol) Centiloids	Longitudinal	■
Amyloid (Flutemetamol) Visual read	Longitudinal	■
Amyloid (Flutemetamol) raw iamges	Longitudinal	■
FDG PET result	Transversal	■
FDG PET raw images	Transversal	■

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CSF

VARIABLE	TYPE	STUDY
Aβ42	Longitudinal	
Aβ40	Longitudinal	
Aβ42/40 ratio	Longitudinal	
p-tau	Longitudinal	
t-tau	Longitudinal	
ATN classification	Longitudinal	
IL-6	Longitudinal	
α-Synuclein	Longitudinal	
GFAP	Longitudinal	
Neurogranin	Longitudinal	
NfL	Longitudinal	
S100	Longitudinal	
sTREM2	Longitudinal	
YKL40	Longitudinal	
N-p-tau217	Longitudinal	
Mid-p-tau231	Longitudinal	

Please note that this may not be an exhaustive, updated list of CSF biomarkers determined

BLOOD

VARIABLE	TYPE	STUDY
Aβ42	longitudinal	
Aβ40	longitudinal	
APOE4	transversal	
GFAP	transversal	
NfL	transversal	
p-tau	transversal	
Fatty Acid	transversal	

Please note that this may not be an exhaustive, updated list of blood-based biomarkers determined

SAMPLES

VARIABLE	TYPE	STUDY
Plasma	Longitudinal	
Serum	Longitudinal	
Whole Blood	Longitudinal	
DNA	Longitudinal	
CSF	Longitudinal	
Citrate	Longitudinal	
PAXgene	Transversal	