University of Pittsburgh

Development, Characterization, and Validation of Genetically Engineered Marmoset Models of Neurodegenerative Disease

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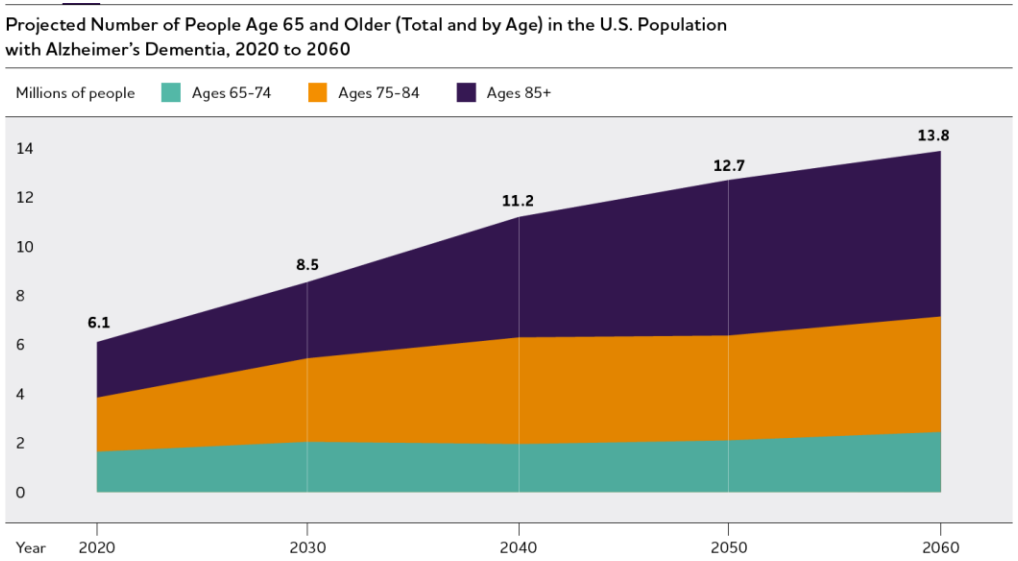
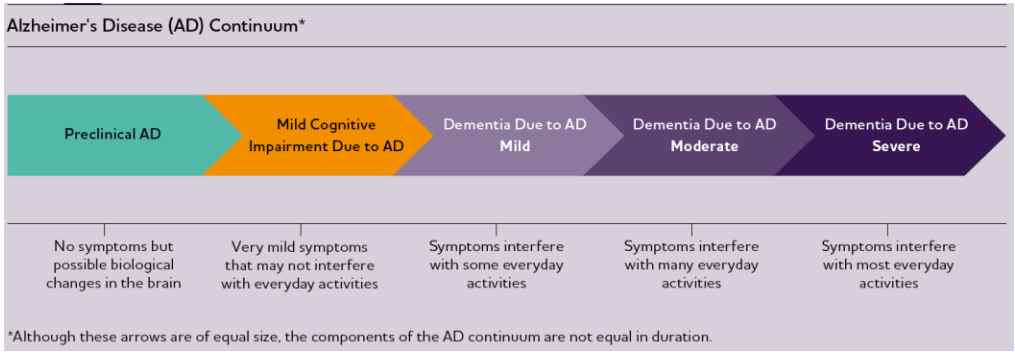
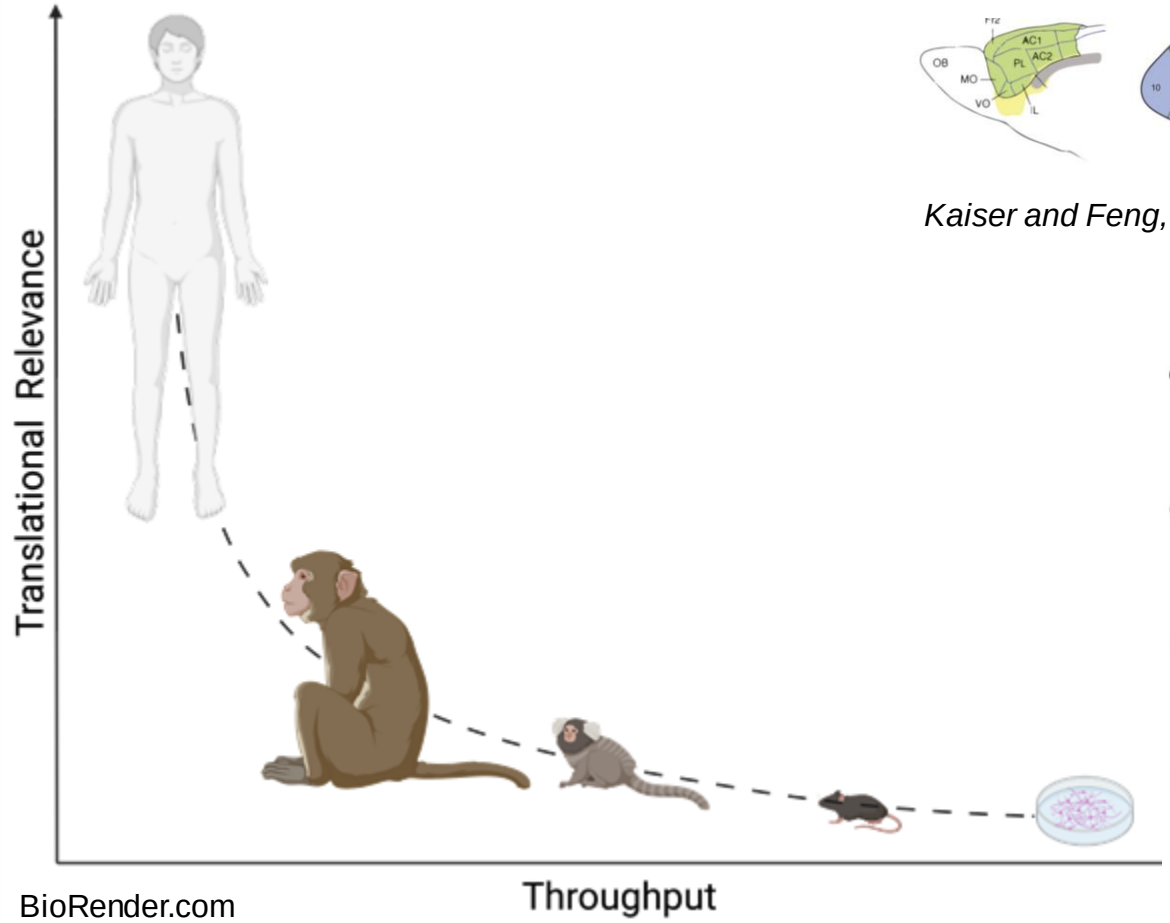
The Need for Better Animal Models of Neurological Disorders

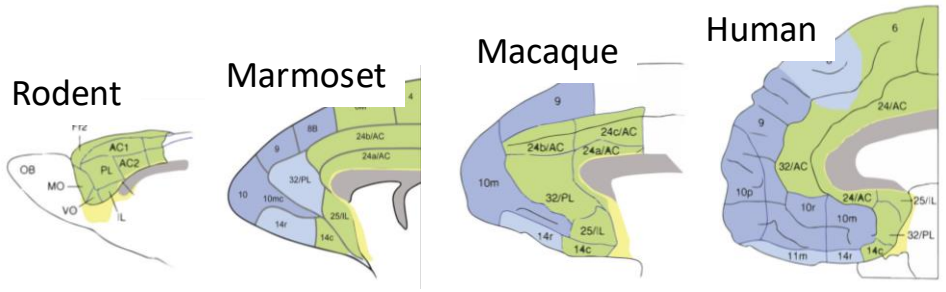
- Neurological disorders are a leading cause of disability worldwide, affecting ~100 million Americans (https://www.ninds.nih.gov)
- Case example: Alzheimer's Disease, the most common cause of dementia
  - 2022: 6.5 million Americans → 13.8 million by 2060
  - Current costs: $321 Billion
  - 99.6% failure rate of clinical trials
  - No disease-modifying drugs
- ***There's an urgent need for developing better animal models of AD***



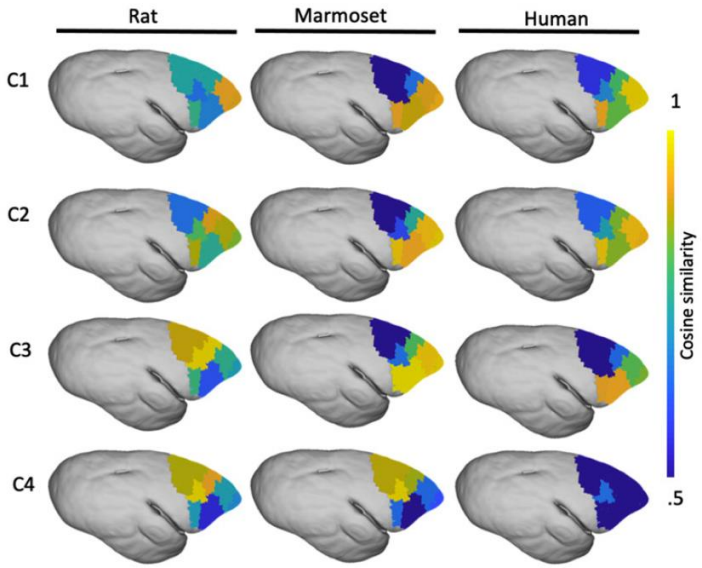


Nonhuman primates bridge the translational gap between human studies and rodent models of AD





Kaiser and Feng, Nat Med. (2015)

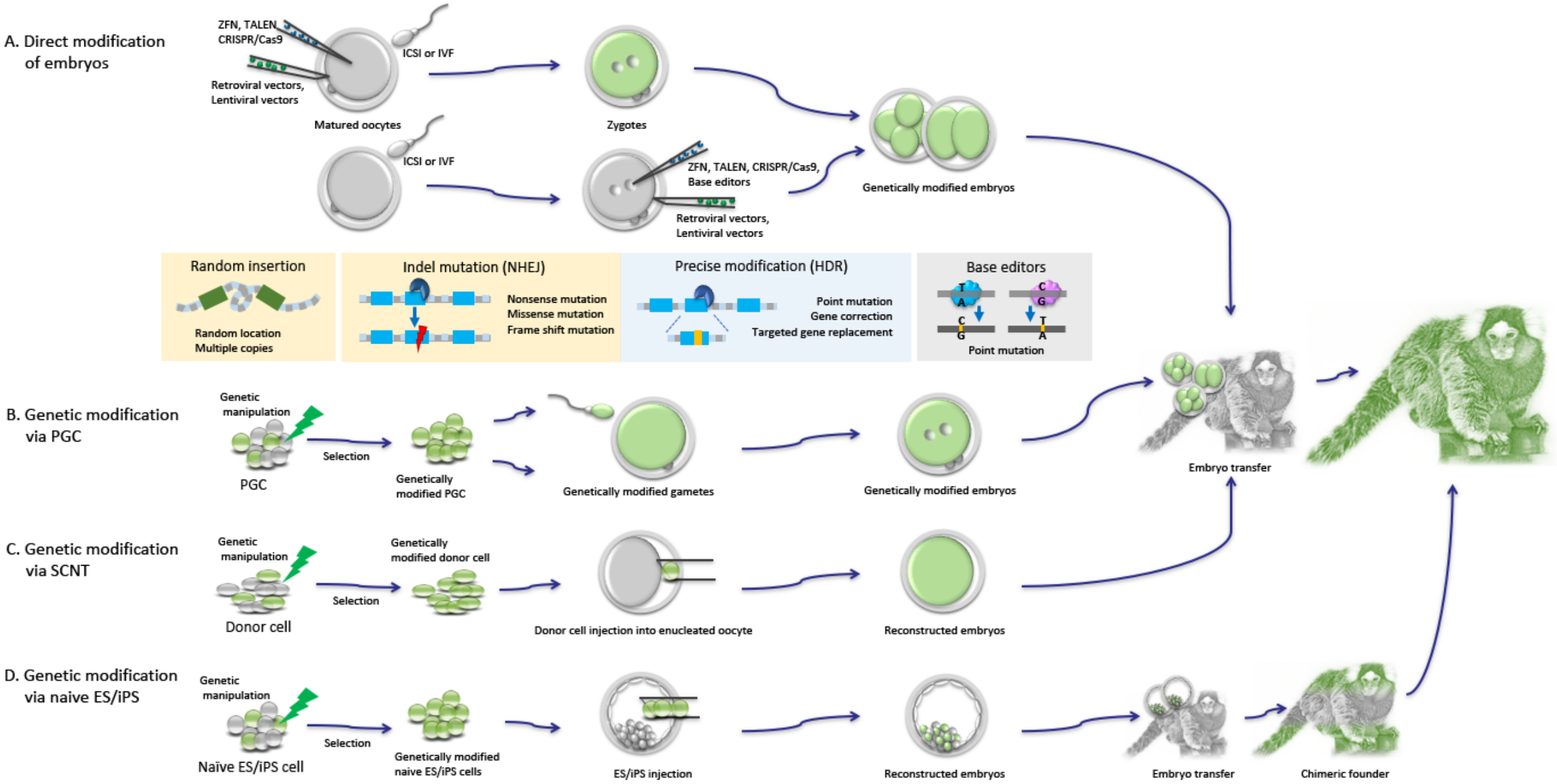


Schaeffer et al., PNAS (2020)

AD Gene	Marmoset-Human %	Mouse-Human %
ABCA7	90	78
APH1B	93	81, 84
APOE	91	71
BCAM	92	72
BCL3	96	80
BTNL2	86	62
CBLC	86	71
CD33	79	40, 50, 55
CLPTM1	99	95
CLU	93	76
CR1	15	8
EPHA1	91	88
IL34	88	67
LRCH4	93	85
MARK4	49	98
MS4A3	79	57
MS4A6A	75	47, 47 ,39
PICALM	95	98
PILRA	72	64
SORL1	98	93
TOMM40	75	93
TREM2	90	54

Improved marmoset-human sequence homology in AD-relevant loci

Gene-Editing Strategies



Transgenic NHP Models

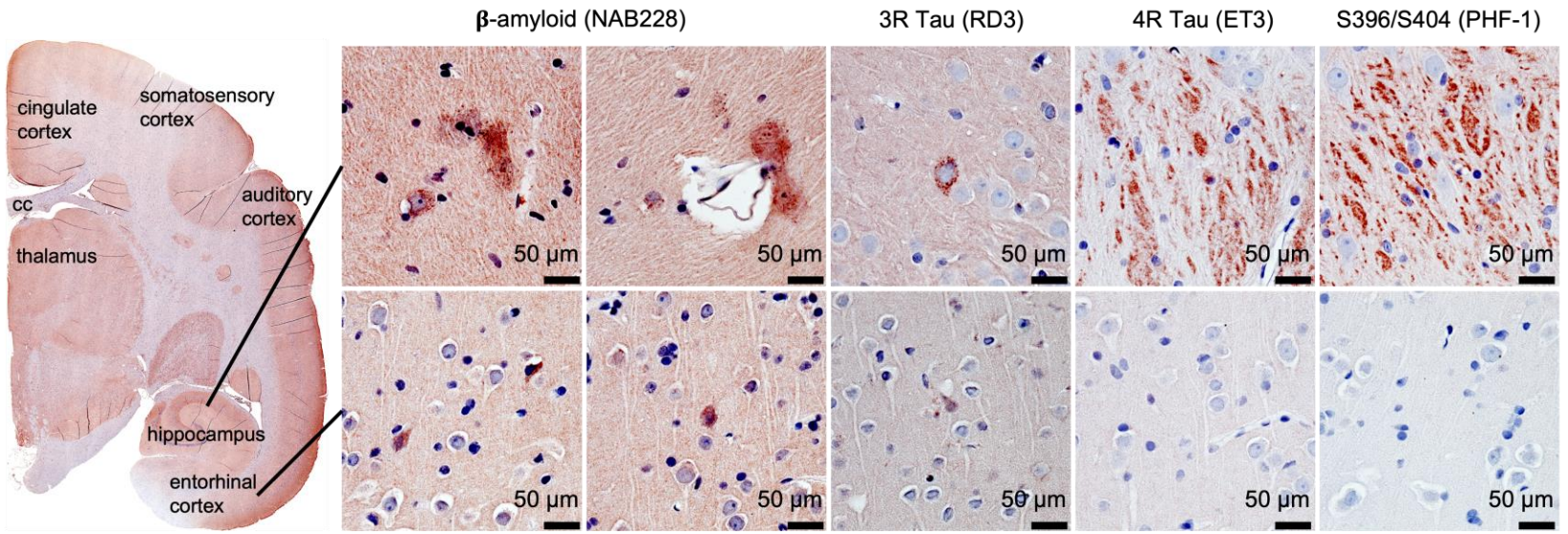
Transgene	Application	Species	Genetic manipulation	Outcome	Reference
GFP	Reporter gene	Rhesus macaque	Injection of retroviral vectors into oocytes followed by ICSI	- One live transgenic offspring - Germline transmission not verified	Chan et al., 2001
Human mutant huntingtin gene	Huntington's disease	Rhesus macaque	Injection of lentiviral vectors into oocytes followed by ICSI	- Five live transgenic offspring with clinical features of Huntington's disease: variable extents of motor dysfunction, chorea and dystonia - Germline transmission confirmed in a follow up study	Yang et al., 2008 Moran et al., 2015
GFP	Reporter gene	Common marmoset	Injection of lentiviral vectors into embryos	- Five live transgenic offspring - Germline transmission confirmed in offspring	Sasaki et al., 2009
EGFP	Reporter gene	Rhesus macaque	Injection of SIV vectors into embryos	- Two live transgenic offspring with mosaic expression of the transgene	Niu et al., 2010
Mutant a-synuclein (A53T)	Parkinson's disease	Rhesus macaque	Injection of lentiviral vectors into oocytes followed by ICSI	- Six live transgenic offspring expressing mutant a-synuclein - Subtle cognitive defects and anxiety like behaviors - Germline transmission not verified	Niu et al., 2015
Human MeCP2	Autism	Cynomolgus macaque	Injection of lentiviral vectors into oocytes followed by ICSI	- Autism-like disorder - Behavioral abnormalities: repetitive circular locomotion, reduction in social interactions, impairment of cognitive functions - Germline transmission confirmed using testicular tissue xenografting	Liu et al., 2016
GFP	Reporter gene	Cynomolgus macaque	Injection of lentiviral vectors into oocytes or embryos	- Lentivirus injection into oocytes before fertilization achieved homogenous expression of GFP throughout the entire body	Seita et al., 2016
GCaMP	Functional reporter gene	Common marmoset	Injection of lentiviral vectors into embryos	- Five live transgenic offspring - Stable and functional GCaMP expression in several different tissues - Germline transmission confirmed	Park et al., 2016
Human mutant ataxin 3-120Q	Polyglutamine diseases	Common marmoset	Injection of lentiviral vectors into embryos	- Seven live transgenic offspring expressing polyQ expanded ataxin 3 - Gradual progression of neurological symptoms and motor impairment - Germline transmission confirmed in offspring	Tomioka et al., 2017
Human mutant ataxin 3-120Q	Polyglutamine diseases	Common marmoset	Injection of lentiviral vectors into embryos	- Mutant human ataxin 3 gene expression controlled by tet-on system - Germline transmission confirmed in offspring	Tomioka et al., 2017

Gene-Edited NHP Models

Edited Gene	Application	Species	Genetic manipulation	Outcome	Reference
Nr0b1, Ppar-r, and Rag1	Proof of concept for CRISPR/Cas9	Cynomolgus macaque	Co-injection of Cas9 mRNA and multiple sgRNAs into single-cell embryos	- Simultaneous disruption of Ppar-r, and Rag1 genes - Germline transmission confirmed in follow-up study	Niu et al., 2014 Chen et al., 2015
MECP2	Autism	Rhesus / Cynomolgus macaque	Injection of circular TALEN plasmid into single-cell embryos	- One live MECP2 mutant offspring - Male embryonic lethality of MeCP2 mutation shown	Liu et al., 2014
MECP2	Autism	Cynomolgus macaque	Injection of TALEN mRNA into single-cell embryos	- One MECP2 mutant male neonate that did not survive	Liu et al., 2014
Nr0b1, Ppar-r, and Rag1	Adrenal hypoplasia congenita (AHC) Hypogonadotropic hypogonadism (HH)	Cynomolgus macaque	Co-injection of Cas9 mRNA and multiple sgRNAs into single-cell embryos	- Cas9 targeted Nr0b1-deficient male monkey fetus displayed defects in adrenal gland development and abnormal testis morphology	Kang et al., 2015
p53	Proof of concept for CRISPR/Cas9	Cynomolgus macaque	Co-injection of Cas9 mRNA and sgRNA into single-cell embryos	- Single-step live p53 biallelic mutant monkeys	Wan et al., 2015
dystrophin	Duchenne muscular dystrophy (DMD)	Rhesus macaque	Co-injection of Cas9 mRNA and sgRNA into single-cell embryos	- Nine live offspring with multiple mutations of dystrophin gene - Muscle changes similar to early stage DMD patients	Chen et al., 2015
MCPH1	Microcephaly	Cynomolgus macaque	Injection of TALEN mRNA into single-cell embryos	- One live monkey carrying biallelic MCPH1 mutation - Microcephaly, hypoplasia of the corpus callosum, upper limb spasticity	Ke et al., 2016
IL2RG	Severe Combined Immunodeficiency	Common marmoset	Injection of ZFN or TALEN mRNA into single-cell embryos	- Nine neonates exhibiting mutations in the IL2RG gene - Immunodeficient phenotypes included lack of thymus, reduced T-cell and natural killer cell count in cord blood samples - Germline transmission confirmed in germ cells	Sato et al., 2016
Prrt2	Proof of concept for CRISPR/Cas9	Cynomolgus macaque	Co-injection of Cas9 mRNA and sgRNA into single-cell embryos	- Complete Prrt2 knockout monkey - Injection of Cas9 mRNA with multiple adjacent sgRNAs that target only a single key exon of the target gene	Zuo et al., 2017
MECP2	Autism	Rhesus / Cynomolgus macaque	Injection of circular TALEN plasmid into single-cell embryos	- Four additional live MECP2 mutant offspring - Complex behavioral abnormalities, including fragmented sleep, increased stereotypic behavior and reduced social interaction	Chen et al., 2017
BMAL1	Circadian rhythm disorders	Cynomolgus macaque	Co-injection of Cas9 mRNA and multiple sgRNAs into single-cell embryos	- five macaque monkeys with BMAL1 mutations in both alleles cloned without mosaicism, with nuclear genes identical to that of the fibroblast donor monkey.	Liu et al., 2019

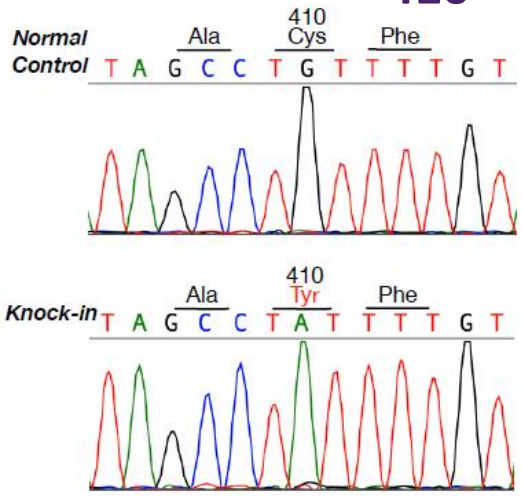
Marmosets as a Model for Alzheimer's Disease

- Genetically diverse and small: 300-600 grams adult body weight
- Life span: 12+ years; age at maturity: 18 months
- Gestation: 144 days; multiple offspring every 6 months
- Spontaneous presentation of Aβ; identical sequence homology to human
- Sophisticated behavioral repertoire
  - Social behavior, diurnal activity
  - Age-dependent changes in motor, sensorimotor, hearing, vision, cognitive function
- Amenable to genetic engineering
  - **PSEN1-ΔE9** marmosets (Sasaguri et al AAIC 2020, 2021; Sato et al BioRxiv 2020)



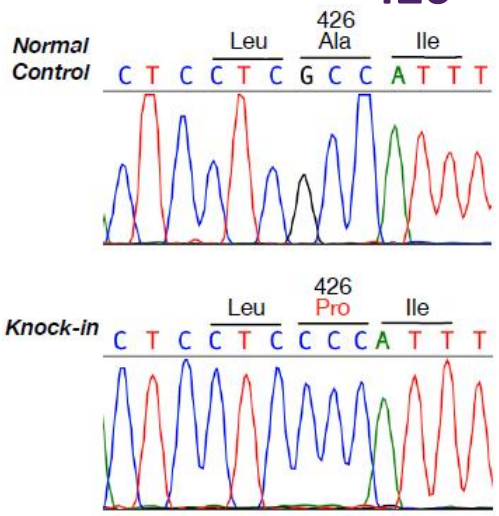
CRISPR Gene Editing of Presenilin 1 Mutations in Marmosets

F0: PSEN1-C_{410}Y



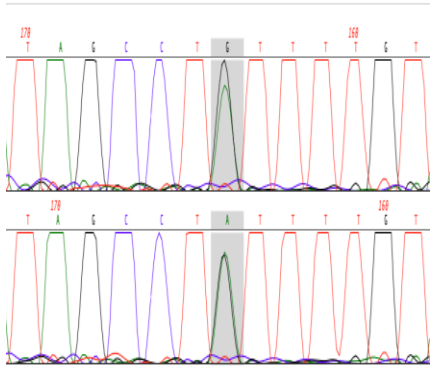
N=3 males, N=2 females

PSEN1-A_{426}P



N=1 male, N=2 females

F1:



N=1 male, N=1 female

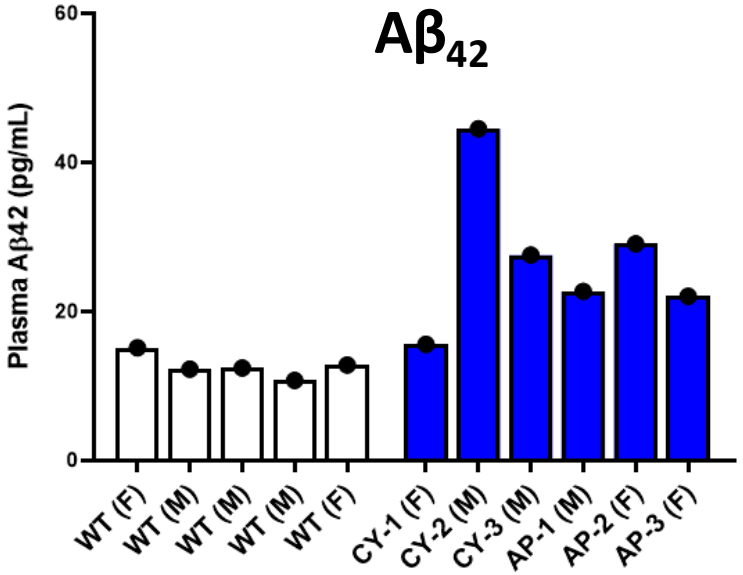
ID	Sex	PSEN1 Mutation	Genotype	Mosaic
M27-20	F	C410Y	KI/D5/KID1/WT	Yes
M28-20	M	C410Y	KI/KI	No
M31-20	M	C410Y	KI/KI	No
M32-20	M	C410Y	KI/+1	Yes
M154-20	F	C410Y	KI/D1/+2/?	Yes
M155-20	-	C410Y	WT/WT	-
M215-21	M	C410Y	D2/D5+14	No
M216-21	F	C410Y	Multiple Indels	Yes
M217-21	M	C410Y	KI/D2	No
-	-	A426P	KI/KID2	No
-	-	A426P	KI/KID1	No
-	-	A426P	KI/KID1	No
M101-20	M	A426P	KI/D20	No
M186-20	F	A426P	KI/Indel	No
M2-21	F	A426P	KI/D7	No

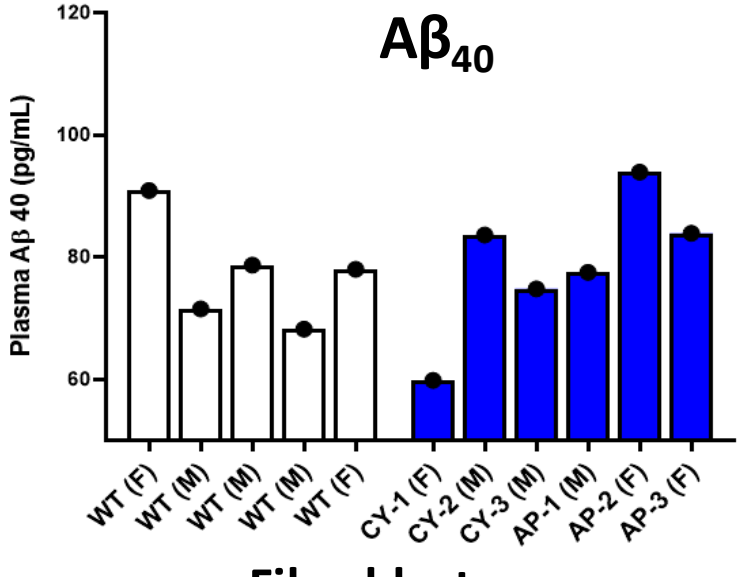
Table 1: Summary of PSEN1 founder marmosets created with CRISPR gene editing. KI=knockin; Δ=deletion; +=insertion; #=number of base pairs (bp); sub=substitution; indel=65bp imperfect duplication plus 14bp insertion.

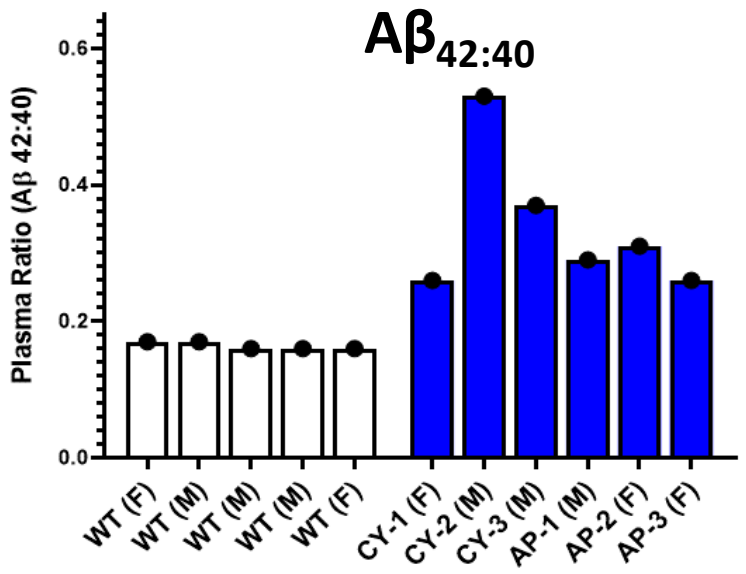
- Low incidence of mosaicism
- Germline transmission confirmed in two offspring

Elevated Levels of Aβ in Plasma and Fibroblasts of PSEN1 Marmosets

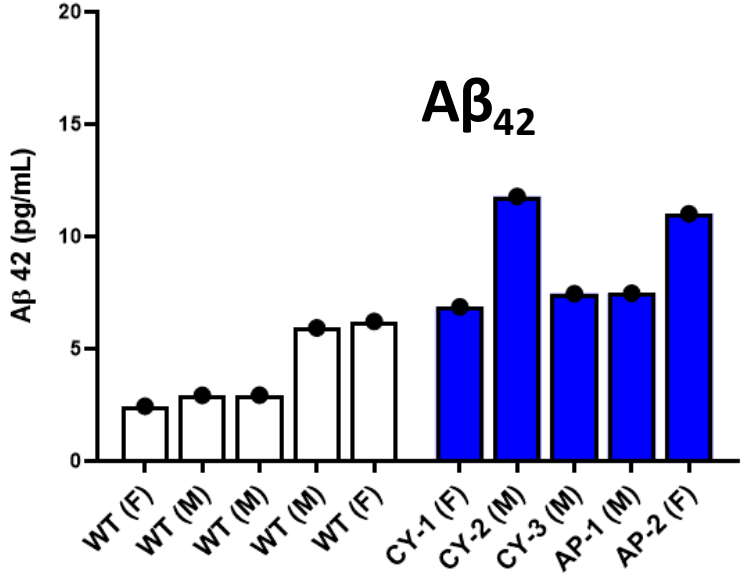
Plasma

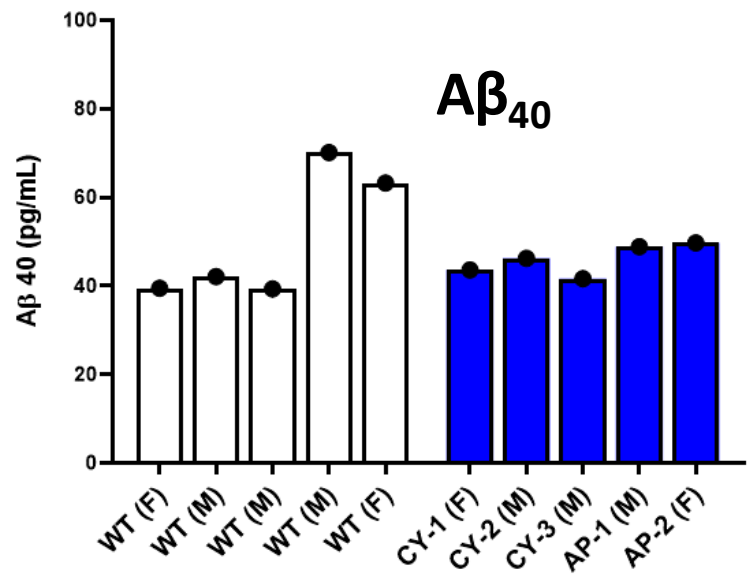


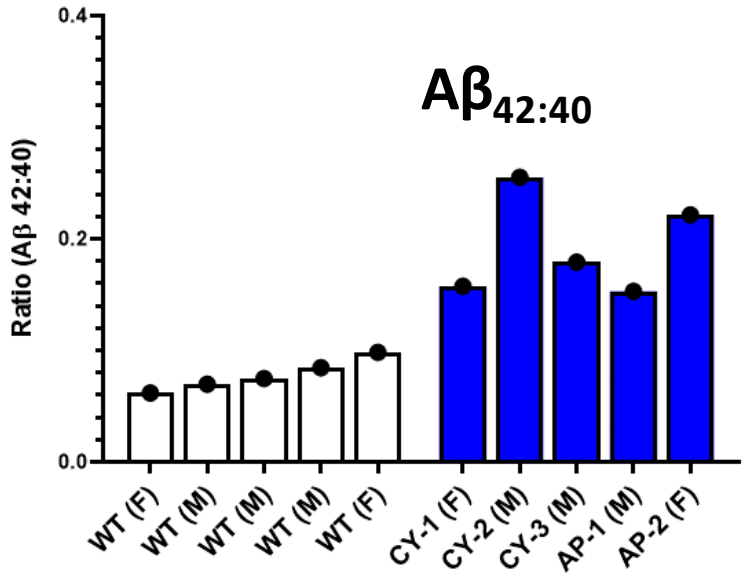




Fibroblasts

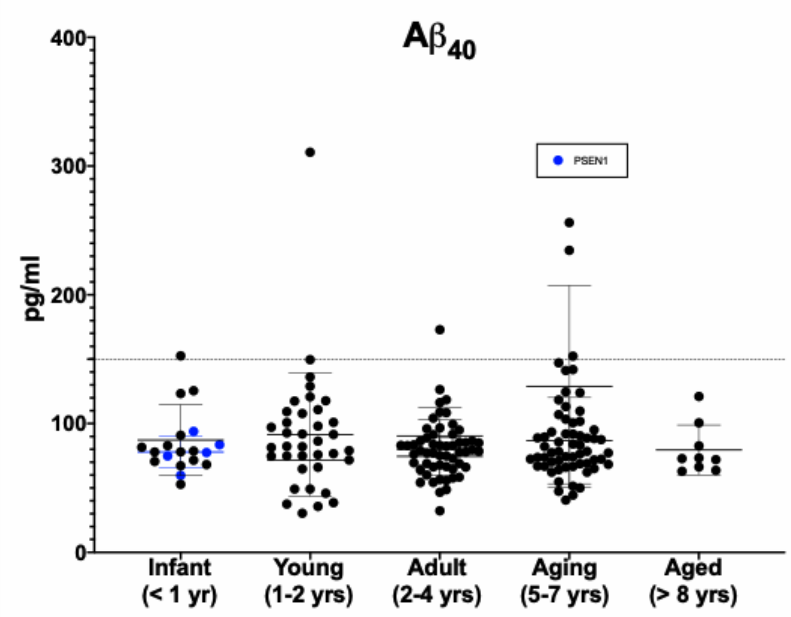


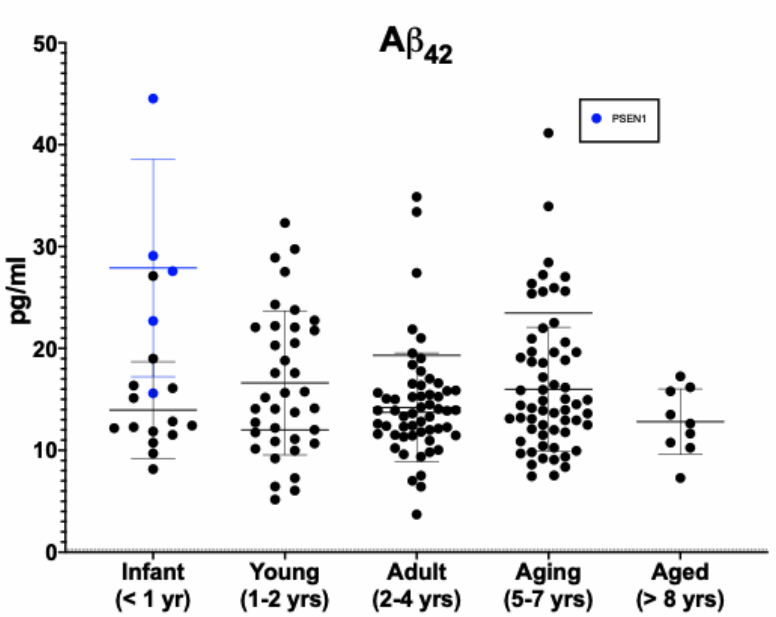


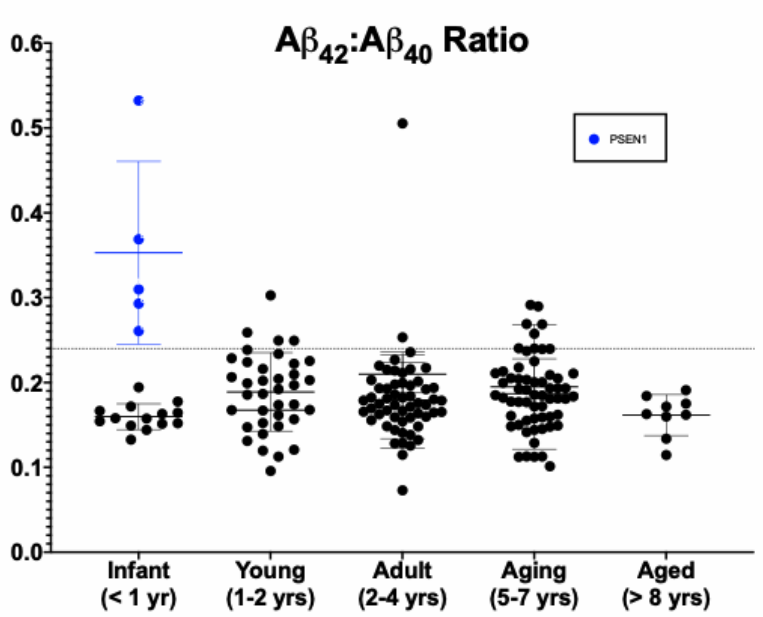


MesoScale Discovery 4G8 Aβ ELISA

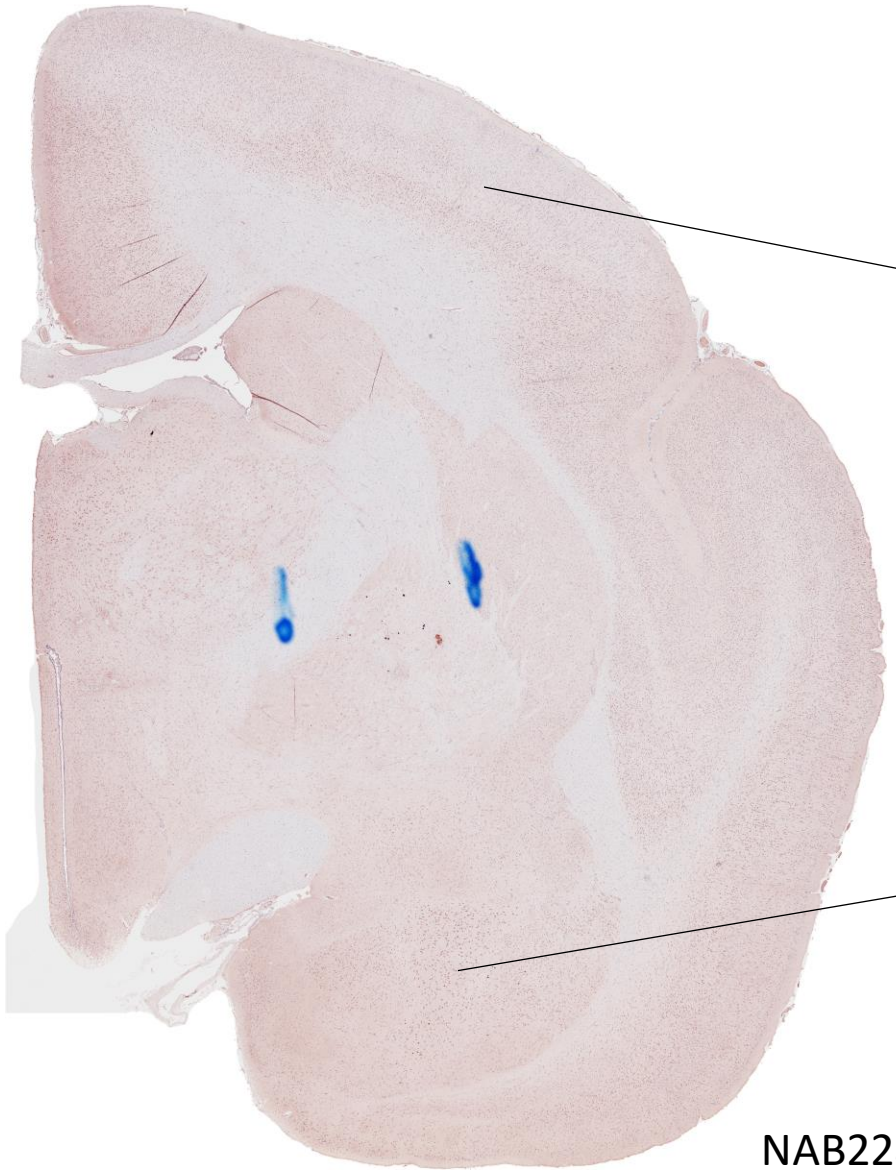
Comparison of Plasma Aβ of PSEN1 KI marmosets relative to normal aging controls



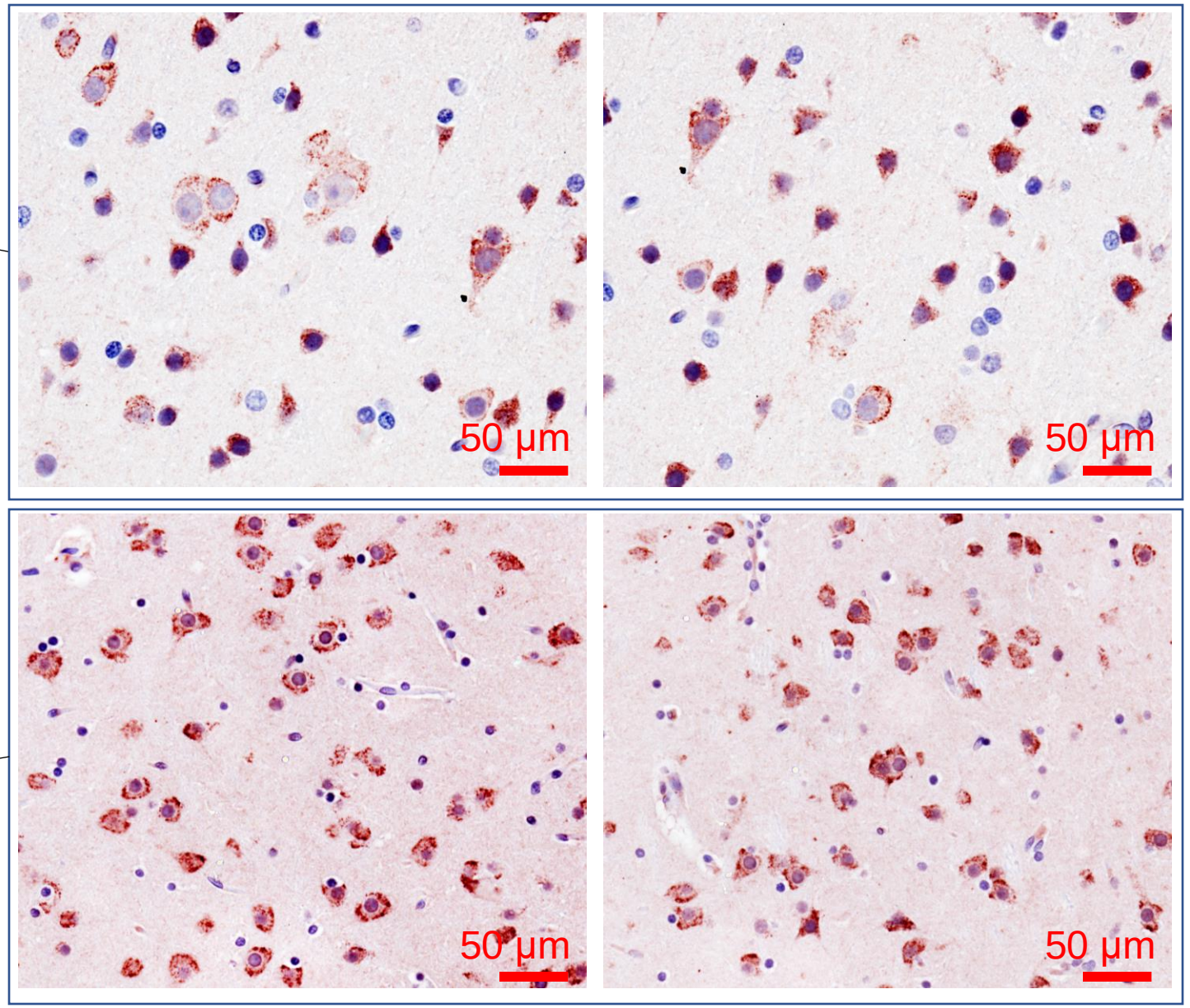




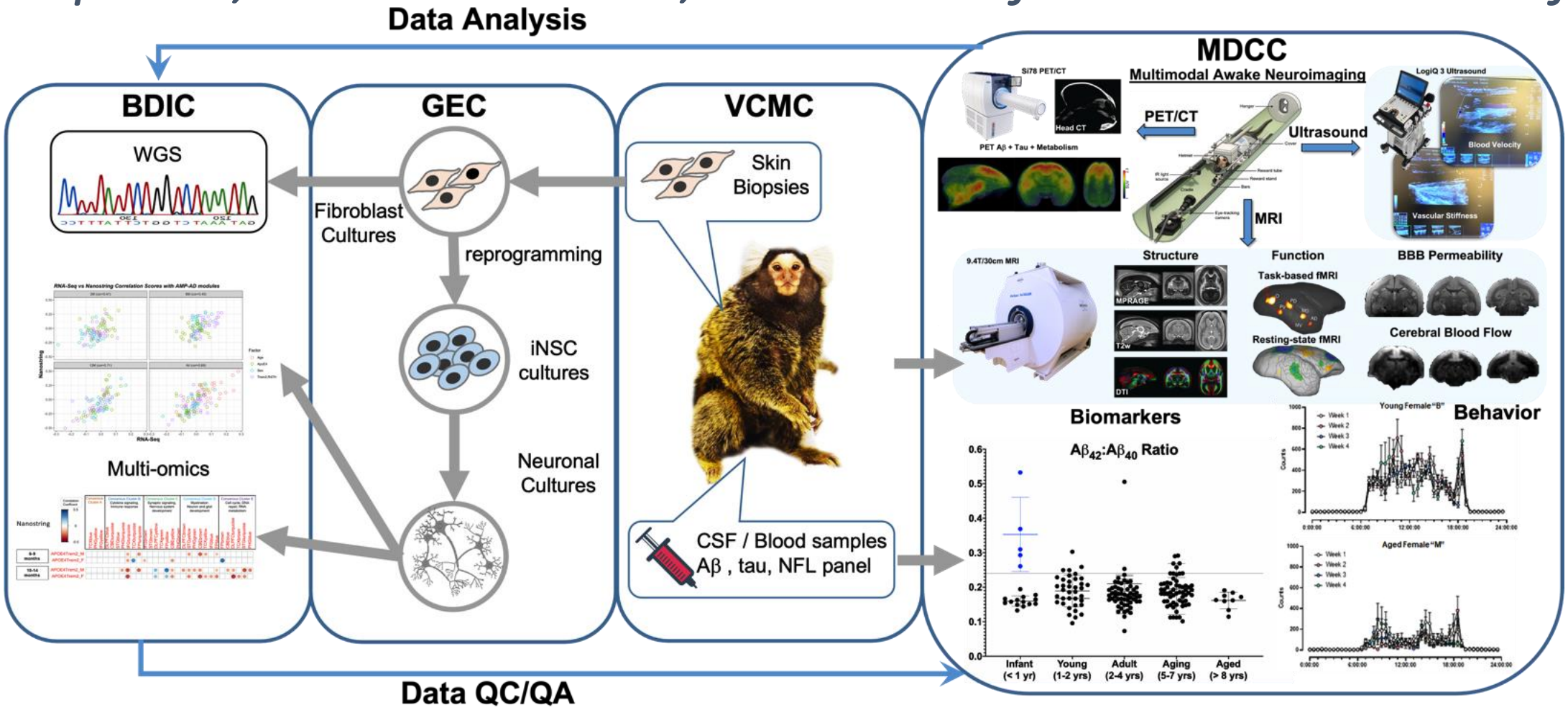
Presence of Aβ in the brain of a homozygous PSEN1 marmoset



NAB228



Development, Characterization, Validation of Marmoset Models of AD

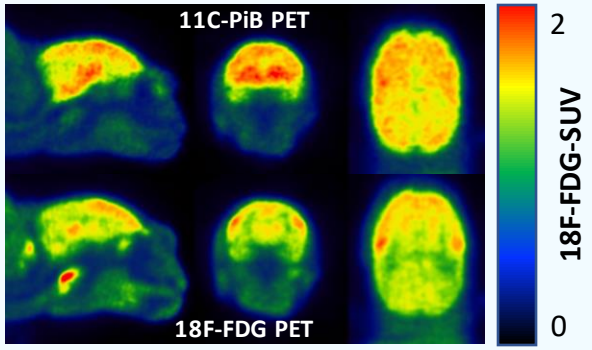


- Longitudinal assessment of marmosets using non-invasive neuroimaging, behavioral and cognitive assessment, and fluid-based biomarkers, in line with clinical assessment of AD patients
- Use of skin-derived fibroblasts and induced neuronal cultures for WGS, transcriptomics, and proteomics

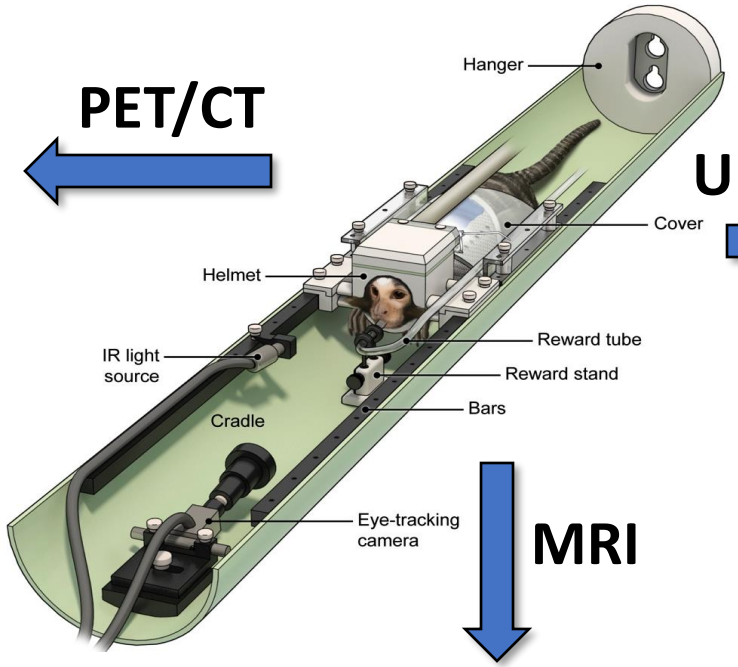
Multimodal Neuroimaging of Awake Marmosets

Si78 PET/CT

PET Aβ + Metabolism



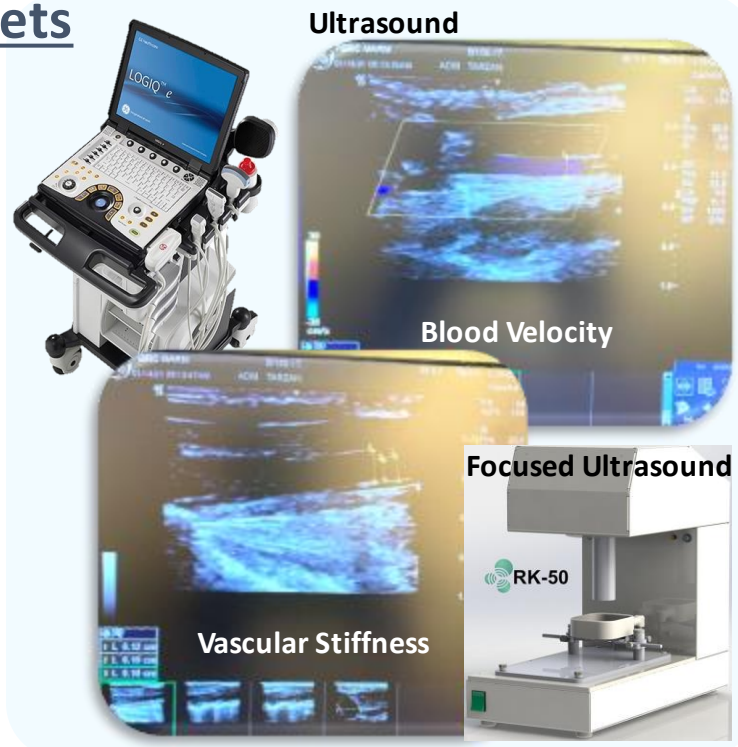
PET/CT



Ultrasound

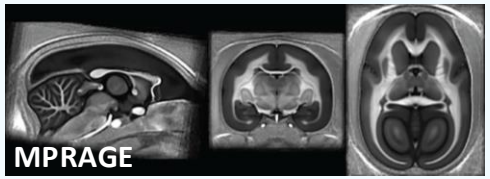
MRI

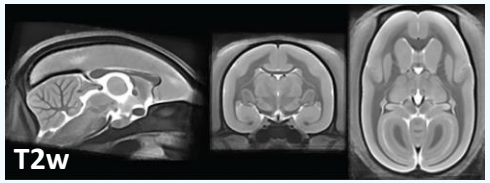
Ultrasound

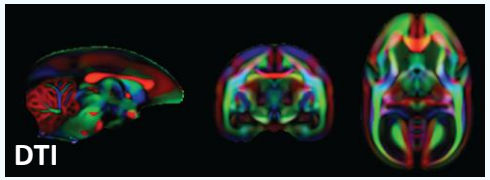


9.4T/30cm MRI

Structure

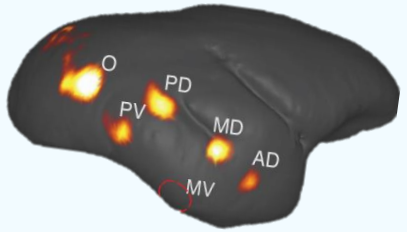




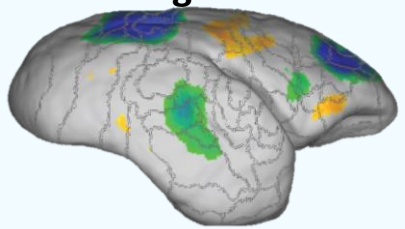


Function

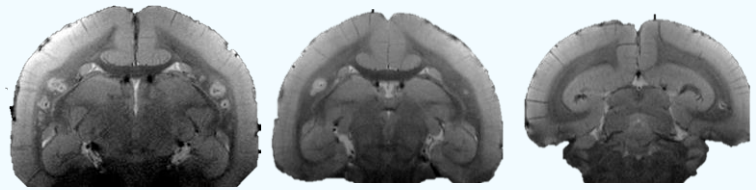
Task-based fMRI



Resting-state fMRI

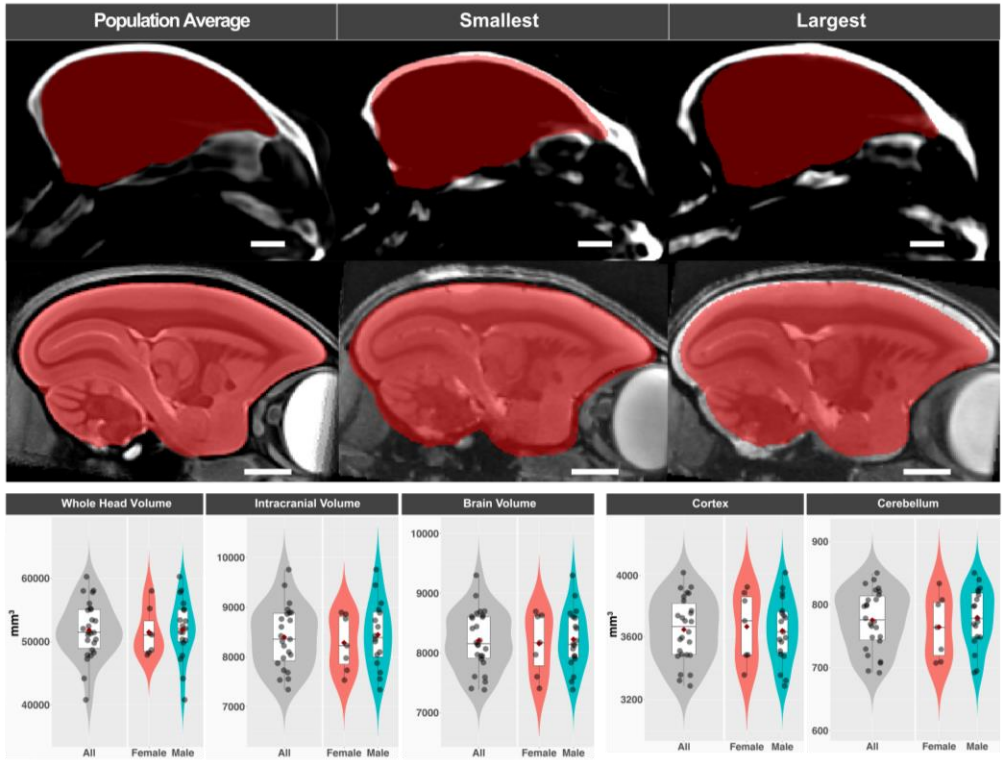


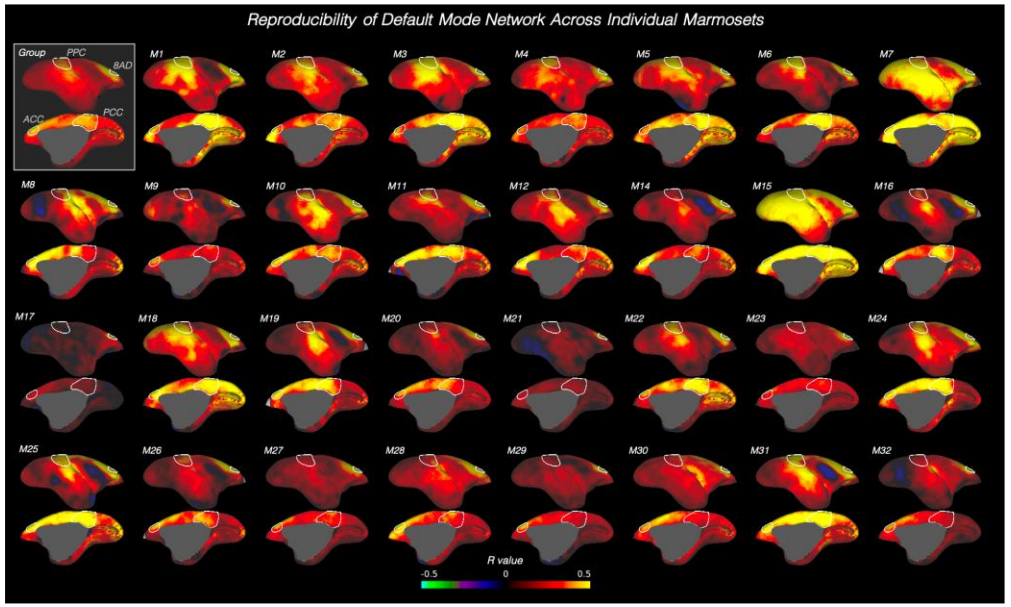
BBB Permeability

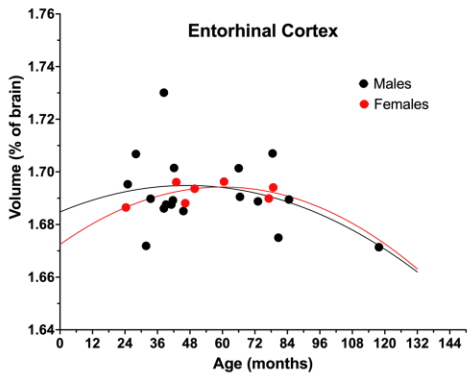


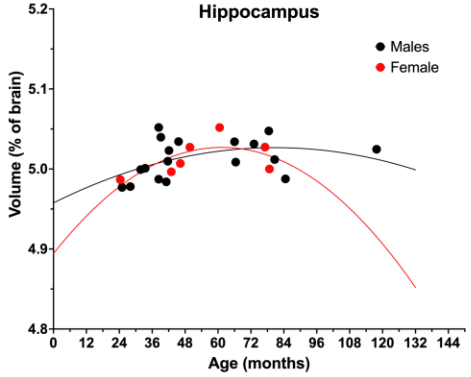
Cerebral Blood Flow

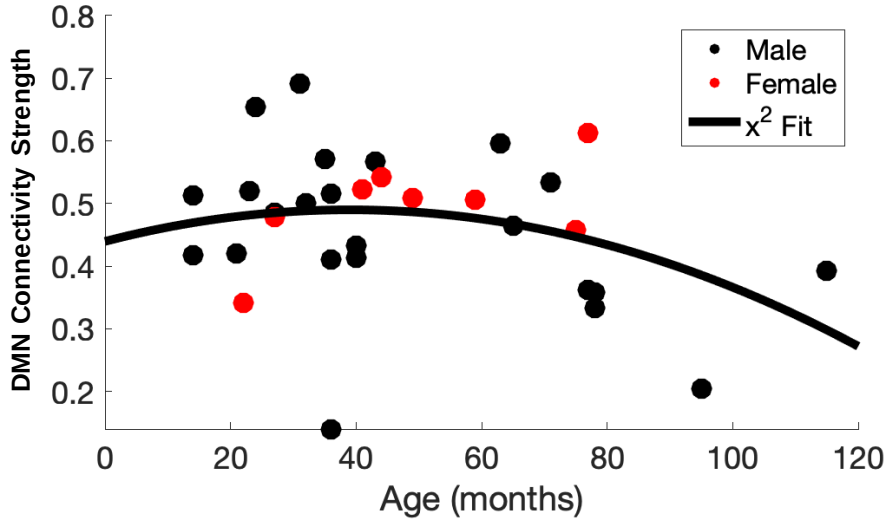
Anatomical and Functional Age Trajectories of the Marmoset Brain



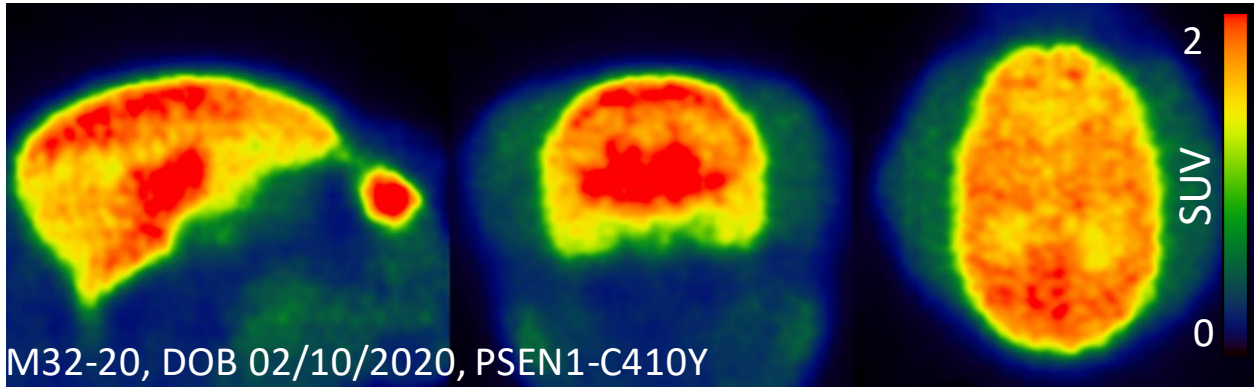


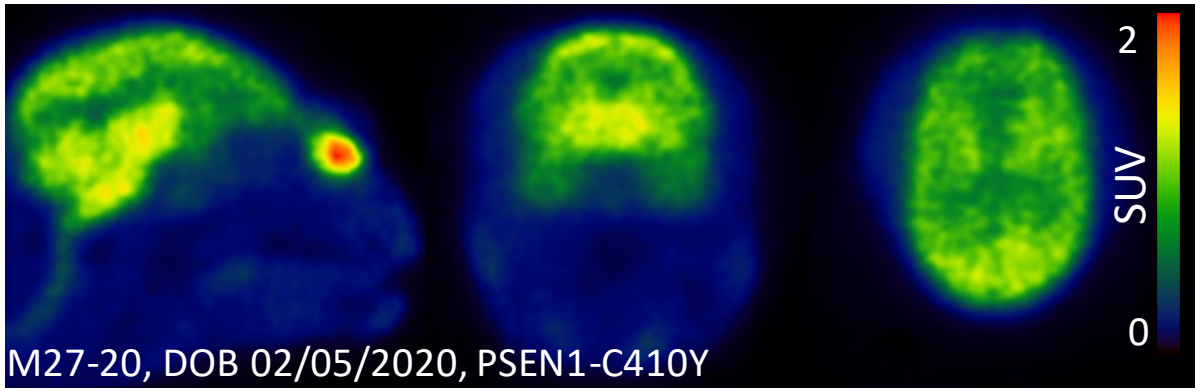


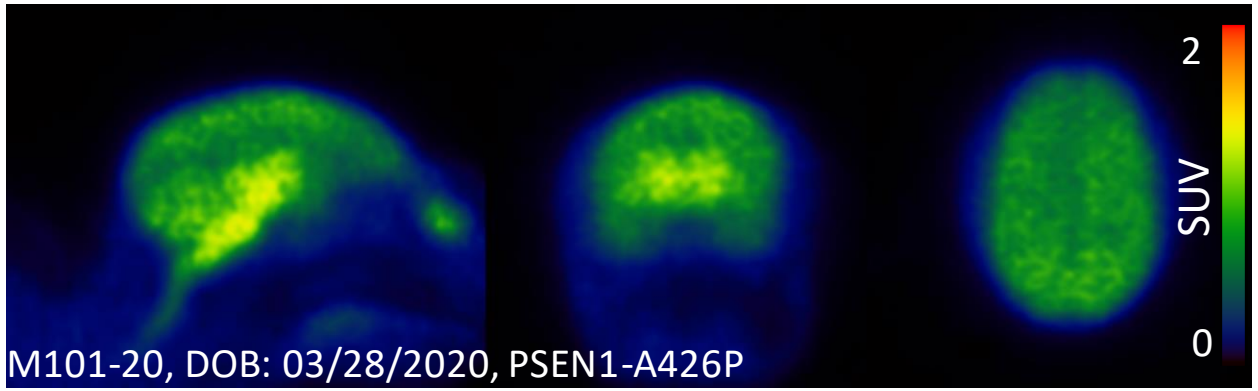


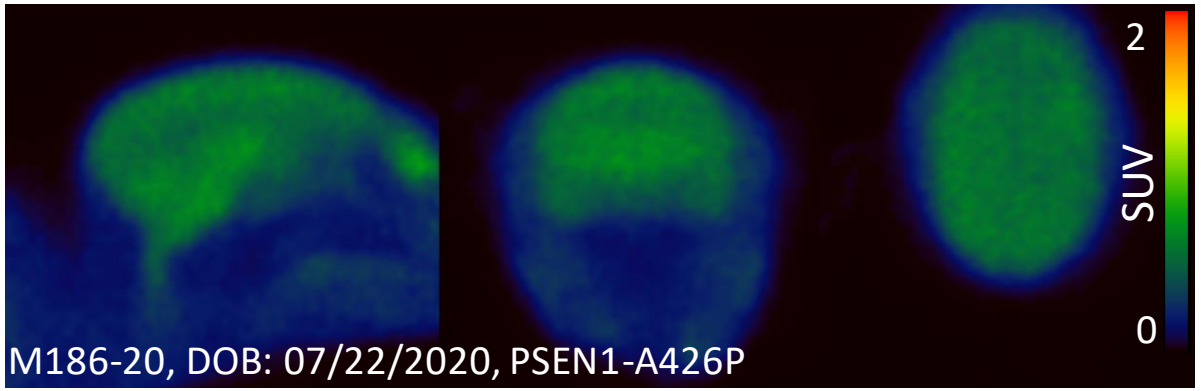


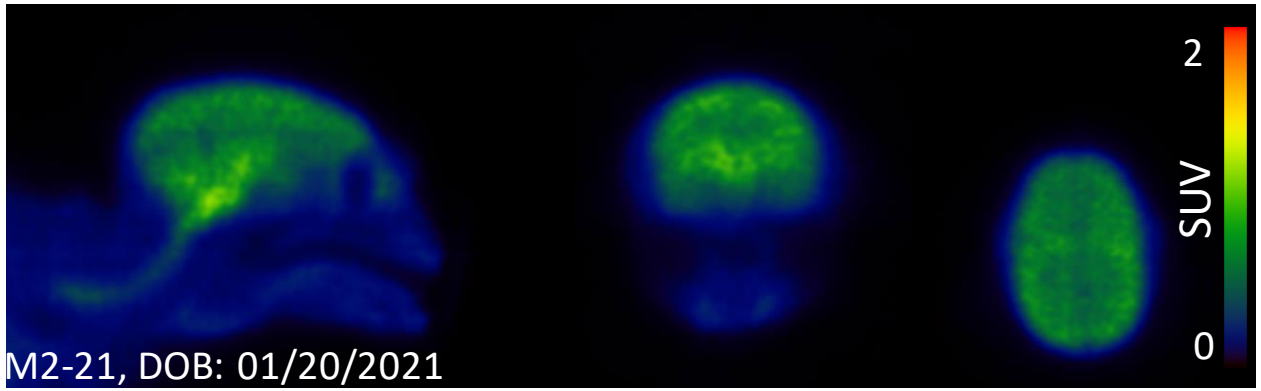
^{11}C-PiB PET-Amyloid in PSEN 1 Marmosets

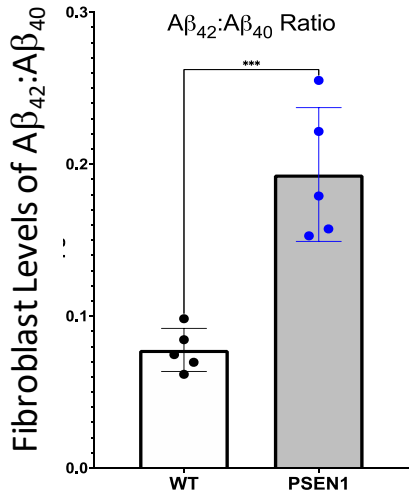












Conclusions

- Marmosets have many advantages as a model of aging and aging-related diseases
- We successfully generated gene-edited marmosets containing point mutations in PSEN1 that lead to early-onset, familial Alzheimer's disease in humans.
- **Germline transmission from PSEN1 mutants!**
- The time course, signs and pathology should mirror the natural onset and progression of the more common sporadic form of AD.
- PSEN1 KI marmosets can be studied from birth throughout lifespan via longitudinal multimodal measures (neuroimaging, behavior, biomarkers) in line with clinical disease staging and may help identify the earliest primate-specific events that are the root cause of disease.

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