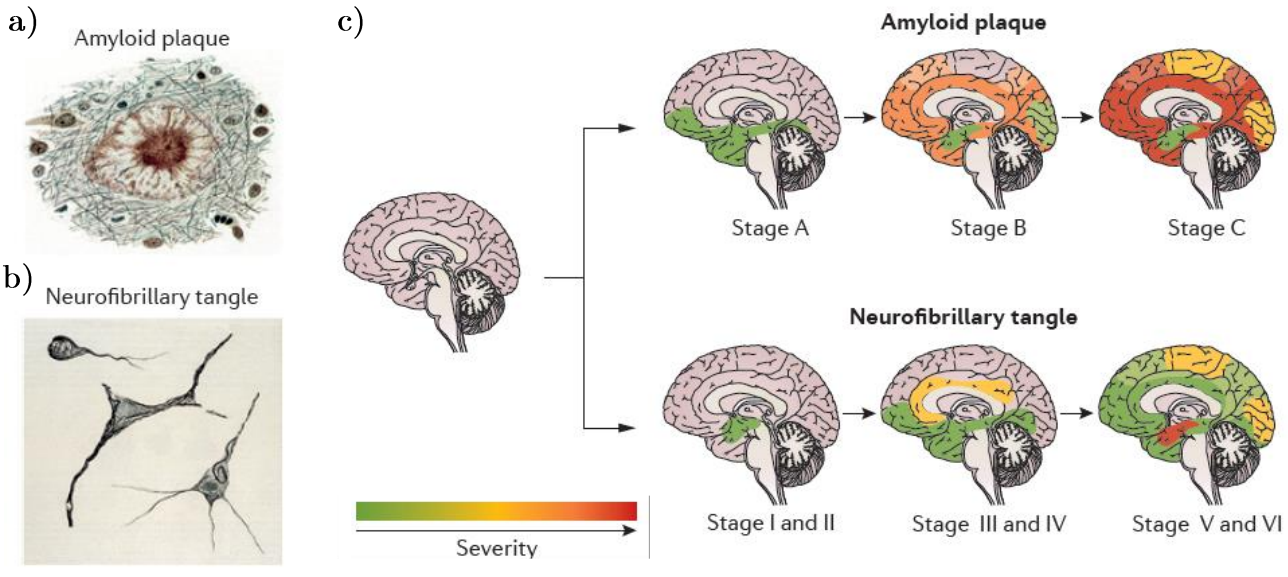
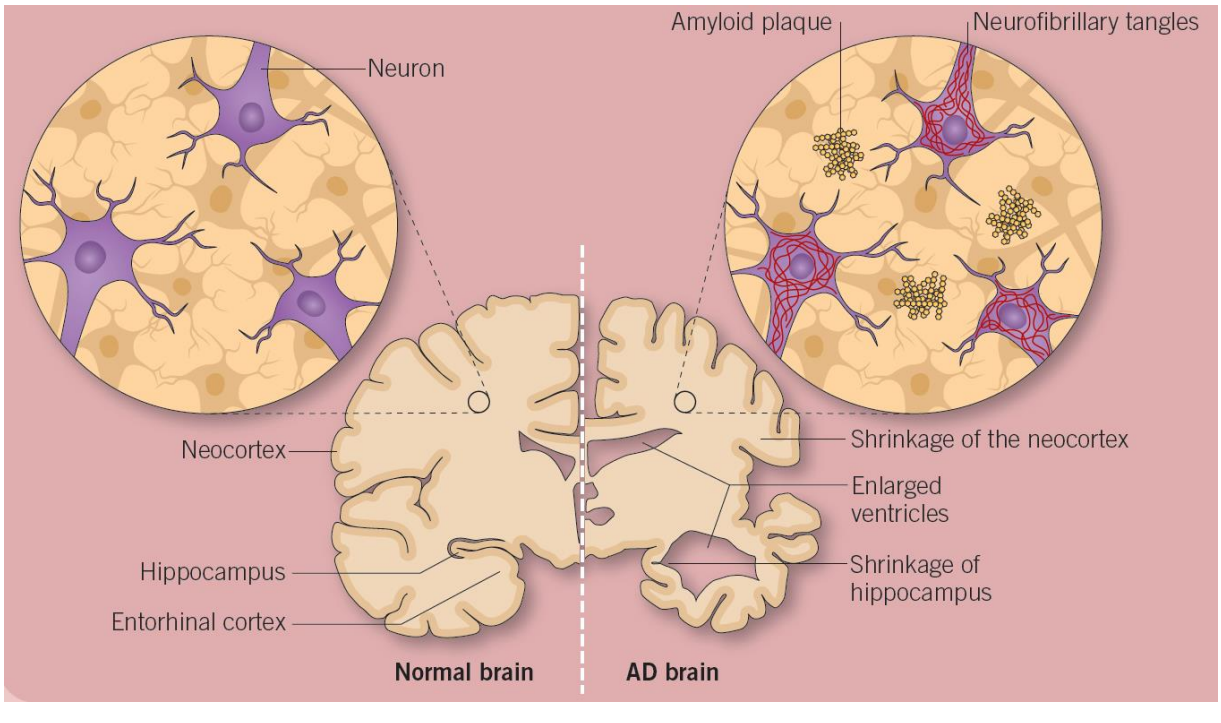
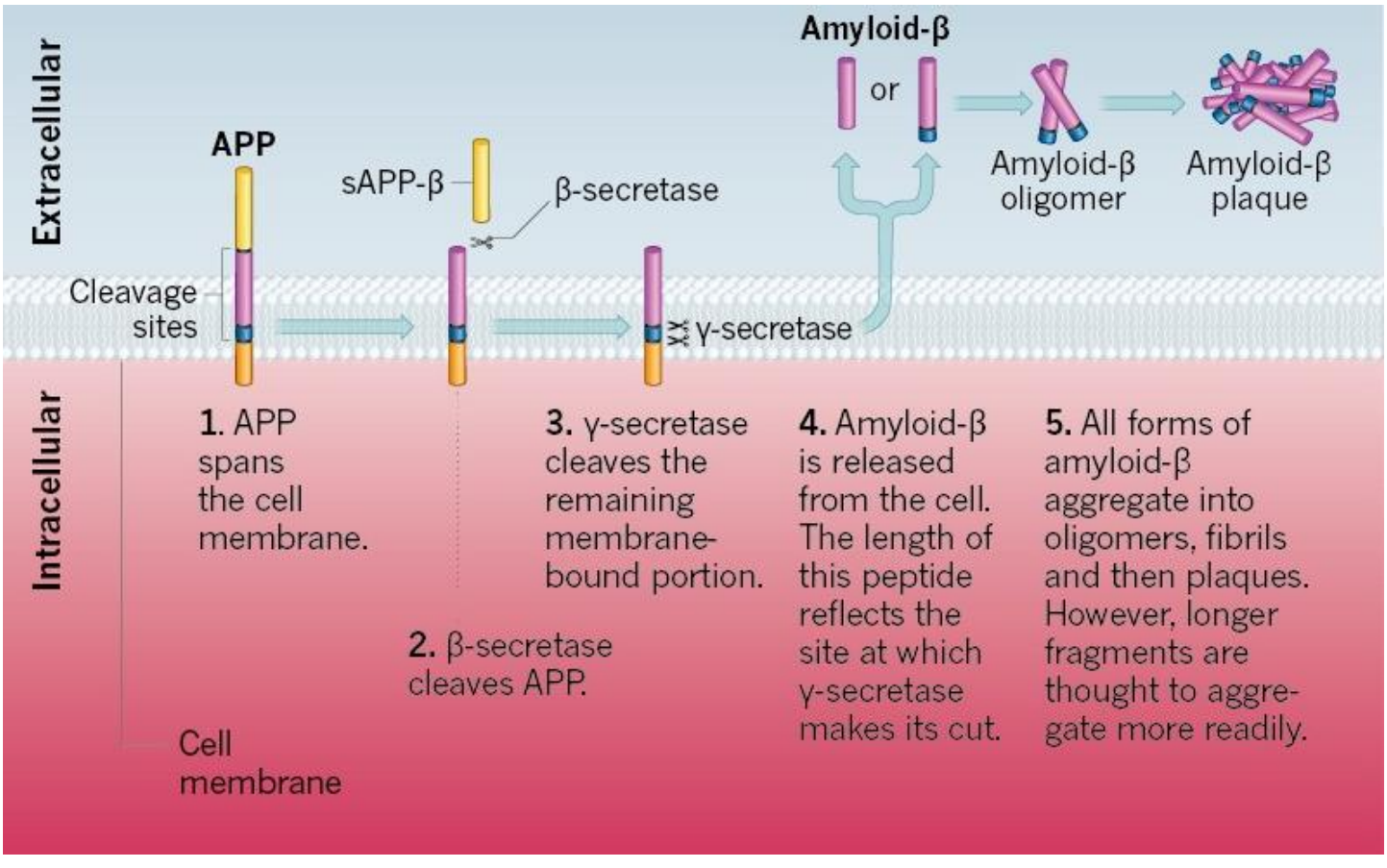
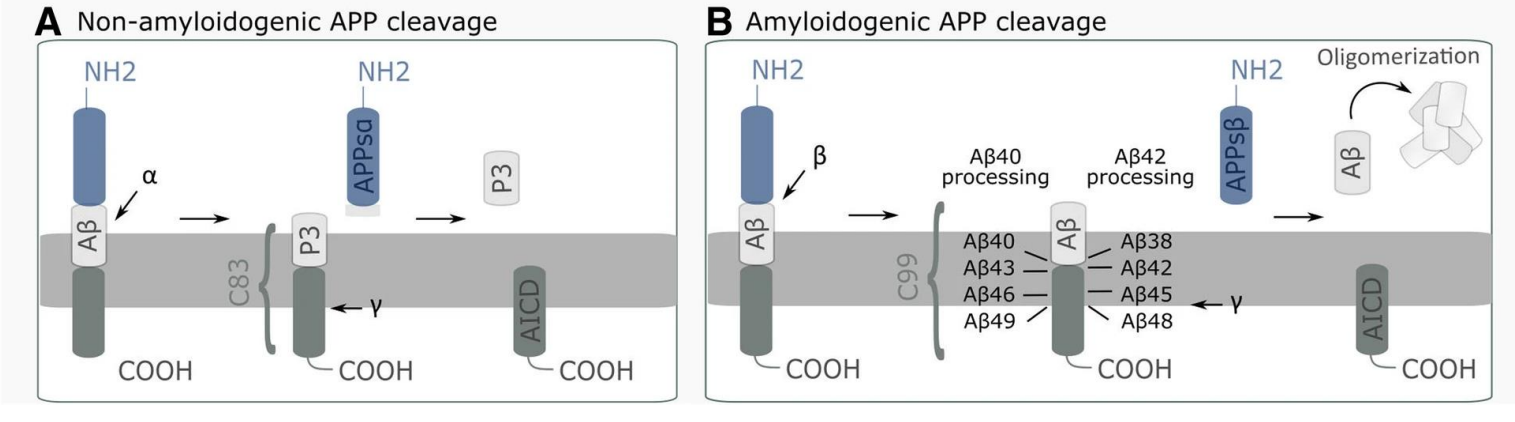
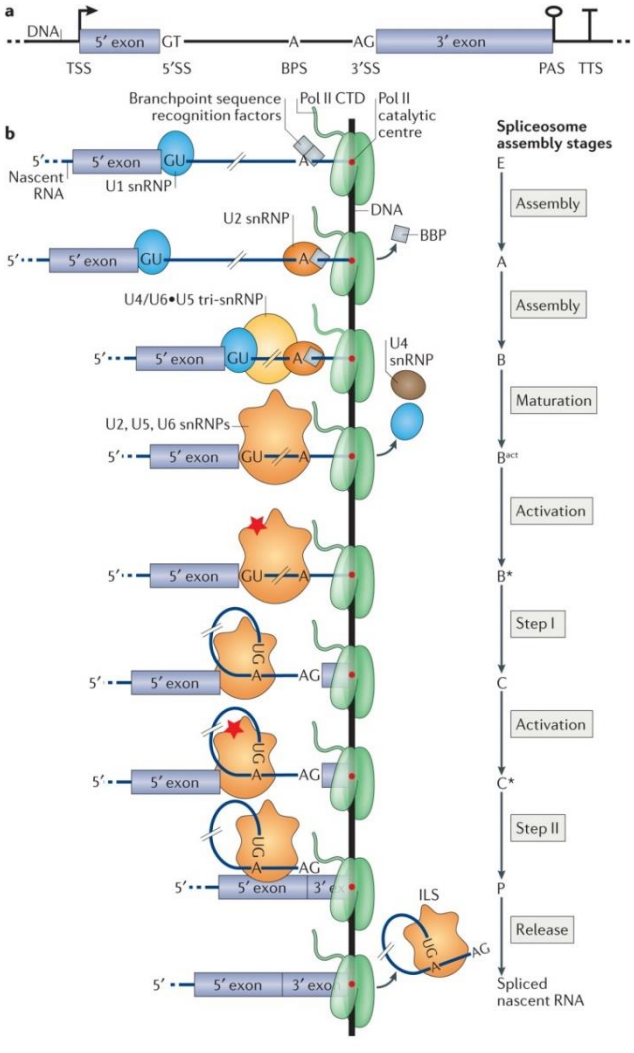
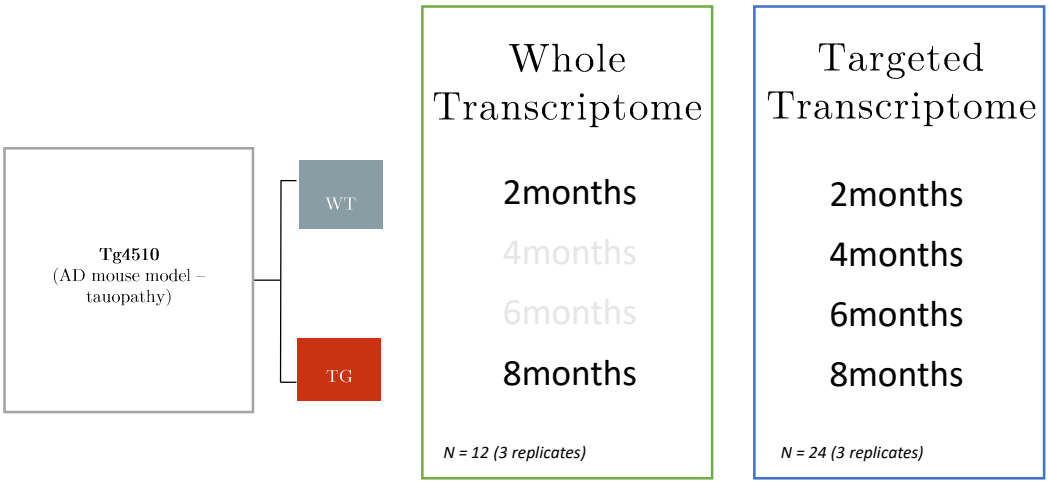

a)
Amyloid plaque
b)
Neurofibrillary tangle
c)
Amyloid plaque
Stage A
Stage B
Stage C
Neurofibrillary tangle
Stage I and II
Stage III and IV
Stage V and VI
Severity

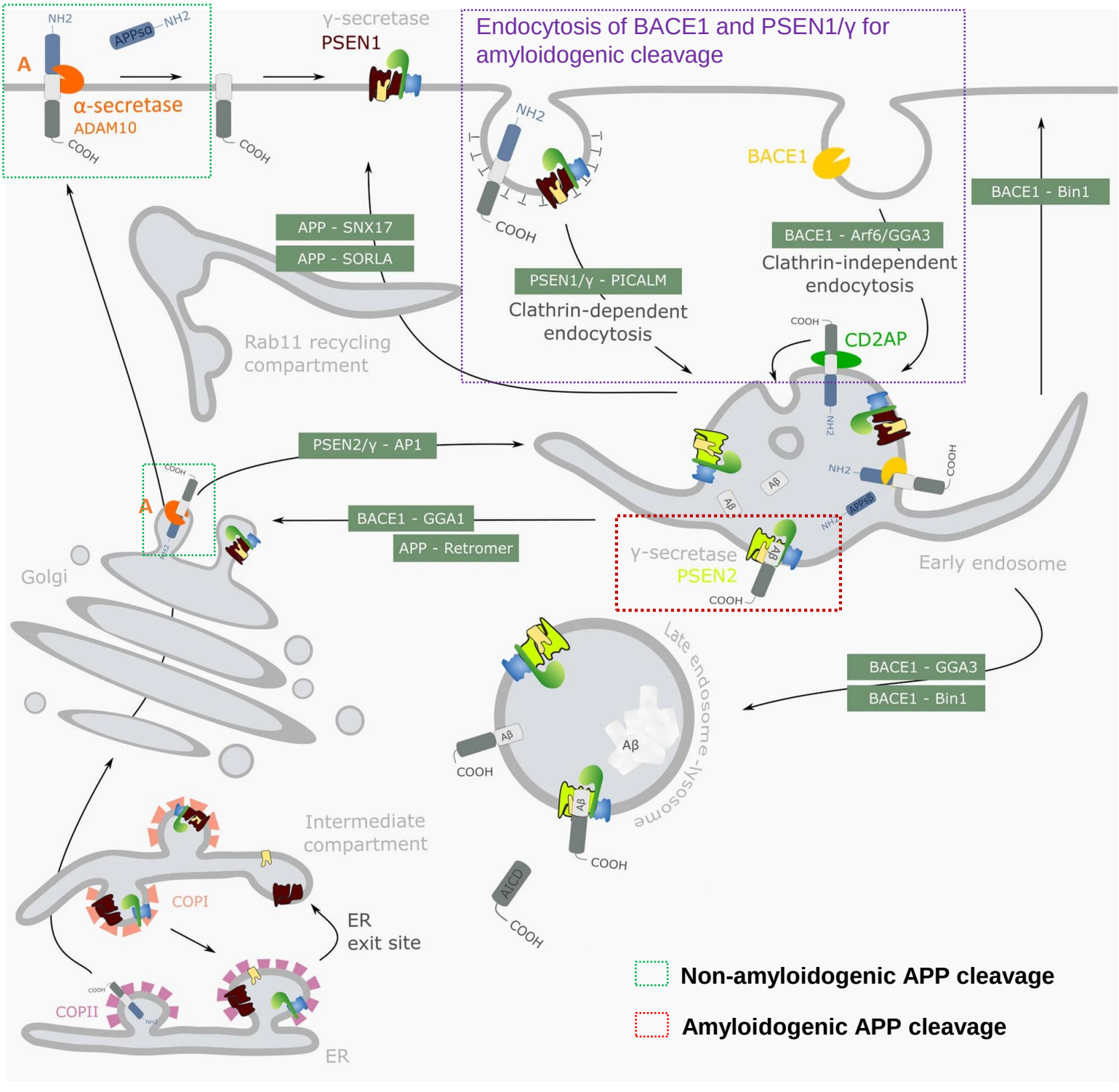

Amyloid plaque
Neurofibrillary tangles
Neuron
Neocortex
Hippocampus
Entorhinal cortex
Shrinkage of the neocortex
Enlarged ventricles
Shrinkage of hippocampus
Normal brain
AD brain

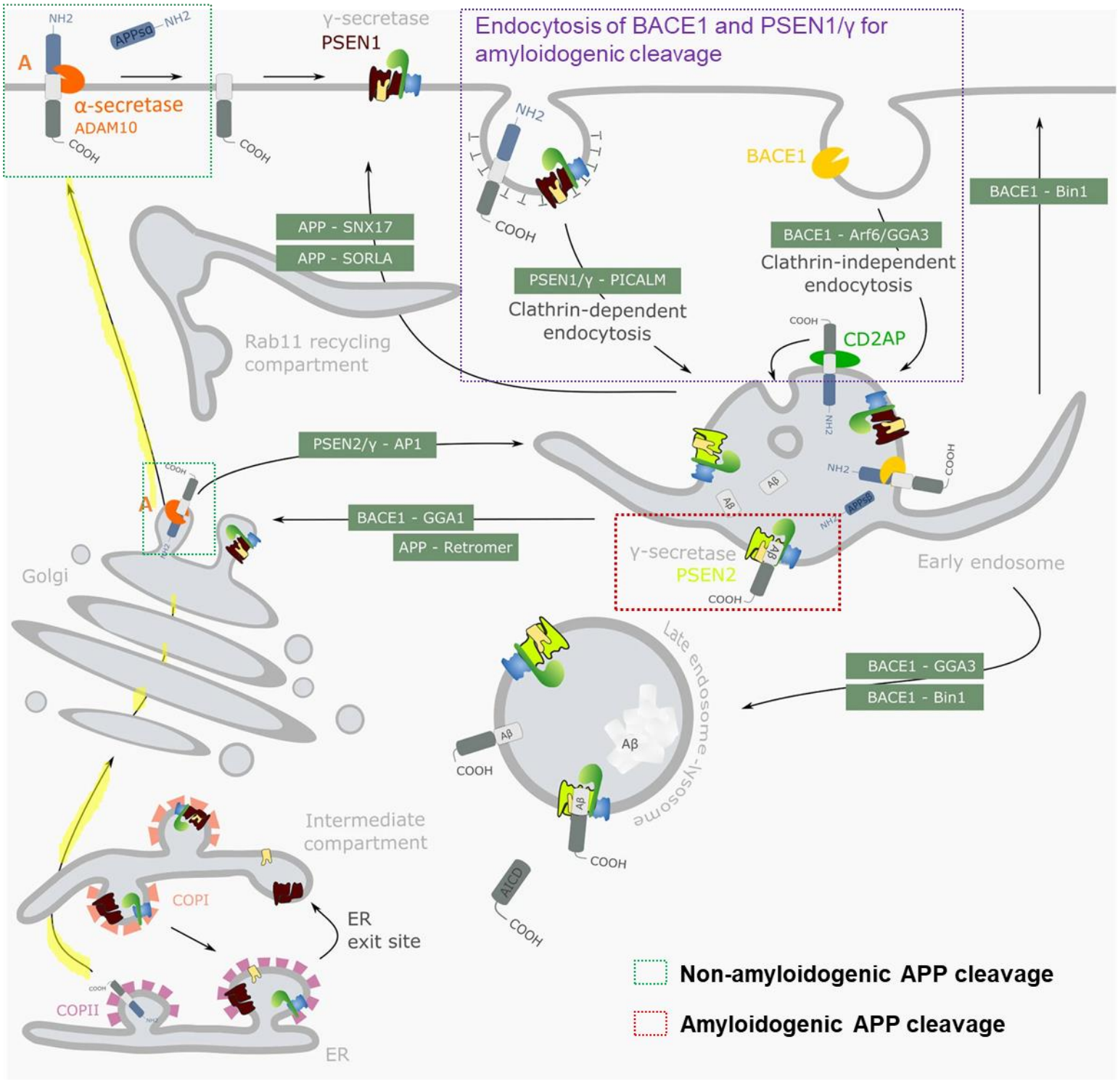

Extracellular
Intracellular
APP
sAPP-β
β-secretase
Cleavage sites
γ-secretase
Amyloid-β
or
Amyloid-β oligomer
Amyloid-β plaque
1. APP spans the cell membrane.
2. β-secretase cleaves APP.
3. γ-secretase cleaves the remaining membrane-bound portion.
4. Amyloid-β is released from the cell. The length of this peptide reflects the site at which γ-secretase makes its cut.
5. All forms of amyloid-β aggregate into oligomers, fibrils and then plaques. However, longer fragments are thought to aggregate more readily.
Cell membrane


A Non-amyloidogenic APP cleavage
NH2
NH2
α
Aβ
APPsα
P3
P3
C83
γ
AICD
COOH
COOH
COOH
B Amyloidogenic APP cleavage
NH2
NH2
Oligomerization
β
Aβ40 processing
Aβ42 processing
APPsβ
Aβ
Aβ
C99
Aβ40
Aβ43
Aβ46
Aβ49
Aβ38
Aβ42
Aβ45
Aβ48
γ
AICD
COOH
COOH
COOH

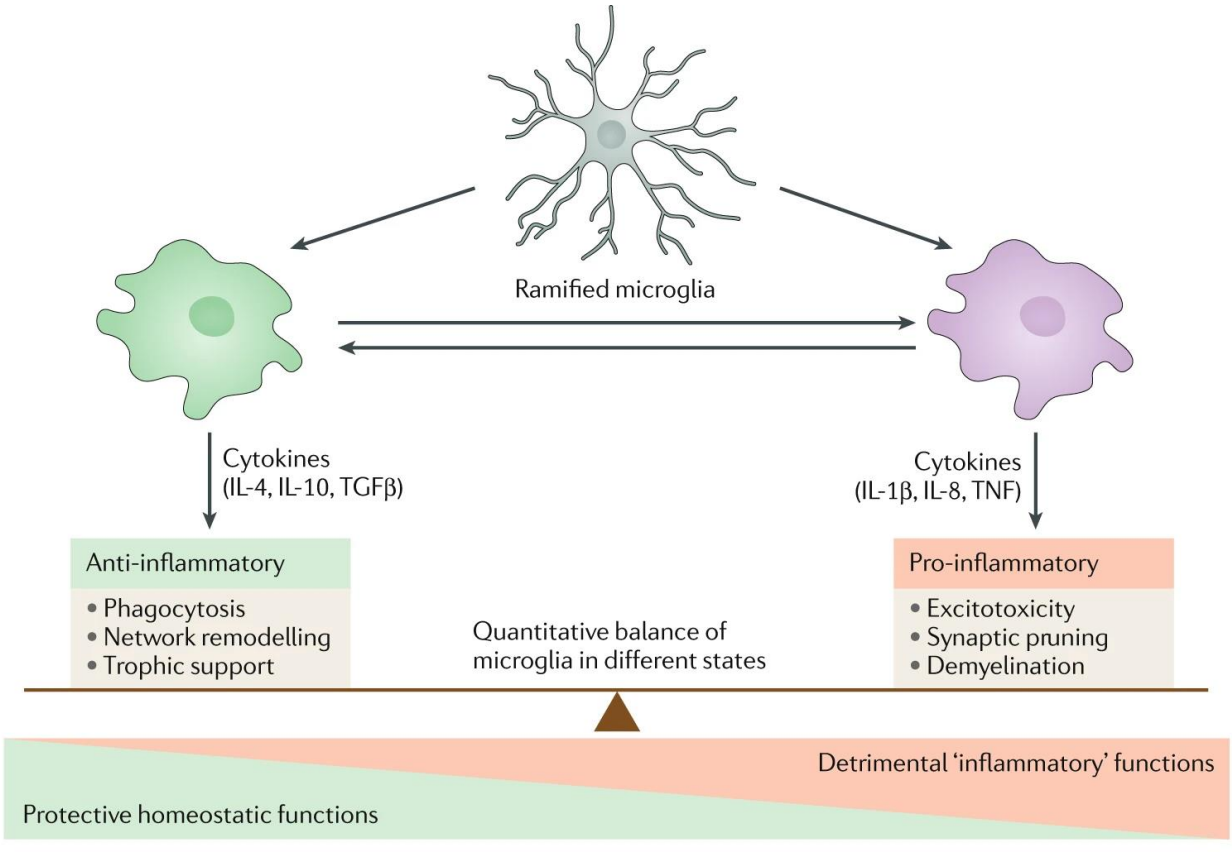

a
DNA
5′ exon
GT
A
AG
3′ exon
TSS
5′SS
BPS
3′SS
PAS
TTS
b
Branchpoint sequence recognition factors
Pol II CTD
Pol II catalytic centre
Spliceosome assembly stages
5′
Nascent RNA
5′ exon
GU
U1 snRNP
A
DNA
U2 snRNP
BBP
U4/U6•U5 tri-snRNP
U4 snRNP
U2, U5, U6 snRNPs
UG
AG
ILS
3′ exon
Spliced nascent RNA
E
Assembly
A
Assembly
B
Maturation
Bact
Activation
B*
Step I
C
Activation
C*
Step II
P
Release


Tg4510
(AD mouse model – tauopathy)
WT
TG
Whole Transcriptome
2months
4months
6months
8months
N = 12 (3 replicates)
Targeted Transcriptome
2months
4months
6months
8months
N = 24 (3 replicates)

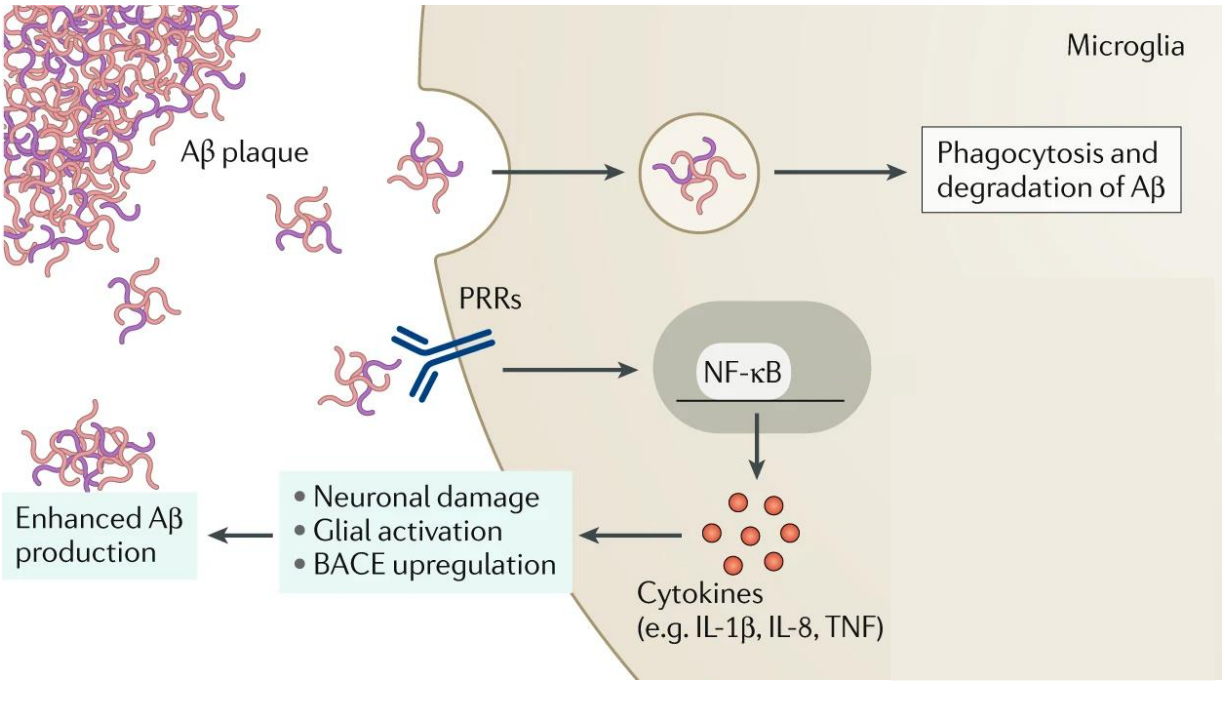

Endocytosis of BACE1 and PSEN1/γ for amyloidogenic cleavage
α-secretase
ADAM10
γ-secretase
PSEN1
BACE1
APP - SNX17
APP - SORLA
PSEN1/γ - PICALM
Clathrin-dependent endocytosis
BACE1 - Arf6/GGA3
Clathrin-independent endocytosis
BACE1 - Bin1
CD2AP
Rab11 recycling compartment
PSEN2/γ - AP1
BACE1 - GGA1
APP - Retromer
γ-secretase
PSEN2
Golgi
Early endosome
Late endosome - lysosome
BACE1 - GGA3
BACE1 - Bin1
Intermediate compartment
COPI
COPII
ER exit site
ER
Non-amyloidogenic APP cleavage
Amyloidogenic APP cleavage


NH2
APPS0
NH2
A
α-secretase
ADAM10
COOH
COOH
γ-secretase
PSEN1
Endocytosis of BACE1 and PSEN1/γ for amyloidogenic cleavage
NH2
COOH
BACE1
BACE1 - Bin1
APP - SNX17
APP - SORLA
BACE1 - Arf6/GGA3
Clathrin-independent endocytosis
PSEN1/γ - PICALM
Clathrin-dependent endocytosis
Rab11 recycling compartment
COOH
CD2AP
NH2
COOH
PSEN2/γ - AP1
A
COOH
NH2
BACE1 - GGA1
APP - Retromer
Aβ
Aβ
APPS0
NH2
γ-secretase
PSEN2
Aβ
COOH
Early endosome
Golgi
Late endosome - lysosome
BACE1 - GGA3
BACE1 - Bin1
Aβ
COOH
Aβ
Aβ
COOH
AICD
COOH
Intermediate compartment
COPI
ER exit site
COPII
ER
Non-amyloidogenic APP cleavage
Amyloidogenic APP cleavage

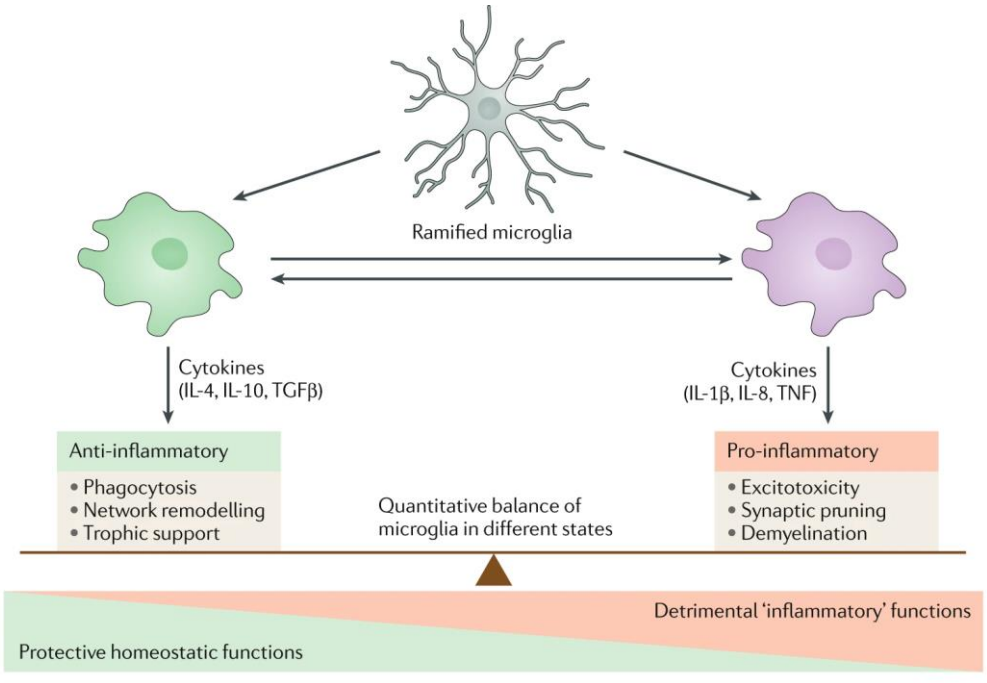
a)


Ramified microglia
Cytokines
(IL-4, IL-10, TGFβ)
Cytokines
(IL-1β, IL-8, TNF)
Anti-inflammatory
• Phagocytosis
• Network remodelling
• Trophic support
Pro-inflammatory
• Excitotoxicity
• Synaptic pruning
• Demyelination
Quantitative balance of
microglia in different states
Detrimental 'inflammatory' functions
Protective homeostatic functions

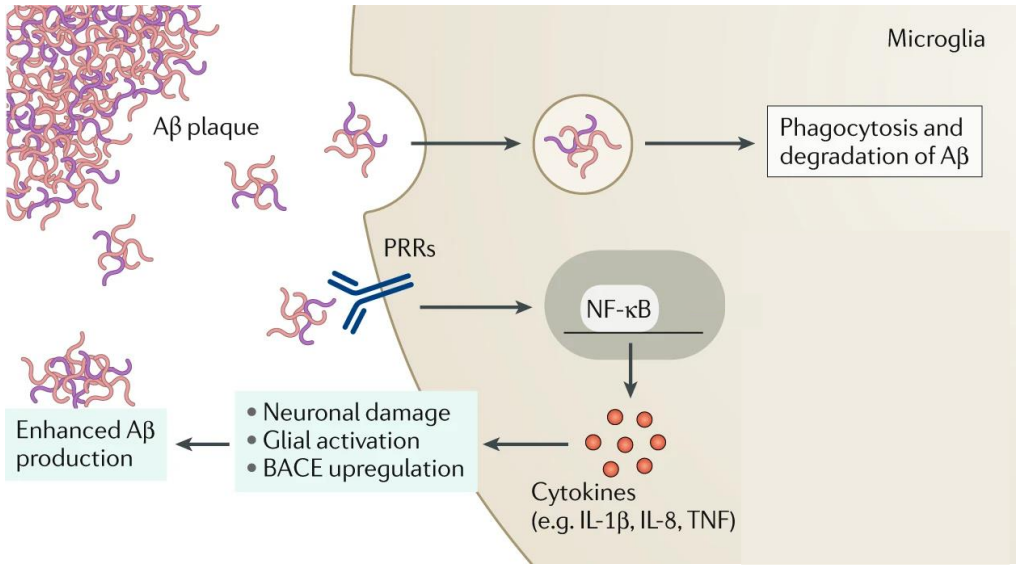
b)


Microglia
Aβ plaque
Phagocytosis and
degradation of Aβ
PRRs
NF-κB
Enhanced Aβ
production
• Neuronal damage
• Glial activation
• BACE upregulation
Cytokines
(e.g. IL-1β, IL-8, TNF)

a)


Ramified microglia
Cytokines
(IL-4, IL-10, TGFβ)
Cytokines
(IL-1β, IL-8, TNF)
Anti-inflammatory
• Phagocytosis
• Network remodelling
• Trophic support
Quantitative balance of
microglia in different states
Pro-inflammatory
• Excitotoxicity
• Synaptic pruning
• Demyelination
Detrimental 'inflammatory' functions
Protective homeostatic functions

b)


Microglia
Aβ plaque
Phagocytosis and
degradation of Aβ
PRRs
NF-κB
Enhanced Aβ
production
• Neuronal damage
• Glial activation
• BACE upregulation
Cytokines
(e.g. IL-1β, IL-8, TNF)

Constitutive Splicing

Alternative Splicing

Skipped Exon (SE)

Mutually Exclusive (MX)

Intron Retention (IR)

Alternative 5’ Splice Sites (A5’)

Alternative 3’ Splice Sites (A3’)

Alternative First Exon (AF)

Alternative Last Exon (AL)

stitutive Splicing

rnative Splicing

ped Exon (SE)

ually Exclusive (MX)

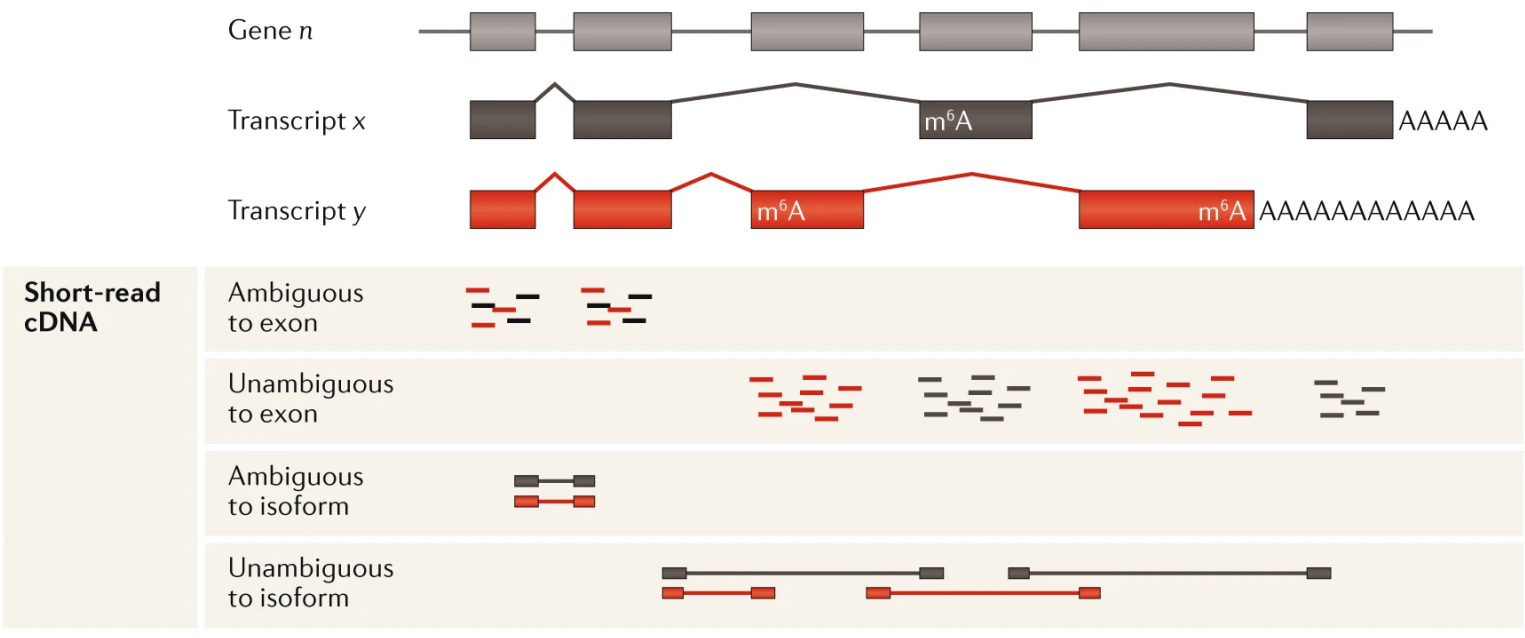
n Retention (IR)

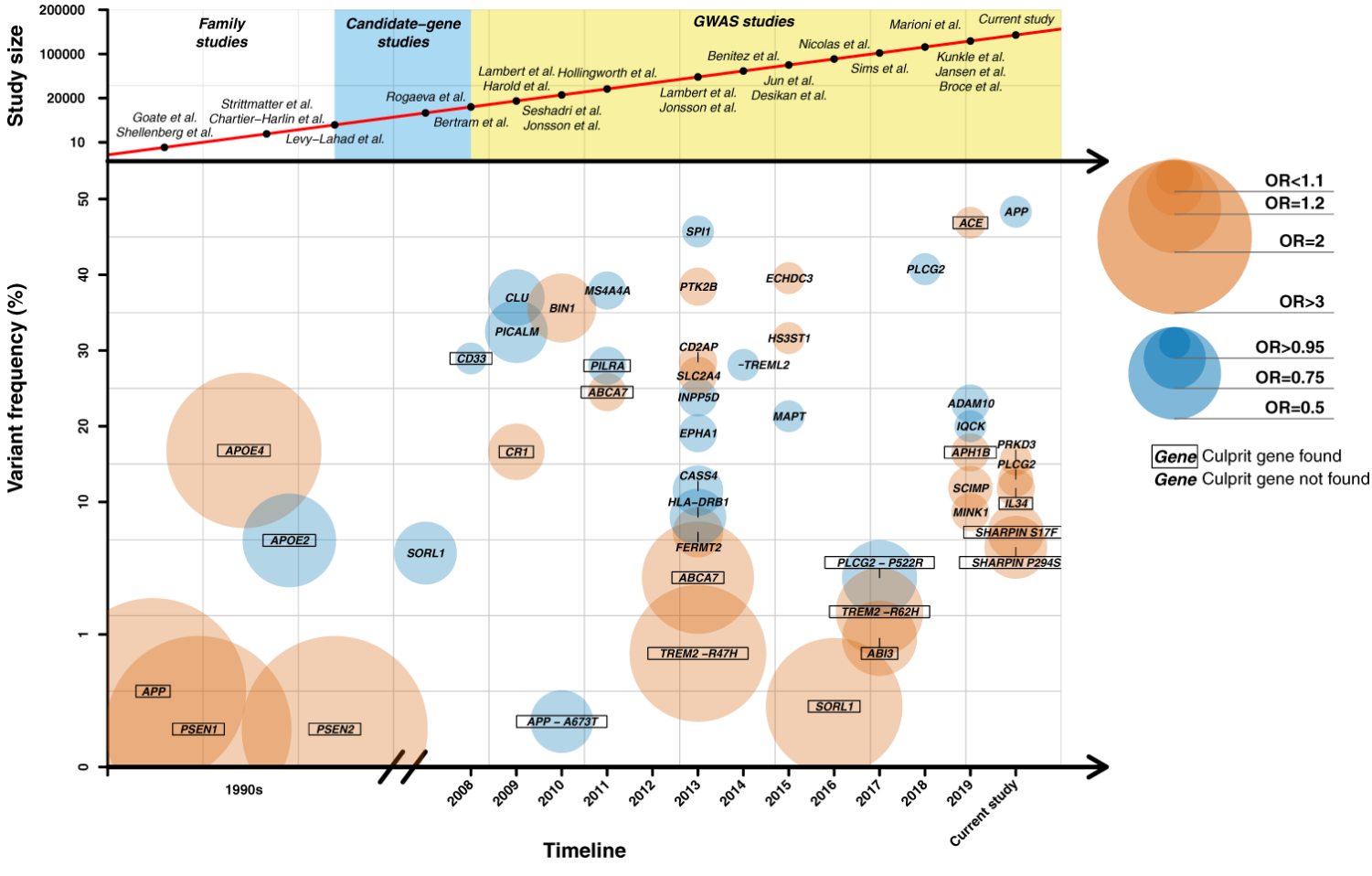
rnative 5’ Splice Sites (A5’)

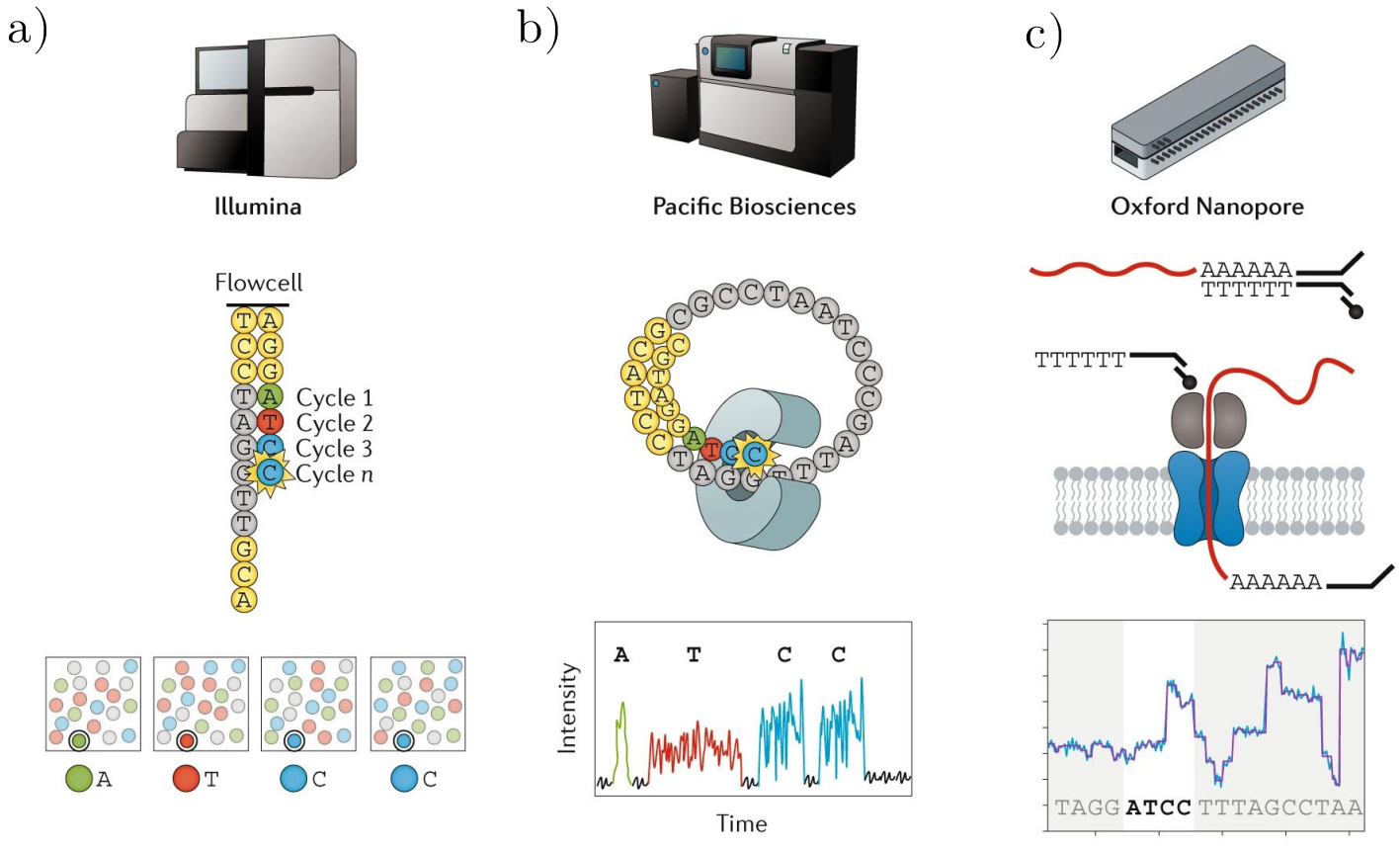
rnative 3’ Splice Sites (A3’)

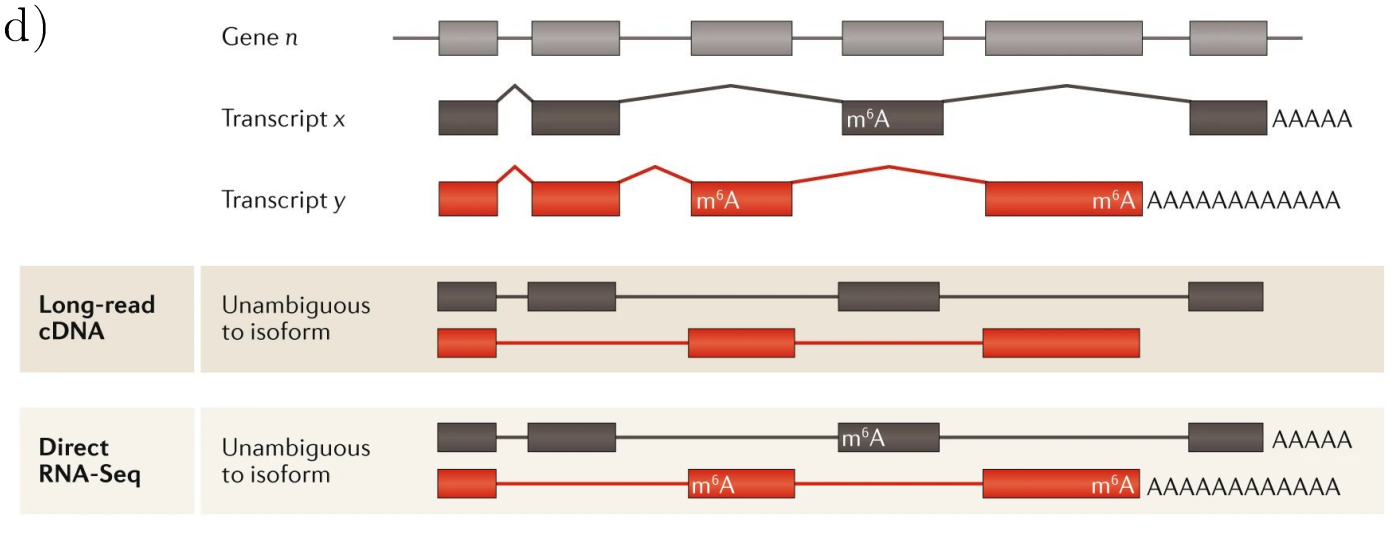
rnative First Exon (AF)

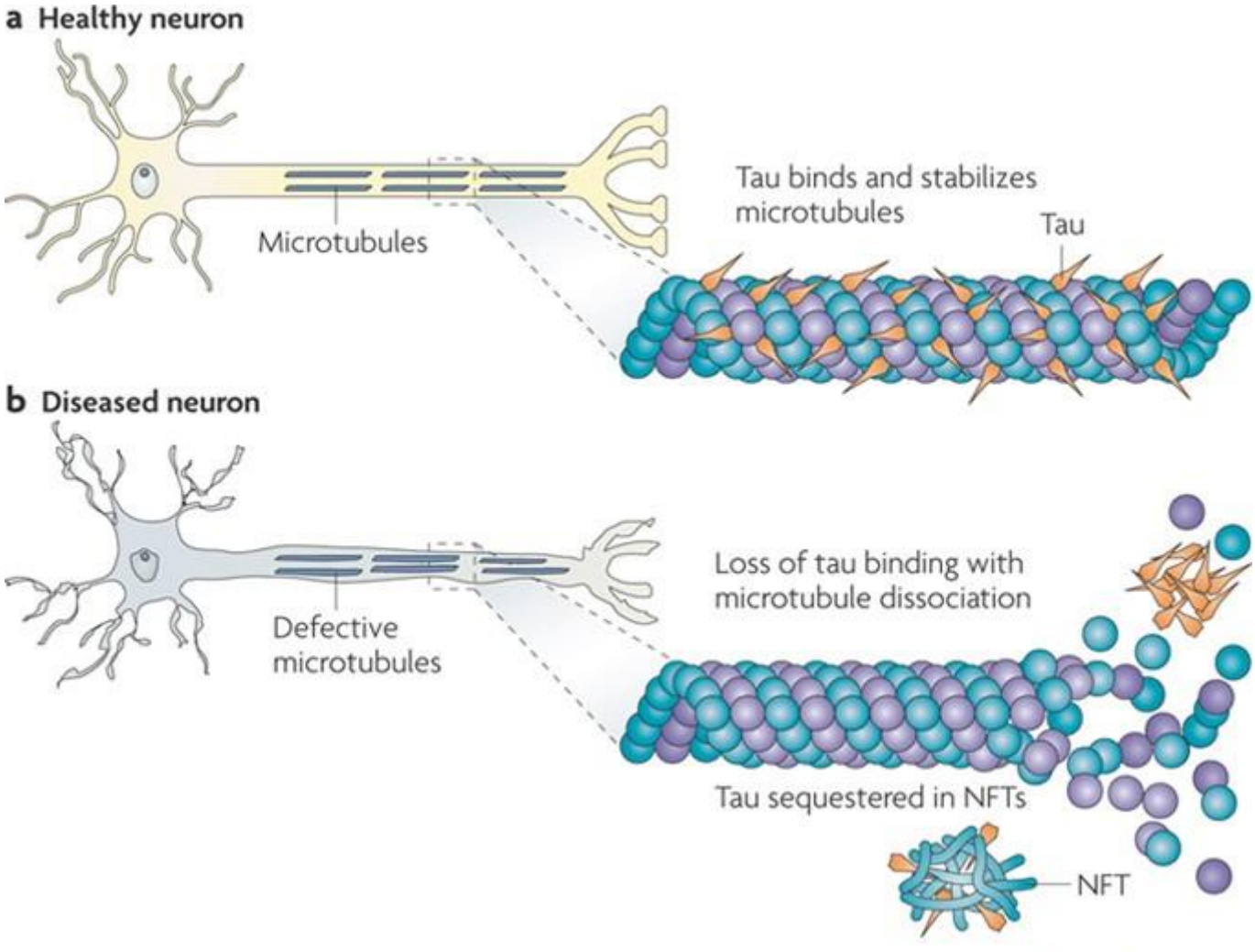
rnative Last Exon (AL)

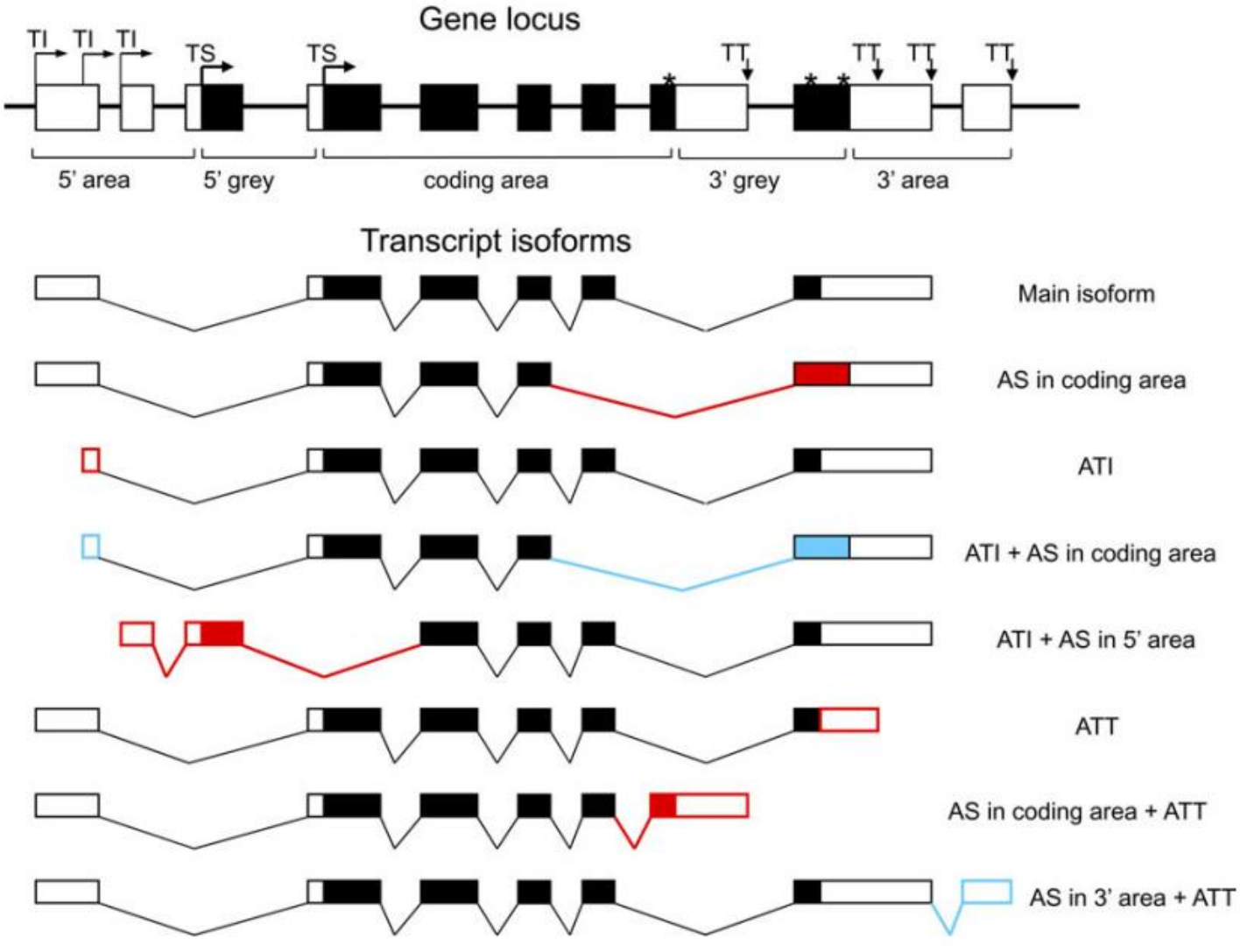

Gene n
Transcript x
m6A
AAAAA
Transcript y
m6A
m6A
AAAAAAAAAAAA
Short-read cDNA
Ambiguous to exon
Unambiguous to exon
Ambiguous to isoform
Unambiguous to isoform


Study size
200000
100000
20000
10
Family studies
Candidate–gene studies
GWAS studies
Goate et al.
Shellenberg et al.
Strittmatter et al.
Chartier–Harlin et al.
Levy–Lahad et al.
Rogaeva et al.
Bertram et al.
Lambert et al.
Harold et al.
Hollingworth et al.
Seshadri et al.
Jonsson et al.
Lambert et al.
Jonsson et al.
Benitez et al.
Jun et al.
Desikan et al.
Nicolas et al.
Sims et al.
Marioni et al.
Kunkle et al.
Jansen et al.
Broce et al.
Current study
Variant frequency (%)
50
40
30
20
10
1
0
APOE4
APOE2
APP
PSEN1
PSEN2
SORL1
CLU
BIN1
PICALM
CD33
CR1
MS4A4A
PILRA
ABCA7
APP – A673T
SPI1
PTK2B
CD2AP
SLC2A4
INPP5D
EPHA1
CASS4
HLA–DRB1
FERMT2
ABCA7
TREM2 –R47H
ECHDC3
HS3ST1
–TREML2
MAPT
PLCG2 – P522R
TREM2 –R62H
ABI3
SORL1
PLCG2
ACE
APP
ADAM10
IQCK
APH1B
SCIMP
MINK1
PRKD3
PLCG2
IL34
SHARPIN S17F
SHARPIN P294S
1990s
2008
2009
2010
2011
2012
2013
2014
2015
2016
2017
2018
2019
Current study
Timeline
OR<1.1
OR=1.2
OR=2
OR>3
OR>0.95
OR=0.75
OR=0.5
Gene Culprit gene found
Gene Culprit gene not found


a)
Illumina
Flowcell
Cycle 1
Cycle 2
Cycle 3
Cycle n
A
T
C
C
b)
Pacific Biosciences
Intensity
A T C C
Time
c)
Oxford Nanopore
AAAAAA
TTTTTT
TTTTTT
AAAAAA
TAGG ATCC TTTAGCCTAA


d)
Gene n
Transcript x
m6A
AAAAA
Transcript y
m6A
m6A
AAAAAAAAAAAA
Long-read cDNA
Unambiguous to isoform
Direct RNA-Seq
Unambiguous to isoform
m6A
AAAAA
m6A
m6A
AAAAAAAAAAAA


a Healthy neuron
Microtubules
Tau binds and stabilizes microtubules
Tau
b Diseased neuron
Defective microtubules
Loss of tau binding with microtubule dissociation
Tau sequestered in NFTs
NFT


Gene locus
TI
TI
TI
TS
TS
TT
TT
TT
TT
5' area
5' grey
coding area
3' grey
3' area
Transcript isoforms
Main isoform
AS in coding area
ATI
ATI + AS in coding area
ATI + AS in 5' area
ATT
AS in coding area + ATT
AS in 3' area + ATT