# Physiological roles of amyloid precursor protein *in vivo* - zebrafish as a model

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To those/us, who cannot sleep

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## ABSTRACT

Amyloid-beta precursor protein (APP) is an evolutionarily conserved transmembrane protein expressed in many different tissues. APP belongs to a gene family consisting of two other APP-like proteins (APLP1 and APLP2). APP has been shown to be involved in biological processes such as neurite outgrowth, neuronal migration, synapse formation and plasticity, and cell-cell interactions. APP also plays a central role in the development of Alzheimer's disease (AD). APP's physiological role has been difficult to understand and despite all research is not yet completely understood. The purpose of this thesis was to study the role of APP during early development with zebrafish as the main model system.

We have focused on the zebrafish's Apps and have tried to understand their function with the help of genetic knockout models created using the CRISPR / Cas9 method. We report that *appb* mutants have weakened cell adhesions that give rise to changes in cell organization. We also report that the *appb* mutants are smaller but develop into fertile and healthy adult individuals. We also found defects in the formation of the trigeminal ganglia (TG) and that Appb seems to have a role in cell-cell interaction. The more widespread TG also consisted of fewer nerve cells, indicating that Appb promotes nerve cell formation. Furthermore, our studies demonstrate APP expression in cilia on sensory nerve cells and ependymal cells covering the brain chambers. The conserved expression of APP in ependymal cilia in mice and humans suggest an important and preserved function. Zebrafish with mutated App were found

to have defects in the formation of both cilia and cerebral ventricles. To identify new signalling pathways through which Appb controls these functions, we studied protein changes in *appb* mutants using mass spectrometry. These studies highlight changes that both confirm known and suggest new regulations by *appb*, especially in neural development, cell adhesion and in gene regulation. Finally, we tried to answer the underlying mechanisms behind compensation within the App family. We found that mutations in the *app* genes activate expression of homologous genes *via* so-called transcriptional adaptation.

In conclusion, the findings reported in this thesis showed that App is implicated already in early cellular adhesion and sensory neuronal differentiation processes and is located to several sensory cilia *in vivo*. The use of zebrafish as a model organism allowed us to gain valuable knowledge on the physiological roles of App.

**Keywords**: Amyloid-beta precursor protein, amyloid precursor protein-b, zebrafish, physiological functions, development, trigeminal ganglia, ependyma, cilia, proteomics, translational adaptation

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# SAMMANFATTNING PÅ SVENSKA

Amyloid-beta prekursor protein (APP) är ett transmembranprotein som är evolutionärt konserverat och uttrycks i många olika vävnader. APP tillhör en genfamilj bestående av två andra APP-liknande proteiner (APLP1 och APLP2). APP har visat sig vara inblandad i biologiska processer så som nervcellsutskottens utväxt, neuronal migration, synapsbildning och plasticitet samt cell-cell-interaktioner. APP har även en central roll vid utveckling av Alzheimers sjukdom (AD). Trots all forskning är APPs fysiologiska roll svår att förstå och kunskapsläget är fortfarande ofullständigt. Syftet med denna avhandling var att studera APP fysiologiska roll under tidig utveckling med zebrafisk som huvudsaklig modellorganism.

Vi har fokuserat på zebrafiskens App och har med hjälp av genetiska knockout-modeller försökt förstå dess funktion. Mutationerna skapades med CRISPR/Cas9-metoden. Vi har rapporterat att *appb*-mutanter har försvagad celladhesion vilken ger upphov till förändringar i cellulär organisation. Vi rapporterade också att *appb*-mutanterna är mindre men utvecklas till fertila och friska vuxna individer. Vi fann även defekter i bildningen av trigeminalgangliet (TG) och fann att Appb även här verkar ha en roll i cell-cell-interaktion. TG bestod även av färre nervceller vilket tyder på att Appb främjar nervcellsbildning. Våra studier påvisar APP i cilier på sensoriska nervceller och ependymala celler som täcker hjärnkamrarna. Uttrycket i ependymala celler fanns även i mus och människa vilket tyder på en viktig och konserverad funktion. Zebrafiskar med muterat App påvisade defekter i bildning av både cilier och hjärnventriklar. För att identifiera nya signaleringsvägar via vilka Appb styr dessa funktioner studerade vi proteinförändringar i *appb*-mutanter med hjälp av masspektrometri. Dessa studier belyste förändringar som både bekräftar och föreslår nya *appb*-relaterade reglervägar, särskilt i neural utveckling, celladhesion och vid genreglering. Slutligen försökte vi besvara den underliggande mekanismen bakom kompensation inom App-familjen. Vi fann att mutationer i *app*-generna aktiverade uttryck av homologa gener via så kallad transkriptionell anpassning. I denna avhandling rapporterar vi flera viktiga och nya funktioner hos App och att zebrafisk tillåter unika studier under tidig utveckling.

# LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

I. Banote RK, **Chebli J**, Şatır TM, Varshney GK, Camacho R, Ledin J, Burgess SM, Abramsson A, and Zetterberg H. *Amyloid precursor protein-b facilitates cell adhesion during early development in zebrafish*. 2020. Sci Rep 10(1): 10127

II. **Chebli J**, Rahmati M, Banote RK, Abramsson A, and Zetterberg H. *Amyloid precursor protein-b coordinates the assembly of the trigeminal ganglia in zebrafish*. Manuscript

III. **Chebli J**, Rahmati M. Lashley T, Edeman B, Oldfors A, Zetterberg H, and Abramsson A. The localization of amyloid precursor protein to ependymal cilia in vertebrates and its role in ciliogenesis and brain development in zebrafish. 2021. Scientific Reports 11(1): 19115

IV. Abramsson A, **Chebli J**, Banote RK, Sauer M, Hansson KT, Blennow K, Gobom J and Zetterberg H. *Proteomic analysis of amyloid precursor protein-b mutant zebrafish (Danio rerio) larvae reveals changes in proteins involved in neural development, cell adhesion and gene regulation*. Manuscript

V. Rahmati M, **Chebli J**, Banote RK, Roselli S, Agholme L, Zetterberg H and Abramsson A. *Transcriptional adaptation between zebrafish amyloid precursor protein gene family members*. Manuscript

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# ABBREVIATIONS

| | |
|---|---|
| aa | amino acid |
| AcD | acidic domain |
| AD | Alzheimer’s disease |
| ADAM10 | a disintegrin and metalloproteinase domain-containing protein |
| AICD | APP intracellular domain |
| APH1 | anterior pharynx defective-1 |
| APLP | amyloid precursor-like protein |
| APP | amyloid precursor protein |
| Aβ | amyloid beta |
| BACE | β-site amyloid precursor protein cleaving enzyme |
| BCIP | 5-bromo-4-chloto-3’indolyphosphate p-toluidine salt |
| bp | base pairs |
| BSA | bovine serum albumin |
| CHX | cyclohexamide |
| CNS | central nervous system |
| CRISPR | clustered regularly interspaced short palindromic repeats |
| CSF | cerebrospinal fluid |
| CTF | C-terminal fragment |
| CuBD | copper-binding domain |
| DEP | differentially expressed proteins |
| DIG | digoxigenin |
| DMEM | Dulbecco's modified eagle medium |
| DMSO | dimethyl sulfoxide |
| DNP | 2,4-dinitrophenyl |
| dpf | days post-fertilization |
| DR6 | death receptor 6 |
| DS | Down syndrome |
| EE | early endosome |
| EGFR | epidermal growth factor receptor |

| | |
|---|---|
| EM | embryonic medium |
| EOFAD | early-onset familial Alzheimer's disease |
| EpCAM | epithelial cellular adhesion molecule |
| ER | endoplasmic reticulum |
| EtOH | ethanol |
| EVL | enveloping layer |
| FC | fold change |
| FISH | fluorescent *in situ* hybridization |
| GAPDH | glyceraldehyde 3-phosphate dehydrogenase |
| GO | gene ontology |
| GS | goat serum |
| HBD | heparin-binding domain |
| hpf | hours post-fertilization |
| HRP | horseradish peroxidase |
| ISH | *in situ* hybridization |
| Isl1 | islet 1 |
| KD | knockdown |
| KO | knockout |
| KPI | Kunitz-like protease inhibitor domain |
| LC-MS | liquid chromoatography – mass spectrometry |
| LE | late endosome |
| LOFAD | late-onset Alzheimer's disease |
| LTP | long-term potentiation |
| M-cells | Mauthner cells |
| mmV | maxillomandibular placode nerve |
| MO | morpholinos |
| MS-222 | tricaine, ethyl 3-aminobenzoate methanesulfonate |
| NBT | nitro-blue tetrazolium chloride |
| NMD | nonsense mediated decay |
| NMJ | neuromuscular junction |
| NPH | normal pressure hydrocephalus |

| | |
|---|---|
| nt | nucleotide |
| opV | optic nerve |
| PCR | polymerase chain reaction |
| PEN2 | presenilin enhancer-2 |
| PFA | paraformaldehyde |
| POD | peroxidase |
| PS | presenilin |
| PTC | premature termination codon |
| PTU | 1-phenyl-2-thiourea |
| qPCR | quantitative polymerase chain reaction |
| ROI | region of interest |
| sAPP | soluble APP |
| SBB | Sudan Black B |
| SVZ | subventricular zone |
| TA | translational adaptation |
| TCA | trichloroacetic acid |
| Tg | transgenic |
| TG | trigeminal ganglia |
| TGN | trans-Golgi network |
| TMT | tandem mass tag |
| TrkC | neurotrophic receptor tyrosine kinase |
| Trpa1b | transient receptor potential cation channel, subfamily A, member 1b |
| Trpv1 | transient receptor potential cation channel, subfamily V, member 1 |
| WISH | whole-mount *in situ* hybridization |
| ZFIN | zebrafish information network |

# 1 INTRODUCTION

The tip of the iceberg?

# 1.1 AMYLOID BETA PRECURSOR PROTEIN

Amyloid beta precursor protein (APP) is a type I single-pass transmembrane protein shown to be implicated in multiple biological functions and diseases. APP contains a large extracellular domain (671 aa) and a small intracellular domain (99 aa) that includes the transmembrane region. Cloned over 30 years ago (Goldgaber, Lerman et al. 1987, Kang, Lemaire et al. 1987, Tanzi, Gusella et al. 1987), APP was shown to be encoded by a single gene, *APP,* composed of 18 exons (Yoshikai, Sasaki et al. 1990), localized on the distal arm of chromosome 21 (21q21) (Goldgaber, Lerman et al. 1987). APP has several splice isoforms, of which APP770, APP751 and APP695 are the three major forms, encoding a protein of 770, 751 and 695 amino acids respectively. APP770 and 751 isoforms, with a molecular weight around 100kDa, are expressed in many tissues including skeletal, smooth and cardiac muscles, neuronal tissues, lung, pancreas, kidney, adipose tissue, liver, spleen, intestine, and also in fibroblasts, whereas APP695 isoform is predominantly expressed by the brain and the spinal cord (Kang, Lemaire et al. 1987, Puig and Combs 2013). *In vitro* studies also reported APP770, APP751 and APP695 to be expressed by astrocytes and microglia (Haass, Hung et al. 1991, LeBlanc, Chen et al. 1991). APP expression starts early and increases through development and remain high during maturity and adulthood (Lorent, Overbergh et al. 1995) (reviewed by (van der Kant and Goldstein 2015, Muller, Deller et al. 2017)).

## 1.1.1 STRUCTURE

When firstly reported, APP was described as a platelet protein, resembling a cell surface receptor (Kang, Lemaire et al. 1987). The extracellular part of APP contains explicit domains (see **Figure 1**). The E1 (cysteine-rich) and the E2 (helix-rich) are two dimerization domains enabling homo- and heterodimerization of the N-terminal portion of APP (Soba, Eggert et al. 2005). Both E1 and E2 domains contain a heparin-binding domain (HBD) enabling APP interaction with other proteins, such as extracellular matrix proteins (reviewed by (Reinhard, Hebert et al. 2005, Muller, Deller et al. 2017)). In addition to the HBD, the E1 contains a copper-binding domain (CuBD). Linking the E1 and E2 domains is the flexible acidic domain (AcD). All three major APP splice isoforms contain the E1, E2 and the AcD domains. In addition, a Kunitz-like protease inhibitor domain (KPI) and an Ox-2 antigen domain are contained in the extracellular portion of APP770. APP751 omits the Ox-2 domain and the APP695 isoform omits both the Ox-2 and KPI domains (Tanzi, Gusella et al. 1987, Weidemann, König et al. 1989). Located

partially integrated within the plasma membrane is the amyloid beta (Aβ) domain. The short C-terminal fragment (CTF) contains the APP intra-cellular domain (AICD) which interacts with the lipidic plasma membrane and harbors several phosphorylated sites (Barrett, Song et al. 2012). The AICD is highly conserved among APP isoforms and spans the YENPTY motif.

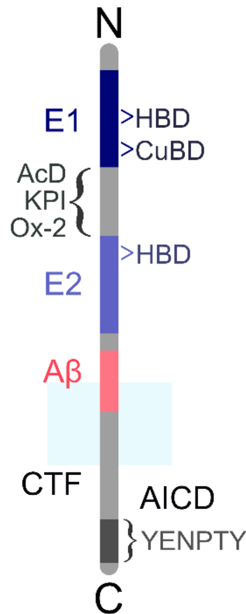


***Figure 1.*** *Structure of amyloid beta precursor protein (APP770). Extracellular domains: E1 domain, acidic domain (AcD), Kunitz-like protease inhibitor domain (KPI), Ox-2 antigen domain, the E2 domain, the heparin-binding domain (HBD), and the copper-binding domain (CuBD). The amyloid beta (Aβ) domain is partially located through the plasma membrane (pale blue). Intracellular domain: APP intracellular domain (AICD) with the YENPTY motif.*

### 1.1.2 CELLULAR TRAFFICKING

APP is synthesized in the endoplasmic reticulum (ER), trafficked through the secretory pathway from the ER to the Golgi and the trans-Golgi network (TGN) and, *via* secretory vesicles, is transported out to the plasma membrane. APP has a short halftime of less than an hour (Weidemann, König et al. 1989) as it is highly processed by endogenous enzymes named secretases (review by (Rajendran and Annaert 2012)). Once located at the plasma membrane, APP is either cleaved by α-secretase (non-amyloidogenic pathway, see section **Canonical pathways**) or internalized and trafficked to the early endosomes (EE), (the amyloidogenic pathway, see section **Canonical pathways**) and cleaved by β-secretase. APP fragments are subsequently trafficked through the late endosome and lysosome pathway or are recycled through the recycling endosome ((reviewed by (Haass, Kaether et al. 2012, van der Kant and Goldstein 2015, Wang, Zhou et al. 2017)).

### 1.1.3 PROTEOLYTIC PROCESSING

The proteolytic processing of APP has been extensively studied and is divided into two main pathways, the canonical and non-canonical, associated with specific secretases, organelles, and cleavage products.

#### Canonical pathways

The canonical processing involves two separate pathways named after the production or not of the Aβ peptide: the non-amyloidogenic pathway and the amyloidogenic pathway (see **Figure 2**).

The non-amyloidogenic pathway is the predominantly cleavage process of APP. This pathway involves alpha (α)-secretases, of which ADAM10 (a disintegrin and metalloproteinase domain-containing protein 10), and gamma (γ)-secretase composed of the catalytic core protein, presenilin 1 or 2 (PS1, PS2), and of nicastrin, presenilin enhancer-2 (PEN2), and anterior pharynx defective-1 (APH1) subunits. α- and γ-Secretases are both involved in the processing of many other substrates including the Notch receptors (De Strooper, Iwatsubo et al. 2012) and cadherins (reviewed by (Haass, Kaether et al. 2012, Hernandez-Sapiens, Reza-Zaldivar et al. 2022)). Proteolytic cleavage is a sequential process. The current view claims that APP, localized at the plasma membrane, is cleaved within the Aβ domain by α-secretase which thus

abolish formation of the amyloid fragment. A soluble APP fragment (sAPPα) is released into the extracellular space and the C-terminal fragment (CTFα), still tethered at the plasma membrane, is then internalized and trafficked to the more acidic intracellular EE, late endosome (LE) and through the lysosome or the TGN (reviewed by (van der Kant and Goldstein 2015)). The γ-secretase then cleaves the CTFα thereby releasing the p3 peptide and the AICD. The AICD is translocated to the cell nucleus where it regulate gene expression (Blennow, de Leon et al. 2006, O'Brien and Wong 2011). The level of APP addressed to the plasma membrane (reviewed by (Muller, Deller et al. 2017)) and the synaptic activity of the cell increase the non-amyloidogenic pathway (Hoey, Williams et al. 2009).

The amyloidogenic pathway on the other hand firstly involves the β-site amyloid precursor protein cleaving enzyme (BACE), expressed by two genes *BACE1* and *BACE2*. BACE1 is the major β-secretase in neuronal tissue and has an optimal enzymatic activity for APP cleavage in the acidic environment of the endosomes (Haass, Schlossmacher et al. 1992, Vassar, Bennett et al. 1999, Huse, Pijak et al. 2000). Once APP is internalized to the acidic EE, it is cleaved by β-secretase upstream (16 amino acids) of the main cleavage site of α-secretase. The sAPPβ is therefore slightly smaller than the sAPPα (O'Brien and Wong 2011, Haass, Kaether et al. 2012) and together represent more than 50% of APP in the brain (Morales-Corraliza, Mazzella et al. 2009). The sAPPβ fragment is trafficked to the plasma membrane and released through recycling endosomes. The remaining CTFβ with an intact Aβ sequence follows the endosomal trafficking, prior to the γ-secretase processing. Then, the γ-secretase cleaves the CTFβ, which releases the Aβ peptide and the AICD (De Strooper, Iwatsubo et al. 2012). The Aβ peptide is either degraded in lysosomes or exits the cytosol through exosomal trafficking or the TGN secretory pathway (Rajendran, Honsho et al. 2006). As the optimal activity of β-secretase is pH-dependent, the amyloidogenic pathway is increased when APP is localized in acidic compartments, such as early endosomes (Das, Scott et al. 2013, Hu, Dammer et al. 2015).

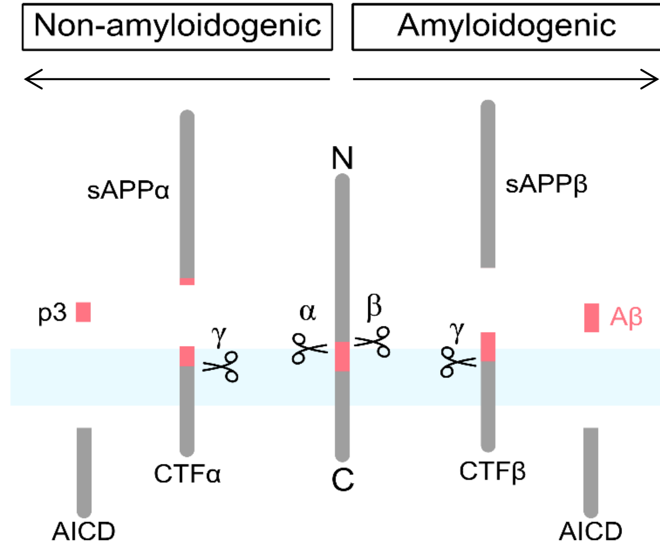


***Figure 2.*** *The canonical proteolytic pathways of APP. The non-amyloidogenic pathway (towards the left side) implicating α- and γ-secretases and the amyloidogenic pathway implicating β- and γ-secretases (towards the right side).*

## Non-canonical pathways

Beyond the canonical pathway, studies have identified additional APP proteolytic pathways implicating other proteases that also can liberate the production of Aβ peptide. The delta (δ)-secretase, a membrane-bound metalloproteinase meprin-β and the eta (η)-secretase all have been shown to have cleavage sites in the N-terminal domain of APP, whereas caspase cleaves in the C-terminal domain of APP (reviewed by (Andrew, Kellett et al. 2016)). The meprin-β is suggested to be a β-secretase with cleavage sites within three APP domains: E1, E2 and the Aβ domains. The meprin-β cleavage pathway gives rise to sAPP fragments of varying lengths that can generate Aβ fragments, once cleaved by the γ-secretase (Jefferson, Causevic et al. 2011). The δ-secretase, known as mammalian asparagine endopeptidase (AEP), is similar to β-secretase, activated by the low-pH in endosomes, and cleave APP at two sites (Zhang, Song et al. 2015). The membrane-bound η-secretase has

been shown to have one cleavage site upstream of the β-secretase cleavage sites (Willem, Tahirovic et al. 2015). A concerted α- and β-secretase cleavages has been previously described, leading to the secretion of truncated Aβ fragments (Portelius, Price et al. 2011). Recently, a BACE2 cleavage at amino acid 34 of the Aβ sequence was reported in trisomy T21 organoids mediating protective mechanisms from amyloidosis (Alic, Goh et al. 2020).

Together, the canonical and the non-canonical pathways result in a wide range of protein fragments, some with specific functions, contributing to the complexity of the APP protein (reviewed by (Andrew, Kellett et al. 2016)). Several of these secretases have been tested as therapeutic targets to prevent or slow down the pathological process of Alzheimer's disease (reviewed by (Mangialasche, Solomon et al. 2010, Mikulca, Nguyen et al. 2014, Miranda, Montiel et al. 2021). However, all have failed or been terminated due to severe side effects. The complex proteolytic pathways of APP add to the complexity of the study of APP physiological functions and the recently described non-canonical pathways suggest that the full role of APP remains unknown.

### 1.1.4 FUNCTIONS IN THE NERVOUS SYSTEM

Redundancy between family members and the production of many isoforms and proteolytic fragments have been considered to hamper the quest of uncovering the physiological roles of APP. Moreover, while some biological and neuronal functions have been established and shown in diverse models, some remain elusive, are species-specific or are newly described (reviewed by (Nicolas and Hassan 2014, van der Kant and Goldstein 2015, Muller, Deller et al. 2017, Guo, Wang et al. 2021).

APP, along with its cleavage products, have been shown to be expressed during brain development (Sarasa, Sorribas et al. 2000) and are involved in neurogenesis and during neuronal development (reviewed by (Nicolas and Hassan 2014)), notably as a cell adhesion molecule (reviewed by (Sosa, Caceres et al. 2017)).

APP function in cell adhesion seemed to have been definitive during the evolution of the neuronal system. In fact, APP is needed for synaptic establishment and stability and APP emergence coincides with the evolution of functional synaptic network in species (Shariati and De Strooper 2013, Baumkotter, Schmidt et al. 2014, Copenhaver and Ramaker 2016). APP is expressed in both pre- and postsynaptic terminals (Yamazaki, Selkoe et al. 1995, DeBoer, Dolios et al. 2014) and is implicated in synaptic adhesion and

in pre- and postsynaptic activity (reviewed by (Ludewig and Korte 2016)). Indeed, APP is shown to be a synaptic adhesion molecule and to be necessary for synaptogenesis in presynaptic motor neurons and in postsynaptic muscles (Wang, Wang et al. 2009). In mice lacking *App*, a lower number of spine was reported (Tyan, Shih et al. 2012). Recently, APP has been shown to have a role in regulating inhibitory neurotransmission *in vivo* (Wang, Wang et al. 2014, Kreis, Desloovere et al. 2021).

APP is implicated in neuronal migration (Copenhaver and Ramaker 2016) and defects in the migration of neurons into the cortical plate have been reported in rats deficient for *App* (Young-Pearse, Bai et al. 2007). APP and its proteolytic products have been reported to modulate neurites length and branching outgrowth and guidance *in vitro* and *in vivo* (Young-Pearse, Chen et al. 2008, Hoe, Lee et al. 2009, Billnitzer, Barskaya et al. 2013).

Studies on APP functions in nervous system also revealed functions associated to specific proteolytic fragments of APP (see **Figure 3**).

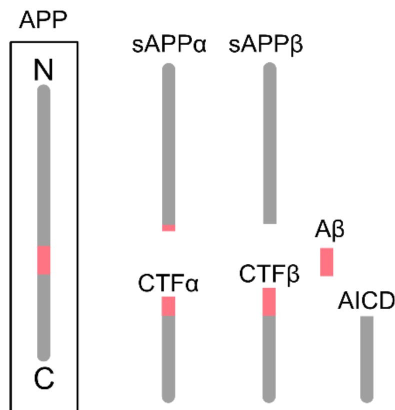


***Figure 3.*** *The full-length APP and some of its proteolytic fragments. N=N-terminal, C=C-terminal.*

The **sAPPα** fragment secreted from the canonical pathways can bind the full-length APP and the amyloid precursor-like proteins (APLPs, see section **Amyloid precursor-like proteins**) to form *cis* or *trans* dimers. sAPPα is described mainly as a neuroprotective fragment, notably against damages in traumatic brain injury (Corrigan, Vink et al. 2012), and neuronal functions have been associated with the fragment (Meziane, Dodart et al. 1998). In *App*$^{-/-}$ mice, sAPPα rescued the long-term potentiation (LTP) and restored spatial learning in adult mice (Ring, Weyer et al. 2007). The activation of LTP and memory by the sAPPα fragment have also been shown in rats, in aged mice, in AD mouse models and in primary hippocampal cultures (Meziane, Dodart et al. 1998, Taylor, Ireland et al. 2008, Moreno, Rose et al. 2015, Fol, Braudeau et al. 2016, Tan, Mockett et al. 2018, Livingstone, Elder et al. 2021). sAPPα has been shown to activate neurogenesis in the adult subventricular zone in mice (Caille, Allinquant et al. 2004) and to stimulate synaptic plasticity (Taylor, Ireland et al. 2008, Hick, Herrmann et al. 2015, Richter, Ludewig et al. 2018). Recently, sAPPα has been shown to act in $Ca^{2+}$ homeostasis regulation (Ludewig, Herrmann et al. 2021). Considerably less is known regarding the **sAPPβ** fragment, the length of which differs to the sAPPα fragment by only 16 amino acids (reviewed by (van der Kant and Goldstein 2015)). Nonetheless, a study showed that sAPPβ induces neural differentiation of human embryonic stem cells (Freude, Penjwini et al. 2011).

The **Aβ** peptide-associated functions is suggested to be concentration-manner. Indeed, picomolar concentrations of Aβ stimulate the LTP, synaptic plasticity and memory (Puzzo, Privitera et al. 2008, Puzzo, Privitera et al. 2011, Gulisano, Melone et al. 2019). On the other hand, higher concentrations have neurotoxic effects notably by inhibiting synaptic plasticity and memory (reviewed by (Sciaccaluga, Megaro et al. 2021)), and can lead to Aβ fragment oligomerization and aggregation as observed in AD (reviewed by (De Strooper and Karran 2016)) (see section **Alzheimer's disease**). More recently, a study reported that the Aβ domain of APP stimulates the neuronal homeostatic synaptic plasticity, which reflects the ability of neurons to adjust their synaptic activity upon negative feedback (Galanis, Fellenz et al. 2021).

The membrane-bound fragments, **CTFα** and **CTFβ,** have been shown to be neurotoxic and to reduce dendritic spines (Bittner, Fuhrmann et al. 2009). CTFβ has been shown to impair neuronal functions in overexpressing models and in AD mice models. Indeed, reduced LTP and memory impairment have been reported (Nalbantoglu, Tirado-Santiago et al. 1997, Berger-Sweeney, McPhie et al. 1999, Mitani, Yarimizu et al. 2012, Tamayev, Matsuda et al. 2012).

The **AICD** fragment is shown to translocate to the cell nuclei and then to act in transcriptional regulation of a wide array of genes (reviewed by (Bukhari, Glotzbach et al. 2017)). The AICD gene target list includes APP itself (von Rotz, Kohli et al. 2004). The highly conserved **YENPTY** motif expressed in the AICD fragment is known to regulate the processing of APP (Perez, Soriano et al. 1999, Ring, Weyer et al. 2007), is involved in the neuromuscular junctions (NMJ) functions and morphology (Weyer, Klevanski et al. 2011) and to bind several proteins containing phosphotyrosine-binding domains, such as kinases and adaptor proteins binding to APP. Among those proteins are Numb, which interacts with Notch (Roncarati, Sestan et al. 2002) and Fe65 (Fiore, Zambrano et al. 1995, Augustin and Kins 2021).

Further functions of APP processing fragments, from both canonical and non-canonical pathways, are reviewed by (Muller, Deller et al. 2017).

### 1.1.5 PROTEIN INTERACTIONS

APP has a cell surface receptor-like structure and its homo- and heterodimerization properties allow APP to interact with many proteins, including itself, in both in *cis*- and *trans*-conformation (Soba, Eggert et al. 2005) (reviewed by (Deyts, Thinakaran et al. 2016)). APP can form homodimers, which have been shown to modulate cell-cell adhesion, synaptogenesis and to increase the synaptic plasticity and stability (Baumkotter, Wagner et al. 2012, Baumkotter, Schmidt et al. 2014) (reviewed by (Reinhard, Hebert et al. 2005, Muller, Deller et al. 2017)).

The extracellular part of APP interacts with molecules implicated in cell adhesion such as N-cadherin, collagen I, integrins, and laminins (Beher, Hesse et al. 1996, Asada-Utsugi, Uemura et al. 2011) and with molecules implicated in neuronal migration such as pancortin, reelin, slit1 and semaphorins (Magdesian, Carvalho et al. 2008, Hoe, Lee et al. 2009, Rice, Townsend et al. 2012, Wang, Li et al. 2017).

APP has been shown to interact with the Notch receptor and the crosstalk between the two proteins is crucial for proper neuronal development (Oh, Ellenstein et al. 2005). The functions of Notch are mainly, but not restricted to, related to neurogenesis and cell fate (reviewed by (Fior and Henrique 2009)). Both APP and Notch are cleaved by the α- and γ-secretases (De Strooper, Annaert et al. 1999, Haass and De Strooper 1999, Kimberly, Esler et al. 2003) and thus compete as secretase substrate (reviewed by (Hitzenberger, Gotz et al. 2020)).

APP can also bind with receptors such as the EGFR to regulate neuronal proliferation (Caille, Allinquant et al. 2004) and the DR6, which interaction triggers axon pruning and neuronal death (Nikolaev, McLaughlin et al. 2009). In addition, APP has been shown to interact with the Frizzled receptor (Magdesian, Carvalho et al. 2008) and recently implicated in Wnt signalling as a Wnt receptor (Liu, Zhang et al. 2021). Thus, APP is involved with several regulatory molecules that all play essential roles during development and tissue homeostasis.

## 1.2 AMYLOID PRECURSOR-LIKE PROTEINS

*APP* is part of a gene family, which includes amyloid precursor-like protein-1 (*APLP1*) and *APLP2* (Goldgaber, Lerman et al. 1987). *APLP1* and *APLP2* are highly conserved through evolution and are expressed in fish, amphibian, mice, non-human primates and in human (review by (Shariati and De Strooper 2013)). The APLPs share similarities with APP such as their type 1 single-pass transmembrane structure and they express the E1 and E2 domains, the AcD domain, as well as the highly conserved AICD domain with the YENPTY motif. The APLP2 spans in addition the KPI domain in contrast to APLP1 (see **Figure 4**). However, one major difference regarding the APLPs is the lack of the Aβ domain (Wasco, Bupp et al. 1992, Wasco, Gurubhagavatula et al. 1993) (review by (Muller, Deller et al. 2017)). APLP1 is predominantly localized to the plasma membrane, whereas APLP2 is rapidly internalized and is thus mainly found in intracellular compartments (Kaden, Voigt et al. 2009). APLP1 is solely expressed in neuronal tissue, whereas APLP2 has a broader expression pattern, similarly to the APP770 (Kaden, Voigt et al. 2009).

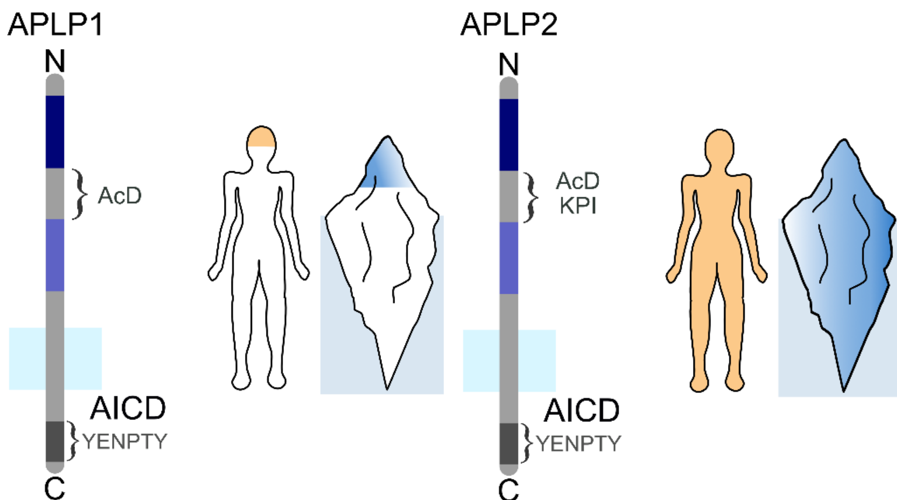


***Figure 4.*** *Structure and expression patterns of APLP1 and APLP2. APLP is predominantly expressed by the brain neuronal tissue, whereas APLP2 has a broader expression pattern. E1 domain (dark blue), acidic domain (AcD), Kunitz-like protease inhibitory domain (KPI), E2 domain (blue), APP intracellular domain (AICD), YENPTY motif (dark grey) and plasma membrane (pale blue). Figure inspired from (van der Kant and Goldstein 2015).*

The expression of APLPs increases during development and, along with APP, the proteins participate in neuromuscular transmission and synaptic plasticity (Lorent, Overbergh et al. 1995) (Weyer, Klevanski et al. 2011, Fanutza, Del Prete et al. 2015, Hick, Herrmann et al. 2015). APLPs are cleaved by α-, β-, and γ-secretases (Eggert, Paliga et al. 2004). The shared sequence homology and expression pattern between APP and APLPs and their similar proteolytic processing surely increase the complexity of the study of their physiological roles.

# 1.3 ALZHEIMER'S DISEASE

Defects in APP expression levels and proteolytic processing can lead to sever cellular damages, developmental defects, pathological states, and increased mortality. The most studied pathology related to APP defects is indisputably Alzheimer's disease (AD).

## 1.3.1 NEUROPATHOLOGY

AD is the most common cause of dementia, characterized by a cognitive state where daily activities are disturbed by impaired memory, language skills, and other cognitive abilities. The World Health Organization reports that around 50 million people worldwide have received a diagnosis of dementia, of which AD counts for more than 75% (https://www.alzint.org/what-we-do/partnerships/world-health-organization/).

Two major forms of AD are currently defined: the early-onset familial AD (EOFAD) and the late-onset sporadic AD (LOAD). The EOFAD clinical symptoms start before age 65 and accounts for less than 1% of all AD cases. Although the cognitive decline and behaviour changes appear later in life (after 65 years of age for the LOAD), the molecular and cellular defects in the brain start to accumulate decades prior to the clinical symptoms (Sperling, Aisen et al. 2011).

The pathological hallmarks of AD are neuronal and synapses loss, hyperphosphorylation of the microtubule-associated protein tau and accumulation of Aβ peptides aggregates, all observed respectively by brain atrophy, intracellular fibrillary tangles and dense senile extracellular plaques. The pathological hyperphosphorylation of tau creates its misfolding, accumulation into tangles, and prevents the protein to act on and stabilize the microtubule assembly of axons and neurites extension (Weingarten, Lockwood et al. 1975, Blennow, de Leon et al. 2006).

The senile amyloid plaques are formed by the deposition and accumulation of Aβ peptides (Masters, Simms et al. 1985). The diffuse and the dense plaques are described as the main types and the dense plaques are associated with synaptic and neuronal loss and with activated microglia and astrocytes (Serrano-Pozo, Frosch et al. 2011). Aβ peptides found are mainly 40- and 42 aa-long fragment ($A\beta_{1-40}$ $A\beta_{1-42}$) and are known to be the main component of the amyloid plaques. High levels of $A\beta_{1-42}/A\beta_{1-40}$ are secreted from cells of patients with EOFAD and the sequestration of the Aβ in the brain of AD

patients is thought to partially explain the lower ratio of $A\beta_{1-42}/A\beta_{1-40}$ found in the cerebrospinal fluid (CSF) on AD patients (Sunderland, Linker et al. 2003). Despite intensive studies, the exact primary events of AD remain elusive and while a cause hypothesis was suggested decades ago, and is questioned, the amyloid cascade is shown to participate in the early events of the pathology.

### 1.3.2 THE AMYLOID CASCADE AND MUTATIONS

Thirty years ago, the amyloid cascade hypothesis was suggested as the primary event leading to AD and is, although highly debated, part of the leading theory (Hardy and Allsop 1991, Hardy and Higgins 1992). The amyloid cascade hypothesis highlights the deposition of Aβ plaques as the primary cause of AD, which is then believed to promote neurofibrillary tangles, cell death and accumulated brain tissue damages, eventually resulting in cognitive decline. The cleavage of APP through the amyloidogenic pathway leads to the production of Aβ peptides, which are normally degraded by a wide range of enzymes (Saido and Leissring 2012). The amyloid cascade hypothesis suggests an imbalance in Aβ production and/or clearance (Hardy and Higgins 1992), causing accumulation of Aβ. The Aβ peptides, mainly the 42 aa-long peptide, are highly prone to aggregate and to form oligomers and Aβ fibrils found in senile plaques.

The hypothesis was also based on the fact that trisomy 21 results in a 3rd copy of the *APP* gene (21q21) and that Down syndrome individuals have a very high risk of developing AD, as they produce more Aβ peptides (Hardy and Allsop 1991).

More than 200 mutations, mostly dominant, in the *APP, PS1* and *PS2* genes modulate APP processing and cause the hereditary form of AD (EOFAD) (https:www.alzforum.org/mutations). The mutations increase the amyloidogenic cleavage pathway of APP and thus the production of Aβ peptides (Citron, Oltersdorf et al. 1992), decrease the affinity of APP towards α-secretase (non-amyloidogenic pathway) or enhance the production of the Aβ42 form, highly prone to aggregate (Goate, Chartier-Harlin et al. 1991, Tanzi 2012, Selkoe and Hardy 2016). A protective mutation in the APP gene (A673T) reduced the β-secretase activity, thus reducing the amyloidogenic cleavage pathway (Jonsson, Atwal et al. 2012).

APP processing and Aβ production are part of the target aim for AD therapeutic strategies (Blennow, de Leon et al. 2006) and while studies focusing on mutations in *APP* and its processing are constantly improving our

understanding of the disease, the knowledge of the full physiological role of APP remains poor and is essential to provide insight of potential outcomes of treatment and their side effects.

# 1.4 ANIMAL MODELS TO STUDY APP

Evolutionary studies on APP revealed that the expression of APP and its homologues coincides with synapse formation during the establishment of the central nervous system. APP and APLPs are expressed in vertebrates including fish (reviewed by (Shariati and De Strooper 2013)), but are absent from prokaryotes. Fruit flies (*Drosophila melanogaster*) and nematodes (*Caenorhabditis elegans*) both express an APP orthologue, APPL and APL-1 respectively. APPL and APL-1 both express the YENPTY motif but lack the Aβ fragment (Daigle and Li 1993, Leyssen, Ayaz et al. 2005). APPL is implicated in axonal outgrowth and in integrity of the neuromuscular junctions buttons (Torroja, Packard et al. 1999), whereas APL-1 seems crucial for *C. elegans* development as its deletion is lethal (Hornsten, Lieberthal et al. 2007).

Most of our current understanding of APP comes from studies in rodents, especially mice. Mice express the *App* and the two *Aplp* genes. The generation of knockdown (KD) and knockout (KO) mouse models allowed the elucidation of several APP related functions. Whereas the single mutants have minor changes and non-severe phenotypes, most double and all triple mutants combinations are embryonically lethal (see **Table 1**) (review by (van der Kant and Goldstein 2015, Muller, Deller et al. 2017)).

Table 1. Lethality associated with knockout mice

| **Genotype** | **Lethality** |
|---|---|
| ***App$^{-/-}$*** | No |
| ***Aplp1$^{-/-}$*** | No |
| ***Aplp2$^{-/-}$*** | No |
| ***App$^{-/-}$ Aplp1$^{-/-}$*** | No |
| ***App$^{-/-}$ Aplp2$^{-/-}$*** | Yes, after birth |
| ***Aplp1$^{-/-}$ Aplp2$^{-/-}$*** | Yes, after birth |
| ***App$^{-/-}$ Aplp1$^{-/-}$ Aplp2$^{-/-}$*** | Yes, after birth |

*App*$^{-/-}$ mice are fertile, smaller and have a reduced body postnatal brain weight compared to wildtypes (Zheng, Jiang et al. 1995, Magara, Muller et al. 1999). Neuronal defects have been reported, which supports the role of *App* in the CNS development. Such defects include a decreased size of their forebrain commissure, impaired dendritic spine density and plasticity (Lee, Moussa et al. 2010, Zou, Crux et al. 2016) and impaired ventricular neurogenesis (Caille, Allinquant et al. 2004). Neurological studies reported decreased locomotor activity, reduced grip strength and learning impairment (Zheng, Jiang et al. 1995, Ring, Weyer et al. 2007). *App*$^{-/-}$ *Aplp2*$^{-/-}$ die shortly after birth with severe neuromuscular synaptic and NMJs deficits (Wang, Yang et al. 2005, Wang, Wang et al. 2009).

Mice are also widely used to investigate APP-related diseases, notably AD. Indeed, more than 200 models of AD are available (see (https://www.alzforum.org/research-models/alzheimers-disease)) (review by (Sasaguri, Nilsson et al. 2017). Mice carrying human mutations in *App* or in genes coding for secretases, exhibiting heavy plaque load and hyperphosphorylation of tau protein are the main models.

## 1.4.1 ZEBRAFISH AS AN *IN VIVO* MODEL

Zebrafish (*Danio rerio*) has gained in popularity in the past three decades as an *in vivo* model to study diseases and the numbers of publications on zebrafish are continuously rising (Ablain and Zon 2013). Zebrafish are small freshwater vertebrates and belong to the teleost family. Genome analysis revealed that more than 70% of human genes have a known zebrafish orthologue (Howe, Clark et al. 2013). Thanks to notably Charles B. Kimmel, Monte Westerfield, and George Streisinger as pioneers of the field, zebrafish are used in various research, such as studies in neuroscience, in toxicology screening, in oncology, and in drug development. Studies using zebrafish also provide insights on neurodegenerative diseases, such as AD (Leimer, Lun et al. 1999, Newman, Musgrave et al. 2007, Wilson and Lardelli 2013, Nery, Eltz et al. 2014, Bhattarai, Thomas et al. 2017, Pu, Liang et al. 2017, Wang, Zhang et al. 2021).

The use of zebrafish enables embryonic studies as early as one-cell zygote, as the fertilization of the eggs is *ex utero*. The developmental stages of zebrafish embryos have been intensively characterized and, under controlled temperature and conditions, are highly predictable (Kimmel 1989, Kimmel, Warga et al. 1990, Kimmel, Ballard et al. 1995). Thus, it is possible to address early developmental defects in zebrafish that would be challenging in rodents.

Similar to mice and rats, zebrafish are used for behavioural studies with several tests being comparable between species (review by (Basnet, Zizioli et al. 2019)).

Zebrafish characteristics provide great advantages for *in vivo* research. Indeed, adult females can normally lay several hundred eggs per breeding and can spawn several times per day. The embryos are practically transparent, and their pigmentation can be halted or prevented pharmacologically to facilitate studies at later time points. Their initial development is rapid as the hatching period ends at 3 days post-fertilization (dpf). Their brain and neural tube segmentations start as early as 14 hours post-fertilization (hpf) and the major organs are developed by 5 dpf (Kimmel, Ballard et al. 1995). Zebrafish genome modification technics are commonly used and described as established methods (methods compiled in (Zebrafish: Methods and Protocols (2016)). A zebrafish-specific database is also available with genetic and genomic data (Zebrafish Information Network (ZFIN) (Ruzicka, Howe et al. 2019)).

## Zebrafish app

Human *APP* and *APLPs* orthologues are present in zebrafish; *app*, *aplp1* and *aplp2* (for nomenclature see **Table 2**). Since the teleost family have been through a partial third duplication of their genome (Gates, Kim et al. 1999, Glasauer and Neuhauss 2014), zebrafish have two homologs of *app*: *appa* and *appb* (Musa, Lehrach et al. 2001, Liao, Wang et al. 2012).

Table 2. Gene and protein nomenclature

| ***Species*** | ***Gene*** | ***Protein*** |
|---|---|---|
| Human | *APP* | APP |
| Mouse | *App* | APP |
| Zebrafish | *appa*, *appb* | Appa, Appb |

At the gene level, *appa* (chromosome 1) encodes Appa with high similarity with the human APP770 isoform and *appb* (chromosome 9) encodes the Appb protein, with high similarity with the CNS-restricted APP695 splice isoform (Joshi, Liang et al. 2009) (see **Figure 5**). The sequences alignment shows a 72% homology between Appa and Appb proteins. Structures and domains are also conserved between species. Protein sequence alignment showed that zebrafish Apps and human APP share more that 60% homology, with a 92% and 100% similarity of the C-terminal part of Appa and Appb respectively (Joshi, Liang et al. 2009). Both Appa and Appb contain the Aβ domain. Although still not shown, the processing of Appa and Appb is likely similar to the human APP as the secretases involved in the processing machinery, such as the α-, β-, and γ-secretase, are expressed in zebrafish (Newman, Musgrave et al. 2007, Wilson and Lardelli 2013).

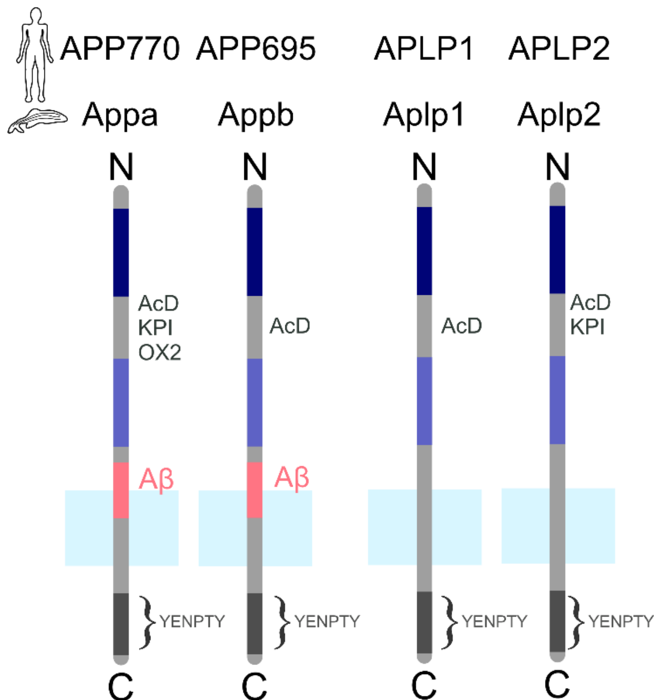


***Figure 5.*** *Human APP and APLPs and zebrafish orthologues. Zebrafish express Appa and Appb paralogues, both containing the Aβ domain (pink), and Aplp1 and Aplp2. YENPTY motif (dark grey) and plasma membrane in pale blue.*

The first study on *appa* and *appb* mRNA expression revealed overlapping patterns during early development as well as expression pattern specific to each paralogues (Musa, Lehrach et al. 2001) (see **Figure 6**).

***Figure 6.*** *Simplified representation of appa (in red) and appb (in green) mRNA expression in 24 hpf zebrafish larvae.*

Both *appa* and *appb* expressions seem to appear around the mid-gastrulation stage of the embryo and their expression, along with the *aplp*s remain through adulthood. *appa* and *appb* are expressed in the developing CNS (Musa, Lehrach et al. 2001), however, during the next hours of development, *appa* expression seems to be the major zebrafish *app* paralogue in the somitogenesis-related tissues, such as the paraxial segmental plate, the presomitic mesoderm and the somites. Contrary, *appb* is shown to be expressed in the neural rod, the developing spinal cord and motor neurons (Musa, Lehrach et al. 2001, Abramsson, Kettunen et al. 2013, Banote, Edling et al. 2016) and seems to be restricted to the neuronal tissue at later stages (Abramsson, Kettunen et al. 2013).

# 2 AIM

## 2.1.1 GENERAL AIM

The overall aim of this thesis was to study the physiological role of the amyloid precursor protein.

## 2.1.2 SPECIFIC AIMS

**Paper I**

To characterize for the first time a zebrafish knockout mutant carrying a homozygous deletion for one of the *app* paralogues (*appb*) and to address *appb* cellular functions.

**Paper II**

To study the implication of *appb* in zebrafish larvae on the assembly of the trigeminal ganglia (TG).

**Paper III**

To report the localization of APP to the ependymal cilia and to analyse function in ependymal ciliogenesis.

**Paper IV**

To characterize the proteomic profile of *appb* $^{-/-}$ zebrafish using a tandem mass tag mass-spectrometry and a molecular approach.

**Paper V**

To address genetic compensation and translational adaptation mechanisms in *appb*-deficient zebrafish.

# 3 MATERIALS

# 3.1 ANIMAL AND CELL MODELS

## 3.1.1 ZEBRAFISH HUSBANDRY AND LINES

Zebrafish (*Danio rerio*) were used as the main *in vivo* model of this thesis (**papers I-V**). Zebrafish husbandry facility is located at the Institute of Neuroscience and Physiology, University of Gothenburg. Zebrafish were maintained in Aquatic Housing System from Aquaneering (San Diego, USA) at 28 °C, under a 14:10 hour light:dark cycle. Zebrafish were fed twice daily, with both dry and live-hatched food. Embryos and larvae, up until 5 days post-fertilization (dpf), were kept in dark incubator at 28.5 °C, in embryonic medium (EM) changed daily, unless stated otherwise. When needed, 0.2 mg/mL tricaine (ethyl 3-aminobenzoate methanesulfonate, MS-222) was added to the EM as an anaesthetic agent and 0.003% PTU (1-phenyl-2-thiourea) to prevent pigmentation.

Zebrafish wild-type lines used were AB and *appb*$^{+/+}$. CRISPR/Cas9-system-generated zebrafish mutants lines used were *appb*$^{26_2/26_2}$ referred as *appb*$^{-/-}$, *appa*$^{21_16/21_16}$ referred as *appa*$^{-/-}$, *appa*$^{21_16/21_16}$ /*appb*$^{26_2/26_2}$ referred as *appa*$^{-/-}$ /*appb*$^{-/-}$, and *appb*$^{P17\Delta}$. Morpholino antisense oligomer (MO) used was *appb* MO (splice acceptor site *appb* MO; 5′-CTCTTTTCTCTCTCATTACCTCTTG-3’) from GeneTools and the transgenic lines *Tg(Trpa1b:GFP)*, *Tg(Isl1(SS):Kaede)* (Pan, Choy et al. 2012) (see **Table 3**).

Table 3: Zebrafish lines used in this thesis

| *Category* | *Genotype* | *Specificity* | *Paper* |
|---|---|---|---|
| *Wildtype* | *AB* | Background fish line | **I, II, III, V** |
| | *appb*$^{+/+}$ | Siblings of *appb*$^{-/-}$ | **I,II,IV,V** |
| *Knockout* | *appb*$^{-/-}$ | Deletion in exon 2 of *appb* | **I,II,IV,V** |

| | | | |
|---|---|---|---|
| | *appa$^{-/-}$* | Deletion in exon 2 of *appa* | **III,V** |
| | *appa$^{-/-}$/appb$^{-/-}$* | Products of *appa$^{-/-}$* and *appb$^{-/-}$* adults breading | **III,V** |
| | *appb$^{P17\Delta}$* | Deletion of exon 1 and upstream sequence of *appb* | **V** |
| *Knockdown-morphant* | *appb* MO | AB zebrafish injected with *appb* MO | **V** |
| *Transgenic* | *Tg(Trpa1b:GFP)* | Trpa1 driving the expression of GFP | **II** |
| | *Tg (Isl1(SS):Kaede)* | Isl1(*SS*) promoter driving the expression of Kaede | **II** |

### 3.1.2 MICE AND HUMAN BRAIN SAMPLES

In **paper III**, to address APP localization to the ependymal cilia in higher vertebrates, adult mice and human brains sections were used for immunohistochemistry.

8–9 week-old C57Bl6/n mouse brains were kindly provided and prepared by Dr Debora Kaminski and Dr Fredrik Sterky, Institute of Biomedicine and The Wallenberg Centre for Molecular and Translational Medicine. Sagittal slices of 16 µm were stored in -80 °C prior to use. Brains sections were selected to stain the lateral, III and the IV brain ventricle.

Neurological healthy human post-mortem brains samples were kindly provided and immunostained by Professor Tammaryn Lashley, and obtained from the Queen Square Brain Bank for Neurological Disorders, UCL Institute

of Neurology, University College London. Brains sections were selected in the caudate nucleus region containing a portion of the lateral ventricle ependyme.

### 3.1.3 HUMAN CELL LINES

In **paper V**, to address mRNA decay effect in human cells, we used a neuroblastoma cell line (SH-SY5Y). SH-SY5Y cells originate from SK-N-SH cell line, where a clone (SH-SY) was isolated from a bone marrow biopsy of a young female with neuroblastoma (Biedler, Helson et al. 1973). The subcloning products, SH-SY5Y cells, are widely used *in vitro* as they exhibit neuron-like characteristics, such as dopaminergic and adrenergic markers and exhibit neurite outgrowth. They can be differentiated into neuron-like cells with neuronal supplements and culture media. Interestingly for this thesis, undifferentiated SH-SY5Y cells express, although to a low level, amyloid precursor protein (APP) and the secretases responsible for its processing (König, Masters et al. 1990). In **paper V,** SH-SY5Y cells were kept undifferentiated and were grown in DMEM and Ham's F-12 (DMEM/F-12) at 37 °C, with 5% $CO_2$ and 20% $O_2$.

### 3.1.4 ETHICAL APPROVALS

All studies (**papers I-V**) were approved by the local ethical committee of University of Gothenburg and by a London Research Ethics Committee at University College London, and followed the guidelines at the facility where samples were collected (**paper III**). All procedures using zebrafish were performed under standardized conditions (Westerfield 2000) and all procedures using animals were performed in accordance with the animal welfare guidelines of the Swedish National Board for Laboratory Animals. Studies using human samples (**paper III**) were also performed in accordance with the declaration of Helsinki (2013). Informed consent for donation of the tissue was obtain from the subject donors or from the next of kin/or legal guardian(s) of the donors. Storage of human samples was made under a license from the Human Tissue Authority.

# 4 EXPERIMENTAL METHODS

# 4.1 GENETIC MUTATIONS

In all papers (**I-V**), zebrafish mutant lines were used, generated with CRISPR/Cas9-mediated gene knockout method (Varshney, Pei et al. 2015). gRNAs synthesis for *appa* and *appb* mutations were done *via* a oligo-based method using DNA oligos. Annealing with a generic DNA oligo for the guide RNA and a DNA polymerase gave rise to the templates used for the *in vitro* transcription. Wild-type AB zebrafish embryos were microinjected into the yolk at the one-cell stage (see **Figure 9)** with the selected gRNA and with Cas9 mRNA. Screened and selected embryos were raised to adulthood and outcrossed with AB background zebrafish.

For *appa* mutagenesis, the selected mutant allele $appa^{21_16}$ exhibits a deletion of 10 base pairs (bp) in exon 2, introducing a frameshift mutation leading to a premature termination codon (**paper III**). For *appb* mutagenesis, two mutations on different alleles were selected: $appb^{26_2}$ and $appb^{26_4}$, both with a frame-shift mutation deleting respectively 5 and 8 bp in exon 2, resulting in a premature termination codon at the 3'-end (**paper I**). Zebrafish mutants ($appa^{-/-}$ and $appb^{-/-}$) were outcrossed with AB until F4 generation to avoid splice isoforms: offspring homozygous wildtypes and mutants were kept and raised to adulthood. For $appa^{-/-}/appb^{-/-}$ line generation, genotyped-confirmed homozygous $appa^{-/-}$ were bred with genotyped-confirmed homozygous $appb^{-/-}$ (**paper III**). For $appb^{P17\Delta}$ mutant, the deletion of the *appb* promoter was intended and the selected gRNA gave rise to a deletion of 972 nucleotides (nt), including the ATG region. Selected embryos were raised to adulthood and outcrossed with AB to obtain F1 generation (**paper V**). Genotypes were confirmed *via* Sanger Sequencing (Sanger, Nicklen et al. 1977, Shendure, Porreca et al. 2011, McGinn and Gut 2013).

## 4.2 ZEBRAFISH MORPHOLOGY

Studies of the gross morphology were done to address developmental morphology phenotypes between the wildtype lines, the morphants, and the mutant lines, accordingly to the previous descriptions (Kimmel, Ballard et al. 1995).

### 4.2.1 EPIBOLY STAGES

In **paper I**, early embryonic developmental rate, accordingly to Kimmel (Kimmel, Ballard et al. 1995), was compared between *appb*$^{-/-}$ and their wildtype siblings. One-cell stage, dome, shield, 75% and 100% epiboly developmental progression stages were timed. Eggs were collected and incubated in warm EM to avoid temperature changes and were kept at 28.5 °C between staging evaluation. Embryos were observed in a brightfield microscope, and the time of the developmental stage were noted, as well as the time of the eggs collection.

### 4.2.2 CELLULAR PROTRUSIONS AND ORGANISATION OF THE EVL

Cellular protrusions in **paper I** were observed in some of the *appb*$^{-/-}$ embryos, close to the enveloping layer (EVL) limits, around 2.25 hpf. Quantification of the frequency of the embryos showing this protrusion phenotypes were done by observing the embryos with a brightfield microscope. The visualization of the cellular organization was done *via* immunofluorostaining of the cellular membrane, using phalloidin dye, at the sphere (4 hpf) and germ ring stages (5.7 hpf), through confocal imaging.

### 4.2.3 BODY LENGTH

The body length of embryos was compared, in **paper I,** at one-cell stage, 24 hpf, 48 hpf, and 72 hpf. Embryos were anaesthetized, with the exception of one-cell stage embryos. Using a brightfield microscope, embryos were imaged, and their body length measured with the imaging software. For the one-cell stage embryos, the length was measured across the yolk and the whole body length of older embryos (24-72hpf) were measured in an apical-caudal way: from the tip (24 hpf) or the front of the head (48-72 hpf) to the end of the tail.

### 4.2.4 CELLULAR AGGREGATION

To address cellular adhesion properties in **paper I**, an aggregation assay was performed. Cells were extracted from *appb*$^{+/+}$ and *appb*$^{-/-}$ whole embryos. To distinguish later the cells genotypes, the embryos were previously microinjected with a fluorescent dye, either red or green (see **Figure 9**). In a sterile environment, cells were strained and seeded in Petri dishes, and incubated at 28.5 °C. Since the aim was to look at how mutant cells would adhere to others to form aggregates, cellular clumps made of wild-type cells only were compared with clumps of mixed wild-type and mutant cells: *appb*$^{+/+}$ + *appb*$^{+/+}$ and *appb*$^{+/+}$ + *appb*$^{-/-}$. Cellular aggregates were analyzed at different time points, as previously described (Schotz, Burdine et al. 2008), to evaluate if the deletion of *appb* affects the adhesion or organization properties.

### 4.2.5 TRIGEMINAL GANGLIA MORPHOLOGY

In **paper II**, we addressed the morphology of the trigeminal ganglia (TG) in. To visualize the TG in whole-mount larvae zebrafish, cells were immunofluorostained against HuC/D (*Elavl3/4*), a pan-neuronal marker.

Using Imaris (Bitplane™) image analysis program, a digital surface was created *via* the *Surface tool*, masking the original HuC/D signal of the TG. To quantify the length of the TG, a region of interest (ROI) was created around the TG surface, generating a rectangular box with $x$, $y$ and $z$ coordinates. Between samples, the $z$ coordinates, corresponding to the depth of the TG, was similar. Therefore, the hypotenuse of this ROI box corresponded to the approximate TG length. Hypotenuse calculation was made from a right triangle equation, with $c$ = hypotenuse: $a^2 + b^2 = c^2$.

The total cell numbers and the number of the sensory neurons subpopulations were calculated using both immunofluorostaining and fluorescent *in situ* methods. Cells were manually counted using Imaris *Spots object tool*.

### 4.2.6 MAUTHNER CELLS

It was previously reported that *appb* knockdown in zebrafish *via* morpholinos (*appb*MO) led to a Mauthner cells (M-cells) defect, as some *appb*MO embryos lack one or both M-cells (Banote, Edling et al. 2016). In **paper V**, we addressed this phenotypic defect in two of our genetic knockout models for *appb*: *appb*$^{-/-}$ and *appb*$^{P17\Delta}$. To visualize the M-cells, we immunostained against

neurofilament RMO433 as described before (Banote, Edling et al. 2016). As the M-cells are located ventrally in the hindbrain, brain larvae dissection prior to imaging is necessary (Turner, Bracewell et al. 2014). Following the last wash of the immunostaining protocol, larvae were dissected on an agarose plate using handmade tools with a dissecting needle. To ensure a better imaging and conserving of the brain tissue integrity, eyes were removed using the dissecting needle, followed by the yolk and the external skin. Then, the jaw along with the epidermis were removed and the hindbrain cleared. Larvae with cleared hindbrain were mounted ventrally for imaging.

### 4.2.7 NEUROEPITHELIUM CILIA LENGTH

In **paper III**, length of the cilia covering the neuroepithelium of the brain ventricle was measured in whole-mounted larvae, using immunofluorostaining signal against acetylated tubulin. Laser scanning confocal imaging was performed from the most dorsal portion of the embryo brain ventricle to ~50µm deep in the ventricle. Using Imaris program, a 3D-representation of the confocal stack was done to visualize all neuroepithelial cilia. Then, using the *Measuring tool* and by selecting the *A-B points* option, all cilia were manually measured, following the fluorescent signal. Total number of cilia and length measured for each cilium were extracted.

### 4.2.8 BRAIN VENTRICLE MORPHOLOGY

To measure brain ventricles size in **paper III**, we used 2 dpf live larvae. Anesthetized embryos were placed on agarose plates, previously molded with rows, and covered with EM + tricaine. Embryos were immobilized on their ventral side, within the rows to stabilize them. With a microinjector, Rhodamine-conjugated dextran was injected in the brain ventricle, at the caudal position of the hindbrain (see **Figure 9**). Injections were confirmed with a wide field fluorescence microscope, and the positive embryos were mounted on their back, in agarose. Confocal imaging was done to image the whole brain ventricle region, easily detectable with the fluorescent rhodamine-dextran dye. Stacked images where processed with Imaris were a digital 3D-surface was created with the *Surface tool*. The volume and area measurement on the surface were extracted. The lengths of specific regions of the ventricles were measured using the *Measuring tool*, where points on the ventricles surface were set manually.

## 4.2.9 RNA EXPRESSION

### *In situ* hybridization

To detect *app* and *aplp* mRNA expression in whole-mount larvae zebrafish in **paper I**, *in situ* hybridization (ISH, or whole-mount WISH) was performed.

Antisense RNA probes, labelled with digoxigenin (DIG) previously described were used (Musa, Lehrach et al. 2001, Combs, Song et al. 2012). Probes were linearized from a DNA template, purified and stored at -20 °C prior to use.

Embryos were dehydrated in MeOH gradients and stored at -20 °C until use. Then, embryos were rehydrated in a decreasing MeOH gradient and permeabilized using proteinase K for 10 min, since embryos were 24 hpf. After rinsing to stop the reaction, embryos were postfix. Prior to the hybridization, the RNA probes were incubated at 70 °C for 5 min. Embryos were then hybridized with specific DIG-probes at 70 °C overnight, followed by the incubation with anti-DIG alkaline phosphatase conjugate antibodies. Then, the detection method used required a dark blue-purple precipitate obtained after the incubation of the hybridized embryos with the combination of nitro-blue tetrazolium chloride (NBT) and 5-bromo-4-chloto-3'indolyphosphate p-toluidine salt (BCIP). NBT/BCIP mix reacts with the alkaline phosphatase of the antibody. When ready, the embryos were flat- or side-mounted and the detection of the RNA signal was done with a brightfield microscope.

### Fluorescent *in situ* hybridization

In **paper III**, we wanted to detect *app*s RNA signals in the whole-mount embryo tissue with a higher resolution, notably to get the expression signal of the brain ventricles from *appa* and *appb*. Thus, fluorescent ISH (FISH) (see **Figure 7**) was performed to detect *appa* and *appb*, as well as *aplp1* and *aplp2*.

As for ISH described above, antisense DIG-probes were used for hybridization. FISH can also be used as co-hybridization method to perform a double detection, with two or three differently labelled-probes, for instance DIG, 2,4-dinitrophenyl (DNP) or fluorescein (Lauter, Söll et al. 2014). However here, a simple hybridization using only one probe per experiment was done for each sample. Next, contrarily to a coloured precipitated obtained with ISH, a fluorescent intense signal is needed for proper microscopy detection.

An antibody against DIG conjugated with a peroxidase (POD) was used. Then to detect the antibody and most importantly to increase the signal, vanillin was added a POD enhancer. The fluorogenic signal was done by the addition of a tyramide signal amplification. In **paper III**, we used FAM-tyramide solution emitting a signal at the wavelength of 488nm. Detection of all *app* gene family members was done by confocal microscopy imaging.

In **paper II**, FISH was used to quantify *trkC* and *trpv1* expressing sensory neurons of the TG. Probes were purified from DNA of PCR products and purified. Here, again a single round of FISH per experiment was performed and a DyLight 633-tyramide, emitting a signal in the far red at 633nm, was used.

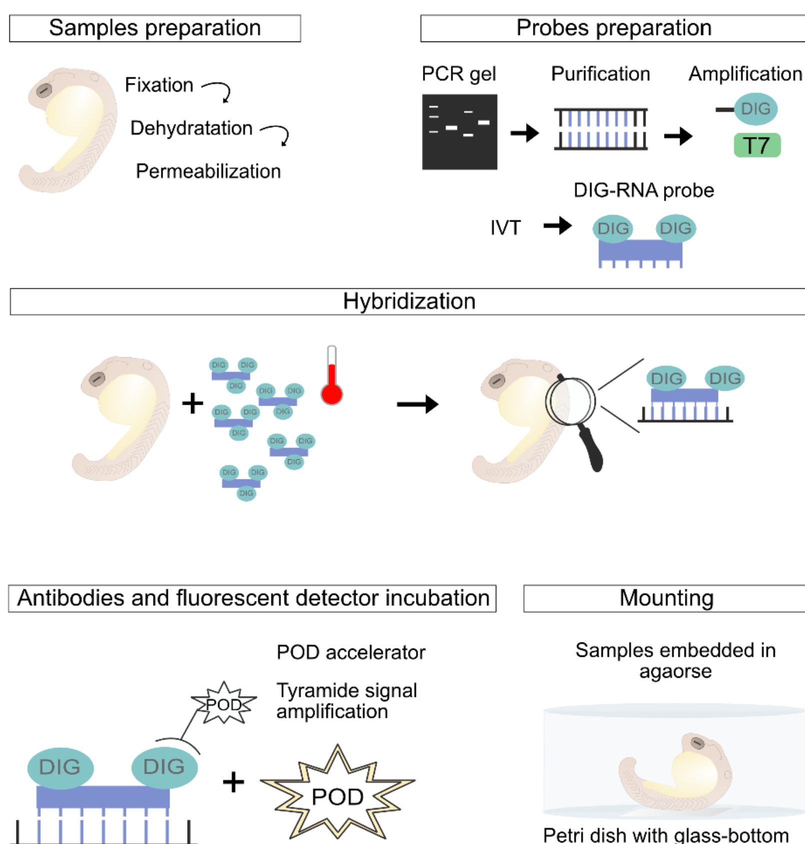


***Figure** 7. Workflow of whole-mount fluorescent in situ.*

### Quantitative Polymerase Chain Reaction

Real-time quantitative polymerase chain reaction (qPCR) was used to detect levels of mRNA in **paper I**, **III**, **IV** and **V**. With the exception of one round using SYBR Green assay (*appa*, **paper V**), all other gene assays tested were done using inventoried TaqMan Gene Expression Assays.

For the genes tested, the TaqMan assays probes had a FAM reporter dye. Elongation Factor 1 Alpha 1, Like 1 (*eef1a1l1*) and Actin, Beta 1 (*actb1*) were both measured as housekeeping genes for relative calculation to avoid changes due to development-associated expression. The qPCR analysis was done by calculating the relative mRNA levels *via* the ΔΔCt method (Livak and Schmittgen 2001). cDNA extracted from a mix of embryos, larvae and adults wild-type zebrafish were pooled and used for calibration. Values were adjusted in relation to the house keeping gene values.

## 4.2.10 PROTEIN EXPRESSION

### Immunostaining

Proteins detection *via* antibodies immunofluorescent was largely used in this thesis, either to detect proteins localization, to count cells, to measure dendrites or to address phenotypes (**paper I**, **II**, **III** and **V**). The detection of the amyloid precursor protein was performed using antibodies with different epitopes (see **Figure 8**).

For whole-mount immunostaining, embryos and larvae were fixed either for 2h or overnight in 4% paraformaldehyde (PFA). For the M-cells staining experiment (**paper V**), 2% trichloroacetic acid (TCA) was used as a fixing agent to avoid embryos to become rigid. Subsequent washes with Triton X-100 allowed the permeabilization process. Embryos were incubated in blocking solution composed of bovine serum albumin (BSA), normal goat serum (GS), Triton X-100 and dimethyl sulfoxide (DMSO) before incubation with primary antibodies. Followed by an incubation was done in the dark with Alexa Fluor secondary antibodies. Fixed and stained embryos were kept in 4 °C, in dark, prior to mounting and imaging. In **paper III**, in addition to whole-mount immunostaining, protein expression was detected on mice and adult

zebrafish brains slices. Cryoslices were kept at -80 °C and rehydrated upon use. In addition to the permeabilization steps, slices were incubated with a dye, Sudan Black B (SBB) in EtOH. Being highly soluble in fat, SSB dye will stain the lipids of the tissue with an intense black colour. As the brain tissue is highly auto-fluorescent, mainly because of its composition high in lipids, SSB step helps to decrease the background noise. Washes, antibodies incubations and mounting followed. Also shown in **paper III**, human brain slices were immunostained, however the detection was done with a secondary antibody conjugated with horseradish peroxidase (HRP).

### Western Blot analysis

Western blot analysis was used to confirm the loss of App proteins in our zebrafish mutants in **paper I** and **III**. Antibody availability for zebrafish proteins is still expanding, and there is a lack of antibodies that specifically recognize zebrafish App and App-like proteins. However, monoclonal antibodies targeting different epitope regions of the human and mice APP are available and some of these cross-react with zebrafish App (see **Figure 8,** for the antibodies used in this thesis).

In **paper I**, to show the loss of Appb protein in the $appb^{-/-}$, we used our G-protein-purified in house-generated rabbit polyclonal antibody (EER15) targeting specifically the zebrafish Appb paralogue. EER15 was generated against the N-terminal half of the Aβ peptide. As a control for quantification analysis, GAPDH signal was used as a loading control.

In **paper III**, to measure the protein levels in $appa^{-/-}appb^{-/-}$, we performed used the combination of a mouse anti-amyloid precursor protein A4 (clone 22C11) and a rabbit anti-amyloid precursor protein monoclonal antibody (Y188), which has a high homology with Appb (100%) and Appa (93%).

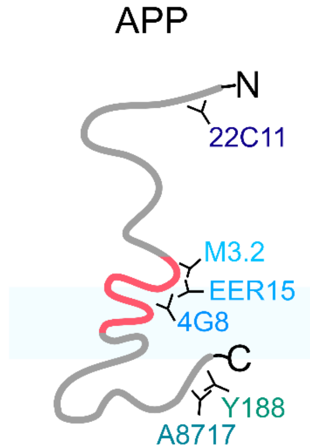


***Figure 8.*** *Epitopes of the antibodies used to detect App. Schematic of App structure with the Aβ peptide sequence in pink and the cellular membrane in pale blue.*

### 4.2.11 MICROINJECTIONS

Microinjections were done with a microinjector apparatus, using a borosilicate injection needle prepared with a micropipette puller. In **paper I**, for the aggregation experiment, to identify wildtype cells from the *appb*$^{-/-}$ cells, embryos were microinjected with two different fluorescent-conjugated dextrans. Injections were performed in the yolk, close to the syncytial layer (*see **Figure 9***) and to ensure a good ratio of successfully coloured cells, no embryos passed four-cell stage were injected, but were discarded. In **paper III**, fluorescent conjugated dextran was once more injected, but this time in 2 dpf embryos. Microinjections were performed in the dorsal side of the hindbrain ventricle, without performing the inner tissue. The injected dextran would fill the brain ventricles without diffusing passed the ependymal layer, making it therefore possible to measure the ventricle lumen of each injected embryos with confocal imaging. In **paper V**, embryos were microinjected with *appb*MO.

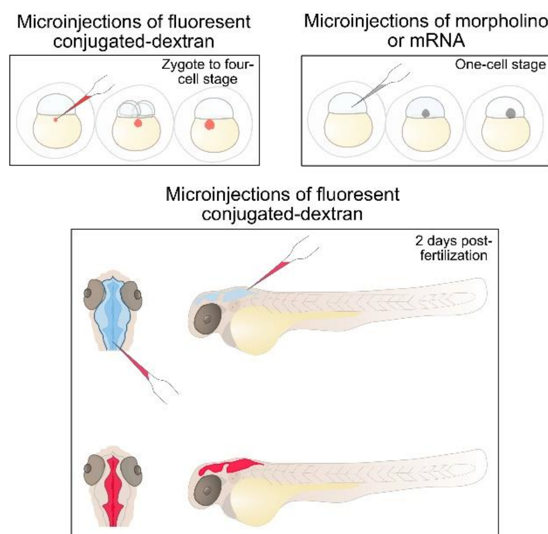


***Figure 9.*** *Types of microinjections in zebrafish embryos. Genomic material or fluorescent dye injected in the yolk, cell or in the brain ventricle of live zebrafish.*

## 4.2.12 TMT LABELLING AND LC-MS/MS

In **paper IV**, in collaboration with Associate Professor Johan Gobom, Department of Psychiatry and Neurochemistry, University of Gothenburg, tandem mass tag (TMT) followed by liquid chromatography mass-spectrometry (LC-MS) were done to identify changes in *appb*$^{-/-}$ zebrafish larvae at the peptidomic level.

To segregate in part peptides found in the CNS and the rest of the body, 3 dpf larvae both *appb*$^{+/+}$ and *appb*$^{-/-}$ were anaesthetised, and head and trunk were dissected. Samples preparation included proteins lysis in buffer containing urea which denatures the proteins and digestion using trypsin. TMT was added to the cleaved peptides. Here, a mix of purchased 10 isobaric different TMT (10plex) were used. The cleaved peptides are then combined with the TMT labels samples for the multiplex step where the proteome reactive group of the TMT bonds to the N-terminus of the peptides. Each tag being isobaric has the same mass, but contain a different mass reporter, due to the different isotope labelling, which is cleaved during the MS.

The mass reporter tag of each TMT is therefore detectable is the early spectrum of the chromatogram. On the same graph are found the peptides separated with the mass over charge ratio with their intensity. Data processing was performed using Proteome Discoverer 2.5 software and peptide identification *via* the Mascot software, using the UniProtKB Swissprot and TrEMBL databases. Conversion of protein to gene ID was done using UniProt and DAVID v6.8 (Database for Annotation, Visualization and Integrated Discovery) database and functional gene ontology annotations was done using PANTHER GO Ontology database.

### 4.2.13 STATISTICAL ANALYSIS

For all experiment of this thesis, statistical analysis was performed using GraphPad Prism software with significance levels set at $\rho<0.05$ (*), $\rho<0.01$ (**), $\rho<0.005$ (***) and $\rho<0.0001$ (****). When measuring two groups with a normal distribution, two-tailed unpaired Student's *t*-test were performed on the samples. Two-way ANOVA analysis was performed for the statistical analysis with the genotype and time variable over the epiboly progression (**paper I**). D'Agostino&Pearson normality test ($P < 0.0001$) and non-parametric two-tailed Mann-Whitney U tests were performed on non-normally distributed samples (**paper III**). In **paper V**, in addition to a $\rho$ value of $< 0.05$, a significant fold change (FC) parameter for protein abundance was set to FC $\geq$ 20%.

# 5 RESULTS AND DISCUSSION

# 5.1 PAPER I

## 5.1.1 RATIONALE

To address physiological functions of *appb*, we used the genomic editing method, CRISPR/Cas9, to generate mutant zebrafish carrying homozygous deletions in *appb*. Knockdown of *appb* have been previously described (Joshi, Liang et al. 2009, Combs, Song et al. 2012, Abramsson, Kettunen et al. 2013, Banote, Edling et al. 2016). and give, depending on knockdown level, defects in notochord formation, motor neurons and neuromuscular junctions (Abramsson, Kettunen et al. 2013) (Combs, Song et al. 2012), and in the Mauthner cells development (Banote, Edling et al. 2016) and even embryonic lethal with severe defects in the convergence-extension process during gastrulation (Joshi, Liang et al. 2009). As the effect of KD declines after 3 dpf combined with the embryonic lethal phenotype of *appb* KD prevents studies at later stages of development we, therefore aimed to investigate the role of *appb* by generating *appb*KO zebrafish lines.

In this paper, we aimed to address the role of *appb* by characterizing phenotypic alterations in the *appb* mutant zebrafish larvae during embryonic development. Although *App* single KO mice (*App*$^{-/-}$) have been subject of several reports, the external development of zebrafish larvae facilitates studies of cellular processes during early development.

## 5.1.2 STUDY RESULTS/DISCUSSION

Our objective in **paper I** was to elucidate *appb* functions and for this purpose we generate *appb* mutant (*appb*$^{-/-}$) zebrafish. To create a stable zebrafish line, carrying homozygous mutations in the *appb* gene, we used the CRISPR/Cas9 technology to create insertions and/or deletions in the first and second exon. Zebrafish with frame shift mutations introducing premature stop codons were selected and here we used two fish line with mutations in the 3'-end of exon 2.

As this was a novel *appb*$^{-/-}$ zebrafish model, we confirmed the mutation through Sanger sequencing and measured the App protein levels in the *appb*$^{-/-}$ larvae and adult brains. As there were no commercially available antibody specific for Appb, we generated an in-house antibody targeting the N-terminal sequence of the Aβ domain. The N-terminal region of App family members show a lower homology than the C-terminal region. We showed that mutants have a significant decrease in the protein levels. As other antibodies

recognizing both Appa and Appb still showed the presence of protein, we concluded that the loss of protein expression corresponds to the Appb levels.

In mice, *App*$^{-/-}$ are viable and fertile with minor phenotypic changes (Heber, Herms et al. 2000). However, a lower body weight, decrease in grip strength and locomotor activity were observed (Müller, Cristina et al. 1994, Zheng, Jiang et al. 1995, Ring, Weyer et al. 2007). Here, we reported that the single deletion of *appb* is partly lethal in zebrafish and resulted in shorter embryos, up until 2 days post-fertilization (dpf), where the *appb*$^{-/-}$ body length equalizes to their wild-type siblings. Previous reports have shown that the deletion or overexpression of *App* resulted in a decrease or a hyperactivity in adult mice, respectively (Ring, Weyer et al. 2007, Rodgers, Born et al. 2012). Here, our behavior study on *appb* larvae revealed no change in swimming speed or distance. The discrepancies between mice and zebrafish could be explained by the age of the models used or due to a compensation by *appa.*

We also noted a delay in the embryonic developmental stages in the *appb* KO embryos. Moreover, during blastula stages we noted that, in around 15% of the *appb*$^{-/-}$, cells were protruding out from the enveloping layer (EVL) and exiting the embryonic core. However, the *appb*$^{-/-}$ embryos show high resilience, as the majority survived and continued their development. As the survival rate is high and as most embryos were only affected with a delayed in the development, this subtle phenotype can be easily missed. To which extent protrusion are present in rodent *App*$^{-/-}$ is difficult to know as these observations are done 2.25 hours post-fertilization.

Early developmental processes including epiboly formation, gastrulation and convergent-extension movements critically depend on cells adhesion (Wallingford, Fraser et al. 2002). The cellular protrusions observed here highly indicate a decreased adhesive property that may give rise to the abnormal cellular EVL organization resulting in cell protrusions. Abnormal EVL morphology and arrangement were previously reported in zebrafish mutants exhibiting defects in cell adhesion, including the adhesion junction protein E-cadherin and EpCAM (epithelial cellular adhesion molecule), which is required for epithelial integrity (Slanchev, Carney et al. 2009, Song, Eckerle et al. 2013). The observed EVL defects in the *appb*$^{-/-}$ embryos further support the implication of App in cell adhesion. The normal expression pattern of E-cadherin, β-catenin and ZO-1 in our mutants suggest that the defects observed does not disturb the distribution of these proteins. However, App may influence their processing through protein interaction or regulate the expression of other adhesion molecules (Soba, Eggert et al. 2005).

The fact that the majority of *appb*$^{-/-}$ embryos are viable and survive despite the cellular protrusion suggests a robustness of cellular organization indicating that other App-family members may also compensate the deletion of Appb. To address if the defects observed in the cell organization were due to defect in cell-cell adhesion, we stimulated shedding of cadherins by ionomycin exposure. Indeed, *appb* mutants were more sensitive to further disruptions in cell adhesion and ionomycin treatment resulted in death in almost all *appb*$^{-/-}$ embryos, whereas the wild-type embryos did show EVL defects, but yet survived.

The extracellular adhesiveness allows cells to adhere and aggregate with other cells. However, differences in adhesive property of cells are partly responsible for creating cell layers. Thus, if cells with different adhesive properties are mixed, will forming cell-aggregates where cells are segregating from each other depending on their adhesiveness. Our results showed no major defects in cellular aggregation as the *appb*$^{-/-}$ cells were participating in forming aggregates, which is supported by the fairly normal development of embryos and larvae. However, deeper analysis revealed that wildtype and *appb*$^{-/-}$ cells started to segregate and were less prone to intermix, compared to a mix of wild-type cells only. Hence, together these results strongly suggest an implication of App in cell-cell adhesion.

Considering App's wide expression pattern and conserved structure it is likely that the lack of severe phenotype in knockout *appb* is due to the presence of the *appa* paralogue and its redundancy of the other *app*-family gene members (review by (Shariati and De Strooper 2013)). To evaluate if *appa*, *aplp1* and *aplp2* RNA levels are modulated by the deletion of *appb*, we used two methods: a semi-quantitative method allowing spatial localization of the mRNA expression *in situ* and a quantitative method. Similar to previous reports in mice (Zheng, Jiang et al. 1995, von Koch, Zheng et al. 1997, Heber, Herms et al. 2000), no significant changes were observed in the *appa* and *aplp*s mRNA levels using qPCR. However, whole-mount *in situ* hybridization showed clear upregulation of *appa* and *aplp2* mRNA expression. The cause to this discrepancy is not clear but may be due to that the latter detect the total mRNA while qPCR only detect the presence of two exons that may be more sensitive to splice alterations or nucleotide variations. An increase of App in *appb*$^{-/-}$ larvae was also supported by western blot analysis of protein level using an anti-APP antibody targeting the C-terminal part of the human protein. In mice, the antibody specificity to APP was demonstrated, but not in zebrafish. However, even if it is impossible to claim the same antibody specificity between the *app*-family members, we can at least state an increase of protein, thought to be Appa. Further studies and experiments are however needed to

address which *app*-family member(s) is/are increased. The minor phenotypes and the increase in *appa* and *aplp2* mRNA levels *in situ* yet suggest shared functions by the *app*s/*aplp*s, acting as compensation (Shariati and De Strooper 2013).

In addition to generate a knockout model for *appb*, we here demonstrated for the first time the implication of Appb in cell-cell adhesion *in vivo*. As App/APP is expressed and implicated through development and at adult stage, it will be interesting to evaluate App/APP-mediated adhesion at later stages.

# 5.2 PAPER II

## 5.2.1 RATIONALE

Through the characterization study of our *appb*$^{-/-}$ zebrafish during development (**paper I,** (Banote, Chebli et al. 2020)), we reported a role of *appb* in cellular adhesion and confirmed the expression of *appb* in the central nervous system (CNS) and in the trigeminal ganglia (TG) of the zebrafish, as also previously shown (Musa, Lehrach et al. 2001). While studying the *appb*$^{-/-}$ larvae, we noted inconsistencies in the shape of the TG between the mutant and wildtype zebrafish.

TG are groups of sensory neurons outside the brain and spinal cord, located on both side of the hindbrain. Their function is to sense stimuli from the environment and relay the information to the CNS. No study previously showed the implication of *appb* in the formation of the TG. We therefore set out to address the implication of *appb* in the TG formation.

## 5.2.2 STUDY RESULTS/DISCUSSION

The size analysis of the TG was the initiating point of this study, as we meant to quantify the observed difference in the TG morphology of our *appb*$^{-/-}$ larvae. Using a pan-neuronal marker we showed that the *appb*$^{-/-}$ larvae exhibit a less condensed and thus longer and more scattered TG through the first days of their development, suggesting TG organization defects.

Previous *in vivo* studies on cell adhesion and TG morphology integrity have highlighted the role of cadherins in the TG aggregation. Indeed, cadherin-2 (*cdh2*), also known as N-cadherin, is expressed in TG in zebrafish (Liu, Kerstetter et al. 2003) and *cdh2* mutants showed a more scattered and looser TG in (Kerstetter, Azodi et al. 2004, LaMora and Voigt 2009). More recently, it has been shown in chick, that the modulation of another cadherin, cadherin-7, also alters the TG organization (Wu and Taneyhill 2019). Furthermore, N-cadherin knockdown also resulted in TG aggregation defects, similar to those observed in the *appb* mutants. The authors also reported the N-cadherin implication in TG formation to be in robo2-dependent manner (Shiau and Bronner-Fraser 2009). Robo2 is a receptor that previously has been shown to act along with its ligand, slit1, in the TG assembly (Shiau, Lwigale et al. 2008). Interestingly, APP has been reported to be a receptor for Slit1 (Wang, Li et al. 2017). Although our current study is too preliminary to identify the molecular pathway leading to a looser TG in the *appb*$^{-/-}$ larvae, it is tempting to speculate

that Appb modulate TG condensation by promoting cell adhesion or through interaction with other cell adhesion molecules such as cadherins or through Robo2/Slit1 pathway.

We wanted to investigate if the elongated shape of the TG was due to a higher number of cells recruited, hence increasing the size of the TG. However, we showed that the number of Islet1 (Isl1) + cells and Isl1/HuC/D+ cells are significantly lower in the TG of *appb*$^{-/-}$ larvae. In vertebrates, the TG sensory neurons are formed by cells originating from two cranial placodes (the optic (opV) and the maxillomandibular (mmV)) and from the cranial neuronal crest. The TG genesis is initiated with the specification of the placodal cells (D'Amico-Martel and Noden 1983, Raible and Kruse 2000, Baker and Bronner-Fraser 2001). Cells will then mature into early-differentiated neurons expressing Isl1 before forming a pool of differentiated neurons. Our result show that *appb*$^{-/-}$ have fewer Isl1+ cells suggesting that App might promote specification of the later differentiation of the TG progenitors. Thus, the increased size of the TG in *appb*$^{-/-}$ does not depend on an increased cell number but is likely due to a change in cell interaction or condensation.

Alterations of the Isl1+ cells population and recruitment has been described before, notably in chick, in the presence of Notch modulators (Lassiter, Ball et al. 2010). In fact, pharmacological inhibition of Notch resulted in an increased differentiation of precursor into Isl1+ cells, as inhibition of the differentiation processes was partially blocked. Contrary, when the Notch downstream signalling was activated, the neuronal differentiation into Isl1+ neurons was abolished (Lassiter, Ball et al. 2010). Thus, although our data are not sufficient to confirm a Notch-implication, they support a Notch-mediated loss of Isl1+ cells. In support, are our previous work showing that Appb act in concert with Notch in neuronal development (Banote, Edling et al. 2016). Here, the hypothesis was that since both Notch and App are γ-secretase substrates, a lower amount of App protein available for the proteases could favour the cleavage of Notch and stimulates its downstream signalling, resulting in a decrease in differentiation of the Isl1+ cells. Alternatively, the lower number of Isl1+ cells could be the direct result of reduced Appb expression and its downstream signalling (Kwak, Marutle et al. 2011). Further experiments are needed to evaluate if Notch signalling pathway is implicated. Such experiment could involve modulating Notch downstream by activators or inhibitors.

The condensation defect in *appb*$^{-/-}$ larvae could also be a reflection of migration defects. Indeed, cellular migration also acts in concert with the neuronal differentiation to form proper TG. Once neurons are differentiated, the placodal cells delaminate and initiate the migration process to the future TG

site (review by (Lassiter, Stark et al. 2014)). The migration process is guided by cells sensing ques in the environment and by their communication with neighboring cells. In fact, the migratory process of TG cells is known as a "chase-and-run" process (Theveneau, Steventon et al. 2013). It is suggested that though chemotaxis, the neural crest cells would chase the placodal cells and forcing them to run upon contact. The hit and run character of the migration was as well highlighted in the robo2/slit1 model, where upon ligation of robo2 with slit1, cells are prone to migrate (Shiau, Lwigale et al. 2008). As APP both is a conserved receptor for slit1 and known to interact with robo2, the TG defects observed in the *appb-/-* could therefore potentially be an App/Slit1-dependent migratory defect. Further studies are needed to understand the processes by which Appb orchestrates TG migration and condensation.

TG functions depend on the specification of somatosensory fate, as the TG sensory neurons should be able to detect and respond to diverse nociceptive stimuli such as heat, chemical and mechanical triggers. To do so, sensory neurons express specific sets of receptors and ion channels to allow cell response to different stimuli and give the cells their neurochemical profile (Lazarov 2002, Marmigere and Ernfors 2007, Basbaum, Bautista et al. 2009). TG sensory neurons can therefore be segregated *via* their stimuli profile. While zebrafish is still an emerging model in this field, researchers have described subpopulations of sensory neurons composing the TG (Pan, Choy et al. 2012, Gau, Poon et al. 2013, Gau, Curtright et al. 2017). Here, to investigate if the loss of *appb* alters differentiation of sensory neurons, we aimed to map the subpopulation of the TG. Proper integrity of the TG can indicate if the zebrafish would response adequately to the exterior environment. Our preliminary data showed that the subpopulation of sensory neurons expressing the transient receptor potential cation channel, subfamily A, member 1b (Trpa1b), the subfamily V, member I (Trpv1), and the neurotrophic receptor tyrosine kinase (TrkC) were not changed in number by the deletion of *appb*. At this point, we cannot exclude that the cell numbers of other TG subpopulations remain intact in the *appb*$^{-/-}$ larvae. In fact, we suspect that the decreased amount of Isl1+cells recruited to the TG results will affect the sensory neurons numbers. Further studies are ongoing to complete the mapping of the families composing the TG in larvae and to address potential behaviour phenotype related to the sensory neurons.

## 5.3 PAPER III

### 5.3.1 RATIONALE

The rationale behind **paper III** emerged while observing whole-mount immunostaining of larvae, as part of **paper II**, and more particularly while examining the cilia. We therefore became interested into this organelle and set out to investigate if App is involved the cilia.

Nearly every vertebrate cell exhibits at least one hair-like structure called primary cilium. The primary cilium is an essential, non-motile organelle emerging from the plasma membrane and formed by microtubules (Amador-Arjona, Elliott et al. 2011). They mainly act as sensory antenna for the control and maintain of vital cellular functions, notably for proper brain development (Whitfield and Chakravarthy 2009, Valente, Rosti et al. 2014, Guo, Otis et al. 2017, Guo, Otis et al. 2019, Park, Jang et al. 2019, Song and Gleeson 2019, Gigante and Caspary 2020). Other cells types, such as lung and kidney epithelium and brain ventricle ependymal cells are decorated with additional motile cilia (review by (Mitchison and Valente 2017)). Ependymal motile cilia create a circulating flow of the CSF in the brain ventricles through their oriented and coordinated beating (Ibanez-Tallon, Pagenstecher et al. 2004). Adequate CSF flow is crucial to maintain transport of nutrients and the clearance of waste metabolites (Sawamoto, Wichterle et al. 2006). Disturbance of CSF flow or obstruction of the ventricular canals have been reported in AD, normal pressure hydrocephalus, and in Down syndrome (LeMay and Alvarez 1990, Del Bigio 1993, Chan and Amin-Hanjani 2010, Ethell 2014, Simon and Iliff 2016, Raveau, Nakahari et al. 2017).

Even if cilia are protruding from the plasma membrane, they have a unique structure and protein content. The ciliary protein recruitment and trafficking are strict processes with only properly targeted proteins localizing to the cilia membrane (Garcia-Gonzalo, Corbit et al. 2011, Williams, Li et al. 2011).

As no previous reports had described a direct implication of APP and otic vesicle, nasal epithelium and brain ependymal cilia, here we aimed at addressing if APP is implicated in ependymal cilia genesis and functions.

### 5.3.2 STUDY RESULTS/DISCUSSION

Through the explorative whole-mount immunofluorescence experiment, we first aimed at studying cilia that are easy to visualize *in situ*. We revealed, for

the first time, the expression of App in motile cilia of the nasal pit and the kino- and stereocilia of the otic vesicle in larvae zebrafish. Both cilia from the olfactory and hearing apparatus relay sensory input from the environment to the CNS. These findings suggest that App might contribute to the stimuli relay starting from the tip of the cilia. Furthermore, the involvement of App in these cilia could perhaps explain the olfactory and hearing defects observed in AD (Doty 2009).

We were intrigued by the fact that App becomes localized to cilia of sensory neurons and then aimed to investigate if App might be involved in motile cilia covering the ependymal cells lining the brain ventricle walls since defects in these cilia potentially could be involved in decreased clearance buildup of Amyloid peptide aggregates. To this end, we then showed that App localizes to the neuroepithelium non-motile cilia in larvae. Through maturation, the neuroepithelial cells develop into the multi-ciliated ependymal cells (Narita and Takeda 2015, Fame, Chang et al. 2016). To confirm App localization into mature motile cilia of the ependyma, we used adult zebrafish brains and showed that App is localized to the cilia membrane. We also showed that APP localization to the ependymal cilia is conserved in adult mice and human. Although previously shown to be expressed by the ependymal cells in rodents and in human, APP localization to the ependymal cilia was, to the best of our knowledge, never reported. APP localization suggests a role of APP in ependymal cilia-related functions. Ependymal cilia participate in the generation of CSF flow (Spassky and Meunier 2017) and it was shown in zebrafish that ependymal cilia stimulates brain ventricles expansion during development (Olstad, Ringers et al. 2019). Our results could suggest that App participates in the brain ventricular development regulated by the ependymal motile cilia.

To address the role of *app* in ciliogenesis and in cilium-related functions, we created a zebrafish with knockout mutations in both *appa* and appb. To this end, we used the previously described zebrafish *appb*$^{-/-}$ (**paper I**) and generated an *appa* mutant with the CRISPR/Cas9 method. We confirmed a frame-shift mutation, creating a deletion in exon 2 of the *appa* gene by Sanger sequencing. The generated *appa*$^{-/-}$*appb*$^{-/-}$ double mutants showed a significant decrease in both *appa* and *appb* mRNA and the loss of App protein. Like the single mutants, *appa*$^{-/-}$*appb*$^{-/-}$ are viable and fertile.

We showed that the deletion of both *appa* and *appb* resulted in longer cilia of the neuroepithelium in larvae. In addition, our results showed that the ventricle volume is smaller in the *appa*$^{-/-}$*appb*$^{-/-}$ larvae. Abnormalities in cilium length can impact cilium movement (Lai, Gupta et al. 2011) and changes in cilium

motility has been linked to aberrant development of the ventricular system (Olstad, Ringers et al. 2019). Therefore, it is thus possible that the length defect in neuroepithelium cilia could explain the ventricular size defects observed. Studies are ongoing to address if loss of App affect cilium movement and CSF flow and if the brain ventricles remain smaller through development.

Previous reports have shown that primary cilia express APP and that the cilium-length is decreased upon exposure to Aβ peptides (Vorobyeva and Saunders 2018). *In vivo* studies in a mouse model modelling AD showed that the length of the primary cilia of the hippocampal dentate granule is reduced (Chakravarthy, Gaudet et al. 2012). Moreover, a study has shown that cultured cells from patients with DS, carrying a third copy of *APP*, have shorter primary cilium (Galati, Sullivan et al. 2018). Thus, these studies together with our results in paper III suggest that increased APP expression result in shorter cilia while loss of App make cilia longer. However, the integrity and motility of the ependymal cilia in DS and AD remain unknown.

To which extent APP participates in the ependymal cilium motility and CSF flow remain unknown. CSF flow allows the distribution of nutrients and signalling molecules along brain ventricles and works to remove metabolite waste, such as Aβ peptides, from the brain parenchyma (Zappaterra and Lehtinen 2012, Abbott, Pizzo et al. 2018). Disrupted CSF flow observed in AD and DS could potentially reflect a defect in motility of the ependymal cilia (LeMay and Alvarez 1990).

Previous reports attribute defects in the CSF flow observed in the pathology of idiopathic normal pressure hydrocephalus to a defect in cilium motility function (Morimoto, Yoshida et al. 2019, Leinonen, Kuulasmaa et al. 2021). Interestingly, like AD and DS, the levels of APP in CSF are decreased in normal pressure hydrocephalus (Jeppsson, Zetterberg et al. 2013, Pyykko, Lumela et al. 2014, Jeppsson, Holtta et al. 2016). The implication of APP in the motility of the ependymal cilia through development and in these pathologies needs further studies.

Similarly to the ventricle size, it will be of interest to measure the number if cilia and cilium length of ependymal cells in ageing zebrafish, as well as in mice and human brain samples with or without changes in APP. Our first investigation on the cilia structure in adult zebrafish revealed no major in the cilium structure. Our data suggest that the internal structure of the cilium

remain intact, regardless of a change in length. Thus, although cilia are longer, the internal structure of the cilia seems to remain intact.

Altogether, we show that App localizes at the ependymal cilia and defects in the brain ventricles size and cilium length are observed in the *appa*$^{-/-}$*appb*$^{-/-}$ zebrafish. Studies are now ongoing to evaluate if the deletion of App results in disruption of ciliary beating and in CSF flow defects *in vivo*.

## 5.4 PAPER IV

### 5.4.1 RATIONALE

Proteomics is used to gain insights into protein abundance in different cell types, fluids, during disease or due to changes associated with mutations. In this manuscript, we pursued the study to find Appb responsive proteins and used *appb* mutant zebrafish larvae for this purpose. Our aim was to identify proteins with changed abundance compared with wildtype zebrafish larvae and to associate them with cellular biological functions and pathways. These data could, not only give molecular insights on previously described Appb functions, such as cell adhesion and neuronal development, but also provide key information for explorative studies to gain additional knowledge on Appb physiological role.

To this end, we used a tandem mass tag (TMT) 10-plex protein labelling combined with liquid chromatograph (LC)-mass spectrometry (MS), to generate lists of peptides found modulated in *appb* mutants. Several reports have analyzed the transcriptomic profile of mouse AD models *or App* knockout mice to study the impact on gene expression in specific cell types (Aydin, Filippov et al. 2011). Here, we aimed to identify proteins and pathways involved with Appb through TMT/LC-MS quantitative proteomics.

### 5.4.2 STUDY RESULTS/DISCUSSION

Here, we identified proteins that are modulated in larvae *appb* mutants in comparison to wildtypes. To decrease the complexity of the data and to segregate the results, we dissected the head from the trunk. From the head samples, we fished out a total of 8328 proteins and 4935 proteins from the trunk samples. Out of them, more than 75% of the trunk proteins were expressed in heads, whereas less than 50% of the proteins identified in the head samples were found in trunk. We identified proteins which levels were changed by more than 20% and reported them as differentially expressed proteins (DEPs). We identified 629 DEPs in head and 322 DEPs were found in trunk samples. These DEPs were assessed to gene ontology (GO) databases in order to link biological and cellular functions. This allowed us to classify the identified proteins in categories between molecular function, biological processes and cellular compartment.

We found that the deletion of *appb* modulated the levels of proteins expressed in head associated to molecular functions and to a greater extent involved in

nucleosomal DNA binding, proton transmembrane transporter activity, and structural constituent of ribosome. In trunk, the majority of DEPs belong to the hydrolase activity and oxidoreductase activity.

In the head samples, the impact of Appb loss on biological process mostly affected proteins related to ATP synthesis couples proton transport, signal transduction, cellular modified amino acid metabolic process, and nucleosome assembly and positioning. The cellular protein-containing complex assembly and translation were the most enriched categories. Through GO databases, we also identified DEPs related to cellular compartments, such as nucleosome for the most enriched categories in the head samples, and cytosol, integral component membrane in trunks. The GO classification gave us a global portrait of the modulated proteins. To identify candidates interacting with or being modulated by Appb, the DEPs were then listed as the most upregulated or downregulated in *appb* mutants. Through a heat-maps segregation we confirmed reproducibility between replicates and identified clusters of up- and down regulated proteins between genotypes confirming the strength of the analysis.

Out of the list of DEPs, we selected candidates further validate. Some DEPs were associated with known APP-implication while most were not previously described to interact with APP. As the antibodies available for zebrafish proteins are limited, we instead used molecular validation through qPCR analysis. From the DEPs list, we selected candidates with some of the highest changes in expression levels and for which a gene expression assay of the region of the peptide was available.

Correlations between protein and mRNA levels are often poor (Koussounadis, Langdon et al. 2015). However, to our surprise, a high percentage of DEPs also showed significant change at the mRNA levels. This could perhaps be due to the selection of the gene assays based on the region where the peptide was identified.

High correlation observed between mRNA and proteins levels are often observed in secreted proteins or cell cycle proteins, suggesting a synchrony. The zebrafish samples used were still at a developmental stage of 3dpf, therefore it is not surprising to find this correlation between levels of expression of proteins involved in cell adhesion and neuronal development.

Of the selected candidates, we identified cell adhesion-related proteins, a function already described in our previous work (**Paper I**, (Banote, Chebli et al. 2020)) to be associated with Appb. Mapre2, Neu3.1 and Nle1 were down regulated in mutants on the proteomic and mRNA levels and Fermt3b was upregulated in the TMT analysis but down in mRNA. Interestingly, *MAPRE2* encodes a microtubule-associated protein, known to promote microtubule stability and formation (Goldspink, Gadsby et al. 2013). Thus, it will be interesting to investigate if similarly low levels of Mapre2 are observed in *appa/appb* mutants (**paper III**) and if the protein is implicated in ependymal cilium structure. However, further studies are needed to identify the pathway through which Appb activates the transcription of those proteins involved in cell adhesion.

The role of APP/Appb in neuronal development and functions has been extensively studied. Here we showed that *cacna1da* levels are decreased in *appb* mutants. The *cacna1da* gene, which encodes the calcium channel Cav1.3, is implicated in neuronal development and functions (Liss and Striessnig 2019) and was previously reported to be modulated by Aβ peptide interaction. We previously showed a crosstalk between Appb and Notch activity (Banote, Edling et al. 2016) and here, we showed that the deletion of *appb* lead to a decrease in *nle1* expression levels, a direct regulator of the Notch signalling pathway (Cormier, Le Bras et al. 2006).

Although further work is needed to complete the validation of DEPs, our report enables future studies to investigate the role of Appb by providing a tool to identify candidates for specific cellular functions and expression.

## 5.5 PAPER V

### 5.5.1 RATIONALE

Genetic knockouts, generated by the CRISPR/Cas9 system, and knockdown models obtained through the use of antisense morpholinos often result in zebrafish larvae with phenotypic differences (Rossi, Kontarakis et al. 2015).

The study of gene function can be hard to assess due to redundancy and compensation by other genes (Shariati and De Strooper 2013). Indeed, when a mutation is created, the ability of an organism to mask genetic defects is referred to as genetic robustness. This tolerance to variation is established at many levels, including both, proteins and pathways. One recently described mechanism is transcriptional adaptation (TA) in which genetic mutations activates gene expression of homologous genes.

*App* is part of a family genes including the *App*-like proteins *Aplp1* and *Aplp2*. In mice, single gene KO exhibits minor phenotypes compared to double and triple KO. This is in contrast with the more severe phenotypes reported in knockdown mice. We and others have described *appb* KD zebrafish (Joshi, Liang et al. 2009, Combs, Song et al. 2012, Abramsson, Kettunen et al. 2013, Banote, Edling et al. 2016) and more recently the *appb* mutant (**Paper I**, (Banote, Chebli et al. 2020)) showing an obvious phenotypic difference between the two *appb*-deficient zebrafish models.

The exact mechanisms behind the phenotypic difference between the models are not well known. However, recent studies showed that mRNA containing a premature translation codon (PTC) activating degradation through nonsense-mediated decay (NMD) involving Upf1, Upf2, and Upf3a (El-Brolosy, Kontarakis et al. 2019) or through genetic compensatory response involving Upf3a and Wrd5 (Ma, Zhu et al. 2019) will trigger upregulation of other homologues genes in the family as a compensatory response. Here, in this manuscript, we aimed to study if the deletion of a single *app* gene could be mask by *appa*, *aplp1* and *aplp2* through compensatory mechanism such as TA and if it is the case, through with pathway.

### 5.5.2 STUDY RESULTS/DISCUSSION

The divergences between the phenotypes of *appb* knockout and knockdown models were at first thought of as off-target effects of the MO or due to the genetic compensation in the knockout models. Here, in the attempt to clarify

the discrepancy, we confirmed the specificity of the *appb*MO by injections into our *appb* mutant and observed no *appb*MO specific effect on the Mauthner cells (M-cells). This result reinforced the possible gene compensation effect, potentially driven by transcriptional adaptation hypothesis. It was previously suggested that mRNA degradation or a PTC was needed to for TA mechanism to be activated, (El-Brolosy, Kontarakis et al. 2019, Ma, Zhu et al. 2019). Here, we wanted to address if mutant *appb* mRNA triggers TA.

According to our hypothesis, MO-induced KD should not change the expression of *appa*, *aplp1* or *aplp2*, while the presence of an *appb* mutant mRNA (**Paper I**) should trigger a TA response to increase expression of *appa* and/or *aplp*s.

Our data confirmed the lack of change in mRNA levels of *appa* and *aplp2* in the *appb* morphants. In addition, we showed and upregulation in all *app* genes family members in our KO models at 24hpf. In our previous report (**Paper I**), the increase in *appa* and *aplp2* was mainly noted by *in situ* through mRNA probe-hybridization, while qPCR analysis only showed a trend of towards increased expression. Increased repetitions and refinement and combination of the techniques allowed us to conclude on these upregulations, which strongly indicate TA. Moreover, here we showed that TA seems to be mostly an event that happens during early development, as gene upregulation is not detectable after 24 hpf. In addition, our data from a human neuroblastoma cell line showed no evidence of TA, suggesting that TA might be species specific or present only in less differentiated cells. Further studies are however needed to confirm this zebrafish-specific TA of *app*s and *aplp*s.

TA has been shown to be activated by PTC-bearing mRNA or through degrading mRNA. In this manuscript, we showed that TA is activated regardless of the protein level. Indeed, our data showed that TA is in fact triggered by the degradation of an unprotected, therefore easy to degrade, mutant *appb* mRNA in a wildtype zebrafish, therefore in the presence of normal level of endogenous Appb protein.

The previously reports on TA suggest that PTC resulting in mRNA degradation trigger TA through nonsense mediated decay, notably through the NMD pathway involving Upf1, Upf2, and Upf3a. Here, we addressed if *appb* mRNA is degraded through NMD and if we could find changes in *appb* decay by inhibiting the Upf1 pathway. Here, *appb* mutant treated with the NMDi14 inhibitor, known to inhibit Upf1, showed a trend towards lowered of *appb* degradation. This likely suggests that Upf1 may not be the main pathway of *appb* mRNA degradation. Inhibition of Upf1 did not downregulate the TA

induced upregulation observed in the *appa* and *aplp* genes. This is likely due to the minor change in *appb* degradation, allow enough mRNA decay to still induce TA.

Data from us and others have shown that TA is triggered by mRNA degradation (with a PTC). To validate the effect of appb mutant RNA we created an appb mutant that do not produce an mRNA transcription by deleting a large part of the promoter and exon 1. We found that contrary to the *appb* $^{26_2}$ mutant, no upregulation of *appa* and *aplp2* were observed. However, *aplp1* levels were increased similar to the *appb* $^{26_2}$ mutant. Thus, while appa and aplp2 expression seem to be regulated by PTC-bearing mRNA decay, the mechanisms behind *aplp1* regulation are not and may instead be driven by Appb protein levels. However, this question remains to be evaluated.

# 6 CONCLUSION

The essential role of APP in biological processes, notably in neuronal functions, is undeniable. However, the ubiquitously expression of APP, its redundancy and the contradictory reports from *in vitro* and *in vivo* studies complicate our understanding.

The implication of APP in pathologies, such as AD, and its position as an important therapeutic target increase the necessity to broaden our knowledge on APP physiological functions.

The work in this thesis contributes to the knowledge in the APP field by characterizing *in vivo* App mutant models, through the association of *Appb* to cellular interactions during early development, by showing APP localization to ependymal cilia and associating a brain ventricular function, and by gaining insight on proteins and pathways modulated by the deletion of *appb*.

We reported that *appb* is important to maintain cellular adhesion properties during blastula formation. The deletion of *appb* is partly lethal and results in defect organization of the enveloping layer, cell protrusions and in a smaller body length until 2 dpf. The *appb* mutants however, developed into healthy and fertile adults, which may be due to upregulation of *app*-family gene members as detected by both RNA expression and protein level analyses.

Further investigation of the *appb* mutant revealed that an impaired trigeminal ganglia assembly, resulting in a less condensed ganglia. Loss of *appb* also negatively modulated the neuronal pool of the TG and is insufficient to affect differentiation of a subset of sensory neurons. Further studies are needed to identify the cellular processes implicated in the defect in TG assembly and to elucidate pathways by which Appb act to promote gangliogenesis.

Proteome analysis confirmed changes in proteins implicated in cell adhesion and neural development, and revealed that the deletion of Appb modulates proteins involved in gene regulation. Molecular validation of the mRNA levels of the proteins are on-going to generate a list of proteins used to explore downstream effectors and proteins interacting with Appb

We also reveal mechanisms by which the App family members compensate for the loss of each other and showed that in early development, the mutations in *app* genes activate mechanism of translational adaptation (TA). TA activates the expression of other family members and the *app* mutation-mediated TA is partly mediated through the nonsense mediating decay.

Finally, we also showed that App localizes to the cilia lining the nasal epithelium and otic vesicle in larvae and to the motile cilia covering the brain ventricle ependymal cells. This localization is conserved through adulthood and was also found in mice and human. This strongly suggests a role of APP in cilia-related functions. Interestingly, we found that the deletion of *appa* and *appb* in zebrafish resulted in defects in ciliary length and brain ventricles size. Further experiments are ongoing to evaluate the role of App in cilia function and the ependymal cilia integrity in AD and other pathologies associated with APP.

We believe that more focus on the physiological roles of APP is needed and that zebrafish is an adequate and relevant model for such studies.

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# 8 REFERENCES

(2013). "World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects." Jama **310**(20): 2191-2194.
(2016). Zebrafish: Methods and Protocols. New York, Humana Press.

Abbott, N. J., M. E. Pizzo, J. E. Preston, D. Janigro and R. G. Thorne (2018). "The role of brain barriers in fluid movement in the CNS: is there a 'glymphatic' system?" Acta Neuropathol **135**(3): 387-407.

Ablain, J. and L. I. Zon (2013). "Of fish and men: using zebrafish to fight human diseases." Trends Cell Biol **23**(12): 584-586.

Abramsson, A., P. Kettunen, R. K. Banote, E. Lott, M. Li, A. Arner and H. Zetterberg (2013). "The zebrafish amyloid precursor protein-b is required for motor neuron guidance and synapse formation." Dev Biol **381**(2): 377-388.

Alic, I., P. A. Goh, A. Murray, E. Portelius, E. Gkanatsiou, G. Gough, K. Y. Mok, D. Koschut, R. Brunmeir, Y. J. Yeap, N. L. O'Brien, J. Groet, X. Shao, S. Havlicek, N. R. Dunn, H. Kvartsberg, G. Brinkmalm, R. Hithersay, C. Startin, S. Hamburg, M. Phillips, K. Pervushin, M. Turmaine, D. Wallon, A. Rovelet-Lecrux, H. Soininen, E. Volpi, J. E. Martin, J. N. Foo, D. L. Becker, A. Rostagno, J. Ghiso, Z. Krsnik, G. Simic, I. Kostovic, D. Mitrecic, S. C. LonDown, P. T. Francis, K. Blennow, A. Strydom, J. Hardy, H. Zetterberg and D. Nizetic (2020). "Patient-specific Alzheimer-like pathology in trisomy 21 cerebral organoids reveals BACE2 as a gene dose-sensitive AD suppressor in human brain." Mol Psychiatry.

Amador-Arjona, A., J. Elliott, A. Miller, A. Ginbey, G. J. Pazour, G. Enikolopov, A. J. Roberts and A. V. Terskikh (2011). "Primary cilia regulate proliferation of amplifying progenitors in adult hippocampus: implications for learning and memory." J Neurosci **31**(27): 9933-9944.

Andrew, R. J., K. A. Kellett, G. Thinakaran and N. M. Hooper (2016). "A Greek Tragedy: The Growing Complexity of Alzheimer Amyloid Precursor Protein Proteolysis." J Biol Chem **291**(37): 19235-19244.

Asada-Utsugi, M., K. Uemura, Y. Noda, A. Kuzuya, M. Maesako, K. Ando, M. Kubota, K. Watanabe, M. Takahashi, T. Kihara, S. Shimohama, R. Takahashi, O. Berezovska and A. Kinoshita (2011). "N-cadherin enhances APP dimerization at the extracellular domain and modulates Abeta production." J Neurochem **119**(2): 354-363.

Augustin, V. and S. Kins (2021). "Fe65: A Scaffolding Protein of Actin Regulators." Cells **10**(7).

Aydin, D., M. A. Filippov, J. A. Tschäpe, N. Gretz, M. Prinz, R. Eils, B. Brors and U. C. Müller (2011). "Comparative transcriptome profiling of amyloid precursor protein family members in the adult cortex." BMC Genomics **12**: 160.

Baker, C. V. and M. Bronner-Fraser (2001). "Vertebrate cranial placodes I. Embryonic induction." Dev Biol **232**(1): 1-61.

Banote, R. K., J. Chebli, T. M. Satir, G. K. Varshney, R. Camacho, J. Ledin, S. M. Burgess, A. Abramsson and H. Zetterberg (2020). "Amyloid precursor protein-b facilitates cell adhesion during early development in zebrafish." Sci Rep **10**(1): 10127.

Banote, R. K., M. Edling, F. Eliassen, P. Kettunen, H. Zetterberg and A. Abramsson (2016). "beta-Amyloid precursor protein-b is essential for Mauthner cell development in the zebrafish in a Notch-dependent manner." Dev Biol **413**(1): 26-38.

Barrett, P. J., Y. Song, W. D. Van Horn, E. J. Hustedt, J. M. Schafer, A. Hadziselimovic, A. J. Beel and C. R. Sanders (2012). "The amyloid precursor protein has a flexible transmembrane domain and binds cholesterol." Science **336**(6085): 1168-1171.

Basbaum, A. I., D. M. Bautista, G. Scherrer and D. Julius (2009). "Cellular and molecular mechanisms of pain." Cell **139**(2): 267-284.

Basnet, R. M., D. Zizioli, S. Taweedet, D. Finazzi and M. Memo (2019). "Zebrafish Larvae as a Behavioral Model in Neuropharmacology." Biomedicines **7**(1).

Baumkotter, F., N. Schmidt, C. Vargas, S. Schilling, R. Weber, K. Wagner, S. Fiedler, W. Klug, J. Radzimanowski, S. Nickolaus, S. Keller, S. Eggert, K. Wild and S. Kins (2014). "Amyloid precursor protein dimerization and synaptogenic function depend on copper binding to the growth factor-like domain." J Neurosci **34**(33): 11159-11172.

Baumkotter, F., K. Wagner, S. Eggert, K. Wild and S. Kins (2012). "Structural aspects and physiological consequences of APP/APLP trans-dimerization." Exp Brain Res **217**(3-4): 389-395.

Beher, D., L. Hesse, C. L. Masters and G. Multhaup (1996). "Regulation of amyloid protein precursor (APP) binding to collagen and mapping of the binding sites on APP and collagen type I." J Biol Chem **271**(3): 1613-1620.

Berger-Sweeney, J., D. L. McPhie, J. A. Arters, J. Greenan, M. L. Oster-Granite and R. L. Neve (1999). "Impairments in learning and memory accompanied by neurodegeneration in mice transgenic for the carboxyl-terminus of the amyloid precursor protein." Brain Res Mol Brain Res **66**(1-2): 150-162.

Bhattarai, P., A. K. Thomas, Y. Zhang and C. Kizil (2017). "The effects of aging on Amyloid-beta42-induced neurodegeneration and regeneration in adult zebrafish brain." Neurogenesis (Austin) **4**(1): e1322666.

Biedler, J. L., L. Helson and B. A. Spengler (1973). "Morphology and growth, tumorigenicity, and cytogenetics of human neuroblastoma cells in continuous culture." Cancer Res **33**(11): 2643-2652.

Billnitzer, A. J., I. Barskaya, C. Yin and R. G. Perez (2013). "APP independent and dependent effects on neurite outgrowth are modulated by the receptor associated protein (RAP)." Journal of Neurochemistry **124**(1): 123-132.

Bittner, T., M. Fuhrmann, S. Burgold, C. K. Jung, C. Volbracht, H. Steiner, G. Mitteregger, H. A. Kretzschmar, C. Haass and J. Herms (2009). "Gamma-secretase inhibition reduces spine density in vivo via an amyloid precursor protein-dependent pathway." J Neurosci **29**(33): 10405-10409.

Blennow, K., M. J. de Leon and H. Zetterberg (2006). "Alzheimer's disease." The Lancet **368**(9533): 387-403.

Bukhari, H., A. Glotzbach, K. Kolbe, G. Leonhardt, C. Loosse and T. Müller (2017). "Small things matter: Implications of APP intracellular domain AICD nuclear signaling in the progression and pathogenesis of Alzheimer's disease." Progress in Neurobiology **156**: 189-213.

Caille, I., B. Allinquant, E. Dupont, C. Bouillot, A. Langer, U. Muller and A. Prochiantz (2004). "Soluble form of amyloid precursor protein regulates proliferation of progenitors in the adult subventricular zone." Development **131**(9): 2173-2181.

Chakravarthy, B., C. Gaudet, M. Ménard, L. Brown, T. Atkinson, F. M. LaFerla, S. Ito, U. Armato, I. Dal Prà and J. Whitfield (2012). "Reduction of the immunostainable length of the hippocampal dentate granule cells' primary cilia in 3xAD-transgenic mice producing human Aβ1-42 and tau." Biochemical and Biophysical Research Communications **427**(1): 218-222.

Chan, M. and S. Amin-Hanjani (2010). Cerebrospinal Fluid and its Abnormalities. Encyclopedia of Life Sciences.

Citron, M., T. Oltersdorf, C. Haass, L. McConlogue, A. Y. Hung, P. Seubert, C. Vigo-Pelfrey, I. Lieberburg and D. J. Selkoe (1992). "Mutation of the beta-amyloid precursor protein in familial Alzheimer's disease increases beta-protein production." Nature **360**(6405): 672-674.

Combs, C., P. Song and S. W. Pimplikar (2012). "Knockdown of Amyloid Precursor Protein in Zebrafish Causes Defects in Motor Axon Outgrowth." PLoS ONE **7**(4).

Copenhaver, P. F. and J. M. Ramaker (2016). "Neuronal migration during development and the amyloid precursor protein." Curr Opin Insect Sci **18**: 1-10.

Cormier, S., S. Le Bras, C. Souilhol, S. Vandormael-Pournin, B. Durand, C. Babinet, P. Baldacci and M. Cohen-Tannoudji (2006). "The murine ortholog of notchless, a direct regulator of the notch pathway in Drosophila melanogaster, is essential for survival of inner cell mass cells." Mol Cell Biol **26**(9): 3541-3549.

Corrigan, F., R. Vink, P. C. Blumbergs, C. L. Masters, R. Cappai and C. van den Heuvel (2012). "sAPPalpha rescues deficits in amyloid precursor protein knockout mice following focal traumatic brain injury." J Neurochem **122**(1): 208-220.

D'Amico-Martel, A. and D. M. Noden (1983). "Contributions of placodal and neural crest cells to avian cranial peripheral ganglia." Am J Anat **166**(4): 445-468.

Daigle, I. and C. Li (1993). "apl-1, a Caenorhabditis elegans gene encoding a protein related to the human beta-amyloid protein precursor." Proc Natl Acad Sci U S A **90**(24): 12045-12049.

Das, U., D. A. Scott, A. Ganguly, E. H. Koo, Y. Tang and S. Roy (2013). "Activity-induced convergence of APP and BACE-1 in acidic microdomains via an endocytosis-dependent pathway." Neuron **79**(3): 447-460.

De Strooper, B., W. Annaert, P. Cupers, P. Saftig, K. Craessaerts, J. S. Mumm, E. H. Schroeter, V. Schrijvers, M. S. Wolfe, W. J. Ray, A. Goate and R. Kopan (1999). "A presenilin-1-dependent gamma-secretase-like protease mediates release of Notch intracellular domain." Nature **398**(6727): 518-522.

De Strooper, B., T. Iwatsubo and M. S. Wolfe (2012). "Presenilins and gamma-secretase: structure, function, and role in Alzheimer Disease." Cold Spring Harb Perspect Med **2**(1): a006304.

De Strooper, B. and E. Karran (2016). "The Cellular Phase of Alzheimer's Disease." Cell **164**(4): 603-615.

DeBoer, S. R., G. Dolios, R. Wang and S. S. Sisodia (2014). "Differential release of beta-amyloid from dendrite- versus axon-targeted APP." J Neurosci **34**(37): 12313-12327.

Del Bigio, M. R. (1993). "Neuropathological changes caused by hydrocephalus." Acta Neuropathol **85**(6): 573-585.

Deyts, C., G. Thinakaran and A. T. Parent (2016). "APP Receptor? To Be or Not To Be." Trends Pharmacol Sci **37**(5): 390-411.

Doty, R. L. (2009). "The olfactory system and its disorders." Semin Neurol **29**(1): 74-81.

Eggert, S., K. Paliga, P. Soba, G. Evin, C. L. Masters, A. Weidemann and K. Beyreuther (2004). "The proteolytic processing of the amyloid precursor protein gene family members APLP-1 and APLP-2 involves alpha-, beta-, gamma-, and epsilon-like cleavages: modulation of APLP-1 processing by n-glycosylation." J Biol Chem **279**(18): 18146-18156.

El-Brolosy, M. A., Z. Kontarakis, A. Rossi, C. Kuenne, S. Gunther, N. Fukuda, K. Kikhi, G. L. M. Boezio, C. M. Takacs, S. L. Lai, R. Fukuda, C. Gerri, A. J. Giraldez and D. Y. R. Stainier (2019). "Genetic compensation triggered by mutant mRNA degradation." Nature **568**(7751): 193-197.

Ethell, D. W. (2014). "Disruption of cerebrospinal fluid flow through the olfactory system may contribute to Alzheimer's disease pathogenesis." J Alzheimers Dis **41**(4): 1021-1030.

Fame, R. M., J. T. Chang, A. Hong, N. A. Aponte-Santiago and H. Sive (2016). "Directional cerebrospinal fluid movement between brain ventricles in larval zebrafish." Fluids Barriers CNS **13**(1): 11.

Fanutza, T., D. Del Prete, M. J. Ford, P. E. Castillo and L. D'Adamio (2015). "APP and APLP2 interact with the synaptic release machinery and facilitate transmitter release at hippocampal synapses." Elife **4**: e09743.

Fior, R. and D. Henrique (2009). ""Notch-Off": a perspective on the termination of Notch signalling." Int J Dev Biol **53**(8-10): 1379-1384.

Fiore, F., N. Zambrano, G. Minopoli, V. Donini, A. Duilio and T. Russo (1995). "The regions of the Fe65 protein homologous to the phosphotyrosine interaction/phosphotyrosine binding domain of Shc bind the intracellular domain of the Alzheimer's amyloid precursor protein." J Biol Chem **270**(52): 30853-30856.

Fol, R., J. Braudeau, S. Ludewig, T. Abel, S. W. Weyer, J. P. Roederer, F. Brod, M. Audrain, A. P. Bemelmans, C. J. Buchholz, M. Korte, N. Cartier and U. C. Muller (2016). "Viral gene transfer of APPsalpha rescues synaptic failure in an Alzheimer's disease mouse model." Acta Neuropathol **131**(2): 247-266.

Freude, K. K., M. Penjwini, J. L. Davis, F. M. LaFerla and M. Blurton-Jones (2011). "Soluble Amyloid Precursor Protein Induces Rapid Neural Differentiation of Human Embryonic Stem Cells." Journal of Biological Chemistry **286**(27): 24264-24274.

Galanis, C., M. Fellenz, D. Becker, C. Bold, S. F. Lichtenthaler, U. C. Muller, T. Deller and A. Vlachos (2021). "Amyloid-Beta Mediates Homeostatic Synaptic Plasticity." J Neurosci **41**(24): 5157-5172.

Galati, D. F., K. D. Sullivan, A. T. Pham, J. M. Espinosa and C. G. Pearson (2018). "Trisomy 21 Represses Cilia Formation and Function." Dev Cell **46**(5): 641-650 e646.

Garcia-Gonzalo, F. R., K. C. Corbit, M. S. Sirerol-Piquer, G. Ramaswami, E. A. Otto, T. R. Noriega, A. D. Seol, J. F. Robinson, C. L. Bennett, D. J. Josifova, J. M. García-Verdugo, N. Katsanis, F. Hildebrandt and J. F. Reiter (2011). "A transition zone complex regulates mammalian ciliogenesis and ciliary membrane composition." Nature Genetics **43**(8): 776-784.

Gates, M. A., L. Kim, E. S. Egan, T. Cardozo, H. I. Sirotkin, S. T. Dougan, D. Lashkari, R. Abagyan, A. F. Schier and W. S. Talbot (1999). "A genetic linkage map for zebrafish: comparative analysis and localization of genes and expressed sequences." Genome Res **9**(4): 334-347.

Gau, P., A. Curtright, L. Condon, D. W. Raible and A. Dhaka (2017). "An ancient neurotrophin receptor code; a single Runx/Cbfbeta complex determines somatosensory neuron fate specification in zebrafish." PLoS Genet **13**(7): e1006884.

Gau, P., J. Poon, C. Ufret-Vincenty, C. D. Snelson, S. E. Gordon, D. W. Raible and A. Dhaka (2013). "The zebrafish ortholog of TRPV1 is required for heat-induced locomotion." J Neurosci **33**(12): 5249-5260.

Gigante, E. D. and T. Caspary (2020). "Signaling in the primary cilium through the lens of the Hedgehog pathway." Wiley Interdiscip Rev Dev Biol **9**(6): e377.
Glasauer, S. M. and S. C. Neuhauss (2014). "Whole-genome duplication in teleost fishes and its evolutionary consequences." Mol Genet Genomics **289**(6): 1045-1060.

Goate, A., M. C. Chartier-Harlin, M. Mullan, J. Brown, F. Crawford, L. Fidani, L. Giuffra, A. Haynes, N. Irving, L. James and et al. (1991). "Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease." Nature **349**(6311): 704-706.

Goldgaber, D., M. I. Lerman, O. W. McBride, U. Saffiotti and D. C. Gajdusek (1987). "Characterization and chromosomal localization of a cDNA encoding brain amyloid of Alzheimer's disease." Science **235**(4791): 877-880.

Goldspink, D. A., J. R. Gadsby, G. Bellett, J. Keynton, B. J. Tyrrell, E. K. Lund, P. P. Powell, P. Thomas and M. M. Mogensen (2013). "The microtubule end-binding protein EB2 is a central regulator of microtubule reorganisation in apico-basal epithelial differentiation." J Cell Sci **126**(Pt 17): 4000-4014.

Gulisano, W., M. Melone, C. Ripoli, M. R. Tropea, D. D. Li Puma, S. Giunta, S. Cocco, D. Marcotulli, N. Origlia, A. Palmeri, O. Arancio, F. Conti, C. Grassi and D. Puzzo (2019). "Neuromodulatory Action of Picomolar Extracellular Abeta42 Oligomers on Presynaptic and Postsynaptic Mechanisms Underlying Synaptic Function and Memory." J Neurosci **39**(30): 5986-6000.

Guo, J., J. M. Otis, H. Higginbotham, C. Monckton, J. Cheng, A. Asokan, K. Mykytyn, T. Caspary, G. D. Stuber and E. S. Anton (2017). "Primary Cilia Signaling Shapes the Development of Interneuronal Connectivity." Dev Cell **42**(3): 286-300 e284.

Guo, J., J. M. Otis, S. K. Suciu, C. Catalano, L. Xing, S. Constable, D. Wachten, S. Gupton, J. Lee, A. Lee, K. H. Blackley, T. Ptacek, J. M. Simon, S. Schurmans, G. D. Stuber, T. Caspary and E. S. Anton (2019). "Primary Cilia Signaling Promotes Axonal Tract Development and Is Disrupted in Joubert Syndrome-Related Disorders Models." Developmental Cell **51**(6): 759-774.e755.

Guo, Y., Q. Wang, S. Chen and C. Xu (2021). "Functions of amyloid precursor protein in metabolic diseases." Metabolism **115**: 154454.

Haass, C. and B. De Strooper (1999). "The presenilins in Alzheimer's disease--proteolysis holds the key." Science **286**(5441): 916-919.

Haass, C., A. Y. Hung and D. J. Selkoe (1991). "Processing of beta-amyloid precursor protein in microglia and astrocytes favors an internal localization over constitutive secretion." J Neurosci **11**(12): 3783-3793.

Haass, C., C. Kaether, G. Thinakaran and S. Sisodia (2012). "Trafficking and proteolytic processing of APP." Cold Spring Harb Perspect Med **2**(5): a006270.

Haass, C., M. G. Schlossmacher, A. Y. Hung, C. Vigo-Pelfrey, A. Mellon, B. L. Ostaszewski, I. Lieberburg, E. H. Koo, D. Schenk, D. B. Teplow and et al. (1992). "Amyloid beta-peptide is produced by cultured cells during normal metabolism." Nature **359**(6393): 322-325.

Hardy, J. and D. Allsop (1991). "Amyloid deposition as the central event in the aetiology of Alzheimer's disease." Trends Pharmacol Sci **12**(10): 383-388.

Hardy, J. A. and G. A. Higgins (1992). "Alzheimer's disease: the amyloid cascade hypothesis." Science **256**(5054): 184-185.

Heber, S., J. Herms, V. Gajic, J. Hainfellner, A. Aguzzi, T. Rülicke, H. von Kretzschmar, C. von Koch, S. Sisodia, P. Tremml, H. P. Lipp, D. P. Wolfer and U. Müller (2000). "Mice with combined gene knock-outs reveal essential and partially redundant functions of amyloid precursor protein family members." J Neurosci **20**(21): 7951-7963.

Hernandez-Sapiens, M. A., E. E. Reza-Zaldivar, A. L. Marquez-Aguirre, U. Gomez-Pinedo, J. Matias-Guiu, R. R. Cevallos, J. C. Mateos-Diaz, V. J. Sanchez-Gonzalez and A. A. Canales-Aguirre (2022). "Presenilin mutations and their impact on neuronal differentiation in Alzheimer's disease." Neural Regen Res **17**(1): 31-37.

Hick, M., U. Herrmann, S. W. Weyer, J. P. Mallm, J. A. Tschape, M. Borgers, M. Mercken, F. C. Roth, A. Draguhn, L. Slomianka, D. P. Wolfer, M. Korte and U. C. Muller (2015). "Acute function of secreted amyloid precursor protein fragment APPsalpha in synaptic plasticity." Acta Neuropathol **129**(1): 21-37.

Hitzenberger, M., A. Gotz, S. Menig, B. Brunschweiger, M. Zacharias and C. Scharnagl (2020). "The dynamics of gamma-secretase and its substrates." Semin Cell Dev Biol **105**: 86-101.

Hoe, H. S., K. J. Lee, R. S. E. Carney, J. Lee, A. Markova, J. Y. Lee, B. W. Howell, B. T. Hyman, D. T. S. Pak, G. Bu and G. W. Rebeck (2009). "Interaction of Reelin with Amyloid Precursor Protein Promotes Neurite Outgrowth." Journal of Neuroscience **29**(23): 7459-7473.

Hoey, S. E., R. J. Williams and M. S. Perkinton (2009). "Synaptic NMDA receptor activation stimulates alpha-secretase amyloid precursor protein processing and inhibits amyloid-beta production." J Neurosci **29**(14): 4442-4460.

Hornsten, A., J. Lieberthal, S. Fadia, R. Malins, L. Ha, X. Xu, I. Daigle, M. Markowitz, G. O'Connor, R. Plasterk and C. Li (2007). "APL-1, a Caenorhabditis elegans protein related to the human beta-amyloid precursor protein, is essential for viability." Proc Natl Acad Sci U S A **104**(6): 1971-1976.

Howe, K., M. D. Clark, C. F. Torroja, J. Torrance, C. Berthelot, M. Muffato, J. E. Collins, S. Humphray, K. McLaren, L. Matthews, S. McLaren, I. Sealy, M. Caccamo, C. Churcher, C. Scott, J. C. Barrett, R. Koch, G. J. Rauch, S. White, W. Chow, B. Kilian, L. T. Quintais, J. A. Guerra-Assuncao, Y. Zhou, Y. Gu, J. Yen, J. H. Vogel, T. Eyre, S. Redmond, R. Banerjee, J. Chi, B. Fu, E. Langley, S. F. Maguire, G. K. Laird, D. Lloyd, E. Kenyon, S. Donaldson, H. Sehra, J. Almeida-King, J. Loveland, S. Trevanion, M. Jones, M. Quail, D. Willey, A. Hunt, J. Burton, S. Sims, K. McLay, B. Plumb, J. Davis, C. Clee, K. Oliver, R. Clark, C. Riddle, D. Elliot, G. Threadgold, G. Harden, D. Ware, S. Begum, B. Mortimore, G. Kerry, P. Heath, B. Phillimore, A. Tracey, N. Corby, M. Dunn, C. Johnson, J. Wood, S. Clark, S. Pelan, G. Griffiths, M. Smith, R. Glithero, P. Howden, N. Barker, C. Lloyd, C. Stevens, J. Harley, K. Holt, G. Panagiotidis, J. Lovell, H. Beasley, C. Henderson, D. Gordon, K. Auger, D. Wright, J. Collins, C. Raisen, L. Dyer, K. Leung, L. Robertson, K. Ambridge, D. Leongamornlert, S. McGuire, R. Gilderthorp, C. Griffiths, D. Manthravadi, S. Nichol, G. Barker, S. Whitehead, M. Kay, J. Brown, C. Murnane, E. Gray, M. Humphries, N. Sycamore, D. Barker, D. Saunders, J. Wallis, A. Babbage, S. Hammond, M. Mashreghi-Mohammadi, L. Barr, S. Martin, P. Wray, A. Ellington, N. Matthews, M. Ellwood, R. Woodmansey, G. Clark, J. Cooper, A. Tromans, D. Grafham, C. Skuce, R. Pandian, R. Andrews, E. Harrison, A. Kimberley, J. Garnett, N. Fosker, R. Hall, P. Garner, D. Kelly, C. Bird, S. Palmer, I. Gehring, A. Berger, C. M. Dooley, Z. Ersan-Urun, C. Eser, H. Geiger, M. Geisler, L. Karotki, A. Kirn, J. Konantz, M. Konantz, M. Oberlander, S. Rudolph-Geiger, M. Teucke, C. Lanz, G. Raddatz, K. Osoegawa, B. Zhu, A. Rapp, S. Widaa, C. Langford, F. Yang, S. C. Schuster, N. P. Carter, J. Harrow, Z. Ning, J. Herrero, S. M. Searle, A. Enright, R. Geisler, R. H. Plasterk, C. Lee, M. Westerfield, P. J. de Jong, L. I. Zon, J. H. Postlethwait, C. Nusslein-Volhard, T. J. Hubbard, H. Roest Crollius, J. Rogers and D. L. Stemple (2013). "The zebrafish reference genome sequence and its relationship to the human genome." Nature **496**(7446): 498-503.

Hu, Y. B., E. B. Dammer, R. J. Ren and G. Wang (2015). "The endosomal-lysosomal system: from acidification and cargo sorting to neurodegeneration." Transl Neurodegener **4**: 18.

Huse, J. T., D. S. Pijak, G. J. Leslie, V. M. Lee and R. W. Doms (2000). "Maturation and endosomal targeting of beta-site amyloid precursor protein-cleaving enzyme. The Alzheimer's disease beta-secretase." J Biol Chem **275**(43): 33729-33737.
Ibanez-Tallon, I., A. Pagenstecher, M. Fliegauf, H. Olbrich, A. Kispert, U. P. Ketelsen, A. North, N. Heintz and H. Omran (2004). "Dysfunction of axonemal dynein heavy chain Mdnah5 inhibits ependymal flow and reveals a novel mechanism for hydrocephalus formation." Hum Mol Genet **13**(18): 2133-2141.

Jefferson, T., M. Causevic, U. auf dem Keller, O. Schilling, S. Isbert, R. Geyer, W. Maier, S. Tschickardt, T. Jumpertz, S. Weggen, J. S. Bond, C. M. Overall, C. U. Pietrzik and C. Becker-

Pauly (2011). "Metalloprotease meprin beta generates nontoxic N-terminal amyloid precursor protein fragments in vivo." J Biol Chem **286**(31): 27741-27750.

Jeppsson, A., M. Holtta, H. Zetterberg, K. Blennow, C. Wikkelso and M. Tullberg (2016). "Amyloid mis-metabolism in idiopathic normal pressure hydrocephalus." Fluids Barriers CNS **13**(1): 13.

Jeppsson, A., H. Zetterberg, K. Blennow and C. Wikkelsø (2013). "Idiopathic normal-pressure hydrocephalus: pathophysiology and diagnosis by CSF biomarkers." Neurology **80**(15): 1385-1392.

Jonsson, T., J. K. Atwal, S. Steinberg, J. Snaedal, P. V. Jonsson, S. Bjornsson, H. Stefansson, P. Sulem, D. Gudbjartsson, J. Maloney, K. Hoyte, A. Gustafson, Y. Liu, Y. Lu, T. Bhangale, R. R. Graham, J. Huttenlocher, G. Bjornsdottir, O. A. Andreassen, E. G. Jönsson, A. Palotie, T. W. Behrens, O. T. Magnusson, A. Kong, U. Thorsteinsdottir, R. J. Watts and K. Stefansson (2012). "A mutation in APP protects against Alzheimer's disease and age-related cognitive decline." Nature **488**(7409): 96-99.

Joshi, P., J. O. Liang, K. DiMonte, J. Sullivan and S. W. Pimplikar (2009). "Amyloid precursor protein is required for convergent-extension movements during Zebrafish development." Developmental Biology **335**(1): 1-11.

Kaden, D., P. Voigt, L. M. Munter, K. D. Bobowski, M. Schaefer and G. Multhaup (2009). "Subcellular localization and dimerization of APLP1 are strikingly different from APP and APLP2." J Cell Sci **122**(Pt 3): 368-377.

Kang, J., H. G. Lemaire, A. Unterbeck, J. M. Salbaum, C. L. Masters, K. H. Grzeschik, G. Multhaup, K. Beyreuther and B. Müller-Hill (1987). "The precursor of Alzheimer's disease amyloid A4 protein resembles a cell-surface receptor." Nature **325**(6106): 733-736.

Kerstetter, A. E., E. Azodi, J. A. Marrs and Q. Liu (2004). "Cadherin-2 function in the cranial ganglia and lateral line system of developing zebrafish." Dev Dyn **230**(1): 137-143.
Kimberly, W. T., W. P. Esler, W. Ye, B. L. Ostaszewski, J. Gao, T. Diehl, D. J. Selkoe and M. S. Wolfe (2003). "Notch and the amyloid precursor protein are cleaved by similar gamma-secretase(s)." Biochemistry **42**(1): 137-144.

Kimmel, C. B. (1989). "Genetics and early development of zebrafish." Trends Genet **5**(8): 283-288.

Kimmel, C. B., W. W. Ballard, S. R. Kimmel, B. Ullmann and T. F. Schilling (1995). "Stages of embryonic development of the zebrafish." Dev Dyn **203**(3): 253-310.

Kimmel, C. B., R. M. Warga and T. F. Schilling (1990). "Origin and organization of the zebrafish fate map." Development **108**(4): 581-594.

Koussounadis, A., S. P. Langdon, I. H. Um, D. J. Harrison and V. A. Smith (2015). "Relationship between differentially expressed mRNA and mRNA-protein correlations in a xenograft model system." Sci Rep **5**: 10775.

Kreis, A., J. Desloovere, N. Suelves, N. Pierrot, X. Yerna, F. Issa, O. Schakman, R. Gualdani, M. de Clippele, N. Tajeddine, P. Kienlen-Campard, R. Raedt, J. N. Octave and P. Gailly (2021).

"Overexpression of wild-type human amyloid precursor protein alters GABAergic transmission." Sci Rep **11**(1): 17600.

Kwak, Y. D., A. Marutle, E. Dantuma, S. Merchant, S. Bushnev and K. Sugaya (2011). "Involvement of notch signaling pathway in amyloid precursor protein induced glial differentiation." Eur J Pharmacol **650**(1): 18-27.

König, G., C. L. Masters and K. Beyreuther (1990). "Retinoic acid induced differentiated neuroblastoma cells show increased expression of the beta A4 amyloid gene of Alzheimer's disease and an altered splicing pattern." FEBS Lett **269**(2): 305-310.

Lai, C. K., N. Gupta, X. Wen, L. Rangell, B. Chih, A. S. Peterson, J. F. Bazan, L. Li and S. J. Scales (2011). "Functional characterization of putative cilia genes by high-content analysis." Mol Biol Cell **22**(7): 1104-1119.

LaMora, A. and M. M. Voigt (2009). "Cranial sensory ganglia neurons require intrinsic N-cadherin function for guidance of afferent fibers to their final targets." Neuroscience **159**(3): 1175-1184.

Lassiter, R. N., M. K. Ball, J. S. Adams, B. T. Wright and M. R. Stark (2010). "Sensory neuron differentiation is regulated by notch signaling in the trigeminal placode." Dev Biol **344**(2): 836-848.

Lassiter, R. N. T., M. R. Stark, T. Zhao and C. J. Zhou (2014). "Signaling mechanisms controlling cranial placode neurogenesis and delamination." Developmental Biology **389**(1): 39-49.

Lauter, G., I. Söll and G. Hauptmann (2014). Sensitive Whole-Mount Fluorescent In Situ Hybridization in Zebrafish Using Enhanced Tyramide Signal Amplification. Brain Development**:** 175-185.

Lazarov, N. E. (2002). "Comparative analysis of the chemical neuroanatomy of the mammalian trigeminal ganglion and mesencephalic trigeminal nucleus." Prog Neurobiol **66**(1): 19-59.

LeBlanc, A. C., H. Y. Chen, L. Autilio-Gambetti and P. Gambetti (1991). "Differential APP gene expression in rat cerebral cortex, meninges, and primary astroglial, microglial and neuronal cultures." FEBS Lett **292**(1-2): 171-178.

Lee, K. J., C. E. Moussa, Y. Lee, Y. Sung, B. W. Howell, R. S. Turner, D. T. Pak and H. S. Hoe (2010). "Beta amyloid-independent role of amyloid precursor protein in generation and maintenance of dendritic spines." Neuroscience **169**(1): 344-356.

Leimer, U., K. Lun, H. Romig, J. Walter, J. Grünberg, M. Brand and C. Haass (1999). "Zebrafish (Danio rerio) presenilin promotes aberrant amyloid beta-peptide production and requires a critical aspartate residue for its function in amyloidogenesis." Biochemistry **38**(41): 13602-13609.

Leinonen, V., T. Kuulasmaa and M. Hiltunen (2021). "iNPH-the mystery resolving." EMBO Mol Med **13**(3): e13720.

LeMay, M. and N. Alvarez (1990). "The relationship between enlargement of the temporal horns of the lateral ventricles and dementia in aging patients with Down syndrome." Neuroradiology **32**(2): 104-107.

Leyssen, M., D. Ayaz, S. S. Hébert, S. Reeve, B. De Strooper and B. A. Hassan (2005). "Amyloid precursor protein promotes post-developmental neurite arborization in the Drosophila brain." The EMBO Journal **24**(16): 2944-2955.

Liao, H. K., Y. Wang, K. E. Noack Watt, Q. Wen, J. Breitbach, C. K. Kemmet, K. J. Clark, S. C. Ekker, J. J. Essner and M. McGrail (2012). "Tol2 gene trap integrations in the zebrafish amyloid precursor protein genes appa and aplp2 reveal accumulation of secreted APP at the embryonic veins." Dev Dyn **241**(2): 415-425.

Liss, B. and J. Striessnig (2019). "The Potential of L-Type Calcium Channels as a Drug Target for Neuroprotective Therapy in Parkinson's Disease." Annu Rev Pharmacol Toxicol **59**: 263-289.

Liu, Q., A. E. Kerstetter, E. Azodi and J. A. Marrs (2003). "Cadherin-1, -2, and -11 expression and cadherin-2 function in the pectoral limb bud and fin of the developing zebrafish." Dev Dyn **228**(4): 734-739.

Liu, T., T. Zhang, M. Nicolas, L. Boussicault, H. Rice, A. Soldano, A. Claeys, I. Petrova, L. Fradkin, B. De Strooper, M. C. Potier and B. A. Hassan (2021). "The amyloid precursor protein is a conserved Wnt receptor." Elife **10**.

Livak, K. J. and T. D. Schmittgen (2001). "Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method." Methods **25**(4): 402-408.

Livingstone, R. W., M. K. Elder, A. Singh, C. M. Westlake, W. P. Tate, W. C. Abraham and J. M. Williams (2021). "Secreted Amyloid Precursor Protein-Alpha Enhances LTP Through the Synthesis and Trafficking of Ca(2+)-Permeable AMPA Receptors." Front Mol Neurosci **14**: 660208.

Lorent, K., L. Overbergh, D. Moechars, B. De Strooper, F. Van Leuven and H. Van den Berghe (1995). "Expression in mouse embryos and in adult mouse brain of three members of the amyloid precursor protein family, of the alpha-2-macroglobulin receptor/low density lipoprotein receptor-related protein and of its ligands apolipoprotein E, lipoprotein lipase, alpha-2-macroglobulin and the 40,000 molecular weight receptor-associated protein." Neuroscience **65**(4): 1009-1025.

Ludewig, S., U. Herrmann, K. Michaelsen-Preusse, K. Metzdorf, J. Just, C. Bold, U. C. Muller and M. Korte (2021). "APPsalpha rescues impaired Ca(2+) homeostasis in APP- and APLP2-deficient hippocampal neurons." Proc Natl Acad Sci U S A **118**(26).

Ludewig, S. and M. Korte (2016). "Novel Insights into the Physiological Function of the APP (Gene) Family and Its Proteolytic Fragments in Synaptic Plasticity." Front Mol Neurosci **9**: 161.

Ma, Z., P. Zhu, H. Shi, L. Guo, Q. Zhang, Y. Chen, S. Chen, Z. Zhang, J. Peng and J. Chen (2019). "PTC-bearing mRNA elicits a genetic compensation response via Upf3a and COMPASS components." Nature **568**(7751): 259-263.

Magara, F., U. Muller, Z. W. Li, H. P. Lipp, C. Weissmann, M. Stagljar and D. P. Wolfer (1999). "Genetic background changes the pattern of forebrain commissure defects in transgenic mice underexpressing the  -amyloid-precursor protein." Proceedings of the National Academy of Sciences **96**(8): 4656-4661.

Magdesian, M. H., M. M. Carvalho, F. A. Mendes, L. M. Saraiva, M. A. Juliano, L. Juliano, J. Garcia-Abreu and S. T. Ferreira (2008). "Amyloid-beta binds to the extracellular cysteine-rich domain of Frizzled and inhibits Wnt/beta-catenin signaling." J Biol Chem **283**(14): 9359-9368.

Mangialasche, F., A. Solomon, B. Winblad, P. Mecocci and M. Kivipelto (2010). "Alzheimer's disease: clinical trials and drug development." Lancet Neurol **9**(7): 702-716.

Marmigere, F. and P. Ernfors (2007). "Specification and connectivity of neuronal subtypes in the sensory lineage." Nat Rev Neurosci **8**(2): 114-127.

Masters, C. L., G. Simms, N. A. Weinman, G. Multhaup, B. L. McDonald and K. Beyreuther (1985). "Amyloid plaque core protein in Alzheimer disease and Down syndrome." Proc Natl Acad Sci U S A **82**(12): 4245-4249.

McGinn, S. and I. G. Gut (2013). "DNA sequencing - spanning the generations." N Biotechnol **30**(4): 366-372.

Meziane, H., J. C. Dodart, C. Mathis, S. Little, J. Clemens, S. M. Paul and A. Ungerer (1998). "Memory-enhancing effects of secreted forms of the beta-amyloid precursor protein in normal and amnestic mice." Proc Natl Acad Sci U S A **95**(21): 12683-12688.

Mikulca, J. A., V. Nguyen, D. A. Gajdosik, S. G. Teklu, E. A. Giunta, E. A. Lessa, C. H. Tran, E. C. Terak and R. B. Raffa (2014). "Potential novel targets for Alzheimer pharmacotherapy: II. Update on secretase inhibitors and related approaches." Journal of Clinical Pharmacy and Therapeutics **39**(1): 25-37.

Miranda, A., E. Montiel, H. Ulrich and C. Paz (2021). "Selective Secretase Targeting for Alzheimer's Disease Therapy." J Alzheimers Dis **81**(1): 1-17.

Mitani, Y., J. Yarimizu, K. Saita, H. Uchino, H. Akashiba, Y. Shitaka, K. Ni and N. Matsuoka (2012). "Differential effects between gamma-secretase inhibitors and modulators on cognitive function in amyloid precursor protein-transgenic and nontransgenic mice." J Neurosci **32**(6): 2037-2050.

Mitchison, H. M. and E. M. Valente (2017). "Motile and non-motile cilia in human pathology: from function to phenotypes." J Pathol **241**(2): 294-309.

Morales-Corraliza, J., M. J. Mazzella, J. D. Berger, N. S. Diaz, J. H. Choi, E. Levy, Y. Matsuoka, E. Planel and P. M. Mathews (2009). "In vivo turnover of tau and APP metabolites in the brains of wild-type and Tg2576 mice: greater stability of sAPP in the beta-amyloid depositing mice." PLoS One **4**(9): e7134.

Moreno, L., C. Rose, A. Mohanraj, B. Allinquant, J.-M. Billard, P. Dutar and O. Arancio (2015). "sAβPPα Improves Hippocampal NMDA-Dependent Functional Alterations Linked to Healthy Aging." Journal of Alzheimer's Disease **48**(4): 927-935.

Morimoto, Y., S. Yoshida, A. Kinoshita, C. Satoh, H. Mishima, N. Yamaguchi, K. Matsuda, M. Sakaguchi, T. Tanaka, Y. Komohara, A. Imamura, H. Ozawa, M. Nakashima, N. Kurotaki, T. Kishino, K. I. Yoshiura and S. Ono (2019). "Nonsense mutation in CFAP43 causes normal-pressure hydrocephalus with ciliary abnormalities." Neurology **92**(20): e2364-e2374.

Muller, U. C., T. Deller and M. Korte (2017). "Not just amyloid: physiological functions of the amyloid precursor protein family." Nat Rev Neurosci **18**(5): 281-298.

Musa, A., H. Lehrach and V. A. Russo (2001). "Distinct expression patterns of two zebrafish homologues of the human APP gene during embryonic development." Dev Genes Evol **211**(11): 563-567.

Müller, U., N. Cristina, Z. W. Li, D. P. Wolfer, H. P. Lipp, T. Rülicke, S. Brandner, A. Aguzzi and C. Weissmann (1994). "Behavioral and anatomical deficits in mice homozygous for a modified beta-amyloid precursor protein gene." Cell **79**(5): 755-765.

Nalbantoglu, J., G. Tirado-Santiago, A. Lahsaïni, J. Poirier, O. Goncalves, G. Verge, F. Momoli, S. A. Welner, G. Massicotte, J. P. Julien and M. L. Shapiro (1997). "Impaired learning and LTP in mice expressing the carboxy terminus of the Alzheimer amyloid precursor protein." Nature **387**(6632): 500-505.

Narita, K. and S. Takeda (2015). "Cilia in the choroid plexus: their roles in hydrocephalus and beyond." Front Cell Neurosci **9**: 39.

Nery, L. R., N. S. Eltz, C. Hackman, R. Fonseca, S. Altenhofen, H. N. Guerra, V. M. Freitas, C. D. Bonan and M. R. Vianna (2014). "Brain intraventricular injection of amyloid-beta in zebrafish embryo impairs cognition and increases tau phosphorylation, effects reversed by lithium." PLoS One **9**(9): e105862.

Newman, M., I. F. Musgrave and M. Lardelli (2007). "Alzheimer disease: amyloidogenesis, the presenilins and animal models." Biochim Biophys Acta **1772**(3): 285-297.

Nicolas, M. and B. A. Hassan (2014). "Amyloid precursor protein and neural development." Development **141**(13): 2543-2548.
Nikolaev, A., T. McLaughlin, D. D. O'Leary and M. Tessier-Lavigne (2009). "APP binds DR6 to trigger axon pruning and neuron death via distinct caspases." Nature **457**(7232): 981-989.

O'Brien, R. J. and P. C. Wong (2011). "Amyloid Precursor Protein Processing and Alzheimer's Disease." Annual Review of Neuroscience **34**(1): 185-204.

Oh, S. Y., A. Ellenstein, C. D. Chen, J. D. Hinman, E. A. Berg, C. E. Costello, R. Yamin, R. L. Neve and C. R. Abraham (2005). "Amyloid precursor protein interacts with notch receptors." J Neurosci Res **82**(1): 32-42.

Olstad, E. W., C. Ringers, J. N. Hansen, A. Wens, C. Brandt, D. Wachten, E. Yaksi and N. Jurisch-Yaksi (2019). "Ciliary Beating Compartmentalizes Cerebrospinal Fluid Flow in the Brain and Regulates Ventricular Development." Curr Biol **29**(2): 229-241 e226.

Pan, Y. A., M. Choy, D. A. Prober and A. F. Schier (2012). "Robo2 determines subtype-specific axonal projections of trigeminal sensory neurons." Development **139**(3): 591-600.

Park, S. M., H. J. Jang and J. H. Lee (2019). "Roles of Primary Cilia in the Developing Brain." Front Cell Neurosci **13**: 218.

Perez, R. G., S. Soriano, J. D. Hayes, B. Ostaszewski, W. Xia, D. J. Selkoe, X. Chen, G. B. Stokin and E. H. Koo (1999). "Mutagenesis identifies new signals for beta-amyloid precursor

protein endocytosis, turnover, and the generation of secreted fragments, including Abeta42." J Biol Chem **274**(27): 18851-18856.

Portelius, E., E. Price, G. Brinkmalm, M. Stiteler, M. Olsson, R. Persson, A. Westman-Brinkmalm, H. Zetterberg, A. J. Simon and K. Blennow (2011). "A novel pathway for amyloid precursor protein processing." Neurobiol Aging **32**(6): 1090-1098.

Pu, Y. Z., L. Liang, A. L. Fu, Y. Liu, L. Sun, Q. Li, D. Wu, M. J. Sun, Y. G. Zhang and B. Q. Zhao (2017). "Generation of Alzheimer's Disease Transgenic Zebrafish Expressing Human APP Mutation Under Control of Zebrafish appb Promotor." Curr Alzheimer Res **14**(6): 668-679.

Puig, K. L. and C. K. Combs (2013). "Expression and function of APP and its metabolites outside the central nervous system." Exp Gerontol **48**(7): 608-611.

Puzzo, D., L. Privitera, M. Fa, A. Staniszewski, G. Hashimoto, F. Aziz, M. Sakurai, E. M. Ribe, C. M. Troy, M. Mercken, S. S. Jung, A. Palmeri and O. Arancio (2011). "Endogenous amyloid-beta is necessary for hippocampal synaptic plasticity and memory." Ann Neurol **69**(5): 819-830.
Puzzo, D., L. Privitera, E. Leznik, M. Fa, A. Staniszewski, A. Palmeri and O. Arancio (2008). "Picomolar amyloid-beta positively modulates synaptic plasticity and memory in hippocampus." J Neurosci **28**(53): 14537-14545.

Pyykko, O. T., M. Lumela, J. Rummukainen, O. Nerg, T. T. Seppala, S. K. Herukka, A. M. Koivisto, I. Alafuzoff, L. Puli, S. Savolainen, H. Soininen, J. E. Jaaskelainen, M. Hiltunen, H. Zetterberg and V. Leinonen (2014). "Cerebrospinal fluid biomarker and brain biopsy findings in idiopathic normal pressure hydrocephalus." PLoS One **9**(3): e91974.

Raible, D. W. and G. J. Kruse (2000). "Organization of the lateral line system in embryonic zebrafish." J Comp Neurol **421**(2): 189-198.

Rajendran, L. and W. Annaert (2012). "Membrane trafficking pathways in Alzheimer's disease." Traffic **13**(6): 759-770.

Rajendran, L., M. Honsho, T. R. Zahn, P. Keller, K. D. Geiger, P. Verkade and K. Simons (2006). "Alzheimer's disease beta-amyloid peptides are released in association with exosomes." Proc Natl Acad Sci U S A **103**(30): 11172-11177.

Raveau, M., T. Nakahari, S. Asada, K. Ishihara, K. Amano, A. Shimohata, H. Sago and K. Yamakawa (2017). "Brain ventriculomegaly in Down syndrome mice is caused by Pcp4 dose-dependent cilia dysfunction." Hum Mol Genet **26**(5): 923-931.

Reinhard, C., S. S. Hebert and B. De Strooper (2005). "The amyloid-beta precursor protein: integrating structure with biological function." EMBO J **24**(23): 3996-4006.

Rice, H. C., M. Townsend, J. Bai, S. Suth, W. Cavanaugh, D. J. Selkoe and T. L. Young-Pearse (2012). "Pancortins interact with amyloid precursor protein and modulate cortical cell migration." Development **139**(21): 3986-3996.

Richter, M. C., S. Ludewig, A. Winschel, T. Abel, C. Bold, L. R. Salzburger, S. Klein, K. Han, S. W. Weyer, A. K. Fritz, B. Laube, D. P. Wolfer, C. J. Buchholz, M. Korte and U. C. Muller (2018). "Distinct in vivo roles of secreted APP ectodomain variants APPsalpha and APPsbeta in regulation of spine density, synaptic plasticity, and cognition." EMBO J **37**(11).

Ring, S., S. W. Weyer, S. B. Kilian, E. Waldron, C. U. Pietrzik, M. A. Filippov, J. Herms, C. Buchholz, C. B. Eckman, M. Korte, D. P. Wolfer and U. C. Muller (2007). "The secreted beta-amyloid precursor protein ectodomain APPs alpha is sufficient to rescue the anatomical, behavioral, and electrophysiological abnormalities of APP-deficient mice." J Neurosci **27**(29): 7817-7826.
Rodgers, S. P., H. A. Born, P. Das and J. L. Jankowsky (2012). "Transgenic APP expression during postnatal development causes persistent locomotor hyperactivity in the adult." Mol Neurodegener **7**: 28.

Roncarati, R., N. Sestan, M. H. Scheinfeld, B. E. Berechid, P. A. Lopez, O. Meucci, J. C. McGlade, P. Rakic and L. D'Adamio (2002). "The gamma-secretase-generated intracellular domain of beta-amyloid precursor protein binds Numb and inhibits Notch signaling." Proc Natl Acad Sci U S A **99**(10): 7102-7107.

Rossi, A., Z. Kontarakis, C. Gerri, H. Nolte, S. Holper, M. Kruger and D. Y. Stainier (2015). "Genetic compensation induced by deleterious mutations but not gene knockdowns." Nature **524**(7564): 230-233.

Ruzicka, L., D. G. Howe, S. Ramachandran, S. Toro, C. E. Van Slyke, Y. M. Bradford, A. Eagle, D. Fashena, K. Frazer, P. Kalita, P. Mani, R. Martin, S. T. Moxon, H. Paddock, C. Pich, K. Schaper, X. Shao, A. Singer and M. Westerfield (2019). "The Zebrafish Information Network: new support for non-coding genes, richer Gene Ontology annotations and the Alliance of Genome Resources." Nucleic Acids Res **47**(D1): D867-d873.

Saido, T. and M. A. Leissring (2012). "Proteolytic degradation of amyloid beta-protein." Cold Spring Harb Perspect Med **2**(6): a006379.

Sanger, F., S. Nicklen and A. R. Coulson (1977). "DNA sequencing with chain-terminating inhibitors." Proc Natl Acad Sci U S A **74**(12): 5463-5467.

Sarasa, M., V. Sorribas, J. Terradoa, S. Climent, J. M. Palacios and G. Mengod (2000). "Alzheimer beta-amyloid precursor proteins display specific patterns of expression during embryogenesis." Mech Dev **94**(1-2): 233-236.

Sasaguri, H., P. Nilsson, S. Hashimoto, K. Nagata, T. Saito, B. De Strooper, J. Hardy, R. Vassar, B. Winblad and T. C. Saido (2017). "APP mouse models for Alzheimer's disease preclinical studies." EMBO J **36**(17): 2473-2487.

Sawamoto, K., H. Wichterle, O. Gonzalez-Perez, J. A. Cholfin, M. Yamada, N. Spassky, N. S. Murcia, J. M. Garcia-Verdugo, O. Marin, J. L. Rubenstein, M. Tessier-Lavigne, H. Okano and A. Alvarez-Buylla (2006). "New neurons follow the flow of cerebrospinal fluid in the adult brain." Science **311**(5761): 629-632.

Schotz, E. M., R. D. Burdine, F. Julicher, M. S. Steinberg, C. P. Heisenberg and R. A. Foty (2008). "Quantitative differences in tissue surface tension influence zebrafish germ layer positioning." HFSP J **2**(1): 42-56.

Sciaccaluga, M., A. Megaro, G. Bellomo, G. Ruffolo, M. Romoli, E. Palma and C. Costa (2021). "An Unbalanced Synaptic Transmission: Cause or Consequence of the Amyloid Oligomers Neurotoxicity?" International Journal of Molecular Sciences **22**(11).

Selkoe, D. J. and J. Hardy (2016). "The amyloid hypothesis of Alzheimer's disease at 25 years." EMBO Mol Med **8**(6): 595-608.

Serrano-Pozo, A., M. P. Frosch, E. Masliah and B. T. Hyman (2011). "Neuropathological alterations in Alzheimer disease." Cold Spring Harb Perspect Med **1**(1): a006189.

Shariati, S. A. M. and B. De Strooper (2013). "Redundancy and divergence in the amyloid precursor protein family." FEBS Letters **587**(13): 2036-2045.

Shendure, J. A., G. J. Porreca, G. M. Church, A. F. Gardner, C. L. Hendrickson, J. Kieleczawa and B. E. Slatko (2011). "Overview of DNA sequencing strategies." Curr Protoc Mol Biol **Chapter 7**: Unit7 1.

Shiau, C. E. and M. Bronner-Fraser (2009). "N-cadherin acts in concert with Slit1-Robo2 signaling in regulating aggregation of placode-derived cranial sensory neurons." Development **136**(24): 4155-4164.

Shiau, C. E., P. Y. Lwigale, R. M. Das, S. A. Wilson and M. Bronner-Fraser (2008). "Robo2-Slit1 dependent cell-cell interactions mediate assembly of the trigeminal ganglion." Nat Neurosci **11**(3): 269-276.

Simon, M. J. and J. J. Iliff (2016). "Regulation of cerebrospinal fluid (CSF) flow in neurodegenerative, neurovascular and neuroinflammatory disease." Biochim Biophys Acta **1862**(3): 442-451.

Slanchev, K., T. J. Carney, M. P. Stemmler, B. Koschorz, A. Amsterdam, H. Schwarz and M. Hammerschmidt (2009). "The epithelial cell adhesion molecule EpCAM is required for epithelial morphogenesis and integrity during zebrafish epiboly and skin development." PLoS Genet **5**(7): e1000563.

Soba, P., S. Eggert, K. Wagner, H. Zentgraf, K. Siehl, S. Kreger, A. Lower, A. Langer, G. Merdes, R. Paro, C. L. Masters, U. Muller, S. Kins and K. Beyreuther (2005). "Homo- and heterodimerization of APP family members promotes intercellular adhesion." EMBO J **24**(20): 3624-3634.

Song, Q. and J. G. Gleeson (2019). "Primary Cilia and Brain Wiring, Connecting the Dots." Developmental Cell **51**(6): 661-663.

Song, S., S. Eckerle, D. Onichtchouk, J. A. Marrs, R. Nitschke and W. Driever (2013). "Pou5f1-dependent EGF expression controls E-cadherin endocytosis, cell adhesion, and zebrafish epiboly movements." Dev Cell **24**(5): 486-501.

Sosa, L. J., A. Caceres, S. Dupraz, M. Oksdath, S. Quiroga and A. Lorenzo (2017). "The physiological role of the amyloid precursor protein as an adhesion molecule in the developing nervous system." J Neurochem **143**(1): 11-29.

Spassky, N. and A. Meunier (2017). "The development and functions of multiciliated epithelia." Nature Reviews Molecular Cell Biology **18**(7): 423-436.

Sperling, R. A., P. S. Aisen, L. A. Beckett, D. A. Bennett, S. Craft, A. M. Fagan, T. Iwatsubo, C. R. Jack, Jr., J. Kaye, T. J. Montine, D. C. Park, E. M. Reiman, C. C. Rowe, E. Siemers, Y. Stern, K. Yaffe, M. C. Carrillo, B. Thies, M. Morrison-Bogorad, M. V. Wagster and C. H. Phelps

(2011). "Toward defining the preclinical stages of Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease." Alzheimers Dement **7**(3): 280-292.

Sunderland, T., G. Linker, N. Mirza, K. T. Putnam, D. L. Friedman, L. H. Kimmel, J. Bergeson, G. J. Manetti, M. Zimmermann, B. Tang, J. J. Bartko and R. M. Cohen (2003). "Decreased beta-amyloid1-42 and increased tau levels in cerebrospinal fluid of patients with Alzheimer disease." Jama **289**(16): 2094-2103.

Tamayev, R., S. Matsuda, O. Arancio and L. D'Adamio (2012). "β- but not γ-secretase proteolysis of APP causes synaptic and memory deficits in a mouse model of dementia." EMBO Molecular Medicine **4**(3): 171-179.

Tan, V. T. Y., B. G. Mockett, S. M. Ohline, K. D. Parfitt, H. E. Wicky, K. Peppercorn, L. Schoderboeck, M. F. B. Yahaya, W. P. Tate, S. M. Hughes and W. C. Abraham (2018). "Lentivirus-mediated expression of human secreted amyloid precursor protein-alpha prevents development of memory and plasticity deficits in a mouse model of Alzheimer's disease." Mol Brain **11**(1): 7.

Tanzi, R. E. (2012). "The genetics of Alzheimer disease." Cold Spring Harb Perspect Med **2**(10).

Tanzi, R. E., J. F. Gusella, P. C. Watkins, G. A. Bruns, P. St George-Hyslop, M. L. Van Keuren, D. Patterson, S. Pagan, D. M. Kurnit and R. L. Neve (1987). "Amyloid beta protein gene: cDNA, mRNA distribution, and genetic linkage near the Alzheimer locus." Science **235**(4791): 880-884.

Taylor, C. J., D. R. Ireland, I. Ballagh, K. Bourne, N. M. Marechal, P. R. Turner, D. K. Bilkey, W. P. Tate and W. C. Abraham (2008). "Endogenous secreted amyloid precursor protein-alpha regulates hippocampal NMDA receptor function, long-term potentiation and spatial memory." Neurobiol Dis **31**(2): 250-260.

Theveneau, E., B. Steventon, E. Scarpa, S. Garcia, X. Trepat, A. Streit and R. Mayor (2013). "Chase-and-run between adjacent cell populations promotes directional collective migration." Nat Cell Biol **15**(7): 763-772.

Torroja, L., M. Packard, M. Gorczyca, K. White and V. Budnik (1999). "The Drosophila beta-amyloid precursor protein homolog promotes synapse differentiation at the neuromuscular junction." J Neurosci **19**(18): 7793-7803.

Turner, K. J., T. G. Bracewell and T. A. Hawkins (2014). "Anatomical dissection of zebrafish brain development." Methods Mol Biol **1082**: 197-214.

Tyan, S. H., A. Y. Shih, J. J. Walsh, H. Maruyama, F. Sarsoza, L. Ku, S. Eggert, P. R. Hof, E. H. Koo and D. L. Dickstein (2012). "Amyloid precursor protein (APP) regulates synaptic structure and function." Mol Cell Neurosci **51**(1-2): 43-52.

Valente, E. M., R. O. Rosti, E. Gibbs and J. G. Gleeson (2014). "Primary cilia in neurodevelopmental disorders." Nat Rev Neurol **10**(1): 27-36.

van der Kant, R. and L. S. Goldstein (2015). "Cellular functions of the amyloid precursor protein from development to dementia." Dev Cell **32**(4): 502-515.

Varshney, G. K., W. Pei, M. C. LaFave, J. Idol, L. Xu, V. Gallardo, B. Carrington, K. Bishop, M. Jones, M. Li, U. Harper, S. C. Huang, A. Prakash, W. Chen, R. Sood, J. Ledin and S. M. Burgess (2015). "High-throughput gene targeting and phenotyping in zebrafish using CRISPR/Cas9." Genome Research **25**(7): 1030-1042.

Vassar, R., B. D. Bennett, S. Babu-Khan, S. Kahn, E. A. Mendiaz, P. Denis, D. B. Teplow, S. Ross, P. Amarante, R. Loeloff, Y. Luo, S. Fisher, J. Fuller, S. Edenson, J. Lile, M. A. Jarosinski, A. L. Biere, E. Curran, T. Burgess, J. C. Louis, F. Collins, J. Treanor, G. Rogers and M. Citron (1999). "Beta-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE." Science **286**(5440): 735-741.

von Koch, C. S., H. Zheng, H. Chen, M. Trumbauer, G. Thinakaran, L. H. van der Ploeg, D. L. Price and S. S. Sisodia (1997). "Generation of APLP2 KO mice and early postnatal lethality in APLP2/APP double KO mice." Neurobiol Aging **18**(6): 661-669.

von Rotz, R. C., B. M. Kohli, J. Bosset, M. Meier, T. Suzuki, R. M. Nitsch and U. Konietzko (2004). "The APP intracellular domain forms nuclear multiprotein complexes and regulates the transcription of its own precursor." J Cell Sci **117**(Pt 19): 4435-4448.

Vorobyeva, A. G. and A. J. Saunders (2018). "Amyloid-beta interrupts canonical Sonic hedgehog signaling by distorting primary cilia structure." Cilia **7**: 5.

Wallingford, J. B., S. E. Fraser and R. M. Harland (2002). "Convergent extension: the molecular control of polarized cell movement during embryonic development." Dev Cell **2**(6): 695-706.

Wang, B., H. Li, S. A. Mutlu, D. A. Bowser, M. J. Moore, M. C. Wang and H. Zheng (2017). "The Amyloid Precursor Protein Is a Conserved Receptor for Slit to Mediate Axon Guidance." eNeuro **4**(3).

Wang, B., Z. Wang, L. Sun, L. Yang, H. Li, A. L. Cole, J. Rodriguez-Rivera, H. C. Lu and H. Zheng (2014). "The Amyloid Precursor Protein Controls Adult Hippocampal Neurogenesis through GABAergic Interneurons." Journal of Neuroscience **34**(40): 13314-13325.

Wang, P., G. Yang, D. R. Mosier, P. Chang, T. Zaidi, Y. D. Gong, N. M. Zhao, B. Dominguez, K. F. Lee, W. B. Gan and H. Zheng (2005). "Defective neuromuscular synapses in mice lacking amyloid precursor protein (APP) and APP-Like protein 2." J Neurosci **25**(5): 1219-1225.

Wang, X., J. B. Zhang, K. J. He, F. Wang and C. F. Liu (2021). "Advances of Zebrafish in Neurodegenerative Disease: From Models to Drug Discovery." Front Pharmacol **12**: 713963.

Wang, X., X. Zhou, G. Li, Y. Zhang, Y. Wu and W. Song (2017). "Modifications and Trafficking of APP in the Pathogenesis of Alzheimer's Disease." Front Mol Neurosci **10**: 294.

Wang, Z., B. Wang, L. Yang, Q. Guo, N. Aithmitti, Z. Songyang and H. Zheng (2009). "Presynaptic and postsynaptic interaction of the amyloid precursor protein promotes peripheral and central synaptogenesis." J Neurosci **29**(35): 10788-10801.

Wasco, W., K. Bupp, M. Magendantz, J. F. Gusella, R. E. Tanzi and F. Solomon (1992). "Identification of a mouse brain cDNA that encodes a protein related to the Alzheimer disease-associated amyloid beta protein precursor." Proc Natl Acad Sci U S A **89**(22): 10758-10762.

Wasco, W., S. Gurubhagavatula, M. D. Paradis, D. M. Romano, S. S. Sisodia, B. T. Hyman, R. L. Neve and R. E. Tanzi (1993). "Isolation and characterization of APLP2 encoding a

homologue of the Alzheimer's associated amyloid beta protein precursor." Nat Genet **5**(1): 95-100.

Weidemann, A., G. König, D. Bunke, P. Fischer, J. M. Salbaum, C. L. Masters and K. Beyreuther (1989). "Identification, biogenesis, and localization of precursors of Alzheimer's disease A4 amyloid protein." Cell **57**(1): 115-126.

Weingarten, M. D., A. H. Lockwood, S. Y. Hwo and M. W. Kirschner (1975). "A protein factor essential for microtubule assembly." Proc Natl Acad Sci U S A **72**(5): 1858-1862.

Westerfield, M. (2000). "The Zebrafish Book : A Guide for the Laboratory Use of Zebrafish." http://zfin.org/zf_info/zfbook/zfbk.html.

Weyer, S. W., M. Klevanski, A. Delekate, V. Voikar, D. Aydin, M. Hick, M. Filippov, N. Drost, K. L. Schaller, M. Saar, M. A. Vogt, P. Gass, A. Samanta, A. Jaschke, M. Korte, D. P. Wolfer, J. H. Caldwell and U. C. Muller (2011). "APP and APLP2 are essential at PNS and CNS synapses for transmission, spatial learning and LTP." EMBO J **30**(11): 2266-2280.

Whitfield, J. F. and B. R. Chakravarthy (2009). "The neuronal primary cilium: driver of neurogenesis and memory formation in the hippocampal dentate gyrus?" Cell Signal **21**(9): 1351-1355.

Willem, M., S. Tahirovic, M. A. Busche, S. V. Ovsepian, M. Chafai, S. Kootar, D. Hornburg, L. D. Evans, S. Moore, A. Daria, H. Hampel, V. Muller, C. Giudici, B. Nuscher, A. Wenninger-Weinzierl, E. Kremmer, M. T. Heneka, D. R. Thal, V. Giedraitis, L. Lannfelt, U. Muller, F. J. Livesey, F. Meissner, J. Herms, A. Konnerth, H. Marie and C. Haass (2015). "eta-Secretase processing of APP inhibits neuronal activity in the hippocampus." Nature **526**(7573): 443-447.

Williams, C. L., C. Li, K. Kida, P. N. Inglis, S. Mohan, L. Semenec, N. J. Bialas, R. M. Stupay, N. Chen, O. E. Blacque, B. K. Yoder and M. R. Leroux (2011). "MKS and NPHP modules cooperate to establish basal body/transition zone membrane associations and ciliary gate function during ciliogenesis." J Cell Biol **192**(6): 1023-1041.

Wilson, L. and M. Lardelli (2013). "The Development of an in vivo γ-Secretase Assay using Zebrafish Embryos." Journal of Alzheimer's Disease **36**(3): 521-534.
Wu, C. Y. and L. A. Taneyhill (2019). "Cadherin-7 mediates proper neural crest cell-placodal neuron interactions during trigeminal ganglion assembly." Genesis **57**(1): e23264.

Yamazaki, T., D. J. Selkoe and E. H. Koo (1995). "Trafficking of cell surface beta-amyloid precursor protein: retrograde and transcytotic transport in cultured neurons." J Cell Biol **129**(2): 431-442.

Yoshikai, S., H. Sasaki, K. Doh-ura, H. Furuya and Y. Sakaki (1990). "Genomic organization of the human amyloid beta-protein precursor gene." Gene **87**(2): 257-263.

Young-Pearse, T. L., J. Bai, R. Chang, J. B. Zheng, J. J. LoTurco and D. J. Selkoe (2007). "A Critical Function for -Amyloid Precursor Protein in Neuronal Migration Revealed by In Utero RNA Interference." Journal of Neuroscience **27**(52): 14459-14469.

Young-Pearse, T. L., A. C. Chen, R. Chang, C. Marquez and D. J. Selkoe (2008). "Secreted APP regulates the function of full-length APP in neurite outgrowth through interaction with integrin beta1." Neural Dev **3**: 15.

Zappaterra, M. W. and M. K. Lehtinen (2012). "The cerebrospinal fluid: regulator of neurogenesis, behavior, and beyond." Cell Mol Life Sci **69**(17): 2863-2878.

Zhang, Z., M. Song, X. Liu, S. Su Kang, D. M. Duong, N. T. Seyfried, X. Cao, L. Cheng, Y. E. Sun, S. Ping Yu, J. Jia, A. I. Levey and K. Ye (2015). "Delta-secretase cleaves amyloid precursor protein and regulates the pathogenesis in Alzheimer's disease." Nature Communications **6**(1).

Zheng, H., M. Jiang, M. E. Trumbauer, D. J. Sirinathsinghji, R. Hopkins, D. W. Smith, R. P. Heavens, G. R. Dawson, S. Boyce, M. W. Conner, K. A. Stevens, H. H. Slunt, S. S. Sisoda, H. Y. Chen and L. H. Van der Ploeg (1995). "beta-Amyloid precursor protein-deficient mice show reactive gliosis and decreased locomotor activity." Cell **81**(4): 525-531.

Zou, C., S. Crux, S. Marinesco, E. Montagna, C. Sgobio, Y. Shi, S. Shi, K. Zhu, M. M. Dorostkar, U. C. Muller and J. Herms (2016). "Amyloid precursor protein maintains constitutive and adaptive plasticity of dendritic spines in adult brain by regulating D-serine homeostasis." EMBO J **35**(20): 2213-2222.