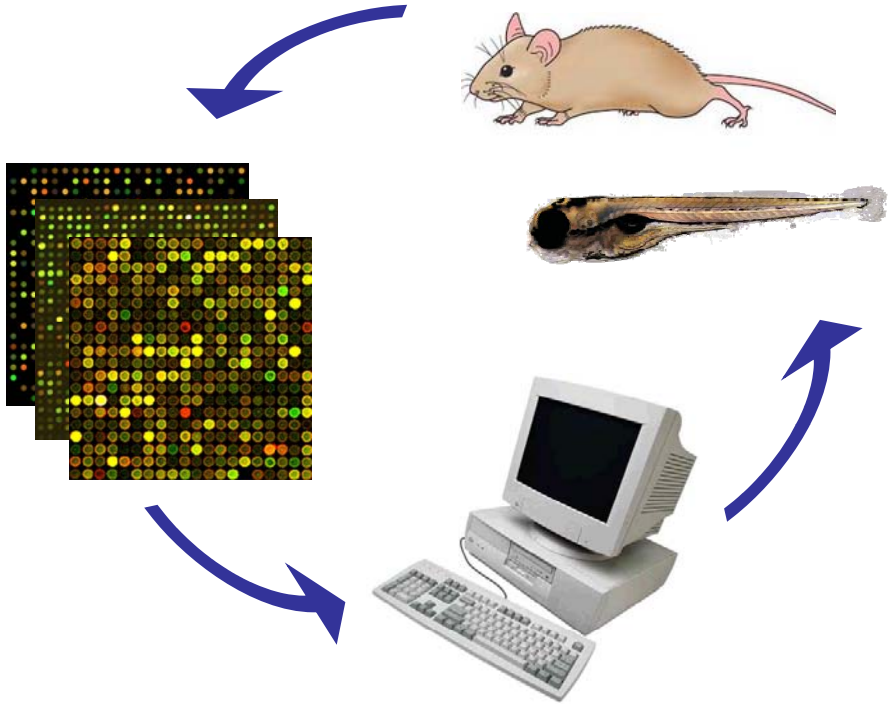
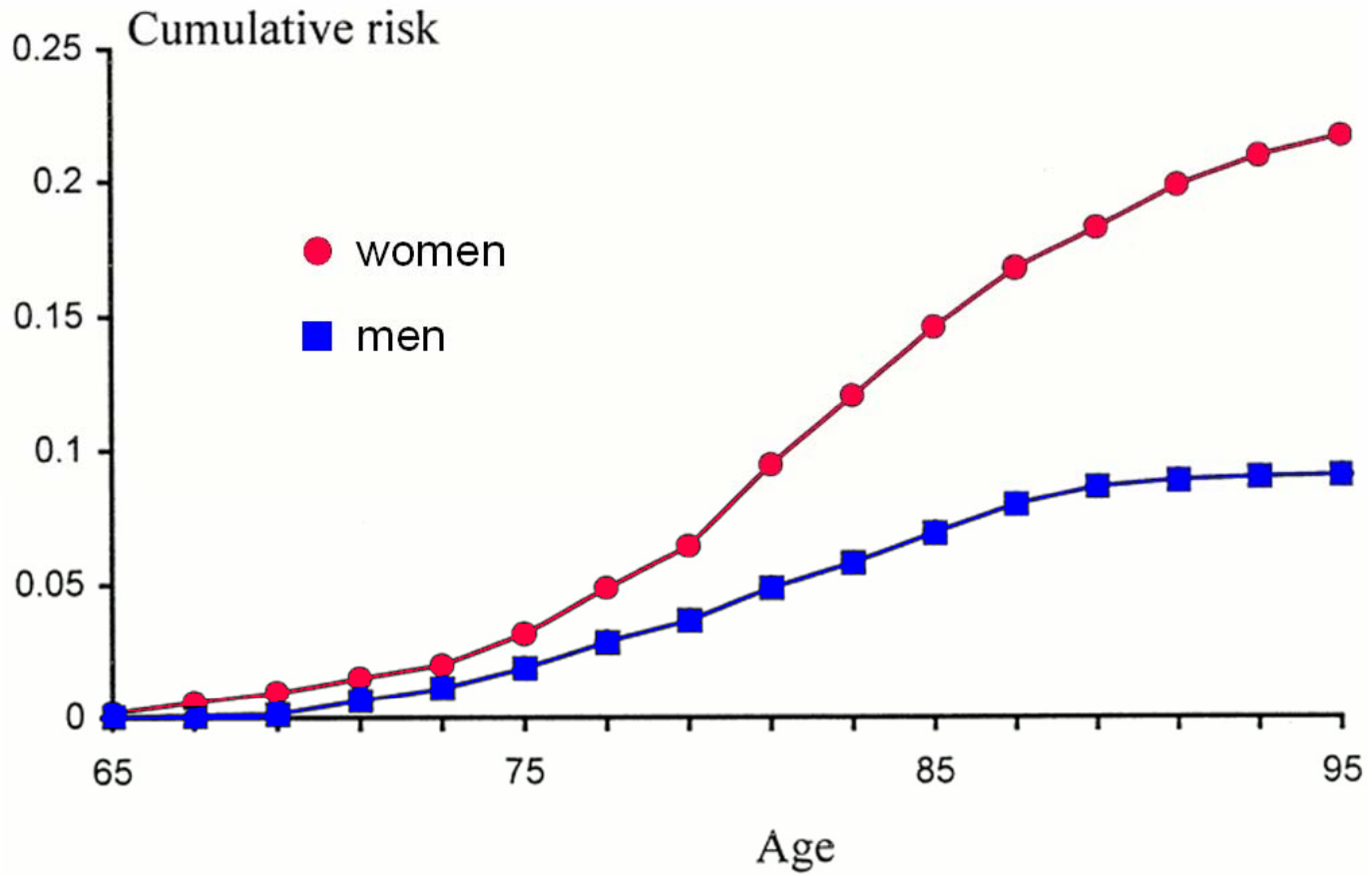


# Age-related gender differences in brain expression levels of tau-interacting ubiquitin-specific peptidase 9 and possible implications for Alzheimer's disease

Speaker: Enrico Glaab, Luxembourg Centre for Systems Biomedicine

# Motivation: Age-dependence of gender-differences in AD

**Sex-specific cumulative risk for a 65-year-old to develop AD by 95 years of age.**




**Adapted from Andersen K et al. Neurology 1999;53:1992-1992**

# Overview of used data sources and analyses

1) Microarray gene expression data from the **Human Brain Transcriptome (HBT) Project** (1340 *post-mortem* samples,16 brain regions,15 age groups)

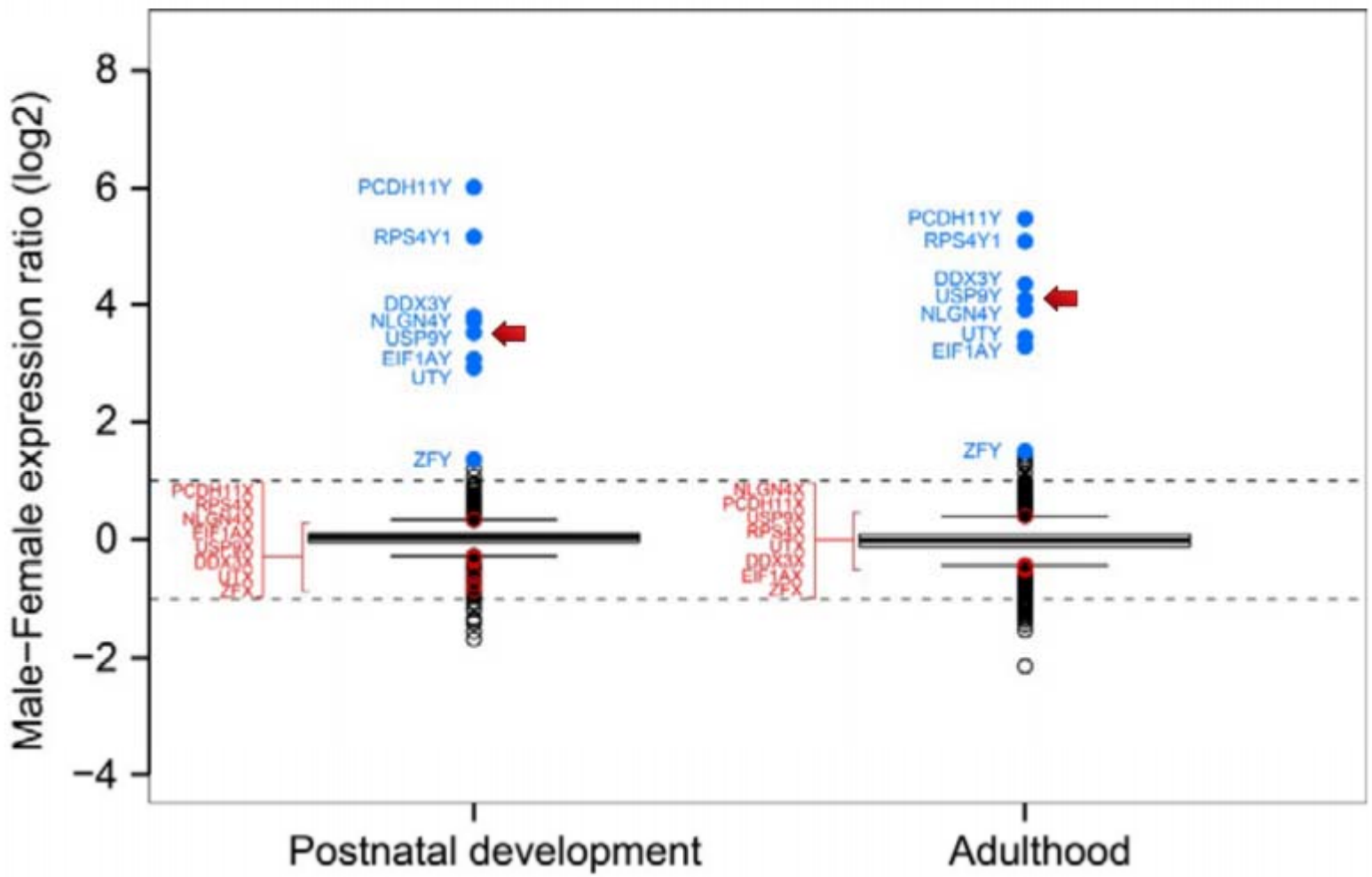
→ used to identify genes with significant gender-differences in expression levels during adult aging

2) Microarray gene expression data from the **late-onset AD study by Zhang *et al*.**, *Cell*, 2013 (171 male AD samples and 213 male controls, 216 female AD samples and 87 female controls; *post-mortem* brain samples covering *dorsolateral cortex*, *visual cortex* and *cerebellum*)

→ used to identify genes with gender-specific significant differential expression between AD samples and controls

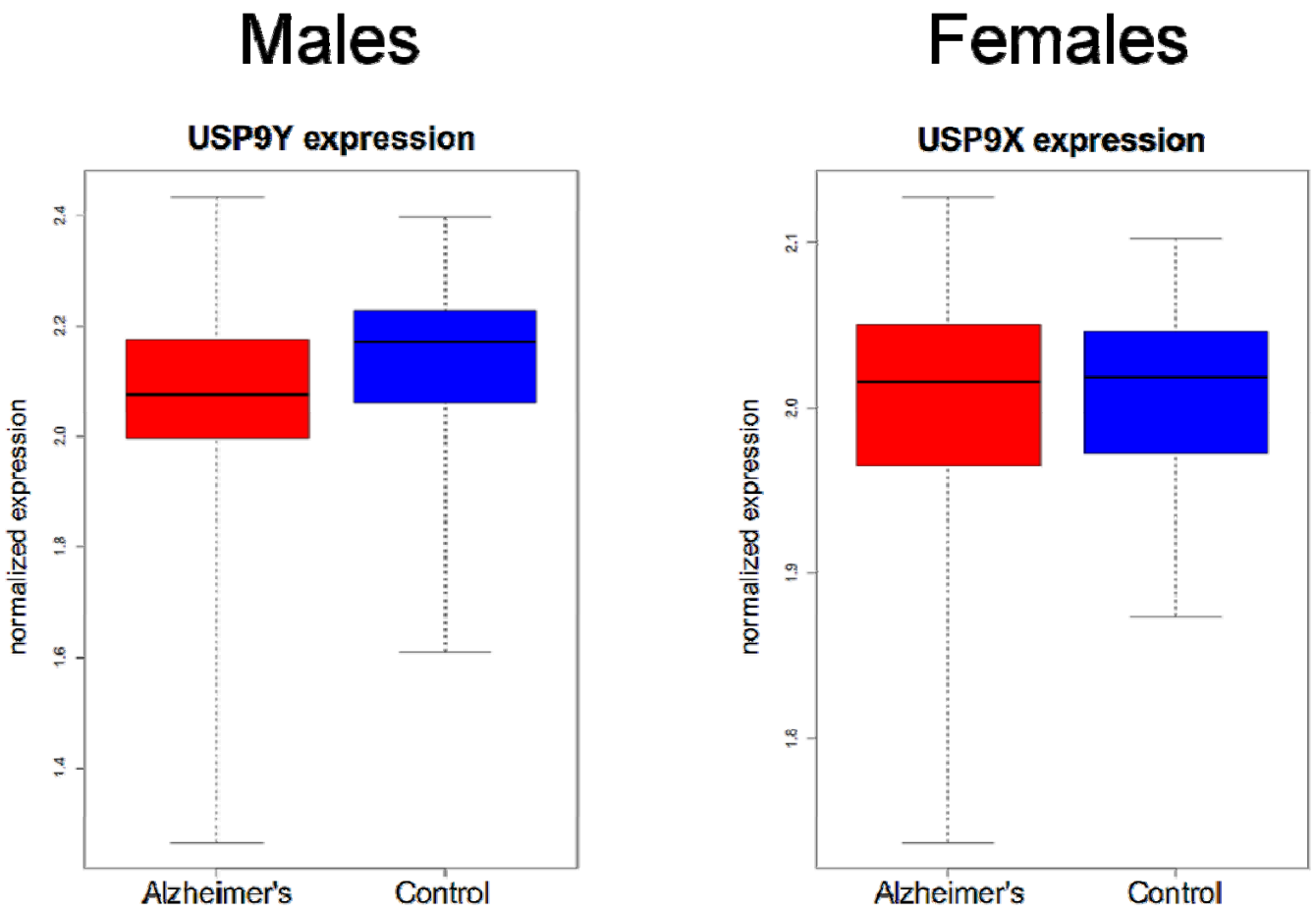
3) Protein interaction **network analysis**, **pathway analysis** and **literature mining** for the candidate gene **Y-chromosomal ubiquitin-specific peptidase 9** (***USP9Y***), derived from the integration of the results from 1) and 2)

# Genes with large male-female expression ratios in the brain




HBT dataset: USP9Y belongs to the genes with the highest male-female expression level ratios across 16 human brain regions (difference not compensated by USP9X)

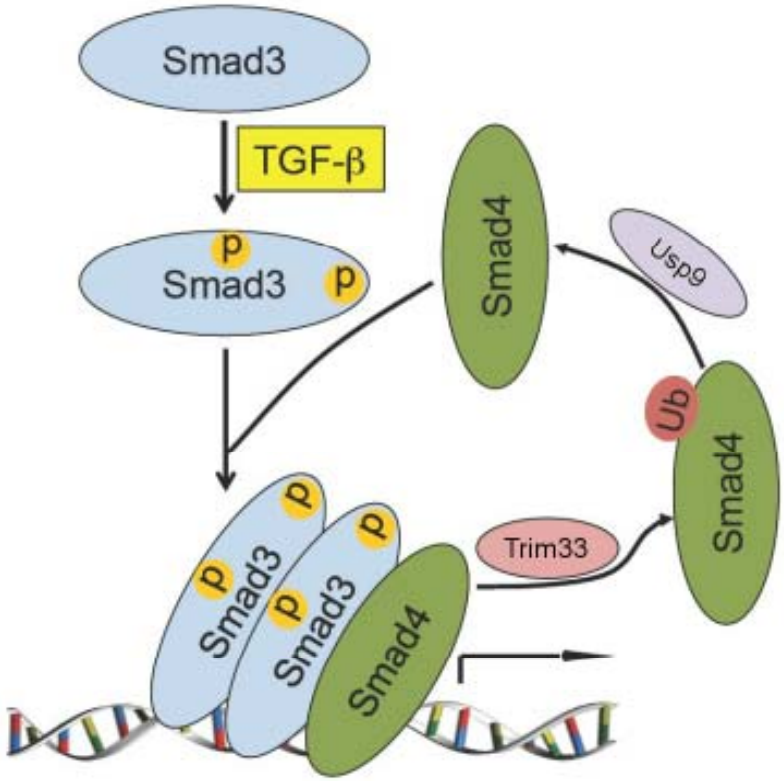
# Gender-specific significant deregulation of USP9 in AD




Zhang *et al*. Alzheimer's dataset: USP9Y is significantly down-regulated in male AD samples vs. male controls (left box plot, adj. p-value: $1.69 \cdot 10^{-5}$), while no signifcant deregulation is observed for USP9X in females (right box plot).

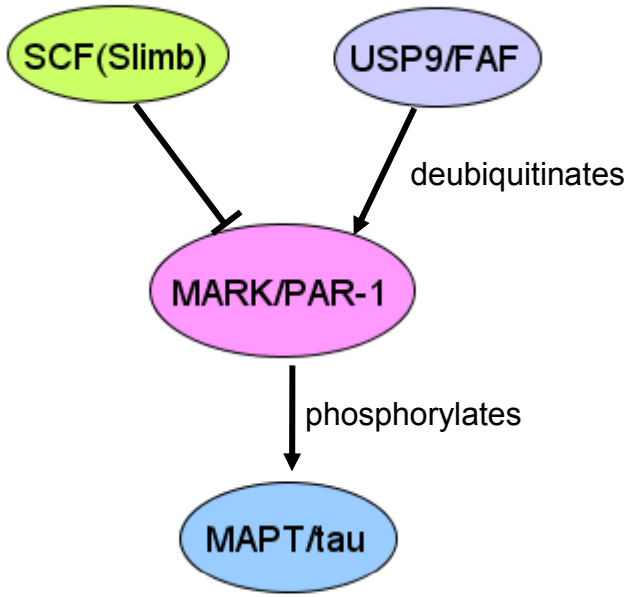
# Regulatory roles of USP9 as a deubiquitinase

**1) Role of USP9 in TGF-Beta signalling**



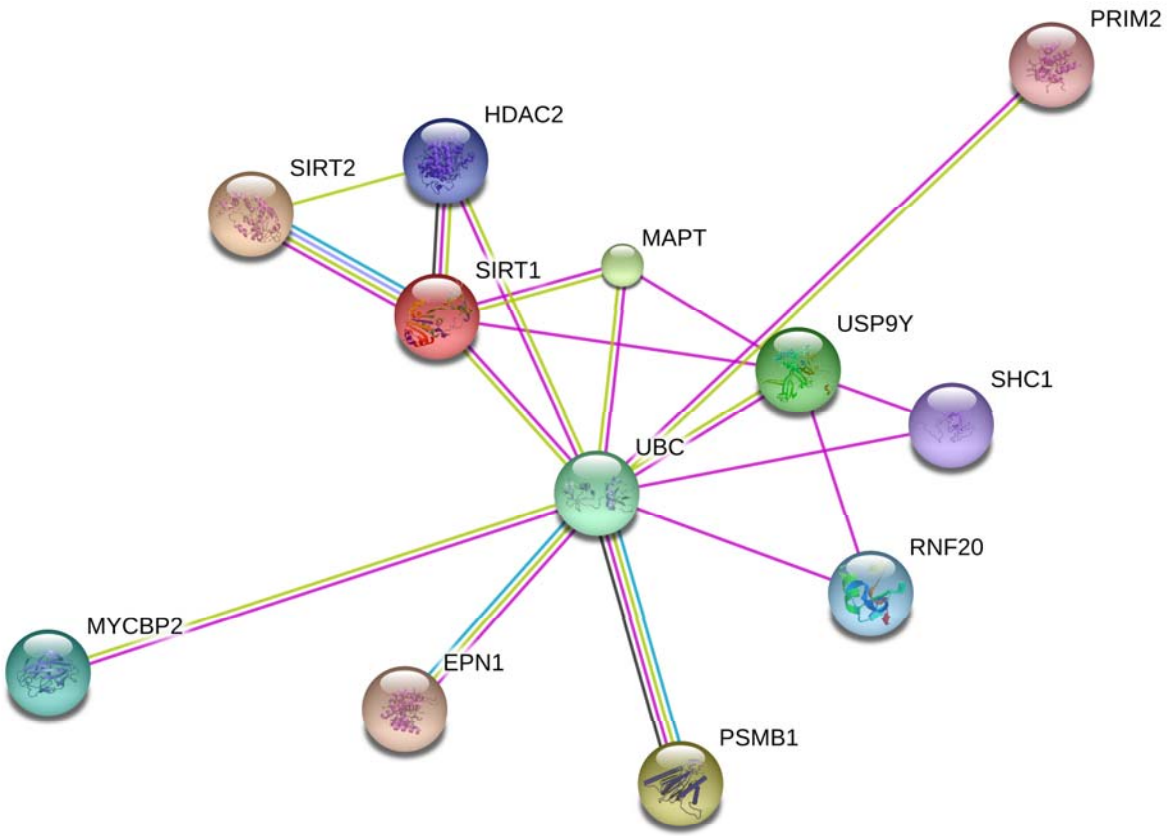

Adapted from Tang and Zhang, Cell & Bioscience, 2011

**2) Role of USP9 in MAPT regulation (Drosophila model)**




Regulation according to the drosophila model by S. Lee et al., Nature Communications, 2011

# Protein interaction network analysis for USP9Y




Public protein-protein interaction databases report interactions of USP9Y with the microtubule-associated protein tau (MAPT) and SIRT1, which have been linked to AD by numerous studies (magenta-colored interactions in the molecular network are supported by at least one source of experimental evidence).

# Literature mining results for USP9X/Y

- Over-expression of the USP9 homologue *faf* in fly neurons induces **synaptic overgrowth**, a BMP/TGF-Beta related phenotype (McCabe et al., *Neuron*, 2004)

- Loss of USP9X in mice leads to disruptions in **cortical architecture**, **hippocampal development** and **TGF-Beta-mediated axonogenesis** (Stegeman et al., *PLoS ONE*, 2013)

- USP9X interacts with, deubiquitinates, and stabilizes the **survival motor neuron (SMN) protein**, low levels of which cause the neurodegenerative disorder Spinal Muscular Atrophy (Han et al., *The Journal of Biological Chemistry*, 2012)

- USP9X interacts *in vivo* with and deubiquitinates α-synuclein (SNCA), a key protein in the pathogenesis of Parkinson's disease, and **knockdown of USP9X promotes accumulation of monoubiquitinated SNCA** species and enhances the formation of toxic SNCA inclusions upon proteolytic inhibition (Rott et al., *PNAS*, 2011)

- Multiple sources of evidence point to a **ubiquitin-dependent degradation of MAPT**, but the contribution of this and other degradation pathways to tau levels in AD still has to be assessed (Schmidt and Finley, *Biochimica et Biophysica Acta*, 2013; Lee et al., *Progress in Neurobiology*, 2013)

# Research plan (1)

1) **Mining of further public data sources (~3 months, Enrico Glaab)**:

- analysis of USP9 sequence variations in ADNI genome sequencing data and GWAS data (at SNP-, gene- and pathway-level)
- statistical analysis of public omics data for AD animal models (comparing USP9X/Y expression for males vs. females in controls and for AD challenges)

2) **Cell culture model perturbation analyses (~3 months, Paul Antony)**:

- select one of three cell lines (SH-SY5Y, HeLa or MEF) depending on the confirmed expression of USP9, MAPT and SIRT1
- perform qPCR expression measurements for USP9, MAPT and SIRT1 to obtain a reference for the perturbation experiments
- test 5 shRNAs for USP9 knockdown, select the best 3 using flow cytometry
- perform microarray expression profiling (one readout after 24h) with 5 replicates for each of the following settings: 1) samples with baculovirus-delivered shRNA (for each of the 3 best shRNAs); 2) samples without virus; 3) samples with virus without shRNA; 4) samples with virus with scrambled shRNA

# Research plan (2)

3) **Zebrafish perturbation analyses (5 months, Alexander Crawford)**:

- use morpholino knockdowns of *usp9* in zebrafish larvae and viral over-expression of *usp9* with a transposon-based vector
- Western blot and qPCR analysis to investigate deregulations in *mapt* and *sirt1* in response to perturbations
- phenotypic assessment of usp9 over-expression effects in zebrafish AD model using *in-vivo* imaging (current approach of choice: tauopathy model by Paquet *et al*., J. Clin. Invest., 2009)

4) **Mouse model analyses (6 months, Manuel Buttini and Pierre Garcia)**:

- compare 2 genotypes (wildtype, APP-mutated mice) and 2 time-points (young: 6-8 months, old: 16-18 months), considering male mice only
- qPCR analysis with 4 brain samples per condition to assess expression levels of USP9Y, SIRT1 and MAPT, comparing young vs. old and APP-mutated vs. wildtype mice
- characterize samples for neuronal markers synaptophysin and MAP-2

# Outlook

**Long-term outlook**:

- Successful accomplishment of the above research plan and publication of the results would pave the way for further research as part of a long-term collaborative grant application (e.g. a 3-year CORE grant by the Luxembourg Fonds National de la Recherche)

- Potential partners based on previous collaborations: Helmholtz Center in Munich, Germany; Mouse Clinical Institute close to Strasbourg, France

- Goal: Long-term time series analysis for the expression of USP9X/Y, SIRT1, MAPT in aging male and female APP-mutated and wildtype mice, focusing on a strain with known increased AD-susceptibility for females