CONCLUDING REMARKS.

The great scientific efforts in understanding AD etiology during the last century have revealed that we are facing a highly complex and heterogeneous disorder with devastating consequences to society. The further molecular understanding of AD pathogenesis opens new insights on the development not only of disease modifying therapies but also of novel biomarkers that may help to predict AD in the earliest possible stage. In this thesis, we aimed to unravel novel hallmarks of AD with a potential involvement in the development of this disorder using proteomics candidates as starting point. In addition, we evaluated whether the novel AD signatures were reflected in CSF and thus, their potential as early biomarkers. Several studies in the last years have unraveled multiple potential AD biomarkers that still need to be validated[107,108]. Given the complexity of this disorder, it is conceivable that a combination of multiple markers including neurochemical molecules and imaging patterns will likely help to accurately predict AD, and thus a synergistic collaboration between scientists from different disciplines is needed. In addition, the development of more sensitive techniques together with the standardization of protocols and the definition and classification of the non-demented control groups (are subjective memory complaints cases optimal controls?) will likely promote the development of predictive diagnostic tools.

The most important findings of this thesis are:

- BRI2 is increased in early stages of AD associated with amyloid plaques (chapter 3).
- BRI2 processing enzymes are changed in AD (chapter 3).
- The BRI2-APP binding is present in human tissue and reduced in AD patients. (chapter 3).
- BRI2 changes correlated with decreased concentration and activity of IDE, an enzyme involved in Aβ degradation (chapter 4)
- Full length BRI2 ectodomain is less efficient on delaying Aβ fibrillation than BRI2 BRICHOS domain (chapter 5).
- Full length BRI2 ectodomain can induce an apoptotic response and promote tau truncation (chapter 5).
- SPPL2b is present in AD post-mortem tissue but absent or remarkably lower in control cases (chapter 6).
- SPPL2b increase started in very early stages of AD and is associated with the classical protein aggregates in AD and other misfolding dementias (chapter 6).
- SPPL2b is a novel tau binding protein (chapter 6).

- SPPL2b was decreased in AD CSF and correlated with the concentration of $A\beta_{42}$ and cognitive decline (chapter 6).
- BRI2 CSF cannot differentiate AD from healthy controls but correlates with the concentration of t-Tau, p-Tau, $A\beta_{40}$ and specific inflammatory and vascular markers (chapter 8).
- Increase of agrin in AD human tissue is not reflected in CSF but it is influenced by age in the demented cases (chapter 9)

FUTURE PERSPECTIVES: WHAT'S NEXT?

SPPL2b and BRI2 as potential etiological factors of AD.

In this thesis we have extensively analyzed the expression of both BRI2 and SPPL2b in post-mortem tissue unraveling the presence of two new characteristics in AD. However, it would be important to further explore the human AD neuropathology and perform a thorough characterization of the specific BRI2 and SPPL2b forms that are modified in AD. Knowing the specific characteristics of those proteins in AD (i.e. glycosylation, aggregation, sequence) will help for the development of sensitive techniques accurately detecting the pathological forms *in vivo*. In addition, taking into account the consequences that SPPL2b activity may have on its substrates, it is important to develop tools able to determine whether the increase on SPPL2b reflects also an increase of SPPL2b activity. Those tools can be then also used to perform a high throughput screening of pharmacological compounds able to bind/inhibit SPPL2b.
Although the data outlined in this thesis revealed that both BRI2 and SPPL2b are promising novel potential contributors to the pathogenesis of AD, we cannot ignore that the studies performed are primarily observational (with the exception of chapter 5). Thus, the current results set the basis for future *in vitro* and *in vivo* experiments, which will give mechanistic insights into the consequences of the SPPL2b increase and BRI2 changes. In this sense, SPPL2b overexpressing cell lines are already available and a mouse model overexpressing SPPL2b is currently being developed. The combination of human material, cell and animal models will help to unravel whether BRI2 and SPPL2b play indeed an important role in AD pathogenesis.

On the road to early AD diagnosis.

The SPPL2b changes found in CSF and tissue support the potential of this protein as an early AD biomarker, but more sensitive and specific techniques are needed. Noteworthy, the pathological changes occurring within the brain are not always reflected into the CSF

as seen not only in this thesis but also in numerous previous studies (i.e. serum amyloid P[109] or C-reactive protein[110] among others). Therefore, considering that SPPL2b reactivity was nearly absent in non-demented controls, it would be also interesting to study the potential of imaging techniques such as positron emission tomography (PET) to measure SPPL2b changes *in vivo*.