Sex differences in Alzheimer’s disease: what we know and what we do

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So there is male and female typical behavior: Are there male typical and female typical diseases???

The biological purpose of these differences is: Reproduction
Many diseases affect men and women differently

Sex is a biological factor in health and disease

Most striking example: **Cardiovascular disease**

Clear sex differences in disease risk between men and women
- Affects more males than females at reproductive age
- Different symptoms
- Different response to treatments
- Very little is still known about these sex differences

Meyer MR et al., Hypertension, 2006
Despite obvious sex differences, why do we know so little about these?

Why are these not studied more?

Sex differences

Disease risk – Etiology — Disease progression —> Sex stratified treatment
Sex and gender differences are understudied

Adds cost and time to the research project!!

• If mostly males or females are affected why study sex differences?
• All trials and treatments need to double - expensive
• The scope of the *in vitro* model need to include XX and XY cells
• Epidemiological and clinical studies would need to be stratified by sex
  – Reduces sample sizes with potential to give inconclusive results
• What about XY chromosomal aberration genotypes, or transgenders??
• How to incorporate gender aspects in the research project?
Sex as a biological factor in health and disease

Most striking example: **Cardiovascular disease**

Clear sex differences in disease risk between men and women
- Affects more males than females at reproductive age
- Different symptoms
- Different response to treatments
- Differences are lost to a large extent after menopause
- Sex hormones are likely involved
- In fact women are at higher risk of dying of a cardiovascular event than men

Meyer MR et al., Hypertension, 2006
What about sex differences in neurological diseases?

Well, we do know that there are clear sex and gender differences in behavior.

Is this because male and females brains are different?
Sex hormones

**SRY gene on the Y chromosome (XY)**
- Development of male testes
- Production of testosterone

**Lack of SRY gene (XX)**
- Default development of ovaries
- Production of estrogen

**Androgen receptor**

**Aromatase enzyme**

**Testosterone**

**Estrogen**
- (Estradiol, E2)
- Estrogen receptor alpha (ERα)
- Estrogen receptor beta (ERβ)
- GPER1
Estrogen and testosterone in neurodevelopment

- **Sex hormone Levels**
  - Birth
  - Puberty
  - Development of gonads
  - Secondary sex characteristics
  - Implantation
  - Neurogenesis (late first trimester)

- **Masculinization or feminization of the brain**

- **Activation of SRY gene**

- **Organizational, physical, lasting changes in different brain regions**

- **T levels**
  - E2 levels
So, on a neurosignalling level there are significant sex differences between the male and female brain.
What about sex differences in neurological diseases?

Pinares-Garcia et al, Brain Sci., 2018
Alzheimer’s disease (AD)

• Most common type of dementia
  • 50 million diagnosed cases, ca 60-70% of all dementia
  • 11% of people aged 65+ have AD
  • 32% of people aged 85+ have AD

• Cause of AD is unknown, except for <5% of cases with known genetic mutations (familial AD)

• > 95% of AD cases are sporadic, where ca 70% of all cases appear to be inherited

• Complex interaction between genes, disease history and environment

• Characterized by misfolded amyloid beta depositions in and around synapses (amyloid plaques) that leads to intraneuronal misfolded Tau-tangles inside neurons, extensive neuroinflammation and neuronal death

• No cure!
Alzheimer’s disease progression

- **Mild Cognitive Impairment**
  - Duration: 7 years
  - Disease begins in Medial Temporal Lobe
  - Symptoms: Short-term memory loss
  - Amyloid pathology: ++
  - Tau pathology: (+)
  - Neuroinflammation: +

- **Mild Alzheimer’s**
  - Duration: 2 years
  - Disease spreads to Lateral Temporal & Parietal Lobes
  - Symptoms include: Reading problems, Poor object recognition, Poor direction sense
  - Amyloid pathology: ++
  - Tau pathology: +
  - Neuroinflammation: ++

- **Moderate Alzheimer’s**
  - Duration: 2 years
  - Disease spreads to Frontal Lobe
  - Symptoms include: Poor judgment, Impulsivity, Short attention
  - Amyloid pathology: +++
  - Tau pathology: ++
  - Neuroinflammation: +++

- **Severe Alzheimer’s**
  - Duration: 3 years
  - Disease spreads to Occipital Lobe
  - Symptoms include: Visual problems
  - Amyloid pathology: +++
  - Tau pathology: +++
  - Neuroinflammation: ++++
Risk factors in AD

- APOE
- (Cardio-) Metabolic disorders
- Age
- Gender
- Genes
- Family history
- Head trauma
- Brain abnormalities
- Smoking
- High blood pressure
- Limited physical activity
- Lack of mental activity
- Obesity

Alzheimer's Risk Factors
Risk factors in AD: Apo-E4

- **APOE4** allele status is the most common genetic risk factor for *sporadic* AD (14%)
- Apo-E is mainly expressed in astrocytes in the brain and participates in cholesterol transport and in proteolysis of Amyloid beta in the brain
- Polymorphic **APOE, APOE4**, is not as effective in these processes
- Females heterozygous for **APOE4** have a 4-fold higher risk of developing AD than heterozygous men. Not known why.

Liu CC et al, *Nat Neuro* 2013
Apo-E4
Other factors?

Pinares-Garcia et al, *Brain Sci.*, 2018
Epidemiological data

**AD Facts & Figures:**

1) In USA: 3.2 M women and 1.7 M men have AD (2014)
   - Being female is a risk factor for AD
   - Females have a higher incidence of developing AD. However, no excess risk in USA – regional differences!

2) Women live on average longer than men

3) Women born before ~1950 have lower education than men

4) Men who survive middle age cardiovascular events are healthier and less prone to develop AD (“survival bias”)
   - This is controversial!

5) Women with *APOE4* genotype have a higher risk of developing AD compared with men with the same genotype
   - *Estrogen* has been suggested to be involved!

![Bar chart showing lifetime risk of developing AD at age 45 and 65](chart.png)

Alzheimer’s disease Facts & Figures. Alzheimer & Dementia, 2018
Wait. How could estrogen be involved in AD?

Sex hormone Levels
- Birth
- Puberty
- Development of gonads
- Implantation
- Neurogenesis (late first trimester)

Masculinization or feminization of the brain

How is the female brain affected by a sudden E2 drop here?

We don’t really know, but we know that T and E2 are neuroprotective.

Organizational, physical, lasting changes in different brain regions
1. E2 regulates ApoE

- ApoE participates in amyloid beta degradation and brain cholesterol transport
- ApoE levels in mice change according to estrus cycle
- Different effects in different brain regions
- Not known how – likely indirect effects of E2 on ApoE

2. E2 is antioxidative

- E2 and progesterone (P4) decreases mitochondrial electron leakage and thereby lowers brain mitochondrial lipid peroxidation.
- E2 upregulates expression of key antioxidant enzymes in the brain.

3. E2 modulates BDNF expression

**BDNF (Brain-derived neurotrophic factor) promotes:**

- Neuronal survival
- Neuronal growth and differentiation
- Neurogenesis (from neural stem cells)
- Synapse formation
- Long-term memory

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**ERE = Estrogen response element in promoter region**

4. E2 is anti-inflammatory

Selective ERβ agonist (LY3201) decreased reactive pro-inflammatory microglia (iNOS expressing) in a mouse model of Multiple Sclerosis (PLP treatment)

Wu WF et al, PNAS, 2013
Is menopause a risk factor for AD?

Sex hormone Levels

- Birth
- Development of gonads (late first trimester)
- Neurogenesis (late first trimester)
- Menopause

T levels

E2 levels

How is the female brain affected by a sudden E2 drop here?

E2 is neuroprotective

Organizational, physical, lasting changes in different brain regions
Is menopause a risk factor for AD??

Looking at the extreme cases: surgical menopause (oophorectomy) & dementia risk

Oophorectomy is a risk factor for dementia (HR: 1.46), especially if performed at a younger age (HR: up to 4.61)

Rocca WA et al, Neurology, 2007
Could hormone replacement therapy (HRT, ERT) at menopause lower the risk of AD and dementia later in life?

In such case, when and how should HRT be given?

Several variables to take into consideration:
- HRT type (e.g. 17β-estradiol or conjugated equine estrogens, ±progestins)
- Route of administration (oral, vaginal, or dermal)
- Timing
- Duration
- Interaction with other sex-biased risk factors? – e.g. cardiometabolic disease
- Interaction with other gender-biased risk factors? – e.g. education, smoking

What about possible side effects of HRT?
- Increased risk for endometrial and breast cancer
- Increased risk for thromboembolism

Overall benefits must be weighed against overall risks!
Some studies exist on hormone replacement therapy (HRT, ERT) and AD risk later in life

About 20 epidemiological studies have been performed


Quite big heterogeneity...
Why the heterogenous results??

Larger cohort size points to positive effects of HRT

Prospective longitudinal studies show stronger beneficial effects of HRT than retrospective case-control studies

Studies displaying results as relative risks (eg prospective studies) show stronger beneficial effects

Somewhat stronger beneficial effects observed in newer studies

Why the heterogenous results??

What about HRT (E2) interaction with other sex-biased effects??
Associations with cardiometabolic events?
Parity?
Apo-E4?

What about gender-biased effects??
Contraceptives?
Smoking?
Education?

We don’t know!

How does E2 interact with AD risk on a mechanistic level??

We don’t really know that either!

Prospective longitudinal studies show stronger beneficial effects of HRT than retrospective case-control studies
Larger cohort size predicts outcome

Studies displaying results as relative risks (eg prospective studies) show stronger beneficial effects
Somewhat stronger beneficial effects observed in newer studies

Song Y-J, et al, Fornt Neuorsci, 2020
But wait! Menopause occurs ca 20-30 years before any signs of cognitive impairment!

What is the consequence of menopausal E2 loss on markers of AD pathology??

Jack et al., 2010
Estrogen and Androgen receptors in the adult brain

Expression:

- Estrogen Receptor alpha (ERα)
- Estrogen Receptor beta (ERβ)

Cortical areas:
- Cortex
- Thalamus
- Hippocampus

Subcortical areas:
- Hypothalamus
- Limbic system (mood, emotions)
- Cognition (memory, learning)
- Reproduction (HPG-axis)

- Pituitary Gland
- Amygdala
- Female AD mice have a more aggressive AD pathology than male mice
- Ovariectomy may worsen pathology in frontal cortex
- Differences in neuroinflammatory response may be one contributing factor for these sex differences (lessons from ischemic stroke)

Still much is not known
- Other factors? Cholesterol metabolism?
- Species differences! Sex hormonal signaling in mice is different from humans
The complexity of human AD cannot be fully addresses only using experimental models.

**What about E2 interaction with other sex-biased effects??**
- Associations with cardiometabolic events?
- Parity?
- Apo-E4?

**What about gender-biased effects??**
- Contraceptives?
- Smoking?
- Education?
"Combined cohorts of menopausal women – Studies of register-based health outcomes in relation to hormonal drugs"

- Pools over **80 000** Swedish postmenopausal women from different cohorts (**unique power!**)  
- Detailed and unique information of HRT use (baseline 1987-2002)  
- Type, route of administration, timing, duration  
- Information on possible confounding variables exist, such as:  
  - Age at baseline, level of education, smoking status, body mass index, level of physical activity, age at menopause onset, type of menopause, parity (number of given births), contraceptives (oral, vaginal or transdermal), alcohol consumption, hypertension, diabetes, family history of cardiovascular disease, and dyslipidemia  
- Genotype of most participants exist (Apo-E, other genes)

**Participants are followed over time (prospective)** through linkage to Swedish national registers regarding incidence of chronic disease: AD and non-AD dementia, Cardiometabolic disease (CVD, CHD)
Are AD treatment strategies different for men and women today?

There is no difference in treatments given to men and women at similar stage of disease.

There is no difference in preventive recommendations given to men and women.

There is no difference in how dementia is diagnosed between men and women. (women can perform better on verbal memory test despite presence of pathology)

Why?

Most animal experiments performed on male mice.

Species differences: Rodent sex hormone signaling is different from human.

AD etiology has a complex interaction with biological and environmental factors which affects one sex more than the other.

More integrative research is needed!
ER signaling group at BioNut, KI

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Soon hiring PhD student and postdoc!