ApoE4 - The Most Prevalent Genetic Risk Factor for Alzheimer’s Disease is a promising untapped Therapeutic Target

Danny Michaelson
Professor of Neurobiology
Faculty of Life Sciences
Tel Aviv University
Choice of Alzheimer’s Disease Therapeutic Targets

Current Approach:
Focus on AD Pathology and Genetics of Familial AD

2002-2012 clinical trials:
413 trials, 244 compounds no success.

Ongoing clinical trials
Current clinical trials focus, among others, on Aβ, Tau & inflammation.

β-Amyloid
Presenilins
Tau

Senile Plaque
Tangles
Choice of Alzheimer’s Disease Therapeutic Targets

A single suitable for all “magic bullet“ approach will probably not work

Alternative Approach: Focus on Genetic risk Factors of Sporadic AD

Criteria: High prevalence and important clinical impact.
Pathways to Alzheimer's disease

Risk of Alzheimer's disease

- Very rare
- Medium
- High

Frequency in the population

- Very common

Genes:
- PSEN1
- PSEN2
- APP
- APOE4
- TREM2
- MS4A
- CR1
- PICALM
- BIN1
- CD2AP
- EPHA1
- CLU
- ABCA7
- CD33
Choice of Alzheimer’s Disease Therapeutic Targets

A single suitable for all “magic bullet“ approach will probably not work

Alternative Approach:
Focus on Genetic risk Factors of Sporadic AD

Criteria: High prevalence and important clinical impact.

Apolipoprotein E4 (apoE4)
Outline of Talk

1. ApoE4 and Alzheimer’s Disease
2. The mechanisms underlying the pathological effects of apoE4
3. ApoE4 directed therapy
Apolipoprotein E (ApoE)

- **N-terminal domain**
- **C-terminal domain**

**Position**

<table>
<thead>
<tr>
<th></th>
<th>112</th>
<th>158</th>
</tr>
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<tbody>
<tr>
<td>ApoE2</td>
<td>Cys</td>
<td>Cys</td>
</tr>
<tr>
<td>ApoE3</td>
<td>Cys</td>
<td>Arg</td>
</tr>
<tr>
<td>ApoE4</td>
<td>Arg</td>
<td>Arg</td>
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- **112 Arg (E4) / Cys (E3)**
- **158 LDL receptor binding**
Adapted from Rebeck & Hyman (1999) in Alzheimer’s Disease (Terry et al eds.), p340.
ApoE4 Dependent Brain Pathology in AD

- Aβ Deposition
- Neuronal Plasticity/Repair
- Inflammation
- Vascular Impairment
Outline of Talk

1. ApoE4 and Alzheimer’s Disease
2. The mechanisms underlying the pathological effects of apoE4
3. ApoE4 directed therapy
The Molecular Mechanisms Underlying the Pathological Effects of ApoE4

ApoE4

- ApoE4 Catabolism
- ApoE Receptors
- Targets (e.g., Autophagy, inflammation)
- Signaling (e.g., VEGF)
- Amyloid Cascade
- Lipid Metabolism

Neurodegeneration, Vascular Pathology
Outline of Talk

1. ApoE4 and Alzheimer’s Disease
2. The mechanisms underlying the pathological effects of apoE4
3. ApoE4 directed therapy
   (i) Models
   (ii) Approaches
ApoE4 Mice are Cognitively Impaired (Morris and Object recognition)

Morris water maze

Object Recognition

Sample-object exposure

Novel-object test

Delay (eg. 1 h)

Time to platform (sec.)

Time near new object / total time

Time (days)

Time to platform (sec.)

ApoE3

ApoE4

*
ApoE4 Mice are Cognitively Impaired (Fear conditioning)
ApoE4 induces AD-related pathology

Aβ42

Tau (AT8)

ApoE3

ApoE4

ApoE3

ApoE4

Liraz O et al; Mol Neurodegener. 2013
ApoE4 affects excitatory (Vglut1) and inhibitory (VGaT) synapses in the hippocampus.
The apoE4 phenotype in heterozygote female mice

![Graphs showing the levels of Aβ, Tau(AT8), Tau(AT180), VGlutT, VGaT, and ApoER2 in ApoE3, ApoE4, and ApoE3/4 phenotypes.](image)
ApoE4 is hypolipidated relative to apoE3

Boehm-Cagan et al; J.Neuroscience, 2014
Outline of Talk

1. ApoE4 and Alzheimer’s Disease

2. The mechanisms underlying the pathological effects of apoE4

3. ApoE4 directed therapy
   (i) Models
   (ii) Approaches
Development of ApoE4 directed therapy

The Gene level: Convert apoE4 to apoE3

The protein level: block apoE4 and/or reverse impairments

Down stream mechanisms (eg signaling, VEGF)
Is ApoE4 “Bad” or Does It Lack “Good” properties of ApoE3?

Structural Possibilities:

ApoE3

Gain

ApoE4

Loss (e.g. lipids)

Level

Pathological Implications:

Gain and/or loss of function

loss of function
Gain vs. Loss of Function Effects of ApoE4

Gain of Function ("not present in ApoE Knockout mice")

Loss of Function (Similar to ApoE Knockout mice)
ApoE4 Targeted Therapeutic Approaches

1. Counteract gain of toxicity effects of apoE4 with mAbs

2. Compensate for loss of lipidation
   - Upregulation of ABCA1
   - Direct Treatment with ABCA1 Agonist
Counteract Gain of Toxicity of ApoE4 with mAbs

- Preparation of anti-apoE4 mAbs
- In vivo proof of concept direct i.c.v injection of the mAb
- Assessment of therapeutic efficacy following i.p application

Readout:
- Brain apoE/IgG
- Behavioral Impairements
- AD related pathology
- ApoE related parameters
- Synaptic pathology

Ishai Luz
In-Vivo Binding of i.p. injected Anti-ApoE4 mAbs to Brain ApoE4

Immunohistochemical Co-Localization

ApoE

ELISA

IgG Bound to ApoE
The Effects of i.p. injected Anti-ApoE4 mAbs on ApoE4-Driven Aβ42 and Phospho-Tau Levels

**Tau hyperphosphorylation (AT8)**

- **PBS**
- Non Specific mAbs
- Anti ApoE4 mAb

**Aβ42**

- Control (PBS)
- mAb 9D11

**Results**

- ApoE3
- ApoE4

- PBS
- Non-Specific mAb
- Anti ApoE4 mAb (9D11)
# Counteracting the Pathological Effects of ApoE4 with Anti-apoE4 mAbs

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ApoE4 Targeted Therapeutic Approaches

1. Counteract gain of toxicity effects of apoE4 with mAbs

2. Compensate for loss of lipidation
   - Upregulation of ABCA1
   - Direct Treatment with ABCA1 Agonist
Upregulation of the Expression and Lipidation of ApoE

Liver X Receptors

Bexarotene

LXR, RXR

LXRE

NCOR/SMRT

HDAC3

CBP/p300

LXR Target Genes

ABCA1

ABCG1

ApoE
Upregulation of the Expression and Lipidation of ApoE

Liver X Receptors

LXRE

NCOr/SMRT

HDAC3

LXR RXR

CBP/p300

LXR Target Genes

ABCA1
ABCG1
ApoE

LXR RXR

CS-6253

ABCA1 agonist

CT domain of apoE

216 244 major lipid-binding region 272

EVRAKLEEGDOQGIRLEAEFOIRLKWEEPLVE

234 270
The ABCA1 agonist CS-6253 accumulates in the brain and co-localizes with astrocytes.
The effects of ABCA1 agonist CS-6253 on the levels and lipidation of ApoE

**Total ApoE (SDS gels)**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>CS-6253</th>
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<tbody>
<tr>
<td>ApoE3</td>
<td></td>
<td></td>
</tr>
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<td>ApoE4</td>
<td></td>
<td></td>
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**Native ApoE gels**

- **ApoE3**
  - Control
  - CS-6253

- **ApoE4**
  - Control
  - CS-6253

**Native ApoE gels**

- **High M.W.**
  - Control
  - CS-6253

- **Mid M.W.**
  - Control
  - CS-6253

- **Low M.W.**
  - Control
  - CS-6253

ApoE3, ApoE4
Reversal of the AD related pathological effects of apoE4 by ABCA1 agonist CS-6253 (female mice)
# Counteracting the pathological effects of ApoE4

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<th>Increased Lipidation by ABCA1 agonist</th>
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Development of directed therapy ApoE4

The Gene level: Convert apoE4 to apoE3

The protein level: block apoE4 and/or reverse impairments

Down stream mechanisms (eg signaling, VEGF)
VEGF

Angiogenic factor

Neurotrophic factor

Vascular Endothelial Growth Factor and Angiogenesis

The neuroprotective function of vascular endothelial growth factor (VEGF)

Kalina Gora-Kuplas, Jadwiga Jośko

Chair and Department of Environmental Medicine and Epidemiology, Medical University of Silesia, Zabrze, Poland
ApoE4 down-regulates the VEGF system

Salomon-Zimri et al; JAD 2016
Up-regulation of VEGF by Intra-hippocampal injection of VEGF expressing lentivirus

*In collaboration with Prof. Danni Offen’s group, the Felsenstein Medical Research Center*
LV-VEGF reverses apoE4-driven neuronal and synaptic brain pathology

**Synaptic pathology:** VGlut1

**ApoE receptor:** ApoR2

**Neurogenesis:** Doublecortin
## Counteracting the Pathological Effects of ApoE4

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<th>Phenotype</th>
<th>VEGF treatment</th>
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<tr>
<td>Cognitive Impairment</td>
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An integrated view of the anti ApoE4 therapeutic approaches

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<td>Treatment</td>
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<tr>
<td>ABCA1 agonist</td>
<td>reversal</td>
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</tr>
<tr>
<td>αApoE4 mAb</td>
<td>no change</td>
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<tr>
<td>VEGF vector reversal</td>
<td>reversal</td>
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Physiological response

Pathological response

Reversal of VEGF and ApoE4 lipidation phenotype

Increase lipidation

VEGF Vector

E4 E3 like

Physiological response

Pathological response

VEGF

Anti ApoE4 mAb

E4
Conclusion: The apoE4 tree of knowledge is ready to be harvested
Collaborators

Dani Offen (Tel Aviv)
Eliezer Masliah (San Diego)
Tobias Hartmann (Homborg)
Aviv Shaish (Tel Hashomer)

Acknowledgments

Artery Ltd
LIPIDIDIET 7th EU Program
The Eichenbaum Foundation
Israel Science Foundation
US-Israel BSF
German-Israeli Foundation
But apoE4 is Understudied
Oil and Water do not Mix and Apolipoproteins are at the Interface
Why is apoE4 is Understudied ? And how should this be changed
Base Pair editing  A Crisper modification shown to convert apoE4 (Arg at 158) to ApoE3 (Cys at 158) in cell cultures with very high efficiency

Figure 1

Effect size

50.0

High

3.0

Intermediate

1.5

Modest

1.1

Low

Rare alleles causing Mendelian disease

Low-frequency variants with intermediate effect

Rare variants of small effect very hard to identify by genetic means

Few examples of high-effect common variants influencing common disease

Common variants implicated in common disease by GWA

Allele frequency

Very Rare

Rare

Low frequency

Common
ApoE4 is a Risk Factor for Other Diseases

- Cerebral Amyloid Angiopathy (CAA)**
- Traumatic Brain Injury **
- Cerebrovascular disease*
- Vascular Dementia*
- Multiple Sclerosis*
- Amyotrophic Lateral Sclerosis (ALS)*

The ABCA1 agonist CS-6253 counteracts ApoE4-driven behavioral deficits

Morris water maze

Probe test

Novel object recognition (24h)
Development of ApoE4 directed therapy

The Gene level: Convert apoE4 to apoE3

The protein level: block apoE4 (eg by mAbs); reverse impairments (eg correction of hypolipidation)

Down stream mechanisms (eg signaling, VEGF)
The effect of LV-VEGF treatment on VEGF expression in young naïve mice

**VEGF**

- **ApoE3**
- **ApoE4**

**LV-GFP**

**LV-VEGF**

**Naive**

**LV-VEGF CA3 (a.u.)**

- **Naive**
- **LV-GFP**
- **LV-VEGF**

**VEGF-R2**

- **ApoE3**
- **ApoE4**

**Naive**

**LV-GFP**

**LV-VEGF**

**Hif-1α**

- **ApoE3**
- **ApoE4**

**Naive**

**LV-GFP**

**LV-VEGF**

**HIF-1α (a.u.)**

- **Naive**
- **LV-GFP**
- **LV-VEGF**

*** ***
## Counteracting the pathological effects of ApoE4

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Choice of Alzheimer’s Disease Therapeutic Targets

Current Approach:
Focus on AD Pathology and Genetics of Familial AD

Senile Plaque
Tangles

β-Amyloid
Presenilins
Tau

2002-2012: 413 trials, 244 compounds no success yet
Will a single magic bullet approach work in AD

1. AD is a syndrome and not a single disease: most cases are mixed

2. The occurrence of a single common upstream pathological factor is thus open to discussion (GWAS cholesterol, inflammation, membrane traffic; AD develops over 20 years)

Conclusion: Although theoretically it may be possible to develop a magic bullet. This is far from certain and there is a need for alternative approaches.
Alternative Approach: Focus on Genetic risk Factors of Sporadic AD

Criteria: High prevalence and important clinical impact.

Apolipoprotein E4 (apoE4)
ApoE4 affects excitatory (Vglut-1), but not inhibitory (GAD67) synapses in the hippocampus.
Inhibition of cholesterol efflux by anti apoE4 mAb 9D11