How meta-analysis launched my career in PD research

Xuemei Huang, M.D., Ph.D
Professor and Vice Chair of Neurology
2015-02-06
Parkinson’s disease

- First described in 1817 by James Parkinson as “Shaking Palsy.”
- Current affect more than 1 million Americans
- Cardinal Signs
  - *Resting tremor*
  - *Bradykinesia-slowness*
  - *Rigidity-stiffness*
  - *Postural/Gait disorder*
The exact cause of PD is unknown - many factors interact to make the PD pie

Identify the problem or knowledge gap
Factors associated with PD risk

**Higher Risk:**
- Strong evidence:
  - Age: >50 yr
  - Gender: Male > Female
  - Family history
- Some evidence (controversial)
  - Exposure to toxins
  - Concussion
- Initial reports (rumor)
  - Physicians
  - higher education

**Lower Risk:**
- Strong evidence
  - Younger, female
  - Cigarette smoking
  - Drinking coffee
- Some evidence (controversial)
  - ? African-American
  - Drinking alcohol
- Initial reports (rumor)
  - Construction workers
  - lower education?
A clinical observation during my fellowship year 2001-2002

- Parkinson’s disease patients are “relatively healthy” cardiovascularly
- Supported by literature

Make the observation & find out what is known!
“Untraditional” hypothesis ca. 2002

- Low LDL-cholesterol may be associated with PD susceptibility.
Cholesterol & Biological Membranes

Cholesterol

Justify the reason for the hypothesis
Cholesterol - Precursor of Critical Hormones

DHEAS

Sulfotransferase?

Sulfatase?

DHEA

3β-HSD?

DHEAS

P450c17?

P450ssc*

CHOLESTEROL

PREGNENOLONE

3β-HSD?

PROGESTERONE

PROGESTERONE

DOC

CORTICOSTERONE

ALDOSTERONE

P450aldo?

11β-Hydroxylase?

DOC

DHT

5αR

17β-HSD

TESTOSTERONE

5αR

17β-HSD

Aromatase

ESTRADIOL

ESTRONE

5α-DHP

3α-THP

5α,3α-THP

17β-HSD
CoQ10 and cholesterol synthesis share same pathway

CoQ10 -\rightarrow\text{HMG-CoA reductase} -\rightarrow\text{mevalonate} -\rightarrow\text{isopentenyl pyrophosphate} -\rightarrow\text{geranyl pyrophosphate} -\rightarrow\text{farnesyl pyrophosphate} -\rightarrow\text{squalene}

\begin{align*}
\text{acetyl-CoA} + \text{acetoacetyl-CoA} & \rightarrow \text{HMG-CoA} \\
\text{mevalonate} & \rightarrow \text{isopentenyl pyrophosphate} \\
\text{geranyl pyrophosphate} & \rightarrow \text{farnesyl pyrophosphate} \\
\text{farnesyl pyrophosphate} & \rightarrow \text{squalene}
\end{align*}
Seek “the wisdom of expert”
(Pierre Morell, lipid expert at UNC)

- Cholesterol in brain is important for synapse genesis.

- Cholesterol is also important for membrane function/lipid rafting.

- How about APOE?

The expert may become your mentor, advisor…collaborator
Apolipoprotein E & cholesterol levels, neurodegeneration

- There are three types of APOE alleles in the general population
  - $\varepsilon_2$: generally considered as “good” allele; associated with
    - lower LDL-cholesterol
    - lower risk of dementia/Alzheimer’s disease.
    - Associated with longevity
  - $\varepsilon_3$: most common allele
  - $\varepsilon_4$: generally considered as “bad” allele; associated with
    - higher LDL-cholesterol
    - higher risk of dementia/Alzheimer’s disease

Do my homework!
Is there a role APOE in Parkinson’s disease?

- Numerous studies have been done (results perceived as inconsistent).
- Majority of authors reported that there is no statistically significant association between APOE alleles and Parkinson’s disease.
Hypothesis

• If lower LDL-cholesterol is associated with PD, then …… Then..
  – APOE 2, not APOE 4, should be associated with PD.

Think, read, and think again!!
Approach

• Re-test this hypothesis using meta-analysis.

• Find a mentor/collaborator (Charlie Poole, UNC)
Meta-analysis methodology

Databases

- Medline (PubMed)
- Biosis Previews
- ISI Web of Science

Key words search

- “Parkinson”
- “apoE” or “apolipoprotein”

Retrieve Articles (123)

Inclusion Criteria

1. Case control studies with clear defined clinical or pathologically cases
2. Only include one set result per author group (31 papers)
3. Genotype frequency from publication or via author contact (2-3 emails)

Total of 22 independent studies including 2,157 cases and 7,831 controls
Meta-analysis methodology: Data extraction

- Necessary data (available in most papers)
  - *Sample size*
  - *ApoE genotype information*
  - *Age of subjects*
  - *Gender of the subjects*
  - *Year of publications*
  - *Country of the study*
  - *Test of Hardy-Weinberg equilibrium in the control group.*

- Desirable data (not available in most papers)
  - *Ethnicity of subjects*
  - *Age of onset*
  - *Clinical subtypes*
  - *Pathological characteristics*
Meta-analysis methodology
-Funnel Plots of Estimated Odd Ratios

Relatively symmetrical distributions suggest no publication bias.
Meta-analysis Methodology
Forest Plots of Estimated Odd Ratios

- ε2
  - Han (1994)
  - Harrington (1994)
  - Marder (1994)
  - Rubensztein (1994)
  - Koller (1995)
  - Martinoli (1995)
  - Egensperger (1996)
  - Helisalmi (1996)
  - Morris (1996)
  - Whitehead (1996)
  - Ballering (1997)
  - Durr (1997)
  - Yamamoto (1997)
  - Grasbon-Frodl (1999)
  - Kruger (1999)
  - Olivieri (1999)
  - Harhangi (2000)
  - Goetz (2001)
  - Eerola (2002)
  - Parsian (2002)
  - Schulte (2003)
  - Summary

- ε3

- ε4

- Log OR (95% CI)
APOE ε2 is associated with PD!!

Total of 22 independent studies including 2,157 cases and 7,831 controls

Huang et al, Neurology, 2004
Nine case control studies were not included in the analyses

- Three studies from USA, four from Europe, two from Asia.
- Only allele frequency was reported, from which genotype information cannot be deduced.
- Authors could not provide data or did not respond to several contacts.

- … but seven of the nine studies reported higher $\epsilon2$ frequency among PD than controls.
Conclusion

Huang et al, 2004, Neurology

• Unlike Alzheimer’s disease, it is the ε2 allele, not ε4, allele that is positively associated with sporadic Parkinson disease.
• APOE isoforms may have differential effects on the different neurodegenerative diseases.

Meta-analysis can be a great starting point for an academic career:
Cheap, high impact
Give you an overview of the field
Give you’re the foundation to build upon
Is there a role of APOE in developing dementia in Parkinson’s population?

• Numerous studies have results perceived as inconsistent and conflicting.

• Hypothesis:
  – Conflicting results may reflect heterogeneity of test population, and multiple overlapping etiologies of dementing process in Parkinson’s population.

• Approach:
  – Systemically review the published literature and performed a meta-analysis on suitable studies to examine the relative prevalence of dementia in Parkinson’s population in relation to APOE genotype.
Meta-analysis methodology: Source data

Data base
- Medline (PubMed)
- Biogenesis Previews
- ISI Web of Science
- Jan 1966-Dec, 2003

Key words search
- "Parkinson"
- "apoE" or "apolipoprotein"
- "Dementia"

Retrieve Articles (71)

Inclusion Criteria
1. Case control studies with clear defined clinical or pathologically PD cases.
2. Dementia was diagnosed with clearly described clinical or pathological criteria.
3. Only include one set result per author group
4. Genotype frequency information either contained from publication or obtained from original author

Total of 9 independent studies including
295 PD cases without dementia and 163 PD cases with dementia
Funnel Plots of Estimated Odd Ratios

There seems to be publication bias & some evidence of heterogeneity especially for ε4 allele.
Forrest Plots of Estimated Odd Ratios

The $\varepsilon_4$ allele seems to be associated with higher prevalence of dementia in PD.
Summary

- Our analysis suggests that publication bias and heterogeneity of study subjects (the age of subjects, geographic locale, and diagnostic criteria) all influence the final estimation of OR of dementia prevalence in the PD population.
- Although the data suggest that the APOE ε4 allele seems to be associated with higher prevalence of dementia in the PD population, further study is warranted using standard diagnostic criteria, clear recording of subject ethnicity, age of onset of subject PD and dementia, and control of geographic location.

Huang et al, 2006, Arch. Neurology

There is no negative data in science. All well-designed research should be published whether or not the hypothesis was supported.
Follow-up studies

• Initial reports of low total/LDL-cholesterol linked to high occurrence and faster clinical progression in PD, and imaging biomarkers:
  – Huang et al, 2007, Movement Disorders, First case control
  – Huang et al, 2008, Movement Disorders, Honolulu Asian Men Health follow-up prospective study
  – Huang et al, 2011, Plos One, DATATOP study,
  – Du et al, 2012, Plos One, MRI Imaging study

• Confirmed by many others
  – De Lau et al., 2006, AJE, Rotterdam Study
  – Simon et al, 2007, Neurology, Health professional follow-up
  – Mollenhauer et al, Neurology, 2013, case-control study in de nova patients.

• Contradicted by few
  – Hu et al, 2008, Neurology, Finnish Study

• Started intensive debate on the role of statins in PD
Latest Finding

Mov. Disord. (in press, 2015)

Research Article

Statins, Plasma Cholesterol, and Risk of Parkinson’s Disease: A Prospective Study

Xuemei Huang, MD, PhD,1* Alvaro Alonso, MD, PhD,2 Xuguang Guo, PhD,3 David M. Umbach, PhD,4 Maya L. Lichtenstein, MD,1 Christie M. Ballantyne, MD,5 Richard B. Mailman, PhD,6 Thomas H. Mosley, PhD,7 and Honglei Chen, MD, PhD8

Statin use may be associated with a higher PD risk, whereas higher total cholesterol may be associated with lower risk. These data are inconsistent with the hypothesis that statins are protective against PD.
Acknowledgements

• Collaborators at:
  – “The Parkinson’s Study Group”
  – “The Honolulu Asian Aging Project”
  – “The Atherosclerosis Risk in the Community (ARIC) Project”

• Support
  – NHLBI
  – NIEHS Intramural Program
  – Penn State Dean’s Feasibility Grant

• Pierre Morell (1941-2003)
• William Koller (1945-2005)
• Charlie Poole
• Richard Mailman
• Honglei Chen

... and many others
The End
What’s ahead in the next decades?

A new era of statins as cure-all miracle drugs?

or

Statin-induced increases in the prevalence of Parkinson’s disease?

Before we should be “holding on to statins for PD”, more studies are needed!