Experimental Approaches in Nutrigenetics #1

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Outline

How to generate promising NGx hypotheses

Exploration of well-understood nutrient-gene interactions

NGx study methods: How a nutrigenetic story develops

Repeatability and other factors influencing reliability
Hypothesis Development
Hypothesis development

Any ideas for this one?

![Bar chart showing protein requirement distribution](chart.png)
Good research ideas can come from

linear extension of previous study results

predictive models integrating prior knowledge

unbiased investigation methods

scrutiny of unexplained outliers, distribution or variance
Any ideas for this one?

Available requirement data suggest multiple genetic and non-genetic influences that cannot be reduced to a simple bell-shaped curve...

... a better fit is with a combination of several bell curves, suggesting that each describes a different group.
Vitamin B12
Hypothesis development

Unbiased search for genetic associations
Carriers of the FUT2 non-secretor allele 461G (rs601338) do not secrete H-type antigens and are more likely to harbor *H. pylori*. The resulting inflammation diminishes the capacity to produce intrinsic factor.

The incomplete recovery of vitamin B12 from hepato-biliary circulation due to the lack of intrinsic factor then depletes stores and lowers the B12 concentration in blood.
GWAS: what else?

TCN1  transcobalamin

CUBN  cubilin

MUT  methylmalonyl-CoA mutase
Hypothesis development

What next?

Review nearby pathways and related mechanisms

Consider known phenotypes with gene defects

Remember the possibility of gain-of-function variants

Search for knock-outs and other transgenic models

Align with other findings and possibly omics data
Hypothesis Exploration
Types of NGx studies

In-vitro studies (transfected cells)

Animal studies (crossbreeding, transgenic)

Twin studies

Family studies

Population studies (case-control, cohort)
Vitamin D and Multiple Sclerosis
Summary of the population data:
Vitamin D appears to reduce the risk to develop multiple sclerosis

Ascherio & Munger, 2016
Vitamin D and MS

Initial report of link with rare variants in CYP27B1

Whole genome sequenced in 43 probands, one from each family with 4 or more members affected by MS.

<table>
<thead>
<tr>
<th>Patient Demographics</th>
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<tbody>
<tr>
<td>Group</td>
</tr>
<tr>
<td>43 probands</td>
</tr>
<tr>
<td>3,046 trios</td>
</tr>
<tr>
<td>844 affected sib pairs</td>
</tr>
</tbody>
</table>

F = female; M = male; MS = multiple sclerosis; SD = standard deviation.

43 families with 4 or more affected individuals from more than 30,000 families participating in the Canadian Collaborative Project on the Genetic Susceptibility to Multiple Sclerosis

Ramagopalan et al., 2011
Vitamin D and MS

Initial report of link with rare variants in CYP27B1

Out of more than 340 candidate genes in 57 regions 3 different variants were identified in 3 families, one of them (rs118204009) in CYP27B1 in one family.

CYP27B1

25(OH)D3 → 1,25(OH)2D3

The other two were in CBLB and IL7R

<table>
<thead>
<tr>
<th>Group</th>
<th>Sex Ratio (F:M)</th>
<th>Relapsing-Remitting MS at Onset, %</th>
<th>Mean Age at Onset, yr (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>43 probands</td>
<td>1.4:1</td>
<td>100</td>
<td>29.8 (5.9)</td>
</tr>
<tr>
<td>3,046 trios</td>
<td>2.3:1</td>
<td>73</td>
<td>30.4 (10.7)</td>
</tr>
<tr>
<td>844 affected sib pairs</td>
<td>2.5:1</td>
<td>74</td>
<td>31.4 (11.1)</td>
</tr>
</tbody>
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F = female; M = male; MS = multiple sclerosis; SD = standard deviation.

43 families with 4 or more affected individuals from more than 30,000 families participating in the Canadian Collaborative Project on the Genetic Susceptibility to Multiple Sclerosis

Ramagopalan et al., 2011
Later studies failed to replicate the finding in other cohorts

2608 MS vs. 1987Ctrls Belgium/Italy Barrizone et al., 2013
495 MS multiplex families vs. 3583 Ctrl Ban et al., 2013
3269 MS vs. 3577 Ctrl European families Cortes et al., 2013
999 MS vs. 397 Ctrl in Austria Rheinthaler et al., 2014
But then discovery of same rs118204009 variant cosegregating in Canadian family with 5 affected members.
Experimental autoimmune encephalomyelitis in mice

EAE can be induced by immunizing mice with complete Freund's adjuvant, M. tuberculosis and B. pertussis.

Treatment with 0.1 µg 1,25(OH)2D3 daily after the immunization suppresses symptoms in most animals.

Chiuso-Minigucci et al., 2015
Experimental autoimmune encephalomyelitis in mice

EAE can be induced by immunizing mice with complete Freund's adjuvant, M. tuberculosis and B. pertussis.

Treatment with 100 ng 1,25(OH)2D3 daily after the immunization suppresses symptoms in most animals.

What would combination of the model with a genetic modification show?

Chiuso-Minigucci et al., 2015
Experimental autoimmune encephalomyelitis in mice

Inactivation of the CYP27B1 gene does not have the expected effect on EAE development in the immunized mice.

Wang Y et al., 2016
On the other hand ...

IFN-gamma is necessary for proper VDR function. Genetic inactivation of the gene (knock-out) makes the immunized mice more dependent on optimal 1,25(OH)2D3 supplies to protect against EAE development.

Spanier et al., 2015
Mendelian Randomization study 25-OH Vitamin D

14,498 cases and 24,091 controls
International Multiple Sclerosis Genetics Consortium

Mokry LE et al., 2015
Mendelian Randomization study 25-OH Vitamin D

- **DHCR7** rs12785878 2.68 (1.92–3.74)
- **CYP2R1** rs10741657 1.62 (1.17–2.26)
- **GC** rs2282679 1.52 (0.98–2.36)
- **CYP24A1** rs6013897 3.70 (1.64–8.35)

**Summary**

2.02 (1.65–2.46) \(7.7 \times 10^{-12}\) 63\% (0–88)

14,498 cases and 24,091 controls

International Multiple Sclerosis Genetics Consortium

Mokry LE et al., 2015
More Hypothesis Exploration
Functional investigation of gene variants

Transient transfection of host cells (COS, yeast, etc.) allows the expression of inserted gene variants and the functional analysis of the resulting products.

Example:
Functional exploration of MTHFR variants and the corresponding allozymes after expression of 70 kb DNA segment in COS cells.
Why most findings are false
### Why most findings are false

**How certain are we?**

<table>
<thead>
<tr>
<th></th>
<th>Most certain</th>
<th>Least certain</th>
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<tbody>
<tr>
<td><strong>Classic PKU</strong></td>
<td>Low Phe</td>
<td>Na⁺ &lt;1600mg</td>
</tr>
<tr>
<td><strong>Low Phe</strong></td>
<td>High Tyr</td>
<td></td>
</tr>
<tr>
<td><strong>HFE 282YY</strong></td>
<td>Low Iron</td>
<td></td>
</tr>
<tr>
<td><strong>Phlebotomy</strong></td>
<td>Phlebotomy</td>
<td></td>
</tr>
<tr>
<td><strong>MTHFR 677TT</strong></td>
<td>150% Folate</td>
<td></td>
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<tr>
<td><strong>ACE DD</strong></td>
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Repeatability of initially reported genotype-nutrient interactions is often disappointingly poor.
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due to diverse kinds of bias including

- selection bias
- information bias
- publication bias
- population stratification
- low penetrance
Repeatability of initially reported genotype-nutrient interactions is often disappointingly poor.

The finding may be just spurious altogether.

Follow-up studies may not be sufficiently powered to replicate a modest effect (e.g. 1.3 fold difference).

Variation in dietary exposure may be too great.

It may depend on a particular nutrition pattern, lifestyle or environmental exposure.

It may be repeatable only in a subgroup because of their particular genetic pattern or other genetic context.

Effect of saturated fat intake on body fat

Genotype ApoA2 -265 CC frequency is about 15% in the US
The Proteus Phenomenon
Population stratification

The common occurrence of a predisposing factor (e.g. poverty) in a subgroup with high genotype frequency (TT) can give the spurious appearance of a strong genotype-nutrient interaction (TT * folate $\rightarrow$ blood pressure)
Penetrance: probability of a phenotype occurring in carriers

1.0: phenotype always associated with genotype
0.1: phenotype seen once in 10 genotype carriers

Nutrition and other exposures may increase penetrance

Hemochromatosis penetrance in most men with HFE 282 YY is about 0.2-0.3

Heavy alcohol abuse may increase that penetrance to > 0.5
Questions?