Nutrigenetics and personalised/stratified approaches to the provision of dietary advice

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Dept of Nutrition, Norwich Medical School
To cover....... 

• What is nutrigenetics?

• Genes, genetic variation, monogenic vs. polygenic disorders

• Tools and approaches for identifying common genetic determinants of disease risk and response to diet

• apoE genotype and response to fish oil n-3 PUFA

• The penetrance of genotype is not homogenous

• Nutrigenetics, the future:
  
  * A useful public health tool or an expensive research exercise
Susceptible genotype
Deleterious exposure in utero

Positive exposure in utero
Protective genotype

High risk lifestyle (including diet)
AGE

disease
health

Health Pendulum

Pendulum graph showing the balance between deleterious and protective factors, highlighting the influence of genotype and lifestyle on health and disease outcomes. Mathers 2004
What is nutrigenetics?

The interactive impact of genetic variation and individual dietary components/overall diet composition on health status

Health status

Genotype

tissue status

Diet composition

Currently ‘one size fits all’ generic approach to dietary recommendations (adherence poor/limited efficacy in certain individuals)

<table>
<thead>
<tr>
<th>Recommendation</th>
<th>Why?</th>
<th>Are we meeting it?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fruit &amp; Veg 5-a-day (5 x 80g)</td>
<td>↓ cancer, CVD and other chronic diseases</td>
<td>2.8 portions/d X</td>
</tr>
<tr>
<td>Oily fish Min. 140g/week (Min 450mg EPA+DHA/d)</td>
<td>↓ CVD risk</td>
<td>42g/week ~ 200mg EPA+DHA/d X</td>
</tr>
<tr>
<td>Fat Average 35% E</td>
<td>↓ CVD and energy density of diet</td>
<td>Average 35% E √</td>
</tr>
<tr>
<td>Saturated fat Average 11% E</td>
<td>↓ CVD</td>
<td>Average 13% E X</td>
</tr>
<tr>
<td>NSP (fibre) Average 18g/d</td>
<td>Improve CVD gut health, ↓ CVD risk</td>
<td>Average ~ 13g/d X</td>
</tr>
<tr>
<td>Refined sugars &lt; 11% E (~60g/d)</td>
<td>↓ Dental caries</td>
<td>Up to 19% E X</td>
</tr>
<tr>
<td>Salt Average 6g/d</td>
<td>↓ Hypertension/CVD risk</td>
<td>Average 9.5g/d X</td>
</tr>
<tr>
<td>Vitamins &amp; Minerals Dietary reference values (DRVs)</td>
<td>To prevent deficiency &amp; promote optimum health</td>
<td>Various X √</td>
</tr>
<tr>
<td>Alcohol 3-4 units a day M, 2-3 units a day F</td>
<td>Minimise risk of liver damage, CVD, cancer &amp; injuries</td>
<td>60% M and 44% F exceed limits X</td>
</tr>
</tbody>
</table>
Moving towards a more personalised/stratified approach to dietary recommendations

Fig. 2. Scheme of the potential of genetics, nutrigenetics and pharmacogenetics in health maintenance and treatment of diseases. The potential advantages of genotype-based personalised nutrition are: start early; personalised therapy; improved motivation.
Genetic discoveries timeline

<table>
<thead>
<tr>
<th>Discovery of DNA</th>
<th>Sequencing of human genome</th>
<th>Application of genetic information in health management</th>
</tr>
</thead>
<tbody>
<tr>
<td>1953</td>
<td>2001</td>
<td>2001+</td>
</tr>
</tbody>
</table>

- Genetic therapy
- Genetic engineering
- Eugenics
- Genetic profiling
- Understanding of gene(s)-environment-disease associations

3 billion base pairs in 23 sets of chromosomes
20,000-22,000 genes
Genetic variation

Genetic information

- **99%+ homogenous**

- **<1% variable**
  - chromosomal disorder
  - insertions or deletions: indels
  - copy number variation
  - repeat sequences
  - single nucleotide polymorphisms (SNP)

<table>
<thead>
<tr>
<th>changes in protein structure</th>
<th>↑↓ expression of gene</th>
</tr>
</thead>
</table>

- metabolic differences
  - differences in risk of diseases
  - differences in response to environmental factors, including diet
Single gene (monogenic) disorders

• Single gene
• 10,000 distinct disorders currently recognised to be single gene
• Dominant, recessive, X-linked
• Examples include:
  - **Sickle cell anaemia** (occurs in 1 in 400 African-Americans (in US))
  - **Huntington's disease** (occurs in 1 in 20,000 individuals)
  - **Phenylketonuria** (1 in 15,000 births (US))
  - **BRCA1 gene & breast cancer** (occurs in 1 in 800 people)
  - **Familial hypercholesterolaemia** (occurs in 1 in 500 individuals)

• Often limited (or no) ability to modify penetrance by diet
**Single nucleotide polymorphisms (SNPs)**

- most common genetic determinants of polygenic disorders
- single base pair (bp) changes
- >90% of all genetic variation
- SNP every 100-300bp
- ~5x10^6 SNPs in the human genome
- ?? functional SNP in human genome
Research tools to identify common genetic variation associated with (1) disease risk and (2) response to environment

- **Candidate-gene studies** (prospective or retrospective genotyping) 1980+
- **Genome-wide analysis studies** (GWAS) 2005+
- **Sequencing** (Targeted, whole genome) recent+
Genome wide association studies (GWAS)

- Take group of cases vs. matched controls
- Typically analyse for approximately 100,000-1,000,000 tagging SNPs across the genome
- Generally only coverage of approximately 80% genome
- Identify gene regions which may predict information on linkage disequilibrium (LD) from the HapMap project
- Do not identify functional gene or SNP
- As of December 2010, over 1200 human GWAS have examined over 200 diseases and traits, and found almost 4000 SNP associations
- Led to the identification of the fat mass and obesity associated (FTO) gene
- To date use in identifying molecular pathways of disease but not risk predictors SNPs
- Generally no dietary data collected
Example of GWAS ‘discovery’: FTO gene and obesity risk
Genetic variation uncovered today, poor predictive value

40-70% risk of excess weight predicted by genetics
Only 0.9% explained by 12 most common obesity SNPs
Example of *Candidate gene* approach: *apoE* genotype and n-3 PUFA

- Liver (80-90%), brain and macrophages
- Lipid transporter
- 84 gene variants described to date
- most well described E2, E3, E4
  - E2  Cys 112, Cys 158
  - E3  Cys 112, Arg 158
  - E4  Arg 112, Arg 158

Typical % apoE phenotype; Caucasian

RR CVD 0.98

RR CVD 1.5

%
Plasma lipid modulatory impact of fish oil fatty acids: *ALP Study*

3g EPA+DHA/d, 6 weeks, cross-over

DHA rather than EPA associated with LDLC raising effect

Table 3. Multiple linear regression analysis of changes in platelet lipids as dependent variables with changes in plasma phospholipid eicosapentaenoic and docosahexaenoic acid as independent variables*†

<table>
<thead>
<tr>
<th></th>
<th>Δ EPA</th>
<th>Δ DHA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Δ LDL-cholesterol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coefficient (SEM)</td>
<td>-0.230 (0.211)</td>
<td>0.754 (0.319)</td>
</tr>
<tr>
<td>Adjusted model $R^2$</td>
<td>0.293</td>
<td>0.159</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ Fasting TG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coefficient (SEM)</td>
<td>-5.2 (-0.3)</td>
<td>-9.5 (5.8)</td>
</tr>
<tr>
<td>Adjusted model $R^2$</td>
<td>0.188</td>
<td>0.116</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ TG AUC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coefficient (SEM)</td>
<td>-0.748 (0.349)</td>
<td>0.014 (0.531)</td>
</tr>
<tr>
<td>Adjusted model $R^2$</td>
<td>0.190</td>
<td>0.976</td>
</tr>
<tr>
<td>P</td>
<td>0.046</td>
<td>0.609</td>
</tr>
<tr>
<td>Δ TG Incremental AUC‡</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coefficient (SEM)</td>
<td>-131 (88)</td>
<td>140 (133)</td>
</tr>
<tr>
<td>Adjusted model $R^2$</td>
<td>0.155</td>
<td>0.304</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ Peak TG (0–480 min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coefficient (SEM)</td>
<td>-1.38 (0.57)</td>
<td>0.634 (0.861)</td>
</tr>
<tr>
<td>Adjusted model $R^2$</td>
<td>0.171</td>
<td>0.471</td>
</tr>
<tr>
<td>P</td>
<td>0.027</td>
<td></td>
</tr>
<tr>
<td>Δ Fasting NEFA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coefficient (SEM)</td>
<td>-7.36 (31.81)</td>
<td>-85.99 (46.01)</td>
</tr>
<tr>
<td>Adjusted model $R^2$</td>
<td>0.620</td>
<td>0.098</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ NEFA AUC (270–480 min)¶</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coefficient (SEM)</td>
<td>-29512 (10890)</td>
<td>5175 (16434)</td>
</tr>
<tr>
<td>Adjusted model $R^2$</td>
<td>0.246</td>
<td>0.757</td>
</tr>
<tr>
<td>P</td>
<td>0.015</td>
<td></td>
</tr>
</tbody>
</table>

EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; chol, cholesterol; LDLox, Cu-induced oxidation of LDL; TG, triacylglycerol; AUC, area under the curve; NEFA, non-esterified fatty acid.

* For details of subject and procedures, see p. 436.
† A negative coefficient indicates an inverse relation.
‡ Calculated by the trapezoidal rule.
§ Calculated as AUC minus the baseline value.
¶ µmol/l per 210 min.

Leigh-Firbank et al., British Journal of Nutrition 2002;:435-45
Plasma lipid impact of EPA+DHA supplementation is highly heterogeneous: 
*ALP* study


Range
LDL-C: -49% to +87%
TG: -114% to +62%
Greatest increases in LDL-C in apoE4 carriers


Leigh-Firbank et al., British Journal of Nutrition 2002;:435-45
Impact of EPA vs. DHA on plasma lipid concentration and lipoprotein metabolism and composition in E3/E3 vs. E4 carriers

Design: Double-blind placebo controlled cross-over trial with placebo (P), EPA-rich oil (ERO, 3.3g EPA/day) and DHA-rich oil (DRO, 3.7g/day) in random order

Participants: male, age= 44y, BMI 25.1 kg/m2, TC=5.80mmol/l, TG= 1.51mmol/l, HDL-C= 1.51mmol/l

Fasting blood sample: plasma lipids, plasma apolipoproteins, lipoprotein (VLDL1, VLDL2, IDL, LDL, HDL) size distribution and composition plasma fatty acid composition

Biokinetic study: - 6mg/kg 5,5,5 \textsuperscript{2}H\textsubscript{3} leucine (d\textsubscript{3} leucine)
- blood samples: 15min, 30min, 45min, 1h, 1.5h, 2h, 2.5h, 3h, 4h, 5h, 6h, 7h, 8h, 10h, 12h, d1, d2, d3, d4, d5, d6, d7, d8, d9, d10, d11, d12, d23, d14
- VLDL1, VLDL2, IDL, LDL- metabolism (synthesis, flux, pool size, FTR, FCR etc)
DHA-rich oil supplementation is associated with an increase in both TC and LDL-C, but only in apoE4 genotype

Olano-Martin E, et al., *Atherosclerosis* 2010;209:104-110
Mechanisms underlying the ↑ LDL-C following DHA supplementation in E4 carriers

1. *Ex vivo* cell culture
   Enrichment of VLDL from apoE4 individuals with DHA, increased their uptake via the LDLR, thereby reducing LDL uptake and ↑ LDL-C uptake (Olano Martin et al., 2010)

2. Biokinetic study using 5,5,5\(^{2}\)H\(_{3}\) leucine lipoprotein labelling
   DHA supplementation in E4 carriers increases the hepatic production of VLDL2 which are the precursors of LDL (Caslake *et al.*, unpublished)
FINGEN Study (2003-2007)

1. Examine the responsiveness of ~ 40 CHD risk indicators of CHD on responsiveness to low(er) dose fish oil intervention
2. Examine impact of age/gender/apoE genotype on response to treatment

Reading, Southampton, Newcastle, Glasgow

ApoE2 males (20-70y) n=45
ApoE2 females (20-70y) n=45
ApoE3 males (20-70y) n=45
ApoE3 females (20-70y) n=45
ApoE4 males (20-70y) n=45
ApoE4 females (20-70y) n=45

~20% in each of 20-29y, 30-39y, 40-49y 50-59y, 60-70y

No evidence of greater increase in LDL-C in E4 carriers at doses < 2g per day of EPA+DHA

Caslake MJ et al., American Journal of Clinical Nutrition 2008;88:618-629
Possible implications of EPA&DHA*apoE genotype interactions on dietary n-3 PUFA recommendations

‘At high intakes (>2g/day): EPA- rather than DHA-rich oils recommended in apoE4 individuals’?

AHA recommendations

• 2 portions of fish/wk, preferably oily
• 1g EPA+DHA/d for those with CHD
• 2-4g per day as TG lowering agent

Mozaffarian and Rimm, JAMA, 2006
The impact of common gene variants on the response of biomarkers of cardiovascular disease (CVD) risk to increased fish oil fatty acids intakes

Jacqueline Madden, Christine M. Williams, Philip C. Calder, Georg Lietz, Elizabeth A Miles, Heather Cordell, John C. Mathers, Anne Marie Minihane

*Annual Review of Nutrition, 2011;203-234*

**FINGEN2: Genetic determinants of physiological response to ↑ EPA/DHA**

- Plasma fatty acid status
- TG,
- TC/HDL-C,
- RQUICKI (Insulin, glucose, NEFA)
- NO/ET-1 ratio,
- inflammatory index (cytokines, CRP)
1. Current criticism of published nutrigenetic literature - inconsistent findings

+ Unpublished SATGENE data which will be presented

Sarkkinen et al., 1998, AJCN
Genotype*diet*phenotype: impact of sex

Unpublished DISRUPT data (UK) and findings from an independent Spanish cohort which will be presented
True inconsistencies (i.e. bad science) or insightful....... 

The penetrance of genotype with respect to response to diet change (i.e. genotype*diet* phenotype* interactions) is likely to be affected by:

- Gender
- Age
- Ethnicity/epistasis
- Adiposity
- Health status/baseline levels of phenotype of interest
- Drug use
- Alcohol intake
- Physical activity

- Favourite Colour
- Last book you read
- Whether you watch Eurovision Song Contest
- What letter your surname begins with
Genotype*diet*phenotype: impact of ethnicity

African American...no interaction in whites

Whites...no interaction in African Americans

Volcik KA, et al., AJCN 2008
2. Current criticism of published genetic literature - effect size of individual SNPs is small

‘Need to look at combinations of SNPs’

<table>
<thead>
<tr>
<th>Gene/Polymorphism(s)</th>
<th>Risk Genotype</th>
<th>No of studies</th>
<th>Size of effect</th>
<th>Frequency of risk</th>
<th>Genotype (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>APOE, Gln415 → Lys</td>
<td>A+</td>
<td>15 (1816)</td>
<td>1.32 (1.14–1.54)</td>
<td>26.7</td>
<td>50.0</td>
<td>Chiodini et al. 2003</td>
</tr>
<tr>
<td>NO35, G1a296 Asp/G1a298</td>
<td>Asp296/Asp298</td>
<td>14 (1096)</td>
<td>1.31 (1.13–1.51)</td>
<td>10.73</td>
<td>43.5</td>
<td>Casa et al. 2004</td>
</tr>
<tr>
<td>APOE, E2, E3, E4</td>
<td>E4+</td>
<td>48 (15402)</td>
<td>1.42 (1.29–1.56)</td>
<td>22.6</td>
<td>28.4</td>
<td>Song et al. 2004</td>
</tr>
<tr>
<td>ACE, Insertion/Deletion</td>
<td>DD</td>
<td>43 (14202)</td>
<td>1.22 (1.11–1.35)</td>
<td>28.4</td>
<td>38.4</td>
<td>Morgan et al. 2003</td>
</tr>
<tr>
<td>SERPINE1 5G/4G</td>
<td>4G4G</td>
<td>7 (2813)</td>
<td>1.20 (1.04–1.39)</td>
<td>31.0</td>
<td>50.0</td>
<td>Boekholdt et al. 2001</td>
</tr>
<tr>
<td>MTHFR, C677T</td>
<td>TT</td>
<td>40 (11102)</td>
<td>1.14 (1.01–1.28)</td>
<td>12.3</td>
<td>67.2</td>
<td>Kerk et al. 2002</td>
</tr>
<tr>
<td>ITG12B, P1A2</td>
<td>A2+</td>
<td>34 (6173)</td>
<td>1.13 (1.02–1.26)</td>
<td>26.9</td>
<td>38.4</td>
<td>Morgan et al. 2003</td>
</tr>
<tr>
<td>PON1, Q192R</td>
<td>R192+</td>
<td>44 (10106)</td>
<td>1.15 (1.09–1.22)</td>
<td>21.2</td>
<td>42.8</td>
<td>Wheeler et al. 2004</td>
</tr>
<tr>
<td>LPL, Ser/Ter S447X</td>
<td>CC</td>
<td>4 (22532)</td>
<td>1.25 (1.00–1.43)</td>
<td>80.0</td>
<td>50.0</td>
<td>Witrup et al. 1999</td>
</tr>
<tr>
<td>CETP TaqIB</td>
<td>B1+</td>
<td>7 (7684)</td>
<td>1.24 (1.09–1.45)</td>
<td>67.6</td>
<td>50.0</td>
<td>Boekholdt et al. 2003</td>
</tr>
</tbody>
</table>
## Combined impact of multiple genes
*(Drenos F et al., 2007)*

<table>
<thead>
<tr>
<th>Number of risk genes</th>
<th>Frequency (% population)</th>
<th>Odds ratio OR</th>
<th>Impact on risk of CVD</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.8</td>
<td>0.50</td>
<td>↓ 50%</td>
</tr>
<tr>
<td>1</td>
<td>7.0</td>
<td>0.62</td>
<td>↓ 38%</td>
</tr>
<tr>
<td>2</td>
<td>20.8</td>
<td>0.77</td>
<td>↓ 23%</td>
</tr>
<tr>
<td>3</td>
<td>29.9</td>
<td>0.94</td>
<td>↓ 6%</td>
</tr>
<tr>
<td>4</td>
<td>24.4</td>
<td>1.17</td>
<td>↑ 17%</td>
</tr>
<tr>
<td>5</td>
<td>12.3</td>
<td>1.44</td>
<td>↑ 44%</td>
</tr>
<tr>
<td>6</td>
<td>4.0</td>
<td>1.77</td>
<td>↑ 77%</td>
</tr>
<tr>
<td>7</td>
<td>0.8</td>
<td>2.18</td>
<td>↑ 218%</td>
</tr>
<tr>
<td>8</td>
<td>0.1</td>
<td>2.70</td>
<td>↑ 270%</td>
</tr>
<tr>
<td>9</td>
<td>.007</td>
<td>3.33</td>
<td>↑ 333%</td>
</tr>
<tr>
<td>10</td>
<td>.0002</td>
<td>4.10</td>
<td>↑ 410%</td>
</tr>
</tbody>
</table>
Nutrigenetics, the future

- Need to appreciate that penetrance of genotype and genotype*diet interactions are not homogeneous
- More extensive phenotyping (in particular dietary information in GWAS studies)
- More widespread use of exon and whole genome sequencing to identify gene variants with lower allele frequency
- Wider consideration of combinations of genes
- Nutrigenetics is in its relative infancy!
‘Now this is not the end. It is not even the beginning of the end. but it is, perhaps, the end of the beginning’
A very big thank you to..............

• All study participants

• Dr Chris Armah, Mrs Jilly Grew, Dr Kim Jackson, Dr Bettina Kofler, Mrs Jan Luff, Prof Julie Lovegrove, Dr Esti Olano-Martin, Prof Christine Williams, Prof Parveen Yaqoob, University of Reading

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• Prof Bruce Griffin, University of Surrey

• Prof John Mathers and Dr Georg Lietz, Dr Peter Curtis, University of Newcastle

• Prof Chris Packard and Prof Muriel Caslake, University of Glasgow