The effect of FADS genotypes, fatty acids, and fish intake on mental development in children

Results from the ALSPAC study

Eva Lattka

Colin D Steer, Pauline M Emmett, Norman Klopp, Thomas Illig and Berthold Koletzko

www.nutrimenthe.eu
Main objective: Interaction of fish intake and genetic variation in determining child behaviour
Main objective: Analysis of genetic polymorphisms as co-variables in determining interaction of nutrition and mental health
The Avon Longitudinal Study of Parents and Children (ALSPAC), www.bristol.ac.uk/alspac

- a contemporary cohort of British children followed from before birth
- 14,000 pregnant women living in Avon in South West England enrolled April 1991 to Dec 1992
- long-term health research project, children have been followed ever since
- information collected by self-completion questionnaires, medical records, biological samples, hands-on measurements
- well-phenotyped; large biobank
Introduction

Hypothesis of workpackages 2 and 8 in NUTRIMENTHE

Lancet 2007; 369: 578–85

Maternal seafood consumption in pregnancy and neurodevelopmental outcomes in childhood (ALSPAC study): an observational cohort study

Joseph R Hibbeln, John M Davis, Colin Steer, Pauline Emmett, Imogen Rogers, Cathy Williams, Jean Golding

- IQ at age 8 years measured by Weschler Intelligence Scale for Children IIIUK (WISC-IIIUK) (n=5449)
- Maternal fish eating at 32nd week of pregnancy: none, low (<340 g/week) & high (>340 g/week) intakes
- Confounders: maternal education, age, smoking, housing, sex of child, breastfeeding
Introduction

Hypothesis of workpackages 2 and 8 in NUTRIMENTHE

Low maternal seafood intake is associated with increased risk of suboptimum outcomes for verbal IQ, prosocial behaviour, fine motor, communication, and social development scores.

➢ **Hypothesis:** LC-PUFA (and especially docosahexaenoic acid, DHA) contained in seafood might be responsible for beneficial effects of high fish intake.

➢ **DHA accumulates in brain during fetal development. Sea food is one major source of DHA.**
Introduction

Hypothesis of workpackages 2 and 8 in NUTRIMENTHE

Main questions:

1) Does dietary intake of LC-PUFA influence behavioural and cognitive outcomes in children?

2) Is there an interaction with genetic effects?
Introduction

Function of the delta-5 and delta-6 desaturase (*FADS1* and *FADS2*)

Omega-6 Pathway

18:2 n-6

\[ \rightarrow \]

18:3 n-6

\[ \rightarrow \]

20:3 n-6

\[ \rightarrow \]

20:4 n-6

\[ \rightarrow \]

20:5 n-3

\[ \rightarrow \]

22:4 n-6

\[ \rightarrow \]

22:6 n-3

\[ \rightarrow \]

neuronal development

Omega-3 Pathway

18:3 n-3

\[ \rightarrow \]

18:4 n-3

\[ \rightarrow \]

20:4 n-3

\[ \rightarrow \]

20:5 n-3

\[ \rightarrow \]

22:4 n-6

\[ \rightarrow \]

22:6 n-3

\[ \rightarrow \]

neuronal development

strong inflammatory eicosanoids

essential membrane constituents

essential fatty acids from nutrition

light inflammatory eicosanoids

20:4 n-6

and other LC-PUFAs

22:6 n-3

and other LC-PUFAs
Introduction

The FADS gene cluster

SNP (single nucleotide polymorphism) = single base pair exchange
Introduction

Association of *FADS* polymorphisms with fatty acid levels

→ **minor alleles** of *FADS* polymorphisms associated with
  - increased levels of desaturase substrates
  - decreased levels of desaturase products

→ effect observable in **different tissues**
  - serum and plasma phospholipids (e.g. Schaeffer et al.; Malerba et al.; Tanaka et al.)
  - erythrocyte membrane phospholipids (e.g. Rzehak et al.; Koletzko, Lattka et al.)
  - breast milk (e.g. Xie et al.; Lattka et al.)
  - adipose tissue (Baylin et al.)
  - cord plasma (Lattka et al., in preparation)

→ genetically determined variance for arachidonic acid: up to 30%!
→ genetic effects on % FA / total FA amounts comparable to nutritional effects in intervention studies
**Introduction**

*FADS* polymorphisms associate with intermediate and complex phenotypes

**Intermediate phenotypes**
- Serum phosphatidyl cholines
  - (Gieger et al., Illig et al.)
- Blood total cholesterol
  - LDL, HDL
  - Triglycerides
  - (e.g. Kathiresan et al., Sabatti et al., Tanaka et al.)
- Fasting glucose
  - Insulin secretion
  - (e.g. Dupuis et al., Ingelsson et al.)
- Resting heart rate
  - (Eijgelsheim et al.)

**Complex phenotypes**
- Type 2 diabetes mellitus
  - (Dupuis et al.)
- Coronary artery disease
  - (e.g. Martinelli et al., WTCCC, Kwak et al.)
- Allergies
  - (e.g. Singmann et al., Rzehak et al., Standl et al.)
- Mental disorders, IQ
  - (e.g. Brookes et al., WTCCC, Caspi et al., Steer et al.)
**Introduction**

*FADS polymorphisms associate with intermediate and complex phenotypes*

**intermediate phenotypes**
- Serum phosphatidyl cholines
  - (Gieger et al., Illig et al.)
- Blood total cholesterol, LDL, HDL, triglycerides
  - (e.g. Kathiresan et al., Sabatti et al., Tanaka et al.)
- Fasting glucose, insulin secretion
  - (e.g. Dupuis et al., Ingelsson et al.)
- Resting heart rate
  - (Eijgelsheim et al.)

**complex phenotypes**
- Type 2 diabetes mellitus
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- Mental disorders, IQ
  - (e.g. Brookes et al., WTCCC, Caspi et al., Steer et al.)

**FADS SNPs**
Introduction

Identification of a functional relevant variant in the FADS2 gene promoter

Genetic association

Strong association of SNPs in the FADS (fatty acid desaturase) gene cluster with LC-PUFA and lipid levels in several studies

Bioinformatic prediction

Functional in vitro studies

Lattka et al J Lipid Res 2010 51(1):182-91
Methods and data
Available data in the ALSPAC cohort

- Genotyping of **18 SNPs** spanning the complete \( FADS \) gene cluster in more than 10,000 mothers and their children of the ALSPAC cohort

- Fatty acid analysis of **maternal red blood cell phospholipids** taken after 20 weeks’ gestation

- Fatty acid analysis of **umbilical cord plasma** taken at birth

- **Child IQ** at age 8 years assessed by WISC-III\(^{UK}\) (verbal and performance subtests)

- **Maternal sea food consumption** at 32 weeks’ gestation obtained by a detailed food frequency questionnaire (mothers were asked about food eaten nowadays and were given a list of 50 foods to tick; several separate questions for meat, fish, vegetables, fruit, breakfast cereals and so on)

- Statistical analysis (linear regression, interaction analysis) done by Colin Steer from Bristol University
Results – Genetic associations with fatty acids

FADS genotypes are associated with DHA concentrations in pregnant women

Background:

- DHA is considered particularly important for brain and retina development
- Associations between FADS genotypes and DHA levels have been scarce in previous studies

Objective:

Explore the relation between FADS genotypes and red blood cell (RBC) fatty acid (FA) amounts in >4000 pregnant women participating in the ALSPAC study

Design:

Linear regression analysis of 17 FADS SNPs with RBC phospholipid FAs of 6711 samples from 4457 women of white ethnic origin obtained throughout pregnancy (mean ± SD gestational age: 26.8 ± 8.2 wk)
Results – Genetic associations with fatty acids

FADS genotypes are associated with DHA concentrations in pregnant women

Results:

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Regression coefficients from analyses of log n-6 and n-3 fatty acid amounts as a percentage of total fatty acids on FADS single nucleotide polymorphisms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FADS1</td>
</tr>
<tr>
<td></td>
<td>rs174548</td>
</tr>
<tr>
<td>Omega-6</td>
<td>18:2</td>
</tr>
<tr>
<td></td>
<td>18:3</td>
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<tr>
<td></td>
<td>20:2</td>
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<td>20:3</td>
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<td>20:4</td>
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<td>22:4</td>
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<td>22:5</td>
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<tr>
<td>Omega-3</td>
<td>18:3</td>
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<td>20:5</td>
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<td></td>
<td>22:5</td>
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<td></td>
<td>22:6</td>
</tr>
<tr>
<td>Ratios</td>
<td>AA to LA</td>
</tr>
<tr>
<td></td>
<td>EPA to ALA</td>
</tr>
<tr>
<td>n</td>
<td>6565</td>
</tr>
</tbody>
</table>

^ SEs are in parentheses. AA, arachidonic acid; LA, linoleic acid; EPA, eicosapentaenoic acid; ALA, a-linolenic acid. Fatty acids and ratios were standardized to have a variance of one.

*P < 0.05, **P < 0.01, ***P < 0.001.

- Associations independent of 8 potential confounders (multiple pregnancy, parity, maternal smoking at 32 wk gestation, gestation, maternal age, maternal prepregnancy BMI, a measure of family adversity, diet)

Koletzko et al AJCN 2011 93(1):211-9
Conclusion:

- DHA amounts in RBC phospholipids of pregnant women are determined by \textit{FADS} genotypes.
- Maternal \textit{FADS} genotype might affect the child’s DHA supply during pregnancy.
- This effect is independent of diet.
Results – Genetic associations with fatty acids

**FADS genotypes are associated with cord blood fatty acid concentrations**

**Background:**

- Fetal supply with LC-PUFA during pregnancy is provided by materno-fetal placental transfer via the umbilical cord

- Maternal *FADS* genotypes influence the fatty acid composition in maternal blood, but the influence on cord blood fatty acids has not been investigated until now

**Objective:**

Investigate the influence of maternal and child *FADS* genotypes on the amounts of LC-PUFA in umbilical cord plasma phospholipids as indicator of fetal fatty acid supply during pregnancy

**Design:**

Linear regression analysis of 11 umbilical cord plasma n-6 and n-3 fatty acids with 17 *FADS* SNPs in over 2000 mothers and children; additional multivariable analysis (adjust the maternal genotype for the child genotype and vice versa) to estimate the dominant genetic influence on cord plasma fatty acid composition
Results: Genetic associations with fatty acids

FADS genotypes are associated with cord blood fatty acid concentrations

- Most maternal and child genotypes are associated with all n-6 fatty acids, except for 18:3n-6 (gamma-linolenic acid)
- Minor alleles are associated with higher amounts of desaturase substrates and lower amounts of desaturase products
- Significant associations also with 18:3n-3 (alpha-linolenic acid) and 22:6n-3 (docosahexaenoic acid)
- Associations independent of 9 potential confounders (child gender, multiple pregnancy, parity, maternal smoking at 32 wk gestation, gestation, maternal age, maternal prepregnancy BMI, a measure of family adversity, diet)
- Multivariable analysis: cord fatty acid levels determined to a greater extent by fetal or maternal metabolism?
### Results: Genetic associations with fatty acids

**FADS genotypes are associated with cord blood fatty acid concentrations**

#### Results:

<table>
<thead>
<tr>
<th>SNP rs174548</th>
<th>Maternal adjusted for child genotype</th>
<th>Child adjusted for maternal genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Omega-6</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18:2</td>
<td>0.190 (0.037)*****</td>
<td>0.044 (0.038)</td>
</tr>
<tr>
<td>18:3</td>
<td>0.046 (0.039)</td>
<td>0.002 (0.041)</td>
</tr>
<tr>
<td>20:2</td>
<td>0.209 (0.036)*****</td>
<td>0.025 (0.038)</td>
</tr>
<tr>
<td>20:3</td>
<td>0.253 (0.036)*****</td>
<td>0.368 (0.037)*****</td>
</tr>
<tr>
<td>20:4</td>
<td>-0.093 (0.038)*</td>
<td>-0.219 (0.039)*****</td>
</tr>
<tr>
<td>22:4</td>
<td>-0.053 (0.038)</td>
<td>-0.156 (0.039)*****</td>
</tr>
<tr>
<td>22:5</td>
<td>0.002 (0.039)</td>
<td>-0.154 (0.040)*****</td>
</tr>
<tr>
<td><strong>Omega-3</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18:3</td>
<td>0.039 (0.037)</td>
<td>0.090 (0.038)*</td>
</tr>
<tr>
<td>20:5</td>
<td>-0.048 (0.037)</td>
<td>-0.004 (0.038)</td>
</tr>
<tr>
<td>22:5</td>
<td>-0.002 (0.038)</td>
<td>-0.017 (0.039)</td>
</tr>
<tr>
<td>22:6</td>
<td>-0.086 (0.038)*</td>
<td>-0.094 (0.039)*</td>
</tr>
<tr>
<td><strong>N</strong></td>
<td>1938</td>
<td></td>
</tr>
</tbody>
</table>

- Maternal genotypes mainly associated with precursor n-6 PUFA
- Child genotypes mainly associated with n-6 products
- DHA levels equally associated with maternal and child genotype

Regression coefficients from analyses of log n-6 and n-3 fatty acid levels as a percentage of total fatty acid levels on maternal (child) SNP rs174548 adjusted for the child (maternal) genotype. *p<0.05, **p<0.01, ***p<0.001. Fatty acids have been standardized to have a variance of one. Standard errors are reported in parentheses.
Conclusion:

- Fatty acid amounts in cord blood are dependent on maternal and child genotype
- *FADS* genotypes might be important for fetal fatty acid status
- Contribution of fetal fatty acid metabolism to fetal n-6 LC-PUFA status?
  - this is in contrast to common belief
  - animal experiments support this hypothesis (Ravel et al 1985: increase of delta-5 desaturase enzymes in rat fetal liver close to term, fetus relies less on provision of LC-PUFA from maternal organism)
  - what about earlier stages of pregnancy?
- DHA amounts are dependent on both maternal and child metabolism
From fatty acids to complex phenotypes

dietary intake of LC-PUFAs

e.g. Krauss-Etschmann et al

FADS genotype

e.g. Koletzko et al

fatty acid status

behavioural and cognitive outcomes in infants and children

e.g. Brookes et al

e.g. Hibbeln et al
Background:

- Maternal fish intake had been associated with child IQ previously (Hibbeln et al 2007)

Objective:

Investigate the influence of maternal blood fatty acid levels on child IQ at age 8 years

Design:

Regression analysis of 13 saturated, 11 monounsaturated, 8 omega-6, and 7 omega-3 maternal fatty acids with child IQ in 2750 mother-child pairs; dietary habits of mothers asked by a food frequency questionnaire
Results: Dietary associations with maternal fatty acids

- Fish eating associated with DHA

Unadjusted associations of maternal fatty acids with child IQ

- Negative associations (p<0.001) with 16:1n-7 (palmitoleic acid), 18:1n-9 (oleic acid and trans isoform)
- Positive associations (p<0.01) with 18:2n-6 (linoleic acid), 20:2n-6 (eicosadienoic acid) & 22:6n-3 (DHA)
Results – Fatty acid associations with IQ
Maternal fatty acid levels and child IQ at age 8 years

However:

Adjusted associations of maternal fatty acids with child IQ

- Adjusted for same factors as previous work (Hibbeln et al. Lancet 2007; 369: 578–85): Maternal education & age, housing, crowding, smoking & alcohol in pregnancy, life events, family adversity score, parity, sex, breastfeeding, parenting at 6 m, gestation blood sample
- All associations became null on adjustment

Conclusion:

- Previous work - positive association of fish eating with child IQ (adjusted)
- No associations with maternal blood FAs after adjustment in our analysis → DHA not the missing link?? Association between fish intake and IQ still confounded??
- Fish contains many other nutrients which could influence IQ
  - e.g. Iodine, Vitamin D, Selenium
From fatty acids to complex phenotypes

e.g. Hibbeln et al

dietary intake of LC-PUFAs

e.g. Krauss-Etschmann et al

FADS genotype

e.g. Koletzko et al

fatty acid status

e.g. Brookes et al

behavioural and cognitive outcomes in infants and children
Results – Gene-nutrition interactions

FADS genotypes modify the effect of breastfeeding on child IQ

Background:

- Breastfeeding is important for child cognitive development, possibly via the biological action of LC-PUFA contained in breast milk

- Study by Caspi et al: FADS2 SNP moderates the association between breastfeeding and IQ

Study by Caspi et al: FADS2 SNP moderates the association between breastfeeding and IQ

 Objective:

Replicate the findings of Caspi et al in the ALSPAC cohort

Caspi et al PNAS 2007 104(47):18860-5

Steer et al Plos One 2010 5(7):e11570
**Results – Gene-nutrition interactions**

**FADS genotypes modify the effect of breastfeeding on child IQ**

**Design:**
- Regression and interaction analysis of *FADS* genotype and breastfeeding on IQ at age 8 years as major outcome in 5934 children from ALSPAC

**Results:**
- No genetic main effect of the tested polymorphisms with IQ
- Interaction between SNP rs174575 and breastfeeding was observed such that breastfed minor GG children performed better than their formula fed counterparts by an additional 5.8 points [1.4, 10.1] (interaction p = 0.0091)
- Adjustment for 7 potential confounders attenuated the interaction effect by about 10%

Steer et al Plos One 2010 5(7):e11570
Conclusion:

- No replication of Caspi results
- Breastfed children have similar IQs irrespective of genotype
- Biologically plausible: Breast milk contains preformed DHA, therefore all children should benefit from breastfeeding
- Children homozygous for the minor allele show the biggest differences between feeding groups and benefit most from breastfeeding
- Further studies are required to replicate these findings

Steer et al Plos One 2010 5(7):e11570
Conclusions and outlook

- Maternal and child FADS genotypes are determinants of fatty acid levels in pregnant women and cord blood.

- Fetal metabolism seems to contribute more to fetal fatty acid status than previously expected.

- Obviously no causal relationship between maternal fatty acid levels and child IQ → what is the missing link between fish intake and child IQ??

- Gene-nutrition interaction between breastfeeding and child FADS genotypes: minor allele carriers benefit most from breastfeeding in regard to later IQ.

- Outlook: replication required; interaction studies on maternal and child fish intake with FADS genotype; IQ not the optimal measure?
Gene-nutrition interactions →

- Explanation of inconsistent findings
- Individual dietary recommendations

Future...
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Thanks for your attention!