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Diversity vs Globalization:
A Nutritional Challenge for a Changing Europe

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

Results from the ALSPAC study

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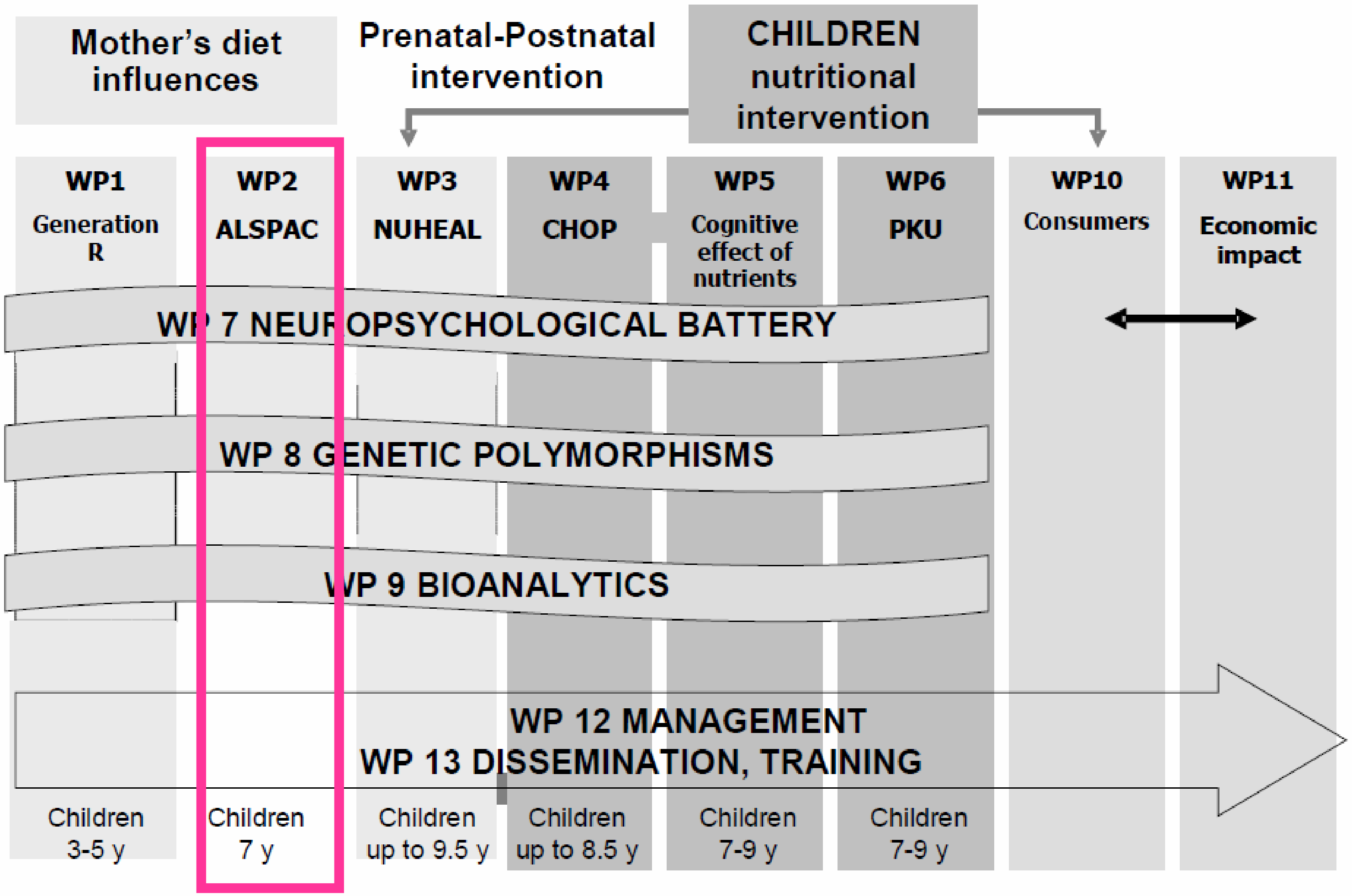
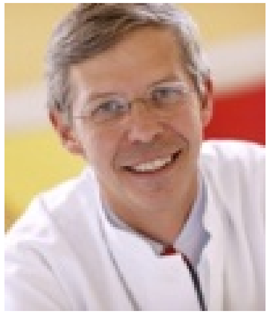
Introduction

Workpackage 2 in NUTRIMENTHE



WP leader

Pauline Emmett



Berthold Koletzko

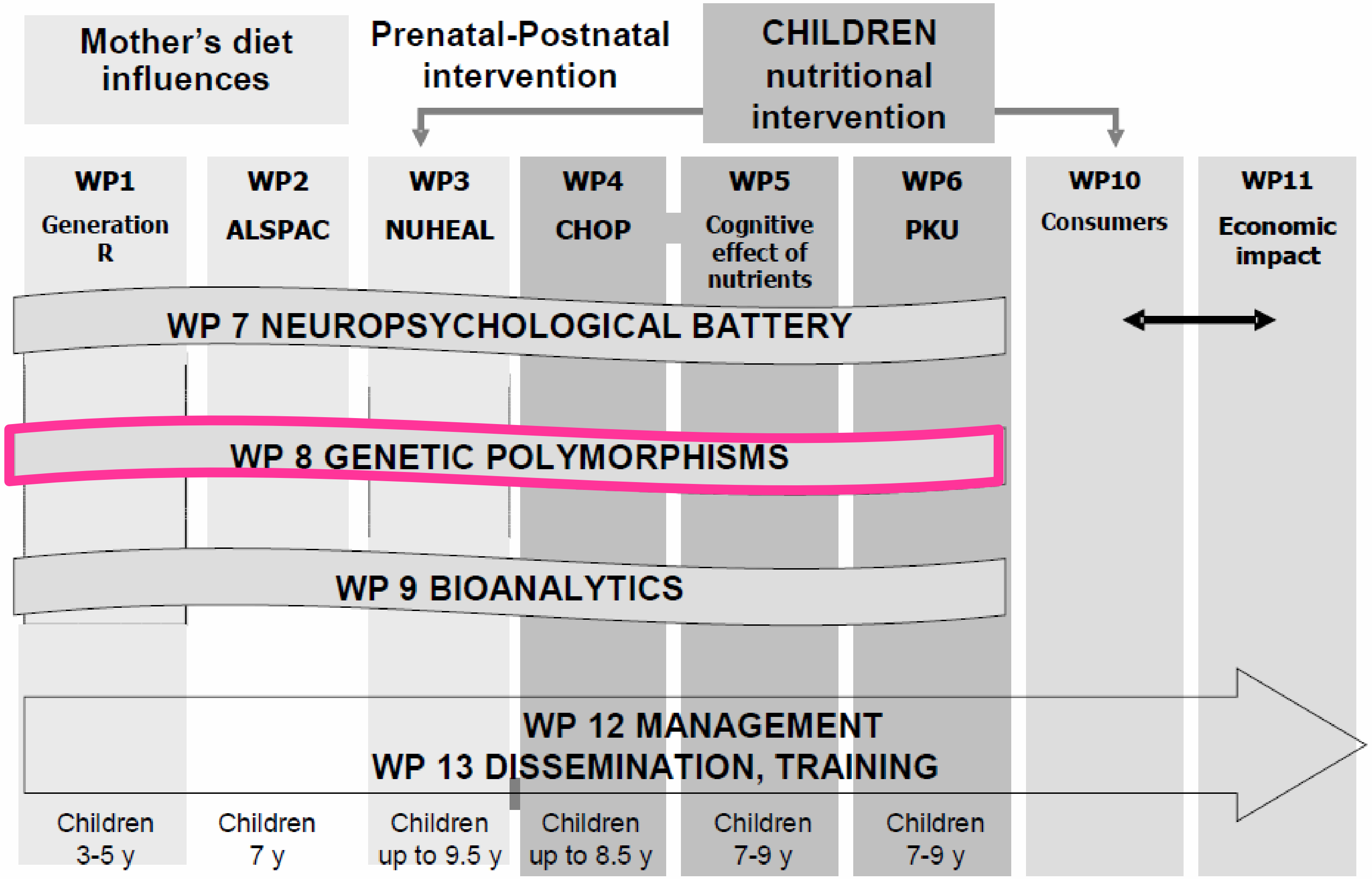
Colin Steer

Jean Golding
Sue Ring
Joe Hibbeln

Main objective: **Interaction of fish intake and genetic variation in determining child behaviour**

Introduction

Workpackage 8 in NUTRIMENTHE



WP leader

Thomas Illig

Norman Klopp

Eva Lattka

<u>Main objective:</u> Analysis of genetic polymorphisms as co-variables in determining interaction of nutrition and mental health

The ALSPAC study

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

Hypothesis of workpackages 2 and 8 in NUTRIMENTHE

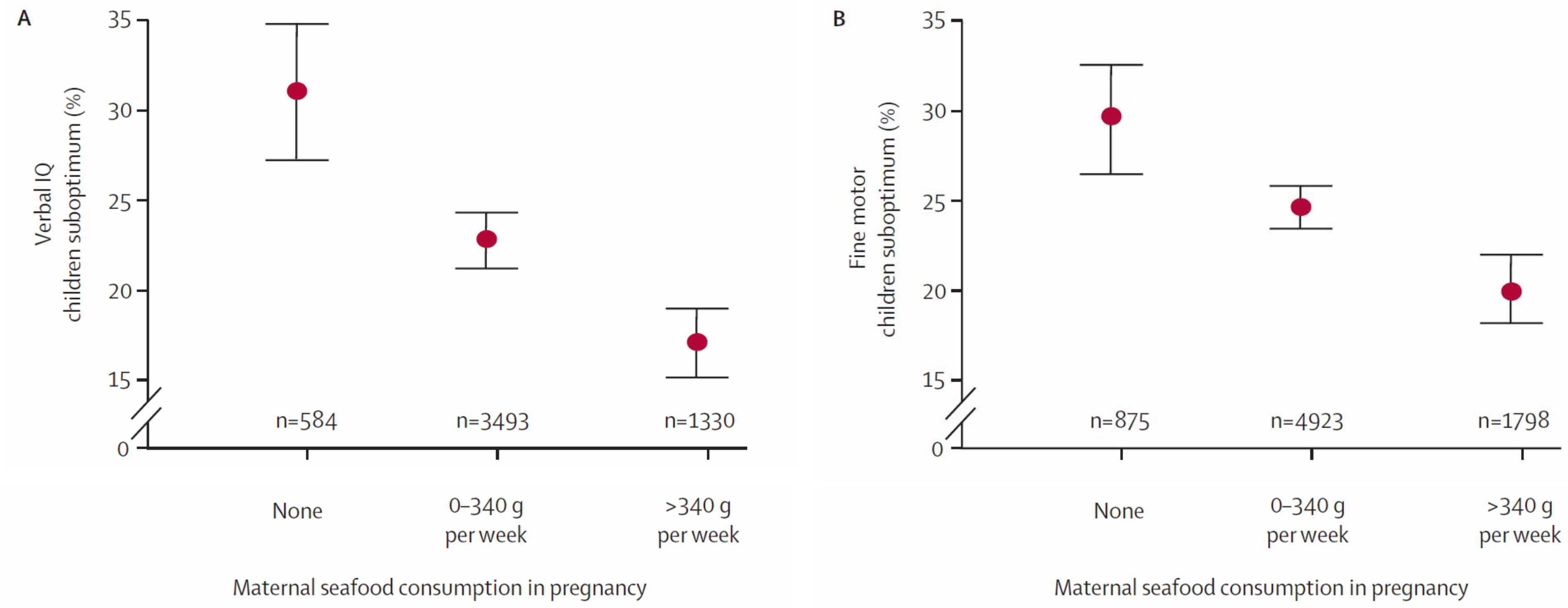
Lancet 2007; 369: 578–85

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 III[UK] (WISC-III[UK]) (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

Hypothesis of workpackages 2 and 8 in NUTRIMENTHE

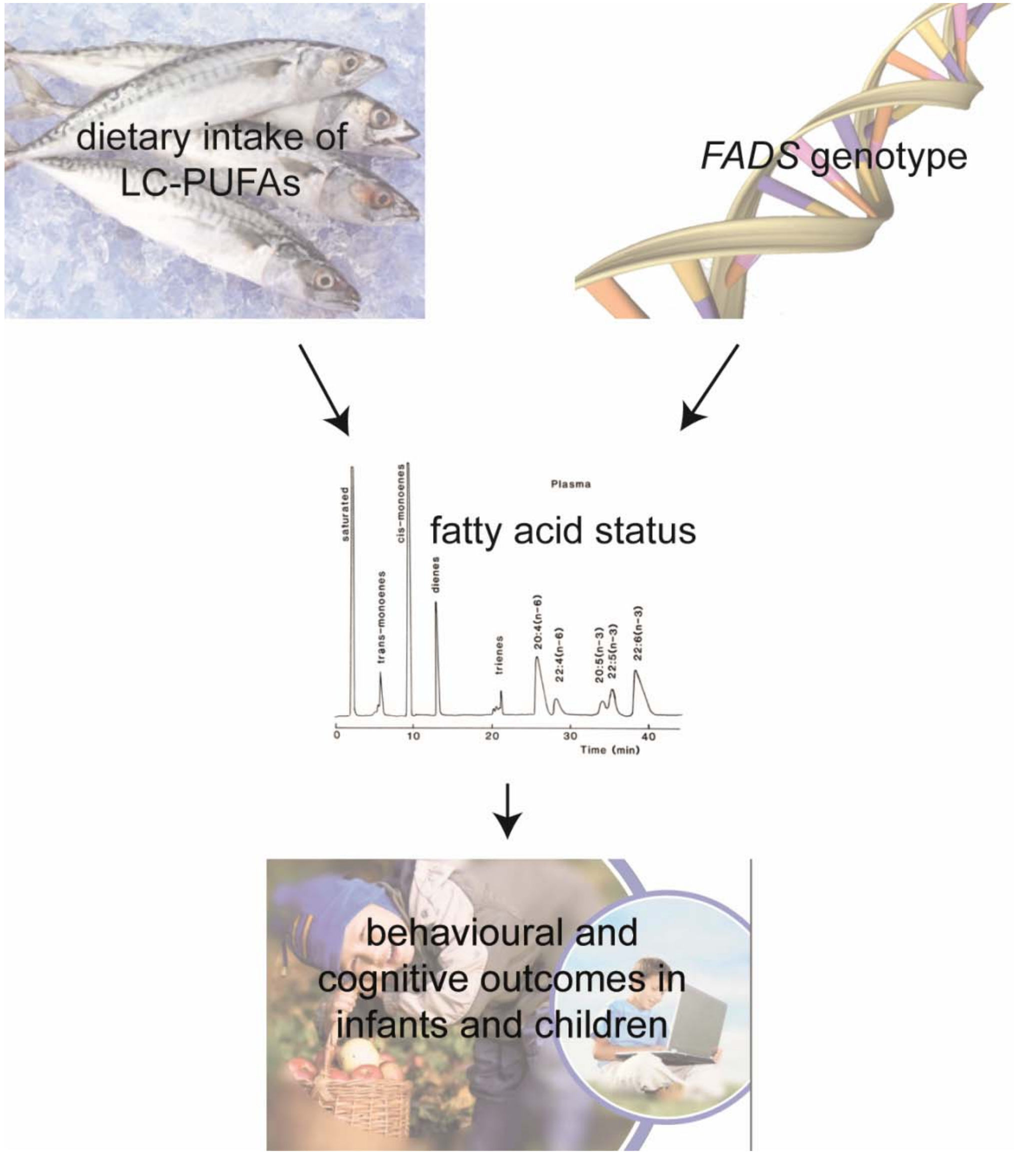


Low maternal seafood intake is associated with increased risk of suboptimum outcomes for verbal IQ, prosocial behaviour, fine motor, communication, and social development scores.

➤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.

Hypothesis of workpackages 2 and 8 in NUTRIMENTHE

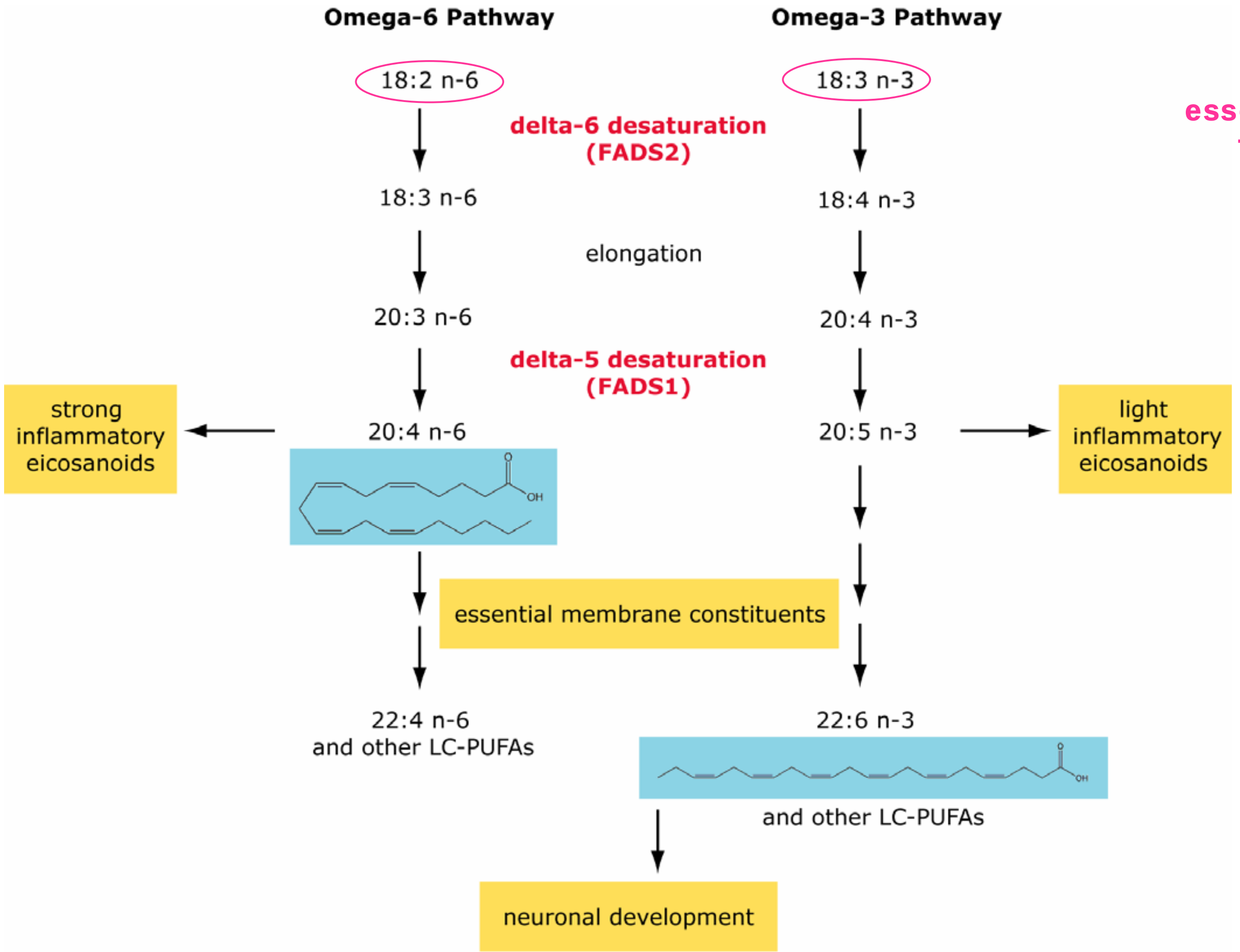


Main questions:

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

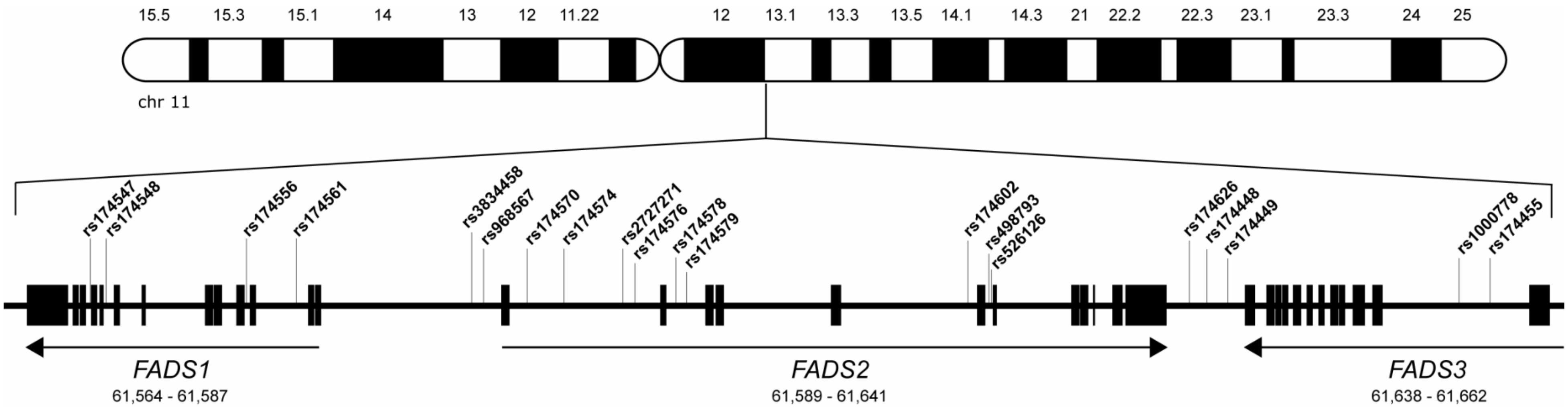
2) Is there an interaction with genetic effects?

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

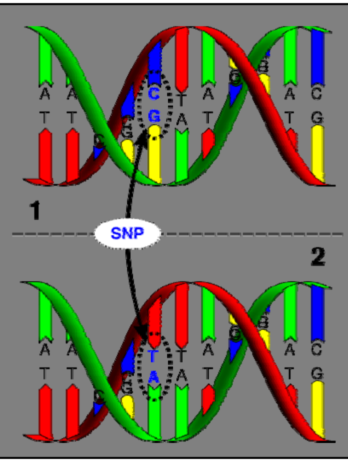


essential fatty acids
from nutrition

The *FADS* gene cluster

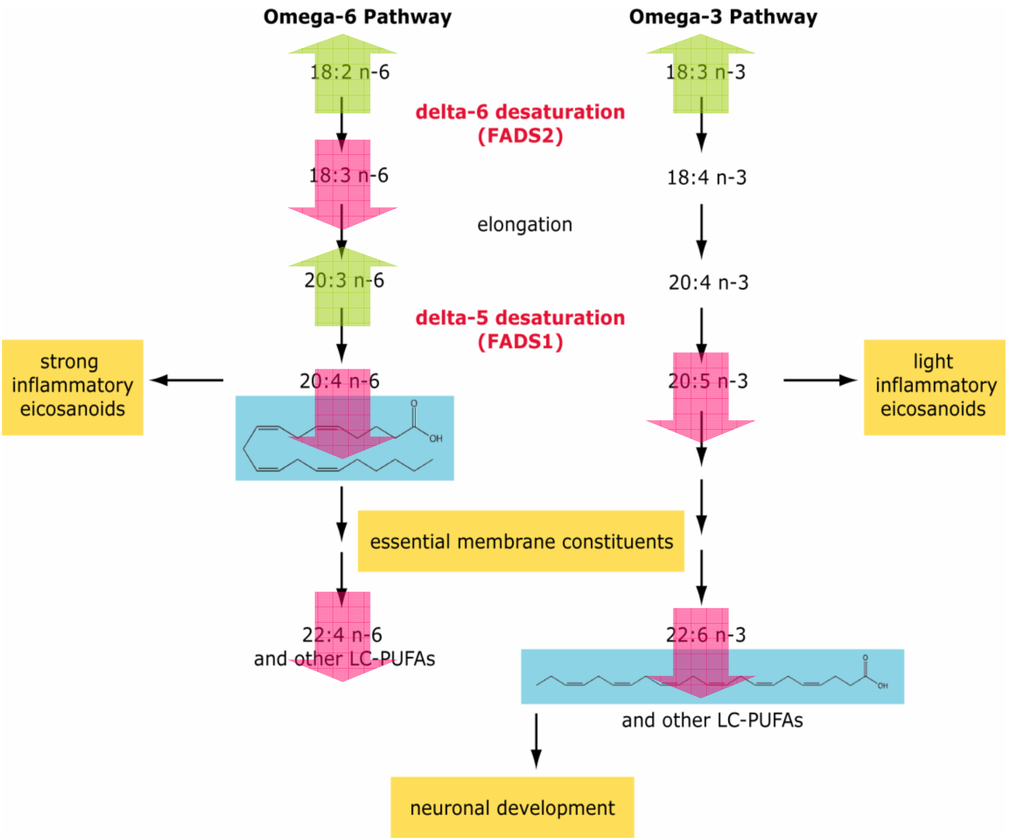


SNP (single nucleotide polymorphism) =
single base pair exchange



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**

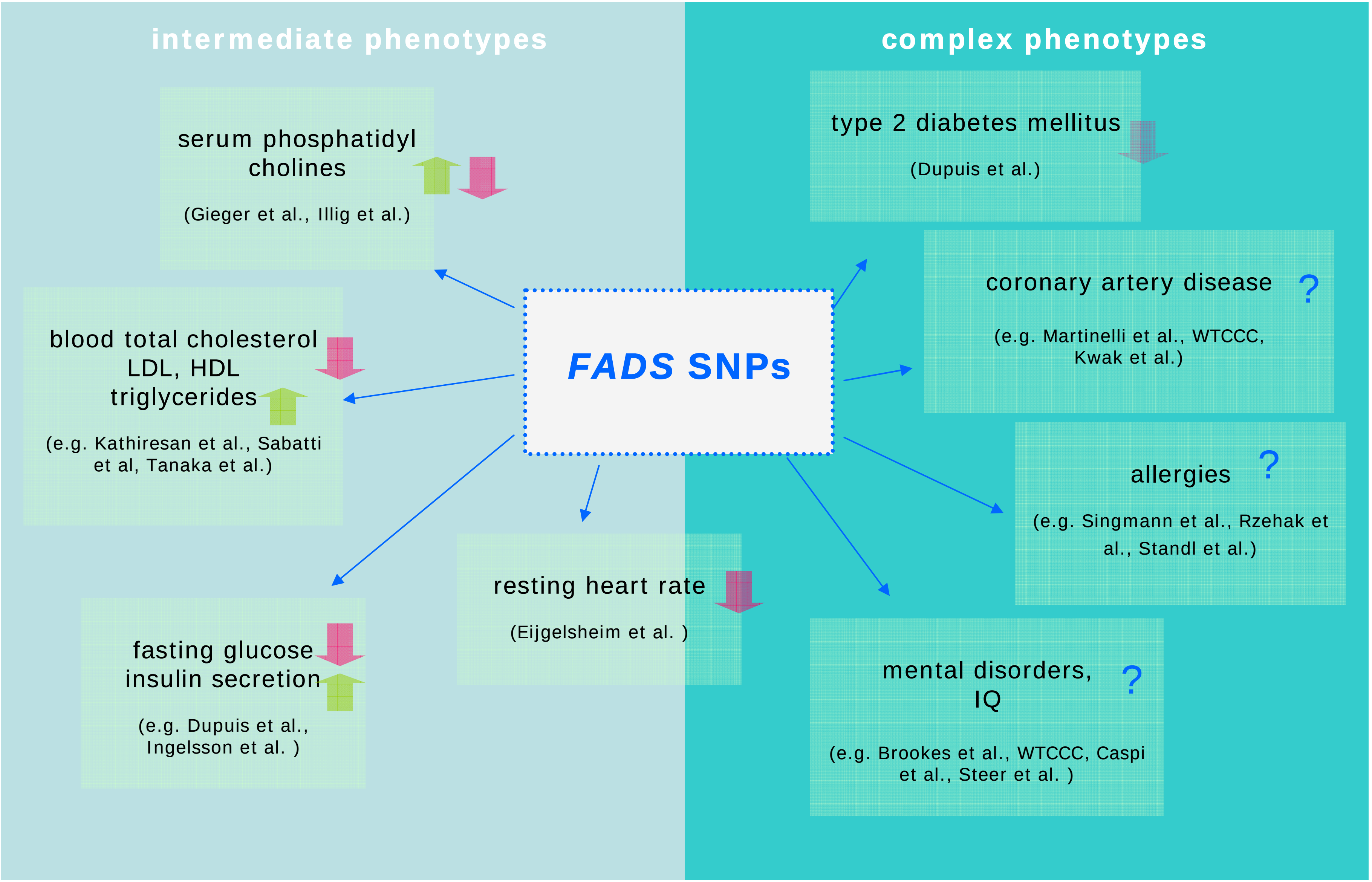
→ effect observable in **different tissues**

- 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)

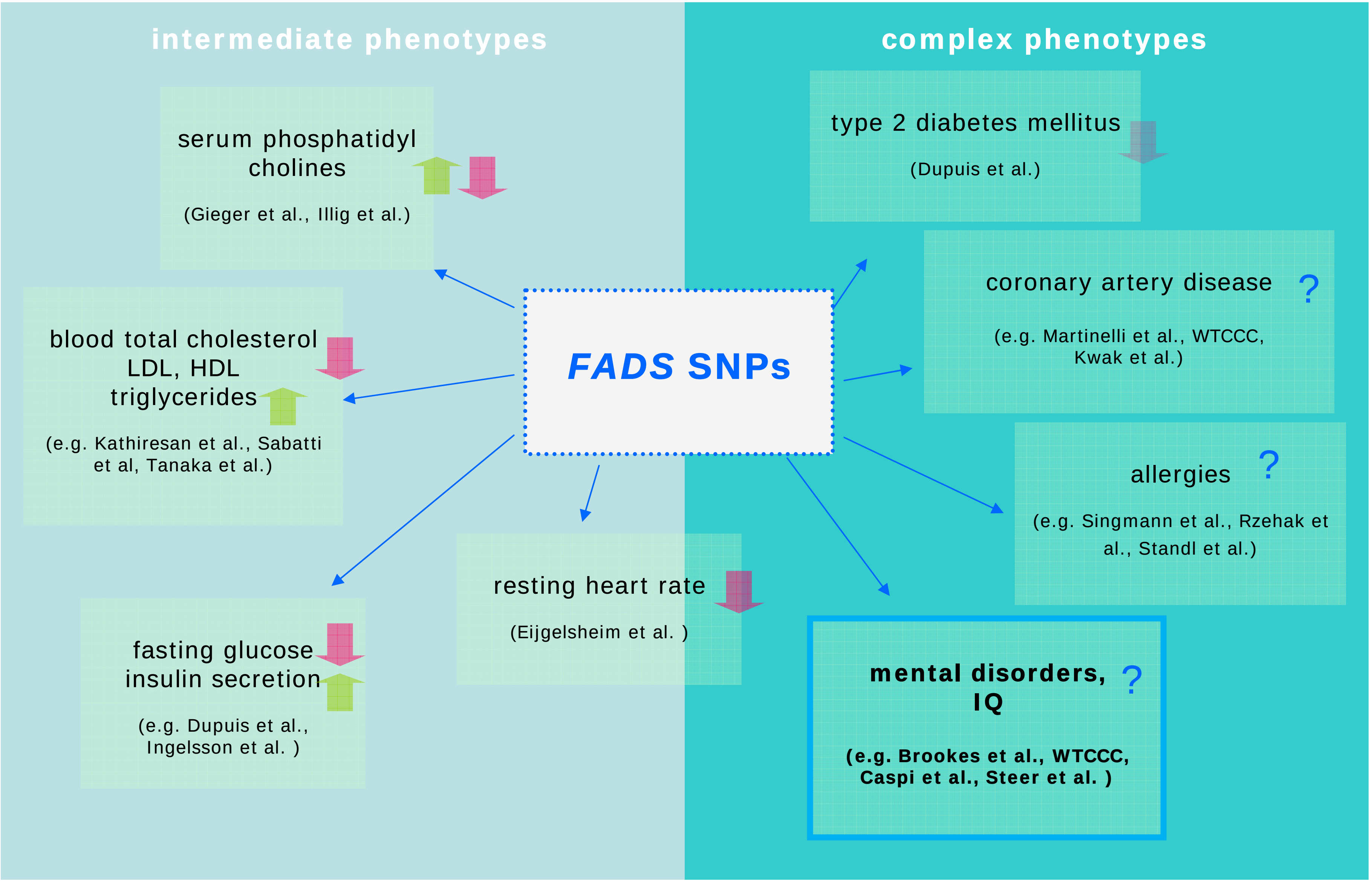
→ genetically determined variance for arachidonic acid: up to 30%!

→ genetic effects on % FA / total FA amounts comparable to nutritional effects in intervention studies

FADS polymorphisms associate with intermediate and complex phenotypes



FADS polymorphisms associate with intermediate and complex phenotypes

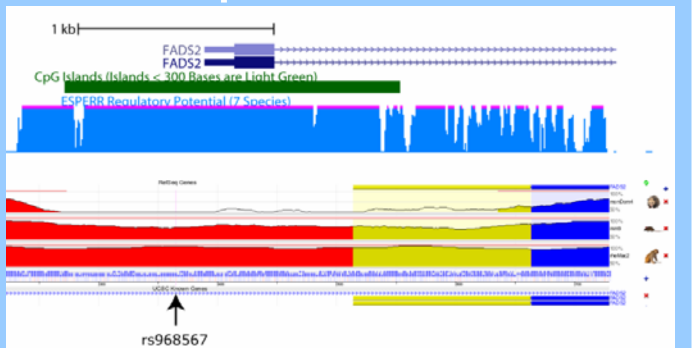


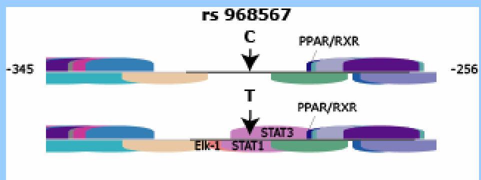
Identification of a functional relevant variant in the *FADS2* gene promoter

Genetic association

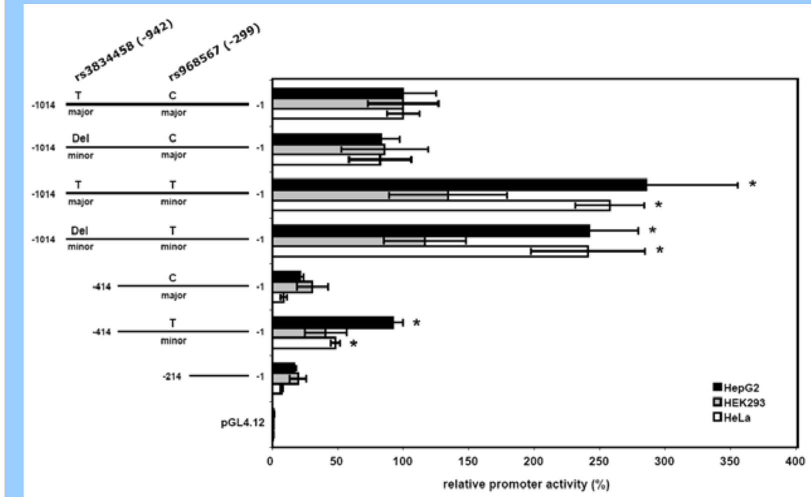
Strong association of SNPs in the *FADS* (fatty acid desaturase) gene cluster with LC-PUFA and lipid levels in several studies

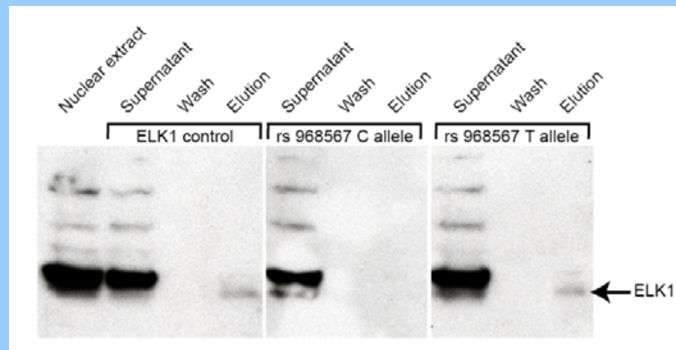
Bioinformatic prediction





Functional *in vitro* studies





Lattka et al J Lipid Res 2010 51(1):182-91

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)

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

FADS genotypes are associated with DHA concentrations in pregnant women

Results:

TABLE 2
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[1]

	FADS1			Intergenic		*FADS2*	
	rs174548	rs174556	rs174561	rs3834458	rs968567	rs174570	rs174574
Omega-6							
18:2	0.110 (0.020)***	0.112 (0.020)***	0.108 (0.020)***	0.106 (0.019)***	0.018 (0.024)	0.150 (0.027)***	0.113 (0.019)***
18:3	−0.047 (0.020)*	−0.036 (0.020)	−0.035 (0.020)	−0.037 (0.020)	0.012 (0.024)	−0.079 (0.027)**	−0.042 (0.019)*
20:2	0.104 (0.021)***	0.110 (0.021)***	0.104 (0.021)***	0.116 (0.020)***	0.011 (0.025)	0.172 (0.028)***	0.115 (0.020)***
20:3	0.336 (0.020)***	0.347 (0.021)***	0.345 (0.021)***	0.316 (0.020)***	0.403 (0.025)***	0.089 (0.029)**	0.305 (0.020)***
20:4	−0.154 (0.021)***	−0.151 (0.021)***	−0.155 (0.021)***	−0.151 (0.020)***	−0.131 (0.025)***	−0.129 (0.029)***	−0.147 (0.020)***
22:4	−0.148 (0.020)***	−0.151 (0.020)***	−0.154 (0.020)***	−0.140 (0.020)***	−0.144 (0.025)***	−0.100 (0.028)***	−0.134 (0.020)***
22:5	−0.141 (0.021)***	−0.134 (0.021)***	−0.137 (0.021)***	−0.140 (0.020)***	−0.085 (0.025)***	−0.166 (0.028)***	−0.146 (0.020)***
Omega-3							
18:3	0.079 (0.021)***	0.084 (0.021)***	0.081 (0.021)***	0.075 (0.020)***	0.013 (0.025)	0.071 (0.028)*	0.073 (0.020)***
20:5	−0.074 (0.021)***	−0.069 (0.021**	−0.074 (0.021)***	−0.075 (0.021)***	−0.091 (0.026)***	−0.037 (0.029)	−0.068 (0.020)***
22:5	−0.039 (0.021)	−0.036 (0.021)	−0.039 (0.021)	−0.036 (0.021)	−0.059 (0.026)*	−0.015 (0.029)	−0.035 (0.020)
22:6	−0.092 (0.021)***	−0.092 (0.021)***	−0.096 (0.021)***	−0.097 (0.020)***	−0.089 (0.025)***	−0.073 (0.028)**	−0.092 (0.020)***
Ratios							
AA to LA	−0.262 (0.021)***	−0.259 (0.021)***	−0.262 (0.021)***	−0.256 (0.020)***	−0.175 (0.025)***	−0.255 (0.028)***	−0.254 (0.020)***
EPA to ALA	−0.165 (0.020)***	−0.165 (0.020)***	−0.166 (0.020)***	−0.161 (0.020)***	−0.111 (0.025)***	−0.117 (0.028)***	−0.151 (0.019)***
n	6565	6524	6560	6558	6666	6583	6554

[1] 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

FADS genotypes are associated with DHA concentrations in pregnant women

Conclusion:

- DHA amounts in RBC phospholipids of pregnant women are determined by *FADS* genotypes
- Maternal *FADS* genotype might affect the child's DHA supply during pregnancy
- This effect is independent of diet

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

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

FADS genotypes are associated with cord blood fatty acid concentrations

Results:

- 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:

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

- 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.

FADS genotypes are associated with cord blood fatty acid concentrations

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?

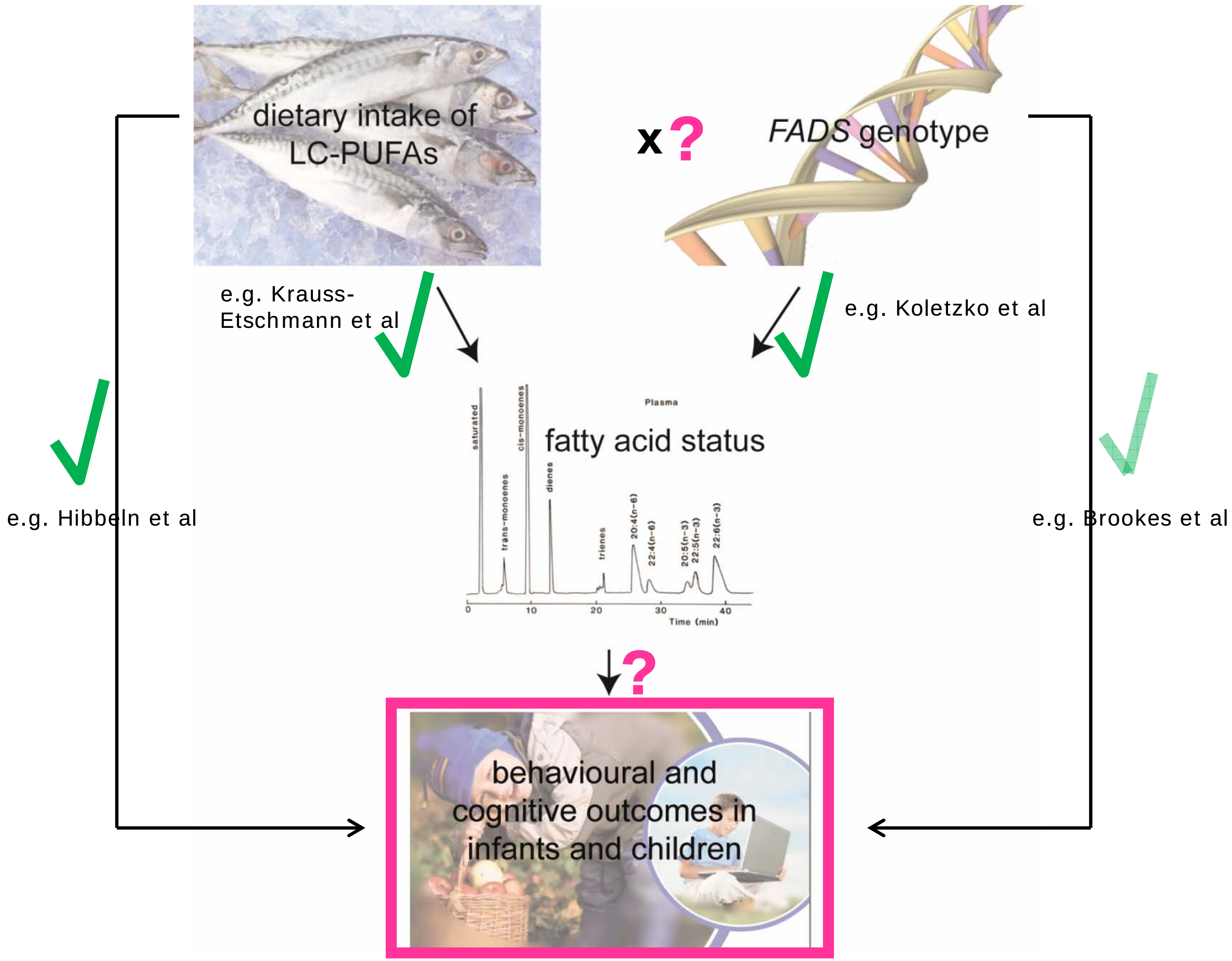
  → this is in contrast to common belief

  → 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



Maternal fatty acid levels and child IQ at age 8 years

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

Maternal fatty acid levels and child IQ at age 8 years

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)

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

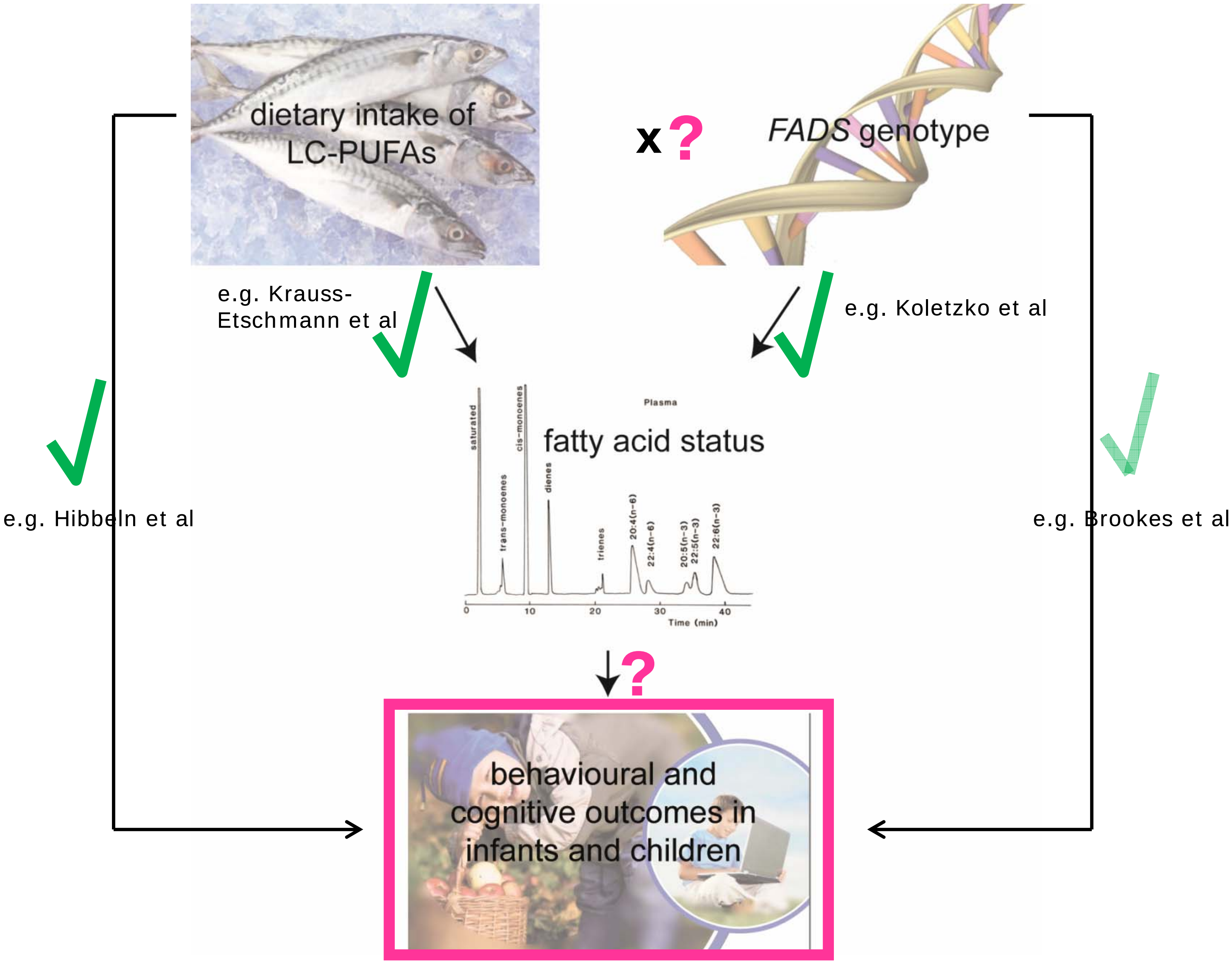
Conclusion:

➢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

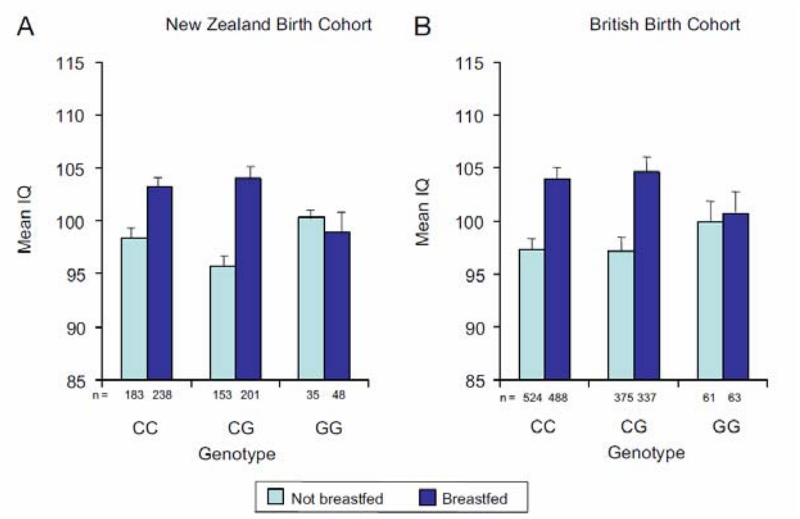
From fatty acids to complex phenotypes



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



→ children homozygous for the minor allele do not benefit from breastfeeding

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

Objective:

Replicate the findings of Caspi et al in the ALSPAC cohort

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

Results – Gene-nutrition interactions

FADS genotypes modify the effect of breastfeeding on child IQ

Design:

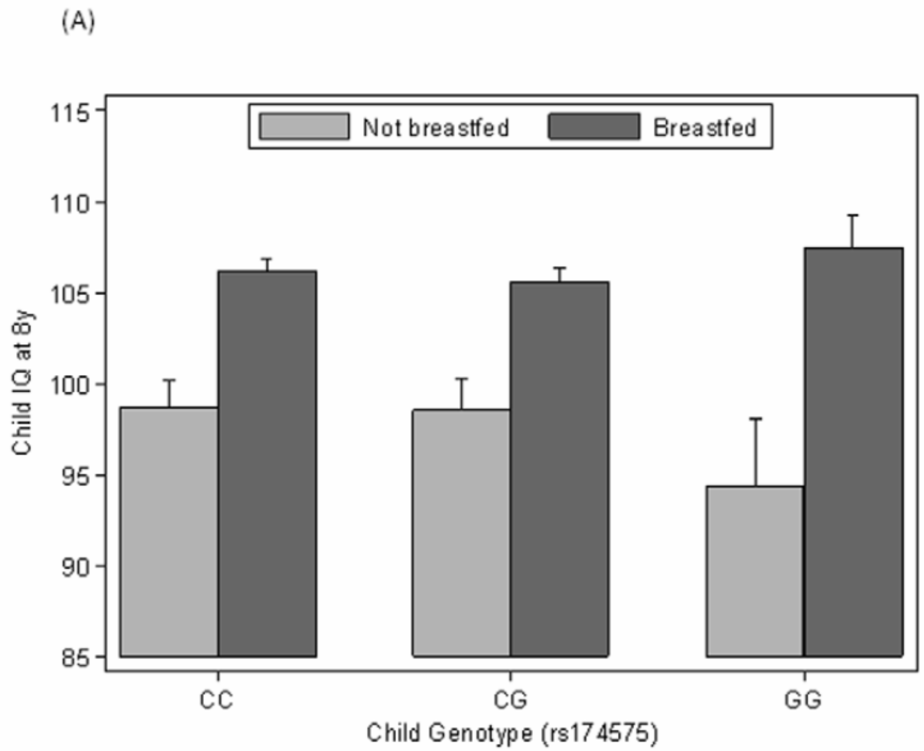
➢ Regression and interaction analysis of *FADS* genotype and breastfeeding on IQ at age 8 years as major outcome in 5934 children from ALSPAC

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

FADS genotypes modify the effect of breastfeeding on child IQ

Conclusion:

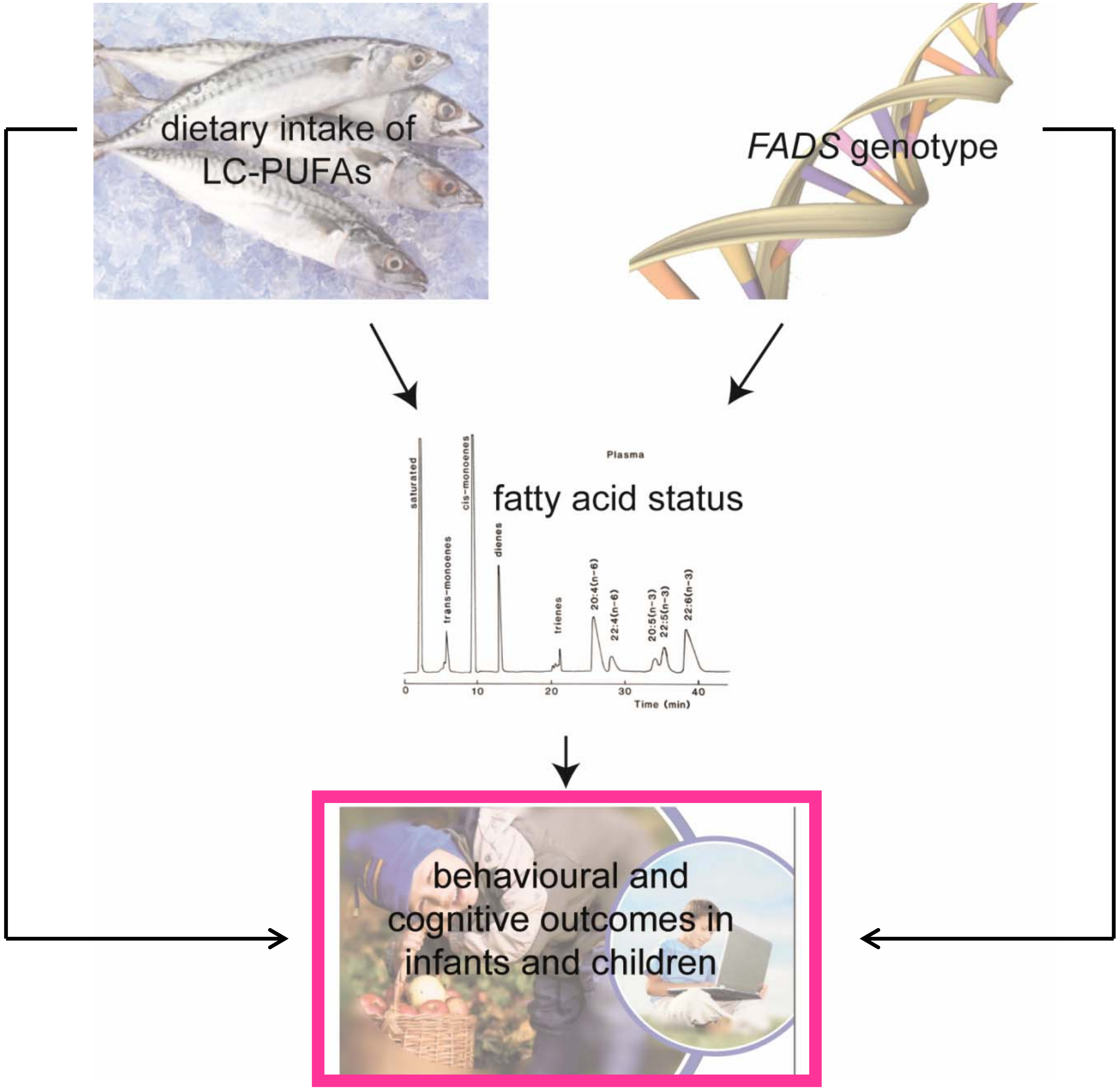
- No replication of Caspi results
- Breastfed children have similar IQs irrespective of genotype
- Biologically plausible: Breast milk contains preformed DHA, therefore all children should benefit from breastfeeding
- Children homozygous for the minor allele show the biggest differences between feeding 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?

Future…



Gene-nutrition interactions →

- Explanation of inconsistent findings
- Individual dietary recommendations

Acknowledgements

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Thanks for your attention!