# Maternal Influence on Toddler Cognitive Development: Genetic and Epigenetic Factors

Carol L. Cheatham,[1,2] Daniel S. Lupu,[1,3] Mihai D. Niculescu [1,3]
[1]Nutrition Research Institute, North Carolina Research Campus, Kannapolis, NC
[2]Department of Psychology, University of North Carolina at Chapel Hill
[3]Department of Nutrition, University of North Carolina at Chapel Hill


## Background

- Docosahexaenoic acid (DHA, 22:6n-3) is the main n-3 fatty acid in the brain, and it is directly implicated in long-term potentiation (LTP: Itokazu, Ikegaya, Nishikawa, & Matsuki, 2000), a form of synaptic plasticity that is utilized by neurons in the formation of a memory trace.
- The effect of DHA on cognitive abilities is elusive: fewer than 40% of trials find any effect (Simmer, Patole, & Rao, 2011).
- During the fetal and neonatal periods, the developing brain is dependent on maternal sources of DHA (Innis, 2008), which can be either dietary (exogenous) or synthesized from alpha-linolenic acid (LNA, 18:3n-3; endogenous).
- The delta-6 desaturase involved in the endogenous synthesis of DHA is the rate-limiting enzyme in n-3 and n-6 synthesis. Importantly, *FADS2* expression is up-regulated during pregnancy (Rodriguez-Cruz et al., 2011), but dietary intake increases DHA levels in plasma, but not in milk, in carriers of minor alleles (Molto-Puigmarti et al., 2010).
- Thus, maternal transfer of DHA to the fetus and infant may be under genetic control irrespective of dietary intake, and maternal genetic influence is an important consideration in infant development.
- Also, gene-nutrient interactions are mediated by epigenetic modifications such as DNA methylation (Niculescu & Lupu, 2011). Maternal nutrition influences fetal brain development (Niculescu & Lupu, 2009) and the infant epigenome (Chmurzynska, 2010).
- We explored how maternal genetic and epigenetic factors relate to early brain development and subsequent cognitive development.

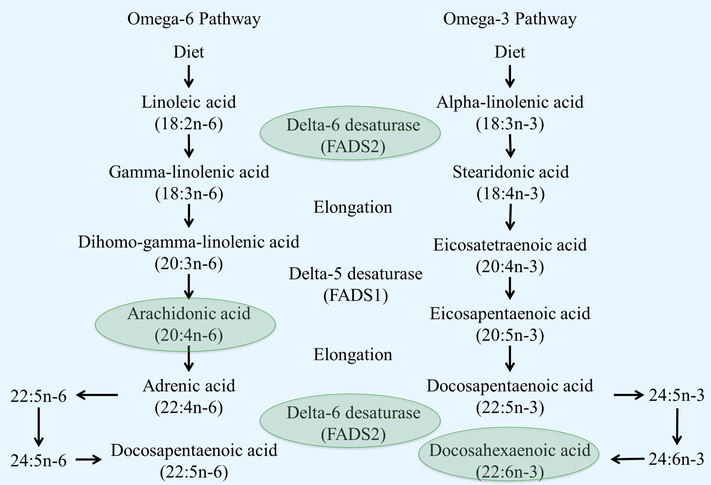
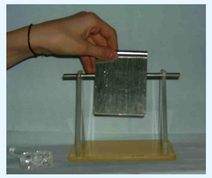



Figure 1. Fatty acid metabolic pathway in which the omega-3 and omega-6 fatty acids compete for the desaturases needed. A single nucleotide polymorphism, *FADS2* rs174575, has been related to lower levels of DHA in plasma and breastmilk (Zie & Innis, 2008).

## Participants and Method

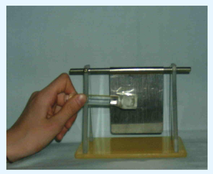
- Typically-developing toddlers (n=66) were enrolled at 16 months of age in the context of a larger study. Only baseline data are reported here.
- Toddlers participated in an elicited imitation paradigm in which they were exposed to four 3-step events. Recall was tested immediately on two events, after 20 minutes on two events, and after a week on all four events.
  - Two outcome measures were coded from the videotapes by one trained coder. A score was given for the number of target actions completed (max = 3), and a score was given for the number of pairs of target actions completed in the intended order (max = 2).
- Blood samples were collected from natural mothers and toddlers. Lipids were extracted from plasma and analyzed using gas capillary chromatography.
- Genomic DNA was extracted from blood using a Qiacube robot (Qiagen, Valencia, CA, USA) using the QIAamp DNA Blood Mini Kit according to the manufacturer's protocol. Genotyping for the rs174575 variant (located at 61,602,003 in the *FADS2* intron) was performed in duplicate using a validated TaqMan allelic discrimination assay (assay ID C_2575522_20, Applied Biosystems by Life Technologies, Grand Island, NY, USA) on an Eppendorf Mastercycler epgradient S (Eppendorf, Hamburg, Germany), with automated pipetting performed by a QiAgility robot (Qiagen, Valencia, CA, USA).
- The DNA methylation status of the CpG island within *FADS2* promoter was determined in triplicate by bisulfite pyrosequencing in a 75 bp CpG island starting at 61,595,750 in exon 1, chromosome 11 spanning the promoter to intron 1 and containing 9 CpGs.



**Example of 3-step Event**

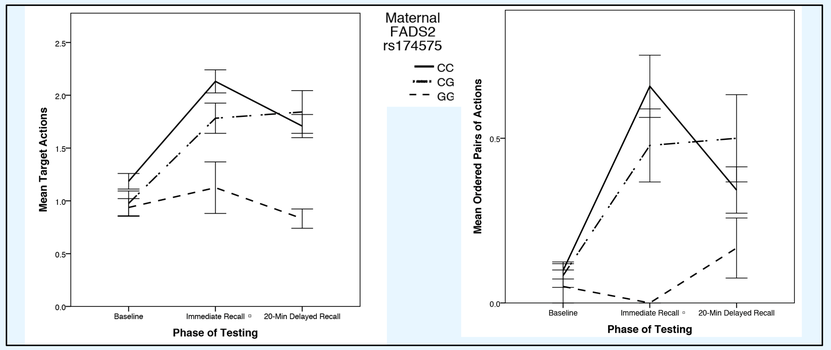
Build a Gong

Step 1 – Put on the bar
Step 2 – Hang the bell
Step 3 – Ring the gong



## MANOVA

- **Target Actions**
  - Main effect of maternal genetics, F (2, 64) = 6.50, p < 0.005
    - Toddlers of CC and CG mothers outperformed toddlers of GG mothers.
  - Main effect of time of testing, F (2, 117) = 16.32, p < 0.0001
    - Toddlers did better at test (immediate and 20-min delay) than at baseline
  - Significant interaction (see figure, top right), F (4, 117) = 3.15, p < 0.01
- **Ordered Pairs**
  - Main effect of maternal genetics, F (2, 64) = 3.4, p < 0.05
    - Toddlers of CC and CG mothers outperformed toddlers of GG mothers.
  - Main effect of time of testing, F (2, 115) = 8.85, p < 0.0005
    - Toddlers did better at test (immediate and 20-min delay) than at baseline
  - Interaction (see figure, top right), F (4, 115) = 2.02, p < 0.10




## Stepwise Regression with Methylation Variables

- **Target Action**
  - Immediate – predicted by maternal genotype, plasma LNA, & toddler methylation, R-sq = 0.28
  - Delayed – predicted by maternal genotype and maternal methylation, R-sq = 0.26
- **Ordered Pairs**
  - Immediate – predicted by maternal genotype, plasma LNA, and toddler methylation, R-sq = 0.26
  - Delayed – predicted by maternal methylation, R-sq = 0.21

## Discussion

- The toddlers interacted with the props as expected with performance better after the demonstration than before.
- When analyses were performed with child genotype as the grouping variable, there was not a significant difference between the groups on any outcome variable (data not shown).
- Toddlers of GG mothers had lower scores at baseline, meaning that they were either not as engaged in the task or were not as good at discovery of affordances as other toddlers.
- Even as late as 16 months of age, maternal differences in fatty acid metabolism is related to the toddlers' ability to attend to modeling, to encode a memory, and/or to retrieve a memory.
- When maternal methylation of the promoter region of the *FADS2* gene was high, toddler performance on delayed recall was low. This finding indicates a potential role for DHA in the neural circuit supporting delayed recall (temporo-frontal).
- When methylation is high, the gene may not be expressed, and in the case of *FADS2*, the supply of desaturase is presumably low. Mothers with the GG genotype have lower DHA levels in pregnancy (Xie & Innis, 2008) and presumably confer lower levels of DHA to the fetus relative to mothers with the other genotypes (CC & CG).
- Longitudinal work will determine the extent to which maternal factors are integral to the cognitive abilities of children.

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