

## Long-chain polyunsaturated fatty acids, homocysteine at birth and fatty acid desaturase gene cluster polymorphisms are associated with children's processing speed up to age 9 years.

### *SNP selection and genotyping*

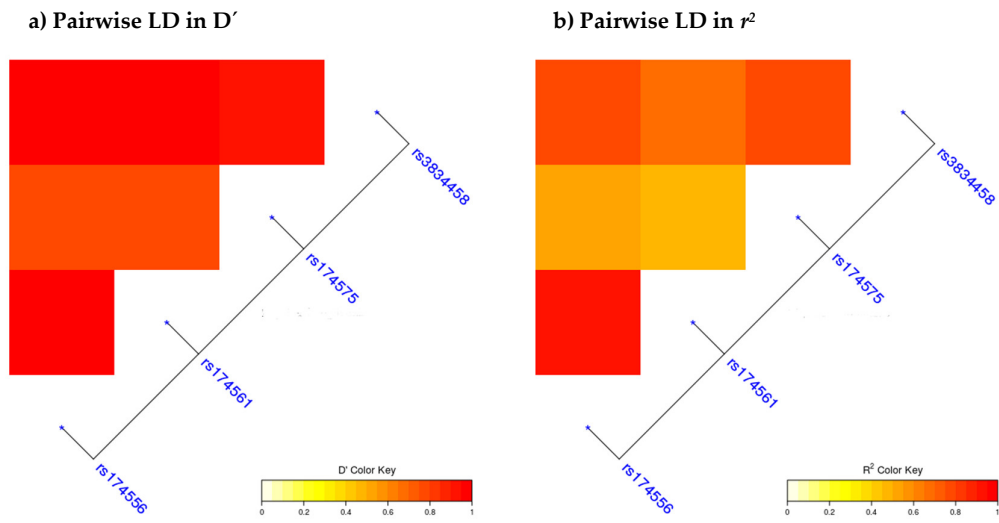
Genotyping of SNPs (single-nucleotide polymorphisms) from the *FADS* gene cluster was performed with the iPLEX method (Sequenom, San Diego, CA, USA) (see main text for more information). Initially, seventeen SNPs from the *FADS1* (rs174548, rs174556 and rs174561), *FADS2* (rs174570, rs174574, rs174575, rs174576, rs174578, rs174579, rs174602, rs498793, rs968567, rs2727271 and rs3834458) and *FADS3* (rs174448, rs174449 and rs174455) gene cluster from the NUHEAL mothers were genotyped (**Supplemental Table 1**). Specific SNPs, including rs174556, rs174561, rs3834458, rs174548, rs174574 and rs174578, were selected due to its relation with FA levels [1,2], while 11 remaining SNPs were included according to its localization in *FADS* gene cluster on chromosome 11. Based on the linkage disequilibrium data from Lewontin's  $D'$  ( $D' > 0.9$ ) and pairwise-squared correlations  $r^2$  ( $r^2 > 0.7$ ), 4 SNPs out of the initial 17 SNPs (*FADS1* rs174556 and rs174561, *FADS2* rs174575 and rs3834458) were considered for further analyses (**Supplemental Figure 1**). Hardy-Weinberg (*H-W*) equilibrium for the genotypes was calculated using the statistical software R (Version 3.2.2, package "genetics"). Deviations from the *H-W* equilibrium were analyzed by Fisher's exact test (**Supplemental Table 2**), where *FADS1* rs174561 was discarded due to its low significance ( $p = 0.016$ ). To test the linkage disequilibrium, Lewontin's  $D'$  and pairwise squared correlations  $r^2$  were also performed (**Supplemental Table 3**). Finally, we have selected the *FADS1* rs174556 as a representative of the block and were included in our following analysis.

**Supplemental Table 1.** Characteristics of the 17 analysed variants in *FADS 1/2/3* gene cluster of NUHEAL population

SNP	Possible Functional Region	Alleles (Major/Minor) and Frequency 0/1	Number (%) of subjects with Genotype			<i>p</i>
			0	1	2	
rs174548	<i>FADS 1</i>	G/C (0.71/0.28)	75 (0.54)	50 (0.36)	15 (0.10)	0.148
rs174556	<i>FADS 1</i>	G/A (0.74/0.26)	80 (0.57)	47 (0.34)	13 (0.09)	0.128
rs174561	<i>FADS 1</i>	A/G (0.76/0.24)	81 (0.61)	38 (0.29)	13 (0.10)	<b>0.016</b>
rs174570	<i>FADS 2</i>	C/T (0.89/0.11)	112 (0.79)	27 (0.19)	2 (0.01)	0.672
rs174574	<i>FADS 2</i>	C/A (0.65/0.35)	65 (0.46)	56 (0.39)	21 (0.15)	0.138
rs174575	<i>FADS 2</i>	C/G (0.71/0.29)	74 (0.52)	53 (0.37)	15 (0.11)	0.230
rs174576	<i>FADS 2</i>	C/A (0.67/0.33)	67 (0.48)	54 (0.38)	20 (0.14)	0.128
rs174578	<i>FADS 2</i>	T/A (0.67/0.33)	67 (0.48)	54 (0.38)	20 (0.14)	0.128
rs174579	<i>FADS 2</i>	C/T (0.78/0.22)	92 (0.64)	42 (0.29)	10 (0.07)	0.135
rs174602	<i>FADS 2</i>	T/C (0.75/0.25)	78 (0.55)	59 (0.41)	6 (0.04)	0.266
rs498793	<i>FADS 2</i>	C/T (0.52/0.48)	37 (0.26)	74 (0.52)	32 (0.22)	0.739
rs968567	<i>FADS 2</i>	C/T (0.85/0.15)	103 (0.72)	37 (0.26)	3 (0.02)	1
rs2727271	<i>FADS 2</i>	A/T (0.91/0.09)	116 (0.82)	24 (0.017)	1 (0.01)	1
rs3834458	<i>FADS 2</i>	T/Z (0.68/0.32)	68 (0.49)	55 (0.39)	17 (0.12)	0.249
rs174448	<i>FADS 3</i>	T/C (0.59/0.41)	50 (0.35)	71 (0.49)	23 (0.16)	0.864
rs174449	<i>FADS 3</i>	T/C (0.60/0.40)	50 (0.35)	70 (0.49)	22 (0.16)	0.862
rs174455	<i>FADS 3</i>	T/C (0.60/0.40)	50 (0.35)	69 (0.49)	23 (0.16)	1

*p*: level of significance according to Fisher exact test; Bold: *p* < 0.05

0= Homozygous major, 1= Heterozygous, 2 = Homozygous minor; *FADS*: Fatty Acid Desaturase.

**Supplemental Figure 1.** Pairwise LD measured in  $D'$  (a) and  $r^2$  (b) for selected maternal *FADS* SNPs

**Supplemental Table 2.** Characteristics of the 4 selected variants in *FADS 1/2* gene from the initial 17 SNPs analyzed in the NUHEAL population

SNP	Supplementation group	Alleles (Major/Minor) and Frequency 0/1	Number (%) of subjects with Genotype			<i>p</i>
			0	1	2	
<i>FADS1</i> rs174556		G/A (0.74/0.26)	80 (0.57)	47 (0.34)	13 (0.09)	0.127
	FO	G/A (0.67/0.33)	22(0.41)	13(0.52)	4(0.07)	0.671
	5-MTHF	G/A (0.66/0.34)	12 (0.38)	12 (0.56)	5 (0.06)	0.591
	FO+5-MTFH	G/A (0.73/0.27)	22 (0.68)	10 (0.27)	-	0.538
	Placebo	G/A (0.79/0.21)	24(0.62)	12(0.35)	4 (0.04)	1
<i>FADS1</i> rs174561		A/G (0.76/0.24)	81 (0.61)	38 (0.29)	13 (0.10)	<b>0.016</b>
	FO	A/G (0.68/0.32)	22 (0.43)	12 (0.50)	4 (0.07)	0.671
	5-MTHF	A/G (0.67/0.13)	12(0.40)	10 (0.53)	5 (0.07)	0.616
	FO+5-MTFH	A/G (0.75/0.25)	23 (0.68)	7 (0.32)	0	1
	Placebo	A/G (0.80/0.20)	24 (0.64)	9(0.32)	4 (0.04)	1
<i>FADS2</i> rs174575		C/G (0.71/0.29)	74 (0.52)	53 (0.37)	15 (0.11)	0.230
	FO	C/G (0.71/0.29)	21 (0.48)	14 (0.45)	5 (0.07)	0.274
	5-MTHF	C/G (0.75/0.25)	12 (0.45)	14 (0.45)	3(0.10)	1
	FO+5-MTFH	C/G (0.73/0.27)	20 (0.50)	11 (0.45)	3 (0.05)	0.385
	Placebo	C/G (0.75/0.25)	21 (0.54)	14 (0.42)	4 (0.04)	0.448
<i>FADS2</i> rs3834458		T/Z (0.68/0.32)	68 (0.49)	55 (0.39)	17 (0.12)	0.249
	FO	T/Z (0.64/0.34)	18 (0.38)	15(0.55)	2 (0.07)	0.413
	5-MTHF	T/Z (0.66/0.34)	11 (0.38)	13 (0.56)	5 (0.06)	0.591
	FO+5-MTFH	T/Z (0.75/0.25)	19 (0.55)	2 (0.41)	2 (0.05)	1
	Placebo	T/Z (0.73/0.27)	20 (0.50)	15 (0.46)	5 (0.04)	0.631

*p*: level of significance obtained from Hardy-Weinberg Equilibrium Test; Bold: *p* <0.05

0= Homozygous major; 1= Heterozygous; 2=Homozygous minor; 5-MTHF: 5-Methyltetrahydrofolate; *FADS*: Fatty Acid Desaturase; FO: Fish-oil.

**Supplemental Table 3.** Pairwise linkage disequilibrium analysis in maternal selected *FADS* SNPs

	rs174561	rs174575	rs3834458		rs174561	rs174575	rs3834458		rs174561	rs174575	rs3834458			
rs174561	<i>n</i>	119	125	125	rs174561	<i>n</i>	-----	119	118	rs174575	<i>n</i>	-----	-----	125
	<i>D</i>	0.184	0.146	0.184		<i>D</i>	-----	0.137	0.170		<i>D</i>	-----	-----	0.187
	<i>D'</i>	0.999	0.782	0.999		<i>D'</i>	-----	0.779	0.999		<i>D'</i>	-----	-----	0.919
	<i>r</i>	0.959	0.719	0.867		<i>r</i>	-----	0.687	0.832		<i>r</i>	-----	-----	0.868
	<i>X</i> <sup>2</sup>	219.29	129.26	187.96		<i>X</i> <sup>2</sup>	-----	112.43	163.31		<i>X</i> <sup>2</sup>	-----	-----	188.51
	<i>p</i>	<0.001	<0.001	<0.001		<i>p</i>	-----	<0.001	<0.001		<i>p</i>	-----	-----	<0.001

*p*: level of significance obtained  $1 - \beta = \Phi(z - z_{1-\alpha/2}) + \Phi(-z - z_{1-\alpha/2})$  ,  $z = \frac{\mu_A - \mu_B}{\sigma \sqrt{\frac{1}{n_A} + \frac{1}{n_B}}}$   
 from correlation analysis. Bold: *p* <

0.05

*D*: coefficient of linkage disequilibrium; *D'*: normalizing *D*; *n*: number of cases; *r*: correlation coefficient; *X*<sup>2</sup>: Chi-square

**Statistical Analysis**

Power calculation was performed for the present analysis using the following formula [3]:

Formula Power calculation →

where:  $\sigma$  is standard deviation;  $\Phi$  is the standard Normal distribution function;  $\Phi^{-1}$  is the standard Normal quantile function;  $\alpha$  is Type I error and  $\beta$  is Type II error, meaning  $1-\beta$  is power.

**Supplementary Table 4** shows the results obtained, where *z* indicates power calculation, considering  $\alpha= 0.05$  and  $\beta= 0.2$  (80%).

**Supplemental Table 4.** Power calculation obtained for processing speed evaluation in NUHEAL children

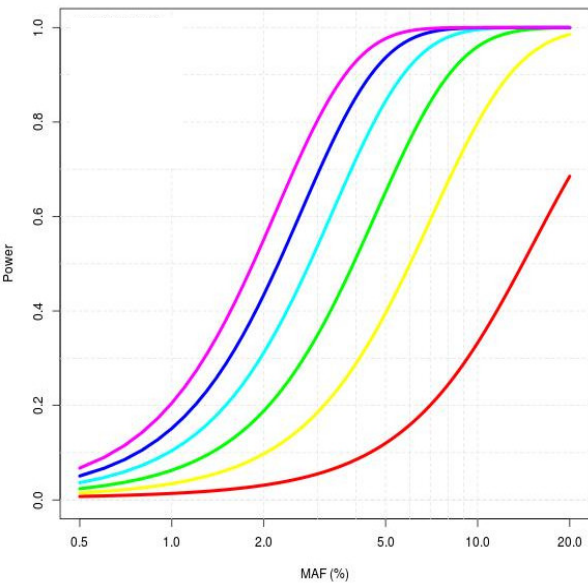
Pooled			
Speed processing tasks at 7.5 years	Intervention group	Placebo group	<i>z</i>
	(FO+5MTHF+MIX)		

SDMT – hits	22.87 ± 6.22	24.66 ± 6.38	0.80
CCTT-1 (sc)	86.12 ± 41.12	99.88 ± 50.77	0.80
STROOP Test – hits -1 (Word-Reading)	60.13 ± 14.85	59.34 ± 16.41	0.80
STROOP Test – hits -2 (Color-Naming)	41.30 ± 8.12	41.95 ± 8.41	0.80

Values expressed as mean ± standard deviation (SD).

5-MTHF: 5-Methyltetrahydrofolate; CCTT: Children Color Trails test; FO: Fish-oil; MIX: FO + 5-MTHF; SDMT: Symbol Digit Modalities test; z= power calculation

Interestingly, power calculation for genetic study was also calculated using the package “PowerEQTL software). For this considered an unbalanced different ranges of 100, our-case 143, α value of 0.05 SNPs in FADS cluster. As show **Figure 2 and Table 5**, a of 90% was analyzed SNPs, rs174570 and



v0.1.3” (R purpose, we one-way ANOVA with sample size (50, 200, 250 and 300), and 17 analyzed 1/2/3 gene in **Supplemental Supplemental** statistical power obtained for except for rs2727271.

**Supplemental**

estimation, α=0.05, SNPs=17 (one-way unbalanced ANOVA). X-axis represents the power calculation, and y-axis shows the percentage of minor allele frequency. Red: sample size (n) of 50 subjects; yellow: n = 100; green: sample size in the present study (n= 143); light blue: n= 200; dark blue: sample size of 250 subjects; violet: n= 300.

**Figure 2.** Power

**Supplemental Table 5.** Power calculation obtained for genetic study in NUHEAL children

SNPs	Possible Functional Region	Alleles Frequency (Major/Minor)	Power Calculation
rs174548	<i>FADS1</i>	G/C (0.73/0.27)	>0.90
rs174556	<i>FADS1</i>	G/A (0.74/0.26)	>0.90
rs174561	<i>FADS1</i>	A/G (0.75/0.25)	>0.90
rs174570	<i>FADS2</i>	C/T (0.90/0.10)	>0.80
rs174574	<i>FADS2</i>	C/A (0.68/0.32)	>0.90
rs174575	<i>FADS2</i>	C/G (0.73/0.27)	>0.90
rs174576	<i>FADS2</i>	C/A (0.68/0.32)	>0.90
rs174578	<i>FADS2</i>	T/A (0.67/0.33)	>0.90
rs174579	<i>FADS2</i>	C/T (0.81/0.19)	>0.90
rs174602	<i>FADS2</i>	T/C (0.77/0.23)	>0.90
rs498793	<i>FADS2</i>	C/T (0.55/0.45)	>0.90
rs968567	<i>FADS2</i>	C/T (0.83/0.17)	>0.90
rs2727271	<i>FADS2</i>	A/T (0.89/0.11)	>0.80
rs3834458	<i>FADS2</i>	T/Z (0.70/0.30)	>0.90
rs174448	<i>FADS3</i>	T/C (0.69/0.31)	>0.90
rs174449	<i>FADS3</i>	T/C (0.69/0.31)	>0.90
rs174455	<i>FADS3</i>	T/C (0.68/0.32)	>0.90

*FADS*: Fatty Acid Desaturase; SNP: Single-Nucleotide polymorphism.





**Supplemental Table 6.** Pairwise comparison of predictive margins of the mixed effects logistic regression model.

7.5 years						
	FO vs 5-MTHF	FO vs FO+5-MTHF	FO vs Placebo	5-MTFH vs FO+5-MTFH	5-MTFH vs Placebo	FO+5-MTFH vs Placebo
<b>CCTT-1 (sc)</b>						
Contrast	-10.077	-5.546	-1.68	4.53	8.39	-3.86
95% CI	(-27.434, 7.29)	(-22.66, 11.57)	(-17.48, 14.12)	(13.23, 23.30)	(-8.10, 24.89)	(-20.11, 12.38)
<i>p</i> -value	0.255	0.526	0.835	0.617	0.319	0.641
<b>STROOP Test – hits 1 (Word-reading)</b>						
Contrast	1.05	-0.77	-2.32	-1.82	-3.38	1.55
95% CI	(-5.61, 7.72)	(-7.35, 5.80)	(-8.40, 3.75)	(-8.66, 5.009)	(-9.74, 2.97)	(-4.70, 7.81)
<i>p</i> -value	0.756	0.818	0.453	0.600	0.291	0.627
<b>STROOP Test – hits 2 (Color-naming)</b>						
Contrast	0.37	4.44	1.09	4.07	0.72	3.35
95% CI	(-4.07, 4.81)	(0.06, 8.83)	(-2.95, 5.14)	(-0.47, 8.62)	(-3.50, 4.95)	(-0.81, 7.51)
<i>p</i> -value	0.870	<b>0.04</b>	0.595	0.07	0.737	0.115
<b>SDMT – hits</b>						
Contrast	-0.73	-0.97	1.64	-0.24	2.37	-2.61
95% CI	(-4.53, 3.06)	(-4.72, 2.77)	(-1.81, 5.09)	(-4.13, 6.64)	(-1.23, 5.98)	(-6.17, 0.93)
<i>p</i> -value	0.705	0.610	0.352	0.903	0.197	0.149
9 years						
<b>CCTT-1 (sc)</b>						
Contrast	-3.33	-28.99	-34.68	-25.65	-31.65	5.99
95% CI	(-34.89, 28.19)	(-60.09, 2.11)	(-63.69, -6.28)	(-57.93, 6.62)	(-61.62, -1.68)	(-23.51, 35.51)
<i>p</i> -value	0.756	<b>0.06</b>	<b>0.017</b>	0.119	<b>0.038</b>	0.69
<b>STROOP Test – hits 1 (Word-reading)</b>						
Contrast	-1.36	-0.13	1.03	1.49	2.39	-0.90
95% CI	(-8.22, 5.49)	(-6.86, 7.22)	(-5.37, 7.44)	(-5.56, 8.55)	(-4.07, 8.87)	(-7.52, 5.71)
<i>p</i> -value	0.696	0.971	0.752	0.678	0.478	0.789
<b>STROOP Test – hits 2 (Color-naming)</b>						
Contrast	-0.95	2.56	1.68	3.52	2.63	0.88
95% CI	(-5.54, 3.66)	(-2.13, 7.26)	(-2.61, 5.97)	(-1.20, 8.24)	(-1.68, 6.95)	(-3.55, 5.32)
<i>p</i> -value	0.683	0.285	0.443	0.144	0.231	0.696
<b>SDMT – hits</b>						
Contrast	-1.89	-0.45	3.72	1.43	5.61	-4.17
95% CI	(-5.87, 2.08)	(-4.48, 3.56)	(0.04, 7.39)	(-2.65, 5.52)	(1.87, 9.36)	(-7.97, -0.38)
<i>p</i> -value	0.350	0.823	<b>0.047</b>	0.491	<b>0.003</b>	0.031

5-MTHF: 5-Methyltetrahydrofolate; **CCTT-1**: Children Color Trials Test; CI: confidence interval; FO: Fish-oil; sc: seconds; **SDMT**: Symbol Digit Modalities Test. Bold:  $p < 0.05$

**Supplemental Table 7.** Pairwise comparison of predictive margins of the mixed effects logistic regression model

7.5 years						
	FO vs 5-MTHF	FO vs FO+5-MTFH	FO vs Placebo	5-MTFH vs FO+5-MTFH	5-MTFH vs Placebo	FO+5-MTFH vs Placebo
<b>CCTT-1 (sc) p75</b>						
Contrast	0.06	0.21	0.23	0.14	0.16	-0.02
95% CI	(-0.12, 0.26)	(-0.002, 0.42)	(0.03, 0.42)	(-0.09, 0.37)	(-0.05, 0.38)	(-0.25, 0.25)
<i>p-value</i>	0.491	<b>0.05</b>	<b>0.018</b>	0.239	0.141	0.857
<b>STROOP Test – hits 1 (Word-reading) p50</b>						
Contrast	0.21	0.06	0.15	-0.14	-0.06	-0.08
95% CI	(-0.09, 0.52)	(-0.24, 0.37)	(-0.13, 0.44)	(-0.30, 0.33)	(-0.36, 0.24)	(-0.39, 0.22)
<i>p-value</i>	0.182	0.680	0.309	0.380	0.703	0.585
<b>STROOP Test – hits 2 (Color-naming) p75</b>						
Contrast	0.09	0.46	0.33	0.36	0.23	0.13
95% CI	(-0.38, 0.58)	(0.06, 0.85)	(-0.11, 0.78)	(-0.03, 0.43)	(-0.21, 0.67)	(-0.19, 0.67)
<i>p-value</i>	0.694	<b>0.023</b>	0.150	0.07	0.304	0.439
<b>SDMT - hits p50</b>						
Contrast	0.03	-0.02	0.16	-0.06	0.12	-0.19
95% CI	(-0.19, 0.26)	(-0.25, 0.20)	(-0.06, 0.39)	(-0.29, 0.17)	(-0.10, 0.36)	(-0.42, 0.03)
<i>p-value</i>	0.763	0.817	0.152	0.599	0.273	0.098
9 years						
<b>CCTT-1 (sc) p50</b>						
Contrast	-0.15	-0.25	0.03	-0.09	0.18	-0.28
95% CI	(-0.42, 0.10)	(-0.49, -0.007)	(-0.27, 0.29)	(-0.31, 0.12)	(-0.05, 0.43)	(-0.50, -0.06)
<i>p-value</i>	0.246	<b>0.043</b>	0.815	0.399	0.127	0.011
<b>STROOP Test – hits 1 (Word-reading) p75</b>						
Contrast	0.11	0.18	0.13	0.07	0.02	0.04
95% CI	(-0.20, 0.42)	(-0.12, 0.48)	(-0.16, 0.43)	(-0.26, 0.40)	(-0.29, 0.35)	(-0.26, 0.35)
<i>p-value</i>	0.495	0.246	0.367	0.676	0.875	0.780
<b>STROOP Test – hits 2 (Color-naming) p50</b>						
Contrast	-0.08	0.14	0.19	0.22	0.27	-0.04
95% CI	(-0.57, 0.40)	(-0.33, 0.61)	(-0.27, 0.65)	(-0.22, 0.67)	(-0.15, 0.71)	(-0.46, 0.36)
<i>p-value</i>	0.735	0.554	0.421	0.320	0.214	0.821
<b>SDMT - hits p75</b>						
Contrast	0.16	0.18	0.28	0.016	0.12	-0.105
95% CI	(-0.05, 0.39)	(-0.05, 0.42)	(0.07, 0.50)	(-0.24, 0.27)	(-0.12, 0.36)	(-0.36, 0.15)
<i>p-value</i>	0.147	0.132	<b>0.009</b>	0.903	0.327	0.418

5-MTHF: 5-Methyltetrahydrofolate; *CCTT-1*: Children Color Trials Test; CI: confidence interval; FO: Fish-oil; *SDMT*: Symbol Digit Modalities Test. Bold:  $p < 0.05$

## Supplemental References

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