Effect of gestational oily fish intake on the risk of allergy in children may be influenced by FADS1/2, ELOVL5 expression and DNA methylation

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**Abstract**

**Background:** Evidence suggests that prenatal exposure to n-3 long-chain polyunsaturated fatty acids (LCPUFA) reduces the incidence of allergic disease in children. LCPUFAs are produced from dietary precursors catalyzed by desaturases and elongases encoded by the FADS1/2 and ELOVL5 genes. DNA methylation regulates gene activity and fatty acid supplementation could alter DNA methylation (DNA-M) at these genes. We investigated whether DNA-M and expression of the FADS1/2 and ELOVL5 genes were associated with allergy in children and gestational fish intake. We studied 170 participants from the Isle of Wight 3rd Generation Cohort, UK. Phenotype data and exposure was assessed by questionnaires. Genome-wide DNA-M in cord blood samples was quantified using the Illumina Infinium HumanMethylation450 and EPIC Beadchips. Five SNPs (single-nucleotide polymorphisms) in the FADS gene cluster and one SNP in ELOVL5 were genotyped in offspring. FADS gene expression in offspring cord blood was determined.

**Results:** Gestational fish intake was significantly associated with increased methylation of cg12517394 ($P = 0.049$), which positively correlated with FADS1 mRNA levels ($P = 0.021$). ELOVL5 rs2397142 was significantly associated with eczema ($P = 0.011$) and methylation at cg11748354 and cg24524396 ($P < 0.001$ and $P = 0.036$, respectively). Gestational fish intake was strongly associated with elevated DNA-M at cg11748354 and cg24524396 ($P = 0.029$ and $P = 0.002$, respectively) and reduced ELOVL5 mRNA expression ($P = 0.028$).

**Conclusion:** The association between induced FADS1/2 and ELOVL5 DNA-M and reduced gene expression due to gestational fish intake provide a mechanistic explanation of the previously observed association between maternal LCPUFA intake and allergy development in early childhood.

**Keywords:** Allergy, FADS, ELOVL, Fish intake, DNA methylation, Pregnancy

**Background**

Maternal diet during pregnancy is a potentially important determinant of intrauterine development and linked to fetal physiologic adaptations and early immune system programming [1]. To maintain fetal development, nutrients are transported to the fetus across the placenta [2]. Exposure to omega-6 (n-6) and omega-3 (n-3) polyunsaturated fatty acids (PUFAs) during pregnancy has been found to be associated with allergic outcomes in infants or children [3]. Maternal PUFA supplementation during pregnancy has been reported to reduce childhood asthma at age 16 [4] and to reduce the absolute risk of persistent wheeze or asthma and airway inflammation in offspring at 36 months after birth [5]. Oily fish consumption, a major source of n-3 PUFAs, contains higher amount of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) which has been shown to be
protective against allergic diseases [6]. Fish intake has also been demonstrated to diminish the risk of asthma at 18 months [7]. In contrast, maternal shellfish consumption during the first trimester has been shown to increase the risk of wheezing, eczema and food allergy, while fatty fish consumption has been associated with increased risk of eczema in offspring [8, 9]. However, a recent large prospective study could not substantiate the previously observed beneficial association between fish and seafood consumption in pregnancy and development of asthma and allergic rhinitis symptoms in children up to 8 years of age [10]. In contrast, a meta-analysis of six studies revealed an association between fish oil supplementation during pregnancy and reduced risk of sensitization to food allergens at first year [11].

Long-chain PUFAs (LCPUFAs), produced from their dietary precursors (omega-3 and omega-6), are catalyzed by desaturases and elongases which are encoded by the FADS1/2 and ELOVL2/5 genes. A number of studies have found an association between FADS gene variants and immune-related outcomes. For example, maternal FADS genetic variation, through a higher infant supply of LCPUFA, has been found to be associated with a decrease in the production of IL-5, IL-10, and IL-17 in the infant [12–16]. Carriers of the minor alleles of FADS genetic variants and their respective haplotypes have been demonstrated to have lower levels of desaturase products and a lower prevalence of allergic rhinitis and atopic eczema [16]. An observational study showed that the FADS SNP rs3834458 was significantly associated with serum LCPUFA levels, with individuals homozygous for the del/del variant shown to have a decreased level of arachidonic acid (AA) and increased alpha-linolenic acid (ALA) to DHA ratio in high fish-eating mothers [17]. Previously, it has been reported that carriers of the minor alleles of FADS SNPs, including rs3834458, tend to have a lower blood composition of LCPUFA, particularly AA [18]. Although the evidence is not conclusive, it is conceivable that minor alleles of FADS genes produce a lower proportion of desaturase products and therefore less AA, reducing the risk of asthma.

Increased DNA methylation (DNA-M) at FADS promoter regions was associated with lower gene expression levels in minor homozygote carriers [19], and differences in DNA-M associated with the development of asthma during childhood has been reported [20, 21]. There are suggestions that DNA-M may regulate FADS activity [22] and that fatty acid supplementation can induce altered methylation of specific CpG loci in FADS2 and ELOVL5 [23]. A murine study reported differing levels of FADS2 promoter methylation in the liver tissue from offspring exposed to linoleic acid during gestation [24]. Recently, allele-specific methylation was reported between rs174537 and DNA methylation in FADS region in leukocyte and CD4+ cells [25].

Given these interrelations between maternal diet, genotype, offspring DNA-M, and asthma, we hypothesized that maternal fish intake may modulate offspring epigenetic programming, regulating fatty acid desaturase and elongase activities, and that this may modulate later health outcomes, in particular childhood wheeze and eczema.

Results

The descriptive characteristics of the cohort are presented in Table 1. There were no substantial differences in the prevalence of maternal smoking during pregnancy, maternal history of asthma, maternal history of eczema, maternal socioeconomic status, child eczema, child wheeze, and maternal oily fish intake between the total cohort and those who were randomly selected for the DNA-methylation analysis (Table 1). For each FADS and ELOVL5 association analysis, 170 methylation-phenotype pairs, 157 methylation-gene expression pairs and phenotype-gene expression pairs, 123 methylation-genotype, phenotype-genotype, and gene expression-genotype pairs were available (Fig. 1).

Three FADS1 and FADS2 SNPs (rs174537, rs174556, rs174575) and one ELOVL5 SNP (rs2397142) were genotyped and used for further analysis as these were not in linkage disequilibrium (r² ≤ 0.80). Associations of these SNPs with the prevalence of wheeze and eczema were assessed as shown in Table 2. Among these, the ELOVL5 variant rs2397142 was associated with eczema (P = 0.011). The eczema prevalence was higher in minor allele carriers than in homozygous major allele carriers.

A total of 39 CpG sites spanning the genomic region of the FADS cluster and 27 CpG sites in ELOVL5 were analyzed for association with wheeze and eczema. Methylation levels of cg00786201 and cg25448062 in the FADS cluster showed an association with eczema, and methylation at cg01400685 was found to be associated with wheeze (P < 0.05, respectively; Additional file 1). Also, methylation levels of cg18564099 in ELOVL5 showed an association with eczema (P = 0.046; Additional file 1).

Maternal oily fish intake was correlated with significantly increased DNA-M level at cg12517394 in FADS (P = 0.049, Table 3).

In addition, maternal oily fish intake was strongly associated with elevated DNA-M at cg11748354 and cg24524396 in ELOVL5 (P = 0.029 and P = 0.002, respectively, Table 4) and reduced ELOVL5 mRNA expression (P = 0.028, Table 5).

A genotype-dependent interaction was identified between ELOVL5 SNPs and CpG sites (cg11748354 × rs2397142 and cg24524396 × rs2397142 (P < 0.001 and P = 0.036, respectively; Additional file 2). When the correlation between methylation levels and gene expression levels in cord blood were tested, DNA-M at cg12517394 was significantly correlated with FADS1 gene expression...
levels ($P = 0.021$, Additional file 3). 

$FADS1$ gene expression levels were significantly different according to the $FADS$ SNPs ($P < 0.05$, Additional file 4), but not in $FADS2$ and $ELOVL5$.

**Table 1** Characteristics of subjects with available methylation data compared to the participants of the total

|                          | Total participants $n = 436$ (%) | Participants with DNA-M data $n = 170$ (%) | $P$ value |
|--------------------------|----------------------------------|---------------------------------------------|-----------|
| Maternal smoking during pregnancy |                     |                                              |           |
| Yes                      | 146 (36.6)                       | 61 (37.0)                                   | > 0.999   |
| No                       | 253 (63.4)                       | 104 (63.0)                                  |           |
| Maternal history of asthma |                     |                                              |           |
| Yes                      | 64 (18.4)                        | 27 (18.2)                                   | > 0.999   |
| No                       | 284 (81.6)                       | 121 (81.8)                                  |           |
| Missing                  | 88                               | 22                                          |           |
| Maternal history of eczema |                     |                                              |           |
| Yes                      | 143 (41.6)                       | 65 (40.4)                                   | 0.846     |
| No                       | 201 (58.4)                       | 96 (59.6)                                   |           |
| NA                       | 92                               | 9                                            |           |
| Child eczema             |                     |                                              |           |
| Yes                      | 58 (15.8)                        | 29 (18.7)                                   | 0.442     |
| No                       | 308 (84.2)                       | 126 (81.3)                                  |           |
| Missing                  | 70                               | 15                                          |           |
| Child wheeze             |                     |                                              |           |
| Yes                      | 67 (18.4)                        | 32 (20.6)                                   | 0.625     |
| No                       | 298 (81.6)                       | 123 (79.4)                                  |           |
| Missing                  | 71                               | 15                                          |           |
| Maternal fish intake     |                     |                                              |           |
| Never                    | 159 (61.9)                       | 70 (61.4)                                   | 0.801     |
| 1–2 or less per month    | 74 (28.8)                        | 32 (28.1)                                   |           |
| 1–3 per week             | 21 (8.2)                         | 11 (9.6)                                    |           |
| 4 or more times per week | 2 (0.8)                          | 0 (0)                                       |           |
| Uncertain                | 1 (0.4)                          | 1 (0.9)                                     |           |
| Missing                  | 179                              | 52                                          |           |
| Maternal socioeconomic status |                     |                                              |           |
| Below low                | 77 (19.3)                        | 25 (15.1)                                   | 0.273     |
| Low                      | 97 (24.3)                        | 54 (32.5)                                   |           |
| Medium Low               | 114 (28.5)                       | 48 (28.3)                                   |           |
| Medium                   | 61 (15.3)                        | 24 (14.5)                                   |           |
| High                     | 51 (12.8)                        | 16 (9.6)                                    |           |
| NA                       | 36                               | 3                                           |           |

Data are $n$ (%), unless otherwise indicated. Difference between groups was assessed using Yates’ continuity correction.

DNA-M: DNA methylation, NA: not applicable

**Discussion**

This study investigated the effects of oily fish intake during the third trimester of pregnancy on the allergic outcomes of the infants by using a subset of umbilical cord blood samples from Isle of Wight (IoW) $F_2$ generation. This is the first study to explore associations between $FADS$ and $ELOVL$ SNPs, gene expression, DNA methylation, child allergy, and gestational oily fish intake in the same cohort. Maternal fish intake could increase mean maternal circulating omega-3 levels and increased cord blood fatty acids that potentially prevent inflammation [26].

Our first major finding was a significantly increased cord blood methylation of cg12517394 in the $FADS$ cluster and DNA methylation at $ELOVL5$, cg11748354, and cg24524396 with gestational oily fish intake, whereas oily fish intake during pregnancy reduced $ELOVL5$ mRNA expression in the cord blood. In previous studies, supplementation with n-3 LCPUFA was shown to increase methylation levels at CpGs in $FADS2$ and $ELOVL5$, which were negatively associated with the level of the $FADS2$ and $ELOVL5$ transcripts in non-atopic adults consuming n-3 LCPUFA for 8–12 weeks [23]. In contrast, it was reported that maternal fish oil supplementation during the second half of pregnancy had small or no effects on DNA-M of infants [27, 28]. In vivo, feeding rats a fish oil-enriched diet for 9 weeks induced lower $FADS2$ mRNA expression and increased methylation of specific CpG loci in $FADS2$ [29]. Together, these findings suggest that methylation at these loci directly regulates $FADS$ and $ELOVL5$ transcription, and n-3 LCPUFA intake can induce changes in methylation levels in specific genes.
Our second major finding was a genotype-dependent methylation of cg11748354 and cg24524396 related to ELOVL5 rs2397142. A previous meta-analysis in liver methylation data, a highly relevant tissue for PUFA metabolism, identified a strong effect of FADS rs174537 on the methylation status of one or more critical CpG sites in the FADS gene cluster [19]. The most significant association was observed with cg27386326 ($p = 2.69 \times 10^{-29}$) and four other sites, including cg16213375, cg10515671, cg03805684, and cg19610905 [22]. We did not observe genotype-dependent methylation of FADS1. However, we identified higher gene expression levels for FADS1 in the cord blood with positive correlation with methylation at cg12517394 that are likely to be influenced by FADS genetic variants.

Finally, rs2397142 in ELOVL5 showed significant association with eczema in this study. The frequency of GG genotype of this polymorphism was higher in children with eczema. In a previous study, children from mothers with low fatty fish consumption during pregnancy had higher risk of eczema [8]. Furthermore, children with atopic eczema had lower ELOVL5 mRNA levels in their blood when compared to healthy controls [30]. These findings indicate that maternal lower fatty acid levels during pregnancy directly associated with decreased ELOVL5 mRNA expression in children and increase the risk of eczema, especially in children carrying GG genotype of rs2397142.

Our study had some limitations. Data on DNA-M and gene expression were only available from the cord blood and not from the liver samples, the major site of fatty acid metabolism. In addition, only proxy measures of maternal LCPUFA intake in the form of self-reported oily fish intake were available. We were unable to determine the ration of omega-6 to omega-3 in this cohort. This has previously been indicated as a possible risk factor for the development of atopic dermatitis and allergic rhinitis [31] and should be addressed in future studies. Nonetheless, the SNP-DNA-M gene expression associations observed largely support previous observations. Finally, this study was underpowered to investigate weaker links between maternal oily fish intake and clinical outcomes in children; however, there was a trend for a lower incidence of early life wheeze in the group whose mothers consumed oily fish compared with those who did not.

Conclusions
We report that infants of mothers supplemented with higher oily fish intake during the last trimester of

### Table 2 Prevalence of wheeze and eczema in offspring stratified by genotype

| Gene   | SNPs   | Genotypes | Wheeze ever | $P$ value | Eczema ever | $P$ value |
|--------|--------|------------|-------------|------------|-------------|------------|
| FADS1  | rs174537 | GG         | 11 (21.6)   | > 0.999    | 10 (19.6)   | 0.849      |
|        |        | GT         | 11 (22.0)   |            | 12 (23.5)   |            |
|        |        | TT         | 3 (25.0)    |            | 2 (16.7)    |            |
| FADS1  | rs174556 | CC         | 11 (20.0)   | 0.669      | 11 (20.0)   | 0.939      |
|        |        | CT         | 11 (24.4)   |            | 11 (23.9)   |            |
|        |        | TT         | 1 (12.5)    |            | 2 (25.0)    |            |
| FADS2  | rs174575 | CC         | 12 (19.4)   | 0.614      | 12 (19.4)   | 0.939      |
|        |        | CG         | 10 (23.8)   |            | 10 (23.3)   |            |
|        |        | GG         | 3 (33.3)    |            | 2 (22.2)    |            |
| ELOVL5 | rs2397142 | CC       | 9 (20.0)    | 0.569      | 4 (8.9)     | 0.011      |
|        |        | CG         | 14 (25.9)   |            | 18 (32.7)   |            |
|        |        | GG         | 2 (14.3)    |            | 2 (14.3)    |            |

Data are $n$ (%). Chi-square test was applied with Yates’ correction.

### Table 3 Association of maternal fish intake with cord blood FADS DNA methylation

| CpG        | Oily fish intake (28 weeks) | $P$ value |
|------------|-----------------------------|-----------|
|            | No ($n = 103$)              | Yes ($n = 11$) |
| cg00614641 | 0.088 ± 0.013               | 0.079 ± 0.010 | 0.022   |
| cg07999942 | 0.880 ± 0.013               | 0.870 ± 0.020 | 0.033   |
| cg12517394 | 0.037 ± 0.007               | 0.040 ± 0.005 | 0.049   |

Data are mean (SD). $P$ values calculated using the Mann-Whitney U test.

### Table 4 Association of maternal fish intake with cord blood ELOVL5 DNA methylation

| CpG        | Oily fish intake (28 weeks) | $P$ value |
|------------|-----------------------------|-----------|
|            | No ($n = 103$)              | Yes ($n = 11$) |
| cg10410213 | 0.608 ± 0.025               | 0.079 ± 0.020 | 0.010   |
| cg11748354 | 0.146 ± 0.045               | 0.191 ± 0.073 | 0.029   |
| cg24524396 | 0.093 ± 0.011               | 0.106 ± 0.012 | 0.002   |

Data are mean (SD). $P$ values calculated using the Mann-Whitney U test.
Early wheezing was defined if symptoms of smoking during pregnancy was ascertained at birth. White fish, shellfish, and usual consumption, before pregnancy, during 24th and 28th week of gestation [36]. White fish, shellfish, and oily fish consumption was evaluated with women reporting their frequency of consumption on a 5-point scale: “never,” “rarely (1–2 or less per month),” “occasionally (1–3 per week),” “4 or more times per week,” and “uncertain.” Mackerel, salmon, sardines, pilchards, herring, kipper, whitebait, trout, crab, and fresh tuna were classified as oily fishes. We considered oily fish intake only in the current study because of its high omega-3 contents.

Table 5 Gestational oily fish intake on offspring gene expression

| Oily fish intake (28 weeks) | P value |
|----------------------------|---------|
| No (n = 81)                 | Yes (n = 9) |
| FADS1 mRNA                 | 6.77 ± 0.42 | 6.68 ± 0.52 | 0.691 |
| FADS2 mRNA                 | 12.13 ± 0.80 | 12.66 ± 0.90 | 0.090 |
| Intergenic mRNA            | 8.57 ± 0.59 | 8.95 ± 0.62 | 0.104 |
| ELOVL5 mRNA                | 8.47 ± 0.43 | 7.93 ± 0.63 | 0.028 |

Data are mean (SD). P values calculated using the Mann-Whitney U test.

Methods

Study population

Children born on the Isle of Wight (IoW) between 1989 and 1990 (n = 1536) were recruited to prospectively study the natural history of asthma, allergy, and obesity. Informed consent was obtained from the parents (1st generation, IoW F0), and after exclusion, 1456 participants were recruited (2nd generation, IoW F1) as the IoW birth cohort [32]. The recruitment of newborns for the IoW 3rd Generation (IoW F2) study started in April 2010, and the samples used in this study are from infants born between April 2010 and May 2018. In total, 436 newborns have been recruited such that at least one of their parents is in the IoW F1 [33]. In this study, we have used cord blood samples from a subset of IoW F2 generation.

Clinical data collection

In the F2 generation, maternal history of asthma and smoking during pregnancy was ascertained at birth. Early wheezing was defined if symptoms of “wheeze” were reported by parents to occur between cold/infections at least once at either the 3-, 6-, or 12-month follow-up after birth. Childhood eczema information was also collected at 3, 6, and 12 months and defined as chronic or chronically relapsing, itchy dermatitis lasting more than six weeks with characteristic morphology and distribution [34, 35].

Assessment of fish and shellfish intake

Participant’s mothers were asked to fill out a validated food frequency questionnaire that inquired about their usual consumption, before pregnancy, during 24th and 28th week of gestation [36]. White fish, shellfish, and

SNP selection for FADS1, FADS2, and ELOVL5 genes

Five candidate SNPs—FADS1 (Entrez Gene 3992) rs174537, rs174545, and rs174556; intergenic between FADS1-FADS2 (Entrez Gene 9415) rs3834458; and FADS2 rs174575 and ELOVL5 rs2397142—were selected based on evidence of association with LCPUFA proportions in human plasma, tissues, and milk [37], or because the SNPs had been suggested to play an important role in regulation of FADS1/2 activity because of their location near potential regulatory regions [16]. Three of the five SNPs (rs174537, rs174545, and rs3834458) are from the same haplotype block (r² > 0.80). Therefore, in our analysis, we have only used four unrelated SNPs: rs174537, rs174545, rs174556, and rs2397142. For the IoW F2 genotyping was conducted using cord blood DNA samples (n = 123) with the genome-wide Illumina OmniExpressExome-8 Kit, and all six SNPs were imputed using the 1000 genome reference panel [38].

DNA methylation

DNA was extracted from the cord blood samples of IoW F2 over six batches, using a standard salting out procedure [39]. The DNA concentration was determined by PicoGreen quantitation. One microgram of DNA was bisulphite-treated for cytosine to thymine conversion using the EZ 96-DNA methylation kit (Zymo Research, Irvine, CA, USA), following the manufacturer’s standard protocol. Genome-wide DNA-M was assessed in 130 samples using the Illumina Infinium HumanMethylation450 BeadChip (Illumina, Inc., San Diego, CA, USA) and using the Illumina Infinium MethylationEPIC BeadChip array for 63 samples. Arrays were processed using a standard protocol as previously described [40], with multiple identical control samples assigned to each bisulphite conversion batch to assess assay variability, and samples were randomly distributed on microarrays to control against batch effects. The degree of DNA methylation at each CpG site was recorded as a beta value, ranging from 0 (no methylation) to 1 (completely methylated). One sample was excluded due to maternal blood contamination. The CPACOR pipeline [41] was used to pre-process the methylation 450k and EPIC data for quality control, and batch correction was done using ComBat [42]. In the final analysis, 170 samples were used with
complete methylation information. The methylation levels of 39 CpG sites spanning the genomic region of the FADS cluster and 27 CpGs of the ELOVL5 were selected.

**Gene expression**

FADS and ELOVL5 gene expression in the cord blood (n = 157) was determined by total RNA extracted from the cord blood using SurePrint G3 Human Gene Expression Microarrays (GeneSpring Technology 39,494) [43]. Microarray data was processed using limma [43] in the R statistical computing environment, and background correction was performed using normal-exponential convolution (normexp) function [44]. Data was converted to log2-transformed data for further analysis. Filtering was performed to remove low expressed probes that are close to the background level. Negative control probes were also removed from the data.

**Statistical analysis**

To assess whether our analytic sample (170 DNA samples) was representative of the total cohort available (n = 436), we compared the characteristics of the two subsets using chi-squared tests. Distribution and prevalence of wheeze and eczema according to genotype were compared using the χ² test with Yates' continuity correction. The genotype frequency of candidate SNPs was examined for a significant departure from the Hardy-Weinberg equilibrium using a χ² test. The methylation levels of the FADS cluster and ELOVL5 were tested for association with wheeze and eczema up to 1 year using Mann-Whitney U tests to compare independent samples. SNP genotype-dependent methylation and genotype-dependent gene expression were analyzed using the Kruskal-Wallis non-parametric test to determine the difference between three genotype groups on continuous variables. A Spearman correlation test was used to analyze the relationship between DNA methylation levels and gene expression. Comparison of DNA-M and gene expression levels between oily fish intake status at 28 gestational weeks were tested by the non-parametric two-sample Mann-Whitney U test. Bonferroni correction was not undertaken as these were a priori hypothesis instead of a random set of CpGs and genotypes. Under this situation, multiple testing adjustments may not be necessary as suggested by Rothman [45].

All statistical analyses were performed using IBM SPSS Statistics, Version 21.0 (IBM Corp., Armonk, NY, USA), except for preprocessing of DNA methylation data, which was done using R statistical package [41]. The P values of < 0.05 were considered to indicate significance.

### Additional files

- **Additional file 1:** Association of wheeze and eczema with cord blood DNA methylation. (XLSX 9 kb)
- **Additional file 2:** Genotype-dependent methylation. (XLSX 9 kb)
- **Additional file 3:** Change in relative mRNA expression compared to change in the methylation status of FADS1 methylation cg12517394. (XLSX 8 kb)
- **Additional file 4:** Gene expression according to the FADS genotypes. (XLSX 9 kb)

### Abbreviations

| Abbreviation | Description |
|--------------|-------------|
| AA           | Arachidonic acid |
| ALA          | α-Linolenic acid |
| DHA          | Docosahexaenoic acid |
| DNA-M        | DNA methylation |
| ELOVL5       | Fatty acid elongase |
| EPA          | Eicosapentaenoic acid |
| FADS         | Fatty acid desaturase |
| IL           | Interleukin |
| IoW          | Isle of Wight |
| LCPUFA       | Long-chain polyunsaturated fatty acids |
| mRNA         | Messenger RNA |
| SNP          | Single-nucleotide polymorphism |

### Consent for publication

The authors declare that they have no competing interests.

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