**Characterization of the effects of age and childhood maltreatment on *ELOVL2* DNA methylation**

**Supplementary Information**

1. **Sample selection for DNA methylation analyses:**

A total of *N=*4376 women who just gave birth at the Ulm University Hospital were approached within the first week after parturition and informed about the study. *N=*533 women agreed to participate. As described in the methods of the main manuscript, the final epigenetic cohort included 117 mothers and 113 newborns. Mothers included in the epigenetic analyses (*N=*117) did not differ from mothers who were not included (*N=*416) in terms of maternal age, sex, and weight of their newborns, ethnicity, chronic illnesses, lifetime psychological diagnosis or medication during pregnancy (all *p-*values>.05). Table S1 describes the demographic and biological characteristics of our cohort when the moderate CTQ cutoff is used.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Table 1.** Demographic and biological characteristics using the moderate cut off for the CTQ | | | | | |
|  | | Whole study cohort | CM+ | CM- |  |
|  | | (*N*=117) | (*N=*33)j | (*N=*84) | Statisticsa |
| *N* Caucasian maternal ethnicity b (%) | | 115 (98.3) | 33 (100) | 82 (97.6) | χ*2(1)=*0.01, *p*=.92 |
| *N* higher education (%) | | 68 (58.1) | 14(42.4) | 54 (64.3) | χ*2(1)=*5.41, *p*=.07 |
| *N* living in partnership (%) | | 115 (98.3) | 33 (100) | 82 (96.6) | χ*2(1)=*0.01*, p=* .92 |
| Categorized monthly household income c (Mean(SD)) | | 7.3 (1.8) | 6.8(1.9) | 7.5 (1.7) | *W=* 1614*, p=* .06 |
| *N* female sex of infant d (%) | | 52 (45.2) | 17 (51.5) | 35 (41.7) | χ*2(1)=*0.40, *p*=.53 |
|  | |  |  |  |  |
| Mean lymphocyte cell count (*SD*) in % | | 18.7 (4.8) | 19.5(4.3) | 18.4 (5.1) | *t(105)*=-1.02, *p=.*31 |
| Mean monocyte cell count (*SD*) in % | | 5.9 (1.5) | 5.8 (1.4) | 6.0 (4.7) | *t(105)=-0.63, p*=.53 |
|  | |  |  |  |  |
| Mean birthweight d  (*SD*) in *g* | | 3387 (497) | 3254 (533) | 3472 (454) | *t(111)=*2.19, *p*=.03 |
| Mean gestational aged  (*SD*) in weeks | | 39.5 (1.4) | 39.9 (1.9) | 39.7 (1.1) | *W=*1524, *p*=.13 |
| N caesarean section d,e (%) | | 33 (28.7) | 7 (21.9) | 26 (32.5) | χ*2(1)=*1.25, *p*=.53 |
| N smokers during pregnancy d (%) | | 10 (8.7) | 5 (5.9) | 5 (15.6) | χ*2(1)=*1.5, *p=*.22 |
|  |  |  |  |
| Self-reported psychiatric diagnosis (lifetime) | |  |  |  |  |
| Depressive disorder *(N (%)* | | 12 (10.2) | 4 (12.1) | 8 (9.5) | χ*2(1)<*0.001*, p= 1* |
| Anxiety disorder *f (N (%))* | | 7 (6.0) | 4 (12.1) | 3 (3.6) | χ*2(1)=*1.72*, p= .*19 |
| Eating disorder *(N (%))* | | 2 (1.7) | 2 (3.4) | 0 | χ*2(1)=*0.53, *p*= .47 |
| Adjustment disorder *(N (%))* | | 2 (1.7) | 0 | 2 (2.4) | χ*2(1)=*0.01*, p= .*92 |
| Other psychiatric diagnosis *(N (%))* | | 6 (5.1) | 3 (5.2) | 3 (5.1) | χ*2(1)<*0.001*, p= 1* |
|  |  |  |  |
| Chronic illnesses g | |  |  |  |  |
| Thyroid dysfunction *(N (%))* | | 19 (16.2) | 3 (9.1) | 16 (19.0) | χ*2(1)=*1.1*, p= .*30 |
| Allergy *(N (%))* | | 17 (14.5) | 3 (9.1) | 14 (17.9) | χ*2(1)=*0.57*, p=.* 45 |
| Neurodermatitis *(N (%))* | | 3 (2.6) | 1 (3.0) | 2 (2.4) | χ*2(1)<*0.001*, p=*.99 |
| Diabetes *(N (%))* | | 3 (2.6) | 1 (3.0) | 2 (2.4) | χ*2(1)<*0.001*, p=*.99 |
|  |  |  |  |
| Medication during pregnancy | |  |  |  |  |
| Corticosteroids h *(N (%))* | | 5 (4.3) | 0 (0) | 5 (5.9) | χ*2(1)=*0.85*, p= .*36 |
| L-thyroxin *(N (%))* | | 24 (20.5) | 5 (15.1) | 19 (22.6) | χ*2(1)=*0.42*, p=.* 52 |
| Antibiotics *(N (%))* | | 11 (9.4) | 3 ( 9.1) | 8 (9.5) | χ*2(1)<*0.001*, p=.* 98 |
| Progesterone *(N (%))* | | 4 (3.4) | 2 (6.1) | 2 (2.4) | χ*2(1)=*0.18, *p=.*67 |
|  | |  |  |  |  |
| Mean CTQ sum score *(SD)j* | | 33.6 (10.8) | 40.2 (12.1) | 27.1 (1.9) | t*(115)=*-8.16, *p<*.0001 |
| Emotional abuse i (*N* (%)) | | - | 10 (30.3) |  |  |
| Physical abuse i (*N* (%)) | | - | 6 (18.2) |  |  |
| Sexual abuse i (*N* (%)) | | - | 13 (39.4) |  |  |
| Emotional neglect i (*N* (%)) | | - | 15 (45.5) |  |  |
| Physical neglect i (*N* (%)) | | - | 6 (18.2) |  |  |

|  |  |  |  |
| --- | --- | --- | --- |
| Note. Group differences calculated with chi-square tests for binomial and t-tests for continuous variables. |  |  | |
| SD= standard deviation; CM= Childhood maltreatment; CTQ= C*hildhood Trauma Questionnaire*; CTQ sum score= childhood maltreatment load. | | | |
| a Main effect of the CTQ moderate classification (*t*-tests or *chi-square* tests). | | | |
| b One study participant of Brazilian origin and one of North-American origin.  c One CM- and one CM+ mother did not provide income data. Monthly household income in €was ranged between 1 and 9 as follows: 1<400; 2:400-1000; 3:1000-1500; 4:1500-2000; 5: 2000-2500; 6: 2500-3000; 7:3000-3500; 8: 3500-4000; and 9>4000. | | | |
| d For gestational and neonatal characteristics, only mother-infant dyads were included: *NCM*-=81; *NCM*+=32 | | | |
| e Included planned (*NCM*-=22, *NCM*+=6) and emergency (*NCM*-=4, *NCM*+=1) forms of caesarean section | | | |
| f One woman from each CM group had a diagnosis of depression and anxiety disorder | | | |
| g One CM- women had asthma, neurodermatitis, and allergy; one CM- had diabetes and thyroid dysfunction; one CM- woman had an allergy and thyroid dysfunction. | | | |
| h Only taken medication with more than one occurrence are included | | |
| i Amount of women with at least moderate experiences in this CTQ subscale. | | |

1. **CTQ dichotomization**

The Childhood Trauma Questionnaire (CTQ) consists of 28 items subdivided into five classes, namely physical, emotional and sexual abuse, and physical and emotional neglect. There is a five-point Likert scale for each item (1 = “never” to 5 = “very often”). The sum of the five items for each subscale ranges from 5 to 25. To allow the dichotomization into two groups, a cutoff was established at mild (low) trauma experience for each subscale, following the widely used Bernstein and Fink (1998) cutoff criteria:

**Table S2**: Bernstein & Fink cutoff criteria for the CTQ.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | None | Low | Moderate | Severe |
| Emotional Abuse | 5-8 | 9-12 | 13-15 | 16+ |
| Physical Abuse | 5-7 | 8-9 | 10-12 | 13+ |
| Sexual Abuse | 5 | 6-7 | 8-12 | 13+ |
| Emotional Neglect | 5-9 | 10-14 | 15-17 | 18+ |
| Physical Neglect | 5-7 | 8-9 | 10-12 | 13+ |

Consequently, the women who reached at least mild trauma in one of those five subclasses were defined as CM+ according to the mild CTQ cutoff, whereas CM- did not experience any type of trauma. This explains why a mother scoring, e.g. 9 for emotional neglect and 5 for the rest of the subscales, would have a CTQ sum score of 29 but would be classified as CM*-* according to the mild cutoff.In contrast, a woman reporting 5 in all subscale except 6 for sexual abuse would score a CTQ of 26 and would be classified as CM+.

1. **Sub-study: using pyrosequencing to test *ELOVL2* 5’ end**

The final cohort for the sub-study was N=116 mothers and 112 infants after the exclusion of one mother from which not enough biological material was available. Using duplicates, the DNAm of the *ELOVL2* region that has previously been described as a biomarker for age (Garagnani et al., 2012; Bacalini et al., 2017) was analyzed using pyrosequencing technique (PyroMark ID; Qiagen). The DNAm value (in %) of the duplicates for each subject was averaged. One maternal sample with missing values in more than 50% of the CpG units was excluded.

1. **Mass array specifications**

The mass array method for DNA methylation quantification consists on a bisulfite treatment of genomic DNA prior to a PCR-based amplification is performed, and followed by a reverse transcription and enzymatic cleavage with RNase. The resulting fragments are then analyzed using the MALDI-TOF technology (Suchiman et al., 2015). The special nature of this technique does not allow the discrimination between enzyme-restricted fragments of DNA with the same molecular weight and thus these cannot be measured independently. Thus, depending on the cleavage pattern, the DNAm values are given for individual CpG sites or multiple CpG sites that are analyzed together as CpG units. Exon 1 of the *ELOVL2* gene could not be measured by mass array because the enzymatic cleavage with RNase would have generated too big fragments, which could not be read by MALDI-TOF. RNase cuts the RNA strand after each adenine (A) within the sequence. Because of the high density of aligned CpG units within the exon 1, the fragments generated would exceed or not reach the length threshold for mass identification. Alternatively, we used pyrosequencing, which does not include the RNase cleavage step.Pyrosequencing quantifies non-converted (methylated) Cytosines using a luciferin/luciferase-based method (Delaney et al. 2016).

1. **Data cleaning of DNAm analyses using mass array**

Two criteria were applied for data processing in order to assure a high quality of raw data: CpG units with missing values in more than 30% of the samples were removed (CpG 11 and CpG 41.42.43.44). Thereafter, 39 CpG sites remained for *ELOVL2* intron 1. For a graphical representation of the assessed *ELOVL2* CpG sites see Figure 1. Furthermore, samples with missing values in more than 50% of the CpG units were also excluded. This exclusion step resulted in a final *ELOVL2* intron 1DNAm dataset of 110 mothers and 107 infants.

1. ***ELOVL2* gene expression assessment**

Total RNA was purified from PBMC cell pellets using the Qiagen RNeasy Kit (Qiagen), quantified with a Qubit spectrophotometer and eluted in RNase-free water according to the manufacturer’s instructions. cDNA was synthesized using a high-capacity cDNA reverse transcription Kit (Thermo Fischer Scientific,) following the manufacturer’s instructions. The assessment of gene expression by Real-time qPCR analyses was performed on a QuantStudio 6 (Life technologies) with TaqMan gene expression arrays (*Hs00366363\_m1,* Thermo Fischer Scientific).

A first run with 5 maternal and 5 newborns samples showed no signal on *ELOVL2* specific amplification. A positive control experiment showed that the gene expression of the housekeeping genes succinate dehydrogenase complex, subunit A (*SDH*) and Importin 8 (*IPO8*) was detectable. Following runs with greater sample number confirmed that *ELOVL2* gene expression in PBMC was under the limit of detection.

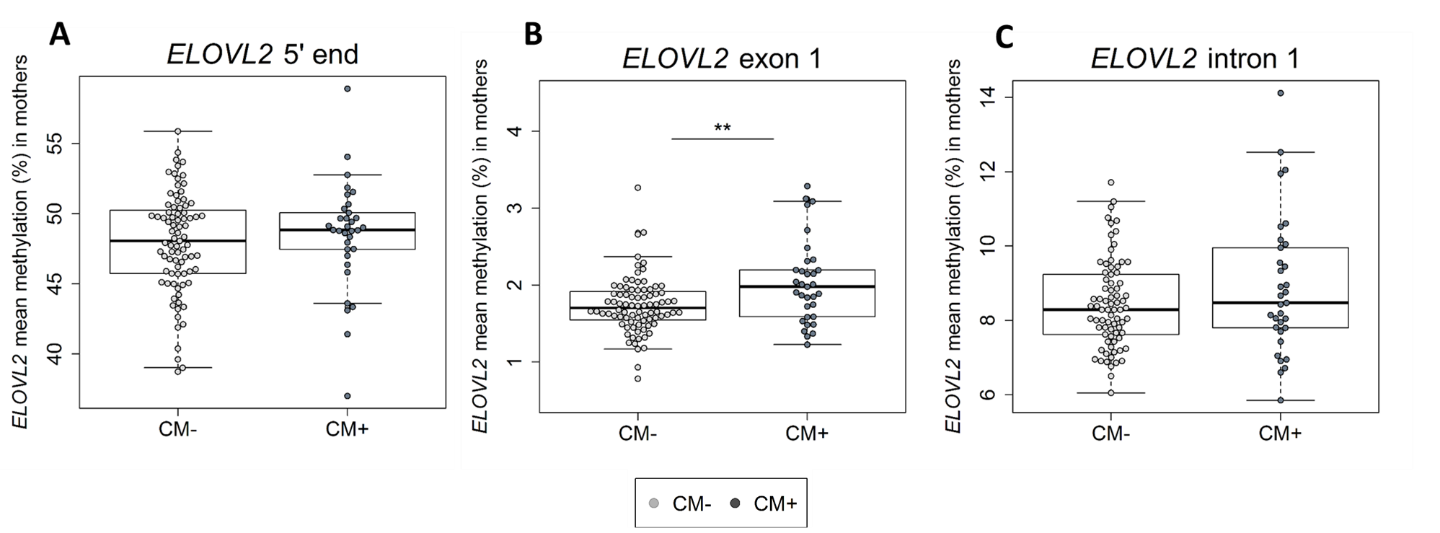
1. **Results from CM-associated group comparison analyses**

There were no CM-groups differences in the *ELOVL2* 5’UTR methylation when the mild cutoff criteria was used to classify mothers in CM+ and CM- or when the moderate CTQ cutoff was used (Figure S1 A, Table S3). Single unit analyses showed no differences between DNAm of any of the 9 CpG between CM+ and CM- mothers, regardless the cutoff used for CM classification (all *p-*values > .05).

The *ELOVL2* exon 1 mean methylation (mean ± SD = 1.8 ± 0.4 %) was significantly associated with CM status: when using a mild CTQ cutoff, CM+ mothers (2.0 ± 0.5 %) showed significantly increased *ELOVL2* exon 1 mean DNAm levels compared to CM-mothers (1.7 ± 0.3 %; Table S3). If CM experiences had been categorized using the moderate CTQ cutoff criterion, group differences on *ELOVL2* exon 1 methylation would have remained significant (Figure S1 B, Table S3). The analyses of DNAm at specific CpG units within *ELOVL2* exon 1 showed that CpG 2, CpG 3, CpG 4, CpG 6 and CpG 8 were significantly higher methylated in CM+ mothers. These results remained significant after correction for multiple comparisons (all *p*FDR <.05). When the moderate CTQ cutoff was used, the CM-associated group differences in the targeted CpG units remained significant with the exception of CpG6, which was reduced to a trend (Figure S2). After adjusting for the three covariates in the same model (age and relative cell counts of monocytes and lymphocytes) group differences of single CpGs remained significant when the mild CTQ cutoff was used (Table S4). When the moderate CTQ cutoff was used, however, the statistical differences were reduced to a trend after correction for multiple comparisons (Table S4).

The mean DNAm across *ELOVL2* intron 1 did not differ between CM+(8.6 ± 1.6 %) and CM- women when the mild CTQ cutoff was used to classify the CM status (8.5± 1.2 %, Table S2). These results would have remained unchanged if the moderate cutoff criterion of the CTQ had been used to classify women in CM+ compared to CM- (Figure S1 C, Table S3)*.* No significant differences in methylation levels were found at any individual CpG unit (original *p* values>.05).

Regarding group-analyses in the offspring, DNAm did not differ between children from CM+ and children from CM- mothers in any of the targeted regions of the *ELOVL2* gene. The group comparison results using both, mild and moderate cutoff of the CTQ, are described in table S3.

**Figure S1.** Association between moderate CM status and the mean DNA methylation of *ELOVL2* 5’ end (*N=*116),exon 1 (*N=*117), and intron 1 (*N=*110) in mothers.

**Table S3:** Group comparison analyses with the mild and moderate CTQ cutoff.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Mothers** | | | | **Newborns** | | | |
|  | **Milda** | | **Moderateb** | | **Mildc** | | **Moderated** | |
|  | *Statistic* | *p* | *Statistic* | *p* | *Statistic (βCM)* | *p* | *Statistic (βCM)* | *p* |
| ***ELOVL2* 5'UTR** | *F*=1.007 | 0.32 | *F=*1.76 | 0.19 | *βCM=-.*03 | 0.76 | *βCM=-.*05 | 0.59 |
| ***ELOVL2* exon 1** | *βCM=.*31 | **0.001** | *βCM=.*25 | **0.01** | *βCM=.*09 | 0.93 | *βCM=.*09 | 0.40 |
| ***ELOVL2* intron 1** | *βCM=.*02 | 0.82 | *βCM=.*03 | 0.76 | *βCM=*-.02 | 0.83 | *βCM*=-.12 | 0.25 |

*βCM*=standardized coefficient for childhood maltreatment is reported for not-normally distributed data. For the analyses in mothers, the covariates age and relative cell counts of monocytes and lymphocytes were accounted for. For newborns, sex of the infant and gestational age in weeks were included as covariates.

a N=58 CM+ vs N=59CM-

b N=33 CM+ vs N=84CM-

c N=55 newborns from CM+ mothers vs N=58 newborns from CM- mothers

d N=32 newborns from CM+ mothers vs N=81 newborns from CM- mothers

**Figure S2.** Moderate CM status and DNA methylation of CpG sites within the *ELOVL2* exon 1

Corresponding *p-*values are listed at Table S3. \*p<.05; \*\* *p* <.01; \*\*\* *p* <.001.

CM+: women with reported moderate childhood maltreatment, CM-: mothers with mild or none history of childhood maltreatment.

**Table S4.** Effects of the CM and maltreatment load on the targeted CpG sites within the *ELOVL2* exon 1 in mothers.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Mild CTQ cutoff** | | | **Moderate CTQ cutoff** | | |  |  |  |
|  | **CM+ (n=58) vs CM- (n=59)** | | | **CM+ (n= 33) vs CM- (n=84)** | | | **CTQ sum score** | | |
|  | *βCM* | *p* | *p*corrected | *βCM* | *p* | *p*corrected | *βCTQ* | *p* | *p*corrected |
| **CpG 1** | 0.01 | 0.59 | 0.76 | 0.01 | 0.29 | 0.32 | 0.08 | 0.34 | 0.49 |
| **CpG 2** | 0.24 | **0.01** | **0.04** | 0.19 | **0.04** | 0.10 | 0.04 | 0.69 | 0.76 |
| **CpG 3** | 0.27 | **0.004** | **0.03** | 0.22 | **0.03** | 0.09 | 0.21 | **0.03** | 0.10 |
| **CpG 4** | 0.31 | **<.001** | **0.02** | 0.25 | **0.009** | 0.09 | 0.13 | 0.17 | 0.34 |
| **CpG 5** | 0.13 | 0.19 | 0.35 | 0.11 | 0.24 | 0.30 | 0.04 | 0.69 | 0.76 |
| **CpG 6** | 0.27 | **0.005** | **0.03** | 0.18 | 0.06 | 0.12 | 0.16 | 0.11 | 0.28 |
| **CpG 7** | 0.15 | 0.13 | 0.29 | 0.13 | 0.19 | 0.28 | 0.11 | 0.26 | 0.43 |
| **CpG 8** | 0.28 | **0.003** | **0.03** | 0.22 | **0.02** | 0.09 | 0.19 | **0.05** | 0.14 |
| **CpG 9** | 0.11 | 0.30 | 0.46 | -0.11 | 0.07 | 0.12 | 0.03 | 0.77 | 0.76 |
| **CpG 10** | 0.04 | 0.69 | 0.76 | -0.01 | 0.90 | 0.90 | 0.04 | 0.72 | 0.77 |

*βCM*=standardized coefficient for childhood maltreatment; *p=original p*-values before FDR correction;

*p*corrected=*p-*values after FDR correction. The reported analyses in this table include the covariates age and relative cell counts of monocytes and lymphocytes.

**References:**

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