**Supplementary material for:**

**Association between DNA methylation of the *KITLG* gene and cortisol levels under stress: a replication study.**

Joanna Wriggleswortha, Marie-Laure Ancelinb , Karen Ritchieb,c, Joanne Ryan a,b\*

**Supplementary Table 1.** Unadjusted linear regression models for the association between cortisol levels measured under basal conditions (non-stress) and *KITLG* DNA methylation.

|  |  |  |  |
| --- | --- | --- | --- |
| ***KITLG* DNA methylation** | **Morning cortisol**  | **Evening cortisol** | **Diurnal cortisol (AUC)** |
|  | **ß (SE)** | **p** | **t** | **ß (SE)** | **p** | **t** | **ß (SE)** | **p** | **t** |
| CpG 1 (n=88) | -4.60 (2.80) | 0.11 | -1.64 | 1.30 (4.12) | 0.75 |  0.31 | -23.11 (36.27) | 0.53 | -0.64 |
| CpG 2.3 (n=107) | -0.60 (0.71) | 0.40 | -1.61 | -0.13 (1.10) | 0.12 |  0.04 | -5.08 (9.66) | 0.60 | -0.82 |
| CpG 4 (n=90) | 2.63 (3.75) | 0.48 |  0.70 | -0.92 (5.44) | 0.87 | -0.17 | 11.95 (48.37) | 0.80 |  0.25 |
| CpG 5 (n=135) | 8.10 (4.31) | 0.06 |  1.73 | 5.73 (6.83) | 0.40 | 0.86 | 96.70 (58.28) | 0.09 |  1.62 |
| CpG 6.7.8 (n=93) | 5.00 (3.04) | 0.10 |  1.64 | -2.30 (4.42) | 0.60 | -0.52 | 18.92 (39.48) | 0.63 |  0.48 |
| CpG 9 (n=92) | 2.90 (2.75) | 0.29 |  1.06 | -0.12 (3.97) | 0.98 | -0.03 | 19.44 (35.27) | 0.58 |  0.55 |
| CpG 22 (n=91) | 7.87 (8.48) | 0.36 |  0.93 | 13.76 (12.4) | 0.27 |  1.11 | 151.43 (109.53) | 0.17 |  1.38 |

ß: the beta coefficient from the linear regression model; SE: Standard Error.

The exact number of participants with data for each CpG site varies, and is indicated.

**Supplementary Table 2.** Univariate linear regression models for the association between cortisol levels measured under stress conditions and *KITLG* DNA methylation.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| ***KITLG* DNA methylation** | **Morning cortisol**  |  | **Evening cortisol** |  | **Total cortisol (AUC)** |  |
|  | **ß (SE)** | **p** | **t** | **ß (SE)** | **p** | **t** | **ß (SE)** | **p** | **t** |
| CpG 1 (n=88) | -2.43 (2.72) | 0.37 | -0.89 | -2.42 (3.86) | 0.53 | -0.63 | -33.97 (34.95) | 0.33 | -0.97 |
| CpG 2.3 (n=107) | -1.85 (0.67) | 0.007 | -2.77 | -0.93 (1.03) | 0.37 | -0.90 | -19.43 (8.87) | 0.03 | -2.19 |
| CpG 4 (n=90) | -0.27 (3.63) | 0.94 | -0.07 | 0.69 (5.06) | 0.89 |  0.14 | 2.93 (46.31) | 0.95 |  0.06 |
| CpG 5 (n=135) | -0.31 (4.23) | 0.94 | -0.07 | 3.60 (6.06) | 0.55 |  0.59 | 23.06 (54.39) | 0.67 |  0.42 |
| CpG 6.7.8 (n=93) | -0.30 (3.17) | 0.92 | -0.10 | -3.87 (4.39) | 0.38 | -0.88 | -29.20 (40.21) | 0.47 | -0.73 |
| CpG 9 (n=92) | -2.78 (2.63) | 0.29 | -1.06 | -2.09 (3.64) | 0.57 | -0.57 | -34.04 (33.49) | 0.31 | -1.02 |
| CpG 22 (n=91) | -4.95 (7.74) | 0.52 | -0.64 | 5.00 (10.8) | 0.65 |  0.46 | 0.32 (99.58) | 0.99 |  0.00 |



**Supplementary Figure 1. The KITLG gene, indicating the previously identified probe and the assay used in this study.**

UCSC Genome Browser’s view of the KITLG gene (Ch37hg19). Blue and black boxes represent possible transcripts for the *KITLG* gene. Arrows point to the direction of transcription. Green box represents the CpG island. The orange square highlights the probe/CpG site identified in *Houtenpen et al (2016*), and corresponding to CpG 1 in our study. As indicated by the arrows, all other CpG units are located upstream from this corresponding probe/site. The approximate position of the assay used in our study is indicated by the red box.