The fetal origins of adult disease, the evidence and mechanisms
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Associations between DNA methylation of a glucocorticoid receptor promoter and acute stress responses in a large healthy adult population are largely explained by lifestyle and educational differences

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ABSTRACT

Background: Glucocorticoids are the key regulators of the biological stress response and act by binding to glucocorticoid receptors (GR). Expression of GR is altered by DNA methylation. Methylation patterns in GR promoters have been shown to be highly variable between individuals, but little is known about the functional consequences of this variation for the acute stress response. The present study investigated associations between methylation status of the GR 1-C promoter and cortisol, cardiovascular and perceived stress responses to a psychosocial stress protocol in a large healthy adult population.

Methods: A total of 725 overall healthy men and women, aged 55-60 years, participated in a standardized psychosocial stress protocol consisting of three different stressors. At different stages during the stress protocol, salivary cortisol levels, continuous blood pressure and heart rate (HR) levels as well as perceived stress were measured. Stress reactivity was calculated as the increase between basal and peak measurements. Methylation status of the GR 1-C promoter was assessed in DNA isolated from peripheral blood samples using a methylation sensitive PCR assay for 675 of the 725 participants.

Results: A decrease in methylation of the GR 1-C promoter was associated with a decrease in stress reactivity as indicated by lower cortisol and lower HR reactivity. A 1% decrease in GR 1-C methylation corresponded with a cortisol decrease by 0.14% (95% CI: 0.03 to 0.25, p = 0.02) and an HR decrease by 0.10 bpm (0.03 to 0.16, p = 0.003). Adjusting for sex, lifestyle and education largely abolished these associations. A decrease in methylation of the GR 1-C promoter was also associated with an increase in stress perception as indicated by higher perceived stress (0.03 points [0.00 to 0.06, p = 0.05]), lower perceived performance (-0.03 points [-0.05 to -0.01], p = 0.02), and lower perceived control (-0.03 points [-0.05 to 0.00], p = 0.04). After adjusting for sex and educational level the associations were no longer statistically significant. GR 1-C methylation status was not associated with blood pressure responses to the stress protocol.

Discussion: Although effects were small, variation in methylation status in the GR 1-C promoter was associated with physical and perceived acute stress responses. Interestingly, these associations could largely be explained by differences in lifestyle and education.
INTRODUCTION

The biological stress response is largely regulated by steroid hormones called glucocorticoids, of which cortisol is the most important in humans. Glucocorticoids act by binding to glucocorticoid receptors (GR) and mineralocorticoid receptors (MR), which are expressed widely across the body. The GR, encoded by the NR3C1 gene located on chromosome 5q31, is expressed in almost every cell in the body. Besides regulating the stress response it regulates developmental processes, immune responses and metabolism. Responsiveness to glucocorticoids is to a large extent determined by the expression level of the GR, which is controlled by a variety of mechanisms, including DNA methylation. In DNA methylation, a methyl group is added to the 5′ position of cytosine in CpG dinucleotides. Methylation of CpG rich clusters, termed CpG islands, which often span the promoter regions of genes, is associated with transcriptional repression, whereas hypomethylation of CpGs is associated with transcriptional activity.

Methylation patterns in GR promoters have been shown to be highly variable between individuals but little is known about the functional consequences of this variation for the biological response to psychosocial stress. Oberlander et al. showed that in a group of 82 neonates, increased methylation of GR promoter region 1-F was associated with increased cortisol responses to a stress protocol, consisting of a visual information processing task, performed at three months of age. These study results suggest that individual variation in methylation patterns of the GR leads to differences in responses to stressful situations. In the present study, we hypothesized that individual variation in methylation status of the GR 1-C promoter (1-C being a promoter broadly expressed in many tissues including the brain) at an adult age is associated with differences in physical and perceived stress responses to a psychosocial stress protocol. To test this hypothesis, we used data from the Dutch Famine Birth Cohort Study in which both stress responses as well as GR 1-C promoter methylation status were assessed. Results from this study so far showed that prenatal exposure to undernutrition was associated with increased blood pressure responses to stress, but no associations with cortisol responses or GR 1-C promoter methylation status were found (unpublished data).

METHODS

Participants and selection

Participants were selected from the Dutch Famine Birth Cohort. For this cohort, we included all singleton babies born alive in the Wilhelmina Gasthuis (a teaching hospital in Amsterdam, the Netherlands) between 1 November 1943 and 28 February 1947. We excluded those whose birth records were not available (1%) or those who were born prematurely (8.9%, gestational age below 259 days). In all, 2414 men and women were included, of whom the population registry of Amsterdam traced 2155 (89%). Of these, 160 babies had not been registered in Amsterdam at
birth, 328 people had died, 213 people had emigrated, 157 people refused permission to record their address, 125 people were not traceable to a current address, and eight people requested their address to be removed from the study’s database. In 2002, we invited all 1423 eligible cohort members to participate in a large study which included a psychosocial stress protocol and blood withdrawal. The study was approved by the local Medical Ethics Committee and carried out in accordance with the Declaration of Helsinki. All procedures were carried out with the adequate understanding and written consent of the subjects. The consent form included information stating that part of the blood sample taken would be used for genetic analyses associated with chronic diseases and that for this purpose DNA would be anonymously stored in the hospital.

**General study parameters**

A research nurse performed a standardized interview in which information was obtained about socio-economic status (SES), educational level, medical history, lifestyle and use of medication. We asked the participants to rate his or her level of physical activity (1 = very active, 2 = active, 3 = little active). Educational level was measured on a 10-point scale (1 = primary education not completed, 10 = university completed). We defined current SES according to ISEI (International Socio-Economic Index)-92, which is based on the participant’s or their partner’s occupation, whichever status is higher⁹. Values in the ISEI-92 scale ranged from 16 (low status) to 87.

**Methylation**

DNA material was extracted from a fasting blood sample. Methylation status of the GR 1-C promoter was assessed using methylation-sensitive polymerase chain reaction (PCR). For analysis of GR 1-C promoter methylation, genomic DNA (400ng) was incubated with the methylation sensitive restriction endonucleases AciI and HinfI as instructed by the manufacturer (New England Biolabs, Hitchin, Hertfordshire, UK). The resulting DNA was amplified using real time PCR, which was performed in a total volume of 25μl with SYBR® Green Jumpstart Ready Mix (Sigma) as described by the manufacturer. To control for the amount of DNA in each reaction, primers specific for the human PPARα exon 7 were used, this region does not contain an AciI or HinfI cleavage site, as an internal control gene (Table 1). Primers were also designed to amplify the CpG island spanning the GR 1-C promoter⁵,ⁱ⁰.

Cycle parameters were 94°C for 2 min, then 40 cycles of 95°C for 30s, 60°C for 1 min and 72°C for 1 min. All cycle threshold values were normalised to the internal control and each sample analysed in duplicate and values expressed relative to the control gene. Single bands of the correct size were verified by gel electrophoresis.
Table 1 Primer sequences used in methylation-sensitive PCR.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward primer</th>
<th>Reverse primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>GR 1-C promoter</td>
<td>ATTTTCGAGCTCGTGCTCTG</td>
<td>CGGACCGAGATAAAACACT</td>
</tr>
<tr>
<td>PPARα exon 7</td>
<td>CGAGCTTATAGGGCCATATTC</td>
<td>AGGGAGATATCATGTCATCCAG</td>
</tr>
</tbody>
</table>

Psychological stress protocol

The stress protocol was performed in the afternoon (between 1200h and 1400h), about an hour after participants had eaten a light lunch. The protocol started with a 20-min baseline period, followed by three 5-min psychological stress tests (Stroop test, mirror-tracing test and speech test). The Stroop test and the mirror-tracing test were each followed by a 6-min recovery period. The speech test was followed by a 30-min recovery period. The Stroop test was a computerized colour-word conflict challenge. After a short introduction, participants were allowed to practise until they grasped the meaning of the test. A mistake or exceeding the response time limit of 5 sec was automatically followed by a short beep. In the mirror-tracing test a star had to be traced that could only be seen in mirror image (Lafayette Instruments Corp, Lafayette, IN, USA). Every divergence from the line of the star induced a short beep. The participants were allowed to practise one circuit of tracing. Participants were instructed to give priority to accuracy over speed and were told that most people could perform five circuits of the star without divergence from the line. Prior to the speech test, participants listened to an audio taped pre-recorded scenario in which they were told to imagine a situation in which they were falsely accused of pick pocketing. Participants were instructed to give a 3-min response to the accusations and were given 2 min to prepare the response. The response was recorded on video. Participants were told that the number of repetitions, eloquence and persuasiveness of their performance would be marked by a team of communication-experts and psychologists.

Continuous blood pressure (BP) and heart rate (HR) recordings were made using a Finometer or a Portapres Model-2 (Finapres Medical Systems, Amsterdam, the Netherlands). We designated six periods of 5 min each as measuring periods. The periods were defined as follows: baseline (15 min into the baseline period), Stroop, mirror-tracing, speech test (including preparation time), recovery 1 (5 min after completing the speech test), and recovery 2 (25 min after completing the speech test). We calculated mean systolic blood pressure (SBP), diastolic blood pressure (DBP) and HR for each measuring period. Saliva samples were collected using Salivettes (Sarstedt, Rommelsdorf, Germany) at seven time points during the protocol: at 5 and 20 min in the baseline period; at 6 min after completion of the Stroop test; at 6 min after completion of the mirror-drawing test; and at 10, 20 and 30 min after completion of the speech test. Saliva was extracted by centrifuging the Salivettes and was stored at -80°C until analysis. Salivary cortisol concentrations were measured using a time-resolved immunofluorescent assay (DELFIA)11. This
assay had a lower detection limit of 0.4 nmol/l, an inter-assay variance of 9-11% and an intra-assay variance of less than 10%.

Perceived stress questionnaires had to be filled out after each of the three stress tests. The questionnaires consisted of six questions (How relaxed did you feel during performance of the stress task?; How stressed did you feel during performance of the stress task?; How difficult did you find the task?; Did you feel committed to the task?; How well did you perform?; How much did you feel in control). The answers had to be given on a 7-point scale with scores ranging from 1 (not at all) to 7 (very much).

**Statistical analyses**
Baseline cortisol was calculated as the mean of the first and the second cortisol concentration measured during the baseline period. The highest average SBP, DBP, and HR value of the 5 min measuring periods and the highest of the seven cortisol values were designated as the peak response during the stress protocol. The increase from baseline to this peak value was designated as stress reactivity. We calculated a total perceived stress score by adding the scores on the questionnaires performed after each stress task.

We applied linear regression analysis to analyse associations between potential confounders and methylation status of the GR 1-C promoter as well as to analyse associations between methylation status and stress outcomes. To investigate whether potential associations between methylation status of the GR 1-C promoter and stress outcomes were influenced by general and lifestyle variables we adjusted the regression models for sex, educational level, smoking behaviour and physical activity level after looking at the association in a univariate way. We started with a crude model and additionally adjusted for potential confounding variables. Methylation status of the GR 1-C promoter was highly skewed to the right with a number of large outliers. Because the outliers did seem clinically relevant, we included them in the analyses. We log transformed methylation status to approach a normal distribution. We did the same for cortisol concentrations, which were also highly skewed to the right. We report effect sizes resulting from these analyses as unit change per 1% change in methylation status in case of normally distributed variables (SBP, DBP, HR and the perceived stress variables) and as percentage change per 1% change in methylation status in case of cortisol. For table 3, we split the methylation values into quartiles and report stress outcomes according to these quartiles. We also report p-values of linear regression analyses using the methylation quartiles as determinant. We considered differences to be statistically significant if p-values were ≤ 0.05. We used SPSS 16.0 (SPSS Inc, Chicago, IL) to perform the statistical analyses.
RESULTS

Characteristics of the study population
A total of 725 of the 1423 invited cohort members completed the stress protocol. We were able to determine the methylation status of the GR 1-C promoter in 675 of these 725 participants. Of the 675 included individuals, 47% were men (Table 2). The mean age of the study population was 58 years (SD 1 year).

Table 2 General, lifestyle and medication use characteristics in the study population.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>GR 1-C methylation (range)</td>
<td>0.064 (0.003 – 20.266)</td>
</tr>
<tr>
<td><strong>General</strong></td>
<td></td>
</tr>
<tr>
<td>Sex (% male)</td>
<td>47</td>
</tr>
<tr>
<td>Age (years)</td>
<td>58 (1)</td>
</tr>
<tr>
<td>Education b, c</td>
<td>4 (2)</td>
</tr>
<tr>
<td>SES</td>
<td>50 (14)</td>
</tr>
<tr>
<td><strong>Lifestyle</strong></td>
<td></td>
</tr>
<tr>
<td>Current smoking (%)</td>
<td>24</td>
</tr>
<tr>
<td>Alcohol consumption (glasses p/week) b</td>
<td>6 (14)</td>
</tr>
<tr>
<td>Activity level</td>
<td>2.2 (0.6)</td>
</tr>
<tr>
<td><strong>Medication use</strong></td>
<td></td>
</tr>
<tr>
<td>Use of systemic corticosteroid therapy (%)</td>
<td>8</td>
</tr>
<tr>
<td>Use of antihypertensive medication (%)</td>
<td>24</td>
</tr>
<tr>
<td>Use of antidepressants or anxiolytics (%)</td>
<td>12</td>
</tr>
<tr>
<td>Use of oral contraceptives (%)</td>
<td>2</td>
</tr>
<tr>
<td>Use of HRT (%)</td>
<td>4</td>
</tr>
</tbody>
</table>

Data are given as means (SD) and percentages, except where given as median or median (interquartile range); HRT = hormone replacement therapy; b, c Educational level measured on a 10-point scale (1 = primary education not completed, 10 = university completed).

Methylation of GR 1-C promoter
Table 2 shows that methylation status of the GR 1-C promoter ranged from 0.003 to 20.266 with a median value of 0.064 (IQR 0.087). Men and women’s methylation status did not differ significantly (p = 0.39) and age was not significantly associated with methylation status (p = 0.33). Education was significantly positively associated with GR 1-C methylation status (p = 0.03), whereas SES was not (p = 0.51). Current smoking was negatively associated (p < 0.001) and
physical activity level was positively associated ($p < 0.001$) with methylation status. Consumption of alcohol was not associated with methylation status ($p = 0.98$), nor was use of systemic corticosteroids ($p = 0.80$), antihypertensive medication ($p = 0.50$), antidepressant or anxiolytics ($p = 0.58$), oral contraceptives ($p = 0.89$) or hormone replacement therapy ($p = 0.37$).

**Stress reactivity**

SBP, DBP, HR and cortisol values increased in response to all three psychological stress tests. Mean SBP, DBP and HR responses to the stress protocol peaked during the speech task. Stress reactivity was 37% (48 mmHg [95% CI: 46 to 49]) for the SBP response, 32% (21 mmHg [21 to 22]) for the DBP response and 16% (12 bpm [11 to 12]) for the HR response. The mean cortisol response peaked during the first recovery period after the speech test. Stress reactivity was 42% (geometric mean 1.7 nmol/l [1.5 to 1.8]).

**Methylation and stress outcomes**

Table 3 shows that methylation of the GR 1-C promoter was significantly positively associated with physical stress reactivity. With 1% decrease in GR 1-C methylation, cortisol levels decreased by 0.14% (95% CI: 0.03 to 0.25, $p = 0.02$) and HR decreased by 0.10 bpm (0.03 to 0.16, $p = 0.003$). However, when adjusting for sex, educational level, smoking behaviour and physical activity, the associations diminished enormously and were by far no longer statistically significant. Cortisol change after adjustment was -0.08% (-0.19 to 0.04, $p = 0.19$) and HR change after adjustment was -0.04 bpm (-0.11 to 0.02, $p = 0.21$). This was mainly due to the adjustment for smoking and activity level. SBP ($p = 0.57$) and DBP ($p = 0.87$) reactivity were not associated with methylation status of the GR 1-C promoter.

GR 1-C methylation was significantly associated with three of the six self perceived stress variables. It was negatively associated with perceived stress (0.03 points increase per 1% decrease in methylation status [95% CI: 0.00 to 0.06, $p = 0.05$] and positively with perceived performance (-0.03 points decrease [-0.05 to -0.01], $p = 0.02$) and control (-0.03 points decrease [-0.05 to 0.00], $p = 0.04$). Adjusting for sex and educational level diminished the strength of the associations with all perceived variables, which were no longer statistically significant: perceived stress (0.02 points [0.00 to 0.05, $p = 0.10$), perceived performance (-0.02 points [-0.04 to 0.00], $p = 0.09$), and control (-0.02 points [-0.04 to 0.01], $p = 0.16$). Adjustment for educational level contributed more to the abolishment of the associations than adjustment for sex.
**Table 3** Stress test outcomes according to quartiles of GR 1-C methylation status.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>All</th>
<th>Q1</th>
<th>Q2</th>
<th>Q3</th>
<th>Q4</th>
<th>( p )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortisol (nmol/l) (^a)</td>
<td>609</td>
<td>3.8 (1.8)</td>
<td>3.7</td>
<td>3.6</td>
<td>3.7</td>
<td>4.0</td>
<td>.14</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>665</td>
<td>128.1 (21.1)</td>
<td>128.3</td>
<td>127.9</td>
<td>127.3</td>
<td>129.1</td>
<td>.80</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>665</td>
<td>66.2 (11.8)</td>
<td>66.6</td>
<td>66.5</td>
<td>65.9</td>
<td>65.6</td>
<td>.38</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>664</td>
<td>74.1 (10.5)</td>
<td>73.3</td>
<td>73.9</td>
<td>74.4</td>
<td>74.9</td>
<td>.14</td>
</tr>
<tr>
<td><strong>Stress reactivity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortisol (nmol/l) (^a)</td>
<td>433</td>
<td>1.7 (3.7)</td>
<td>1.5</td>
<td>1.6</td>
<td>1.6</td>
<td>2.2</td>
<td>.04</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>665</td>
<td>47.8 (20.8)</td>
<td>48.5</td>
<td>46.8</td>
<td>46.6</td>
<td>49.3</td>
<td>.76</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>665</td>
<td>21.4 (9.1)</td>
<td>21.6</td>
<td>20.9</td>
<td>21.4</td>
<td>21.6</td>
<td>.89</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>664</td>
<td>11.6 (9.3)</td>
<td>10.7</td>
<td>10.4</td>
<td>11.5</td>
<td>13.9</td>
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<tr>
<td><strong>Perceived stress</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Relaxedness</td>
<td>625</td>
<td>11.5 (3.8)</td>
<td>11.4</td>
<td>11.3</td>
<td>11.4</td>
<td>11.8</td>
<td>.40</td>
</tr>
<tr>
<td>Stressfulness</td>
<td>626</td>
<td>11.1 (4.0)</td>
<td>11.5</td>
<td>11.1</td>
<td>10.9</td>
<td>10.7</td>
<td>.08</td>
</tr>
<tr>
<td>Difficulty</td>
<td>625</td>
<td>14.4 (3.5)</td>
<td>14.8</td>
<td>14.3</td>
<td>14.7</td>
<td>14.0</td>
<td>.09</td>
</tr>
<tr>
<td>Commitment</td>
<td>625</td>
<td>14.7 (4.1)</td>
<td>14.8</td>
<td>14.7</td>
<td>14.9</td>
<td>14.5</td>
<td>.60</td>
</tr>
<tr>
<td>Performance</td>
<td>623</td>
<td>9.2 (3.4)</td>
<td>8.7</td>
<td>9.4</td>
<td>9.1</td>
<td>9.6</td>
<td>.04</td>
</tr>
<tr>
<td>Control</td>
<td>625</td>
<td>10.1 (3.6)</td>
<td>9.5</td>
<td>10.3</td>
<td>10.0</td>
<td>10.4</td>
<td>.06</td>
</tr>
</tbody>
</table>

Data are given as means (SD), except where given as \(^a\) geometric mean (geometric SD); \( p \)-values for difference between the quartiles.

**DISCUSSION**

We found that lower level of methylation of the GR 1-C promoter in peripheral blood was associated with lower physical stress reactivity in terms of lower cortisol and HR responses to a psychosocial stress protocol and with higher levels of perceived stress variables. Interestingly, these associations largely disappeared after adjusting for sex, educational level, smoking behaviour and physical activity, where the latter two had the largest effect. This suggests that variation in GR 1-C methylation only has a small functional effect on the stress response and that the remainder of the effect can be explained by differences in lifestyle and educational level.

Little is known about the functional effects of variation in methylation status in GR-promoters on the biological stress response. However, existing data does seem to support the present findings of a positive association between methylation status and stress reactivity. The previously mentioned study by Oberlander et al. showed in neonates that increased methylation of GR promoter 1-F was associated with increased cortisol responses to a stress protocol\(^4\). In addition, Weaver et al. showed in rats who received little care of their mothers early in life that high
methylation status of the exon 1, GR promoter (the homolog of the 1-F region in humans) was associated with lower GR expression and higher corticosterone stress reactivity\textsuperscript{12}, which was suggested to be a consequence of reduced glucocorticoid negative feedback sensitivity\textsuperscript{13}.

The observation of an association between educational level and an adverse lifestyle (smoking and low physical activity) and methylation pattern of the GR-1C promoter is in itself an interesting one. Epigenetic patterns have been shown to be rather stable, but there is increasing evidence that changes over time do take place\textsuperscript{14}. Several environmental factors have been suggested to affect this process, including circumstances during embryonic development as well as factors in adult life such as diet, smoking and exposure to environmental pollution\textsuperscript{15}. Other factors than those observed in the present study may play a role in the observed association between environmental factors and GR-1C methylation. For example, educational level and physical activity may both be strongly associated with diet, of which several components such as folate and methionine have been shown to be associated with DNA methylation\textsuperscript{15}.

While we found that an adverse lifestyle was associated with lower methylation of the GR 1-C promoter, at the same time lower physical stress responses were observed in those with low GR-1C methylation. These associations may seem contradictory, but fit in a growing body of evidence that blunted stress reactivity may be associated with adverse health. In a previous report on the present cohort, we have shown that lower cortisol and cardiovascular stress responses are associated with increased symptoms of depression and anxiety and poor subjective health perception\textsuperscript{16,17}. Others have shown similar results\textsuperscript{18-20}. Several biological explanations can be put forward. Blunted reactivity could be due to a failing stress response as a consequence of prolonged stress. Another explanation may be that a diminished stress response could have adverse immunological consequences. Under certain circumstances, the biological stress response upregulates the immune system, which is beneficial for health\textsuperscript{21}. A decreased stress response may be less able to do so. Besides this, cortisol is needed to eventually suppress the inflammatory response. A lack of cortisol may thus lead to a prolonged inflammatory state with negative effects on health.

Lower methylation status of the GR 1-C promoter was also associated with increased perception of stress: those with lower levels of methylation felt more stressed during the stress protocol, felt they had performed less well on the stress tasks and felt less in control. Again, this seems at odds with the decreased levels of cortisol and HR reactions to the protocol. However, we showed the same in the above referenced studies where symptoms of depression and anxiety and poor self-perceived health were also associated with increased stress perception and decreased physical responses\textsuperscript{16-17}. This discrepancy between experienced stress and physiological stress is not unique. Several studies have found only small or no associations between self-perceived stress /emotions and biological stress in the form of cortisol and cardiovascular responses\textsuperscript{22-24}. Different explanations can be put forward. The physical stress response may be dysfunctional. Individuals may not be aware of their own emotions or individuals may not be sincere in reporting their thoughts and emotions.
A number of limitations to the present study have to be pointed out. We did not assess GR 1-C expression levels, which clearly would have added relevant information to the study. We assessed methylation status of the GR 1-C promoter in peripheral blood and do not know whether this is comparable to methylation status in different parts of the body implicated in the functional regulation of the stress response. We have applied a methylation-sensitive PCR technique to assess DNA methylation of the GR 1-C promoter, this measures the average methylation range across the promoter region analysed. It is unknown whether the same results would have been achieved when we would have applied the nowadays commonly used bisulfite pyrosequencing technique, which can measure the methylation status of individual CpGs. We therefore suggest that the present results should be replicated in a study in which GR 1-C methylation status is assessed by the more sensitive pyrosequencing method. Finally, effects of GR 1-C methylation status on stress responses were statistically significant, but small. Only about 1% of the variation in the stress reactivity data was explained by methylation status. However, given all the limitations referred to above and the fact that we only measured methylation status in one exon of eight translated exons known, it may be seen as remarkable that we found such associations at all.

Major assets of our study include a population based design and a large, well-described study population, enabling us to investigate the potential confounding/mediation by a set of different variables including basal characteristics, lifestyle variables and use of medication. An additional strength is the use of a psychological stress protocol with different types of stressors: cognitive and social stressors. Unlike a physiological stress test, a psychological stress protocol is able to activate stress responses above the hypothalamic level where the limbic structures process cognitive and affective information^25.

In conclusion, we found evidence for associations between methylation status of the GR 1-C promoter and biological and perceived stress responses. Interestingly, these associations could largely be explained by lifestyle and education.
REFERENCES


