

Increased methylation of glucocorticoid receptor gene (*NR3C1*) in adults with a history of childhood maltreatment: a link with the severity and type of trauma

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Childhood maltreatment, through epigenetic modification of the glucocorticoid receptor gene (*NR3C1*), influences the hypothalamic–pituitary–adrenal axis (HPA axis). We investigated whether childhood maltreatment and its severity were associated with increased methylation of the exon 1_F *NR3C1* promoter, in 101 borderline personality disorder (BPD) and 99 major depressive disorder (MDD) subjects with, respectively, a high and low rate of childhood maltreatment, and 15 MDD subjects with comorbid post-traumatic stress disorder (PTSD). Childhood sexual abuse, its severity and the number of type of maltreatments positively correlated with *NR3C1* methylation ($P = 6.16 \times 10^{-8}$, 5.18×10^{-7} and 1.25×10^{-9} , respectively). In BPD, repetition of abuses and sexual abuse with penetration correlated with a higher methylation percentage. Peripheral blood might therefore serve as a proxy for environmental effects on epigenetic processes. These findings suggest that early life events may permanently impact on the HPA axis through epigenetic modifications of the *NR3C1*. This is a mechanism by which childhood maltreatment may lead to adulthood psychopathology.

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Introduction

Exposure to early life adverse events, including abuses and neglect, significantly increases the risk of psychopathology in adulthood.^{1,2}

In humans as well as in animals, response to stress, and particularly to childhood trauma, is mediated by the hypothalamic–pituitary–adrenal (HPA) axis, which includes release of corticotrophin-releasing factor and secondarily results in secretion of glucocorticoids.³ Early life stress has been shown to induce persistent changes in corticotrophin-releasing factor neurotransmission and sustains alteration of the HPA axis.^{4,5} Hyperactivity as well as decreased activity of the HPA axis in response to stress has been demonstrated in humans with a history of childhood maltreatment.^{4–6} It has been speculated that the direction of such HPA alterations may depend on the characteristics of the stressor such as the type, duration, intensity or timing.^{7,8} Nevertheless, although the main role of this HPA axis is to prevent over-response to stress,⁹ prolonged exposure to elevated levels of glucocorticoids has damaging effects on the developing brain, which may secondarily lead to behavioral problems later in life.^{10–13}

Recent studies point to the epigenetic mechanisms as a process by which environmental factors may alter the gene expression involved in control of the HPA axis. In rodents, epigenetic modification of specific exon 1_F in the promoter

region of the glucocorticoid receptor (GR) gene (*NR3C1*) has been linked to early maternal care and secondarily to effects on hippocampal GR expression.^{14,15} In rats, Weaver *et al.*¹⁴ showed that this promoter region of *NR3C1* is regulated through early experience including the quality of maternal care. This epigenetic programming of GR expression inhibits the activation of HPA activity,¹⁶ which may in part explain how early maternal care influences HPA axis stress response in the offspring.^{14,17}

To our knowledge, only three studies performed in humans to date—and only one of them in adults—have investigated the relationship between methylation status of the exon 1_F *NR3C1* promoter, the human homolog of the rat exon 1_F *NR3C1* promoter and early life adverse events. All three reached the same conclusion: higher methylation status was found in subjects who experienced early life adverse events.^{18–20} McGowan *et al.*¹⁹ showed increased methylation of the exon 1_F *NR3C1* promoter in the hippocampus of suicide victims with a history of childhood abuse compared with control samples, which was associated with decreased levels of GR mRNA, as well as mRNA transcripts bearing the GR 1_F splice variant. Neither the hippocampal GR expression nor the methylation status of the exon 1_F *NR3C1* promoter was altered in suicide victims with no history of abuse. This result suggests a specific effect of child abuse independent of

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suicide on methylation status and GR mRNA expression. Adding to the hypothesis of early environmental effect on *NR3C1* epigenetic status in humans, Oberlander *et al.*¹⁸ found that prenatal exposure to increased third trimester maternal depressed/anxious mood was associated with increased methylation of *NR3C1* in newborns (in genomic DNA from cord blood mononuclear cells) and increased salivary cortisol stress responses at 3 months. Finally, Radtke *et al.*²⁰ showed an increased methylation of *NR3C1* promoter in children exposed years earlier as a fetus to stress during their mother's pregnancy.

We undertook this study to examine whether childhood maltreatment, with a primary focus on childhood sexual abuse, was associated with increased *NR3C1* promoter methylation in a sample of borderline personality disorder (BPD) subjects expected to have a high rate of childhood traumas (sexual, physical as well as emotional) and a sample of mildly abused and neglected subjects suffering from major depressive disorder (MDD) and a sample of subjects suffering from MDD with comorbid past/current PTSD. As some data moreover suggest that higher severity and type of abuse are risk factors for worse mental health outcome in adulthood²¹ (see the Supplementary Information for additional references), we also expected the severity of the maltreatment (intensity of the trauma, age at onset, number of type of abuses/neglects and their repetition) to be positively correlated with the percentage of methylation.

Materials and methods

Samples. A total of 101 subjects suffering from BPD were recruited in a specialized center using intensive dialectical behavior therapy as the core treatment for this disorder.²² Briefly, patients were referred by their physician or other medical services for suicidal or deliberate self-harm behaviors. All participants received psychopharmacological treatment, which was refined by a psychiatrist if necessary before and during follow-up. Blood monitoring to ascertain compliance was systematically done at entry in the program. All patients were assessed for Axis I psychiatric diagnoses using the French version of the Diagnostic Interview for Genetic Studies²³ and for BPD by the Screening Interview for Axis II Disorders BPD part.²⁴ Only subjects fulfilling DSM-IV criteria for BPD were accepted in the program. Each participant was interviewed by either a trained psychiatrist or a psychologist before acceptance into treatment. Before 1 week the commencement of intensive dialectical behavior therapy each subject completed the Beck Depression Inventory II²⁵ to assess the current severity of depression symptoms and the Childhood Trauma Questionnaire (CTQ), which examines five types of trauma in a self-report assessment: sexual abuse, physical abuse, physical neglect, emotional abuse and emotional neglect. Scores range from 5 to 25 for each type of trauma. According to Bernstein and Fink,²⁶ thresholds or cutoff scores have been set for each type of trauma at four levels of maltreatment: none, low, moderate and severe.

In addition to the CTQ, each participant was clinically questioned about the existence or not of sexual or physical

abuses during childhood and adulthood. If abuses were reported, the following items were then systematically explored: age of the abuse, duration of the abuse, frequency of the abuse, perpetrator of the abuse, nature of the abuse and the number of abuses throughout life. Each case was then discussed with the team program,²² and a final report on abuse was then made. According to the literature, we classified the abuses as follows:²¹ frequency of abuses (day, month, week or year), number of consecutive abuses (repetition of abuses: one, two or more), relationship to the perpetrator (family member or not), child vs adolescent/adulthood abuses, sexual abuse with or without penetration.

Demographic and additional clinical data were obtained from a standard questionnaire given to all participants before entering the program. These data were checked against notes from psychiatric, medical and surgical units.

As BPD subjects were expected to have a high rate of abuses and neglects (thus methylation in subjects with no maltreatment would be difficult to ascertain due to the ceiling effect) and to suffer from MDD and PTSD comorbidities, we decided to contrast our BPD sample with two other samples. First, 99 subjects with a low rate of abuses and neglect were selected from a cohort of >1500 subjects suffering from various psychiatric disorders and for which CTQ was available. These subjects were recruited from consecutive admissions to the psychiatric university hospitals of Montpellier (France) and Geneva (Switzerland) between 1994 and 2010. Subjects were assessed for psychiatric diagnoses using either the Diagnostic Interview for Genetic Studies or the Mini International Neuropsychiatric Interview.²⁷ All of them fulfilled the CTQ and a part the Beck Depression Inventory short form (Beck Depression Inventory-Short Form; 13 items). All subjects with a diagnosis of MDD without past/current PTSD who reported no sexual abuse, no physical abuse and neglect and no emotional abuse were chosen. 'No emotional neglect' was not considered as an inclusion criterion, as only 49 subjects among the 1500 patients failed to report neglect of this kind in addition to failing to report any of the other abuses or neglect (see Table 1). Second, 15 MDD subjects with past/current PTSD were chosen from the above sample of 1500. The sole selection criteria were suffering from MDD and having a past/current diagnosis of PTSD based on the Diagnostic Interview for Genetic Studies.

The study protocol was approved by the Research Ethics Committees in each center, and written informed consent was obtained for each subject.

NR3C1 methylation status. For the BPD subject, blood samples were systematically collected 1 week before the commencement of the program. For MDD subjects blood samples were collected just after the interviews. DNA was extracted from blood white cells (Nucleon extraction kit, Amersham Life Science, Dubendorf, Switzerland).

We analyzed a portion of the exon 1_F *NR3C1* promoter, which was equivalent to the one reported by Oberlander *et al.*¹⁸ to exhibit methylation modulation in response to antenatal maternal mood and reported by McGowan *et al.*¹⁹ to exhibit methylation differences in suicide victims with a history of sexual abuse. It should be noted that this region

Table 1 Clinical and demographic characteristics of BPD subjects and MDD subjects with or without PTSD

	BPD subjects (N = 101)		MDD subjects with low rate of maltreatment (N = 99)		MDD subjects with past/current PTSD (N = 15)		BPD vs MDD		BPD vs MDD+PTSD		MDD vs MDD+PTSD	
	Mean	s.d.	Mean	s.d.	Mean	s.d.	P	P	P	P		
Age	30.76	9.74	41.63	12.81	37.33	10.46	<0.0001	0.02	0.22	—	—	—
Severity of depression (BDI-II)	34.42	11.78	—	—	—	—	—	—	—	—	—	—
Severity of depression (BDI-SF) ^a	21.39 ^b	7.42	14.03	7.77	22.60	5.59	<0.0001	0.74	0.02	—	—	—
Number of SCID-II BPD criteria	6.81	1.76	—	—	—	—	—	—	—	—	—	—
	N	%	N	%	N	%	P	P	P	P	P	P
Female	95	94.06	64	64.65	11	73.33	<0.0001	0.01	0.51	—	—	—
<i>Axis I main diagnosis</i>												
MDD	74	73.26	99	100.00	15	100.00	<0.0001	0.21	—	—	—	—
BD-I	4	3.96	—	—	—	—	—	—	—	—	—	—
BD-II	15	14.85	—	—	—	—	—	—	—	—	—	—
SZA	8	7.92	—	—	—	—	—	—	—	—	—	—
Past/current alcohol use disorder ^a	57	61.29	19	21.11	3	25.00	<0.0001	0.02	0.76	—	—	—
Past/current substance use disorder ^a	48	51.61	5	6.49	1	16.67	<0.0001	0.10	0.35	—	—	—
Past/current PTSD ^a	25	26.88	0	0.00	15	100.00	<0.0001	<0.0001	<0.0001	—	—	—
History of suicide attempt	70	72.16	96	96.97	14	93.33	<0.0001	0.078	0.48	—	—	—
<i>CTQ</i>												
Sexual abuse	52	55.32	0	0.00	4	44.44	<0.0001	0.53	<0.0001	—	—	—
Physical abuse	54	55.10	0	0.00	6	66.67	<0.0001	0.47	<0.0001	—	—	—
Physical neglect	48	48.98	0	0.00	5	55.56	<0.0001	0.61	<0.0001	—	—	—
Emotional abuse	86	87.76	0	0.00	9	100.00	<0.0001	0.25	<0.0001	—	—	—
Emotional neglect	83	84.69	50	51.02	8	88.89	<0.0001	0.70	0.03	—	—	—
None	5	5.32	49	49.49	0	0.00	<0.0001	0.41	<0.0001	—	—	—
<i>Psychopharmacological treatment</i>												
Antidepressant	39	38.61	70	70.71	6	0.40	<0.0001	0.81	0.02	—	—	—
Neuroleptics	40	39.60	4	4.04	1	6.67	<0.0001	0.01	0.64	—	—	—
Mood stabilizers	14	13.86	1	1.01	0	0.00	0.00	0.12	0.70	—	—	—
None	17	16.83	28	28.28	9	60.00	0.09	<0.0001	0.01	—	—	—

Abbreviations: BD, bipolar disorder; BDI-SF, Beck Depression Inventory-Short Form; BPD, borderline personality disorder; CTQ, Childhood Trauma Questionnaire; MDD, major depressive disorder; PTSD, post-traumatic stress disorder; SCID-II, Screening Interview for Axis I Disorders; SZA, schizoaffective disorder.
^aMissing values encountered.
^bFor BPD the 13 items corresponding to BDI-SF were extracted from the BDI-II.

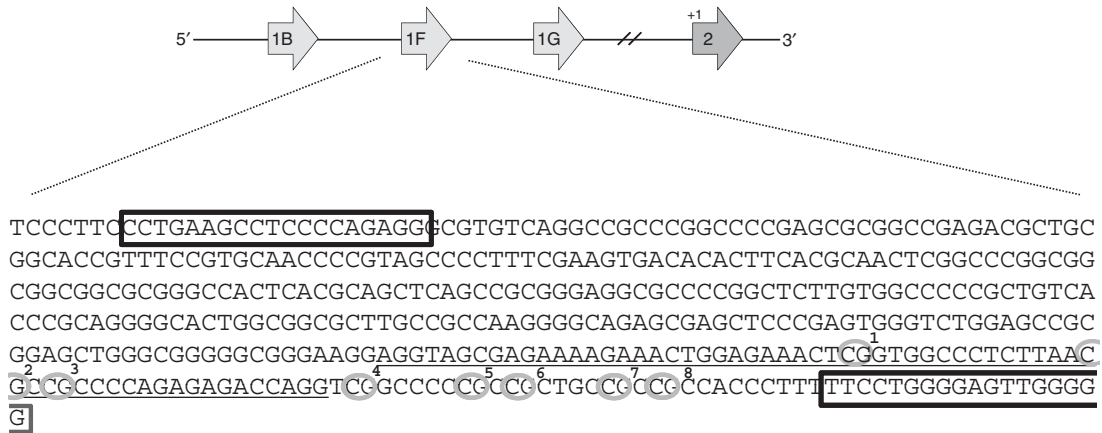


Figure 1 The region from nucleotide –3492 to –3082 (numbers relative to the translation start site considered as +1) of the 5'-end of the *NR3C1* gene. Underlined: exon 1F; square boxes: position of the oligonucleotides used for amplification of bisulfite-treated DNA; encircled: CpGs analyzed.

contains a nerve growth factor inducible protein A-binding site that regulates gene transcription.

The degree of methylation at each CpG site was determined using Pyro Q-CpG Software (Biotage AB, Uppsala, Sweden). Details of the bisulfite treatment and of the PCR amplification of bisulfite-treated DNA for subsequent pyrosequencing are available in the Supplementary Information.

Eight CpG sites were analyzed and the percentage of total methylation was calculated (Figure 1). These eight CpGs correspond to CpGs 6–13, described by Oberlander *et al.*¹⁸ and are located immediately downstream to those described by McGowan *et al.*¹⁹ CpG1 in our study is located 20 nucleotides downstream of what McGowan *et al.* described as CpG 39. All samples were analyzed in duplicate and the mean percentage was then calculated and used for the current analyses. All duplicates with 3% error or more between the two measures were either re-analyzed and used if <3% error was then observed between the two new measures or were excluded from the analyses ($N=7$) if the 3% error or more was still present. It should be noted that, in a previous study, the results obtained showing a small ($\leq 3\%$) difference in methylation in two difference conditions were double checked by comparing the technique used above, that is, DNA bisulfite treatment and PCR amplification followed directly by pyrosequencing, with PCR amplification followed by amplicon subcloning and sequencing. The results were found to be similar and confirmed that pyrosequencing is a technique permitting to detect small percentages of difference with sensitivity as good as the cloning sequencing technique.²⁸ Samples were processed and analyzed blindly with respect to demographic variables, psychiatric diagnoses and childhood maltreatment history.

Statistics. The analyses were carried out in two sequential steps, starting with the better-characterized sample of BPD expected to be enriched for childhood trauma history and for which a large panel of potential confounding factors was available. Confounding factors (see below) were assessed for association with methylation status and with each of the childhood maltreatments using either the linear regression or

the χ^2 test, respectively. Any variable showing an association reaching a P -value of 0.05 or below was then used as a covariate in a linear regression model. Methylation status was used without any transformation as it was normally distributed, in the BPD and the whole sample, according to the one-sample Kolmogorov–Smirnov test.

As type of childhood trauma has previously been shown to be an important correlate of adult psychopathology,¹⁸ each type of maltreatment as measured by the CTQ (sexual abuse, emotional and physical abuses/neglects) was first analyzed separately as a binary outcome (yes or no). We started the analyses with childhood sexual abuse as this has been the most consistently and most widely investigated childhood maltreatment.¹⁸ If significantly associated with methylation status we then ascertained whether the severity of the individual trauma (using CTQ cutoff scores described above) as a continuous variable was associated with methylation status. Second, and based on evidences from the literature,^{29,30} we investigated whether the number of types of childhood maltreatment (by summing the different maltreatments reported by the CTQ) as a continuous variable was associated with methylation status. Finally, and only for the BPD sample, association between clinically recorded abuses and methylation status was tested.

The same methodology was then applied to the whole sample (MDD + BPD samples). Results of regression models are presented as standardized regression coefficients (b), which can be interpreted as effect size; however, values in the text represent the mean percentage of methylation with the corresponding s.d. The threshold of significance was set at $P \leq 0.05$.

Results

BPD sample. Table 1 displays the clinical and demographic characteristics of the BPD subjects. Most of them were female (94.06%), aged around 30 years old (30.76, s.d. = 9.76), suffering from comorbid MDD (73.26%), and from alcohol (61.3%) and substance use disorders (51.6%).

One fourth of the BPD patients presented a diagnosis of current or past PTSD. The mean number of BPD DSM-IV items was 6.81 (s.d. = 1.76) as measured by the Screening Interview for Axis II Disorders. Only 5 out of the 101 BPD subjects reported no childhood maltreatment.

Childhood maltreatment, methylation status and potential confounding factors. There was no association between methylation status and any of the potential clinical and demographic confounding factors. Gender, primary Axis I diagnosis, past/current alcohol/substance use disorder, past/current PTSD, history of suicide attempt, severity of depression and of BPD and current pharmacological treatment were all associated with childhood maltreatment to a certain degree and with childhood sexual abuse in the majority of cases (see Supplementary Information). These variables were therefore used as covariates in the analyses.

Effect of childhood maltreatment on NR3C1 methylation. There was a significant association between childhood sexual abuse and methylation status, with sexually abused subjects being more highly methylated (0.141 (s.d. = 0.02)) than non-sexually abused subjects (0.128 (s.d. = 0.02); $b = 0.41$; $P = 0.011$). The association was still significant after adjusting for all the significant confounding factors ($b = 0.45$; $P = 0.015$). After adjustment for confounding factors, increased severity of childhood sexual abuse was significantly associated with increased NR3C1 methylation (none = 0.128 (s.d. = 0.02); low = 0.138 (s.d. = 0.03); moderate = 0.148 (s.d. = 0.02); severe = 0.137 (s.d. = 0.02); $b = 0.13$; $P = 0.039$; Figure 2a).

There was no association between childhood physical abuse and methylation status.

There was a significant association between childhood physical neglect and methylation status, with physically

neglected subjects being more highly methylated (0.141 (s.d. = 0.02)) than non-physically neglected subjects (0.129 (s.d. = 0.02); $b = 0.38$; $P = 0.017$). The association was still significant after adjusting for all the significant confounding factors ($b = 0.41$; $P = 0.015$; Supplementary Figure S1).

There was no association between childhood emotional abuse or neglect and methylation status.

After adjustment for confounding factors, there was a significant association between the number of types of childhood abuse and neglect and methylation status ($b = 0.12$; $P = 0.034$; Figure 3a).

After adjustment for confounding variables, there was a significant trend in percentage of methylation from no clinically recorded childhood sexual abuse (0.131 (s.d. = 0.02)) to sexual abuse without penetration (0.137 (s.d. = 0.02)) and to sexual abuse with penetration (0.141 (s.d. = 0.01); $b = 0.22$; $P = 0.02$). The number of abuses over a subject's life span (repetition of abuse) was also significantly associated with methylation status after adjustment for confounding factors. Those with one reported abuse had less methylation (0.131 (s.d. = 0.02)) than those reporting two or more abuses (0.141 (s.d. = 0.02) ($b = 0.28$; $P = 0.043$). (See Table 2 for details on the clinical evaluation of physical/sexual abuse).

Whole sample. Almost all the clinical and demographic variables distinguishing BPD subjects from MDD subjects (see Table 1) were significantly associated with methylation status and were therefore used as covariates in the following analyses (see the Supplementary Information).

Effect of childhood maltreatment on NR3C1 methylation. There was a significant association between sexual abuse and NR3C1 methylation ($b = 1.10$; $P = 1.98 \times 10^{-12}$) showing that sexually abused subjects had higher methylation status (0.138 (s.d. = 0.02)) than

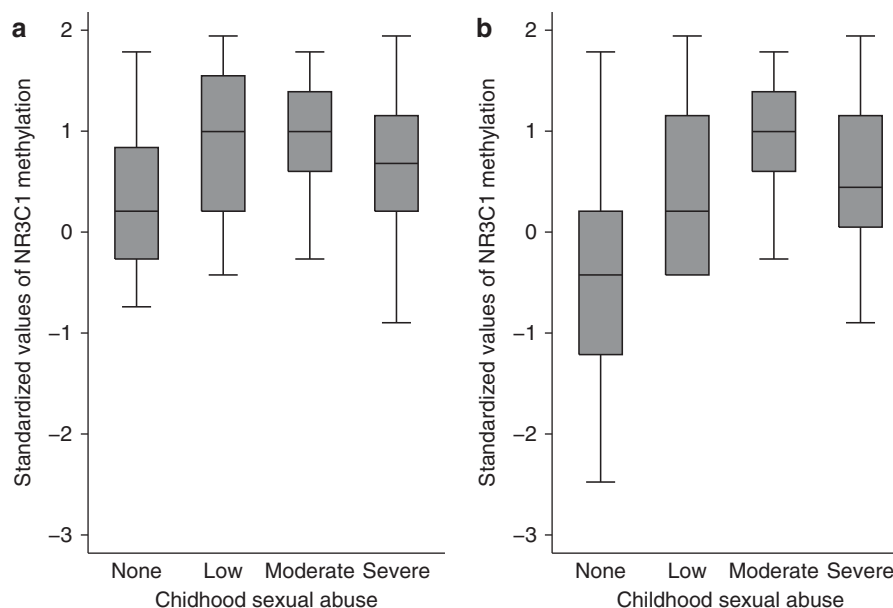


Figure 2 Box-plot of standardized methylation rate according to severity of sexual abuse in borderline personality disorder (BPD) subjects (a) and in the whole sample (b).

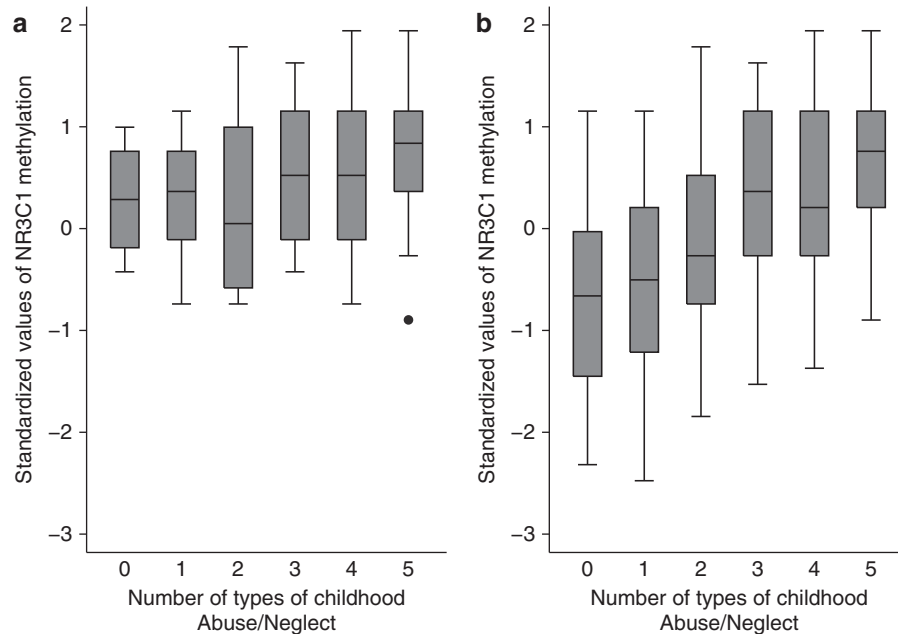


Figure 3 Box-plot of standardized methylation frequency according to the number of types of childhood abuse and neglect in BPD subjects (a) and in the whole sample (b).

Table 2 Evaluation of abuse in 75 physically and sexually abused borderline personality disorder (BPD) subjects

	Mean	s.d.
Age at first abuse	6.93	5.31
Duration of abuses (years)	7.03	6.37
	N	%
Frequency of abuses		
Day	11	15.07
Month	15	20.55
Week	31	42.47
Year	16	21.92
Number of consecutive abuses		
1	38	50.67
2	30	40
3 or more	7	9.33
Perpetrator of the first abuse		
First-degree relatives	47	62.66
Second-degree relatives	11	14.66
Close relatives (friends, neighbor)	13	17.33
Others (priest, doctor, unknown)	4	5.33
Perpetrator of the second abuse (N = 36)		
First-degree relatives (including husbands)	10	27.78
Second-degree relatives	3	8.33
Close relatives (friends, neighbor)	16	44.44
Others (priest, doctor, unknown)	7	19.44
Childhood sexual abuse (< 14 years old)		
Without penetration	19	25.33
With penetration (oral, vaginal or anal)	17	22.66
Adolescence/Adulthood sexual abuse (≥ 14 years old)		
Without penetration	5	6.66
With penetration (oral, vaginal or anal)	24	32
Childhood physical abuse	46	61.33
Adolescence/Adulthood physical abuse	23	30.66

non-sexually abused subjects (0.103 (s.d. = 0.03); Figure 2b). The association was still significant after adjustment for all the significant confounding factors ($b = 1.01$; $P = 6.16 \times 10^{-8}$). After adjustment for confounding factors, there was a significant trend from no childhood sexual abuse (0.103 (s.d. = 0.03)), to low severity of sexual abuse (0.132 (s.d. = 0.03)) to moderate severity (0.147 (s.d. = 0.02)) and to severe sexual abuse (0.134 (s.d. = 0.02); $b = 0.36$, $P = 5.18 \times 10^{-7}$).

Interestingly, when Figure 2a is compared with Figure 2b it is obvious that, after adjustment for confounding factors, there is a significant difference in methylation status between BPD subjects and MDD subjects when those without sexual abuse are considered ($b = 0.95$; $P = 5.99 \times 10^{-7}$).

There was a significant association between childhood physical abuse and methylation status ($b = 0.79$; $P = 8.91 \times 10^{-7}$). This association, which shows higher methylation in physically abused subjects (0.131 (s.d. = 0.02)) than in non-physically abused subjects (0.106 (s.d. = 0.03)), was still significant after adjusting for confounding factors ($b = 0.61$; $P = 0.003$; Supplementary Figure S1).

There was a significant association between physical neglect and NR3C1 methylation ($b = 0.95$; $P = 3.34 \times 10^{-9}$) in the same direction as in the BPD sample (0.135 (s.d. = 0.03) vs 0.105 (s.d. = 0.03); Supplementary Figure S1).

There was a highly significant association between childhood emotional abuse and NR3C1 methylation ($b = 1.06$; $P = 5.11 \times 10^{-15}$) showing that emotionally abused subjects had higher methylation status (0.135 (s.d. = 0.03)) than non-emotionally abused subjects (0.098 (s.d. = 0.03); Supplementary Figure S1). The association was still significant after adjusting for all the significant confounding factors ($b = 1.18$; $P = 7.24 \times 10^{-10}$).

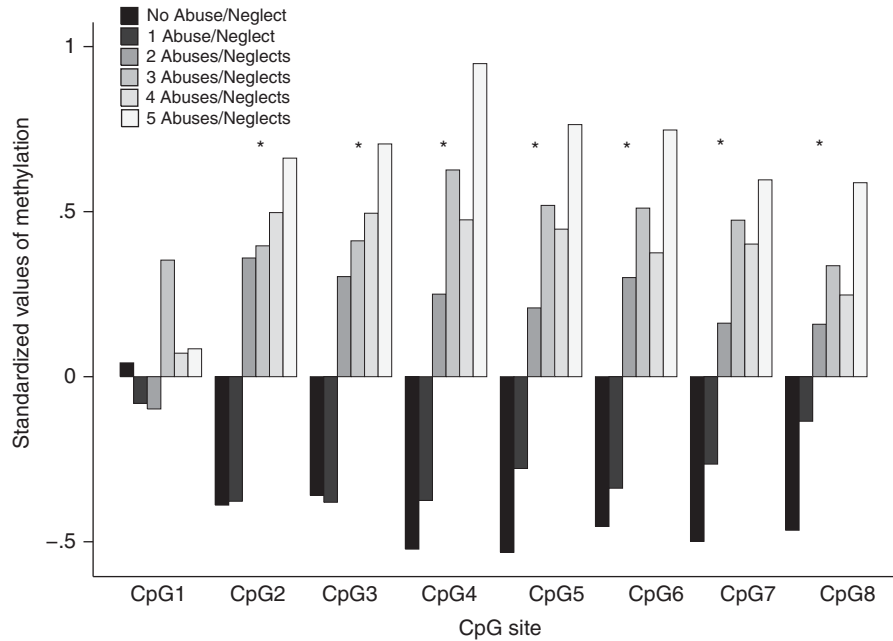


Figure 4 Methylation of the exon 1_F NR3C1 promoter region, showing the standardized value of methylation observed at each CpG site according to the number of types of childhood abuse and neglect in the whole sample. (* $P < 1 \times 10^{-6}$).

There was a significant association between childhood emotional neglect and methylation status ($b = 0.56$; $P = 0.0002$) showing higher methylation in emotionally neglected subjects (0.118 (s.d. = 0.03)) than in non-emotionally neglected subjects (0.101 (s.d. = 0.03)) that was still significant after adjustment for confounding factors ($b = 0.44$; $P = 0.008$). As is obvious from Supplementary Figure S1, emotional neglect positively associated with methylation status, high neglect predicting higher methylation status (from 0.101 (s.d. = 0.03) to 0.130 (0.03) $P = 2.71 \times 10^{-6}$).

There is an obvious association between the number of types of abuse and neglect and NR3C1 methylation status from 0.096 (s.d. = 0.03) to 0.141 (s.d. = 0.02; $b = 0.30$; $P = 4.94 \times 10^{-15}$; cf. Figure 3b). This was still significant after adjustment for confounding factors ($b = 0.31$; $P = 1.25 \times 10^{-9}$).

With the exception of CpG1, all CpGs showed an association with the number of types of abuse and neglect in the same direction than the one observed for the whole methylation at a level of significance of $P < 1 \times 10^{-6}$ (Figure 4).

Discussion

According to our primary hypothesis, we showed that childhood sexual abuse was associated with increased NR3C1 promoter methylation in the peripheral blood. Moreover, we also found that the severity of childhood abuses and neglect (repetition of abuses, number of types of abuse and neglect, types of abuses) positively correlated with NR3C1 promoter methylation.

In a recent paper, McGowan *et al.*¹⁹ showed that childhood sexual abuse was associated with increased methylation of the exon 1_F NR3C1 promoter in the brain of suicide victims. This paper was the first to highlight such an association

opening the way for the next step: are we able to see a similar change in the peripheral blood of living patients in whom less recollection bias should come into play? Exploring the same region as McGowan *et al.*,¹⁹ we suggest that what was primarily observed in the brain is true of the peripheral blood. We have added therefore to the growing body of evidence linking childhood maltreatment to the permanent alteration of the HPA axis and specifically here to epigenetic modifications of the NR3C1 promoter region. Glucocorticoids are the final effectors of the HPA axis and exert their activity in part via GR. The effect of negative feedback of glucocorticoids on adrenocorticotrophic hormone secretion is to limit excessive glucocorticoid exposure.⁹ Interestingly, childhood abuse has been associated with an increased adrenocorticotrophic hormone response to stress.³¹ It is hypothesized here that excessive methylation of the NR3C1 promoter region as a consequence of childhood maltreatment permanently disturbs the expression and thus the availability of the GR in the brain and thus alters normal glucocorticoid negative feedback on adrenocorticotrophic hormone.^{14,15,17} This long-lasting effect on the HPA axis is possibly one mechanism by which early adverse events may have an impact on adult psychopathology.^{3,5,31-34} Hypothetically, this is one mechanism linking childhood maltreatment to the development of adulthood BPD.

With the exception of CpG1, methylation at all the other CpGs showed an association with childhood maltreatment. The sites we explored were located a few nucleotides downstream of the specific ones reported by McGowan *et al.*¹⁹ to have increased methylation levels in subjects with childhood sexual abuse. These results, as well as those obtained by Oberlander *et al.*¹⁸ could suggest that differences in methylation levels may occur only at specific sites in the exon 1_F NR3C1 promoter, sites located near the nerve growth

factor inducible protein A recognition elements. This clearly requires investigation in further studies.

Our data showed that other forms of abuse and neglect, such as physical neglect, may have an effect on gene methylation and that the number of types of abuse/neglect strongly correlated with methylation percentage. Childhood maltreatment research has primarily focused on the effect of childhood sexual abuse and physical abuse whereas childhood emotional abuses and neglects as well as physical neglects have received little attention. Several studies are now suggesting that childhood emotional abuse may be as predictive of risk for adult psychopathology as sexual and physical abuse.³⁵ Interestingly, Teicher *et al.*^{29,30} have shown that exposure to multiple forms of maltreatment had a greater effect than additive deleterious effect.

The association between repetition of abuses and *NR3C1* methylation status is of particular interest, as most of the data suggest that exposure to abuse and other early life events is associated with an increased risk for subsequent abuse.³⁶ This raises the question as to whether *NR3C1* epigenetic modifications are the cause or consequences of the abuses. Ideally, a sample consisting of subjects who were sexually abused or maltreated in their childhood but have no adult psychopathology may help to answer this question.

We showed that the type of sexual abuse (sexual abuse with or without penetration), and the severity of these abuses were associated with increased methylation status. It has been shown that the total brain volume positively correlated with age of onset of trauma-related PTSD and negatively correlated with the duration of abuse,³⁷ suggesting that severity of abuse is a strong correlate of biological modification in humans. Some data have shown that sexual abuse with penetration, severity and certain type of abuses (the use of force or threats, longer duration and frequency of abuse, repetition of abuses) are linked to worse outcome in adulthood.²¹ Our data are therefore concordant with this literature and suggest that the degree of *NR3C1* promoter methylation may be a marker of the severity of these abuses.

Surprisingly, BPD patients with no sexual abuse as well as those with no childhood maltreatment have higher *NR3C1* methylation than MDD subjects with the same reported abuses and neglect in infancy. This could be related to the fact that BPD could be *per se* linked to higher methylation status than MDD alone, or that other environments were not carefully considered in this study, while explaining the higher percentage of methylation in this population. Maternal anxious/depressed mood during pregnancy has been shown to correlate with *NR3C1* methylation in newborns.¹⁸ Moreover, maternal exposure to intimate partner violence during pregnancy predicted the methylation status of *NR3C1* in adolescent children.²⁰ The very early environment, which was not assessed in our study, may also explain higher methylation in BPD subjects. In the rodent model, variations in early maternal care are associated with differences in HPA stress response in the offspring and with epigenetic alterations in gene expression.^{14,17} Moreover, increased HPA stress reactivity in the offspring of mothers exhibiting decreased levels of maternal care appears to be mediated by decreased hippocampal *NR3C1* expression.^{17,38} BPD subjects often

have dysfunctional parents who sometimes suffer from BPD themselves.³⁹ The hypothesis that BPD subjects have higher methylation status than MDD because of a very early adverse environment makes sense.

Although the severity of BPD correlated with sexual abuse (see Supplementary Information), it did not correlate with methylation percentage and thus we cannot conclude that methylation status is an indirect extrapolation of the severity of psychopathology in adulthood. The link may be more complex, as genes not explored in this study may be at play in the construction of this complex phenotype.⁴⁰

Another limitation is that we used peripheral blood cells as study material. Although it has been shown that *NR3C1* exon 1_F is detected in peripheral blood (in CD19 B lymphocytes and plasmacytoid dendritic immune cells),⁴¹ this results and those from previous studies⁴⁰ imply that childhood maltreatment modulates its methylation in the same way as in the brain. Study of methylations of the same CpGs in peripheral blood and brain tissues from the same subjects are required to confirm that peripheral blood may be used as a proxy for methylation processes occurring in the brain.

Finally, it remains to be demonstrated that *NR3C1* exon 1_F methylation is also associated with decreased transcription in peripheral blood, as previously shown by McGowan *et al.*⁴⁰ in human brain.

Nevertheless, and as suggested by Philibert *et al.*,⁴² major life stressors should impact both on peripheral and central tissues and result in correlated changes across multiple cell types. Despite these limitations, it is not unreasonable to hypothesize that our findings in peripheral blood might reflect changes in cell brains.

GR has an important role in multiple physiological and pathophysiological processes including response to glucocorticoids as treatment used in a number of inflammatory, autoimmune and neoplastic diseases. If and how the methylation processes observed in our study have an impact on peripheral GR functions, such as treatment response, requires further investigations.

This is the first human study to show an association between a wide range of childhood trauma and the methylation status of the promoter and exon 1_F of the gene for GR (*NR3C1*) in peripheral blood in adults. Moreover, it shows for the first time the link between methylation status and the severity and type of childhood maltreatment. Although this result may be striking, it is in line with some data suggesting that the severity of childhood abuses is associated with poor mental health outcome in adulthood and indicating that the degree of *NR3C1* promoter methylation may be a surrogate marker of maltreatment severity. The quality of parental care and of early life environment has developmental effects on brain development¹² including disturbance of the HPA axis and exerts a broad impact on later mental health, including the risk of psychopathology. Our findings temptingly suggest that epigenetic processes might mediate the effects of these early environments on psychopathology into adulthood through effects on HPA axis activity.

Conflict of interest

The authors declare no conflict of interest.

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