The link between parental psychological control, depressive symptoms and epigenetic changes in the glucocorticoid receptor gene (*NR3C1*)

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Abstract

Aims: This paper examines the relationship between parental Psychological Control (PC) and depressive symptoms in adolescents and assesses whether this relationship was mediated by DNA methylation, focusing on the glucocorticoid receptor gene (*NR3C1*), which plays a crucial role in HPA-axis functioning and is linked to environmental stress and depression. This is among the very few studies that looked at the relation between DNA methylation, environmental stress and depression in family trios.

Methods: The study cohort consisted of 250 families: father, mother and a biologically related adolescent (adolescents (48.9% boys), mean age: 15.14, SD= 1.9; mean age mothers: 45.83, SD= 4.2; mean age fathers: 47.77, SD= 4.7). Depressive symptoms and PC were measured in adolescents and in both parents. DNA methylation levels in *NR3C1* were examined in all participants.

Results: Depressive symptoms in adolescents were predicted by PC of both mothers and fathers. Moreover, maternal depressive symptoms were associated with maternal PC, and fathers' depressive symptoms and PC. In fathers, only the level of their self-reported PC was associated with their depressive symptoms. There was no relation between adolescents' DNA methylation and depressive symptoms or the level of parental PC. Yet, there was a significant association between maternal depressive symptoms and maternal epigenetic patterns in *NR3C1*.

Conclusions: These findings highlight the need for more research in order to better understand the biological and contextual mechanisms through which parenting and parental emotional well-being is related to the development of psychopathology.

Keywords: NR3C1, depressive symptoms, family, epigenetic, parenting

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Introduction

Depression is among the most commonly diagnosed mental health disorders worldwide (1) and is associated with a number of adverse social and health outcomes (2, 3). Epidemiological studies have shown that the onset of depression is preceded by the number and severity of adverse stressful life events in both adolescents and adults (4, 5, 6, 7). Furthermore, the family environment has been shown to play a crucial role in the development and maintenance of depression in youth (8, 9, 10), but also in adults (11, 12, 13).

Indeed, it has been shown that specific parenting behavior, such as high Psychological Control (PC), as well as increased parental depressive symptoms, are associated with elevated depressive symptoms in adolescents (14, 15, 16, 17). Depressive symptoms in parents have been shown to relate to increased parental hostility and disengagement (i.e., less warmth and more withdrawal) (18). Therefore, PC and increased parental depressive symptoms may be important contextual stressors for developing children (19, 20).

On the biological level, the response to contextual stressors is regulated by two major stress response systems, among which the Hypothalamic–Pituitary–Adrenal (HPA) axis plays a crucial role (21). Changes in HPA-axis functioning have been implicated in the pathogenesis of depression in both children and adults (22, 23, 24). Interestingly, it has also been shown that parental PC is associated with dysregulation of the HPA-axis in children (25), which suggests that parental PC can negatively affect the development of the HPA-axis and can serve as a risk factor for developing depression later in life.

In recent years, epigenetic modifications in stress response system related genes have been proposed as a possible mechanism linking adverse social environment and biological adjustments to the development of depression (6, 26, 27, 28). One of the most studied epigenetic modifications is DNA methylation, which is considered to be a part of broader epigenetic changes (29). More specifically, it has been shown that the DNA methylation status of the glucocorticoid receptor gene (*nuclear receptor subfamily 3, group C, member 1: NR3C1*), that encodes the glucocorticoid receptor (GR), which plays a crucial role in the HPA-axis functioning), is associated with depression, childhood adversities, social stress, and abnormal stress reactivity (30, 31, 32, 33).

There are still multiple gaps within the literature on the effects of parenting practices, parental and child depressive symptoms and the possible underlying epigenetic mechanisms.

First, the field of behavioral epigenetics is relatively new (34) and findings in this area have often been inconsistent (35, 36).

Second, parenting practices may also relate to the emotional and mental well-being of parents themselves (37). Studies have indicated that parenting has been associated with depressive symptoms in the parents of young children (38, 39). In addition, increased levels of parental stress have been linked to changes in cortisol regulation and health complications in mothers (40, 41). However, the literature on the effect of parenting on parental mental well-being is still scarce and most published studies focus on the parents of younger children and much less is known about this effect when children move into adolescence – a period associated with increased parental stress (42). This particularly holds for fathers, as they are often neglected in studies on the intergenerational transmission of depression where the focus is primarily on mothers (43, 44).

Finally, despite the dearth of research on fathers in this area, there is evidence to suggest that increases in both paternal and maternal depressive symptoms tend to co-occur during the early years of child life (45, 46). Whether this is also the case in adolescence is largely unknown, again stressing the need for more research on the effects of depressive symptoms and parental practices of both parents within the family context.

Therefore, in the current study we, examined associations between depressive symptoms of mothers, fathers and their adolescent child, psychological control, and patterns in DNA methylation of the *NR3C1* gene in parents and children using data from a cross-sectional study of 263 families (i.e., mother, father, and biological child).

Specifically, we investigated the following hypotheses:

First, we expected that adolescents' depressive symptoms would be predicted by (a) parental depressive symptoms, (b) psychological control of both parents and (c) adolescents' DNA methylation of the *NR3C1* gene. Second, we expected that depressive symptoms of parents would be predicted by (a) depressive symptoms of the partner and child, (b) self-reported parental psychological control and (c) DNA methylation of the *NR3C1* gene of the parent. For parents, models were tested for mothers and fathers separately. Finally, we expected that for both adolescents and their parents, the relationship between parental PC and depressive symptoms might be mediated by their own *NR3C1* DNA methylation (i.e., high level of parental PC will lead to changes in *NR3C1* DNA methylation associated with depressive symptoms).

Materials and Methods

Participants

The study cohort consisted of 263 Belgian Dutch-speaking families with a biologically related adolescent (adolescents: 48.9% boys). The families were recruited from a community sample across Flanders, Belgium. Exclusion criteria were families with members who had a serious medical illness (e.g., cancer, recent physical injury or physical disability). This study was approved by the ethical committees of the KU Leuven and Ghent University, for details please see our previous report (47).

Measurements

Depressive symptoms

Depressive symptoms were measured in adolescents with the Children's Depression Inventory (CDI) (48) and in both parents with the Beck Depression Inventory-II (BDI) (49). Responses were summed with higher total scores indicating greater levels of depressive symptoms.

Psychological control

Parents- and adolescent-reported degree of psychological control towards the adolescents was measured with the self-assessment Psychological Control Scale (PCS), (50), 8-items. The PCS is widely used to assess the extent to which parents engage in psychologically controlling parenting behaviors (i.e., love withdrawal and guilt induction). Greater levels indicate higher parental psychological control. The mean score was calculated and used in further analysis.

DNA methylation in the glucocorticoid receptor gene (NR3C1)

DNA methylation level in (a part of) exon 1F of the *NR3C1* gene was analyzed via Pyrosequencing. This region was reported to exhibit variation in methylation patterns in relation to depression, childhood adversities, social stress, posttraumatic stress disorder and stress reactivity (31, 51, 52, 53, 54, 55). In this study we have used DNA from saliva. It was shown that methylation levels generated using salivary DNA resemble DNA methylation patterns in the brain and blood, which makes saliva a suitable material for analysis in studies of psychiatric traits (78).

First, DNA was extracted from saliva samples (Oragene DNA sample collection kit; DNA Genotek Inc., Canada) and bisulfite converted following the manufacturer's protocol (EZ-96 DNA Methylation Kit (Zymo Research, USA)). Next, the levels of methylation at eight CpG sites of the *NR3C1* were determined using Pyrosequencer (56). Protocols for the PCR amplification and Pyrosequencing analysis were adapted from Perroud and colleagues (57). Details are available in the *Supplementary Information*.

Statistical analysis

Scores on the CDI, BDI and DNA methylation values were skewed towards the lower levels, therefore they were log transformed.

First, correlation analyses were used to examine associations between depressive symptoms, perceived psychological control and DNA methylation in *NR3C1*. Results are presented in the *Supplementary Information*. Next, the individual effects of family members depressive symptoms, psychological control and the level of the DNA methylation on self-reported depressive symptoms were tested using linear regression models.

In all regression models, age was entered as a covariate, as it has been associated with both depressive symptoms and DNA methylation. All statistical analyses were carried out with SPSS 21.0 software and in R (58, 59).

Results

Descriptive statistics

Descriptive statistics of CDI, BDI, PC and demographics are reported in Table 1. The descriptive statistics of the mean DNA methylation for adolescents, mothers and fathers for the eight CpG sites of the *NR3C1* are available in the *Supplementary Information*. Girls had elevated levels of depressive symptoms compared to boys ($t = -2.07$; $p < 0.03$). No other mean level differences were found on any other of the study variables.

Table 1 *Descriptive statistics of the main study variables*

Variable	*N*	*Mean*	*Min*	*Max*	*SD*
Age adolescents	*251*	15.1	11.74	18.8	1.98
Age mothers	*246*	45.7	33.84	56.55	4.28
Age fathers	*247*	47.7	33.49	62.74	4.75
CDI scores, adolescents	267	9.06	0	35	5.55
BDI scores, mothers	267	6.59	0	48	6.70
BDI scores, fathers	266	5.51	0	40	5.77

PC, reported by father	*251*	1.93	1	4	0.56
PC, reported by mother	*251*	1.97	1	3.75	0.54
PC of father, reported by adolescent	*251*	1.7	1	3.88	0.59
PC of mother, reported by adolescent	*249*	1.6	1	4.25	0.66

Note. PC – Phycological Control reported by parents; CDI – depressive symptoms of adolescents; BDI – depressive symptoms.

Depressive symptoms in adolescents, Parental PC and depressive symptoms

For adolescents, correlation analysis revealed a significant positive association between depressive symptoms and PC of both parents as reported by adolescents (for boys, fathers PC: $r = .32$, $p < 0.01$, mothers PC: $r = 0.19$, $p < 0.05$; for girls, fathers PC: $r = 0.34$, $p < 0.01$, mothers PC: $r = 0.32$, $p < 0.01$). There was no association between adolescents' methylation of *NR3C1* and parental PC as reported by adolescents or adolescents' depressive symptoms (see Supplementary Information for the full zero order correlation tables). These results indicate that basic requirements for mediation were not fulfilled.

Next, we examined if adolescent' depressive symptoms could be predicted by parental psychological control as reported by the adolescent and by parental depressive symptoms (see Table 2.1 and Table 2.2; scatterplots of the association between depressive symptoms and PC are available in *Supplementary Information)*.

Overall models for paternal as well as maternal effects on adolescent depressive symptoms were significant ($F[7.3, 4] = 186$, $p < 1.56\times10^{-5}$; $F[5, 4] = 199$, $p < 0.0007$). Results revealed that when predicting adolescent depressive symptoms, there was a significant effect of both PC of mother ($\beta = 0.20$, $p = 0.003$) as well as PC of father ($\beta = 0.26$, $p = 0.0002$). However, there was no effect of parental depressive symptoms on the level of adolescents' depressive symptoms.

Table 2.1 *Regression results predicting depressive scores of adolescents from maternal PC reported by adolescent and self-reported depressive symptoms of mother*

Predictor	*β*	*beta* 95% CI [LL, UL]	*r*	*p-value (predictors)*	Model Fit
Age (ado)	0.18	[0.05, 0.32]	0.20	0.007	*p-value = 0.0007*
Gender	0.10	[-0.03, 0.23]	0.09	0.145	$R^2 = 0.091$
PC (ado) of mother	0.20	[0.06, 0.33]	0.22	**0.003**	*Adjust.* $R^2 = 0.073$
BDI, mothers	0.04	[-0.09, 0.17]	0.04	0.564	*F-stat.: 5 on 4 and 199 DF*

Note. PC (ado) – Phycological Control reported by adolescents; *beta* indicates the standardized regression weights; *r* represents the zero-order correlation; *LL* and *UL* indicate the lower and upper limits of a confidence interval, respectively.

Table 2.2 *Regression results predicting depressive scores of adolescents from paternal PC reported by adolescent and self-reported depressive symptoms of father*

Predictor	*beta*	*beta* 95% CI [LL, UL]	*r*	*p-value (predictors)*	Model Fit
Age (ado)	0.15	[0.01, 0.28]	0.16	0.033	p-value = 1.56×10^{-5}
Gender	0.21	[0.08, 0.35]	0.19	0.002	R^2 = 0.135
PC (ado) of father	0.26	[0.12, 0.40]	0.26	0.0002	Adjust. R^2 = 0.118
BDI, father	0.09	[-0.05, 0.22]	0.07	0.192	*F-stat.: 7.3 on 4 and 186 DF*

Parental depressive symptoms, Parental PC, and depressive symptoms of spouse and child

For both parents, correlation analysis revealed no association between DNA methylation and PC as reported by parents. For fathers, there was no association between depressive symptoms and DNA methylation. For mothers, there were a negative correlation between depressive scores and DNA methylation at one specific CpG site, CpG47 (r = -0.18, p < 0.01), (see Supplementary Information for the full zero order correlation tables, as well as for schematic representation of *NR3C1* gene structure and CpG numbering).

In the following models, we investigated if depressive symptoms of parents can be predicted by their parental attitude, by depressive symptoms of their spouse and, in case of mothers, by DNA methylation status at CpG47. Results of linear regression models for parental depressive symptoms are available in Table 3.1 and Table 3.2.

In brief, overall models were significant (predicting depressive symptoms of mother: F[6.65, 6] = 152, p = 6.84×10^{-6}; of father: F[2.3, 5] = 161, p = 0.047). For both parents the self-reported level of PC was associated with their depressive symptoms and this effect was much stronger for mothers (for mothers: β = 0.32, p = 9.43×10^{-6}; for fathers: β = 0.18, p = 0.022). For mothers, depressive symptoms of spouse (β = 0.32, p = 0.023) as well as the level of PC of the partner (β = -0.17, p = 0.039) and the level of DNA methylation at CpG47 (β = -0.20, p = 0.005) were also significantly associated with maternal depressive symptoms. For fathers, depressive symptoms of spouse showed a trend towards significance (β = 0.16, p = 0.052). For both parents, the level of adolescents' depressive symptoms was not predictive of parental depressive symptoms.

Table 3.1 *Regression results predicting depressive symptoms of mothers from self-reported PC; depressive symptoms of father and self-reported PC of father*

Predictor	*beta*	*beta*	*r*	*p-value*	Model Fit

		95% CI [LL, UL]		*(predictors)*	
Age, mother	0.06	[-0.09, 0.21]	0.02	0.375	p-value = 6.84×10^{-6}
PC, mother	0.32	[0.20, 0.49]	0.31	**9.43×10^{-6}**	R^2 = 0.198
BDI, father	0.17	[0.02, 0.32]	0.14	**0.023**	Adjust. R^2 = 0.166
PC, father	-0.17	[-0.31, -0.01]	-0.11	**0.039**	*F-stat.: 6.25 on 6 and 152 DF*
CpG47	-0.20	[-0.35, -0.06]	-0.23	**0.005**	
CDI (adolescent)	-0.07	[-0.23, 0.08]	-0.02	0.336	

Note. PC – Phycological Control reported by parents; BDI – depressive symptoms; CpG47 – DNA methylation at CpG47; CDI – depressive symptoms of adolescents; beta - indicates the standardized regression weights; r - represents the zero-order correlation; LL and UL indicate the lower and upper limits of a confidence interval, respectively.

Table 3.2 *Regression results predicting depressive symptoms of father from self-reported PC; depressive symptoms of mother and self-reported PC of mother*

Predictor	*beta*	*beta* 95% CI [LL, UL]	*r*	*p-value (predictors)*	Model Fit
Age, father	-0.15	[-0.31, 0.00]	-0.12	0.050	p-value = 0.047
PC, father	0.18	[0.03, 0.34]	0.15	**0.022**	R^2 = 0.066
BDI, mother	0.16	[-0.00, 0.32]	0.11	**0.052**	Adjust. R^2 = 0.037
PC, mother	-0.10	[-0.27, 0.06]	0.00	0.219	*F-stat.: 2.3 on 5 and 161 DF*
CDI (adolescent)	-0.06	[-0.10, 0.21]	-0.02	0.464	

Mediational analyses

We also ran a mediation analysis (60), where we looked if the effect of psychological control on depressive symptoms was mediated by the sum score of *NR3C1* DNA methylation. The models were built separately for mothers, fathers and adolescents. For mothers, we also looked at the possible mediating effects of DNA methylation at CpG47, as this CpG site was significantly associated with the level of maternal depressive symptoms. The results showed no evidence for mediation effects, for details please see *Supplementary Information*.

Discussion

Although a reasonable consensus exists about the importance of family for mental well-being of both children and parents, only a limited number of studies have focused on family trios, especially when it comes to the potential involvement of biological mechanisms sensitive to social environment, such as epigenetic modifications. To our knowledge, no previous research has looked at the level of DNA methylation in the *NR3C1* gene, depressive symptoms and the level of Psychological Control in both children and parents.

Research has quite consistently shown that parental depression negatively affects children's mental health and socio-emotional development (9, 61). Yet, in this study we found no evidence for direct associations between levels of depression in adolescents and their parents. The absence of such associations could be due to a number of reasons. First of all, the majority of studies on the influence of parental depression mostly focus on young children (i.e., babies or toddlers) of depressed mothers, where parental depressive symptoms during early years of development were linked to long lasting negative outcomes in children (46, 62, 63). However, much less is known about the influence of current parental depressive symptoms on adolescents' mental well-being. Our results suggest that during adolescence this influence might not be strong and other factors contribute to youngsters' depressive symptoms. Secondly, given the non-clinical nature of our sample and skewness of CDI and BDI variables, it is worth noting that the association between parental and adolescents' depressive symptoms might be different in clinical cohorts.

Next, we looked at the relation between the level of parental psychological control and adolescents' depressive symptoms. Parental psychological control is a well-known risk factor for children's and adolescents' depressive symptoms (17) and is associated with increased contextual stress. In the developmental and parenting literature most studies focus on the influence of mothers and therefore the studies regarding father-child relationship are rare (61). One of the results in our study was that psychological control of both mother and father significantly predicted the depressive symptoms of adolescents, which is in line with a recent study highlighting the importance of paternal parenting for adolescents (47). This finding is important, as it supports the need for inclusion of fathers in both research and parental training programs, as the contribution of the father to adolescent mental well-being might have been largely underestimated. (64).

In previous studies it has been shown that parents' attitude towards their child is not independent from their mental well-being (65). Therefore, we also investigated the relationship between the level of depressive symptoms of both parents and their level of psychological control. We found that increased parental psychological control was associated with higher level of depressive symptoms in parents. Interestingly, this association was much stronger for mothers and was relatively weak for fathers. This finding might reflect that, for mothers parenting and the interaction with a child have a strong impact on mental well-being, even when the child transitioned to adolescence.

In our sample, depressive symptoms of a spouse were associated with depressive symptoms of their partner. In addition, for mothers the level of psychological control of their partner was also significantly associated with maternal depressive symptoms. These findings support the notion that the individual family environment can affect one's mental well-being, in line with family system theory (66, 67, 68, 69). Also, this emphasizes the need to include data from both parents in psychological, behavioral as well as molecular genetic research.

In this context, over the past decades a rapidly growing literature suggests that epigenetic mechanisms, such as DNA methylation, in stress system related genes are associated with depression, adverse life events and early life stress (35, 70, 71, 72). However, this literature is mostly based on animal studies, and research in humans appears inconsistent.

In the current study we did not find associations of adolescents' depressive symptoms with methylation of *NR3C1* (1F). This is in contrast to findings from other studies (31, 72). We also found no association between the level of parental psychological control and DNA methylation patterns in the analyzed part of the *NR3C1* gene. This may mean that parental psychological control does not directly elicit the level of stress that can lead to the epigenetic changes in the *NR3C1* gene, but rather contributes to a general level of contextual stress. This also suggests that broader measurement of contextual stress might be required to capture the level of stressful life experiences that can trigger the epigenetic changes in the stress system related genes. Such measurement may consist of a score that would represent not only parent-child interactions, but also other aspects of a person's immediate environment, such as relationships with friends or the level of daily stress.

In this study, lower DNA methylation at one CpG site (CpG47) in the *NR3C1* gene was associated with severity of depressive symptoms in adult women. Theoretically, this result may be related to the gender-specific physiological mechanisms in depression (73, 74) and in biological stress response pathways (75), which is in line with general knowledge of gender differences in vulnerability to depression (1).

Interestingly, major depressive disorder (MDD) patients had lower DNA methylation at two CpG sites (*CpG45 and CpG46 as numbered in our study*) and changes in the DNA methylation level were associated with hippocampal subfield volume (32). In the same study lower DNA methylation at CpG47 was associated with perceived stress in healthy subjects. Similar findings of lower *NR3C1* DNA methylation (*CpGs39–47 average*) were reported for MDD patients (33). In another study, lower level of DNA methylation at the same *NR3C1* region

that was analyzed in our study was associated with a history of childhood adversity and current or past depressive or anxiety disorders in a general population (76). Our results are in line with evidence from the aforementioned studies, which may suggest that reduced DNA methylation at some CpG sites in the *NR3C1* gene, might be associated with depressive symptoms.

However, it should be kept in mind that findings regarding the direction of DNA methylation effects in depression and stress research are mixed. A number of studies indicate increased *NR3C1* DNA methylation levels in association with depression and environmental stress (for details please see the following reviews: 6, 35). This inconsistency might be related to the complexity of *NR3C1* gene regulation and might point to the fact that this gene is regulated in a more site-specific manner.

It is important to note, that the findings of the present study should be considered within the context of its limitations. Notably the use of self-reports to assess both depressive symptoms and psychological control can introduce certain types of re-call bias and the cross-sectional design, does not allow to draw conclusions about the direction of causality. Next, in this study there was no data on lifetime history of parental depression. Therefore, it might be interesting to examine the influence of lifetime parental depressive symptoms on their parental practices and adolescents' mental well-being. Moreover, considering the previous findings on epigenetics and lifetime history of depression, it also will be meaningful to examine associations between lifetime parental depression and the level of DNA methylation in the *NR3C1* gene, especially for mothers. We also did not measure the level of contextual stress (such as work-related stress for parents or level of daily hassles) which could be contributory. Lastly, it is important to note, that the DNA methylation levels were only analyzed in a relatively small part of the *NR3C1* gene and therefore, it is difficult to generalize our finding towards the epigenetic modifications in the whole *NR3C1* gene. Lastly, it is worth mentioning that, biologically, *NR3C1* DNA methylation is relatively low and therefore, analysis of this region is more prone to technical errors. However, it was also shown that the level of DNA methylation in the part of the *NR3C1* gene analyzed in our study is associated with gene expression (33, 52, 77), suggesting that even small changes in the level of DNA methylation can affect gene regulation. Still, more studies are needed to validate these results.

To sum up, in the current study parental psychological control of both father and mother is predictive of the level of adolescent's depressive symptoms, but parental depressive symptoms are not. In addition, we showed that in parents their depressive symptoms are

associated with the self-reported level of psychological control, as well with the depressive symptoms of the spouse. This association was much stronger for mothers, for whom depressive symptoms were also related to epigenetic modifications of the *NR3C1* gene.

In conclusion, through this study, we address questions regarding associations between parental psychological control, the level of depressive symptoms, and the *NR3C1* DNA methylation in families with adolescents. Future studies with longitudinal designs and more in-depth measurements of contextual stress should be considered in order to understand the role of epigenetic mechanisms in the development and maintenance of depressive psychopathology.

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