The Effects of Stressful Life Events on Epigenetic Modification: An Association Between the FKBP5 gene and the Onset of Mental Disorders

A Systematic Review and a longitudinal Data Analysis

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Abstract: This dissertation consists of two parts. This first part serves as a literature review integrating studies measuring DNA methylation in a variety of genes following recent and/or chronic life adversity in children and adults. The objective was to understand if the experience of stressful life events is related to DNA methylation. The results confirm the epigenetic influence of stress, although its degree varies depending on the individual regardless of his/her developmental stage. The second part is devoted to analyzing data from a unique longitudinal cohort of Swedish twins at two-time points to see if life adversity is related to changes in methylation in the *FKB5* gene. **Results:** The findings suggest that stressful events change the course and methylation in the *FKBP5* gene (predominantly around the CpG site rs15929276) and that this could provoke mental dysfunction. **Conclusions & recommendations:** There seems to be a reciprocal causality between psychopathology and the 'gene X environment' interaction. We need to better understand the complex interplay between internal and external influences in order to ameliorate preventive interventions based on sufficient genetic and mental history screening.

Introduction

The discourse regarding the interaction between stressful life events and genetic variation has been in the spotlight for more than six decades (Dick, 2011). For instance, it has been suggested that early stressful experiences may interact with our genetic composition and thereby alter the susceptibility for developing psychiatric disorders (Belsky & Pluess, 2009). Consequently, we know that some psychological traits, including the stress response, is regulated by both nature (genetics) and nurture (life events) (Zannas & West, 2014). Moreover, research shows that stress-induced epigenetic modification can be transmitted to subsequent generations even if these individuals (from these generations) are not directly exposed to any stressful events (Versmissen, Lennep, & Sijbrands, 2014).

Several theories have been proposed to explain the phenomenon of epigenetic alternation following early life adversity. In 1960, Paul Meehl introduced the *diathesis-stress model* to explain the onset and heritability of schizophrenia (McCutcheon, 2006). According to this model, there must be a genetic predisposition for vulnerability to a certain disorder. When one who carries a psychotic predisposition (an amalgam of alleles and environmental influences) confronts a very stressful situation (sexual abuse or sudden loss for example) he/she may develop schizophrenia or another psychotic-spectrum disorder (Walker & Diforio, 1997). In other words, a combination of environmentally detrimental factors with a certain (mal)inherited gene or with a dysfunctional and complex genetic composition may unfold a full-blown mental disorder (Belsky & Pluess, 2009).

The term *epigenetics* was first introduced by Conrad Waddington in 1942 to define the processes by which genotype gives rise to phenotype (Jobe & Zhao, 2017). This concept refers to functional and operational changes in the DNA due to phenotypical variations (observable differences) following, for instance, life adversity (Kanherkar, Bhatia-Dey, & Csoka, 2014). At the molecular level, the term “epigenetics” reveals gene regulatory mechanisms that are mediated by biochemical modifications of genomic DNA (Zannas & West, 2014). The most well-known and well-studied epigenetic transcriptional regulatory mechanism is called DNA-methylation. Methylation is a process whereby adding or deleting a methyl group to the DNA, the activity of that DNA segment can be altered (Berger, Kouzarides, Shiekhattar, & Shilatifard, 2019). This process is central to this dissertation.

There are two main approaches to uncover an epigenetic predisposition to disease or potentially methylated DNA segments: 1. The first one is based on targeting candidate genes (CG) and 2. the other one is based on testing the entire genome (genome-wide association (GWA)) (Amos, Driscoll, & Hoffman, 2011). The former has the advantage of exhibiting high statistical power but lacks the ability to track down new genes or the combination of specific genes, whereas the latter (GWA), is effective to target new genetic combinations, however, it is not supported by high statistical power (Amos et al., 2011). In this dissertation, I want to examine the associations between DNA-Methylation and environmental stressors on a gene-wide scale (e.g. including the total genetic coverage and not only single nucleotide polymorphism-SNP's), which will combine the strengths of both candidate gene and genome-wide methods.

This dissertation consists of two parts with two respective research questions that lead to an overall conclusion. The first part of this dissertation serves as a systematic review in which I broadly describe which genes or CpG sites have been most frequently reported to be susceptible to the effects of stress. Specifically, we strive to integrate studies measuring the overall effects of life stressors on the entire genome. I hypothesize that stressful events may provoke epigenetic effects that persist into adulthood. Thus, the first research question is: *"Is the experience of stressful life events related to DNA methylation in adults"?*

The fundamental contribution of genetic susceptibility and environmental risk factors to the development of mental disorders has been well established (Dick, 2011). Therefore, the purpose of this dissertation is to further highlight the necessity of considering the interaction between environmental and genetic factors. This interaction is crucial for a correct diagnosis as well as for targeting specific genes that are more vulnerable to environmental influences. Thus, the mental health care community (researchers and practitioners) can benefit from a potential classification of genes that are vulnerable to aversive events and, thus, formulate better treatment or intervention plans that facilitate the prevention, the prognosis or the rehabilitation of a given disorder. Albeit that stressors that occur early in development have more enduring effects than stressors that occur in adulthood (Zannas & West, 2014), it is also important to estimate the runoff of recent life adversity. Therefore, since most epigenetic studies consider only early life stressors, the relevance and utility of this dissertation lie in the inclusion of studies investigating genetic modification following both early and chronic life adversity in adults. Since studies on recent

stressors are largely lacking, there is a growing need to track down epigenetic effects throughout the development (daily stressors or mental disorders). In addition, since most molecular studies on gene X environment interaction has focused on rodents, this paper will only integrate studies on human samples in the first part and will analyze epigenetic data from actual patients in the second part.

The second part is devoted to investigating a specific gene that has been found to be susceptible to the effects of environmental stressors. I decided to examine a co-chaperone of the glucocorticoid receptor (CG) that seems to be susceptible to external influences and to the development of mental disorders, namely, the *FKBP5* gene (Provencal1 & Binder, 2015). This specific gene has been found to be sensitive to environmental stressors and susceptible to the development of mental disorders like schizophrenia, bipolar disorder and post-traumatic stress disorder (PTSD) (Klengel & Binder, 2014). Specifically, several single nucleotide polymorphisms (SNPs) in *FKBP5*, have been shown to interact with childhood abuse to predict adult PTSD symptoms (Zannas & West, 2014). Thus, this study will strive to add upon the findings of pre-existing research and further specify the influence of epigenetics by providing statistical analysis on a unique longitudinal cohort. Briefly, the purpose of this second part is to empirically confirm the hypothesis that carrying the *FKBP5* gene serves as a predisposition to experience mental discomfort following life adversity and to bridge the gap between the study of molecular processes and stress vulnerability in humans.

To do so, I analyze longitudinal data gathered from a population-based twin study from Sweden. I hypothesize that stressful events regulate the methylation of the *FKBP5* gene which, in turn, may trigger the onset of mood and/or other mental disorders. Specifically, the second question I strive to answer is: *"Do aversive life events change the course and methylation of the FKBP5 gene resulting in mental dysfunction"?*

This dissertation concludes by discussing the contribution of the integrated studies in response to the first research question and by interpreting the results of the statistical analysis of the longitudinal study in response to the second research question.

Method (Part 1)

To answer the first research-question a systematic literature search was conducted. This review attempts to do two things: (a) Investigate the possibility of environmentally-induced DNA methylation in adults and (b) list a set of genes that are most susceptible to life adversity. There were several criteria for the selection of the research articles used for this review: 1. The core theme of every article must be the environment X gene interaction. 2. Both empirical research and review articles were used. The quality of the literature articles was assessed by the number of the integrated studies and the quality of the empirical articles was assessed based on the relevance and utility of the specific experiments in response to the research question. 3. The selected studies must examine the effects of epigenetics in the entire genome (GWA). 4. The integration of up-to-date articles is essential for the review part of this dissertation and, thus, studies conducted before 2010 were excluded. This did not apply for the introduction. 5. All studies must entail human samples. 6. The studies included must target the influence of epigenetics on mental and not on physical health. 7. The majority of life stressors have to be either recent or chronic. In addition, two studies must examine early adversity for comparison. 8. All articles must be in English. The assessment of the articles prior to selection was done by reading the abstract and discussion sections. When these two sections were not informative enough, further screening of the articles was conducted.

The search engines used were *SCOPUS* and *OVID.* The search was done by using the Boolean operator. The keywords below were linked to the subjects of epigenetics and DNA methylation caused by life adversity. The search terms used were:

(TITLE ((epigen OR methyl* OR genes) AND (stress OR environ* OR advers*) AND (adult OR neur* OR plastic*) AND (stressor OR methyl* OR envir*) AND (inter* OR gene* OR disor*)) AND TITLE-ABS-KEY ((epigen* OR methyl*) AND (stress OR 'life event')) OR ((plastic* OR brain OR modifi* OR epige*) OR (diath* OR gene OR stress OR interac*)) OR (recent OR adult OR chron* OR envir*)) AND PUBYEAR > 2010 AND DOCTYPE (ar) AND (LIMIT-TO (SUBJAREA, "PSYC")) AND (LIMIT-TO (SUBJAREA, "NEURO")) AND (LIMIT-TO (LANGUAGE, "English"))*

To provide a clear overview of the selection procedure, the figure below presents the precise numbers of the search results. Herein, you can see the total number of the relevant articles from both databases, the selected and non-selected studies, as well as the articles used for this review:

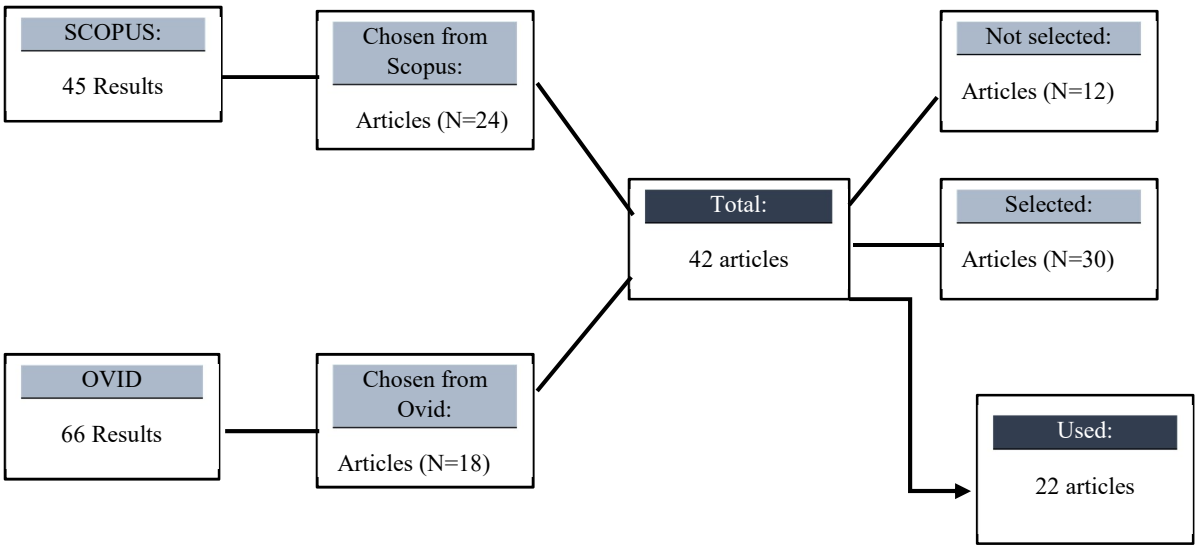

Figure 1. *Search results from Scopus and Ovid (March 2019).*

A total of 30 articles were reviewed for this dissertation. The post-search screening process was divided into two parts, one for the introduction (and discussion) and one for the literature review. For the introduction, the most important subjects were: the general theory of environment X gene interaction, an introduction to the diathesis-stress model, an introduction to the possible ways of measuring genetic modification (CG & GWA). For the review, I integrated studies measuring the effects of stress on epigenetics, studies measuring DNA methylation following trauma and finally, studies demonstrating the different functions of genes due to stress resulting in different genetic expressions. Specifically, I included two studies on children, three in adults and one measuring chronic stressors throughout the development (mainly adolescence). After the final division, single-case studies and book chapters were excluded and 22 articles, that met all the aforementioned criteria, were used for the introduction, discussion (16) and part 1 (6) of this dissertation. All 16 articles used for the introduction & discussion are literature reviews and all 6 used for the first part (review) are empirical studies. The remaining 8 articles were disqualified due to little or disoriented information. Furthermore, articles that focused on histone modification instead of DNA-methylation were also excluded. Last but not least, 7 more articles (not mentioned in the literature search) were used for the method of part 2 (SATSA cohort).

Results (Part 1)

The studies presented herein exhibit the overall effects of either early or chronic stress to the genome of traumatized and healthy adults while pretreating the most detectable genes *(see Table 1).* Kang, Kim, Choi, So, and Kim (2019) examined whether epigenetic changes in *FKBP5* are associated with stress exposure among veterans with combat trauma. They selected the *rs1360780* polymorphism on the *FKBP5* locus (Kang et al., 2019). 50% of the sample had already developed PTSD. The results show that veterans carrying the T allele, exhibit *FKBP5* methylation levels that are positively correlated with the severity of PTSD symptoms (Kang et al., 2019). There were significant main and interaction effects between the genotype and PTSD status on the *FKBP5* methylation level and, thus, we can infer that allele-specific DNA methylation level of *FKBP5* is involved in PTSD pathophysiology (Kang et al., 2019). Based on these findings, it is evident that intense stress resulting from complex trauma (war veterans with PTSD) can trigger epigenetic mechanisms and contribute to the onset or to the deterioration of a mental disorder in adults. Similarly, another study examined the interaction between the dopamine receptor D4 gene *(DRD4)* and the family environment of children with attention-deficit-hyperactivity disorder (ADHD) (Martel et al., 2010). They divided 548 kids into two groups (ADHD and non-ADHD) and collected data on their parents (via questionnaires) and perceived parenting behavior (via interviews from the children) as well as DNA samples. The results indicate that the interaction between *DRD4* genotype and inconsistent parenting was significant in predicting inattentive ADHD symptoms (Martel et al., 2010). In congruence with the previous study (confirming the contribution of epigenetics to pathophysiology), this study demonstrates that the *DRD4* gene is also responsible for 'pathogenesis' by interacting with environmental processes to shape the development of self-regulation abilities related to ADHD (Martel et al., 2010).

Apart from PTSD and ADHD, another disorder of even higher prevalence is ‘Major Depressive Disorder’ (MDD). In addition, the Catechol-O-methyl transferase *(COMT)* gene mainly determines prefrontal dopaminergic activity and has been linked to the development or maintenance of depression (Na et al., 2018). Consequently, Na et al. (2018) investigated the impact of *COMT* gene methylation status on prefrontal connectivity in a group of MDD patients and in a control group. Specifically, they measured *COMT* gene methylation and polymorphisms *(Val158Met)* by collecting peripheral blood samples (Na et al., 2018). According to the results,

patients with MDD exhibit significantly lower *COMT* methylation than control individuals (Na et al., 2018). This means that people who suffer daily stressors (MDD) exhibit lower prefrontal connectivity and, thus, impaired decision making. We can conclude that stress-related alterations in dopaminergic neurotransmission have negative effects for depressed individuals. This study is relevant to this dissertation because it is the first one to describe the impact of *COMT* gene methylation on prefrontal structural connectivity in adults with major depression.

Another gene that is susceptible to the risk of psychopathology is the human glucocorticoid (GR) receptor gene *NR3C1*. Only a few studies have investigated the environment X NR3CI interaction in humans. One study measured the effects of childhood adversity (maltreatment and parental loss) to the degree of methylation of the gene *NR3C1* (Tyrka, Price, Marsit, Walters, & Carpenter, 2012). They found that early parenting-induced stressors are associated with the methylation of the promoter region of *NR3CI* which, in turn, provokes alternations in the function of the hypothalamic-pituitary-adrenal (HPA) axis (Tyrka et al., 2012). Such alternations combined with epigenetic effects of the *NR3CI* gene early in development can predispose individuals to develop disorders like depression and PTSD (Tyrka et al., 2012). However, the function of this gene in response to stressors later in life remained a question. Consequently, Van der Knaap et al. (2014) conducted a longitudinal study examining *NR3C1* methylation following stressful events between birth and adolescence in a sample of "in risk" for psychopathology individuals. They found that the experience of multiple stressful life events (SLEs) and exposure to traumatic experiences was associated with higher *NR3C1* methylation rates in adolescents. What is striking is that, in contrast with previous findings, they found that SLEs in adolescence was a greater predictor for higher *NR3C1* methylation than perinatal or childhood stress. Thus, this outcome is in accordance with the relevance of this dissertation and supports its purpose to integrate more recent or chronic stressors to the study of epigenetics.

After consulting the studies above, it is apparent that environmental influences (combat trauma, parental behavior, daily stressors, SLEs) and genes interact to provoke, maintain or worsen the symptoms of a mental disorder (PTSD, ADHD, MDD, etc.). However, the last two studies provided conflicting results regarding the degree of DNA methylation in different developmental stages. Therefore, there is a growing need to better understand the relationship between both early and recent life stressors to genetic methylation and mental dysfunction. Dumanand and Canli

(2015) gathered a sample of healthy individuals to investigate the interaction between early, chronic and recent life stressors and the serotonin transporter-linked polymorphic region *(5-HTTLPR)* located in the promoter region of the serotonin transporter gene *(SLC6A4)*. This gene has been systematically found to be associated with mood or psychotic disorders (Dumanand & Canli, 2015). They administered questionnaires for all three types of stressors and gathered DNA samples. The results show that both early and recent life adversity modulate *5HTTLPR* methylation, however, different people react alternatively to stressors depending on their susceptibility or resilience to psychopathology (Dumanand & Canli, 2015). Thus, we can confirm that life adversity (at every stage) may influence epigenetic changes to the genome and trigger symptoms of mental disturbance in some people. Finally, the table below presents a list of genes that, based on this review as well as on previous findings, have proven to be vulnerable to stress.

Table 1.

List of genes susceptible to environmental stressors displayed in this review

Authors	Sample	Stressor	Assessment	Gene	Interaction	Finding
Kang et al. (2019)	Veterans	Combat trauma (PTSD)	Questionnaires, PTSD diagnosis and DNA sample	FKBP5 *(rs136078)*	Significant interaction between the *FKBP5* genotype and PTSD status	Allele-specific DNA methylation level of *FKBP5* is involved in PTSD
Martel et al. (2010)	ADHD kids	Invalidated parenting	Questionnaires, ADHD diagnosis and DNA sample	DRD4	Significant interaction between *DRD4* and inconsistent parenting	*DRD4* methylation predicts inattentive ADHD symptoms
Na et al. (2018)	MDD adults	Daily stressors	MDD diagnosis and DNA sample	COMT *(Val158Met)*	Patients with MDD showed significantly lower	Depressed individuals exhibit lower prefrontal

					COMT methylation	connectivity and impaired decision making
Tyrka et al. (2012)	Healthy kids	maltreatment and parental loss	Interview, questionnaires and DNA sample	NR3CI	Parenting-induced stressors provoke methylation of the promoter region of *NR3CI*	*NR3CI* methylation disturbs the HPA axis which influences the onset of PTSD and/or depression
Van der Knaap et al. (2014)	"In risk" teenagers	Stressful life events (SLEs)	Interviews and DNA sample for children and questionnaires for parents	NR3CI	Exposure to traumatic experiences increases *NR3C1* methylation	Adolescence is equally sensitive to epigenetic modification
Dumanand and Canli, (2015)	Healthy adults	All possible stressors throughout the development	Questionnaires and DNA sample	5HTTLPR *(SLC6A4)*	Both early and recent life adversity modulate *5HTTLPR* methylation	Stress provokes changes to the genome and mental disturbance regardless of age in some people

To conclude, and in response to the first research question, we can confirm that the experience of stressful life events is related to DNA methylation in adults, although its degree varies depending on the individual. In addition, these studies show that environmental stressors may influence the genome equally regardless of the developmental stage (Dumanand & Canli, 2015).

Method & materials (Part 2)

This study used methylation data of participants from the SATSA study (Swedish Adoption Twin Study of Aging; part of the Swedish Twin Registry-STR), a population-based national registry of old twins reared together or apart (Pedersen, 2005). The data collection took place between 1986 and 2000. This unique longitudinal cohort provides an opportunity to understand individual biological and psychological differences in aging (Pedersen, 2005).

Participants

Blood samples were collected from 288 participants (39.93% men) at two time-points (average age 66 and 73) in order to investigate the DNA methylation of the FKB5 gene following life adversity (before visit 2). All participants have provided written informed consent (Wang et al., 2018).

Methylation data

For each sample, 200 ng of DNA was bisulfite converted using the EZ-96 DNA MagPrep methylation kit. The Infinium HumanMethylation450 BeadChips by the University College London was used and DNA methylation levels of 485,512 CpGs were measured for each sample (Wang et al., 2018). In addition, raw methylation data were processed by using the R package RnBeads (Wang et al., 2018). Data for cellular compositions were estimated by the Houseman method using a blood cell reference panel (Wang et al., 2018). Furthermore, the Sammon mapping method was used to remove technical variance and preserve the original data structure (Wang et al., 2018). Last but not least, functional annotation of age-associated CpG sites was used. Finally, genotype data were generated by using the Illumina PsychChip (Wang et al., 2018).

FKBP5 gene

Using the UCSC genome browser (based on the assembly of NCBI36/Hg18), we defined the location of the FKBP5 gene as follows: chromosome 6, from basepair 35,649,345 to basepair 35,764,692. In total 22 CpG sites were identified in this genomic location.

Life events

The SATSA measure for life events was based on the 'Social Readjustment Rating Scale' (Saudino, Pedersen, Lichtenstein, Mcclearn, & Al, 1997). This scale targets periodic major life events (such as financial problems, severe illness of souse, physical abuse and/or the experience of loss, etc.) and it was administered at both time points. It includes 25 events and the subjects report whether each event has occurred or not. If yes, they rate their severity and intensity.

Covariates

Phenotypes used in this study include chronological age, sex, zygosity, cancer, cardiovascular disorder, psychiatric history, smoking habits, and BMI. Cancer and CVD were assessed by medical health records (Harris, Pedersen, Mcclearn, Nesselroade, & Plomin, 1992). Lifetime tobacco use was assessed by a series of questions in a self-reported questionnaire (Kendler, Thornton, & Pedersen, 2000). To target, identify and measure the psychiatric history of each twin we administered the 'State-Trait Personality Inventory' (STPI) to assess anxiety, the 'Older American Resources and Services' (OARS) for the depression subscale and the 'Mini-Mental State Examination' (MMSE) to assess cognitive decline caused by aging (Petkus, Gatz, Reynolds, & Pedersen, 2018). Heights and weights were reported by the twins themselves on questionnaires. To check for reliability, we weighted the subjects as well and calculated the correlation coefficient between measured and reported values (Stunkard, Harris, Pedersen, & Mcclearn, 1990). This way we obtained the most reliable BMI index.

Statistical analysis

Initially, a table with the baseline characteristics of the subjects was generated. We first conducted a *Pearson's correlation* between the differences of all CpG sites and life events before visit 2 to detect a possible association of the number of life events before visit 2 with the differences in methylation levels. Second, to investigate the potential influence of the number of the life events before visit 2 in the differences of the twenty-two sites a *MANOVA* test was performed. The dependent variables were all the 22 differences of the sites and the independent variables were the number of life events, gender, cancer, cardiovascular disorder, psychiatric history, and smoking habit and as covariates the BMI index and age at visit 2. The third and last step was to conduct a *Multiple regression model* to predict the difference in expression of the affected site(s) in response

to the dependent variables (difference of the affected CpG site '*Dcg15929276'*) and independent variables the number of life events- life stressors before visit 2, the BMI index, the dummy variables of cancer, cardiovascular disorder, psychiatric history, years follow – up in between measures, the participants' gender, and the smoking habit. The backward method was used in order to estimate the most suitable regression model.

Results (part 2)

Baseline characteristics of Sample and Life events

Table 1
Participants' characteristics

	N	%
Gender		
Female	173	60.07%
Male	115	39.93%
Cancer		
No	196	95.15%
Yes	10	4.85%
Cardiovascular Disorder		
No	115	55.83%
Yes	91	44.17%
Psychiatric history		
No	131	63.59%
Yes	75	36.41%
Smoking habit		
Never smoker	229	79.51%
Former smoker	9	3.13%
Current smoker	50	17.36%
Age and BMI index	**M**	**SD**
Age at visit 1	66.57	9.08
Age at visit 2	73.02	9.29
Years of follow-up in between two measures	6.46	2.54
BMI ((htcm/100)^2)	25.91	4.14

Table 2

Number of reported life events before visit 2

	N	%
.00	22	9.2
1.00	25	10.5
2.00	43	18.1
3.00	50	21.0
4.00	23	9.7
5.00	17	7.1
6.00	21	8.8
7.00	16	6.7
8.00	8	3.4
9.00	7	2.9
10.00	3	1.3
11.00	2	.8
12.00	1	.4
Total	238	100,0

Table 2 shows that 9.2% of the participants had zero life events before visit 2, 49.6% of the participants had 1 -3 life event before visit 2, 32.4% of the participants had 4 – 7 life events and the rest 8.8% of the participants had 8 -12 life events before visit 2. The average of reported events was 19.8.

Pearson's correlation

All CpG sites in this analysis start with the letter D which stands for the difference in methylation. There is no statistically significant relationship among the number of life events before visit 2 with the differences of all 22 CpG sites *(See appendix A).*

MANOVA analysis

The analysis shows that the number of the life events before visit 2 *[Wilks' Lambda = .915, F(22, 122) = .508, p = .966]*, age at visit 2 *[Wilks' Lambda = .851, F(22, 122) =.952 , p =.529]*, cancer *[Wilks' Lambda =.853 , F(22, 122) = .938, p =.546]*, cardiovascular disorder *[Wilks' Lambda = .790, F(22, 122) = 1.448, p =.106]*, BMI index *[Wilks' Lambda = .835, F(22, 122) =1.078 , p =.379]* and smoking habit *[Wilks' Lambda =.681 , F(22, 122) = 1.154, p = .249]* were not statistical significant. However, the analysis resulted that they were statistical significant main effects for sex *[Wilks' Lambda = .750, F(22, 122) = 1.821, p =.022]* and psychiatric history *[Wilks' Lambda = .737, F(22, 122) = 1.950 , p = .012]*. In addition we have to mention that in the case of the Dcg15929276 site the number of life events before visit 2 had a significant effect *F(1, 141) = 2.843, p = .094* (at 10% significant level) *(See appendix B)*.

Regression analysis

Table 3

Multiple regression model predicting Dcg15929276

	Unstandardized Coefficients		Standardized Coefficients		
	B	Std. Error	Beta	t	p
(Constant)	.060	.066		.911	.364
psychiatric history	-.153	.076	-.154	-2.009	.046
Sum of reported life events before visit 2	-.025	.014	-.140	-1.832	.069

The full regression model was statistical significant *F (2, 167) = 4.453, p = .013,* R^2 *= .051.* In the model, there was not any autocorrelation problem (Durbin Watson index = 2.088, acceptable values 1 – 3) or any multicollinearity problem *(VIF values = 1.032<10)*. As it can be seen, two variables were statistical significant: the number of life events – life stressors *(b =-0.025, p = 0.069*, marginal significant at 5% and significant at 10%) and psychiatric history *(b = -0.153, p = 0.046)*. The psychiatric history had the most significant impact on the Dcg15929276 since it has the highest absolute value of Beta *(0.154)*. Also, both predictor variables had a negative effect

on the CpG 15929276 site. More precisely, participants that have psychiatric history have lower Dcg15929276 by 0.153 units compared to people without a psychiatric history. Finally, for one life event increase in someone's life, there is a decrease of -0.025 units of the Dcg15929276.

Discussion

This study attempted to do two things: one, to present a list of genes susceptible to environmental stressors by integrating studies investigating the gene X environment interaction and two, to analyze available data on methylation levels of the *FKB5* gene following life adversity.

Regarding the first research goal, we can confirm that stressful experiences alter the expression of certain genes that may result in mental dysfunction. Specifically, the function of five genes (*FKBP5, DRD4, COMT, NR3CI*, and *5HTTLPR)* was assessed in response to either daily stressors (in the form of mental disorders like PTSD, ADHD, and MDD) or to specific stressful experiences in samples of adults, children or individuals experiencing chronic stress throughout their development. This review is one of the first, to our knowledge, to examine epigenetic influences following early, chronic and recent stressors in adults. The results demonstrate that stressful life events may trigger DNA methylation in adults although its degree varies depending on the individual (Dumanand & Canli, 2015). Moreover, it seems that environmental stressors may influence the genome equally regardless of the developmental stage. However, since the direct effects of recent stressors on acute development are largely lacking, we should interpret these results with great caution. Although it is logical to conclude that both early and chronic life adversity leads to epigenetic changes in adults, the extent to which recent stressful experiences instantly affect the function of a certain gene remains to be seen and constitutes an important objective for future research. It is important to mention that the genes presented in *table 1* have been systematically found to be vulnerable to environmental and developmental influences. Thus, this study adds upon previous findings and further validates the effects of the environment on the genome. However, this first part (review) is not free of limitations. Three of the included studies considered mental disorders as daily stressors (intrusive symptoms in PTSD, Invalidated parenting in ADHD and rumination in MDD) and used their symptoms to measure neuroplasticity instead of using concrete stressful experiences. Thus, it might be premature to conclude that chronic stressors, outside the spectrum of anxiety and mood disorders, may have the same epigenetic

effects on healthy subjects. Consequently, future research should define other aspects of daily life (bullying, exclusion, dysfunctional household, etc.) as chronic stressors instead of psychopathology.

The second part was devoted on finding an association between life adversity (measured at two time-points) and *FKB5* methylation levels, taking into account several critical covariates (gender, cancer, cardiovascular disorder, smoking habit, BMI index and age at visit) in a sample of 288 Swedish twins (Wang et al., 2018). The findings show that the number of life events before visit 2 had a significant effect only for one CpG site (Dcg15929276) suggesting that the accumulation of stressful events plays a secondary role to the development of a mental disorder. Indeed, several lines of evidence suggest that minor environmental stressors may induce epigenetic alterations that do not manifest themselves directly to the individual but get transmitted to subsequent generations (Kubota, 2016). Furthermore, the results demonstrate the significant effect of sex and psychiatric history to all 22 sites. In other words, being a woman and/or having a high load for psychiatric history affects the methylation of all the 22 CpG sites of the *FKB5* gene following life stressors. This outcome (regarding the role of the gender) is in line with previous findings which suggest that women have a greater susceptibility to surrounding stress and somatic illness that might contribute to the development of a mental disorder (Sandanger, Nygård, Sørensen, & Moum, 2004). On the other hand, this outcome is important because it contradicts the belief that the female hormone estradiol plays a protective role against the development of psychosis (Huber et al., 2001). Since the Dcg15929276 site is most commonly affected, we examined its relationship with all the covariates. Only two variables significantly affect the functionality of this CpG site. In congruence with the previous analysis, participants who reported a greater number of life events – life stressors before visit 2 had a decrease of Dcg15929276 activity. Respectively, participants with a higher load for psychiatric history had lower Dcg15929276 activity. Thus, being a woman with a psychiatric history represents a clear vulnerability to environmentally-induced neuroplasticity. According to these results and in response to the second research question, we can firmly conclude that traumatic events change the course and expression of the *FKBP5* gene and may provoke mental dysfunction. Specifically, 'psychiatric history' is by far the most important criterion linking external influences to genetic modification specifically for the *FKBP5* gene. Thus, there is an ambiguous causality between psychopathology and the 'gene X environment interaction' expressed in two notions. The notion

that psychopathology emerges after neurochemical changes caused by stress and the notion that pre-existing psychopathology mediates these changes following stress exposure. What remains to be seen is the exact relationship between the two notions as well as which of the two is more accurate. For example, in one study, Dogan, Lei, Beach, Brody, and Philibert (2016) found that substance abuse (alcohol and tobacco dependency) also affects the expression of the cg15929276 site of the *FKBP5* gene in subjects from the FACHS cohort (Family and Community Health Study), which in turn moderates the severity of the withdrawal symptoms. Thus, subsequent research should further focus on the "chick and egg" paradox portraying the process of 'gene X environment' interaction.

A particular strength of this dissertation rests on the inclusion of both a literature review, on environmentally-induced neuroplasticity, as well as on the inclusion of a specific and unique longitudinal data analysis. This combination provides a better overall understanding of the 'gene X environment interaction' in theory and in practice. On the other hand, this data analysis has two main limitations. First, the data had some inconsistencies that had to be corrected. For example, one participant reported having a younger age during the second visit (that was a typo-mistake and was corrected before the analysis). Second, subjects were old enough in both time-points (average of 66,5 to 73 years) and, thus, we cannot interpret the results in a life-course perspective but only focus strictly on the effects of aging (and the events that come with it regardless of older stressors) on the genome.

As a closing statement, research should focus on how to best integrate epigenetic findings to preventive interventions based on the list of genes that are vulnerable to environmental influences *(Table 1 of part 1)* as well as on how to conceptualize the 'gene X environment interaction' as a moderator of psychopathology. DNA screening and mental-history data gathering are necessary during the intake of a neuropsychiatric clinic for a faster and more reliable diagnosis which, in turn, will facilitate the prognosis and rehabilitation process of a given individual regardless of his/her developmental stage.

Appendix A

Correlation among the differences of sites and life events before visit 2 (first eleven)

	Dg14284211	Dcg08586216	Dcg19014730	Dcg00862770	Dcg00140191	Dcg10913456	Dcg16012111	Dcg07843056	Dcg01294490	Dcg20813374	Dcg00130530
Dg14284211	1										
Dcg08586216	-,069	1									
Dcg19014730	,066	,169**	1								
Dcg00862770	-,161**	-,076	-,197**	1							
Dcg00140191	,021	,139*	-,088	-,024	1						
Dcg10913456	-,043	,030	-,010	,008	,170**	1					
Dcg16012111	,039	-,274**	-,016	,180**	,023	-,030	1				
Dcg07843056	-,063	-,006	-,134*	,181**	,136*	,046	-,047	1			
Dcg01294490	-,151*	-,095	-,132*	,238**	,084	,105	,115	,260**	1		
Dcg20813374	,082	,057	-,150*	,011	-,012	-,007	-,001	-,015	,065	1	
Dcg00130530	,082	,062	,020	-,092	,074	-,111	-,041	-,007	,011	,150*	1
Dcg03591753	-,132*	-,176**	-,150*	,031	,004	-,041	-,021	,225**	,012	,021	-,133*
Dcg15929276	-,122*	-,049	-,063	,130*	-,030	,066	,153**	-,055	,082	-,078	-,029
Dcg23416081	,083	-,126*	,119*	-,088	,023	-,007	-,040	,076	-,053	-,060	-,032
Dcg00052684	,011	-,127*	,047	,038	,009	,006	-,012	,009	,137*	-,019	,068
Dcg06937024	-,034	-,028	-,104	,050	-,134*	-,001	,073	,051	,022	-,003	-,137*
Dcg11845071	,060	,147*	,276**	-,240**	-,016	,302**	-,054	-,199**	-,127*	-,067	-,057
Dcg00610228	-,070	-,261**	-,186**	,242**	,045	-,046	,376**	,020	,266**	,061	-,043
Dcg07485685	,077	,148*	,028	,034	-,013	-,053	-,052	,001	-,020	-,017	,092
Dcg17030679	-,113	-,163**	-,151*	,043	-,006	,052	,039	,016	,106	,002	,023
Dcg25114611	,071	-,161**	-,193**	-,024	-,054	-,113	,166**	-,017	-,032	,075	,036
Dcg19226017	,049	,075	,052	,016	,113	,078	-,026	,063	,042	-,005	,046
Sum of reported life events before visit 2	-,093	-,105	-,088	,021	-,039	-,026	,074	,036	-,015	-,015	-,174**

Correlation among the differences of sites and life events before visit 2 (last eleven)

	Dcg03591753	Dcg15929276	Dcg23416081	Dcg00052684	Dcg06937024	Dcg11845071	Dcg00610228	Dcg07485685	Dcg17030679	Dcg25114611	Dcg19226017
Dg14284211											
Dcg08586216											
Dcg19014730											
Dcg00862770											
Dcg00140191											
Dcg10913456											
Dcg16012111											
Dcg07843056											
Dcg01294490											
Dcg20813374											
Dcg00130530											
Dcg03591753	1										
Dcg15929276	,045	1									
Dcg23416081	,083	-,088	1								
Dcg00052684	,100	-,049	-,096	1							
Dcg06937024	-,011	,057	,017	-,022	1						
Dcg11845071	-,045	-,147*	,048	-,060	-,089	1					
Dcg00610228	,058	,120*	-,089	,082	,106	-,115	1				
Dcg07485685	-,025	,156**	,030	-,116*	,158**	-,013	,023	1			
Dcg17030679	,066	,111	-,027	,073	,036	-,128*	,021	,001	1		
Dcg25114611	,004	,156**	-,036	-,035	,104	-,081	,217**	-,103	,120*	1	
Dcg19226017	,067	-,164**	-,027	,014	-,113	,042	,042	-,046	,019	-,088	1
Sum of reported life events before visit 2	,102	-,107	,059	,012	,025	-,034	,027	,031	-,050	-,024	,077

Appendix B

Multivariate Tests[a]

Effect		Value	F	Hypothesis df	Error df	Sig.
Intercept	Pillai's Trace	,181	1,204[b]	22,000	120,000	,258
	Wilks' Lambda	,819	1,204[b]	22,000	120,000	,258
	Hotelling's Trace	,221	1,204[b]	22,000	120,000	,258
	Roy's Largest Root	,221	1,204[b]	22,000	120,000	,258
BMI	Pillai's Trace	,165	1,078[b]	22,000	120,000	,379
	Wilks' Lambda	,835	1,078[b]	22,000	120,000	,379
	Hotelling's Trace	,198	1,078[b]	22,000	120,000	,379
	Roy's Largest Root	,198	1,078[b]	22,000	120,000	,379
sumleq.2	Pillai's Trace	,085	,508[b]	22,000	120,000	,966
	Wilks' Lambda	,915	,508[b]	22,000	120,000	,966
	Hotelling's Trace	,093	,508[b]	22,000	120,000	,966
	Roy's Largest Root	,093	,508[b]	22,000	120,000	,966
AGE.2	Pillai's Trace	,149	,952[b]	22,000	120,000	,529
	Wilks' Lambda	,851	,952[b]	22,000	120,000	,529
	Hotelling's Trace	,174	,952[b]	22,000	120,000	,529
	Roy's Largest Root	,174	,952[b]	22,000	120,000	,529
CANCER	Pillai's Trace	,147	,938[b]	22,000	120,000	,546
	Wilks' Lambda	,853	,938[b]	22,000	120,000	,546
	Hotelling's Trace	,172	,938[b]	22,000	120,000	,546
	Roy's Largest Root	,172	,938[b]	22,000	120,000	,546
CVD	Pillai's Trace	,210	1,448[b]	22,000	120,000	,106
	Wilks' Lambda	,790	1,448[b]	22,000	120,000	,106
	Hotelling's Trace	,265	1,448[b]	22,000	120,000	,106
	Roy's Largest Root	,265	1,448[b]	22,000	120,000	,106
Psych_his	Pillai's Trace	,263	1,950[b]	22,000	120,000	,012
	Wilks' Lambda	,737	1,950[b]	22,000	120,000	,012
	Hotelling's Trace	,358	1,950[b]	22,000	120,000	,012
	Roy's Largest Root	,358	1,950[b]	22,000	120,000	,012
SEX	Pillai's Trace	,250	1,821[b]	22,000	120,000	,022
	Wilks' Lambda	,750	1,821[b]	22,000	120,000	,022
	Hotelling's Trace	,334	1,821[b]	22,000	120,000	,022
	Roy's Largest Root	,334	1,821[b]	22,000	120,000	,022
smoking.1	Pillai's Trace	,338	1,119	44,000	242,000	,293
	Wilks' Lambda	,681	1,154[b]	44,000	240,000	,249

	Hotelling's Trace	,439	1,188	44,000	238,000	,209
	Roy's Largest Root	,360	1,981[c]	22,000	121,000	,010
CANCER * CVD	Pillai's Trace	,169	1,109[b]	22,000	120,000	,347
	Wilks' Lambda	,831	1,109[b]	22,000	120,000	,347
	Hotelling's Trace	,203	1,109[b]	22,000	120,000	,347
	Roy's Largest Root	,203	1,109[b]	22,000	120,000	,347
CANCER * Psych_his	Pillai's Trace	,235	1,680[b]	22,000	120,000	,041
	Wilks' Lambda	,765	1,680[b]	22,000	120,000	,041
	Hotelling's Trace	,308	1,680[b]	22,000	120,000	,041
	Roy's Largest Root	,308	1,680[b]	22,000	120,000	,041
CANCER * SEX	Pillai's Trace	,000	.[b]	,000	,000	.
	Wilks' Lambda	1,000	.[b]	,000	130,500	.
	Hotelling's Trace	,000	.[b]	,000	2,000	.
	Roy's Largest Root	,000	,000[b]	22,000	119,000	1,000
CANCER * smoking.1	Pillai's Trace	,097	,588[b]	22,000	120,000	,925
	Wilks' Lambda	,903	,588[b]	22,000	120,000	,925
	Hotelling's Trace	,108	,588[b]	22,000	120,000	,925
	Roy's Largest Root	,108	,588[b]	22,000	120,000	,925
CVD * SEX	Pillai's Trace	,112	,690[b]	22,000	120,000	,842
	Wilks' Lambda	,888	,690[b]	22,000	120,000	,842
	Hotelling's Trace	,127	,690[b]	22,000	120,000	,842
	Roy's Largest Root	,127	,690[b]	22,000	120,000	,842
CVD * smoking.1	Pillai's Trace	,309	1,004	44,000	242,000	,472
	Wilks' Lambda	,712	1,008[b]	44,000	240,000	,465
	Hotelling's Trace	,374	1,013	44,000	238,000	,457
	Roy's Largest Root	,262	1,442[c]	22,000	121,000	,108
Psych_his * SEX	Pillai's Trace	,091	,544[b]	22,000	120,000	,950
	Wilks' Lambda	,909	,544[b]	22,000	120,000	,950
	Hotelling's Trace	,100	,544[b]	22,000	120,000	,950
	Roy's Largest Root	,100	,544[b]	22,000	120,000	,950
Psych_his * smoking.1	Pillai's Trace	,237	,738	44,000	242,000	,887
	Wilks' Lambda	,775	,739[b]	44,000	240,000	,885
	Hotelling's Trace	,274	,741	44,000	238,000	,883
	Roy's Largest Root	,194	1,068[c]	22,000	121,000	,391
SEX * smoking.1	Pillai's Trace	,342	1,135	44,000	242,000	,272
	Wilks' Lambda	,675	1,184[b]	44,000	240,000	,213
	Hotelling's Trace	,456	1,234	44,000	238,000	,164

CVD * Psych_his * SEX	Pillai's Trace	,141	,897[b]	22,000	120,000	,599
	Wilks' Lambda	,859	,897[b]	22,000	120,000	,599
	Hotelling's Trace	,164	,897[b]	22,000	120,000	,599
	Roy's Largest Root	,164	,897[b]	22,000	120,000	,599
CVD * Psych_his * smoking.1	Pillai's Trace	,252	,793	44,000	242,000	,821
	Wilks' Lambda	,763	,788[b]	44,000	240,000	,827
	Hotelling's Trace	,290	,783	44,000	238,000	,833
	Roy's Largest Root	,172	,948[c]	22,000	121,000	,534
CVD * SEX * smoking.1	Pillai's Trace	,092	,550[b]	22,000	120,000	,947
	Wilks' Lambda	,908	,550[b]	22,000	120,000	,947
	Hotelling's Trace	,101	,550[b]	22,000	120,000	,947
	Roy's Largest Root	,101	,550[b]	22,000	120,000	,947
Psych_his * SEX * smoking.1	Pillai's Trace	,078	,459[b]	22,000	120,000	,981
	Wilks' Lambda	,922	,459[b]	22,000	120,000	,981
	Hotelling's Trace	,084	,459[b]	22,000	120,000	,981
	Roy's Largest Root	,084	,459[b]	22,000	120,000	,981
CANCER * CVD * Psych_his * SEX	Pillai's Trace	,000	.[b]	,000	,000	.
	Wilks' Lambda	1,000	.[b]	,000	130,500	.
	Hotelling's Trace	,000	.[b]	,000	2,000	.
	Roy's Largest Root	,000	,000[b]	22,000	119,000	1,000
CANCER * CVD * Psych_his * smoking.1	Pillai's Trace	,000	.[b]	,000	,000	.
	Wilks' Lambda	1,000	.[b]	,000	130,500	.
	Hotelling's Trace	,000	.[b]	,000	2,000	.
	Roy's Largest Root	,000	,000[b]	22,000	119,000	1,000
CANCER * CVD * SEX * smoking.1	Pillai's Trace	,000	.[b]	,000	,000	.
	Wilks' Lambda	1,000	.[b]	,000	130,500	.
	Hotelling's Trace	,000	.[b]	,000	2,000	.
	Roy's Largest Root	,000	,000[b]	22,000	119,000	1,000
CVD * Psych_his * SEX * smoking.1	Pillai's Trace	,102	,620[b]	22,000	120,000	,902
	Wilks' Lambda	,898	,620[b]	22,000	120,000	,902
	Hotelling's Trace	,114	,620[b]	22,000	120,000	,902
	Roy's Largest Root	,114	,620[b]	22,000	120,000	,902

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