University of Verona
Department of Neurosciences, Biomedicine and Movement Sciences
Graduate School of Life and Health Sciences
Doctorate Course of Neuroscience, Psychology and Psychiatry

XXX cycle – 2014

CHILDHOOD TRAUMATIC EXPERIENCES AND MENTAL HEALTH: INVESTIGATING BIOLOGICAL, PSYCHOLOGICAL AND SOCIAL CORRELATES

SSD: MED/25 PSICHIATRIA

Coordinator: Professor Leonardo Chelazzi
Supervisor: Professor Sarah Tosato

PhD Student: Doctor Simona Tomassi

ACKNOWLEDGEMENTS

Firstly, I would like to express my sincere gratitude to my advisor Professor Tosato for the continuous support during my PhD, for her patience, motivation, and knowledge.

My sincere thanks also goes to Professor Ruggeri, Dr. Bonetto, and Dr. Cristofalo, who provided me the opportunity to join their research group. Without their precious support, it would not have been possible to conduct this research.

I thank my fellow labmates Michela, Mara, and Giorgia. In addition, I thank my friends Sara, Betta, Gloria, and Olivia for the support they have shown to me, for all the fun we have had together, but also for the shed tears, we have been sharing for the last four years.

Last but not the least, I would like to thank my family: to my husband, my parents, and my brother for supporting me along the way. This thesis is dedicated to my beloved kids, Francesco and Alessandro.

ABSTRACT

BACKGROUND: Childhood traumatic experiences, including physical and sexual abuse, antipathy, and neglect, are significantly associated with higher risk of developing both mental disorders and medical conditions.

AIM: To elucidate the effects of childhood trauma on health, especially mental health, taking into account some literature-selected biological, psychological, and social correlates.

METHODS: Investigations were conducted in three different sample groups: (1) a group of First Episode Psychosis (FEP) patients belonging to the "Genetics, Endophenotypes and Treatment: Understanding early Psychosis" (GET UP) Research Project; (2) a group of hepatitis C patients included in the "Chronic Inflammation and Depression" Research Project; and (3) a sample of healthy subjects. In these three samples, childhood traumatic experiences were evaluated by the Childhood Experience of Care and Abuse Questionnaire (CECA) questionnaire (Bifulco et al., 2005): physical punishments, sexual unwanted experiences, loss, separation, antipathy, and neglect were investigated. HPA axis functioning, cannabis use, and glucose metabolism were investigated as potentially involved biological correlates. HPA axis functioning was estimated by measuring salivary cortisol levels, as both diurnal cortisol levels and Cortisol Awakening Response (CAR). The Cannabis Experiences Questionnaire (CEQ) (Barkus et al., 2006) evaluated cannabis use. The concentrations of C-peptide, Ghrelin, GIP, GLP-1, Glucagon, Insulin, Leptin, PAI-1, Resistin and Visfatin were determined using Bio-PlexPro™ Human Diabetes Assays (Bio-Rad, CA, USA). Coping strategies, age, gender, education, socio-economic status, and recent severe stressful life events were taken into account as potentially involved psychosocial correlates.

RESULTS: (1) Significant associations between severe sexual abuse and a diagnosis of affective psychosis and between childhood trauma, severe sexual abuse in particular, and lifetime cannabis use were found in FEP subjects. No gender

difference was detected. Moreover, C-peptide and insulin levels were found increased in traumatized FEP subjects. Indeed, we found that C-peptide was higher in patients who experienced childhood trauma (with or without severe life events), while Insulin was higher in patients who reported childhood trauma (with no mention of severe recent stressful life events), in comparison to FEP subjects without traumatic events. (2) In subjects affected by Hepatitis C, childhood trauma, parental antipathy, and neglect were found significantly associated with higher levels of depressive and anxious symptomatology and with greater emotional distress. Exploring different types of trauma, we found that subjects with a history of physical punishments demonstrated significantly increased levels of depression, emotional distress, and fatigue in comparison with subjects not recalling this history. An even stronger association was found between sexual unwanted experiences and levels of depression, anxiety, and emotional distress. Healthy subjects (3) with a history of childhood trauma showed higher lifetime frequency of positive psychotic-like symptomatology, while maternal antipathy was significantly associated with lower lifetime frequency of negative symptoms and lower levels of distress in response to depressive symptoms. Lifetime cannabis use and salivary cortisol levels were explored as potentially mediating factors, but no mediational effect was found. Finally, higher insulin levels were significantly associated in healthy subjects with physical abuse and childhood trauma plus severe life events (SLEs); Body Mass Index (BMI), which was also significantly associated with higher insulin levels, completely mediated the association with physical abuse, while childhood trauma plus SLEs and BMI gave independent contributions to higher insulin levels. Higher PAI-1 levels were found in subjects reporting childhood trauma and SLEs. Finally, physical abuse, severe sexual abuse and childhood trauma plus SLEs were found significantly associated with higher C-peptide levels. BMI completely mediated the association between physical abuse and C-Peptide, while severe sexual abuse or childhood trauma plus SLEs and BMI independently contributed to C-Peptide levels.

CONCLUSION: Our findings confirmed the detrimental consequences of childhood trauma, even at non-clinical level. On the other side, some of our results

evoked a potential increased resilience in subjects with a history of childhood traumatic experiences.

CONTENTS

INTRODUCTION

Childhood traumatic experiences represent a compound phenomenon, which includes physical, sexual, and psychological abuse, and neglect, strikes one child out of 100 in Italy, and is significantly associated with higher risk of developing both mental disorders and medical conditions. The overall aim of this PhD thesis is to investigate the effects of childhood trauma on mental health, taking into account some literature-selected biological, psychological, and social correlates. In details, in chapter 1, the definition of childhood traumatic experiences is given and the magnitude of the phenomenon reported, focusing on worldwide, European and Italian lifetime prevalence rates. Moreover, a brief review of the evidence supporting the well-established association between childhood trauma and the increased risk of developing severe mental disorders (such as psychosis and mood disorders) and medical conditions (like metabolic syndrome, diabetes, and obesity) is reported. The chapter closes with the description of the known biological (HPA axis, cannabis, and epigenetics- here a systematic review on the relationship between childhood trauma and epigenetics published in 2017 is presented), psychological (believes, dissociation, and attachment), and social (gender and poverty) correlates of the association between childhood trauma and severe mental disorder.

Based on this background, the impact of childhood trauma on mental health in relation with several biopsychosocial features has been investigated in three different sample groups: a group of subjects affected by First Episode Psychosis (FEP) (chapter 2), a group of subjects affected by hepatitis C (chapter 3), thus characterized by a state of chronic inflammation and inclined to develop depression, and a sample of healthy subjects (chapter 4).

Chapter 2 includes the description of the "Genetics, Endophenotypes and Treatment: Understanding early Psychosis" (GET UP) Research Project by Ruggeri and collaborators, within whose framework two studies were conducted. The first, published in 2017 in the British Journal of Psychiatry (Tomassi et al., 2017) aimed to investigate in a large sample of FEP patients the influence of childhood trauma on diagnosis and lifetime cannabis use. The second, next to be submitted soon,

explored the association between childhood traumatic experiences and glucose metabolic biomarkers in early psychosis. In both cases, methods are fully described, results reported, and findings discussed.

In chapter 3, I reviewed the literature regarding depression and inflammation, paying particular attention to the role of peripheral pro-inflammatory cytokines and to the known association between inflammatory chronic medical conditions (such as hepatitis C) and depression. Afterwards, the "Chronic inflammation and Depression" Research Project by Tosato and collaborators is described. Then, some preliminary findings about hepatitis C, depression, and risk and resilience factors (a special focus is kept on childhood trauma), recently presented in a symposium at the 6° European Association of Psychosomatic Medicine Conference held in June 2018 in Verona, are reported and discussed.

Finally, the "Healthy Subjects" Research Project by Tosato and collaborators is illustrated in chapter 4. Two studies were conducted using data from this project. The first one is actually under review. It aimed to investigate the association between childhood trauma and psychotic-like experiences in healthy subjects, also taking into account some potentially mediating factors such as lifetime cannabis use and salivary cortisol levels. The second study is also under review. It aimed to explore the association of childhood traumatic experiences and altered glucose metabolism. In both cases, methods are fully described, results reported, and findings discussed.

In general, we found several significant effects of childhood traumatic experiences on mental health in all sample groups and we confirmed the detrimental consequences of trauma, even at non-clinical level. On the other side, some of our findings evoked a potential increased resilience in subjects with a history of childhood traumatic experiences. It seems therefore to suggest an alternative starting point for future research on childhood adversities, which in our opinion, should be more positively resilience-oriented.

CHAPTER 1: CHILDHOOD TRAUMATIC EXPERIENCES

1.1 DEFINITION AND MAGNITUDE OF THE PHENOMENON

Childhood trauma is a complex and heterogeneous phenomenon, significantly conditioned and modulated by bio-psycho-sociocultural elements. The World Health Organization defines childhood trauma as (WHO, 1999):

‘‘*Child maltreatment is the abuse and neglect of people under 18 years of age. It includes all forms of physical and/or emotional ill-treatment, sexual abuse, neglect or negligent treatment or commercial or other exploitation, resulting in actual or potential harm to the child's health, survival, development or dignity in the context of a relationship of responsibility, trust or power. Four types of child maltreatment are generally recognized: physical abuse, sexual abuse, psychological (or emotional or mental) abuse, and neglect.*’’

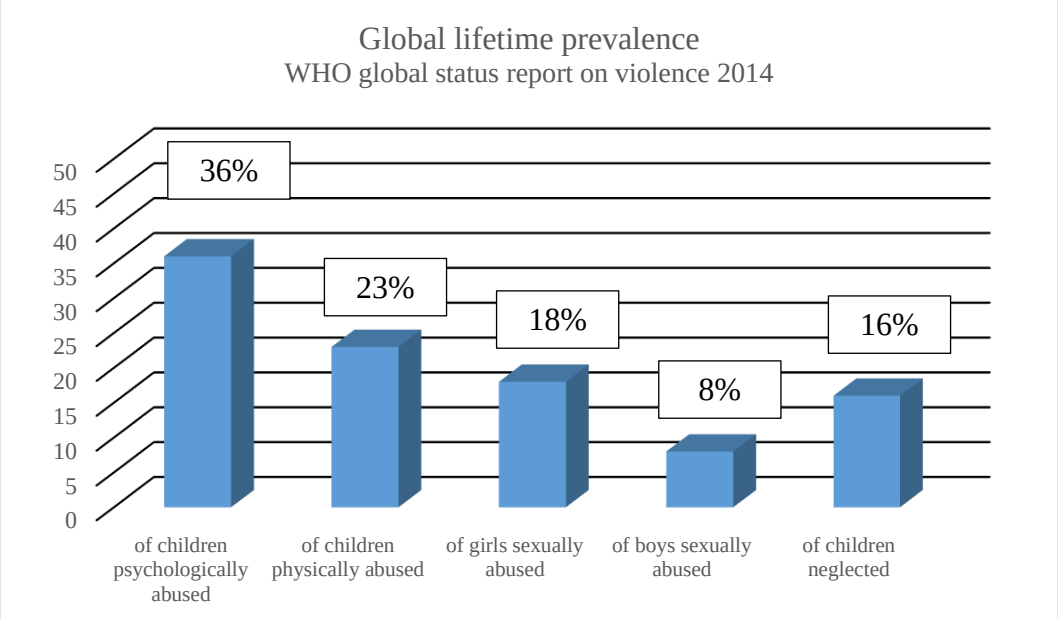
In scientific literature, definitions of childhood trauma are heterogeneous: some focus on adults’ behaviors regardless of the outcome, while others consider abuse to take place if there is harm or the threat of harm to the child. Some require the harm to the child to be intended for the act to be defined as abusive, while others consider as abused also those children inadvertently harmed through the actions of an adult (WHO 2002). Finally, the question remains whether the experience of abuse should be understood as a dichotomous event (which may or may not occur) or whether it should be seen in a dimensional, rather than a categorical context, characterized by distinct qualitative elements, such as frequency of occurrence, severity of the act, and duration in time. Taking into consideration the 1999 WHO definition, this thesis will be focusing on the main four types of maltreatments: physical abuse, sexual abuse, psychological abuse (or antipathy), and neglect. Physical abuse of a child is defined as those acts of commission by a caregiver that cause actual or potential physical harm. Sexual abuse includes those acts where a caregiver uses a child for sexual gratification. Emotional abuse (or antipathy) can be defined as the lack of a caring and supportive environment, and includes: acts restricting child’s movements, denigration, ridicule, threats and intimidation, discrimination, rejection, and other nonphysical forms of hostile treatment. Neglect

refers to the failure of a parent to provide for the development of the child in one or more of the following areas: health, education, emotional development, nutrition, shelter and safe living conditions. Neglect can occur only in cases where reasonable resources are available to the family and it is thus distinguished from circumstances of poverty (WHO, 2002). To investigate the presence of childhood traumatic experiences, I chose the CECA questionnaire (Bifulco et al., 2005), because it is an internationally validated instrument and one of the most applied in this field of research. I have described the questionnaire thoroughly in more than one section of this thesis (see pages 64, 65, 81, 111, 115, 116, 149, 150, 163, 164). The instrument is based on retrospective reports of past events and may therefore imply the risk of recalling bias. Concurrent mental health factors may influence the reporting of traumatic childhood experiences (Colman et al., 2016). Studies that use retrospective reporting (such as the CECA-Q) to estimate associations between childhood adversity and adult outcomes associated with mental health may be biased. On one side, reliance on the retrospective reporting of abuse might then increase the risk of recall bias in studies investigating a sample of psychotic patients (Howard, 1993; Young et al., 2001 in Fisher et al., 2011). On the other side, the reliability of patients affected by psychosis in referring trauma has been clearly demonstrated (Fisher et al., 2011): their reports were shown to be independent from symptomatology, stable over the course of time (test-retest reliability), similar when obtained by different assessment instruments and generally consistent with other sources of information (concurrent validity). It has also been proven that retrospective recall yields to an underestimation of the phenomenon, rather than an overestimation (Hardt & Rutter, 2004). Depressed mood may also increase the likelihood of recall of negative experiences (Jorm et al., 1992; Mechanic et al., 1998; Mandelli et al.,2015), though several studies sustain a certain reliability in retrospective evaluation of adverse life events (Bifulco et al., 2002; Hardt et al., 2004; Dube et al., 2004). We investigated the history of childhood trauma in healthy subjects and in subjects affected by hepatitis C (in this case, the assessment on childhood trauma was performed at baseline, and at that time point, none of the participants was depressed). Even if present a recall bias due to an altered mood, it did not affect our data. Ideally, though, the validity of retrospective self-reports of

childhood abuse should be established by comparison with social services' records of corroborated abuse. However, the approval to obtain corroboration of childhood abuse reports from social services' records was not available for the studies included in this thesis. Nevertheless, these would probably not have been useful anyway for the majority of cases as very few incidents of abuse are ever reported to the authorities (Hardt et al., 2004). Other forms of corroboration (eg, family reports, documentation by different professionals) (Fergusson et al., 1996; Heim et al., 2012) could be be useful and should therefore be included in future research. A more accurate assessment of traumatic childhood experiences would be obviously obtained by prospective, longitudinal study designs (Widom et al., 2007), which unfortunately lack on feasibility and are very expensive in terms of both time and resources.

Every year, about 41,000 children under age 15 are victims of homicide worldwide. More deaths from child maltreatment go unreported — since they are incorrectly documented as due to other causes. Figure 1 shows global lifetime prevalence of childhood trauma (WHO, 2014).

Figure 1: Global lifetime prevalence of childhood trauma



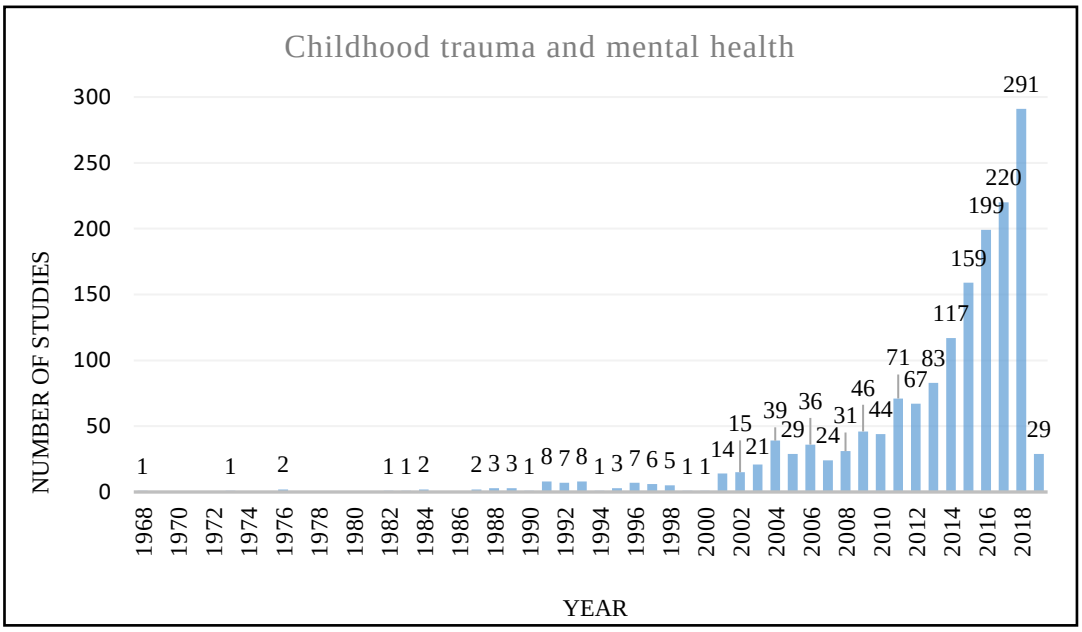
In the United States, 1.2% of children are victims of maltreatments. Among these, 10.0% and 7.0% are victims of physical abuse and sexual abuse, respectively, 11% reports psychological abuse, 60.0% neglect; in Canada the prevalence of child maltreatment is equal to 1.0%; physical, sexual and psychological abuse are reported respectively in 23.0%, 17.0% and 11.0% of cases, while 38.0% of subjects reports being neglected. In Australia, 0.7% of children are victims of abuse: the abuse is physical in 28.0% of cases, sexual in 10.0%, psychological in 34.0%; negligence affects 34.0% of children who are victims of maltreatments (Gilbert et al., 2009). Within the European Region, 852 children under the age of 15 die every year in because of maltreatments. The highest rate of maltreatment-related deaths is found in children under age of four. Nine per cent of European children are victims of sexual abuse (13.4% of girls and 5.7% of boys); 12-23% are victims of physical violence, 15-30% are psychologically abused, and 9% are neglected (WHO, 2013, 2014). According to a recent national survey conducted in 2015, maltreated youths (0-17 years of age) followed by Social Services in Italy are 91,000 (CISMAI-TDH, 2015).

In Italy, almost one children out of 100 (9.5‰) is victim of maltreatments: among those in charge to Social Services, 6.9% and 4.2% refers to be victim of physical and sexual abuse respectively, 13.7% reports a psychological abuse, while 47.0% relates neglect, both physical and emotional (CISMAI-TDH, 2015). Girls or female teenagers (11-17 years of age) and foreign children (20‰ vs 8.5‰ of Italian children) appear to be the most affected (CISMAI-TDH, 2015).

In 1924, The Declaration of the Rights of the Child, also known as the Geneva Declaration of the Rights of the Child, was adopted by the League of Nations (UN, 1924). It was the first document promoting child rights approved by an inter-governmental institution (Buck, 2014); it was adopted by the United Nations in 1946 and implemented in 1959 (UN, 1959). On 20 November 1959, the UN General Assembly adopted a Declaration of the Rights of the Child, based on the structure and contents of the 1924 original, with ten principles, and followed in 1989 by the Convention on the Rights of the Child (UN, 1989). Currently 196 countries are parties to the treaty. The United States signed the convention in 1995, but they have not ratified it yet.

When searching on Pubmed for Childhood Trauma AND Psychosis, 519 papers were found, most of them published starting from the late nineties, the oldest one printed in 1962. Looking at them carefully, it is worth noting that the very first work concerning the association between trauma during childhood and psychosis is actually dated 1996. Since then the research on this field has been spreading as showed in figure 2, which displays results by the searching "Childhood Trauma AND Mental Health".

Figure 2: "Childhood Trauma AND Mental Health" searching results



It is therefore possible to affirm that only quite recently, since the nineties, childhood has been world widely entitled to special care and assistance. Similarly, research on childhood trauma and mental health has seen a steep spread in the last two decades.

1.2 THE ASSOCIATION WITH SEVERE MENTAL DISORDERS

Childhood trauma has serious consequences. The effects can be immediate: victims can suffer serious injuries or die. The consequences can also last a lifetime. Adults who were abused or neglected as children have a higher risk of perpetrating or being a victim of violence, developing mental disorders (including psychosis), becoming obese, using tobacco, drugs, and alcohol (WHO, 2015). Childhood trauma has in fact been globally associated with the development of health problems, specifically with mental and neurological disorders (OR=2.3) (WHO, 2014), as well as with social and behavioral problems (e.g. low life satisfaction: OR=2.4; prostitution: OR=2.5), and impaired cognitive performance (e.g poor academic performance: OR= 1.3; cognitive impairment: OR=1.8). Childhood traumatic experiences have been widely proven to be associated with the development of severe mental disorders, such as schizophrenia and mood disorders (Read et al., 2005; Conus et al., 2010; Alvarez et al., 2011). In the general population, subjects recalling a history of childhood trauma (within their first 17 years of life) show an increased risk to undergo psychotic like experiences in their adulthood (OR 2.78; 95% CI 2.34–3.31) (Varese et al., 2012a). On the other hand, subjects with a diagnosis of severe mental disorder show an increased rate of past childhood trauma, with the rate size varying according to the type of exposure and perpetrator, up to the 47% of cases (Alvarez et al., 2011). A diagnosis of schizophrenia has been associated with a higher frequency of traumatic experiences during childhood. Female affected subjects showed rates of sexual abuse and physical abuse equal to 47.7% and 47.8% respectively, while males referred sexual abuse in 28.3% and physical abuse in 50.1% of cases (Read et al., 2005). A recent systematic review (Bonoldi et al., 2013) summarized evidence regarding the prevalence of childhood traumatic experiences in subjects affected by psychosis, and found that 26% and 39% of patients were sexually and physically abused when children, respectively. Moreover, first episode psychosis (FEP) patients showed a two-fold higher prevalence (85.7% vs 38.7%) of childhood trauma when compared with healthy controls (Mondelli et al., 2010). Severe sexual abuse was reported in 15.0-18.2% of FEP subjects (Neria et al., 2002; Fisher et al., 2010), while severe physical abuse was recalled in 15.2–21.6% (Fisher

et al., 2010; Neria et al., 2002). Similarly, childhood trauma has been proven a relevant risk factor for developing depression in adulthood (Zavaschi et al., 2006; Chapman et al., 2004; Kendler et al., 2004). A recent systematic review and meta-analysis (Mandelli et al., 2015) showed a strong association between emotional abuse (OR = 2.78), or neglect (OR = 2.75), or sexual abuse (OR = 2.42) and depression. A significant association was also found for physical abuse (OR = 1.98). Moreover, exposure to childhood traumatic experiences has been found to be associated with a two-fold risk of both recurrent and persistent depression (Nanni et al., 2011). They predict lifetime suicide attempts in treatment-resistant depression patients (OR 2.79; 95% CI 1.14-6.84) (Tunnard et al., 2014) and they have been related to the presence of psychotic features in mood disorders (Gaudiano & Zimmerman, 2010).

In recent years, findings have converged in support of a negative impact of childhood adversity on illness' features, outcome and course of severe mental disorders, across diagnostic boundaries (Alvarez et al., 2011; Aas et al., 2016). Patients suffering from schizophrenia and with a history of child abuse have, in fact, in comparison with unexposed affected subjects, a younger age of onset (on average their onset occurs four years earlier), with an earlier first admission, and a double hospitalization rate (Alvarez et al., 2011). Childhood trauma has been also associated to a more severe symptomatology with dissociative characteristics, a worse cognitive performance, and a more deteriorated social-relational functioning with a lower quality of life (Alvarez et al., 2011). Moreover, being affected by schizophrenia and having undergone traumatic experiences during childhood has been associated with more severe positive symptoms (i.e. commenting voices, ideas of reference, experiences of insertion and reading of thought and paranoid ideation) (Read et al., 2005).

The presence of childhood traumatic experiences has been associated with greater suicidality. One out of two (50%) exposed subjects also affected by a severe mental disorder, regardless of the type of trauma, have at least one suicide attempt in their life against 26% of the unexposed subjects (Alvarez et al., 2011). If we consider child sexual abuse, the percentage of patients attempting suicide rises to 69%

among subjects affected by schizophrenia (vs. 21% in unexposed) and to 73% of individuals suffering from mood disorders (vs 34% in unexposed) (Alvarez et al., 2011; Daruy-Filho et al., 2011). Finally, in patients with a diagnosis of both schizophrenia and mood disorder, the presence of childhood traumatic experiences has been associated with a higher risk of drug use (Daruy-Filho et al., 2011).

It is worth mentioning that even though the association between childhood traumatic experiences and mental disorders appear strong (Varese et al., 2012a), traumatized children do not develop a clinically significant disorder in the majority of cases (Matheson et al., 2013). There is evidence that protective factors can modify the negative effects of early adverse life circumstances leading people to select functional environments (Werner, 2004, 1992). The term resilience derives from the Latin word “resalio” (i.e. jump or rebound) and found its first application in mathematical and physical sciences, where it indicates the property of a material to resume the original shape after having suffered a stroke. Thereafter, the term was introduced and used in psychology, and the first longitudinal resilience study began in 1955. In psychology, resilience refers to a "dynamic process that includes a positive adaptation to a significantly adverse context ". It is therefore not a quality of the person, but a process, a phenomenon, which occurs due to the interaction between a set of external and internal resources, which can vary over time and within which the subject is only one of the involved elements. Protective factors towards stress have previously been classified into three categories:

1) Protective factors related to the individual (cognitive, emotional, and relational skills): children defined as "resilient" demonstrate in challenging situations to possess good adaptive skills, combining spirit of initiative and the ability to seek help. They seem to be characterized by a facilitating and affectionate temperament; they maintain a locus of internal control (with a good sense of self-efficacy) and an integral self-esteem.

2) Family protective factors (heat, family cohesion, support, and openness to dialogue): a family defined as protective should ensure the presence of at least one adult representing a stable emotional reference, capable of eliciting a sense of founded trust in the child and, as such, representing a valid identification model.

Furthermore, families with a strong religious belief seem to be more protective in difficult times; in fact, they represent a source of stability and provide a shared meaning to cope with in front of an adverse reality.

3) Community-related protective factors (social network, significant leisure-time activities, education): in this case, a role of primary importance seems to be played by the school. The presence, in fact, of a reference teacher, able to advise and support, appears to be strongly protective in demanding situations.

To sum up, protective resilient factors can be attributed to the individual itself, as temperamental characteristics that easily elicit positive responses from caregivers and/or good communication and problem solving skills, or lie within the family (caregivers providing positive role models) and in the community (teachers, caring neighbors and peers) (Werner, 2004). A primary role has also been theorized for contextual and cultural factors (Ungar, 2013) and resilience has been defined as the capacity of both individuals and their environments to interact to overcome childhood adversities (Ungar & Liebenberg, 2011). Political processes, funding, and cultural norms contribute significantly to make it more or less likely a positive adaptation (Leadbeater et al., 2005). It is therefore possible to postulate that subjects that endured childhood maltreatments, whether surrounded by a supportive relational and social context, could also cope with it in an adaptive way (Ungar, 2013; Ungar & Liebenberg, 2011).

Vulnerability, on the other hand, is what in a system determines an increased risk of negative consequences deriving from stressful events or situations. In the late 1970s, Zubin and Spring (Zubin & Spring, 1977) conceptualized the so-called "stress-vulnerability model" for schizophrenia. The model postulates that the vulnerability to psychosis is acquired through a genetic predisposition, which is a necessary condition, but not sufficient for the development of the disease. In order for the disorder to become manifest clinically, in fact, additional pathogenic factors must intervene, with a triggering effect on the onset of the disease. Psychosocial stimuli, particularly in early stages of life, would therefore have a profound impact in determining the risk of developing a mental disorder, contributing to increase (or reduce) the effects of an innate vulnerability (Luthar et al., 2006). The concept of

“risk” always maintains the characteristic of probability, and not of certainty, of causing harm (Luthar et al., 2006). Risk can be internal (such as genetic predisposition or organic pathologies) or external to the individual (family or related societies). Evidence has shown that a biological risk for severe mental disorders exist, mainly due to genetic vulnerability: with regards to schizophrenia, studies on twins have estimated an inheritability of the disorder equal to 60-80% (Sullivan et al., 2003), while for mood disorders the estimate is equal to 6-80% (Wray & Gottesman, 2012). Alongside the genetic vulnerability, it is also possible to identify a risk component due to elements defined as "environmental". In schizophrenia it has been hypothesized that environmental risk factors may have a relative weight of 60% in determining the vulnerability to the disorder. Worth mentioning, as now supported by clear scientific evidence, are: childhood traumatic experiences, growing in urban environment, migrant status, high levels of maternal stress during the perinatal period (pregnancy and childbirth), the advanced paternal age, obstetric complications, and the use of drugs (particularly cannabinoids) in adolescence (Schmitt et al., 2014). With regard to mood disorders, childhood traumatic experiences, obstetric complications (Schmitt et al., 2014), recent stressful life events and poorly supportive socio-family contexts appear to be the significant environmental risk factors involved.

Any reasonable theory that aimed to explain how childhood traumatic experiences might favor later on the development of a severe mental disorder should therefore take into account the integrated bio-psycho-social paradigm. Several paradigms, often integrated, have been developed focusing on diverse elements such as the stress response system, including the hypothalamus-pituitary-adrenal (HPA) axis, cognitive models and schemas, and environmental factors (Larkin & Read, 2008). Great attention has been paid to the effects of childhood trauma on brain development, in particular with respect to the potential damage caused by early adversities to the stress response system (Read et al., 2005). The Traumagenic Neurodevelopmental (TN) Model, a recent evolution of the original conceptualization of the "stress-vulnerability model" for schizophrenia (Zubin & Spring, 1977) represents a genuinely integrated bio-psycho-social approach.

According to this model, early traumatic events would be able to determine an activation of the hypothalamic-pituitary-adrenal axis, a system involved with a role of primary importance in the mechanisms of stress response. In case of abnormal and/or prolonged in time exposure to the trauma, a dis-regulation of the HPA axis itself could take place. The consequence would be the establishment of a condition of emotional hyper-reactivity to the stressors of everyday life, which would represent an element of vulnerability (Van Winkel et al., 2008).

From a psychological point of view, the association between childhood trauma and severe mental disorders has been mainly explored in light of cognitive processes and meaning attribution, dissociative phenomena, and attachment theory. The cognitive models proposed are numerous (Larkin & Read, 2008): their common element appears to be the presence of cognitive-behavioral patterns, acquired during childhood, determined by traumatic experiences, and responsible for the increased vulnerability. The experience of abuse would lead, in fact, to the shaping of negative believes about oneself, the others and the world, such as "I am vulnerable", "others are unreliable" and "the world is threatening/dangerous", which would then condition the reading of reality and daily events and would facilitate the development of a severe mental disorder (Larkin & Read, 2008).

1.3 THE ASSOCIATION WITH MEDICAL CONDITIONS:
Metabolic syndrome, diabetes, and obesity

There is strong evidence indicating that traumatic experiences during critical periods of brain development, such as childhood, can cause long-term, persistent effects on behaviour, increasing the likelihood to negative health outcomes. Childhood trauma has been demonstrated to increase the vulnerability for several medical conditions, including metabolic disorders and type 2 diabetes (Huffhines et al., 2016, Miller et al., 2011).

The physiological response to stress involves the activation of multiple biological systems, comprising the endocrine, metabolic, and immune systems; whereas the acute stress activate adaptive mechanisms, (McEwen, 2003) the long term exposure to stress is associated with long lasting physiological dysregulation, eventually leading to enhanced vulnerability to several kind of diseases (Juster et al., 2010). Confirming the long-term effects of early life stress on the body, several studies (Danese et al., 2009; Dich et al., 2015) have linked childhood trauma to detrimental changes in physiological functions. For instance, it has been shown that victims of childhood sexual and physical abuse are more likely to be obese or to show three or even more symptoms of metabolic syndrome when compared with non-victims (Danese & Tan, 2014). Moreover, it was showed that exposure to maltreatment is associated with higher levels of several biomarkers related to metabolism, including high glycated hemoglobin (Danese et al., 2009). Finally, evidence suggests that subjects who reported an exposure of childhood trauma had an increased risk of the 32% to develop later in life type 2 diabetes (Huang et al., 2015) and of the 20-50% to develop obesity (Thomas et al., 2008).

1.4 BIOLOGICAL CORRELATES OF THE ASSOCIATION BETWEEN CHILDHOOD TRAUMA AND SEVERE MENTAL DISORDERS

HPA axis, cannabis, and epigenetics

HPA axis functioning, cannabis consumption, and epigenetics are some among the biological correlates that have been investigated to better understand the association between being traumatized as children and the later development of a severe mental disorder.

1.4.1 HPA AXIS

An altered HPA axis functioning has been found significantly associated with both childhood trauma and psychosis. HPA axis is a key element of the body system that modulates the response to stress (Lupien et al., 2009): in physiological conditions, corticotrophin releasing hormone (CRH) and vasopressin (AVP) are synthetized in the hypothalamic paraventricular nucleus (PVN). Once released into the portal circulation, they reach the pituitary gland and activate the secretion of adrenocorticotropic hormone (ACTH), which in turn finally stimulates the secretion of glucocorticoids, including cortisol, from adrenal glands. Cortisol then binds its receptors (glucocorticoid receptors or GR and mineralcorticoid receptor or MR) in multiple target tissues, including the Central Nervous System and the HPA axis itself, and it is responsible for a negative feedback of both ACTH and CRH secretion (Pariante & Lightman, 2008). In case, however, of prolonged stimulation this homeostatic mechanism may fail. A flattening of the daily curve of cortisol values was observed with a significant increase in the evening values in association to a chronic stimulation by pro-inflammatory cytokines (Raison et al., 2010a). A chronic stimulation of the HPA axis can be responsible for its malfunction through an inhibitory effect on GR receptors. It has been demonstrated that pro-inflammatory cytokines can reduce the functionality of GR receptors: cytokines were shown to inhibit both cytoplasm-nucleus translocation and DNA binding, and were proven to influence the expression of receptors' isoforms, stimulating the production of a relatively inert isoform (Pace et al., 2007). It would result in increased levels of circulating corticosteroid hormones, due to an altered HPA

cortisol-axis negative feedback mechanism and mediated, at least in part, by glucocorticoid receptor resistance. Both HPA axis hyper- (Danese et al., 2007, 2008, 2009) and hypo-activation (Lupien et al., 2009) and hyper- (Heim et al., 2000) and hypo-reactivity to stress (Klaassens et al., 2009; Carpenter et al., 2007) have been significantly associated to childhood traumatic experiences. HPA-axis dysfunction was also proven significantly associated to severe mental disorders themselves (both psychosis and depression). HPA-axis hyperactivity, characterized by high cortisol levels in basal condition, and a blunted HPA axis response to stress (hypo-reactivity), were in fact both found to characterized psychosis (Borges et al., 2013). Similarly, hyperactivity of the hypothalamic–pituitary–adrenal (HPA) axis with cortisol hyper-secretion (Pariante & Lightman, 2008; Pariante & Miller, 2001) and glucocorticoid resistance (Carvalho et al., 2010) can be consistently identified in depressed patients. Whether HPA axis dysfunction mediates the trauma-severe mental disorder association or represents a trait of illness is still a matter of debate. Nonetheless, several findings (Danese et al., 2007, 2008, 2009) indicate that abnormalities in the stress response system, and the subsequent increased inflammation, originate in childhood and might be considered a 'biological scar' of the early exposure to high levels of stress (Baumeister et al., 2016).

1.4.2 CANNABIS

Cannabis consumption has been found significantly associated with both childhood trauma and psychosis. On one side, childhood traumatic experiences has been associated with a higher risk of substance use (including cannabis) in adolescence and early adulthood in the general population (Madruga et al., 2011). Subjects recalling childhood trauma are in fact more prone to use cannabis: they may develop depressive symptoms and try to self-medicate and/or they often live in disadvantaged environments, where it is easier to get addicted (Houston et al., 2011). On the other side, the association between cannabis consumption and psychotic symptoms is well established in literature, even in the general population (Rössler et al., 2011: Moore et al., 2007; Henquet et al., 2005). It is worth mentioning that childhood trauma has been hypothesized to have a role in modulating and moderating the association between cannabis use and the later

development of psychosis (Houston et al., 2011). The risk for psychosis has been in fact found significantly higher in those who were exposed to both childhood trauma and cannabis use compared to those exposed to only one of the two. The strength of this association was found to be dose-dependent, with higher rates of psychosis as the frequency and severity of traumatic experiences increase (Konings et al., 2011). Despite clear evidence of association between trauma, cannabis, and psychosis, available evidence does not elucidate whether childhood traumatic experiences and cannabis use represent vulnerability factors for psychosis or environmental stressors that trigger the disorder acting on a pre-existing vulnerability. Traumatic experiences and cannabis use could both be vulnerability agents, which decrease the subject's resilience in response to other stressors (i.e., migration) and lead to psychosis. They might represent two different environmental stressors, acting (in an additive or in a multiplicative way) on another type (genetic?) of vulnerability. Finally, childhood trauma could enhance vulnerability and cannabis use acts as an exacerbating agent, or alternatively, vice versa.

1.4.3 EPIGENETICS

Epigenetics has been demonstrated to take a part in transducing environmental experiences in both genome and brain structure modifications, potentially underlining the association between childhood trauma (CT) and the development of psychosis (Heim & Nemeroff, 2001). Epigenetic modifications, like cytosine residues methylation, histone modifications and non-coding RNAs (Chuang and Jones, 2007), determine whether a DNA region is compacted and transcriptionally repressed/silent or open and transcriptionally active. CT could thus influence gene expression and individuals' capacity of adaptation through epigenetic modifications (Korosi et al., 2012). It is not yet possible neither to draw definitive conclusion about the epigenetic modifications due to CT, nor does to clarify why only a subset of people exposed to CT develop a psychosis while the majority does not. Furthermore, it stands unclear among subjects affected by psychosis, to what extent the observed epigenetic modifications are to be ascribable to the CT rather than to the psychosis itself. The main aim of the present review, published in 2017 in Neuroscience and Biobehavioral Reviews (Tomassi & Tosato, 2017), was then to

summarize the evidence relating to the specific epigenetic and gene expression modifications associated with CT in both subjects affected by FEP and healthy samples. Since we aimed also to explore the relative role of psychosis itself in determining these modifications, evidence about FEP and epigenetics/gene expression was also summarized.

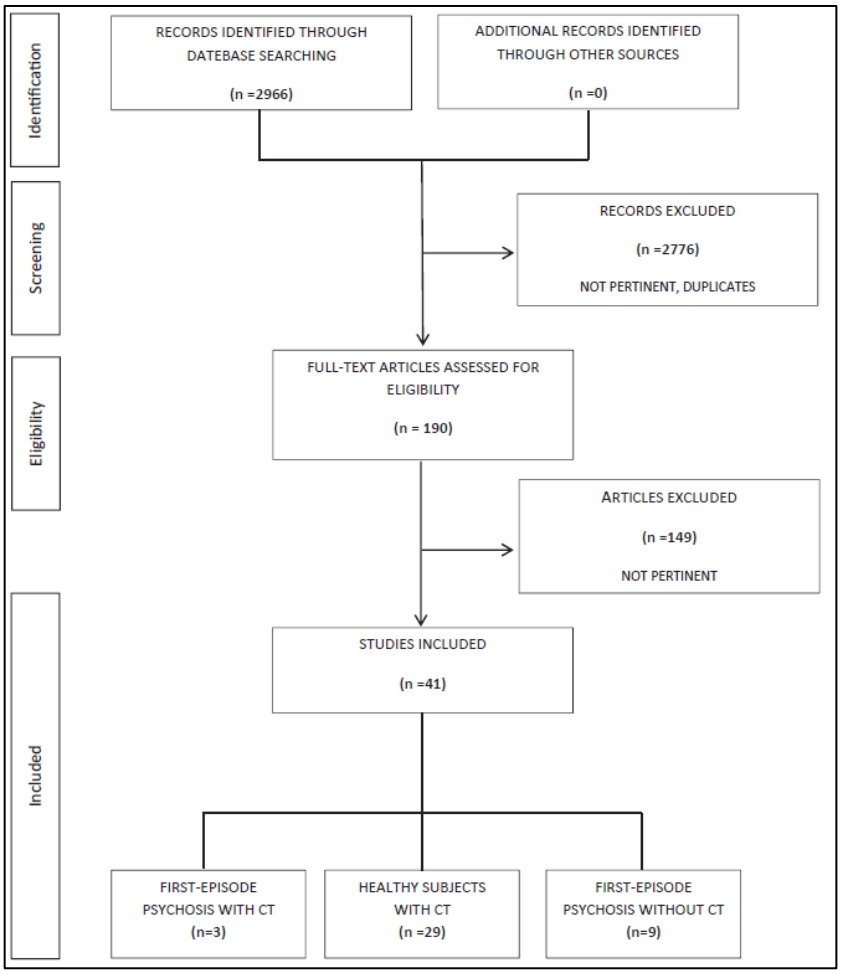
Methods

Search strategy and selection criteria

Based on previous works (Aas et al., 2012; Bifulco et al., 2005), we defined CT as the experience of sexual or physical abuse, neglect, parental loss or separation. We considered healthy subjects those non-affected by any psychiatric disorder. We performed a literature search on PubMed, last updated in December 2016.

Original searches were done for experimental studies using the following search terms: "childhood trauma" OR "child abuse" OR "ABUSE*" OR "childhood sexual abuse" OR "childhood physical abuse" OR "neglect" "childhood parental loss" OR "childhood separation" in combination with the following terms: "DNA methylation" OR "histone modification" OR "microRNA" OR "gene expression" OR "genome wide methylation" OR "genome wide gene expression"; and specifically for the main aim in combination with "first episode psychosis". Finally, for the secondary aim searches were done using the following search terms: "DNA methylation" OR "histone modification" OR "microRNA" OR "gene expression" OR "genome wide methylation" OR "genome wide gene expression" in combination with "first episode psychosis". The database search was restricted to human studies, English language papers. Cross-references from all included articles were also screened. Unpublished studies, conference abstracts or poster presentations were not included. The search yielded a total of 2966 potentially relevant records. After excluding duplicated studies and those that clearly did not pertain to our topic of interest (n=2776), basing on the review of titles or abstracts, the full text of the remaining studies (n=190) was assessed for eligibility. Once removed non-pertinent articles, 41 studies were included (figure 3). Articles were screened and selected by me and checked by my supervisior.

Figure 3: Process of study selection



Studies' characteristics

The main studies' characteristics are presented in Table 1.

Table 1: List of included studies and their main characteristics

Study	Case Group(s)	Sample information:			Tissue
		N	**Age** Mean(SD)/range, year	**Gender M** N(%)	
Alexander et al., 2014	German healthy adults with early trauma	200 Cases N=--	23.7 (2.8); 18-30	100 (50)	Peripheral blood
Beach et al., 2011	Sexually abused by Iowa Adoptee Study (IAS) Sample non-overlapping w Beach et al., 2010	155 Cases* N=15	--	0(0)	Peripheral blood
Beach et al., 2010	Sexually and Physically abused by Iowa Adoptee Study (IAS)	192	35-69	96(50)	Peripheral blood
Bick et al., 2012	Foster placed adolescents	9 Cases N=4	23.7 (2.8)	5 (55.5)	Peripheral blood
Cecil et al., 2016	Maltreated adolescents from the High risk inner city (London) sample	124 Cases N=84	16-24	58 (47)	Buccal cells
Duman & Canli, 2015	Caucasian males (CT as a continuous by CTQ)	105 Cases N=--	28.5 (13.8); 18-77	105 (100)	Peripheral blood
Di Nicola et al., 2013	FEP patients with a history of loss/separation/CSA/CPA.	24	28.1(1.1)	16(66.7)	Peripheral blood
Fusté et al., 2013	FEP patients	28 Cases* N=14 Schizophreniform disorder (N=3), psychosis NAS (N=7), BD (N=4)	25.2 (5.5); 17-40	7 (50)	Peripheral blood
Gouvea et al., 2016	Antipsychotic naïve FEP patients	142 Cases* N=69 (N=53 FEP SCZ) (N=16 FEP w mania)	*26.3 (7.5) FEP w SCZ *25.1 (6.9) FEP w mania	*46 (66.7)	Peripheral blood
Guillemin et al., 2014	Women with an history of childhood chronic physical aggression	19 Cases* N=5	25 (1)*	0 (0)	Peripheral blood
Houtepen et al., 2016	Healthy individuals w CT (CT as a continuous by CTQ)	69 (discovery sample) 45 (blood replication sample)	--	--	Peripheral blood
Janusek et al., 2016	American African men w CT (CT as a continuous by CTQ)	33	20.2 (2.3); 18-25	34 (100)	Peripheral blood
Khulan et al., 2014	Separated from parents Finland males	166 Cases* N=83	*64.0 (2.9)	166 (100)	Peripheral blood

Klengel et al., 2013	Adult subjects with an history of CSA and CPA By 3 different cohorts 1) Grady trauma project Cohort 2) Conte Center Cohort 3) Third cohort (from Metha et al., 2011)	1) cases N=30 with ≥2 CT; controls N=46 2)N=56 with ≥2 CT 3) 129 individuals (CA + risk allele, N = 40; CA + protective genotype, N = 15; no CA + risk allele, N = 60; no CA + protective genotype, N = 14)	1) cases: 41.5 (11.7); controls: 41 (11.9) 2) 28.4(6.7) 3) --	1) cases 8(26.7); Controls 10(21.7) 2) 0 (0) 3) --	Peripheral blood
Kumsta et al., 2016	Romanian adoptees with an (1) extended (6-43 months) or (2) limited (<6 months) experience of deprivation	49 Cases N=16 (1) and N=17 (2)	15 (--)	7 (43.7) (1) 9 (52.9) (2)	Buccal cells
Kuzman et al., 2009	Antipsychotic naïve FEP patients	64 Cases* N=32 All SCZ	*28.1 (7.9)	*8 (25)	Peripheral blood
Lee et al., 2012	FEP patients	52 Cases* N=26 SCZ N=16 (61.5%), Schizoaffective disorder N=1 (3.8%), Schizophreniform disorder N=8 (30.8%), Delusional disorder N=1 (3.8%)	*29.1 (5.1)	*15 (5.3)	Peripheral blood
Levine et al., 2015	Sample from the Health and Retirement Study (HRS)	114 Cases N=--	73.2 (9.5); 51-95	51 (44.7)	Peripheral blood
Misiak et al., 2015	First Episode Schizophrenia (FES) subjects with CT (FES+)	94 N=48 FES (n=18 FES+*) N=46 HS- (no CT)	*25.2 (4.17)	42 (44.7)	Peripheral blood
Mondelli et al., 2011	Childhood traumatized FEP subjs	79 N=49 FEP (9 w CT)	FEP 28.2 (0.9) Controls 27 (0.8)	52(65.8)	Peripheral blood
Naumova et al., 2012	Institutionalized children	28 Cases N=14	8.25 (--); 7-10	19 (67.8)	Peripheral blood
Nishioka et al., 2013	FEP patients	33 Cases* N=18 All SCZ	*22.8 (4.5)	*11 (61.1)	Peripheral blood

Noto et al., 2016	Antipsychotic naïve FEP patients	251 Cases* N=174 SCZ (53.2 %), schizophreniform disorders (17.4 %), brief psychotic disorders (12.0 %), psychosis NAS (10.9 %), psychotic mania (6.5 %).	* 26.19 (7.56); 16-40	*(62.2)	Peripheral blood
Ota et al., 2015	Antipsychotic naïve FEP patients	146 Cases* N=73 SCZ (60.9%), schizophreniform disorder (17.2%), brief psychotic disorder(14.1%) psychosis NAS (7.8%)	*26.1 (7.4)	*45 (61.6)	Peripheral blood
Ota et al., 2014	Antipsychotic naïve FEP patients	102 Cases* N=51 SCZ (59.6%) schizophreniform disorder (21.3%) brief psychotic disorder (12.7%) psychosis NAS (6.4%)	*25.4 (8.0)	*32 (62.7)	Peripheral blood
Provençal et al., 2014	Male with an history of childhood chronic physical aggression	20 Cases* N=8	25.8 (2.9)* 25.4 (2.7) in controls	20 (100)	Peripheral blood
Provençal et al., 2013	Male with an history of childhood chronic physical aggression	20 Cases* N=8	25.8 (2.9)* 25.4 (2.7) in controls	20 (100)	Peripheral blood
Radtke et al., 2015	Maltreated volunteers	46	11-21	18 (39.1)	Peripheral blood
Romens et al., 2015	Physically maltreated children	56 Cases N=18	11-14	30 (53.6)	Peripheral blood
Santoro et al., 2015	Antipsychotic naïve FEP patients	133 Cases* N=66 All SCZ	*25.9 (7.4)	*39 (59.1)	Peripheral blood
Schwaiger et al., 2016	Healthy individuals w CT (CT as a continuous and as categories by CTQ)	60 Cases* N=30	*52.6 (5.5)	*10 (33.3)	Peripheral blood
Shields et al., 2016	Women reporting physical/sexual Childhood Abuse (CA) from the Black Women's Health Study	295 Cases* N=153	*52.8 (--); 43-78	0 (0)	Peripheral blood
Smearman et al., 2016	Traumatized African American (CT as a continuous by CTQ)	389 Cases N=189	41 (12.8); 18-77	115 (29.3)	Peripheral blood

Suderman et al., 2014	Abused male adults from the British Birth Cohort	40 Cases N=12	45 (--)	40 (100)	Peripheral blood
Tyrka et al., 2012	Adults with parental death or desertion/childhood maltreatment	99	27.3 (10.4); 18-59	41(41.4)	Peripheral blood
Van der Knaap et al., 2015	Dutch Adolescents from the Tracking Adolescents' Individual Lives Survey (TRAILS) with traumatic Young Experiences (TYEs)	939 Cases N=--	16.2 (--)	--	Peripheral blood
Van der Knaap et al., 2014	Dutch Adolescents from the Tracking Adolescents' Individual Lives Survey (TRAILS) with traumatic Young Experiences (TYEs)	468 Cases N=--	16.1 (--); 14-18	232 (49.5)	Peripheral blood
Vijayendran et al., 2012	Sexually abused female by Iowa Adoptee Study (IAS)	158 Cases* N=26	*45 (7) 46(8) in controls	0(0)	Peripheral blood
Wankerl et al., 2014	German healthy adults with early trauma	133 Cases N=--	23.8 (3.05); 18-30	70 (52.6)	Peripheral blood
Weder et al., 2014	Maltreated Children (abused and/or neglected)	190 Cases N=94	10.2 (--); 5-14	80 (42)	Saliva
Yang et al., 2013	Maltreated children (physically/ sexually/emotionally abused and/or neglected)	N=192 children Cases N=96 Controls N=96	10.2 (--); 5-14	80 (42)	Saliva

Results

Epigenetic modifications/gene expression and CT in FEP

Only one study (Misiak et al., 2015) investigated genome wide DNA methylation patterns in patients affected by first-episode schizophrenia with a history of CT, collected using Early Trauma Inventory Self Report–Short Form (ETISR-SF) (Bremner et al., 2000). Methylation of two repetitive DNA sequences (long interspersed element–1 or LINE–1 and B–melanoma antigen or BAGE) was assessed using fasting whole peripheral blood samples. Emotional abuse and total trauma score predicted lower LINE–1 methylation in subjects with CT in comparison with those without CT. Conversely, no significant differences were found in BAGE methylation between groups.

We did not find any article exploring gene target DNA methylation, histone modifications, non-coding RNAs or genome wide gene expression in FEP subjects with history of CT.

Two articles (Di Nicola et al., 2013; Mondelli et al., 2011) investigated gene targets gene expression profile in FEP, and assessed CT using a modified version of Childhood Experience of Care and Abuse (CECA) Questionnaire (Bifulco et al., 2005). Peripheral blood samples were collected and RNA isolation performed. The first study (Di Nicola et al., 2013) found no differences in IL1a, IL1b, IL6, IL8, MCP-1, VEGF, EGF,INF-γ and TNFa gene expression between subjects with and without CT, while the second (Mondelli et al., 2011) found a negative correlation between BDNF gene expression and the number of CT. No significant differences were found in IL-6 and TNFa gene expression. Main findings are shown in Table 2.

Table 2: Childhood Trauma and epigenetic modifications/gene expression in First-Episode Psychosis

Authors	Epigenetic assessment(s) /gene expression	Gene(s)	Results
Di Nicola et al., 2013	Gene target gene expression	IL1a, IL1b, IL6, IL8, MCP-1, VEGF, EGF,INF-γ, and TNFa	**NO** significant difference between groups
Misiak et al., 2015	LINE-1 and BAGE methylation	Surrogate measure of global DNA	↓ LINE-1 in association with CT
Mondelli et al., 2011	Gene target gene expression	BDNF, IL6, and TNFa	↓ BDNF in association with CT

↑ = hyper-methylated/expressed; ↓ = hypo-methylated/expressed

Epigenetics modifications/gene expression and CT in healthy samples

Genome wide DNA methylation

Eleven different studies conducted a genome wide DNA methylation analysis in healthy samples with reference to CT: three studies (Cecil et al., 2016; Houtepen et al., 2016; Weder et al., 2014) used the Childhood Trauma Questionnaire (CTQ) (Bernstein et al., 2003; Bernstein & Fink, 1998) to explore the presence of CT (emotional, sexual and physical abuse; emotional and physical neglect), three (Bick et al., 2012; Khulan et al., 2014; Naumova et al., 2012) focused on parental separation only, three (Guillemin et al., 2014; Suderman et al., 2014; Yang et al., 2013) defined the presence of CT (physical and sexual abuse; neglect) on the basis of different types of reporting (self-report, family- and school-report), one (Kumsta et al., 2016) considered severe early life deprivation (abuse and neglect), and one (Provençal et al., 2014) focused on physical abuse only. In seven studies (Bick et al., 2012; Guillemin et al., 2014; Houtepen et al., 2016; Khulan et al., 2014; Naumova et al., 2012; Provençal et al., 2014; Suderman et al., 2014) out of eleven, DNA was extracted from whole peripheral blood samples, in two (Kumsta et al., 2016, Cecil et al., 2016) from buccal cell samples and in the remaining two studies (Weder et al., 2014; Yang et al., 2013) DNA was obtained from saliva. Main findings are summarized in Table 3.

Table 3: Childhood Trauma and Genome wide DNA methylation in healthy samples

Authors	Epigenetic assessment(s)	N probes/genes	Results
Bick et al., 2012	Genome wide DNA methylation	13 500 genes	180/27 000 differentially methylated CpGs sites between groups. Involved 173 genes: 72/173 ↑ and 101/173 ↓ in cases w history of foster care; many involved in immune system functioning, inflammatory response, cell cycle, cell development and differentiation.
Cecil et al., 2016	Genome wide DNA methylation	413 239 probes	34 Differentially Methylated Probes (DMP) for Physical abuse: top ranked cg20000641 ↑ located in the promoter region of PSEN2 7 DMP for Sexual abuse: top ranked cg17106653 ↑ located in the promoter region of glutamate receptor gene GRIN2D 118 DMP for Physical neglect: top ranked cg00691266 ↓ in the body of EVPL 20 for all 3 maltreatment types: top ranked cg08796898 ↑ located in the promoter region of HUWE1; top ranked cg10494379 **(↓)** located in the body of CC2D2A
Guillemin at el., 2014	Genome wide DNA methylation	20 318 genes	430 Differentially Methylated Gene Promoters (DMGP) in association with childhood chronic physical aggression: 77 ↑ and 353 ↓ 25 genes promoters most significantly different; among them NR3C1↓ 74 functional categories enriched in cell signalling and interaction, inflammatory response and behaviour
Houtepen et al., 2016	Genome wide DNA methylation	285 882 probes	**NO** significant differences between groups
Khulan et al., 2014	Genome wide DNA methylation	485 000 probes	**NO** significant differences between groups
Kumsta et al., 2016	Genome wide DNA methylation	382 291 probes	A ~ 600bp Differentially Methylated Region (DMR), including 9 CpGs, found in chromosome 10 ↑ in those severely exposed; it involves the P450 gene, CYP2E1

Naumova et al., 2012	Genome wide DNA methylation	485 000 probes 14 000 genes	914/26 214 differentially methylated CpGs sites between groups: 815/914 ↑ in the institutionalized group comparing with controls. Involved 838 gene promoters: 744/838 ↑ (control of cellular signalling system and immune response) and 94 ↓ (in the institutionalized, many of these genes play a critical role in the development and function of the brain.
Provencal et al., 2014	Genome wide DNA methylation	20 000 genes	448 DMGP in association with childhood chronic physical aggression: 171 ↑ and 277 ↓ 60 genes promoters most significantly different; among them SLC6A3↑
Suderman et al., 2014	Genome wide DNA methylation	20 533 genes	997 DMGP in association with CT: 311 ↑ and 686 ↓, affecting 1141 genes 169/1141 enriched in regulatory functions → 134/169 ↓ 230/1141 enriched in developmental functions → 172/230 ↓ 125/1141 enriched in cell surface receptor linked signal transduction → 46/125 ↓
Weder et al., 2014	Genome wide DNA methylation	> 485 000 probes	**NO** significant differences between groups
Yang et al., 2013	Genome wide DNA methylation	> 485 000 probes	2868 differentially methylated CpGs sites (on all 23 chr) in association with abuse/maltreatment: ↑ methylation values at sites with ↓ methylation values, and ↓ methylation at sites with ↑ methylation values. 45 (2%) CpG sites were in 3=UTR regions, 213 (7%) in 5=UTR regions, 848 (30%) were promoter-associated; 524 (18%) were in intergenic regions; and 1238 (43%) were on the gene body 8 genes had significant methylation differences: CCDC85C, FANK1, FRG1, TMED2, WNT3A, PTPRN2, SLC29A4, and PTPRN. ↓ at cg04111177 CpG site on the body of the NR3C1 gene

↑ = hyper-methylated; ↓ = hypo-methylated

Gene target methylation

Eighteen studies (Alexander et al., 2014; Beach et al., 2011, 2010; Duman & Canli, 2015; Houtepen et al., 2016; Janusek et al., 2016; Klengel et al., 2013; Provençal et al., 2013; Radtke et al., 2015; Romens et al., 2015; Shields et al., 2016; Smearman et al., 2016; Tyrka et al., 2012; Van der Knaap et al., 2015, 2014; Vijayendran et al., 2012; Wankler et al., 2014; Weder et al., 2014) investigated whether the presence of CT would influence gene target methylation: ten (Alexander et al., 2014; Duman & Canli, 2015; Houtepen et al., 2016; Janusek et al., 2016; Klengel et al., 2013; Smearman et al., 2016; Tyrka et al., 2012; Van der Knaap et al., 2015; Wankler et al., 2014; Weder et al., 2014) applied the CTQ (Bernstein et al., 2003; Bernstein and Fink, 1998), seven (Beach et al., 2011, 2010; Provençal et al., 2013; Romens et al., 2015; Shields et al., 2016; Van der Knaap et al., 2014, Vijayendran et al., 2012) defined the presence of CT (sexual and physical abuse) on the basis of different types of reporting (self-report, Child Protective Service- and school-report), and one (Radtke et al., 2015) used a German version of the Modified Adverse Childhood Experience (MACE) interview (physical and sexual abuse; physical and emotional neglect). Only in one study (Weder et al., 2014) out of 18, DNA was obtained from saliva, while in the others DNA was extracted from whole peripheral blood samples. Main findings are summarized in Table 4.

Table 4: Childhood Trauma and gene target methylation in healthy subjects

Authors	Epigenetic assessment	Gene(s)	Results
Alexander et al., 2014	Gene target methylation	SLC6A4 (83 CpG sites)	**NO** significant difference between groups
Beach et al., 2011	Gene target methylation	SLC6A4 (71 CpG sites)	↑(overall mean) in sexually abused females
Beach et al., 2010	Gene target methylation	SLC6A4 (26 CpG sites)	↑(overall mean) in abused; ↑at CpG1 and 3 in abused females
Duman & Canli, 2015	Gene target methylation	SLC6A4 (2 upstream amplicons; 79 CpGs sites)	**NO** significant difference between groups
Van der Knaap et al., 2015	Gene target methylation	SLC6A4	**NO** significant difference between groups
Vijayendran et al., 2012	Gene target methylation	SLC6A4 (16 CpG sites)	Methylation of cg22584138 and cg05016953 was influenced by sex abuse history.
Wankerl et al., 2014	Gene target methylation	SLC6A4 (83 CpG sites)	**NO** significant difference between groups
Weder et al., 2014	Gene target methylation	SLC6A4 (16 CpG sites)	**NO** significant difference between groups
Radtke et al., 2015	Gene target methylation	NR3C1 (exon 1_F) (41 CpGs)	**NO** significant difference between groups in average methylation. ↑ in maltreated at cg17860381
Romens et al., 2015	Gene target methylation	NR3C1 (exon 1_F) (13 CpGs)	↑ in maltreated, at CpG site 3, 6 and 7

Shields et al., 2016	Gene target methylation	NR3C1 (promoter region, CpG island shore)	↑ in abused, w a clear dose – response relation
Tyrka et al., 2012	Gene target methylation	NR3C1 (exon 1_F) (13 CpGs)	↑ at CpG 1 in subjects with loss; ↑ at CpG 3 in maltreated and in subjects with loss
Van der Knaap et al., 2014	Gene target methylation	NR3C1 (3 amplicons and exon 1_F)	↑ in amplicon 1, 2 (which includes exon 1_F) and 3 in traumatized
Weder et al., 2014	Gene target methylation	NR3C1 (promoter region, 41 CpGs)	↓in maltreated
Klengel et al., 2013	Gene target methylation	FKBP5 (glucocorticoid response element or GRE)	↓at intron 7 in CA exposed – risk allele carriers
Weder et al., 2014	Gene target methylation	FKBP5 (both body and promoter region, 34 CpG sites)	↓in maltreated
Provençal et al., 2013	Gene target methylation	IL-1a, IL-6, IL-4, IL-10, and IL-8	48 (↑ **or** ↓**)** methylated regions (none in the promoters of the target genes) associated with physical aggression ↓ IL-6 (involved a region 2042 bp upstream and close to the IL-6 transcription start site TSS)
Janusek et al., 2016	Gene target methylation	IL-6 (promoter region)	↓in greater exposure to CT

Provençal et al., 2013	Gene target methylation	NFkB1, NFAT5, and STAT6	66 (37+19+10) differentially (↑ **or** ↓**)** methylated regions associated with physical aggression
Houtepen et al., 2016	Gene target methylation	KITLG	↑ in cg27512205 in association with CT
Smearman et al., 2016	Gene target methylation	OXTR (18 CpGs)	↑ in cg04523291 and cg02192228 in association with CT
Weder et al., 2014	Gene target methylation	BDNF (gene body, 77 CpG sites)	↓ in maltreated;

↑ = hyper-methylated; ↓ = hypo-methylated

The most explored genes were: SLC6A4 (Alexander et al., 2014; Beach et al., 2011, 2010; Duman & Canli, 2015; Van der Knaap et al., 2015; Vijayendran et al., 2012; Wankler et al., 2014; Weder et al., 2014), NR3C1 (Radtke et al., 2015; Romens et al., 2015; Shields et al., 2016; Tyrka et al., 2012; Van der Knaap et al., 2014; Weder et al., 2014), FKBP5 (Klengel et al., 2013; Weder et al., 2014), and IL-1a, IL-6, IL-4, IL-10 and IL-8 genes (Janusek et al., 2016; Provençal et al., 2013). A single study analyzed KITLG (Houtepen et al., 2016), one explored OXTR (Smearman et al., 2016), one BDNF (Weder et al., 2014), and one NFkB1, NFAT5 and STAT6 (Provençal et al., 2013).

Five studies (Alexander et al., 2014; Duman & Canli, 2015; Van der Knaap et al., 2015; Wankler et al., 2014; Weder et al., 2014) out of the eight exploring SLC6A4 methylation found no significant differences between subjects with and without CT. The remaining studies (Beach et al., 2011, 2010; Vijayendran et al., 2012) showed a significant influence of CT on SLC6A4 methylation. An association was found between changes in SLC6A4 methylation (specifically at CpG residues cg22584138 and cg05016953) and sexual abuse (Vijayendran et al., 2012). Moreover, a hyper-methylation of SLC6A4 characterized sexually and/or physically abused women (Beach et al., 2011, 2010): they showed both an overall hyper-methylation early in the promoter region (CpG1-17) (Beach et al., 2011) and at CpG1 (cg25586514) and CpG3 (cg25586527) (Beach et al., 2010) in comparison with non-abused.

Six studies (Radtke et al., 2015; Romens et al., 2015; Shields et al., 2016; Tyrka et al., 2012; Van der Knaap et al., 2014; Weder et al., 2014) investigated DNA methylation of NR3C1 in relation to CT, and five of them (Radtke et al., 2015; Romens et al., 2015; Shields et al., 2016; Tyrka et al., 2012; Van der Knaap et al., 2014) found an hyper-methylation in traumatized subjects. Women most frequently physically abused showed significantly higher mean percent methylation levels in comparison with non-abused (Shields et al., 2016). A dose–response relation was observed and a trend of increasing methylation levels resulted significantly associated with increased severity of abuse. CT was also shown to increase methylation levels at specific single CpGs sites in exon 1F of NR3C1: hyper-methylation was demonstrated at CpGs 3, 6 and 7 in maltreated children (Romens

et al., 2015), at CpGs 1 and 3 in children whose mother and/or father had died (Tyrka et al., 2012); in amplicon 2 (which covers Exon1F) in sexual abused adolescents (Van der Knaap et al., 2014) and at cg17860381 in maltreated children (Radtke et al., 2015). Conversely, a sixth study (Weder et al., 2014) showed reverse results, finding a significant hypo-methylation at NR3C1 promoter region in association with maltreatment.

Associations between CT and hypo-methylation of FKBP5 (Klengel et al., 2013; Weder et al., 2014), IL-6 (Provençal et al., 2013; Janusek et al., 2016) and BDNF (Weder et al., 2014) and between CT and hyper-methylation of KITLG (Houtepen et al., 2016) and OXTR (Smearman et al., 2016) were also found. Finally, 66 differentially methylated regions (both hyper-methylated and hypo-methylated) were demonstrated in physically abused subjects involving NFkB1, NFAT5 and STAT6 genes (Provençal et al., 2013) in comparison with non-abused.

Histone modifications

We did not find any article exploring this issue.

Non-coding RNA

Four studies (Bick et al., 2012; Guillemin at el., 2014; Provençal et al., 2014; Suderman et al., 2014) explored the methylation of non-coding RNA genes in healthy subjects with reference to CT. Two of them (Guillemin at el., 2014; Suderman et al., 2014) defined the presence of CT (physical and sexual abuse; neglect) on the basis of reporting (self- and school-report), one (Bick et al., 2012) focused on parental separation, and one (Provençal et al., 2014) on physical abuse. In all four, DNA was extracted from whole peripheral blood samples. Main findings are summarized in Table 5.

Table 5: Childhood Trauma and microRNA methylation in healthy samples

Authors	Epigenetic assessment	Gene(s)	Results
Bick et al., 2012	microRNA methylation	110 microRNA	**NO** significant differences between groups
Guillemin at el., 2014	microRNA methylation	400 microRNA	**NO** significant differences between groups
Provencal et al., 2014	microRNA methylation	400 microRNA	12 differentially methylated microRNA promoters in association with childhood chronic physical aggression: 2 ↑ and 10 ↓
Suderman et al., 2014	microRNA methylation	489 microRNA	39 differentially methylated microRNAs promoters in association with CT: 31/39 ↑

↑ = hyper-methylated; ↓ = hypo-methylated

Genome wide gene expression

Only one study (Schwaiger et al., 2016) investigated genome wide gene expression profile in traumatized healthy subjects. CT was defined by a German version of CTQ (Bernstein et al., 2003; Wingenfeld et al., 2010) and included emotional, sexual and physical abuse, emotional and physical neglect. Peripheral blood samples were used for gene expression analysis. The study explored differences in gene expression following stress exposure (induced by the Trier Social Stress Test paradigm) between trauma and no-trauma groups. Out of the 405 transcripts differently expressed at 45' post stress, 77 were up-regulated and 69 down-regulated in the traumatized group, and conversely they did not change their expression in the non-traumatized subjects. Out of the 608 transcripts differentially regulated between the groups at 180' post-stress, 141 were upregulated and 86 down-regulated in the CT group and did not change their expression in the non-CT group. Among differentially expressed genes, several were involved in hormone activity, as steroid binding.

Gene target gene expression

Three studies (Duman & Canli, 2015; Levine et al., 2015; Wankerl et al., 2014) examined gene target gene expression differences between healthy subjects with and without CT. Two studies (Duman & Canli, 2015; Wankerl et al., 2014) applied CTQ (Bernstein et al., 2003; Wingenfeld et al., 2010) while the third (Levine et al., 2015) relied on self-report of physical abuse. In all 3 studies, peripheral blood samples were used. Main findings are summarized in Table 6.

Table 7 highlights the strongest findings, based on reproducibility, about epigenetic modifications/gene expression in association with CT in both FEP and healthy subjects. Notably, replicated findings are not available for methylation and CT in FEP, nor for gene expression and CT in both FEP and healthy subjects.

Table 6: Childhood Trauma and Gene target gene expression in healthy sample

Authors	Epigenetic assessment	Gene(s)	Results
Duman & Canli, 2015	Gene target gene expression	SLC6A4 and NR3C1	**NO** significant difference between groups for both SCL6A4 and NR3C1
Levine et al., 2015	Gene target gene expression	IL1B, IL8, and PTGS2	↑ pro – inflammatory gene expression in traumatized
Wankerl et al., 2014	Gene target gene expression	SLC6A4	↓ in maltreated

↑ = hyper-expressed; ↓ = hypo-expressed

Table 7: Most replicated epigenetic /gene expression modifications in association with CT in both First-Episode of Psychosis and Healthy Subjects

Gene target Methylation in association with CT			
Gene	**↓ or ↑**	**Sample group**	**N of study**
SLC6A4	↑	Healthy subjects	2 (Beach et al., 2011, 2010)
NR3C1	↑	Healthy subjects	5 (Radtke et al., 2015; Romens et al., 2015; Shields et al., 2016; Tyrka et al., 2012; Van der Knaap et al., 2014)
FKBP5	↓	Healthy subjects	2 (Klengel et al., 2013; Weder et al., 2014)
IL-6	↓	Healthy subjects	2 (Provençal et al., 2013; Janusek et al., 2016)

↑ = hyper-methylated; ↓ = hypo-methylated

Epigenetics modifications/gene expression in FEP

Genome wide DNA methylation

Only one study (Nishioka et al., 2013) investigated DNA methylation profile in peripheral blood samples from subjects affected by FEP. Among the 27 578 CpG sites analyzed, the overall DNA methylation level resulted significantly lower in patients in comparison with healthy controls. Hypo-methylation was found at 603 CpG sites: 589 genes resulted involved, mostly related to nucleotide and transcription factor binding.

Gene target methylation

A single target gene was considered: GCH1. Peripheral blood specimens were analyzed. Investigating a total of 37 CpG sites (Ota et al., 2014), both higher mean methylation levels and significant hyper-methylation at 4 CpGs were found in FEP subjects: specifically at CpG13, CpG15, CpG16, and CpG21.

Histone modifications

We did not find any article exploring this issue.

Non-coding RNA

We did not find any article exploring this issue.

Genome wide gene expression

Two studies (Kuzman et al., 2009; Lee et al., 2012) investigated genome wide gene expression profile in FEP subjects in comparison with healthy controls. RNA was extracted from peripheral blood samples. One-hundred and eighty genes were found to be significantly differently expressed (115 up-regulated and 65 down-regulated) in FEP when compared with healthy controls (Kuzman et al., 2009). Out of these 180, a subset of genes was selected to be tested as potential markers of FEP: catenin gene, CTNNB1, RYBP, RFN10, SLC2A3, zinc metallopeptidase gene, NLN-1, MYO1C and DAAM2. Patients showed significantly increased expression of

SLC2A3 and DAAM2 and decreased expression of NLN-1 and MYO1C in comparison with healthy controls. Moreover, a transcriptomic signature of FEP, basing on a 400 genes transcriptomics, was identified and confirmed by three different techniques (Lee et al., 2012).

Gene target gene expression

Finally, six studies (Fusté et al., 2013; Gouvea et al., 2016; Noto et al., 2016; Ota et al., 2015, 2014; Santoro et al., 2015) explored gene target gene expression differences between FEP and healthy controls. Peripheral blood samples were used for gene expression analysis. Twenty-five different genes were investigated: COMT,TNF, DISC1, PAFAH1B1, NDEL1, MBP, AKT1, DGCR8, DICER1, DROSHA, UFD1L, DGCR2, ABAT, TSPO, CHRNA7, CHRNB1, CHRNE, GABRR2, GCH1, GCHFR, TACR2, NRG1, SP1, SP3 and SP4. Three studies (Gouvea et al., 2016; Ota et al., 2015; Santoro et al., 2015) analyzed the same panel of 12 target genes (COMT, TNF, DISC1, PAFAH1B1, NDEL1, MBP, AKT1, DGCR8, DICER1, DROSHA, UFD1L and DGCR2), one (Noto et al., 2016) explored a subset of nine target genes from the same panel (COMT, TNF, DISC1, NDEL1, MBP, AKT1, DICER1, DROSHA, and UFD1L), one (Ota et al., 2014) focused on 11 genes (COMT, ABAT, TSPO, CHRNA7, CHRNB1, CHRNE, GABRR2, GCH1, GCHFR, TACR2 and NRG1) and one study (Fusté et al., 2013) examined SP1, SP3 and SP4. FEP patients showed significantly hyper-expression of MBP, NDEL1 (Gouvea et al., 2016; Noto et al., 2016; Ota et al., 2015), AKT1 and DICER1 (Gouvea et al., 2016) and hypo-expression of COMT, DISC1, DROSHA (Noto et al., 2016) and GCH1 (Ota et al., 2014) when compared with healthy controls.

Main findings are summarized in Table 8.

Table 8: Epigenetic modifications/gene expression in First-Episode of Psychosis

Authors	Epigenetic assessment(s)/ gene expression	Gene(s)	Results
Nishioka et al., 2013	Genome wide DNA methylation	27 578 CpG sites (72·5% inside CpG islands), 14 475 genes	↓ mean levels at all CpG sites in FEP vs healthy controls (HC) ↓ mean levels at CpG sites inside CpG islands in FEP vs HC ↓ at 603 CpG sites (covering 589 genes and located in 96·4% of cases inside CpG islands) in FEP vs HC Top ranked involved genes: CLDN12 and BCDIN3. Enrichment of genes related to the nuclear lumen, to nucleotide binding and transcription factor binding (both genders). Enrichment of mitochondrion-related genes (female only).
Ota et al., 2014	Gene target methylation	GCH1 promoter region (37 CpGs)	↑ mean levels in FEP vs HC ↑ at CpG13, 15, 16 and 21 in FEP vs HC
Kuzman et al., 2009	Genome wide gene expression	22 283 probes, 11 000 genes	180 gene differently expressed in FEP vs HC: ↓ 115/180, ↑ 65/180 Genes with altered expression included genes from different functional groups: transcription/RNA processing, ubiquitin, lipid/glucose/protein metabolism, signal transduction and cytoskeleton. ↑ SLC2A3 and DAAM2 in FEP vs HC; ↓ neurolysin 1 and myosin C in FEP vs HC
Lee et al., 2012	Genome wide gene expression	400 genes	Found a blood-based, gene expression "signature" that classified FEP from HC

Fusté et al., 2013	Gene target gene expression	SP1, SP3, and SP4	**NO** significant difference between FEP and HC
Gouvea et al., 2016	Gene target gene expression	COMT, TNF, DISC1, PAFAH1B1, NDEL1, MBP, AKT1, DGCR8, DICER1, DROSHA, UFD1L, and DGCR2	↑ MBP and NDEL1 in FEP (both SCZ and BD) vs HC ↑ AKT1 and DICER1 in FEP BD vs HC
Noto et al., 2016	Gene target gene expression	COMT, TNF, DISC1, NDEL1, MBP, AKT1, DICER1, DROSHA, and UFD1L	↑ MBP and NDEL1 in FEP vs HC ↓ DROSHA, COMT, and DISC1 in FEP vs HC
Ota et al., 2015	Gene target gene expression	COMT, TNF, DISC1, PAFAH1B1, NDEL1, MBP, AKT1, DGCR8, DICER1, DROSHA, UFD1L, and DGCR2	↑ MBP and NDEL1 in FEP vs HC
Ota et al., 2014	Gene target gene expression	COMT, ABAT, TSPO, CHRNA7, CHRNB1, CHRNE, GABRR2, GCH1, GCHFR, TACR2, and NRG1	↓ GCH1 in FEP vs HC
Santoro et al., 2015	Gene target gene expression	COMT, TNF, DISC1, PAFAH1B1, NDEL1, MBP, AKT1, DGCR8, DICER1, DROSHA, UFD1L, and DGCR2	**NO** significant difference between FEP and HC

↑ = hyper-methylated/expressed; ↓ = hypo-methylated/expressed

Discussion

CT might profoundly influence epigenetic programming (McGowan 2013). Environmental mediation of the epigenome, and aberrant epigenetic regulation, could therefore provide a mechanism for the gene-environment interaction underlying psychosis. The principal aim of the present review was to summarize the evidence relating to the epigenetic modifications associated with CT in both subjects affected by FEP and healthy samples. Despite the known impact of CT on people with psychosis and the attention paid recently to epigenetics and gene expression, available studies were only three. The existing evidence is therefore very limited and no definitive conclusions can be drawn from these findings. Waiting for more robust evidence, findings regarding specific modifications associated with CT in FEP indicate that CT entails global DNA hypo-methylation (Misiak et al., 2015) and reduced BDNF gene expression (Mondelli et al., 2011). CT-associated lower DNA methylation could have a functional relevance to gene regulation and/or be responsible for genomic instability, which has been previously observed in schizophrenia (Shimabukuro et al., 2007; Smith et al., 2010). A similar global DNA hypo-methylation was also found in FEP subjects (Nishioka et al., 2013) when compared with controls, not taking into account the presence of CT. Further investigations are therefore required to elucidate whether the global hypo-methylation is to be considered as an epigenetic consequence of CT or a trait of psychosis. Reduced BDNF mRNA levels associated with CT in FEP (Mondelli et al., 2011) could result in altered neuroplasticity, since FEP subjects were also characterized by a smaller left hippocampal volume. Conversely, no association was found between CT and BDNF gene expression in healthy subjects (Mondelli et al., 2011), standing unclear whether the reduced BDNF gene expression is to be ascribable to a specific effect of CT, independently from the presence of psychosis. We need for large, well-designed case-control studies enrolling FEP both with and without CT to generate biologically relevant information on epigenetic and gene expression modifications.

Findings about CT and epigenetic modifications in healthy subjects could potentially help to shed some light on biological vulnerability factors for psychosis,

especially when conducted in children and adolescents not (or not yet) affected by. Genome wide DNA methylation studies in children and adolescents (Bick et al., 2012; Cecil et al., 2016; Kumsta et al., 2016; Naumova et al., 2012; Yang et al., 2013) showed that differently methylated genes associated with CT were enriched in: neurotoxin metabolism (Kumsta et al., 2016), central nervous system development (Cecil et al., 2016; Naumova et al., 2012; Yang et al., 2013), plasticity and degeneration, DNA repair mechanisms, neuronal signaling and interaction (Cecil et al., 2016; Naumova et al., 2012), immune system and inflammatory response (Bick et al., 2012; Naumova et al., 2012). In adults, differently methylated (Guillemin et al., 2014; Provençal et al., 2014; Suderman et al., 2014) or expressed (Schwaiger et al., 2016) genes associated with CT were enriched in: regulation of chromatin and histone modification, regulation of transcription factor binding, development of multicellular organismal (Suderman et al., 2014), cell signaling (Guillemin et al., 2014; Provençal et al., 2014; Schwaiger et al., 2016; Suderman et al., 2014), and inflammatory response (Guillemin et al., 2014; Provençal et al., 2014; Schwaiger et al., 2016). Alterations in any of these processes might be responsible for an increased vulnerability for psychosis because each of them refer to several pathological models of schizophrenia (Sullivan 2012). The connection between methylation and gene transcription, and between mRNA levels and protein are critical to the hypotheses assuming biological changes as a consequence of CT. A significant hyper-methylation of SLC6A4, NR3C1, KITLG and OXTR promoter regions was found in association with CT in healthy subjects. A hyper-methylation of the gene promoter region is usually associated with a reduced gene expression due to a closer chromatin structure (Schübeler 2015). Thus, it is possible to speculate that CT led to a significant reduction in SLC6A4, NR3C1, KITLG and OXTR gene expression, with a subsequent dysfunction of serotonergic neurotransmission (SLC6A4), stress-reactivity (NR3C1, KITLG) and social behavior and bonding (OXTR). In line with this hypothesis, CT was found associated in healthy subjects with a reduced SLC6A4 gene expression (Wankerl et al., 2014), while to date no evidence supports a reduced NR3C1, KITLG and OXTR gene expression in association with CT. A significant gene promoter hypo-methylation of FKBP5 (Klengel et al., 2013; Weder et al., 2014) and IL-6 (Janusek

et al., 2016; Provençal et al., 2013) was also reported in association with CT in healthy subjects leading hypothetically to an increased transcriptional gene expression (Schübeler 2015). FKBP5 is an important functional regulator of the stress hormone system. It down-regulates the glucocorticoid receptor (GR) complex activity, decreasing ligand binding and impeding complex translocation to the nucleus (Scammell et al., 2001; Wochnik et al., 2005). In normal condition, GR activation mediates the cessation of the stress response once removed the stimulus (Binder 2009). A CT-dependent hypo-methylation in the FKBP5 promoter region (Klengel et al., 2013; Weder et al., 2014) might therefore be linked to an increased gene transcription and to an enhanced GR complex activity down-regulation. It could in turn result in a long-term dysregulation of the stress hormone system and entails effects on brain areas associated with stress regulation. IL-6 is a pro-inflammatory cytokine. IL-6 promoter region hypo-methylation significantly associated with CT (Janusek et al., 2016; Provençal et al., 2013) might result in higher gene expression, and thus contribute to create a pro-inflammatory, stress-vulnerable phenotype. Finally, BDNF gene was found significantly hypo-methylated within its body in healthy subjects with CT (Weder et al., 2014). Being methylation within gene bodies positively correlated with gene expression (Portela and Esteller, 2010), CT could thus be associated with reduced BDNF gene transcription. Significantly increased pro–inflammatory genes gene expression (including IL1B, IL8 and PTGS2) was also demonstrated in association with CT (Levine et al., 2015), in line once more with the hypothesis of a CT-related dysfunction of the stress response system.

Finally, significant differences in terms of miRNAs methylation between healthy subjects with and without CT were observed (Provençal et al., 2014; Suderman et al., 2014). MiRNAs are small, non-coding RNA molecules. Despite the poor understanding of miRNAs expression regulation, it appears reasonable that epigenetics (e.g. methylation) could control miRNAs transcription (Chuang and Jones, 2007). In turn, miRNAs would exert their epigenetic regulatory function by binding target mRNAs, down-regulating mRNAs translation, finally repressing mRNAs expression. Difference in this regulatory function could thus be implicated

in the enhanced vulnerability for psychosis observed in subjects with a history of CT.

Aiming to explore the relative role of psychosis itself in determining epigenetic modifications, we finally reviewed the available knowledge concerning these modifications in FEP. As discussed above, a global DNA hypo-methylation was not only found in FEP subjects with CT (Misiak et al., 2015) but also in FEP subjects when compared with controls, without taking into account the presence of CT (Nishioka et al., 2013). This last finding appears in line with previous results (Melas et al., 2012; Shimabukuro et al., 2007) which demonstrated global DNA hypo-methylation in schizophrenia. It stands therefore unclear whether the global hypo-methylation is to be considered as an epigenetic consequence of CT or a trait of psychosis. Genome wide DNA methylation and genome wide gene expression studies showed that differently methylated or differently expressed genes in FEP, when compare with healthy controls, were related to nucleotide and transcription factor binding, mitochondrion functioning, lipid/glucose/protein metabolism, signal transduction and RNA processing. Such alterations could be considered both a direct (expression of a true biological difference) and an indirect (for example related to environmental exposures) manifestation of the psychosis. FEP subjects showed a significantly increased methylation and reduced GCH1 gene expression (Ota et al., 2014). GCH1 is located in chromosome 14 and it is involved in the tetrahydrobiopterin (BH4) production, which in turn, as a cofactor enzyme, plays an important role in the synthesis of dopamine and serotonin (Richardson et al., 2005). Therefore, GCH1 altered methylation and expression, observed in FEP could potentially entail a neurotransmission dysfunction. Finally, MPB, NDEL1, AKT1 and DICER1 were found hyper-expressed, while DROSHA, COMT, and DISC1 resulted hypo-expressed in FEP in comparison with healthy controls. They are all involved in a variety of CNS functions including neurodevelopment, plasticity and neurotransmission (Gouvea et al., 2016; Noto et al., 2016). Notably, none of the studies investigating epigenetic modifications in FEP, explore the potential role of environmental exposures, such as CT.

In conclusion, childhood trauma appears associated in both FEP and healthy samples with a variety of epigenetics modifications and seems linked to specific

gene expression profiles. Some alterations observed in FEP are most probably ascribable to the psychosis itself, rather than to CT, while some other modifications found in healthy subjects could represent vulnerability factors for psychosis. However, the understanding of how, at a biological level, childhood trauma is translated into a higher risk for psychosis is still a key research challenge.

1.5 PSYCHO-SOCIAL CORRELATES OF THE ASSOCIATION BETWEEN CHILDHOOD TRAUMATIC EXPERIENCES AND SEVERE MENTAL DISORDERS

As aforementioned, from a psychological point of view, the association between childhood traumatic experiences and severe mental disorders has been mainly explored in light of cognitive processes and meaning attribution, dissociative phenomena, and attachment theory.

Garety et al. (2001) have suggested that childhood traumatic experiences can bias attributions of cause towards external sources, eliciting the development of negative schemas about the self and others, and in doing so, contributing to the development of paranoid delusions. The cognitive models proposed to underline the association between childhood traumatic experiences and severe mental disorders have been numerous (Larkin & Read, 2008): their common element appears to be the presence of cognitive-behavioral patterns, acquired during childhood, determined by traumatic experiences, and responsible for the increased vulnerability. The experience of abuse would lead, in fact, to the shaping of negative believes about oneself, the others and the world, such as "I am vulnerable", "others are unreliable" and "the world is threatening/dangerous", which would then condition the reading of reality and daily events and would facilitate the development of a severe mental disorder (Larkin & Read, 2008).

Dissociation can be defined as "*a disruption in the usually integrated functions of consciousness, memory, identity, or perception of the environment*" (American Psychiatric Association, 2000). It has been suggested to mediate, albeit partially, the relationship between trauma and subsequent psychopathology (Gershuny et al., 2004) and has been shown to account for the association between sexual abuse and hallucinations (Perona-Garcelán et al., 2012; Varese et al., 2012b; Chu & Dill, 1990). A dissociative response to childhood trauma can be considered, within the framework of the traumagenic neurodevelopmental model, as the first step of the pathway to psychosis (i.e. hallucinations, delusions and dissociative symptoms) (Read et al., 2001). Causing impaired reality testing, dissociation can in fact favor the experience of psychotic experiences, particularly hallucinations (Kilcommons

& Morrison, 2005), in subjects coping from trauma, particularly regarding sexual abuse (Varese et al., 2012b). The concept of 'Self' described as *"…an organized and interactive system of thoughts, feelings, identities, and motives that (1) is born of self-reflexivity and language, (2) people attribute to themselves, and (3) characterizes specific human beings*" (Owens, 2003), may be important in relation to dissociation and psychosis vulnerability. Several assumptions have been theorized, as for example: "…*dissociative detachment renders individuals vulnerable to psychosis not only because it deprives them of external anchors... In addition, it robs them of internal anchors - the sense of being connected to one's body, a sense of self or identity, and one's own actions. The result may be not only profoundly impaired reality-testing but also severe confusion, disorganization, and disorientation*" (Allen et al., 1997). Particular attention has been paid to the so-called self-concept clarity (Campbell et al., 1996, 2003) defined as "*the extent to which the contents of the self-concept, i.e. the totality of an individual's thoughts and feelings with reference to himself as an object* (Rosenberg, 1979), *are clearly and confidently defined, internally consistent, and temporally stable*" (Campbell et al., 1996).

There is emerging evidence that evokes a role of self-concept clarity within the childhood trauma – dissociation – psychosis pathway, suggesting that levels of trauma-related dissociation would be inversely associated with self-concept integration (Lutz & Ross, 2003; Pollack et al., 2001).

Childhood traumatic experiences have been strongly associated (OR=41.2) with the development of an insecure attachment style (WHO, 2014). Insecure attachment styles, both anxious (preoccupied) and avoidant (dismissing), characterized by difficulties in trusting others, have also been suggested to represent a factor potentially favoring a paranoid attributional style (Bentall & Fernyhough, 2008). This hypothesis is line with findings showing significant association between being raised in institutional care and paranoia (Bentall et al., 2012), and between insecure attachment styles and subclinical paranoia in a non-clinical sample (Pickering et al., 2008). The attachment theory concerns infant emotional bonding and supposes that over time, the child's perception of the interaction with their primary caregivers

would affect the formation of internal working models of the self and others (Bowlby, 1973). The development of secure attachment occurs when a positive working model is formed. In this case, a feeling of security and safety is established and the caregiver is perceived as responsive, accessible, and trustworthy (Bowlby, 1973). Conversely, adverse interactions lead to negative working models of others as unpredictable (anxious/ambivalent) and unavailable (anxious/avoidant) (Gamble & Roberts, 2005). Internal working models have been interpreted as archetypes, applied throughout a lifetime and shaping individuals' understanding and anticipation of others' behaviour (Bowlby, 1973). Four different attachment styles have been outlined (Hazan & Shaver, 1987, Bartholomew & Horowitz, 1991): the secure, the preoccupied, the dismissing, and the fearful style. An individual with a secure attachment style have a positive view of others and the self. The anxious style (preoccupied) reflects a positive view of others and a negative view of the self. Individuals seeks to gain approval and acceptance from others, feeling unworthy of love. On the opposite, the avoidant (dismissing) attachment style reflects a negative view of others and a positive view of the self. Individuals perceived others as untrustworthy and rejecting, and although feeling worthy of love, they avoid close relationships. Finally, the fearful attachment style reflects a negative view of both others and the self: individuals feels unworthy of love and avoids relationships to protect against rejection. Insecure attachment styles may act to increase the likelihood of mental health difficulties compared to patterns of secure attachment (Sroufe, 2005)

Finally, several social correlates, including gender and socio-economic status, also seem to be involved within the association between childhood trauma and severe mental disorder. On one side, a gender difference in stress reactivity has been hypothesized. Women have in fact been shown to be more susceptible to the negative consequences of stress in general (Myin-Germeys et al., 2004) and of early traumatic experiences, in particular (Myin-Germeys et al., 2007). A recent study, described a significant association between childhood sexual abuse and depressive symptoms in women but not in men, and suggested that maltreatments may have a gender effect on the development of mental disorders (Haug et al., 2015). Finally,

although this issue will not be further discussed in this thesis, poverty has to be mentioned as a potential mediator of the association between childhood trauma and psychosis. Poverty and mistreatment of children go hand in hand. Although there is no evidence which shows that poverty causes child maltreatment, poverty and child maltreatment share many similar risk factors, and frequently overlap (Jütte et al., 2014). Moreover, number of studies using varied measures of maltreatment and adult economic outcomes support a specific association between child maltreatment and a range of poverty-related outcomes (Bywaters et al., 2016 – JFR Report): mistreated children are more likely to live in poverty in their adulthood. On the other side, it cannot be denied the importance of poverty as a variable adversely influencing health. In Bridging the Gaps, the World Health Organization (1995) states, '*The world's most ruthless killer and the greatest cause of suffering on earth is extreme poverty*.' It is a well-recognized fact that poverty has important implications for health, and can be considered both a determinant and a consequence of poor mental health (Langner & Michael, 1963). Subjects living in disadvantaged circumstances have been demonstrated to suffer disproportionately from common mental disorders and their adverse consequences (Patel et al., 2010; Campion et al., 2013; Melzer et al., 2004; Patel & Kleinman, 2003). A systematic review showed that the vast majority (70%) of the 115 included studies reported a significant and positive association between poverty and mental disorders (Lund et al., 2010). Low socio-economic status, lower educational and lower social achievement levels, social isolation, economic deprivation and financial debt, stressful, unrewarding and de-personalizing work or unemployment, dangerous environments, and poor marital relationships, can be considered among the social determinants mostly involved (Allen et al., 2014; Murali & Oyebode, 2004). Once again, a significant gender-related difference appears to subsist: being equal the level of household income, women show higher prevalence of mental disorder than men (McManus et al., 2007).

The relationship between poverty and mental disorder is complex. Two main hypotheses have been proposed: social causation ('breeder') and social selection ('drift'). The social causation hypothesis asserts that experiencing greater socio-economic adversities, typical of lower-class living conditions, increases the risk of

subsequent mental illness. According instead to the social selection/drift hypothesis, mental health can influence socioeconomic achievement and lead people to drift down the occupational and social scale, into the lower social class, or never escape poverty (Mossakowski, 2014).

CHAPTER 2: CHILDHOOD TRAUMA AND PSYCHOSIS

2.1 THE GET UP PROJECT RESEARCH

The Genetics, Endophenotypes and Treatment: Understanding early Psychosis – (GET UP) was a large research program, which aims to compare, at 9 months, the effectiveness of a multi-component psychosocial intervention versus treatment as usual (TAU) in a cohort of patients with FEP (Ruggeri et al., 2012). They and their family members were recruited from all public community mental health centers (CMHCs) located in two entire regions of Italy (Veneto and Emilia Romagna), and in the cities of Florence, Milan and Bolzano. GET UP research Program was constituted by four partner projects including "Psychosis early Intervention and Assessment of Needs and Outcome" (GET UP PIANO) Trial and "Genetic data Utilization and Implementation of Targeted drug Administration in the clinical Routine" (GET UP GUITAR) Project.

The GET UP PIANO Trial had a pragmatic cluster randomized controlled design. The randomized units (clusters) were the CMHCs, and the units of observation were the centers' patients and their family members. Patients in the experimental group received TAU plus: 1) cognitive behavioral therapy sessions, 2) psycho-educational sessions for family members, and 3) case management. Patient enrolment took place over a 1-year period. Several psychopathological, psychological, functioning, and service use variables, including the presence of childhood traumatic experiences, were assessed at baseline and at 9-months follow-up.

Several studies were performed exploring data from the GET UP Research Program. With reference to childhood traumatic experiences, the effects of early adversity were investigated in first-episode psychosis patients in terms of diagnosis and lifetime cannabis use (Tomassi et al., 2017), clinical and functional profiles (Ritunnano et al, under review), and glucose metabolism (Tosato et al., in preparation). In this PH thesis, I will report the two studiesI have significantly contributed.

2.2 THE INFLUENCE OF CHILDHOOD TRAUMA ON DIAGNOSIS AND SUBSTANCE USE IN FIRST-EPISODE PSYCHOSIS

Childhood trauma has been associated with first-episode psychosis (Fisher et al., 2010; Mondelli et al., 2010), affective dysfunction (Nanni et al., 1011; Tunnard et al., 2014; Gaudiano & Zimmerman, 2010; Upthegrove et al., 2015), and substance use (Madruga et al., 2011; Houston et al., 2011; Konings et al., 2011). In a recent case-control study, first-episode psychosis (FEP) patients showed a prevalence of physical abuse of 14.0% – 15.2% and a prevalence of sexual abuse of 18.2% (Fisher et al., 2010). In another sample of FEP patients, two-fold higher rates of childhood trauma were reported in cases compared with controls (Mondelli et al., 2010).

Exposure to childhood trauma was found to be associated with a two-fold risk of both recurrent and persistent depression (Nanni et al., 2011); it predicts lifetime suicide attempts in treatment-resistant depression patients (OR 2.79; 95% CI 1.14-6.84) (Tunnard et al., 2014), and it has been related to the presence of psychotic features in mood disorders (Gaudiano & Zimmerman, 2010).

Finally, childhood trauma has been associated with a higher risk of substance use in adolescence and early adulthood in the general population (OR 3.83; 95% CI1.29–11.30) (Madruga et al., 2011) and might also have a role in modulating and moderating the association between cannabis use and the later development of psychosis (Houston et al., 2011). The risk for psychosis is higher in those who have been exposed to both childhood trauma and cannabis use compared to those exposed to only one of the two. The strength of this association was found to be dose-dependent, with higher rates of psychosis as the frequency and severity of traumatic experiences increase (Konings et al., 2011).

Based on the above literature on first-episode psychosis, childhood trauma, affective dysfunction, and cannabis use, we aimed to verify, in a large epidemiologically representative sample of FEP patients, whether subjects who had experienced childhood trauma, when compared with those who had not, show: 1) a psychosis onset characterized by a higher rate of affective psychosis and 2) an increased lifetime rate of substance use.

Methods

This study, published in 2017 in the British Journal of Psychiatry (Tomassi et al., 2017) was conducted within the framework of the "Genetics Endophenotypes and Treatment: Understanding early Psychosis - Psychosis early Intervention and Assessment of Needs and Outcome" (GET UP PIANO) Trial. As aforementioned, the GET UP PIANO Trial was a large, multicenter, randomized, controlled trial comparing an add-on multi-element psychosocial early intervention with 'routine care' for subjects affected by FEP and their relatives, provided within Italian public mental health services. Detailed information on the study design, sample recruitment, and clinical assessment has been reported elsewhere (Ruggeri et al., 2012).

The GET PIANO Trial (Ruggeri et al., 2015) was proposed to all community mental health centres (CMHCs) located across two northern Italian regions (Veneto and Emilia-Romagna) and the urban areas of Florence, Milan, and Bolzano, covering an area of 9,951,306 inhabitants. Of 126 CMHCs, 117 (92.8%, covering 9,304,093 inhabitants) participated. The trial was approved by the Ethics Committees of the coordinating center (Azienda Ospedaliera Universitaria Integrata di Verona) and each participating unit and was registered with ClinicalTrials.gov (NCT01436331).

Subjects

All CMHC professionals were asked to refer potential psychosis cases at first contact during the index period (Apr 1, 2010-Mar 31, 2011) to the study team. Immediately thereafter, a screening questionnaire for psychosis (Jablensky et al., 1992) was administered. The inclusion criteria to ascertain FEP were: (a) age 18–54 years; (b) residence within the catchment areas of CMHCs; (c) presence of at least 1 of the following symptoms: hallucinations, delusions, qualitative speech disorder, qualitative psychomotor disorder, or bizarre or grossly inappropriate behavior; or 2 of the following symptoms: loss of interest, initiative, and drive; social withdrawal; episodic severe excitement; purposeless destructiveness; overwhelming fear; or marked self-neglect; and (d) first lifetime contact with CMHCs, prompted by these symptoms.

Exclusion criteria were: (a) antipsychotic medication (>3 months) prescribed for an identical or similar mental disorder ; (b) mental disorders due to a general medical condition; (c) moderate-to-severe mental retardation assessed by clinical functional assessment; and (d) psychiatric diagnosis other than ICD-10 for psychosis.

Since FEP is generally a phase of high diagnostic instability, the specific ICD-10 codes for psychosis (F1x.4; F1x.5; F1x.7; F20–29; F30.2, F31.2, F31.5, F31.6, F32.3, F33.3) were assigned at 9 months. The best-estimate ICD-10 diagnoses were made by consensus by a panel of clinicians by taking into account all available information gathered in the 9-month follow-up period, as required to apply the Item Group Checklist (IGC) of the Schedule for Clinical Assessment in Neuropsychiatry (SCAN) (Wing et al., 1990).

Eligible FEP patients, identified as those who were sufficiently clinically stable, gave written informed consent (Ruggeri et al., 2015).

Assessment

All FEP patients were assessed by 17 independent researchers who underwent specific training on the use of the standardized instruments and an inter-rater reliability exercise to determine consistency of evaluations between investigators.

Information about childhood trauma and substance use was collected using the Childhood Experience of Care and Abuse-Questionnaire version (CECA-Q) (Bifulco et al., 2005) and the Cannabis Experiences Questionnaire (Barkus et al., 2006), respectively.

Based on previous literature (Bifulco et al., 2005; Aas et al., 2012), severe sexual abuse (SSA) has been defined as "an experience that had to meet at least two of the following criteria: the individual knew the perpetrator; the perpetrator was a relative; the perpetrator lived in the same household; the unwanted sexual experience occurred more than once; the perpetrator touched the child's genitals; the perpetrator forced the child to touch their genitals; the abuse involved intercourse." Severe physical abuse (SPA) has been defined as "a repeated exposure to physical violence from parental figures before age 16 that had to meet at least two of the following criteria: the abuse consisted of being hit with a belt or stick or being punched or kicked; the abuse resulted in an injury, including broken limbs,

black eyes, or bruising; the perpetrator was considered to be out of control." Separation has been defined as a detachment from at least one living relative longer than 6 months within the first 17 years of life; loss was defined as the death of one or both parents during the subjects' childhood. Subjects were defined as "traumatized" if they had experienced at least one trauma: severe sexual abuse, severe physical abuse, separation, and/or loss.

Lifetime substance use, including several types of drugs, was assessed by the Cannabis Experiences Questionnaire (Barkus et al., 2006). Subjects were stratified into two groups: those who had never used substances and those who had used substances at least once in their life.

Description of the sample

Within the GET UP PIANO Trial, 444 FEP patients identified at the intake with a confirmed ICD-10 diagnosis of psychosis at 9 months were assessed. Out of them, 345 (77.7%) [(57.7% male, mean age 29.8 years (SD ± 9.7)] FEP subjects agreed to be interviewed on childhood traumatic experiences and represented the sample of the present study. Socio-demographic features and clinical characteristics of the sample are shown in Table 9. No significant differences were found with regard to socio-demographic or clinical characteristics between subjects who completed the CECA-Q and those who did not (data available from the authors), with the exception of non-Italian nationality, which was more frequent among those who did not complete the CECA-Q ($p=0.010$).

Table 9: Socio-demographics of patients assessed with CECA-Q (n=345)

Gender, *n (%)* M	 199	 (57.7)
Age at first contact with services, *mean (SD)*	29.8	(9.7)
Educational level, *n (%)* **Low** (primary–middle school) **High** (secondary school, university)	 125 211 Missing 9	 (37.2) (62.8)
Marital status, *n (%)* **Unmarried** **In a relation/married** **Widowed/separated/divorced**	 251 62 20 Missing 12	 (75.4) (18.6) (6.0)
Working status, *n (%)* **Unemployed** **Employed** **Student/housewife/retired**	 107 126 103 Missing 9	 (31.8) (37.5) (30.7)
Nationality, *n (%)* **Italian** **Other**	 311 30 Missing 4	 (91.2) (8.8)

Among the 345 FEP subjects who completed the CECA-Q, 80 (23.2%) received an ICD-10 code for affective psychoses, while 265 (76.8%) received an ICD-10 code for non-affective psychoses (N=96, 27.8% of schizophrenia; N=169, 49.0% of non-affective, non-schizophrenic psychosis).

Regarding childhood trauma, in the 345 FEP subjects, 8.5% experienced severe sexual abuse during their childhood, 14.3% reported severe physical abuse, and 20.4% was separated for more than 6 months from at least one of the parental figures and/or lost one of their parents. Thus overall, 37.1% had had at least one traumatic experience during their childhood.

In terms of lifetime substance use, 43.3% out of the 345 FEP subjects reported cannabis use, 20.0% referred cocaine use, and 6.3% recalled heroin use. When looking at combined lifetime use, we found, as expected, that the totality (100.0%) of subjects who referred a lifetime use of heroin had both cocaine and cannabis lifetime use. Similarly, subjects who reported a lifetime use of cocaine also had a lifetime cannabis use in the 96.3% of cases. Finally, among those with a cannabis lifetime use, 52.3% related an exclusive use of cannabis.

Statistical analysis

The association between categorical variables was evaluated by chi-square or Fisher's exact test, where appropriate. Adjustment for gender was performed in univariate logistic regression models, with specific types of traumas as dependent variables and diagnosis and substance misuse as independent variables, respectively. Interaction between gender and trauma was controlled for in all models. All tests were bilateral at $p<0.05$. Analyses were performed by SPSS 22.0 for Windows.

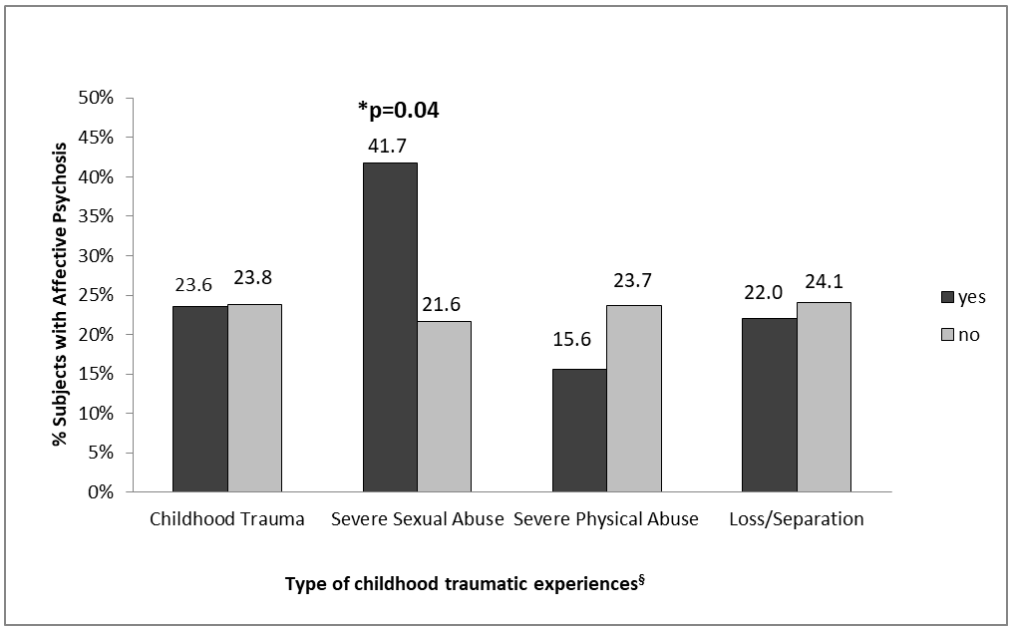
Results

Childhood trauma and diagnosis

As shown in Figure 4, the overall rate of affective psychosis detected in traumatized and non-traumatized subjects was around 24.0% in both groups (23.6% vs 23.8%, respectively; p=1.00). Analysing the specific types of traumas, we found a

significant association between severe childhood sexual abuse and affective psychosis (X2=4.9, p=0.040). In particular, 41.7% of subjects who reported severe sexual abuse had an affective psychosis compared with 21.6% of non-sexually abused subjects; this finding remained significant after adjusting for gender (OR=2.2, CI 95% 1.1–6.2; p=0.03). The interaction between gender and trauma was not significant. In contrast, the percentage of affective psychosis was lower in patients with severe physical abuse than in those without it (15.6% vs 23.7%; p=0.25), although this difference was not significant. Moreover, no significant difference was found in terms of diagnosis between subjects with a history of separation or loss of parents and those without such a history (22.0% vs 24.1%; p=0.85).

Figure 4: Association between childhood traumatic experiences and diagnosis

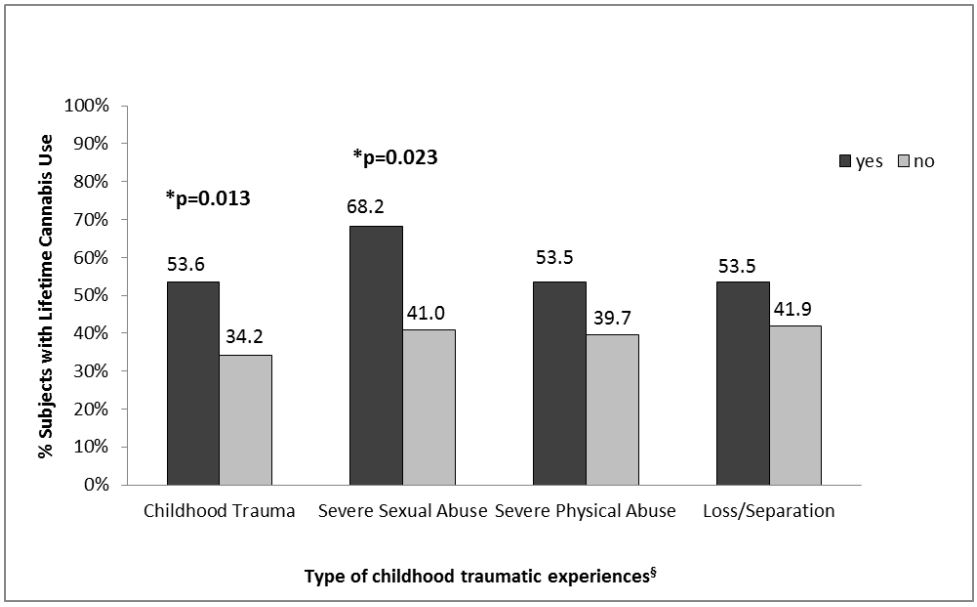


§Childhood trauma (at least 1 among severe sexual abuse; severe physical abuse; loss/separation): 72 out of 194 (56%) subjects with information available
Severe sexual abuse: 24 out of 283 (82%) subjects with information available
Severe physical abuse: 45 out of 315 (91%) subjects with information available
Loss/separation: 50 out of 245 subjects (71%) with information available

Childhood trauma and substance use

Concerning cannabis, about one-half (53.6%) of subjects with traumatic experiences during childhood had lifetime cannabis use compared with 34.2% of those without traumatic experiences (p=0.013) (Figure 5). In particular, cannabis use was significantly more frequent among patients reporting severe sexual abuse compared with those who did not report severe sexual abuse (68.2% vs 41.0%, respectively; p=0.023). This finding remained significant after adjusting for gender (OR=4.6 CI 95% 1.7-12.5; p=0.003). The interaction between gender and trauma was not significant. On the contrary, no significant association was found between severe physical abuse or loss/separation and lifetime cannabis use.

Figure 5: Association between childhood traumatic experiences and lifetime cannabis use



§Childhood trauma (at least 1 among severe sexual abuse; severe physical abuse; loss/separation): 69 subjects out of 180 (52%) with information available
Severe sexual abuse:22 subjects out of 261 (76%) with information available
Severe physical abuse:43 subjects out of 280 (81%) with information available
Loss/separation: 43 subjects out of 215 (62%) with information available

Regarding heroin, no significant difference in lifetime use was found between traumatized and non-traumatized patients (6.3% vs 5.2%, respectively; p=0.74). Instead, severely sexually abused participants reported significantly higher lifetime heroin use compared with non-sexually abused subjects (20.0% vs 4.6%, p=0.020). This finding remained significant after adjusting for gender (OR=12.6 CI 95% 2.7-58.1; p=0.001). Similarly, physically abused individuals reported substantially higher heroin lifetime use when compared with non-physically abused subjects (14.6% vs 4.7%, p=0.030). This finding remained significant after adjusting for gender (OR=3.7 CI 95% 1.2-11.3; p=0.020). The interaction between gender and trauma was not significant. In contrast, no significant association was found between loss/separation and lifetime heroin use (7.9% in exposed vs 6% in non-exposed; p=0.71).

Lastly, no significant difference in lifetime cocaine use (23.4% vs 15.8%; p=0.30) was found between traumatized and non-traumatized subjects. In particular, no significant association was found between severe sexual abuse or loss/separation and lifetime use of cocaine. Conversely, severely physically abused participants showed higher lifetime cocaine use in comparison with non-physically abused patients (31.7% vs 17.4%, p=0.050). This finding gained significance after adjusting for gender (OR=2.3 CI 95% 1.1-4.9; p=0.040). The interaction between gender and trauma was not significant.

Overall, severely abused individuals showed a significantly higher frequency of lifetime substance use (i.e., cannabis, cocaine, or heroin) than non-abused individuals (59.8% vs 39.2%, p=0.008).

Discussion

This is the first study to investigate the relationship between childhood trauma and affective psychosis in a large FEP sample recruited in a ‘real world’ setting. As hypothesized, we found a significant association between affective psychosis and severe sexual abuse and between drug misuse and both severe sexual and severe physical abuse.

In the present study, the frequency of sexual and physical abuse was substantially lower than in previous FEP studies (Fisher et al., 2010; Neria et al., 2002). In fact, severe sexual abuse was reported in 8.5% of our subjects versus 15.0-18.2% in previous works (Neria et al., 2002; Fisher et al., 2010), and similarly, severe physical abuse was reported in 14.3% versus 15.2–21.6% (Fisher et al., 2010; Neria et al., 2002). Variability in the prevalence of abuse has been widely described before. Methodological issues, such as heterogeneity of definition, sample size, and demographic and social context issues, have been also identified as possible reasons for this variability (Fisher & Craig, 2008). The use of a "severe" abuse category, instead of the "abuse" one, might account for differences with other studies. Rates of sexual and physical abuse in our sample were 10.6% and 28.6%, respectively. Regarding sexual abuse, our rate remains substantially lower than others (Neria et al., 2002; Fisher et al., 2010). In contrast, the prevalence of physical abuse is basically in line with the literature. The surge of the rate of physical abuse in our sample might depend on the inclusion in this category of physical punishments (i.e., open-handed smacks) and maltreatments (such as punches and kicks), even when occurring once in a lifetime. The social acceptability of corporal punishments as a method of education and their consequent high frequency within Italian families might therefore explain the data.

Overall, the lower percentage of abused patients in our sample is consistent with a survey carried out in the Italian general population, in which eight children out of 1000 were reported to be victims of maltreatments; specifically, among minors under the care of Child Services, 4.2% was sexually abused, while 6.9% experienced physical maltreatment (CISMAI-TDH, 2015). When compared with rates from the WHO Global Status Violence Prevention, a discrepancy comes to light with Italian rates, which are substantially lower, and other high-income countries' rates (WHO, 2014). In the US, the prevalence of sexual abuse and physical maltreatment was 7.0% and 10.0%, respectively; in Canada the prevalence was 7.0% and 23.0%, in Australia it was equal to 10.0% and 28.0%, respectively. These differences might be due to a more cohesive family and an enhanced social support network, which are common in southern European countries (Bertani et al., 2012), or it could reflect the proneness to silence, connected with the feelings of

shame and stigma, related to the experience of trauma (Taylor, 2015). Nonetheless, comparing our FEP trauma rates (sexual abuse 10.6%, physical abuse 28.6%) with those of the Italian general population (sexual abuse 4.2%, physical abuse 6.9%), the results confirm at least a two-fold increase and are consistent with findings shown previously (Mondelli et al.,2010) .

Affective psychosis and childhood trauma

In our study, severely sexually abused FEP subjects had a five-fold higher likelihood of receiving a diagnosis of affective psychosis. Our results are in line with previous studies, which showed an increased presence of psychotic features in both unipolar depression and bipolar disorder when the subject was abused (Gaudiano & Zimmerman, 2010; Upthegrove et al., 2015; Hammersley et al., 2003). Affective symptoms could mediate the victimization-psychosis association. Our data are consistent with previous studies (Garety et al., 2007; Gracie et al., 2007), which advocate a significant role of negative beliefs about self and others and of depression and anxiety within the pathway from early trauma to psychosis. Most interestingly, only sexual abuse, and not physical abuse, has been proven to be a strong and specific risk factor for mental disorders, including major depression and anxiety disorder (Fergusson et al., 2013), once more supporting our results. A recent study described a significant association between sexual abuse and depressive symptoms in women but not in men, suggesting that maltreatments may have a gender effect on the development of maladaptive self-images and depression (Haug et al., 2015) mediated by a gender difference in stress reactivity. Women have in fact been shown to be more susceptible to the negative consequences of stress in general (Myin-Germeys et al., 2004) and of early traumatic experiences, in particular (Myin-Germeys et al., 2007). In contrast, we did not find any effect of gender on the association between severe sexual abuse and affective psychosis. Our study did not investigate the presence of depressive symptoms but focused on diagnostic category; issues other than gender might therefore be involved and explain the discrepancy with previous evidence.

Substance use and childhood trauma

In our sample, drug use was found to be associated with both severe sexual and severe physical abuse. Traumatized FEP subjects and, in particular, those reporting severe childhood sexual abuse showed significantly higher lifetime cannabis use.

The trauma-cannabis-psychosis association could involve several psychosocial elements, contributing to an enhanced vulnerability (Houston et al., 2011), and various biological factors, including the dopamine neurotransmitter system (Kaufman et al., 2000) and the hypothalamic-pituitary-adrenal axis (Mondelli et al., 2010). First, childhood trauma has been significantly associated with depression, which usually precedes the onset of substance dependence among traumatized people (Douglas et al., 2010). Consistent with this, abused FEP subjects in our sample more frequently received a diagnosis of affective psychosis when compared with those who were not abused. We may therefore hypothesize the role of affective symptoms as mediators/modulators of the trauma-cannabis-psychosis association. Abused people tend to become more frequently depressed and, thus, might more frequently use cannabis to alleviate depressive symptoms or could develop dysfunctional coping strategies, such as self-medication, to reduce trauma-related stress (Houston et al., 2011). Second, social factors might have a meaningful influence: disadvantaged environments, where it is easier to get entrapped into substance dependence, and social adversities, such as a low socioeconomic status and unemployment, have been shown to be significantly associated with both psychosis and childhood trauma (Wicks et al., 2005).

Finally, it is reasonable to hypothesize that abused and non-abused individuals have similar drug misuse patterns but that only the former develop psychosis due to a pre-existing enhanced vulnerability (Houston et al., 2011; Tosato et al., 2013).

Biological factors might also be involved. First, and in line with the "sensitization hypothesis," genetically predisposed subjects, whether exposed to environmental risk factors (as childhood trauma and cannabis use) or not, have been proven to show an increased dopaminergic response to social stressors. It may lead to stable changes in the stress-related dopaminergic response system (Collip et al., 2008) and eventually to a subsequent enhanced vulnerability for psychosis. Second, animal studies have shown an influence of "environmental" stressors on a rat's ΔTHC response (Suplita et al., 2008): under stressful housing conditions (i.e., isolation and

food deprivation), THC administration resulted in an increased dopamine uptake and significant behavioural abnormalities, not observed in control rats (MacLean et al., 1977). Finally, early traumatic experiences have been associated with permanent hypothalamus-pituitary-adrenal (HPA) axis over-reactivity (higher levels of cortisol) to stressors and changes in brain structures (Read et al., 2001).
Moreover, we found that both sexual and physical severe abuse experiences were also significantly associated with heroin lifetime use, while cocaine lifetime use appeared to be associated only with severe physical abuse, which is somewhat in line with results from other non-FEP studies (Banducci et al., 2014).
Our findings seem to be in contrast with a recent study (Duhig et al., 2015) that did not find any association between childhood trauma and use of cannabis or other illicit drugs. This inconsistency might be partially explained by the different time frame used when investigating substance use: the present study explored lifetime use, while the other investigated substance use in the previous month.

Strengths and limitations

GET UP PIANO is the first FEP patient trial performed in a large catchment area, corresponding to nearly 10 million inhabitants. Over 90% of CMHCs completed the study, demonstrating that the subjects were highly representative of the patients treated in the community psychiatric services. We used reliable, internationally validated instruments and adopted conservative cut-off points, previously applied, to identify only the most severe forms of abuse. Moreover, we systematically performed adjustments, excluding the gender effect, known to represent a potential confounder on the association between trauma and psychosis (Fisher et al., 2009).
However, some limitations should also be considered. Reliance on the retrospective reporting of abuse might increase the risk of recall bias. However, the reliability of patients affected by psychosis in referring trauma has been clearly demonstrated (Fisher et al., 2011): their reports were independent from symptomatology, stable over the course of time, and generally consistent with other sources of information. It has also been proven that retrospective recall yields an underestimation of the phenomenon, rather than an overestimation (Hardt et al., 2004).

Despite clear evidence of association between trauma, cannabis, and psychosis, our data do not elucidate whether childhood traumatic experiences and drug misuse represent vulnerability factors for psychosis or environmental stressors that trigger the disorder acting on a pre-existing vulnerability. Traumatic experiences and drug misuses could both be vulnerability agents, which decrease the subject's resilience in response to other stressors (i.e., migration) and lead to psychosis. They might represent two different environmental stressors, acting (in an additive or in a multiplicative way) on another type (genetic?) of vulnerability. Childhood trauma could enhance vulnerability and cannabis use acts as an exacerbating agent, or alternatively, vice versa. Finally, because of the relative rarity of abusive experiences resulting in small numbers, no further adjustments for social factors (such as employment or socioeconomic status) and/or genetic vulnerability (familiarity) have been carried out in the analysis.

In the future, in addition to clarifying whether cannabis and childhood trauma represent vulnerability factors or stressors triggering psychosis, studies should also analyze this association, taking into account other environmental variables and/or biological markers. A population-based, longitudinal prospective study design, with long-term follow-up (from infancy to adulthood) would be most appropriate (Poulton et al., 2015).

Overall, this study suggests that FEP patients exposed to childhood trauma constitute a distinctive subgroup characterized by diverse features in terms of nosology and drug use. It has elucidated, albeit partially, to what extent the presence of childhood trauma affects FEP features. Finally, this study may provide some important hints for specific therapeutic and/or preventive interventions, which might carry within themselves an enhanced impact on illness course, outcomes, and prognosis.

2.3 CHILDHOOD TRAUMA AND/OR STRESSFUL LIFE EVENTS AND METABOLIC DYSFUNCTION IN FIRST-EPISODE PSYCHOSIS

Introduction

People with severe mental disorders, including schizophrenia and bipolar disorder, have increased mortality rates, 2–3 times higher than the general population (Reininghaus et al., 2015) turning into a mortality gap of 10–20 years (Lawrence et al., 2013). Epidemiological evidence suggests that physical illness, including cardiovascular disease and type 2 diabetes, account for the most of the increased mortality risk (Olfson et al., 2015). In details, subjects affected by schizophrenia (Mitchell et al., 2013a, 2013b) or bipolar disorder (Vancampfort et al., 2013, 2015a) are at higher risk to develop metabolic syndrome when compared with the general population. Indeed, subjects affected by schizophrenia have twice the risk of developing type 2 diabetes compared to general population (Stubbs et al., 2015): traditionally, this association has been attributed to the secondary effects of antipsychotics (Vancampfort et al., 2015b) or to sedentary life style or unhealthy dietary regimen associated with negative symptoms of psychosis (Samele et al., 2004). Interestingly, accumulating evidence show abnormal glucose metabolism in drug-naïve subjects with schizophrenia (Spelman et al., 2007). Indeed, higher levels of insulin (Chen et al., 2013, Pillinger et al., 2017), insulin-resistance (Ryan et al., 2003; Verma et al., 2009; Arranz et al., 2004, Chen et al., 2013, Pillinger et al., 2017) and an increased levels of insulin-related peptides (Guest et al., 2010; Guest et al., 2011) as C-peptide (Wu et al., 2013) were found in drug-naïve subjects with first episode of schizophrenia. In bipolar disorder, it has been demonstrated increased levels of some adipokines (like leptin and adiponectin) (Barbosa et al., 2012) and a reduction of glucagon, GLP-1 e ghrelin blood levels associated with increased GIP levels (Rosso et al., 2015), when compared with healthy subjects. Interestingly, the abnormal levels of GIP, GLP-1 and ghrelin found in these patients do not appear to be associated nor with the medications used neither with the presence of diabetes, hypertension or metabolic syndrome (Rosso et al., 2015). Finally, a recent work of our group reported a decreased levels of ghrelin, glucagon

and GLP-1 in first-episode psychosis (FEP) patients at the onset regards to controls and an increased PAI-1 levels (Bocchio-Chiavetto et al., 2018).

Notably, social determinants of health occurring during childhood have been documented to increase the risk for the emergence of both psychosis and metabolic dysfunction, providing a conceptual framework to explain this association. Specifically, the most studied environmental risk factor common to both conditions is childhood trauma (Varese et al., 2012a; Danese & Tan, 2014). Specifically, childhood trauma acting as chronic and severe stress could induce the production of elevated levels of glucose and insulin in blood (Deppermann et al, 2014). In addition, Hypothalamus-Pituitary-Adrenal (HPA) axis interacts with glucose metabolism hormones, like insulin, glucagon, gastric inhibitor peptide (GIP) and glucagon-like peptide-1 (GLP-1) (Chong et al., 2014; Nussdorfer et al., 2000) increasing the likehood to develop metabolic dysfunction. Insulin has an inhibitory activity on HPA axis, while glucagon, GIP and GLP-1 have an enhancing one, inducing the release of corticotrophin release hormone (CRH)/adrenocorticotrophic hormone (ACTH) (Nussdorfer et al., 2000).

The association between FEP and metabolic abnormalities (Perry et al., 2016) is quite well demonstrated as well as the association between childhood trauma and the risk to develop psychosis (Varese et al., 2012a). Nonetheless, up to date only one study (Veru-Lesmes et al., 2018) has investigated the possible role of childhood trauma in the development of glucose metabolism disorders in FEP, finding that FEP patients who were physically abused during childhood have higher levels of glycated hemoglobin when compared with other patients.

Moreover, an excess of recent stressful life events in the year prior to psychosis onset was reported (Mansueto & Faravelli, 2017). Although stressful life events in adulthood have been associated with enhanced vulnerability to develop metabolic disorders (Rutters et al., 2015), type 2 diabetes (Maksimovic et al., 2014), the underlying biological mechanisms are still poor explored and up to date the relationship between recent stressful life events and metabolic disorders in psychosis has not been investigated yet.

Although it is not known whether a different timing in stress exposure could exert differential effects and play different roles in the future development of metabolic

related dysfunctions, the underlying biological processes have not been identified yet.

In the frame of the available knowledge, briefly summarized above, we performed a study to verify, in a large epidemiologically representative sample of FEP patients at their first contact with Italian public mental health services serving a 10 million inhabitant catchment area, whether FEP patients with an history of childhood trauma and/or stressful life events show a different metabolic biomarker profile when compared with non-traumatized subjects.

Methods

This study was conducted within the "Genetics Endophenotypes and Treatment: Understanding early Psychosis (GET UP) Research Program. Detailed information on the study design, sample recruitment and clinical assessment has been reported elsewhere (Ruggeri et al., 2012; 2015).

Based on the WHO 10-Country study (Jablensky et al., 1992), the initial target group comprised people, with potential psychosis, who had had a first contact with any community mental health centres (CMHCs) located in two northern Italian regions (Veneto and Emilia-Romagna) and the urban areas of Florence, Milan, and Bolzano, during the index period (Apr 1, 2010-Mar 31, 2011). Inclusion criteria were: (a) age 18–54 years; (b) residence within the catchment areas of CMHCs; (c) presence of at least 1 of the following symptoms: hallucinations, delusions, qualitative speech disorder, qualitative psychomotor disorder, bizarre, or grossly inappropriate behavior; or 2 of the following symptoms: loss of interest, initiative, and drive; social withdrawal; episodic severe excitement; purposeless destructiveness; overwhelming fear; or marked self-neglect; and (d) first lifetime contact with CMHCs, prompted by these symptoms. Exclusion criteria were: (a) prescribed antipsychotic medication (>3 months) for an identical or similar mental disorder; (b) mental disorders due to general medical condition; (c) moderate-severe mental retardation assessed by clinical functional assessment; and (d) psychiatric diagnosis other than ICD-10 for psychosis.

Eligible FEP patients, identified as those who reached the clinical stabilization, gave written informed consent to be assessed and to give blood sample, after a

complete description of the study. The GET UP project was approved by the Ethics Committees of the coordinating center (Azienda Ospedaliera Universitaria Integrata di Verona) and each participating unit.

Assessment

A comprehensive set of standardized instruments was used to collect clinical and psychosocial information of subjects. In details, psychopathology was assessed by the Positive and Negative Syndrome Scale (PANSS; Kay et al., 1987), depressive symptoms by the Hamilton Rating Scale for Depression, (HAMD; Hamilton, 1960) and symptoms of mania by the Bech-Rafaelsen Mania Rating Scale (BRMRS; Bech et al., 1978). Since FEP is generally a phase of high diagnostic instability, the specific ICD-10 codes for psychosis (F1x.4; F1x.5; F1x.7; F20–29; F30.2, F31.2, F31.5, F31.6, F32.3, F33.3) were assigned at 9 months. The best-estimate ICD-10 diagnoses were made by consensus by a panel of clinicians by taking into account all available information gathered from the point of enrolment into the study, as required to apply the Item Group Checklist (IGC) of the Schedule for Clinical Assessment in Neuropsychiatry (SCAN) (Wing et al., 1990). Lifetime and current substance use, including several types of drugs, was assessed by the Cannabis Experiences Questionnaire (Barkus et al., 2006). Subjects were divided into two groups: those who had never tried cannabis and those who had tried at least once in their life.

Information about childhood trauma was obtained using the Childhood Experience of Care and Abuse-Questionnaire version (CECA-Q) (Bifulco et al., 2005). It elicits information concerning experiences of childhood adversity before age 17, including physical and sexual abuse, separation from at least one of the parental figures longer than 6 months, and death of a parent. For the purpose of this study, we defined childhood trauma (CT) as having experienced at least one event among sexual abuse, physical abuse, separation and/or loss.

The presence of stressful life events (SLEs) were recorded using a semi-structured interview derived from Brown et al. (1973), which was adapted to the Italian population (Faravelli & Ambonetti, 1983). The semi-structured interview recorded the events of the prior year in detail, as well as the timing and the circumstances in

which they occurred. On the basis of these accounts, a couple of assessors evaluated the following: a) whether a given event corresponded to any of the events listed in the Paykel et al. (1971) scale; b) if it was considered severe (i.e., in the top 20 events of the Paykel list); and c) if the life event was "dependent" or "independent." For the purpose of this study, we considered the stressful events judged severe and occurred in the last 12 months (death of a family member, sexual or physical abuse, being accused of having committed a crime, sentence of imprisonment, being exposed to war or natural catastrophes, family breakdown, being removed from home, sentimental breakdown, severe physical illness).

Subjects underwent also a detailed medical examination including tobacco use and current drug therapies. BMI (kg/m2) was calculated: weight was measured without shoes, and in light indoor clothes, using a balance beam scale; height was measured without shoes using a fixed stadiometer.

Biomarker analyses

An anticoagulant-free tube was draw from each subject in the morning after an overnight fast (between 07:00 and 10:00). The tubes were kept at room temperature for 2 h followed by 1 h at 4 °C before serum separation by centrifugation (3000 g for 15 min). Serum samples were then stored at -80 °C until the time of assays.

The concentrations C-peptide, Ghrelin, GIP, GLP-1, Glucagon, Insulin, Leptin, PAI-1 (total), Resistin and Visfatin were analysed by using the Bio-Plex Pro™ Human Diabetes kit (Bio-Rad, CA, USA). In brief, 12.5 µl of each sample were diluted 1:4 with the sample diluents, and then 50 µl of the diluted sample were incubated in a pre-wet filter plate for 1 hour in the dark with the biotinylated detection antibody. Each analyte was detected by the addition of a streptavidin-phycoerythrin solution and quantified using the BioPlex array reader (Bio-Rad, CA, USA). Data acquisition and analysis from the reactions were performed using a Bio-Plex system reader. Standard curves were obtained using as reference the model given by the manufacturer. Single analytes' concentrations were calculated using the Bio-Plex manager software 6.0. All the measurements were double checked and all the specimens were processed with reference to negative controls.

Statistical analysis

Data were described means (standard deviations) for continuous variables and frequencies (percentages) for categorical variables. Differences among groups were evaluated by ANOVA with Bonferroni post-hoc comparisons in case of continuous variables, while Chi-square test was used for categorical variables. The association between adverse events and metabolites' concentrations was performed by linear regression models adjusted for confounders. The procedure was repeated after deleting extreme outliers or applying the Box-Cox transformation to the metabolites' concentrations in order to check if conclusions change.

All p-values were two-tailed with a significance level of 0.05. Statistical analyses were carried out by Stata 13.0 for Windows.

Description of the sample

Within the GET UP Research Project, 211 FEP patients out of 444 (47.5%) accepted to give blood specimens (Bocchio-Chiavetto et al, 2018). By considering the 201 patients for whom it was possible to determine metabolites' concentrations, 192 (95.5%) agreed to be assessed with CECA-Q and 143 (71.1%) were interviewed about stressful life events in the last 12 months. Overall, 139 (69.1%) patients had information on both childhood trauma and stressful life events and constituted the sample to be analyzed. No significant differences were found with regard to socio-demographic and clinical characteristics between subjects with information about metabolites' concentrations (n=201) and those without (n=243) and between subjects assessed with both CECA and stressful life events (n=139) and those not assessed (n=62). Moreover, no differences were found between these two sub-samples in metabolites' concentrations (data available from the Authors). Socio-demographic features and clinical characteristics of the sample are shown in table 10.

Table 10: Socio-demographic and clinical characteristics (n=139)

Variable	Mean (sd) or N (%)
Age at first contact with services (years)	29.9 (9.6)
Gender	
Male	81 (58.3%)
Tobacco use (17 missing)	
Yes	58 (47.5%)
Cannabis use (18 missing)	
Yes	17 (14.0%)
Psychotropic medications (17 missing)	
Yes	112 (91.8%)
Body Mass Index (BMI) (25 missing)	23.4 (3.5)
Diagnosis	
Affective psychosis	37 (26.6%)
Non affective psychosis (No schizophrenia)	62 (44.6%)
Schizophrenia	40 (28.8%)
PANSS at BL (after clinical stabilization) (1 missing)	
Positive symptoms	2.3 (0.8)
Negative symptoms	2.4 (0.9)
General symptoms	2.3 (0.5)
HAMILTON at BL (after clinical stabilization)	17.3 (6.7)
No depression	10 (7.2%)
Mild depression	59 (42.4%)
Moderate depression	52 (37.4%)
Severe depression	18 (12.9%)
BRMRS at BL (after clinical stabilization) (1 missing)	3.0 (3.6)
No mania	120 (87.0%)
Doubtful mania	10 (7.2%)
Hypomania	7 (5.1%)
Moderate mania	1 (0.7%)

Results

The 139 FEP patients assessed with both CECA-Q and stressful life events and with metabolites' blood concentrations were classified with respect to their traumatic experiences as follows: nor CT nor severe stressful events (n=41; 31.1%), no CT but at least one severe life event (n=12; 9.1%), CT but no severe life event (n=46; 34.8%), and both CT and at least one severe life event (n=33; 25.0%). For seven patients we had no enough information in order to classify them.

Comparisons among the four sub-groups with respect to age at first contact with services, gender, tobacco use, cannabis use, psychotropic medications, BMI, diagnosis, psychopathology, depression and mania at baseline did not find significant differences (Table 11), with the only exception of tobacco, whose use was found significantly more frequent among patients who experienced both CT and severe SLEs.

Table 11: Characteristics of patients classified by experienced traumatic events (n=132)

Variable	Mean (sd) or N (%)				
	No CT & no severe SLEs (n=41)	**No CT & severe SLEs (n=12)**	**CT & no severe SLEs (n=46)**	**CT & severe SLEs (n=33)**	**p-value ANOVA or Chi-Square test**
Age at first contact with services (years)	28.5 (10.9)	31.6 (11.5)	30.6 (9.2)	29.6 (7.5)	0.677
Gender					
Male	23 (56.1%)	7 (58.3%)	27 (58.7%)	19 (57.6%)	0.996
Tobacco use (16 missing)					
Yes	12 (32.4%)	6 (60.0%)	19 (48.7%)	21 (70.0%)	0.020
Cannabis use (17 missing)					
Yes	5 (12.8%)	1 (11.1%)	4 (10.8%)	7 (23.3%)	0.490
Psychotropic medications (15 missing)					
Yes	36 (94.7%)	8 (80.0%)	39 (90.7%)	24 (92.3%)	0.521
Body Mass Index (BMI) (23 missing)	23.2 (3.1)	24.8 (4.5)	23.5 (3.4)	23.7 (3.6)	0.606
Diagnosis					
Affective psychosis	10 (24.4%)	3 (25.0%)	14 (30.4%)	9 (27.3%)	0.998
Non affective psychosis (No schizophrenia)	20 (48.8%)	6 (50.0%)	20 (43.5%)	15 (45.5%)	
Schizophrenia	11 (26.8%)	3 (25.0%)	12 (26.1%)	9 (27.3%)	
PANSS at BL (after clinical stabilization) (1 missing)					
Positive symptoms	2.3 (0.8)	2.6 (0.8)	2.3 (0.8)	2.4 (1.0)	0.652
Negative symptoms	2.4 (1.0)	2.6 (1.2)	2.3 (0.7)	2.5 (1.0)	0.648
General symptoms	2.3 (0.6)	2.2 (0.5)	2.3 (0.6)	2.4 (0.5)	0.836
HAMILTON at BL (after clinical stabilization)	16.9 (6.5)	15.4 (6.2)	17.2 (6.9)	18.4 (7.0)	0.580
BRMRS at BL (after clinical stabilization) (1 missing)	2.7 (3.3)	2.2 (4.0)	3.4 (3.8)	3.6 (3.6)	0.538

By considering metabolites' concentrations, only C-peptide and Insulin showed significant differences among the four sub-groups. In detail, C-peptide was higher in both groups of patients who experienced childhood trauma (with or without SLEs) with respect to those ones who did not experience adverse events, while Insulin was higher for patients who declared CT without SLEs with respect to those ones without traumatic events (Table 12, Bonferroni post-hoc comparisons).

Table 12: Metabolites' concentrations of patients classified by experienced traumatic events (n=132)

Metabolite	**Mean (sd)**				
	No CT & no severe SLEs	**No CT & severe SLEs**	**CT & no severe SLEs**	**CT & severe SLEs**	**p-value ANOVA**
C-peptide (3 missing)	0.55 (0.30)[a,b]	0.68 (0.20)	0.85 (0.58)[a]	0.86 (0.61)[b]	**0.020**
Ghrelin (1 missing)	1.26 (0.57)	1.57 (0.45)	1.26 (0.49)	1.44 (0.44)	0.123
GIP (4 missing)	0.15 (0.08)	0.30 (0.47)	0.31 (0.86)	0.17 (0.10)	0.490
GLP-1 (2 missing)	0.35 (0.07)	0.42 (0.06)	0.39 (0.10)	0.39 (0.09)	0.053
Glucagon (2 missing)	0.90 (0.30)	0.94 (0.21)	0.98 (0.34)	0.90 (0.17)	0.534
Insulin (2 missing)	0.29 (0.14)[c]	0.31 (0.12)	0.47 (0.35)[c]	0.44 (0.31)	**0.014**
Leptin (2 missing)	12.17 (10.32)	9.38 (6.71)	10.15 (8.74)	10.09 (8.08)	0.651
PAI-1 (2 missing)	41.97 (17.45)	38.76 (24.05)	47.29 (26.13)	44.54 (22.45)	0.588
Resistin (2 missing)	3.68 (2.59)	3.44 (2.36)	3.96 (2.34)	4.07 (1.94)	0.813
Visfatin (4 missing)	3.91 (2.16)	7.11 (10.21)	4.92 (8.09)	4.66 (3.97)	0.463

[a,b,c] Bonferroni post-hoc comparison $p<0.05$

Multivariate linear regression models estimated the association between each metabolite and the experienced traumatic events by taking into account age, gender and BMI as confounders. They have been chosen by a series of univariate linear regression models with each metabolite as the dependent variable and age, gender, tobacco use, cannabis use, psychotropic medications, BMI, diagnosis, PANSS, Hamilton and BRMRS as independent variables ($p<0.10$; data available from the Authors). The multivariate models showed that experienced traumatic events influenced only the levels of C-peptide and Insulin (Table 13, while Leptin was associated with gender and BMI. No other metabolites showed significant associations with adverse events and the confounders (data available from the Authors).

Table 13: Multivariate linear regression models for metabolites (only those ones significantly associated with at least one independent variable shown)

Independent variables[#]	**β Coefficient (p-value)**		
EXPERIENCED TRAUMATIC EVENTS	**C-Peptide**	**Insulin**	**Leptin**
No CT & no severe SLEs (Ref.) No CT & severe SLEs CT & no severe SLEs CT & severe SLEs	- 0.059 (p=0.727) 0.275 (p=0.019) 0.313 (p=0.018)	- -0.031 (p=0.742) 0.190 (p=0.004) 0.167 (p=0.025)	- -4.634 (p=0.110) -2.628 (p=0.182) -2.462 (p=0.267)
CONFOUNDERS[§]			
Age at first contact with services (years)	-0.010 (p=0.084)	-0.008 (p=0.013)	0.005 (p=0.955)
Male sex (Ref.)	-0.105 (p=0.305)	-0.065 (p=0.257)	-9.407 (p<0.001)
BMI	0.055 (p=0.001)	0.035 (p<0.001)	0.660 (p=0.011)
Number of observations	107	108	107
Prob>F	0.005	<0.001	<0.001
Adj-R-squared	0.116	0.221	0.293

[#] No significant association (p<0.05) for Ghrelin, GIP, GLP-1, Glucagon, PAi-1, Resistin and Visfatin (data available from the Authors)

[§] only variables which resulted significantly associated (p<0.05) with metabolites in univariate linear regression models (data available from the Authors)

Specifically, we found that patients who showed higher C-peptide concentrations were those who: (1) experienced CT (with or without SLEs) and (2) had higher BMI; these variables accounted for 11.6% of the variance. Patients who showed higher Insulin levels were those who: (1) reported CT (with or without SLEs); (2) were younger; and (3) had higher BMI. This model accounted for 22.1% of the variance. Finally, Leptin was higher for females and for higher levels of BMI, with a percentage of variance explained of 29.3.

A secondary data analysis was performed after having deleted the extreme outliers (defined as values more than Q3+3*IQ) for each metabolite (specifically, 2 for C-peptide, 4 for GIP, 3 for Glucagon, 1 for Leptin, 4 for Resistin and 9 for Visfatin) and having re-estimated the regression models. Moreover, another secondary data analysis was performed by applying Box-Cox transformations to the metabolites' distributions. In both cases, the results and the significance remained unchanged (data available from the Authors).

Discussion

To our best knowledge, this is the first study to investigate the relationship between childhood trauma, stressful life events, and glucose metabolic biomarkers' levels in a large FEP sample. C-peptide and insulin levels were increased in traumatized subjects. Indeed, we found that C-peptide was higher in both groups of patients who experienced childhood trauma (those with and those without SLEs) with respect to subjects who did not experience adverse events, while Insulin was higher for patients who declared CT without SLEs with respect to those ones without traumatic events.

The C-peptide is a polypeptide that connects insulin's A-chain to its B-chain in the proinsulin molecule; it is processed and stored in secretory vesicles and released, together with the mature hormone insulin, by β pancreatic cells in response to hyperglycemia. C-peptide physiologic role has not been clarified yet. C-peptide seems to possess anti-inflammatory (Haidet et al., 2012), vasodilatory (Wallerath et al., 2003) and antioxidant (Giebink et al., 2013) effects, exerted throughout both a direct action on endothelial cells and an indirect one on erythrocytes and immune system cells. Insulin, in turn, can be considered the main anabolic hormone of the

body and regulates the metabolism of carbohydrates, fats, and protein (Voet & Voet, 2011) and has been also prove to exert an inhibitory activity on HPA axis (Nussdorfer et al., 2000). Our study is fresh evidence evoking an association between childhood traumatic experiences (alone or in association with recent stressful life events) and C-peptide/insulin levels in a FEP sample. Nonetheless, data coming from an animal study (Balasubramanian et al., 2015) showed a significant association between prenatal stress and higher levels of c-peptide; however, findings were not associated with abnormal calories intake, weight gain or increased fat mass and thus failed to demonstrate a significant association between prenatal stress and adulthood obesity. As aforementioned, insulin has been also prove to exert an inhibitory activity on HPA axis. Studies in psychosis have shown an hyperactivity of the hypothalamic–pituitary–adrenal (HPA) axis, the main biological system involved in mediating the effects of stress (Borges et al., 2013), particularly evident at the illness onset, which is often described as the most distressing time. Several factors have been hypothesized to explain this phenomenon, and among them, a primary role appeared to be played by an increased rate of childhood trauma in people who suffered from psychosis when compared with the general population (Fisher et al., 2009; Nemeroff, 2004; Read et al., 2005). The increase of insulin, and of C-peptide that we found in traumatized FEP subjects might therefore reflect an attempt, even though dysfunctional, to hinder HPA axis hyper activation having insulin an inhibitory activity on HPA axis. The chronic activation of the stress response system is in fact associated with increased visceral adiposity and lean mass reduction. Moreover, glucocorticoid hormones, like cortisol, stimulate gluconeogenesis and facilitate insulin-resistance, whereby chronic HPA axis activation might be at the bottom of the development of a lacking glycemic control (Charmandari et al., 2005). In turn, the increase of fat mass due to glucocorticoids worsens the insulin resistance and the glycemic control, determining a vicious circle consisting in hyper-glycaemia, hyperlipidemia and insulin-resistance (Charmandari et al., 2005). This mechanism might at least partially explains the higher incidence of cardiovascular disease, metabolic abnormalities and immune system dysfunctions in abused subjects (Parvanidou & Chrousos, 2012). Stress response system abnormalities might therefore represent a

common factor implied in both mental and metabolic disorders: medical conditions, so often present in comorbidities with mental disorders, could then be interpreted not only as a consequence of the unhealthy life style and the psychotropic treatments, but also as early signs of a multi-systemic dysfunction (Leboyer et al., 2012). Following this model, childhood traumatic experiences (and perhaps recent stressful life events) would bring about stress response abnormalities, as HPA axis hyper-activation resulting in higher corticosteroids blood levels, and would facilitate the development of medical conditions like diabetes and obesity. In turn, they would exert their own influence on the HPA axis, picturing a dysfunctional vicious circle. Mental disorders and medical conditions appear thus to be associated in a complex and bi-directional relationship. They seem to be influenced reciprocally, in terms of clinical manifestation and therapeutic options: life expectancy of subjects suffering from severe mental illnesses is 30% lower comparing with the one of the general population, cause of the high frequency of untreated medical comorbidities (Fagiolini et al., 2009).

The present study has several strengths. First, FEP patients recruited in the present study belonged to GET UP PIANO Trial (Ruggeri et al., 2012), performed in a large catchment area, corresponding to nearly 10 million inhabitants. Since over 90% of Community Mental Health Services (CMHCs) completed the study, and since no significant differences were found with regard to socio-demographic or clinical characteristics between subjects who gave blood and those which did not and between subjects with data on childhood trauma and stressful life events and those without, our subjects were highly representative of the patients treated in the "real world" CMHS, thus our results could be generalized. Second, we used reliable, internationally validated instruments and adopted conservative cut-off points, previously applied, to identify only the most severe forms of abuse. Thus, our study is, to the best our knowledge, the first attempt to investigate the association between childhood traumatic experiences, recent stressful life events, and glucose metabolic biomarkers' levels in a FEP sample.

Some limitations also need to be acknowledged in our study. First, although our is the largest sample of FEP subjects in which the relationship between childhood trauma, severe stressful life events and metabolic markers has been investigated,

the number of subjects with childhood trauma history was relatively small in size, and thus our findings should be considered preliminary and need to be replicated in larger samples. Second, a substantial number of tests were performed, which means that any Bonferroni correction for multiple testing would be large, thus any significant p-value should be regarded as nominal. Third, as for the majority of the studies on childhood trauma, abuse data rely on retrospective reporting. This limitation has been mitigated by the use of a standardized interview as CECA-Q, which has good reliability and validity both in general and clinical populations. Fourth, it was not possible to explore the full range of variables known to affect glucose metabolism; for example, we did not have data on diet regime or daily physical activity. Moreover, blood samples were not taken to verify the presence/absence of diabetes by an oral glucose tolerance test (OGTT) according to criteria from the American Diabetes Association. Finally, there are the possibility that the association between childhood trauma and/or recent severe stressful life events with plasma levels of glucose related biomarkers could be mediated not only by BMI and smoking, but also by other factors, such as psychosocial elements including low socio-economic levels. Information about the type of the ongoing medication was not considered; concerning this, findings from literature suggest that metabolic abnormalities in subjects suffering from psychosis are independent from medication and pre-existing the beginning of pharmacological treatment.

Conclusion

Our results suggest a significant association between childhood traumatic experiences, stressful life events, and metabolic abnormalities in the first phase of psychosis. Subjects affected by psychosis are known to possess an increased risk to develop a metabolic syndrome (De Hert et al. 2011, Fagiolini et al., 2008). Childhood trauma might therefore represent an additional risk factor for the development of metabolic disorders in patients affected by psychosis. Based on our findings, it would be appropriate and recommended to conduct an accurate and meticulous medical history, which takes into account the presence of childhood trauma, allows settling an early and intensive program of food and nutrition education, and guides the choice of medication.

CHAPTER 3: CHILDHOOD TRAUMA AND DEPRESSION

3.1 HEPATITIS C AND DEPRESSION

The association between depression and inflammation has been the object of several studies in recent years; many research goals have been achieved, many others are still open questions. A central role of peripherally produced pro-inflammatory cytokines (as IL-1, IL-6 and TNFa) has been hypothesized. Peripheral pro-inflammatory cytokines appear in fact to represent among the main mediators of the complex interaction between the peripheral inflammation and the central nervous system (Krishnadas & Cavanagh, 2012). In response to a chronic and/or an abnormal stimulus, the homeostatic purpose of the inflammatory response would be lost and the peripheral cytokines would trigger a dis-adaptive phlogistic state at central level. Neurons, astrocytes, and glia seem therefore responsible for the "conversion" of the peripheral phlogistic state in behavioral outputs (Miller et al., 2009). The mechanisms hypothesized to be responsible for the translation in behavior of biological, biochemical, and molecular alterations include the monoaminergic circuit, the hypothalamic-pituitary-adrenal (HPA) axis functioning and neuroplasticity (Capuron & Miller, 2011). A behavioral pattern expression of an inflammatory state, observed in pre-clinical studies and defined as "sickness behavior"(Mellor & Munn, 1999), in consideration of its affinity with the depressive syndrome, represents the starting point of this field of research. The inflammation-depression hypothesis has been later supported by at least two further orders of evidence: first, it has been shown that depressed subjects have higher levels of pro-inflammatory cytokines (Dowlati et al., 2010, Liu et al., 2012) and of inflammatory indices (PCR) (Krishnadas & Cavanagh, 2012) than the general population; second, subjects suffering from chronic inflammatory disease, such as chronic HCV-related hepatitis (Fábregas et al., 2012; Fontana et al., 2002), are more frequently depressed than healthy subjects. To date, question remains open whether inflammation during depression represents a condition of "state", therefore secondary to the pathology, or of "trait" and potentially involved in the development of the disorder as a predisposing, triggering, and/or maintenance factor. Moreover, it is still to be clarified how biological occurrences in genetics (gene sequence), epigenetics (gene expression) and/or proteomics turn into psychological and

behavioral phenomena. Finally, it remains to be elucidated the intricate network of reciprocity among inflammation (and its biological correlates), environment (such as childhood traumatic experiences and stressful life events), psychological aspects (like stress perception, coping strategies, and level of self-esteem), and depression. The prospective longitudinal study "Chronic inflammation and Major Depression in patients with Chronic HCV-related Hepatitis" aims to contribute to the clarification of these questions. To this end, subjects with chronic HCV-related liver disease were recruited and longitudinally assessed prospectively over the course of 48 weeks, through the collection of both biological and clinical data. The study included a comparison between three groups of subjects: group A, which included subjects about to start interferon therapy at baseline, group B, comprising individuals about to start direct-acting antivirals (DAAs) at baseline, and group C, which included subjects who underwent routine controls for monitoring liver disease. We aimed to investigate the direction of the inflammation-depression association, to explore potentially involved predictors, and elucidate mediation and moderation factors of the association itself.

The term "depression" is not uniquely referable to a single type of depressive disorder. Among the different types of depression, the "inflamed" one is characterized by the presence of an absence of motivation and invalidating fatigue. In this perspective, it is possible that depressed subjects with increased inflammatory indices would suffer from a biologically relevant subtype of depression, in which the immune system (peripheral and central) play a key pathogenic role (Raison & Miller 2011).

Inflammation and Depression

As said before, the role of the inflammation, both peripheral and central, in the pathogenesis of depression has been the subject of several investigations in the last two decades. There is now evidence that proves an important role of the inflammation in the development of depression (Patel, 2013) and that demonstrates an intricate network of interactions between the immune system and the central (the brain) and peripheral nervous system (Krishnadas & Cavanagh, 2012). Inflammatory cytokines, including IL-1, IL-6 and TNFa, seem to represent the main

mediators of this complex interactive network. Cytokines are in fact believed to be responsible not only for the organization of the cellular response to the pathogenic stimulus, but also for the behavioral changes necessary to healing.

In physiological conditions, following a pathogenic stimulus, the cytokine-mediated pro-inflammatory response is temporary and strictly regulated by a balanced anti-inflammatory mechanism. Indeed, under normal conditions, in reaction to an inflammatory stimulus, a neuro-immuno-endocrine anti-inflammatory response develops (involving the sympathetic and parasympathetic peripheral nervous system, the immune system and the endocrine-hormonal system). An adaptive behavioral response also takes place; it is usually temporary and placed under the control of the central nervous system (CNS).

However, whether the phlogistic stimulus becomes chronic and/or excessive it can lead to an out-of-control reaction of the inflammatory system, resulting in the development of immune-endocrine disorders (eg excessive production of proinflammatory cytokines and/or in a malfunction of the hypothalamic-pituitary-adrenal axis) and contributing to the occurrence of non-functional behaviors. They therefore lose their adaptive purpose and sometimes become clinically relevant and attributable to psychiatric syndromes of various kinds, including depression (Krishnadas & Cavanagh, 2012).

Cytokines are relatively large proteins that do not pass the blood-brain barrier (BBB) freely. Nevertheless, there is evidence that they are able to reach the brain through humoral, neural and cellular mechanisms (Capuron & Miller, 2011). So far, there are at least five mechanisms identified, not mutually exclusive, by which cytokines would reach the CNS:

1) Through the BBB at regions characterized by a greater permeability, such as the choroid plexus and the circumventricular organ (Quan & Banks, 2007; Konsman et al., 2002);

2) Through the BBB, active transported by cytokine-specific transporters located on the endothelium (Plotkin et al., 1996; Quan & Banks, 2007; Rivest et al., 2000);

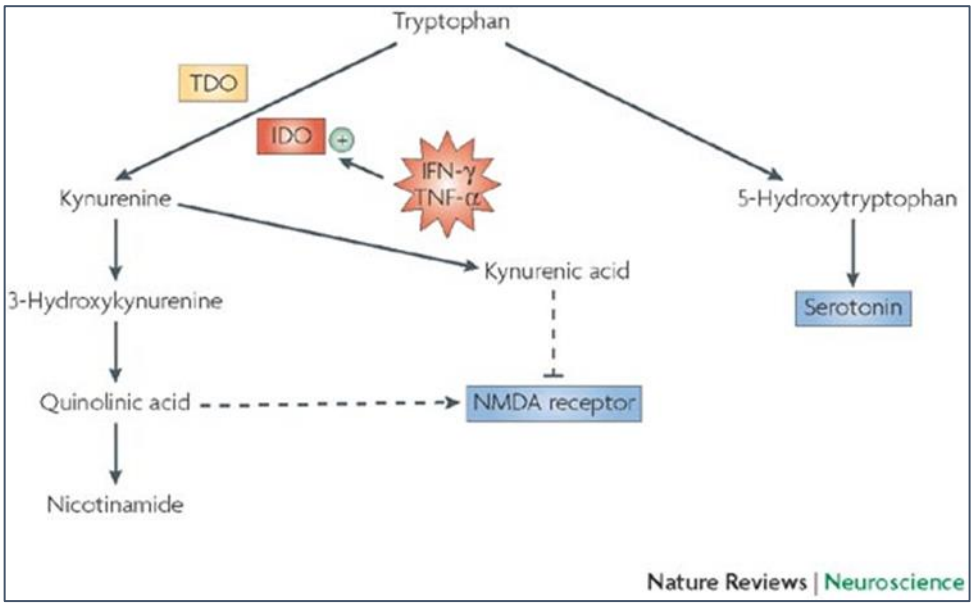
3) Through activation of endothelial cells, with the release of second messengers, such as prostaglandin E2 (PGE2) and nitric oxide (NO), at the level of the cerebral parenchyma (Quan & Banks, 2007);
4) Through the activation of afferent nervous fibers, such as those of the Vagus nerve, which would transmit the cytokine-mediated pro-inflammatory signal (Watkins et al., 1995; Quan & Banks, 2007; Konsman et al., 2002);
5) Through the entrance into the cerebral parenchyma of activated peripheral monocytes (D'Mello et al., 2009).

The activation of these specific mechanisms appears to spread the effect of peripherally produced cytokines on the CNS. The inflammatory cellular network in the brain, consisting of different elements such as neurons, microglia and astrocytes, is therefore activated and an inflammatory state is established. These neural inflammatory subjects, organized in complex systems, seem therefore potentially involved in the development of psychiatric syndromes (Miller et al., 2009).
How a peripheral inflammatory state could result into a mental disorder such as depression through brain inflammation is still a matter of investigation. Several studies have focused on the monoaminergic neurotransmitter system (tryptophan, serotonin and dopamine in the first instance), on the neuroendocrine activity (with a primary role of the hypothalamus-pituitary and adrenal axis functioning), and on neuroplasticity (Capuron & Miller, 2011). Regarding the action of cytokines on monoaminergic neurotransmitters, the "hypothesis of tryptophan depletion" (Mellor & Munn, 1999) postulated that there is a relationship between reduced levels of tryptophan and the development of depression. A subsequent study (Raison et al., 2010b), however, demonstrated the absence of a statistically significant difference in terms of liquor levels of tryptophan between subjects with depression and healthy subjects. An alternative hypothesis has therefore been developed, known as the "neurotoxic hypothesis". According to the latter, the activation of the enzyme indoleamin 2,3-dioxygenase (IDO), due to a long-term or uncontrolled inflammatory stimulus, would provoke an imbalance of the use of

tryptophan in the endothelial cells of BEE favoring the production of Kinurenina (Kyn) (at the expense of the production of serotonin, 5-HT).

IDO is highly inducible by pro-inflammatory cytokines, including interferon-γ (IFN-γ) and tumour necrosis factor-α (TNF-α) (Dantzer et al., 2008) (Figure 6).

Figure 6: Degradation of tryptophan through the kynurenine pathway.



Dantzer et al., 2008. TDO tryptophan dioxygenase; IDO indoleamine 2,3 dioxygenase.

Kynurenine, is readily transported across the blood–brain-barrier into the brain where it can be further metabolized in perivascular macrophages, microglia and astrocytes to generate neuroactive compounds. Kynurenine is degraded along one of two catabolic branches, leading to the formation of either 3-hydroxykynurenine (3-HK) and quinolinic acid (QA) or kynurenic acid (KA) (Krishnadas & Cavanagh,2012). QA is an N-methyl-D-aspartate (NMDA) receptor agonist, it is preferentially produced by microglia and can cause oxidative stress and lipid peroxidation. By contrast, KA is an NMDA receptor antagonist produced by astrocytes and it has been speculated to be neuroprotective (Schwarcz & Pellicciari, 2002). An increased production of QA together with an enhanced production of microglial glutamate (Schwarcz & Pellicciari, 2002), would cause an increase in oxidative stress, with a cytotoxic and a calcium mediated apoptotic effect in the CNS (McNally et al., 2008). An imbalance of kynurenine pathway metabolites might underlie inflammation-associated depressive disorders. Furthermore, cytokines have been shown to influence dopamine (DA) synthesis, release, and re-uptake. In animal studies, intrathecal administration of kinurenic acid (KA) produced a dramatic reduction of extra cellular DA at the striated body (Wu et al., 2007), whereas IFN-a administration resulted in a reduction of tetrahydro-biopterin (4HB) and DA concentration in association with an increase in nitric oxide (NO) (Kitagami et al., 2003). 4HB is a cofactor necessary for the synthesis of both NO and DA. Therefore, an increased synthesis of NO, occurring under an inflammatory drive, could reduce the availability of 4HB and consequently limit the production of DA in significant brain regions.

Regarding the action of the cytokines on the neuroendocrine system, the acute (short duration) administration of cytokines is able to determine the activation of the hypothalamic-pituitary-adrenal (HPA) axis: corticotrophin releasing hormone (CRH) is synthetized in the hypothalamic paraventricular nucleus (PVN). Once released into the portal circulation, it reaches the pituitary gland and activates the secretion of adrenocorticotropic hormone (ACTH), which finally stimulates the secretion of cortisol from adrenal glands. Cortisol in turn binds its receptors (GR and MR) in multiple target tissues, including also the HPA axis itself; at this level,

it is responsible for a negative feedback of both ACTH and CRH secretion (Pariante & Lightman, 2008).

In case, however, of prolonged stimulation this homeostatic mechanism may fail. A flattening of the daily curve of cortisol values was observed with a significant increase in the evening values in association to a chronic stimulation by pro-inflammatory cytokines (Raison et al., 2010a). A chronic stimulation of the HPA axis can be responsible for its malfunction through an inhibitory effect on glucocorticoid (GR) receptors. It has been demonstrated that pro-inflammatory cytokines can reduce the functionality of glucocorticoid (GR) receptors: cytokines were shown to inhibit both cytoplasm-nucleus translocation and DNA binding, and were proven to influence the expression of receptors' isoforms, stimulating the production of a relatively inert isoform (Pace et al., 2007). It would result in increased levels of circulating corticosteroid hormones, due to an altered HPA cortisol-axis negative feedback mechanism and mediated, at least in part, by glucocorticoid receptor resistance.

To conclude, cytokines also appear to exert an effect on neuronal plasticity (neurogenesis) and to influence neuronal circuits. In a context of chronic stress, the levels of pro-inflammatory cytokines has been significantly associated with a decreased production of neuronal growth factors and with a reduced neurogenesis, especially in the hippocampus (Duman & Monteggia 2006). Neuro-imaging studies have also extensively demonstrated how pro-inflammatory cytokines influence neuronal circuits in both cortical and subcortical levels, including an action at the basal ganglia and anterior cingulate cortex (ACC). The former play a fundamental role in determining motor activity and motivating force, while the latter is involved in the modulation of affective responses (such as anxiety and depression), alertness and vigilance (Capuron & Miller 2011).

In summary, it has been hypothesized that peripheral pro-inflammatory cytokines would affect the brain by one or more of the aforementioned mechanisms, which in turn would lead to poorly adaptive behavioral changes. The presence of psychomotor retardation, reduction of appetite and body weight, sleep disturbances,

increased perception of pain, inability to experience pleasure, have been observed in animals affected by microbial infection, and therefore characterized by a chronic inflammatory state a long time ago (Hart, 1988). This set of behavioral characteristics was given the name of "sickness behavior". The "sickness behavior" could be reliably reproduced in the laboratory by the administration of pro-inflammatory cytokines and its effects were reversible through the administration of cytokine antagonists or anti-inflammatory cytokines (such as IL-10) (Dantzer et al., 2008). The "sickness behavior" represented a reliable experimental model that seemed to share some characteristics with the depressive syndrome and led to the hypothesis of a role of inflammation in the pathogenesis of depression (Raison et al., 2006).

This hypothesis was subsequently supported by at least three further orders of evidence:

1) Subjects suffering from Major Depressive Disorder (MDD) (even in the absence of a medical comorbidity) had mean levels of inflammatory indexes significantly higher than healthy subjects did. Specifically, higher concentrations of pro-inflammatory cytokines were found, such as sIL-2R, IL-6 and TNF-α (Dowlati et al., 2010; Liu et al, 2012) in subjects with MDD, both in peripheral blood and in cerebral-spinal fluid.

In addition, approximately one third of those with MDD showed elevated inflammatory indices (PCR) compared to the general population. These increases were usually modest (2-3 times the values observed in the general population) and often within the normal range (Krishnadas & Cavanagh, 2012).

2) Patients suffering from chronic medical conditions characterized by an inflammatory pattern, such as HCV-related chronic liver disease, had a much higher prevalence of psychiatric comorbidity, including depression, when compared to the general population (Fontana et al ., 2002).

MDD is an event that occurs 5-10 times more frequently in individuals with chronic organic disease than in the general population, it worsens prognosis and increases disability (Krishnadas & Cavanagh, 2012). The inflammatory state in a context of organic disease can therefore facilitate the onset of depression in vulnerable and

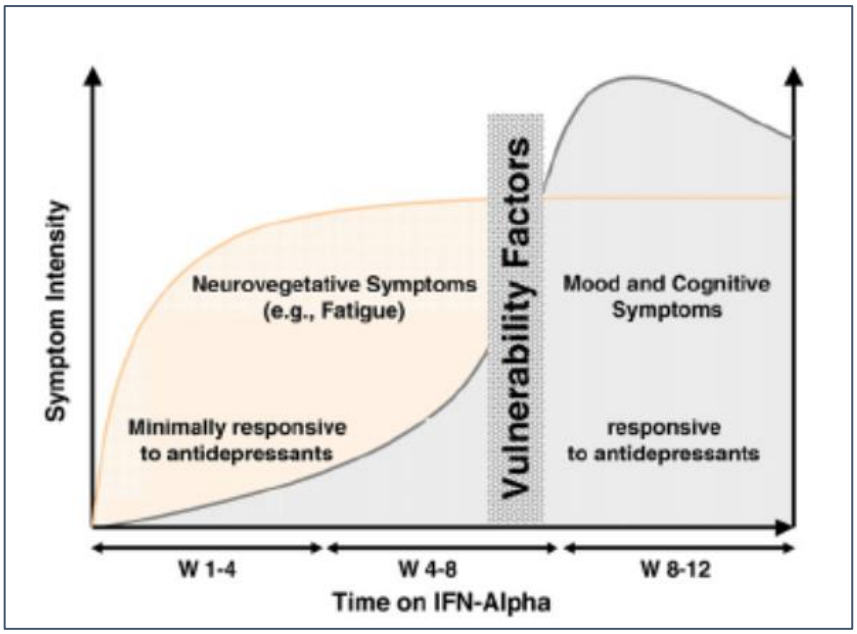
predisposed subjects (genetically and not only), acting as a precipitating factor (Krishnadas & Cavanagh, 2012).

3) Patients suffering from different organic conditions, which were treated with cytokines (including subjects with chronic HCV-related liver disease undergoing interferon treatment), had a significant risk of developing depression during treatment with an estimated prevalence equal to 20-30% of cases (Sockalingam et al., 2011).

Interferon-induced Depression

Interferon treatment was proven to be responsible for the onset of two distinct behavioral syndromes (Figure 7): a neuro-vegetative syndrome and an affective-cognitive syndrome, characterized by different symptomatology and diverse responsiveness to anti-depressants.

Figure 7: The Neuro-vegetative and the Affective-cognitive Syndromes



Capuron & Miller, 2011

The neuro-vegetative syndrome is characterized by the presence of fatigue, psychomotor retardation, loss of appetite and sleep disorders. It occurs early during treatment, already during the first weeks and persists for the entire duration of therapy. It is not responsive to treatment with antidepressants and has been associated with changes in the functioning of basal ganglia and with alterations in DA metabolism.

The affective-cognitive syndrome is characterized by the presence of depressed mood, anxiety, memory and attentional deficit and occurs later in the course of treatment, approximately between 4 and 12 weeks, especially in vulnerable subjects. It is usually well responsive to treatment with antidepressant and it has been associated with tryptophan/serotonin metabolism alterations (Capuron & Miller, 2011).

No one feels better during interferon treatment. However, the same dose of drug, taken following the same therapeutic scheme, produces a wide variability of clinical response, ranging from mild malaise with increased fatigue to cases of severe depression with suicidal ideation. This clinical variability suggests that there are other factors potentially involved. Several so-called vulnerability factors (or

immune response amplifiers) associated with the development of depression during interferon-treatment have been identified, including:
(1) increased serum concentration of TNF-α and / or IL-6 (Wichers et al., 2007);
(2) Reduced diurnal variation of the hypothalamic-pituitary-adrenal axis function (with a flattened cortisol curve) (Raison et al., 2010a);
(3) Increased cortisol levels in the evening (Raison et al., 2010a);
(4) Reduced sensitivity of glucocorticoid (GR) receptors to the negative feedback mechanism by glucocorticoids (Raison & Miller, 2011);
(5) Reduced plasma concentration of BDNF (Wichers et al., 2007),
(6) Reduced plasma concentration of tryptophan (Capuron et al., 2002b),
(7) Increased plasma and liquor concentrations of Kinurenine and quinoline acid (Capuron et al., 2002a).

Therefore, subjects with alterations in the inflammatory system appear to be the subjects that, from a strictly biological point of view, are more at risk of developing depression during interferon-treatment (Raison & Miller, 2011). Moreover, from a clinical point of view, female subjects with low education, with a personal history of depression or other psychiatric disorders and subthreshold depressive symptoms at the beginning of the treatment were proven the most vulnerable (Udina et al., 2012).

3.2 THE RESEARCH PROJECT

Aims

The research project "Chronic inflammation and Depression", still ongoing, evaluates a sample of subjects with chronic hepatitis C, following them for a period of 48 weeks, and aims to elucidate clinically relevant predictors of depression and to better understand the molecular mechanisms involved.

In details, the study aims:

1. To explore the basal inflammatory profile of patients with chronic HCV-related liver disease;
2. To explore whether patients who develop depression during the follow up period would show significantly higher levels of inflammatory bio-markers in comparison with patients not developing depression ("state" bio-markers).
3. To examine whether clinical predictors (including antiviral drug therapy, childhood traumatic experiences, and stressful life events over the last 6 months), genetics, and/or epigenetics would be associated with both changes over time of bio-markers' levels and the development of depression.

Methods

Study design

This is a prospective longitudinal study, evaluating subjects with HCV-related liver disease. Patients considered eligible have been proposed to participate in the study by the attending infectious disease doctor during a routine visit. Those who have agreed to take part to the study have been then evaluated, using a longitudinal perspective design.

After baseline assessment, subjects were divided into three groups:

- GROUP A: subjects with hepatitis C eligible for the treatment with peg-IFN-α of 24 (most of the time), 36 or 48 weeks duration.

- GROUP B: subjects with hepatitis C eligible for the treatment with DAAs of 8 or 12 (most of the time) weeks duration.

- GROUP C: subjects with chronic HCV related liver disease, not about to start any treatment.

Patients about to start antiviral therapy (GROUP A and B) have been evaluated at baseline, before the begin of the treatment, after 4 weeks of treatment, at week 12 (the "end of therapy" time-point for most of the subjects included in group B), at week 24 (only group A, and it was the "end of therapy" time-point for most of the subjects included in this group), and finally at 6 months after the end of the treatment (6-month FU). In case of patients undergoing disease monitoring (GROUP C), the evaluations were made accordingly to the clinical practice and therefore at baseline, 24, and 48 weeks.
Researchers with a clinical background (psychiatrists and psychologists) performed clinical assessments during the regular visits to which patients attended at the Infectious Diseases Department, outpatients Clinic. During these visits blood sampling was also performed.

Subjects

Recruitment process began on October 2013 at the Infectious Diseases Department of the Verona University Hospital and it will terminate on October 2019.

Inclusion criteria

- Patients suffering from chronic HCV-related liver disease who had/had not undergone antiviral treatments previously;
- Age> 18 years

Exclusion criteria

- Patients receiving HAART (Highly Active Anti-Retroviral Therapy) for HIV
- Patients in pregnancy or breastfeeding
- Patients with autoimmune diseases, acute infections or other hepatic diseases of different origin from chronic HCV infection
- Cirrhosis with Child-Pugh B or C stage

- Esophageal varices> F1
- Unstable cardiac pathology
- Pharmacologically not controlled epilepsy
- Current drug addiction or alcoholism
- Patients with current severe depression (ICD-9 296.23 / 296.24 / 296.33 / 296.34 / 296.53 / 296.54) or current psychotic disorder (ICD9 295/297/298)
- Patients with known Cognitive Impairment (MMSE <24/30)

Assessment

Severity of symptomatology

- Depressive and anxious symptomatology were assessed by the Inventory of Depressive Symptomatology (IDS) (Rush et al., 1996) and by the Hospital Anxiety and Depression Scale (HADS) (Zigmond et al., 1983). The IDS is a 30-items self-administered scale that focuses on mood, sleep quality, appetite, psychomotricity, cognitive performance, and sexual interest changes in the last week. The HADS is also a self-administered instrument, validated in a hospital setting, which allows a more general investigation with respect to anxiety-depressive symptomatology;
- Manic symptoms (sleep, appetite, sexual activity, mood quality, psychomotority) were evaluated by the Bech-Rafaelsen Mania Rating Scale (BRMRS) (Bech et al., 1978);
- Fatigue levels were evaluated using the Chalder Fatigue Questionnaire (CFQ) (Chalder et al., 1993). Levels of tiredness, weakness and/or lack of energy in the last month were estimated;
- Health status was investigate by the Standard health status questionnaire (SF-36 V1) (Ware & Sherbourne, 1992) aiming to detect the subject's opinion about his general health.

Socio - demographics and risk factors

- Patient Information Sheet (PIS) allowed a brief collection of socio-epidemiological data;

- An anamnestic file was applied to register any current and/or previous pharmacological treatments;
- Family History for Genetic Studies (FIGS) (NIMH, 1992) assessed the family psychiatric history;
- The presence of stressful life events (SLEs) in the previous year was determined using a modified version of the Life Events Scale Life (Paykel et al., 1971);
- The Perceived Stress Scale (PSS) (Cohen et al., 1983) was used to measure the perception of stress in the last month;
- The Childhood Experiences of Care and Abuse (CECA) (Bifulco et al., 2005) questionnaire assessed the presence of childhood traumatic experiences and concerned adversity before age 17, including physical and sexual abuse, separation from at least one of the parental figures longer than 6 months, death of a parent, antipathy, and neglect;
- The Beliefs about Medication Questionnaire (BMQ) (Horne et al., 1999): explored convictions about the received treatments and opinions regarding medicines in general;
- Social functioning was estimated by the Global Assessment of Functioning Scale (GAF) (APA, 1994);
- The Italian version of the Coping Orientation to Problems Experienced inventory (COPE) (Carver et al., 1989), a self-administered questionnaire, accounted different coping modalities: positive reinterpretation and growth, mental disengagement, focus on and venting of emotions, use of instrumental social support, active coping, denial, religious coping, humor, behavioral disengagement, restraint, use of emotional social support, substance use, acceptance, suppression of competing activities, and planning;
- The Rosenberg self-esteem scale (Rosenberg, 1979) assessed self-esteem: it is a one-dimensional measurement questionnaire of global self-esteem.

Diagnosis (including Substance Use Disorder)

- Any Axis I psychiatric disorder was explored by the Mini International Psychiatric Interview (MINI) (Sheehan et al., 1998);
- The use of alcohol in the last 12 months was evaluated by the Use of alcohol Alcohol Use Disorder Identification Test AUDIT (Babor et al., 1992);
- Lifetime cannabis use was investigated by the Cannabis Experience Questionnaire (CEQ) (Barkus et al., 2006);
- Questionnaire on Nicotine Addiction (Heatherton et al., 1991) explored the habits of smoking tobacco.

Biological Investigation

- One DNA tube was collected only at baseline to analyze candidate genes' variants. They will include IL-6, IL-10, TNF-α genes and genes of the soluble interleukin receptors IL-2, IL-6, INF-α.
- One paxgene tube was collected at baseline, at week 4 (only in group A), 12 (only in groups A and B), 24 (only in group A and C), and 48 (or 6 months after the end of the treatment) to analyze gene expression (mRNA). The investigation of both candidate genes and "whole genome" gene-expression will be performed.
- One serum tube was collected at baseline, at week 4 (only in group A), 12 (only in groups A and B), 24 (only in group A and C), and 48 (or 6 months after the end of the treatment). Serum cytokine concentrations will be evaluated.
- One plasma tube was collected at baseline, at week 4 (only in group A), 12 (only in groups A and B), 24 (only in group A and C), and 48 (or 6 months after the end of the treatment). Plasma analyzes of polyunsaturated fatty acids PUFA will be performed. The plasmatic lipid profile was also estimated.
- Routine test tubes for hemoglobin and total white blood cells, T3, T4, TSH, AST, ALT, viremia (HCV-RNA), creatinine, cryoglobulins and autoantibodies were collected according to the clinical routine.
- Salivary cortisol levels were measured using Salivettes (Sarstedt, Leicester, UK) at baseline. Subjects were instructed to wake up before 10 am, to

collect saliva samples by chewing the cotton roll for 2 min, immediately after awakening (0 min) while still in bed, and 15, 30 and 60 min after awakening. Having breakfast and teeth brushing were to be avoided during the first hour of awakening. Saliva samples were collected again at noon and at 8 pm and subjects were recommended not to eat in the 30 min before sampling. Subjects were instructed to store salivary samples at 4°C. On the arrival to the laboratory, salivary samples were immediately centrifuged at 3000 rpm per ten minutes and stored at -20°C until the assay. Cortisol was measured using a commercially available ELISA kit (Enzo Life Sciences, Farmingdale, NY, USA), following the manufacturer's instruction. All samples were assessed in duplicate. Diurnal cortisol levels and the Cortisol Awakening Response (CAR) were measured using the Area Under the Curve (AUC) respected to the ground calculation (Pruessner,et al., 2003): for the former were taken into account levels at 0 min, 15min, 30min, 60min, noon and 8 pm, while for CAR levels at 0 min to 15, 30, and 60 min after awakening were considered.

As mentioned above, this Research Project is still ongoing. Nonetheless, we have already conducted some preliminary investigation, whose results have been recently presented in the Symposium "Clinical pathways: a tool for improving mental health outcomes in medical settings" at the 6° Annual Scientific Conference of the European Association of Psychosomatic Medicine (EAPM) 27th-30th June 2018 in Verona.

3.3 RESILIENCE AND VULNERABILITY FOR DEPRESSION IN HEPATITIS C PATIENTS

Aims

We conducted a study on a sample of patients affected by Hepatitis C, paying particular attention to resilience and vulnerability factors for depression. In details, we aimed:

- To estimate baseline levels of depression, anxiety, fatigue, and perceived stress;
- To explore whether subjects under INF would show higher levels of depression, anxiety, fatigue, and/or perceived stress than subjects under DAAs at week 12 and 48 or patients not in treatment, at week 24 and 48;
- To explore whether at week 12, 24 or 48, levels of depression, anxiety, fatigue, and/or perceived stress would be associated with childhood traumatic experiences, substance or alcohol use, recent stressful life events (SLEs), personal or family psychiatric history, or coping strategies.

Methods

Subjects

After giving a written consent, eligible subjects have been evaluated, using a longitudinal perspective study design up to 48 weeks. Inclusion and exclusion criteria are reported above. After baseline assessment, they were divided into three groups:

- GROUP A: subjects with hepatitis C eligible for the treatment with peg-IFN-α of 24 (most of the time), 36 or 48 weeks duration.

- GROUP B: subjects with hepatitis C eligible for the treatment with DAAs of 8 or 12 (most of the time) weeks duration.

- GROUP C: subjects with chronic HCV related liver disease, not about to start any treatment.

Assessment

- Patient Information Sheet (PIS) allowed to collect socio-epidemiological data;
- Depressive and anxious symptomatology were assessed by the IDS (Rush et al., 1996) and the HADS (Zigmond et al., 1983). IDS score can range from 0 to 84, with scores categorized as follows: normal (0-13), mild (14-25), moderate (26-38), severe (39-48), and very severe (49-84). HADS score for each subscale (anxiety and depression) can range from 0 to 21 with scores categorized as follows: normal (0-7), mild (8-10), moderate (11-14), severe (15-21). Scores for the entire HADS scale (emotional distress) range from 0-42 (with higher scores indicating more distress).
- Fatigue levels were evaluated using the CFQ (Chalder et al., 1993); This scale was scored "bimodally" with columns representing 0, 0, 1 & 1 and a range from 0 to 11, with a total of 4 or more qualifying for "caseness".
- The PSS-10 (Cohen et al., 1988) was used to measure the perception of stress; each item is rated on a 5-point scale ranging from never (0) to almost always (4). PSS-10 scores are obtained by reversing the scores on the four positive items (4, 5, 7, and 8) and then summing across all 10 items. Scores around 13 are considered average. Scores of 20 or higher are considered high stress.
- The CECA-Q (Bifulco et al., 2005) assessed the presence of childhood traumatic experiences. It comprises information concerning experiences of childhood adversities before age 17, including physical punishments, sexual unwanted experiences, parental separation and loss. Separation has been defined as a detachment from at least one of the relatives, longer than 6 months; whereas, loss was determined whether one or both parents died during subjects' childhood. Childhood trauma (CT) was defined as the experience of at least one among physical punishments, sexual unwanted experiences and parental loss/separation. The questionnaire also assessed the presence of lack of parental care (neglect; cut-off: mother >=22, father >=24) and hostile or cold parenting (antipathy; cut-off mother or father

>=25). Therefore, childhood traumatic experiences (CTE) involve childhood trauma (CT),(including physical punishments, sexual unwanted experiences, parental separation and loss), neglect, and antipathy.

- Lifetime cannabis use was investigated by the Cannabis Experience Questionnaire (CEQ) (Barkus et al., 2006): current and lifetime use of cannabis, cocaine and heroin were investigated;
- The use of alcohol in the last 12 months was evaluated by the AUDIT, (Babor et al., 1992);
- The presence of stressful life events (SLEs) in the previous year was determined using a modified version of the Life Events Scale (LES) (Paykel et al., 1971). Based on a previous works (Lopizzo et al., 2017; Ira et al., 2014), only "severe life events" (i.e. death of a family member, sexual or physical abuse, being accused of having committed a crime, sentence of imprisonment, being exposed to war or natural catastrophes, family breakdown, being removed from home, sentimental breakdown, severe physical illness) were taken into account.
- Any Axis I psychiatric disorder was explored by the Mini International Psychiatric Interview (MINI) (Sheehan et al., 1998);
- The FIGS, (NIMH, 1992) assessed the family psychiatric history: only first degree family history was taken into account;
- The Italian version of the COPE-NVI (Carver et al., 1989; Sica et al., 2008) accounted different coping modalities, as described above. Higher means meant more frequent use.

Patients in GROUP A have been evaluated at baseline, before beginning the treatment, after 4 weeks of treatment, at week 12, at week 24 (it was the “end of therapy” time-point for most of the subjects included in this group), and finally at 6 months after the end of the treatment (48 weeks).

Patients in GROUP B have been evaluated at baseline, before beginning the treatment, after 4 weeks of treatment, at week 12 (it was the “end of therapy” time-point for most of the subjects included in this group), and finally at 6 months after the end of the treatment (48 weeks).

In case of patients undergoing disease monitoring (GROUP C), the evaluations were made accordingly to the clinical practice and therefore at baseline, 24, and 48 weeks.

Table 14 summarizes the assessments at each time points for the three groups.

No biological investigation were performed for the purpose of this study.

Table 14: Assessment plan

Instruments	GROUP A					GROUP B					GROUP C				
	BL	W4	W12	W24	W48	BL	W4	W12	W24	W48	BL	W4	W12	W24	W48
PIS	X	----	----	----	----	X	----	----	----	----	X	----	----	----	----
IDS	X	X	X	X	X	X	X	X	----	X	X	----	----	X	X
HADS	X	X	X	X	X	X	X	X	----	X	X	----	----	X	X
CFQ	X	X	X	X	X	X	X	X	----	X	X	----	----	X	X
PSS	X	X	X	X	X	X	X	X	----	X	X	----	----	X	X
CECA Q	X	----	----	----	----	X	----	----	----	----	X	----	----	----	----
CEQ	X	----	----	----	----	X	----	----	----	----	X	----	----	----	----
AUDIT	X	----	----	----	----	X	----	----	----	----	X	----	----	----	----
LES	X	----	----	----	X	X	----	----	----	X	X	----	----	----	X
MINI	X	----	----	----	----	X	----	----	----	----	X	----	----	----	----
FIGS	X	----	----	----	----	X	----	----	----	----	X	----	----	----	----
COPE	X	----	----	----	----	X	----	----	----	----	X	----	----	----	----

Results

Description of the sample

Socio-demographic characteristics of the sample

To date, 50 subjects affected by Hepatitis C have been included in the study.

The majority of individuals lived with partners and/or offspring (N=35, 70.0%), while 12.0% and 10.0% of them lived alone and with the family of origin, respectively. The remaining 8.0% reported other types of housing arrangements. Socio-demographical characteristics of the sample are shown in table 15.

Table 15: Socio-demographic characteristics of the sample

	Whole sample (N=50)		**Group A (N=21)**		**Group B (N=11)**		**Group C (N=18)**		**p-value**
	Mean or N	**(SD) or %**	**Mean or N**	**(SD) or %**	**Mean or N**	**(SD) or %**	**Mean or N**	**(SD) or %**	
Age (years)	49.6	(13.3)	45.4	(1.8)	58.6	(13.1)	48.9	(14.1)	**0.02**
Gender, (Male)	22	44.0	13	61.9	4	36.4	5	27.8	ns
Marital status:									
- unmarried	8	16.0	3	14.3	1	9.1	4	22.2	ns
- married/in a stable relation	28	56.0	13	61.9	7	63.6	8	44.4	
- separated/divorced	9	18.0	5	23.8	2	18.2	2	11.1	
- widow(er)	5	10.0	0	0.0	1	9.1	4	22.1	
Education									
- low (primary/secondary school)	29	58.0	14	66.7	8	72.7	7	38.9	ns
- high (high school/degree)	21	42.0	7	33.3	3	27.3	11	61.1	
Employment:									
- unemployed	3	6.0	2	9.5	0	0.0	1	5.6	ns
- employed	33	66.0	15	71.4	6	54.5	12	66.7	
- other (student/housewife/retired)	14	28.0	4	19.1	5	45.5	5	27.8	

Clinical profile at baseline

Exploring baseline levels of depression, anxiety, fatigue, and perceived stress, we analysed the caracteristics of the whole sample and then of three groups.

In the whole group, we found that, when assessed by IDS, 33.3% and 4.8% of the subjects showed mild and moderate levels of depression, respectively, while 61.9% scored within the range of normality. When applying the HADS, non-significant levels of depression were found in the vast majority of our sample (90.2%), and only 9.8% resulted mildly/moderately depressed. None of the participants was under antidepressant treatment. Similarly, mild to severe anxiety was detected in 7.2% of participants, while 92.7% of them did not report anxious symptomatology. Significantly higher levels of fatigue in the last month, in comparison with the "usual wellbeing", were reported by 39.0% of subjects. Finally, almost one subjects out of two (47.5%) referred high levels of stress in the last month.

No significant difference was found among groups in terms of levels of depression, anxiety, fatigue, or perceived stress, as shown in table 16.

Table 16: baseline levels of depression, anxiety, fatigue and perceived stress in the whole sample and in groups A, B, and C

	Whole sample (N=50)		Group A (N=21)		Group B (N=11)		Group C (N=18)		p-value
	Mean	**(SD)**	**Mean**	**(SD)**	**Mean**	**(SD)**	**Mean**	**(SD)**	
IDS[1]	12.7	(6.9)	11.9	(7.9)	11.0	(5.3)	14.9	(6.7)	ns
HADS[2]									
- depression	2.8	(3.2)	2.7	(2.9)	2.5	(2.9)	3.0	(3.7)	ns
- anxiety	3.8	(3.1)	2.8	(1.7)	3.4	(3.1)	5.1	(3.8)	ns
- emotional distress	6.5	(5.5)	5.7	(3.7)	6.0	(4.9)	8.1	(7.2)	ns
CFQ[3]	3.2	(3.9)	2.3	(3.1)	2.2	(2.7)	4.9	(4.9)	ns
PSS[4]	19.3	(4.9)	17.9	(5.5)	20.7	(3.2)	19.8	(5.3)	ns

[1] IDS score can range from 0 to 84, with scores categorized as follows: normal (0-13), mild (14-25), moderate (26-38), severe (39-48), and very severe (49-84).

[2] HADS score for each subscale (anxiety and depression) can range from 0 to 21 with scores categorized as follows: normal (0-7), mild (8-10), moderate (11-14), severe (15-21). Scores for the entire HADS scale (emotional distress) range from 0-42, (with higher scores indicating more distress).

[3] a total of 4 or more qualifying for “caseness”.

[4] Scores of 20 or higher are considered high stress.

Risk and resilience factors profile

Childhood traumatic experiences

Overall, 41.7% of the subjects included in the study had at least one childhood traumatic experience among physical punishment (14.6%), sexual unwanted experiences (4.5%), parental loss or/and separation (31.3%). Parental antipathy and neglect were reported by 21.3% and 40.4% of participants, respectively. Mother and father antipathy were detected in 12.2% and 12.8% of individuals, while mother and father neglect were detected in 18.4% and 29.8%, respectively. No significant difference was found among groups in terms of childhood trauma (or mother/father antipathy or neglect) rates.

Substance and alcohol use

In terms of lifetime substance use, 48.0% of subjects reported cannabis use, 32.0% referred cocaine use, and 36.7% recalled heroin use. Current use rates were extremely low, and only a few subjects (two for cannabis, one for cocaine, and three for heroin) admitted current, but sporadic consumption and none of them presented a related disorder or was in charge to a dedicated unit.

Subjects reported to consume alcohol more frequently than once in a month in 38.0% of cases (18.0% had drunk almost daily in the last year). Another 38.0% described themselves as non-drinkers, while 24.0% of subjects referred to drink alcohol less than once in a month. Among subjects admitting to drink, 10.0% reported that people around them (family, physician, or friends) had shown apprehension and expressed concerns regarding their alcohol consumption at some point of their lives. None of the subjects suffered from an alcohol use disorder when included in the study.

No significant difference was found among groups in terms of substance or alchol use rates.

Severe stressful life events

Almost one subjects out of two (47.9%) recalled a severe stressful life events in the last year. No significant difference was found among groups (57.1% in group A,

36.4% in group B, and 43.8% in group C) regarding the frequency of severe stressful life events in the last year.

Personal or family psychiatric history

Subjects recalling at least one episode of Major Depression in their lives were the 24.0% of the sample. No significant difference was found among groups (26.3% in group A, 18.2% in group B, and 29.4% in group C) in terms of personal history.

Twenty (40.0%) participants out of 50 reported a positive family psychiatric history, having at least one first-degree relative affected. No significant difference was found among groups in terms of family psychiatric history.

Coping strategies

Coping strategy profiles for both the whole sample and the three groups are summarized in table 17.

Table 17: Coping strategies profile

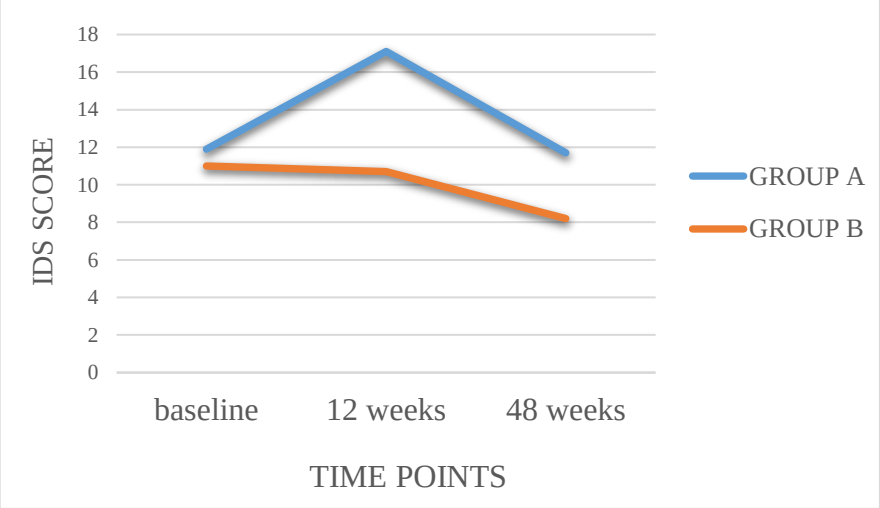
Coping strategies	Whole sample (N=50)		Group A (N=21)		Group B (N=11)		Group C (N=18)		p-value
	Mean	(SD)	Mean	(SD)	Mean	(SD)	Mean	(SD)	
Positive reinterpretation and growth	11.5	(3.0)	11.4	(2.5)	11.4	(4.1)	11.6	(3.2)	ns
Mental disengagement	7.4	(7.4)	7.8	(2.6)	6.0	(2.4)	7.9	(2.1)	ns
Focus on and venting of emotions	7.8	(2.9)	7.4	(2.5)	7.9	(3.2)	8.4	(3.5)	ns
Use of instrumental social support	10.4	(3.0)	9.7	(3.3)	11.0	(3.0)	11.1	(2.7)	ns
Active coping	10.9	(2.1)	0.9	(1.9)	11.6	(2.4)	10.5	(2.3)	ns
Denial	5.8	(2.5)	6.2	(3.1)	4.6	(1.1)	5.8	(1.9)	ns
Religious coping	9.0	(4.1)	7.8	(3.8)	10.2	(3.8)	10.2	(4.6)	ns
Humor	7.6	(2.7)	8.1	(2.8)	8.0	(3.6)	6.5	(1.7)	ns
Behavioral disengagement	5.6	(1.9)	5.8	(1.8)	4.4	(0.5)	6.3	(2.6)	ns
Restraint	9.5	(2.8)	9.5	(2.8)	9.8	(2.9)	9.2	(2.8)	ns
Use of emotional social support	9.7	(3.7)	9.0	(3.9)	9.5	(2.2)	11.0	(4.0)	ns
Substance use	4.6	(1.7)	4.9	(1.7)	4.2	(0.6)	4.6	(2.2)	ns
Acceptance	11.2	(2.9)	10.5	(2.6)	13.6	(2.5)	10.4	(2.6)	0.006
Suppression of competing activities	9.7	(2.7)	9.3	(2.6)	10.6	(3.5)	9.6	(2.3)	ns
Planning	11.1	(3.2)	10.4	(3.2)	12.0	(2.9)	11.5	(3.4)	ns

Levels of depression, anxiety, fatigue, and perceived stress at follow-ups

Group A in comparison with group B at week 12 and 48

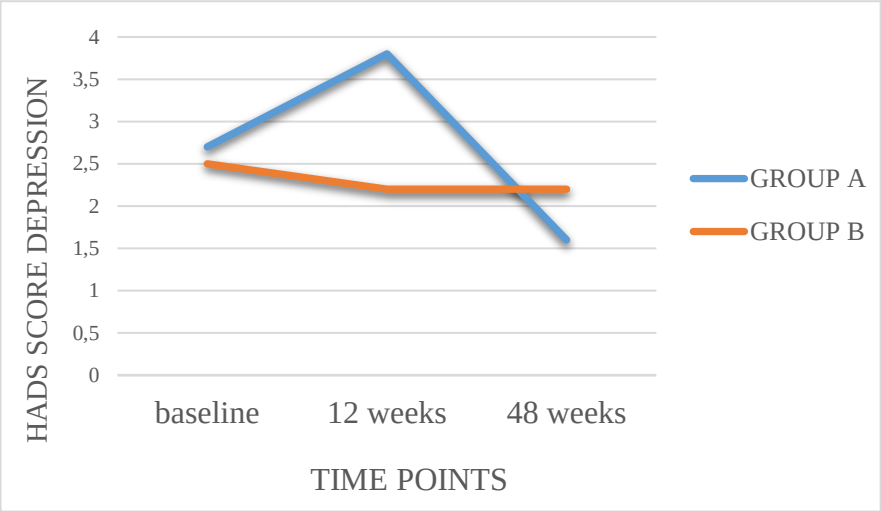
As expected (figure 8), we found within group A an increase in depression rates during the treatment with interferon-alpha, especially at week 12 (IDS mean=17.1, sd=7.8; range= 7-29), while no such change was observed in group B (IDS mean=10.7, sd=5.7; range= 4-20), whose levels of depression actually dropped, although the difference between groups did not reach significance. At baseline, 62.5% of subjects of group A reported no significant symptoms of depression (vs 72.7% in group B), while 31.3% and 6.3% referred mild and moderate symptomatology, respectively (vs 27.3% and 0.0% in group B, respectively). At week 12, only 33.3% of subjects in group A was not depressed at all (vs 66.7% in group B), while 50.0% and 16.7% resulted affected by mild and moderate depression, respectively (vs 33.3% and 0.0% in group B, respectively). At week 48 (6 months after the end of the treatment), rates of mild depression in group A were equal to 46.7% (vs 16.7% in group B), while 53.3% of participants were non depressed (vs 83.3% in group B). Differences between groups did not reach significance.

Figure 8: depression levels (IDS score) in group A vs group B



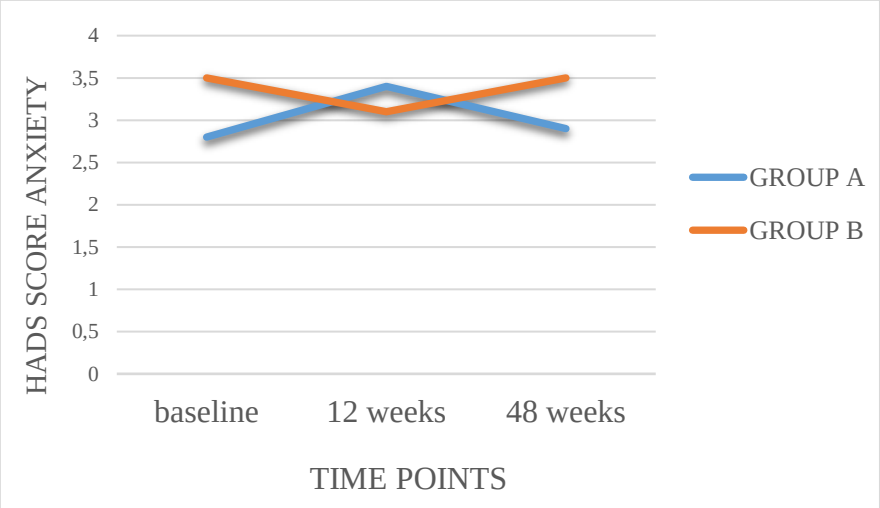
When looking at HADS scores for depression (figure 9), we confirmed the increase in depressive symptomatology at week 12 only in group A (mean=3.8, sd=4.7; range= 0-12 vs mean=2.2, sd=1.7; range= 0-4 in group B), but even in this case the difference did not reach significance.

Figure 9: depression levels (HAD score) in group A vs group B



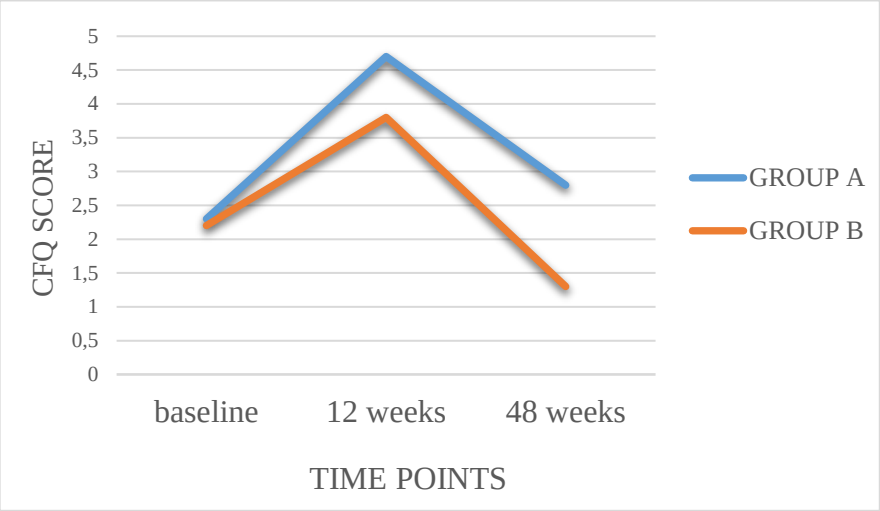
With regards to anxiety symptomatology (see figure 10), levels remained substantially stable during and after the treatment in both groups and did not differ significantly between groups.

Figure 10: Anxiety levels (HADS score) in group A vs group B



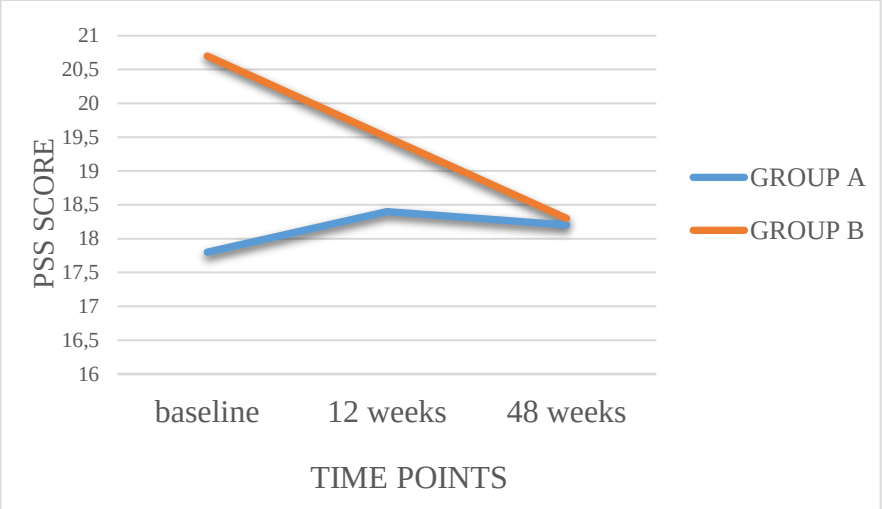
Significant levels of fatigue (including feeling tired, weak or lacking in energy) at week 12 were reported by 45.5% and 33.3% of subjects in group A and B, respectively. At six months after the end of the treatment, levels were decreased in both groups (33.3% in group A and 16.7% in group B) with mean CFQ scores equal to 2.8 (sd=3.6) and 1.3 (sd=2.2) ad shown in figure 11. Difference did not reach significance.

Figure 11: Levels of fatigue in group A vs group B



Finally, we found that levels of perceived stress gradually decreased in group B: mean values were equal to 20.7 (sd=3.2) at baseline, and diminished to 19.5 (sd=4.4) and to 18.3 (sd=3.7) at 12 and 48 weeks, respectively. In group A level of stress remained substantially stable and low (figure 12). No significant difference was however found between groups in term of stress perception during and after the treatment.

Figure 12: Perceived stress in group A vs group B



Group A in comparison with group C at week 24 and 48

We did not find any significant difference in terms of levels of depressive symptomatology between group A and group C at both 24 and 48 weeks FU. Similarly, no difference was found at both time points between group A and C in terms of anxiety, fatigue levels, or perceived stress.

Risk and resilience factors

Finally, we wanted to explore whether at week 12, 24 or 48, levels of depression, anxiety, fatigue, and/or perceived stress would be associated with childhood traumatic experiences, substance or alcohol use, recent stressful life events (SLEs), personal or family psychiatric history, or coping strategies.

Childhood traumatic experiences

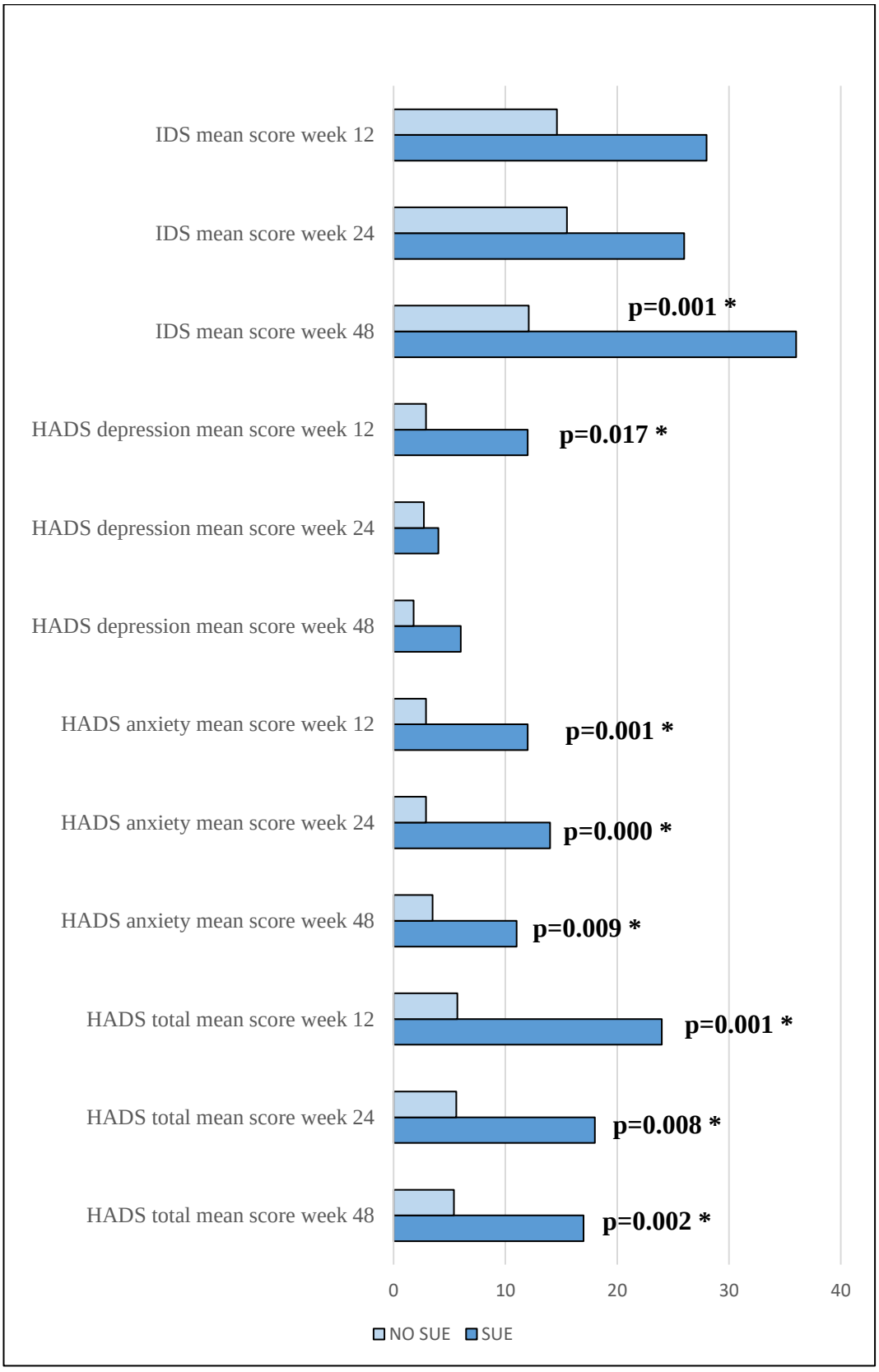
Childhood trauma (i.e. at least one among physical punishments, sexual unwanted experiences, loss, or separation) was found significantly associated with high levels of depressive and anxious symptomatology at week 24. Traumatized subjects showed higher IDS scores (mean=20.1, sd=10.2, p=0.017), and higher HADS depression (mean=3.9, sd=3.1, p=0.042) and anxiety (mean=5.4, sd=4.1, p=0.012) sub-scores, and higher HADS total score (mean=9.3, sd=5.1, p=0.001) in comparison with non-traumatized subjects at week 24 (IDS total score: mean=12.5,

ds=3.1; HADS depression sub-score: mean=1.5, sd=1.6; HADS anxiety sub-score: mean=1.8, sd=1.1; HADS total score: mean=3.4, sd=1.4). Conversely, any significant association was found between childhood trauma and levels of depression or anxiety when looking at week 12 or 48, or between trauma and fatigue or perceived stress, regardless the investigated time point.

Exploring different types of trauma, we found that subjects with a history of physical punishments demonstrated significantly increased levels of depression, emotional distress, and fatigue at week 24 in comparison with subjects not recalling such a history. The IDS mean score at week 24 in subjects physically punished when children was equal to 25.6, sd=7.5 vs a mean of 13.1, sd=4.7 (p=0.000) found in non-punished. Similarly, HADS depression sub-score resulted significantly higher in association with physical punishments (mean=5.8, sd=3.1 in punished vs mean=1.7, sd=1.7 in non-punished, p=0.001). A greater emotional distress was also found in association with physical punishments at week 24: the HADS total score was higher in subjects recalling punishments (mean=10.4, sd=3.8) in comparison with that of subjects non reporting such a history (mean=4.5, sd=4.2, p=0.017). Finally, physical punishments were found significantly associated with higher levels of fatigue (mean CFQ score in cases was equal to 6.8, sd=4.9 vs a mean of 2.9, sd=2.8, p=0.04).

An even stronger association was found between sexual unwanted experiences (SUE) and levels of depression, anxiety, and emotional distress at different time points as shown in figure 13. Any association was instead found between sexual unwanted experiences and fatigue or perceived stress.

Figure 13: Sexual unwanted expericences (SUE) and levels of depression and anxiety



Subjects recalling loss or separation did not differ significantly in terms of levels of depression, anxiety, fatigue or perceived stress regardless the investigated time points when compared with individuals without parental loss or separation.

Parental antipathy was significantly associated with higher levels of depression (IDS mean score in cases was equal to 30.5, sd=10.6 vs mean=13.6, sd=5.8, p=0.002), anxiety (IDS anxiety mean sub-score in cases was equal to 8.5, sd=0.7 vs mean=3.1, sd=3.2, p=0.03), and emotional distress (HADS mean total score in cases was equal to 13.0, sd=4.2 vs mean=5.1, sd=4.4, p=0.025) at week 24.

Finally, parental neglect was found associated with higher levels of perceived stress (PSS mean score in cases was equal to 21.6, sd= 3.9 vs mean=17.9, sd=2.8, p=0.043) at week 12 and higher levels of depression at week 24 (IDS mean score in cases was equal to 23.8, sd=12.8 vs mean=13.2, sd=4.7, p=0.012; HADS depression mean sub-score in cases was equal to 4.8, sd=3.9 vs mean=1.7, sd=1.7, p=0.022).

Substance or alcohol use

Any significant association was found between cannabis or heroin lifetime use, or alcohol use and levels of depression, anxiety, fatigue, or perceived stress regardless the investigated time point. Cocaine lifetime use was found conversely significant associated with higher levels of depression at week 24 (IDS mean score in cases was equal to 24.0, sd=12.8 vs mean=14.4, sd=6.1, p=0.040).

Recent severe stressful life events (SLEs)

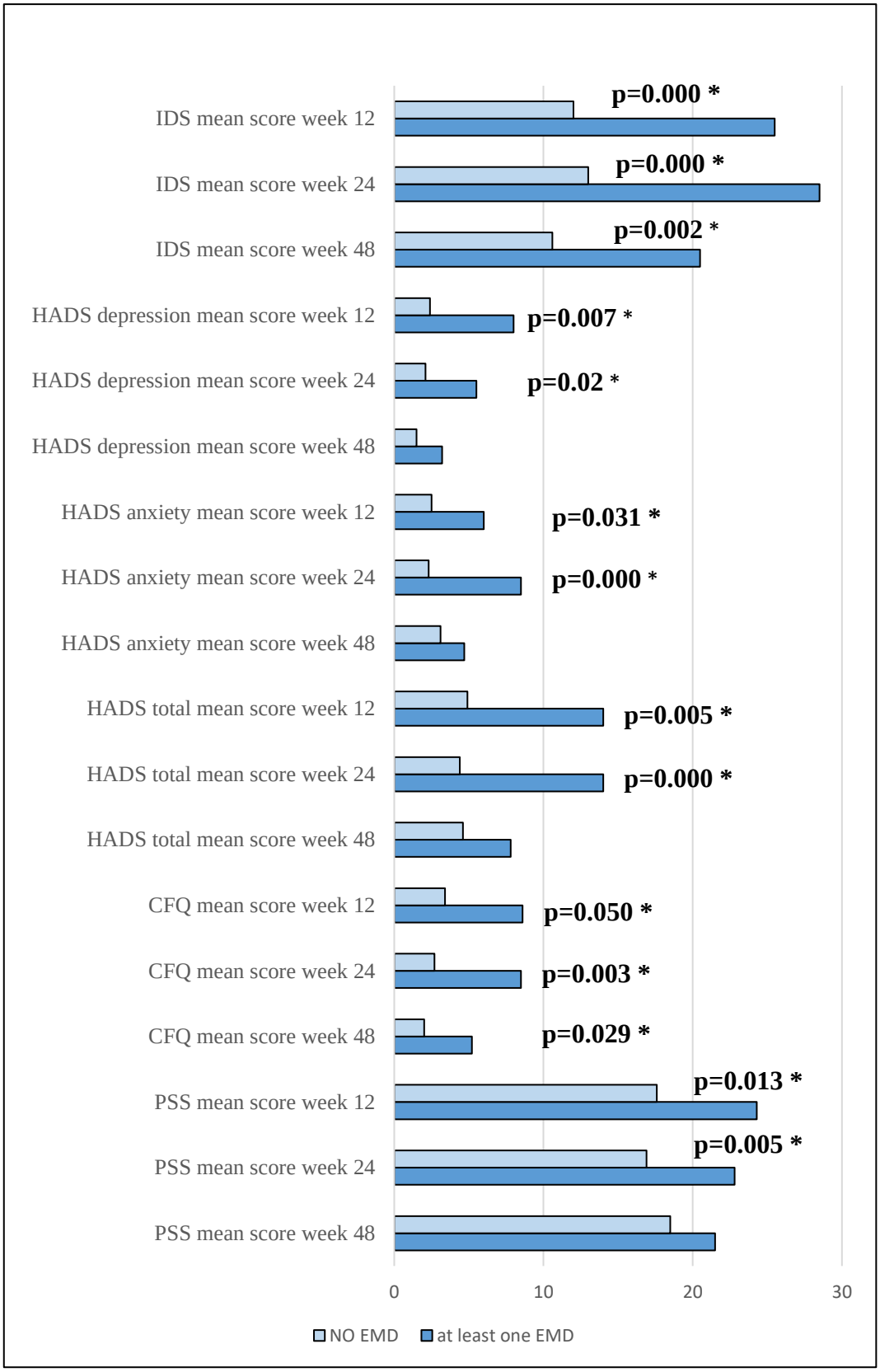
Any significant association was found between recent severe stressful life events and levels of depression, anxiety, fatigue, or perceived stress regardless the investigated time point

Personal or family psychiatric history

We found that having at least one Episode of Major Depression (EMD) in the past was significantly associated with higher levels of depression, anxiety, fatigue, and perceived stress at several time points, as shown in figure 14.

Conversely, any significant association was found between family psychiatric history and levels of depression, anxiety, fatigue, or perceived stress regardless the investigated time point.

Figure 14: Personal psychiatric history and levels of depression and anxiety



Coping strategies

When investigating coping strategies, we found that some (Focus on and venting of emotions, Denial, and Substance use) were significantly associated with higher levels of depression, anxiety, fatigue, and stress, while some others appeared to act as resilience factors (specifically, Mental disengagement and Humor) and were related to lower levels of levels of depression, anxiety, fatigue, and stress at several time points.

In details, Focus and venting of emotions coping strategy resulted associated with higher levels of depression (correlation coefficient =0.469, p=0.037), anxiety (correlation coefficient =0.541, p=0.014), and emotional distress (correlation coefficient=0.471, p=0.036) at week 24. Similarly, Denial coping strategy was associated with increased levels of fatigue at week 48 (correlation coefficient =0.363, p=0.041), and with higher level of depression at week 12 (correlation coefficient =0.469, p=0.049). Substance use coping strategy was also found to enhance both depression levels (correlation coefficient =0.481, p=0.032) and perceived stress correlation coefficient =0.448, p=0.048) at week 24.

Conversely, Mental disengagement and Humor coping strategies were found to play a protective role. Mental disengagement coping strategy and lower levels of perceived stress were found associated at week 24 (correlation coefficient= -468, p=0.037). Humor coping strategy was significantly associated with lower levels of depression at both 24 and 48 weeks (correlation coefficient = -0.605, p=0.005 for HADS depression score at week 24; correlation coefficient = -0.451, p=0.010 for IDS score at week 48). A reduction in levels of anxiety at week 48 (correlation coefficient = -0.363, p=0.041) was also found in association with humor as a coping strategy. Similarly, reduced emotional distress was found associated with Humor coping strategy at both week 24 and 48 (correlation coefficient = -0.449, p=0.047, and correlation coefficient = -0.354, p=0.047, respectively). Finally, decreased level of fatigue at week 12 and 24 (correlation coefficient = -0.604, p=0.010, correlation coefficient = -0.562, p=0.012, respectively), and reduced perceived stress at week 48 (correlation coefficient = -0.397, p=0.027) and Humor coping strategy were also significantly associated.

Discussion

In a sample of subjects affected by chronic Hepatitis C followed up for 48 weeks, we explored levels of depression, anxiety, fatigue, and perceived stress at baseline and at several time points. As expected, we found an increase in depression rates during the treatment with interferon-alpha (group A), especially at week 12, while no such change was observed in subjects treated with DAAs (group B), whose levels of depression actually dropped, although the difference between groups did not reach significance. Similarly, no significant difference was found with regards to levels of anxiety, fatigue, or perceived stress between group A and B (or C, not in treatment) during the 48 weeks of follow up. Our findings appear to be in line with the literature that points out an "illness-effect": patients suffering from chronic medical conditions characterized by an inflammatory pattern, such as HCV-related chronic liver disease, had a higher prevalence of psychiatric comorbidity, including depression, when compared to the general population (Fontana et al., 2002). Although patients undergoing interferon therapy showed increasing levels of depression during the treatment, differences with subjects undergoing DAAs treatment or with subjects not in treatment did not reach significance. On one side, these results appear to support the prevailing of the illness-effect on the treatment-effect. On the other side, they might be ascribable to the small sample size, and thus further investigation could be required.

Risk and resilience factors were also assessed, including childhood traumatic experiences, drug and alcohol use, psychiatric history, and coping strategies, and their influence on subjects' well being was explored during the follow up. We wanted to explore whether levels of depression, anxiety, fatigue, and/or perceived stress would be associated with risk/resilience factors. We found that overall, 41.7% of the subjects had at least one childhood traumatic experience among physical punishment, sexual unwanted experiences, parental loss or/and separation. Parental antipathy and neglect were reported by 21.3% and 40.4% of participants, respectively. Mother and father antipathy were detected in 12.2% and 12.8% of individuals, while mother and father neglect were detected in 18.4% and 29.8%, respectively. When compared to data from the Italian National Survey (CISMAI-TDH, 2015), which found that almost one children out of 100 (9.5‰) is victim of

maltreatments in Italy, these findings, although descriptive, evoke an increased rate of maltreatments in our sample of subjects affected by Hepatitis C. Moreover, if looking at our own data collected in a sample of healthy subjects (Tomassi et al., under revision), the difference appears even more significant: 28.2% (vs 41.7%) of healthy subjects included had at least one childhood traumatic experience. Mother and father antipathy were detected in 7.7% (vs 12.2%) and 10.8% (vs 12.8%) of healthy individuals, while mother and father neglect were detected in 10.3% (vs 18.4%) and 10.8% (29.8%), respectively. An association between suffering from Hepatitis C in adulthood and childhood trauma is suggested. However, a proper case-control study is warrant. We found that childhood trauma (i.e. at least one among physical punishments, sexual unwanted experiences, loss, or separation) was significantly associated with higher levels of depressive and anxious symptomatology and with greater emotional distress at week 24. Exploring different types of trauma, we found that subjects with a history of physical punishments demonstrated significantly increased levels of depression, emotional distress, and fatigue at week 24 in comparison with subjects not recalling such a history. An even stronger association was found between sexual unwanted experiences and levels of depression, anxiety, and emotional distress. Parental antipathy was significantly associated with higher levels of depression, anxiety, and emotional distress at week 24. Finally, parental neglect was found associated with higher levels of perceived stress at week 12 and higher levels of depression at week 24. Our findings are mostly in line with the literature and confirm a significant role for childhood traumatic experiences in predisposing subjects to develop depression and anxiety (Buckman et al., 2018, Tunnard et al, 2014, Nanni et al., 2011, Gaudiano & Zimmerman., 2010). However, to our best knowledge, it is the first study investigating childhood trauma as a potential vulnerability factor for depression, anxiety, and fatigue in a sample of Hepatitis C patients. The effect of childhood trauma in increasing the risk of depressive and anxious symptomatology appears substantially independent from other factors, including HCV treatments. However, further analyses will be needed to validate and corroborate these results. Finally, it is worth reminding, that the study is still ongoing. Several biological investigations have also been conducted, and specimens are now under process.

Thus, we will be soon able to deep our exploration with regards to risk/resilience factors, specifically childhood traumatic experiences, in terms of genetics, epigenetics and proteomics. Any significant association was found between drug lifetime use, alcohol use, or recent severe stressful life events and levels of depression, anxiety, fatigue, or perceived stress regardless the investigated time points. Subjects recalling at least one Episode of Major Depression in their lives were the 24.0% of the sample. We found that having at least one episode of Major Depression in the past was significantly associated with higher levels of depression, anxiety, fatigue, and perceived stress at several time points. These findings are in line with previous literature (Buckman et al., 2018; Raison et al., 2005) and support the need of specific psychosocial and/or pharmacological interventions in this subsample of patients. Conversely, any significant association was found between family psychiatric history and levels of depression, anxiety, fatigue, or perceived stress regardless the investigated time point.

Finally, when investigating coping strategies, we found that some (Focus on and venting of emotions, Denial, and Substance use) were significantly associated with higher levels of depression, anxiety, fatigue, and stress, while some others appeared to act as resilience factors (specifically, Mental disengagement and Humor) and were related to lower levels of levels of depression, anxiety, fatigue, and stress at several time points. Once again, our findings appear coherent with the available evidence (Hardeveld et al., 2010, Beshai et al., 2011, Conradi et al., 2008), support the association between poor coping skills and depression, and extend that notion to subjects affected by Hepatitis C. In addition, our results evoke a potentially increased resilience in a sub-sample of these subjects and support a further investigation, along with the routine medical caring, of the coping strategies profile.

Our study has several strengths. First, it has a longitudinal perspective design, with a follow up period 48 weeks long, which provide an appropriate time-window for the evaluation of the subjects. Second, the presence of the group of hepatitis C subjects in the phase of stationary disease (C, not in therapy) represents an element of methodological accuracy. The comparison between the groups A vs C has allowed in fact to estimate the prevalence of depression during interferon treatment, distinguishing it from the prevalence of depression in the course of chronic hepatitis

C. Similarly, the presence of group B represents one of the major innovation of this study in comparison with previous literature on the field that to our best knowledge has never before investigated DAAs as we did. Third, the study has a collaborative nature: in fact, it has been being conducted in collaboration with the Section of Infectious Diseases of the Verona University Hospital. This aspect has allowed the integration skills, the sharing of knowledge, has ensured the accuracy of the data collected, and has applied in research a collaboration between medical disciplines already tested in the clinic. Finally, subject has been assessed using internationally validated scales by expert researchers with a clinical background (psychiatrists and psychologists).

Some limitations must also be addressed. First, these are preliminary findings and descriptive results obtained in a relatively small sample and therefore should not be interpreted as hypothesis generating. Second, retrospective reporting of abuse might increase the risk of recall bias, although the reliability of referring trauma has been previously demonstrated (Schäfer & Fisher, 2011): reports were consistent with other sources of information and stable over time. Third, the presence of personality disorders was not investigated. It is honest to admit that personality could play a central role in a sample whose rates of substance use disorder as well as the rates of childhood traumatic experiences appear higher than in non-hepatitis C subjects. However, we decided to maintain the focus on severe mental disorders rather than to include a complex element such as personality traits. Childhood trauma and personality disorder represents in fact a wide, different, and quite diffused field of research.

To conclude, among clinical vulnerability factors for depression, anxiety, and fatigue in Hepatitis C subjects, childhood traumatic experiences must be considered to play a significant role. It appears therefore of primary importance to systematically investigate the presence of a history of childhood traumatic events. It could help the clinician to identify the most appropriate therapeutic interventions to offer the patient, which can be both pharmacological and psychosocial (Larkin & Read, 2008).

CHAPTER 4: CHILDHOOD TRAUMA AND HEALTHY SUBJECTS

4.1 THE HEALTHY SUBJECTS GROUP

Background

Clinical and social outcomes of psychosis represent a challenge for clinical practice. Psychoses are characterized by high clinical heterogeneity and wide treatment response variability, in association with individual factors of neuro-psychological, genetic, and environmental nature.

The GxE model

In the last two decades, several studies have tried to elucidate how genetics and psycho-social elements interact with each other in determining the onset and the evolution of psychosis. Despite the effort, a causal association between genetic variants and psychosis has not been demonstrated yet (Allen et al., 2009). On the other hand, epidemiological studies have established the existence of non-genetic risk factors that can contribute to enhance the vulnerability for psychosis, leading to the development of the disorder. Among biological factors, malnutrition (Penner & Brown, 2007), obstetric complications (Cannon et al., 2002;), paternal age at the time of conception (Ek et al., 2015), and maternal infections (Khandaker GM et al., 2013) were investigated. Psychosocial factors were also explored: childhood trauma (Read et al., 2005), stressful life events (Mansueto & Faravelli, 2017), migration to another country (Cantor-Graae & Selten, 2005), urbanity (Padhy et al., 2014) and cannabis use (Henquet et al., 2005) were found to play a role in increasing the vulnerability for psychosis. Psychotic disorders seem therefore to represent "multi-factorial" disorders, in which the genetic and non-genetic factors play an equally important role in determining vulnerability (Van Os & Rutten, 2008).

In this scenario, the gene-environment (GxE) model for psychosis postulates that neither genes nor environmental risk factors are sufficient to determine the onset of the disease, but that their synergistic co-action is necessary. According to this model, the risk of developing a psychotic disorder in response to a certain type of environmental stimulus would be influenced by the GxE interaction. It seems then relevant to note that even the healthy general population shares with psychotic

patients numerous factors that have been considered risky for the development of psychosis, including cannabis use and stressful life events (e.g. childhood trauma). In healthy subjects, however, these predisposing factors were not sufficient to determine the illness onset. Which are the key-factors necessary for an individual to exceed the vulnerability threshold and to develop the disorder? What is the role of genetic factors and biological modifiers?

Endophenotypes: Neurological Soft Signs and neuro-cognitive evaluations

A further promising approach of investigation is based on endophenotypes. An endophenotype (also known as intermediate phenotype) is a quantitative biological trait. It is reliable in reflecting the function of a discrete biological system and is reasonably heritable, and as such is more closely related to the root cause of the disease than the broad clinical phenotype (Gottesman & Gould, 2003; Cannon & Keller, 2006;). This term refers not to symptoms or signs of specific psychiatric disorders, but rather to components of the disease itself, such as alterations in cognitive abilities, cerebral neuroanatomy, and behavior (Mazzoncini et al, 2009). Endophenotypes lay between the genetic variants and the clinical expression of the disorder and can be found at any intermediate level of the pathophysiological process that links the genotype to the final diagnosis (Cannon & Keller 2006). In psychiatry research, the accepted criteria, which a biomarker must fulfill to be called an endophenotype, include (Beauchaine, 2009):

- An endophenotype must segregate with illness in the population.
- An endophenotype must be heritable.
- An endophenotype must not be state-dependent (i.e., manifests whether illness is active or in remission).
- An endophenotype must co-segregate with illness within families.
- An endophenotype must be present at a higher rate within affected families than in the population.
- An endophenotype must be amenable to reliable measurement, and be specific to the illness of interest.

Consequently, due to their intrinsic characteristics, it is not uncommon to find endophenotypes even in healthy individuals of the general population. Their prevalence in healthy subjects is however not adequately documented. At the theoretical level, such endophenotypes could be neutral or even beneficial (Keller and Miller 2006), for example conferring a particular selective advantage under certain circumstances. The endophenotypes that in literature have been associated with psychosis and which were also found in healthy subjects are numerous and heterogeneous. These include neurological abnormalities, such as Neurological Soft Signs (NSS) (Chan & Gottesman 2008), and neurocognitive deficits, such as memory functions, processing speed, attention, verbal reasoning, resolution of visual-spatial problems (Reichenberg et al., 2010).

Aim

The Healthy Subjects Research Project leaded by Tosato S. et collaborators aims to investigate, in a sample of healthy subjects belonging to the general population, a set of environmental, biological, and neuropsychological variables, including endophenotypes, known to be involved in the development of psychosis. The project, once completed, will allow the execution of one, or more, case-control studies, whose results will enrich, with a scientifically relevant contribution, the knowledge concerning psychosis already acquired in the Psychosis Incident Cohort Outcome Study (PICOS) (Lasalvia et al., 2012) and the "GET-UP PIANO – Psychosis: early Intervention and assessment of Needs and Outcome" (Ruggeri et al., 2012, 2015).

Methods

Subjects' recruitment

The project, still ongoing, aims to recruit 435 subjects belonging to general population. Assessments takes place at the Psychiatry Section of the Verona University Hospital. Subjects are recruited on a voluntary base, through leaflets and word of mouth.

The inclusion criteria are:

1) Age >= 18 years

The exclusion criteria are:

1) History of psychotic disorder (schizophrenic spectrum disorders, bipolar I disorder, manic episode, depression with psychotic symptoms)

2) Known intellectual disability (IQ<70);

3) History of neurological disease (eg epilepsy, dementia, cerebrovascular or infectious diseases, trauma or brain tumors);

4) Family history of psychosis (first-degree relative affected).

Eligible subjects receive a full explanation of the aims of the study. Once a written consent is obtained, subjects are assessed through the administration of clinical scales and a battery of neuropsychological/neurological tests, and blood is sampled. Partial participation is foreseen and for this reason, enrolled subjects are allowed to choose whether to carry out the whole assessment (clinical-epidemiological and neuro-psychological evaluations, and blood sampling) or only a part.

Questionnaires

Socio-demographic data are collected according to a subject information sheet, including information about: gender, age, ethnicity, employment, marital status, living status, medical conditions and use of medication. Table 18 shows the instruments used in the socio-demo and clinical assessment.

Table 18: Instruments used in the socio-demo and clinical assessment

Instruments	**Investigated area**
Mini International Neuropsychiatric Interview (MINI) (Sheehan et al., 1998)	Psychiatric disorder in Axis I
Alcohol Use Disorders Identification Test (AUDIT) (Babor et al, 1992)	Alchool use
Cannabis Experience Questionnaire (CEQ) (Barkus et al., 2006)	Drug use
Questionnaire on Nicotine Addiction (Heatherton et al., 1991)	Smoking tobacco
Childhood Experience of Care and Abuse Questionnarie (CECA-Q) (Bifulco et al., 2005)	Childhood traumatic experiences
The Community Assessment of Psychic Experiences (CAPE) (Stefanis et al., 2002)	Psychotic-like Experiences
Parental Bonding Instrument (PBI) (Parker, 1979)	Parenting style
Life Events Scale (Paykel et al., 1971)	Stressful life events
Lewis-Murray Obstetric Complications Scale (Lewis et al., 1989)	Obstetric complications
The Level of Expressed Emotion Scale (LEE) (Cole & Kazarian, 1988)	Expressed Emotion Levels
Hamilton Rating Scale for Depression (HAM-D) (Hamilton, 1960)	Depressive symptomatology
Bech-Rafaelesen Mania Rating Scalen (BRMRS) (Bech et al., 1978)	Manic symptomatology
Manchester Short Assessment of Quality of life (MANSA) (Priebe et al., 1999)	Quality of life
Family Interview for Genetic Studies (FIGS) (NIMH, 1992)	Family Psychiatric History

A full neuro-psychological evaluation is also conducted (details not shown).

Biological Investigation

Subjects are also blood sampled: one DNA tube (for sequencing), one paxgene tube (to investigate gene expression and epigenetics), one serum and one plasma tube (aiming to explore proteomics and glucose/lipid profiles, respectively) are collected. Optionally, salivary cortisol levels are also measured (to evaluate salivary cortisol levels) using Salivettes (Sarstedt, Leicester, UK).

Some studies have been already performed exploring the available data form the Healthy Subjects Research Project. With reference to childhood traumatic experiences, and for the purpose of this thesis, the effects of early adversity were investigated in healthy individuals in terms of psychotic-like experiences, salivary cortisol levels and lifetime cannabis use (Tomassi et al., under review), gene expression and leukocyte telomere length (Lopizzo et al., 2017), and glucose metabolism (Tosato et al., under review). In this PhD thesis, I will report the two studies I have significantly contributed.

4.2 CHILDHOOD TRAUMA AND PSYCHOTIC-LIKE EXPERIENCES IN HEALTHY SUBJECTS

Psychosis might be considered a dimensional phenotype, also expressed at non-clinical levels. The psychosis continuum implies that symptoms usually reported by subjects affected by psychosis can be experienced also by non-clinical samples (Linscott & Van Os, 2013). Indeed, a median annual incidence rate equal to 2.5% and a median prevalence rate of 7.2% of psychotic experiences were found in the general population (Linscott & Van Os, 2013). Then, considering psychosis as a quantitative trait (Stefanis et al., 2002) it is possible to affirm that psychotic-like experiences (PLEs) exist along a continuum in the general population, may similarly be measurable, and share similar risk factors with psychotic disorders, including, among others, childhood traumatic experiences (Kessler et al., 2010; Linscott and Van Os, 2013). Within environmental risk factors, childhood traumatic experiences double the risk of developing psychosis (Bonoldi et al., 2013; Borges et al., 2013; Varese et al., 2012a). Nevertheless, the biological, psychological or environmental mechanisms underlying the association between being traumatized as a child and developing a psychosis or psychotic like experiences later in life are still a matter of debate. A primary role for the stress response system, including the hypothalamic-pituitary-adrenal (HPA) axis, has been hypothesized. Both HPA axis hyper- and hypo-activation (Lupien et al., 2009) and hyper- (Heim et al., 2000) and hypo-reactivity to stress (Klaassens et al., 2009; Carpenter et al., 2007) have been associated to childhood trauma. On the other hand, HPA-axis dysfunction has been significantly associated to psychosis: HPA-axis hyperactivity, characterized by high cortisol levels in basal condition, and a blunted HPA axis response to stress (hypo-reactivity) have been observed in psychosis (Borges et al., 2013). However, whether HPA axis dysfunction mediates the trauma-psychosis association or represents a trait of illness is still unclear. Moreover, HPA axis functioning may be influenced by numerous factors, including age, gender and tobacco (Adam & Kumari, 2009) which could play a confounding role. Smoking cannabis represents another element, which potentially mediates the association between childhood traumatic experiences and psychosis (Tomassi et al., 2017). In fact, the association

between cannabis consumption and psychotic symptoms is well known, even in the general population (Henquet et al., 2005.; Moore et al., 2007; Rössler et al., 2011). Moreover, subjects recalling childhood trauma are more prone to use substances, including cannabis: they may develop depressive symptoms and try to self-medicate themselves (Houston et al., 2011) and they often live in disadvantaged environments, where it is easier to get addicted to substance (Wicks et al., 2005).

Based on the aforementioned knowledge, we conducted a study, to explore the association between childhood traumatic experience and PLEs in a sample of healthy subjects, investigating potentially mediating factors like HPA axis functioning (in terms of both activity and reactivity) and cannabis use. We hypothesized that subjects with a history of childhood traumatic experiences would have more frequent and/or more distressful PLEs, show different saliva cortisol levels, and higher rates of lifetime cannabis use when compared to subjects without childhood traumatic experiences.

Methods

Subjects and questionnaires

Healthy subjects were recruited through notices posted at the Verona University Hospital, Verona (Italy). Individuals presenting a history of neurological or psychiatric diseases, prior traumatic brain injury, or intellectual disability (IQ<70); being pregnant or breastfeeding or under current medications were excluded from the study. The Mini International Neuropsychiatric Interview (Sheehan et al., 1998) was used to exclude any psychiatric disorder in Axis I; and the Structured Clinical Interview for DSM disorders (First et al., 1997) was applied to exclude any psychiatric disorder in Axis II. Information about self-reported BMI and tobacco use were also collected.

The presence of childhood traumatic experiences (CTE) was assessed by the CECA-Q scale (Bifulco et al., 2005). It comprises information concerning experiences of childhood adversities before age 17, including physical punishments, sexual unwanted experiences, parental separation and loss. Separation has been defined as a detachment from at least one of the relatives, longer than 6 months; whereas, loss was determined whether one or both parents

died during subjects' childhood. Childhood trauma (CT) was defined as the experience of at least one among physical punishments, sexual unwanted experiences and parental loss/separation. The questionnaire also assessed the presence of lack of parental care (neglect; cut-off: mother >=22, father >=24) and hostile or cold parenting (antipathy; cut-off mother or father >=25). Therefore, childhood traumatic experiences (CTE) involve childhood trauma (CT),(including physical punishments, sexual unwanted experiences, parental separation and loss), neglect, and antipathy.

The Community Assessment of Psychic Experiences (CAPE) (Stefanis et al., 2002), a 42-items instrument, collected information about lifetime psychotic-like experiences, with regards to both frequency (1 never, 2 sometimes, 3 often, 4 nearly always) and related distress (1 not distressed, 2 a bit distressed, 3 quite distressed, 4 very distressed). Scores were estimated for the positive (20 items), negative (14 items), and depressive (8 items) dimensions by adding up single item scores and weighting for the number of valid answers.

The Cannabis Experiences Questionnaire (Barkus et al., 2006) assessed lifetime cannabis use. Subjects were divided in two groups: those who had never used cannabis and those who had used cannabis at least once in their life.

The investigation was carried out in accordance with the latest version of the Declaration of Helsinki (World Medical Association et al., 2013). Written informed consent of the participants was obtained after the nature of the procedures had been fully explained. The study design was reviewed and approved by the ethic committee of the Verona University Hospital.

Salivary cortisol assessment

Salivary cortisol levels were measured using Salivettes (Sarstedt, Leicester, UK). Subjects were instructed to wake up before 10 am, to collect saliva samples by chewing the cotton roll for 2 min, immediately after awakening (0 min) while still in bed, and 15, 30 and 60 min after awakening. Having breakfast and teeth brushing were to be avoided during the first hour of awakening. Saliva samples were collected again at noon and at 8 pm and subjects were recommended not to eat in the 30 min before sampling. Subjects were instructed to store salivary samples at

4°C. On the arrival to the laboratory, salivary samples were immediately centrifuged at 3000 rpm per ten minutes and stored at -20°C until the assay. Cortisol was measured using a commercially available ELISA kit (Enzo Life Sciences, Farmingdale, NY, USA), following the manufacturer's instruction. The analysis was performed by Dr. Brondino, with the supervision of Professor Politi, at The University of Pavia. All samples were assessed in duplicate. The sensitivity of the assay was 56.72 pg/ml, while the intra- and inter-assay coefficients of variation were 6.6% and 7.8%, respectively. Diurnal cortisol levels and the Cortisol Awakening Response (CAR) were measured using the Area Under the Curve (AUC) respected to the ground calculation (Pruessner et al., 2003): for the former were taken into account levels at 0 min, 15min, 30min, 60min, noon and 8 pm, while for CAR levels at 0 min to 15, 30, and 60 min after awakening were considered.

Statistical analysis

Comparisons between the groups of subjects reporting trauma and no trauma, respectively, were performed by t test in case of continuous variables and Fisher's exact test in case of categorical dummy variables. Linear regressions models were estimated to compare the outcomes of interest between these groups of individuals when putative confounders were taken into account. All tests were bilateral at $p<0.05$. Analyses were performed by using SPSS 22.0 for Windows.

Results

Description of the sample

We recruited 39 subjects from the general population. Socio-demographics and life style characteristics are shown in table 19.

Table 19: Socio-demographics and life style characteristics with respect to childhood trauma (at least one among physical punishment, sexual unwanted experiences, parental loss or/and separation; CECA-Q)

	Total sample	**Childhood trauma**		
Characteristics	**N=39**	**Yes (N=11; 28.2%)**	**No (N=28; 71.8%)**	**p-value (t or Fisher test)**
Age (years), mean (sd)	39.1 (11.0)	41.8 (10.1)	38.0 (11.3)	0.335
Sex Male, N (%)	18 (46.2%)	4 (36.4%)	14 (50.0%)	0.497
Current tobacco Use Yes, N (%)	12 (30.8%)	5 (45.5%)	7 (25.0%)	0.262
Lifetime cannabis use Yes, N (%)	17 (43.6%)	6 (54.5%)	11 (39.3%)	0.482
BMI, mean (sd)	25.3 (4.4)	25.8 (3.2)	25.1 (4.9)	0.683

Overall, 28.2% of the subjects included in the study had at least one childhood traumatic experience among physical punishment, sexual unwanted experiences, parental loss or/and separation. Mother and father antipathy were detected in 7.7% and 10.8% of individuals, while mother and father neglect were detected in 10.3% and 10.8%, respectively (data not shown). No significant differences were found between people who experienced childhood trauma (or mother/father antipathy or neglect) and those ones who did not with respect to socio-demographics and life style characteristics.

Frequencies of psychotic-like experiences and the associated levels of distress are summarized in table 20.

Cortisol Awakening Response (CAR) and Diurnal Cortisol (DC) levels (measured using the AUC) are shown in Table 21.

Table 20: Lifetime psychotic-like experiences: frequency and related distress (CAPE) with respect to childhood trauma (at least one among physical punishment, sexual unwanted experiences, parental loss or/and separation; CECA-Q)

	Total sample	**Childhood trauma**		
CAPE scores, mean (sd)	**N=39**	**Yes (N=11; 28.2%)**	**No (N=28; 71.8%)**	**p-value (t test)**
Frequency score (1-4)				
Positive	1.23 (0.22)	1.35 (0.25)	1.19 (0.20)	**0.050**
Negative	1.52 (0.29)	1.47 (0.36)	1.55 (0.26)	0.480
Depressive	1.66 (0.31)	1.67 (0.21)	1.66 (0.34)	0.898
Distress score (1-4)				
Positive	1.59 (0.54)	1.72 (0.63)	1.51 (0.48)	0.329
Negative	2.00 (0.63)	2.31 (0.87)	1.88 (0.48)	0.055
Depressive	2.59 (0.65)	2.52 (0.85)	2.62 (0.57)	0.676

Table 21: Cortisol Awakening Response (CAR) and Diurnal Cortisol levels with respect to childhood trauma (at least one among physical punishment, sexual unwanted experiences, parental loss or/and separation; CECA-Q)

	Total sample	**Childhood trauma**		
Mean (sd)	**N=39**	**Yes (N=11; 28.2%)**	**No (N=28; 71.8%)**	**p-value (t test)**
Cortisol Awakening Response (AUCg)	71.8 (25.8)	79.2 (37.2)	68.9 (19.9)	0.270
Diurnal Cortisol levels (AUCg)	708.4 (155.9)	689.6 (100.6)	715.7 (173.9)	0.644

Childhood traumatic experiences and PLEs

Regarding the lifetime frequency of PLEs (Table 20), a significantly higher lifetime frequency of positive symptomatology was found in subjects reporting CT (1.35 sd 0.25) in comparison with those not recalling such experience (1.19 sd 0.20) (t test, p=0.050). Conversely, traumatized and non-traumatized subjects did not differ significantly in terms of lifetime frequency of negative or depressive symptomatology.

No significant difference between traumatized and non-traumatized subjects was found for lifetime distress caused by PLEs.

By exploring a history of parental antipathy or neglect, we found that subjects reporting maternal antipathy showed a significantly lower lifetime frequency of negative symptoms than individuals who did not recall such experience (1.19 ds 0.11 vs 1.55 sd 0.28; t test, p=0.037) and significantly lower levels of distress in association with depressive symptoms (1.89 sd 0.84 vs 2.65 sd 0.61; t test, p=0.05). Father antipathy and father or mother neglect did not show any significant association with lifetime frequency and distress levels of PLEs (data not shown).

Childhood Traumatic Experiences and saliva cortisol levels

We did not find any significant difference regarding cortisol levels (both CAR and diurnal cortisol levels) between subjects who recalled a history of CT and those who did not (Table 21). By exploring the most severe type of childhood trauma (i.e. sexual unwanted experiences), we found a significant difference for the levels of CAR, which were higher in subjects with sexual unwanted experiences than in those without such experiences (112.8 sd 74.5 vs 69.6 sd 21.3; t test, p=0.019).

Parental antipathy and neglect did not show any significant association with saliva cortisol levels (data not shown).

Childhood Traumatic Experiences and lifetime cannabis use

Lifetime cannabis use did not differ significantly between traumatized and non-traumatized, although 54.5% of people reporting trauma had used cannabis at least once in their life vs 39.3% of those ones without trauma (Table 19).

Parental antipathy and neglect did not show any significant association with lifetime cannabis use (data not shown).

Association of childhood traumatic experiences with PLEs adjusted for putative confounders

Cortisol levels (both CAR and diurnal levels) and cannabis lifetime use were explored as potential confounders of the association between CT or mother antipathy and PLEs.

CT and the frequency of positive symptoms

Subjects who reported at least one CT showed significantly higher lifetime frequency of positive symptoms in comparison with subjects without a history of CT. The aforementioned association remained significant only when it was adjusted for CAR (Beta coefficient for CT=0.341, p=0.039), although CAR did not significantly affect the frequency of positive symptoms (Beta coefficient for CAR=-0.141, p=0.382).

Maternal antipathy and the frequency of negative symptoms or the distress levels of depressive symptoms

Subjects with a history of maternal antipathy had a significantly lower lifetime frequency of negative symptoms in comparison with individuals not reporting such experience. When adjusting for CAR, diurnal cortisol levels or lifetime cannabis use, this association remained significant (Beta coefficient for mother antipathy was -0.346 with p=0.034, -0.338 with p=0.038 and -0.333 with p=0.041, respectively), although CAR, diurnal cortisol levels or lifetime cannabis use did not significantly affect the frequency of negative symptoms (data not reported).

Moreover, subjects with a history of maternal antipathy had significantly lower levels of distress in response to depressive symptoms with respect to individuals not reporting such experience. After adjusting for CAR, this association remained significant (Beta coefficient for mother antipathy=-0.322, p=0.050), while it lost significance after adjusting for diurnal cortisol levels (Beta coefficient for mother

antipathy=-0.312, p=0.052) or cannabis lifetime use (Beta coefficient for mother antipathy=-0.313, p=0.056). CAR, diurnal cortisol levels or lifetime cannabis use did not result associated with the magnitude of distress associated with lifetime depressive symptoms (data not reported).

Sexual unwanted experiences and CAR

Subjects with a history of sexual unwanted experiences had significantly higher levels of CAR in comparison with individuals not reporting such experiences. When adjusting for possible confounders (sex, age and current tobacco use), this association remained significant (Beta coefficient for sexual unwanted experiences was 0.371 with p=0.021, 0.387 with p=0.023 and 0.358 with p=0.027, respectively), although no confounders significantly affected the CAR levels (data not reported).

Discussion

To the best of our knowledge, this is the first study aiming to explore the effect of childhood traumatic experiences and their association with psychotic-like experiences (PLEs) in a sample of healthy subjects, taking into account the potential confounder effect of HPA axis activity and lifetime cannabis use. We found that subjects with a history of childhood trauma, including at least one among physical punishment, sexual unwanted experiences, loss and separation, showed higher lifetime frequency of positive symptoms. In psychosis, childhood adversities were found significantly associated with both auditory hallucinations and paranoid ideation (Bentall et al., 2012; Dvir et al., 2013). Our findings are in line with the available knowledge and extend the notion that childhood trauma is associated with PLEs (especially positive symptoms), at a non-clinical level. Unfortunately, the association was no more significant after adjusting for diurnal cortisol levels or lifetime cannabis use, while CAR showed no effect. HPA-axis dysfunction was proven significantly associated to both childhood trauma (Carpenter et al., 2007.; Lupien et al., 2009) and psychosis (Borges et al., 2013). In contrast, in our sample we did not observed an association between salivary cortisol levels and CT or lifetime occurrence of positive symptomatology. Interestingly, we found that sexual

unwanted experiences were significantly associated to higher CAR, in line with previous findings (Mondelli et al., 2010; Weissbecker et al., 2006). Trauma spectrum disorders (i.e. psychosis and PTSD) have frequently shown an association with an altered regulation of the HPA axis (Frodl and O'Keane, 2013; Schalinski et al., 2015). As we recruited healthy subjects from the general population, thus without any mental disorder related to trauma, our finding suggests the role of environmental programming for the HPA axis and hints that environmental stressors, such as sexual unwanted experiences, can be significantly related to the neuroendocrine phenotype.

In contrast with previous studies (Houston et al., 2011; Moore et al., 2007; Rössler et al., 2011; Tomassi et al., 2017) we also found that lifetime cannabis use rates did not differ significantly between traumatized and non-traumatized subjects and that no association existed between lifetime cannabis use and lifetime frequency of positive symptoms. There are several explanations for this finding. Firstly, there is evidence that protective factors can modify the negative effects of early adverse life circumstances leading people to select functional environments (Werner, 2004, 1992). These factors can be attributed to the individual itself, as temperamental characteristics that easily elicit positive responses from caregivers and/or good communication and problem solving skills, or lie within the family (substitute caregivers providing positive role models) and in the community (teachers, caring neighbors elder mentors and peers) (Werner, 2004). A primary role has also been theorized for contextual and cultural factors (Ungar, 2013) and resilience has been defined as the capacity of both individuals and their environments to interact to overcome childhood adversities (Ungar and Liebenberg, 2011). Political processes, funding, and cultural norms contribute significantly to make it more or less likely a positive adaptation (Leadbeater et al., 2005). Secondly, we used a cross sectional, lifetime-framed investigation for cannabis use and PLEs, preventing any further exploration in terms of temporal association between cannabis consumption and symptoms. Moreover, prospective studies have previously determined that the risk of psychotic outcomes is specifically increased in those who use cannabis very frequently (Moore et al., 2007). We could not differentiate subjects that used

cannabis frequently, and thus more prone to experience symptoms, from those who just tried a few times or even once in their lives.

Finally, we found that maternal antipathy was significantly associated to lower lifetime frequency of negative symptoms and lower levels of distress in response to depressive symptoms. Once again, it is possible to postulate that subjects that endured mother hostility, whether surrounded by a supportive relational and social context, also coped with it in an adaptive way (Ungar, 2013; Ungar and Liebenberg, 2011). While the first association remained significant when adjusted for salivary cortisol levels and lifetime cannabis use, both considered potential confounders, the second lost significance when adjusted: however we did not find any significant difference regarding cortisol levels or lifetime cannabis use between subjects who reported a history of mother antipathy and those who did not. It is possible to assume that dysfunctional parenting styles and their consequences, in terms of both offspring's vulnerability and resilience, maybe mainly influenced by psychological (e.g. attachment styles, coping strategies, temperamental characteristics,) and environmental (e.g. supportive teachers, religion and faith, peers' relationships) factors (Werner et el., 1992) rather than biological ones, like HPA axis functioning and cannabis use.

Our study has some important limitations. First, because of the study's explorative nature, due to the small sample size, findings should be considered as preliminary and should be replicated in larger samples. Second, the results do not survive multiple testing corrections, which may be due to the small sample size; so they should not be interpreted as hypothesis generating. Third, the cross-sectional study design precludes any claims of causality. Finally, retrospective reporting of abuse might increase the risk of recall bias, although the reliability of referring trauma has been previously demonstrated (Schäfer and Fisher, 2011): reports were consistent with other sources of information and stable over time.

The principal strengths of our study rely on the sound methodology applied at different levels (study design, subjects recruitments, and data collection and analysis) and on the use of internationally validated scales to assess subjects along with an accurate clinical evaluation by expert researchers with a clinical background (psychiatrists and psychologists).

Conclusions

Our explorative study suggests significant effects of childhood traumatic experiences in terms of both symptomatology frequency and related distress in a non-clinical sample. Varying degrees of stress sensitivity possibly mediate how childhood traumatic experiences affect the frequency of PLEs and their related distress. Well-designed, population-based, prospective longitudinal studies are desirable to disentangle this lifelong multi-factors (biological, psychological and environmental) bundle. Optimistically, studies should follow traumatized children, collecting information about potentially involved factors (such as cannabis or other drugs consumption), taking into account biological determinants (including genetics, epigenetics and proteomics) and explore resilience factors (individual's, family, and cultural). Even though traumatized children do not develop a clinically significant disorder in the majority of cases (Matheson et al., 2013), the association between CTE and mental disorders is well known and appear strong (Varese et al., 2012a). On one side, our study confirms a detrimental effect of trauma, even at non-clinical level. On the other side, our findings evoke a potential increased resilience in subjects with a history of CTE, therefore suggesting an alternative starting point for future research on childhood adversities, which should be more positively resilience-oriented.

4.3 CHILDHOOD TRAUMA, RECENT SEVERE LIFE EVENTS, AND BIOMARKERS RELATED TO GLUCOSE METABOLISM IN HEALTHY SUBJECTS.

There is strong evidence indicating that stressful experiences during critical periods of brain development, such as childhood, can cause long-term, persistent effects on behaviour, increasing the likelihood to negative health outcome. In details, childhood trauma, encompassing physical and sexual abuse, increases the vulnerability for mental disorders, including depression and psychosis (Li et al., 2016; Trotta et al., 2015) but also for several medical conditions, including cardiovascular diseases, type 2 diabetes and metabolic disorders (Huffhines et al., 2016, Miller et al., 2011).

The physiological response to stress involves the activation of multiple biological systems, comprising the endocrine, cardiovascular, metabolic, and immune systems; moreover, whereas the acute stress activate adaptive mechanisms, (McEwen, 2003) the long term exposure to stress is associated with long lasting physiological dysregulation, eventually leading to enhanced vulnerability to several kind of diseases (Juster et al., 2010).

Confirming the long-term effects of early life stress on the body, several studies (Danese et al., 2009; Dich et al., 2015) have linked childhood trauma to detrimental changes in physiological functions. For instance, it has been shown that victims of childhood sexual and physical abuse are more likely to be obese or to show three or even more symptoms of metabolic syndrome when compared with non-victims (Danese & Tan, 2014). Moreover, it was showed that exposure to maltreatment is associated with higher levels of several biomarkers related to metabolism, such as the inflammation marker C-reactive protein (hs-CRP) and clustering of metabolic markers, including high glycated hemoglobin, at age 32 (Danese et al., 2009). Moreover, Evidence suggests that subjects who reported an exposure of childhood trauma had an increased risk of the 32% to develop later in life type 2 diabetes (Huang et al., 2015) and of the 20-50% to develop obesity (Thomas et al., 2008).

One of the possible biological mechanisms underlying the association between childhood trauma exposure and the later development of metabolic disorders is

represented by glucose metabolism (Deppermann et al., 2014). In fact, childhood trauma acting as chronic and severe stress could induce the production of elevated levels of glucose and insulin in blood. Moreover, the hypothalamic-pituitary-adrenal (HPA) axis and inflammatory systems, which are both influenced by childhood trauma, interact with other stress mediators, including hormones involved in the glucose metabolism, like insulin, glucagon, secretin, gastric inhibitory polypeptide (GIP) and glucagone-like peptide 1 (GLP-1) (Nussdorfer et al., 2000) to further regulate HPA axis homeostasis. For example, insulin physiologically exerts an inhibitory action on HPA axis tone (Takao et al., 2000), while glucagon, secretin, GLP-1 and GIP enhance its function, stimulating the hypothalamus-pituitary CRH/ACTH release (Nussdorfer et al., 2000). However, only one study (Anderson et al, 2018) analysed the association between childhood trauma and biomarkers plasma levels related to glucose metabolism, specifically insulin, and found no association with physical and sexual abuse. In this context, up to now, as compared to the plenty of the literature proving a role of childhood trauma in enhancing the individual vulnerability to develop a wide range of illnesses, only a few studies have investigated the impact of recent severe stressful life events on health outcomes in the adulthood. Nonetheless, although stressful life events in adulthood have been associated with enhanced vulnerability to develop metabolic disorders (Rutters et al., 2015), type 2 diabetes (Maksimovic et al., 2014), the underlying biological mechanisms are still poor investigated.

Moreover, although it is not known whether a different timing in stress exposure could exert differential effects and play different roles in the future development of metabolic related dysfunctions, the underlying biological processes involved have not been identified yet.

Based on this, in the present pilot study, we aimed to investigate the effect of stress occurred in different periods of time (childhood and adulthood) on a panel of biomarkers related to glucose metabolism. In detail, we measured the plasma levels of Insulin, C-peptide, GIP, PAI-1, Visfatin, GLP-1, Ghrelin, Glucagon, Leptin, and Resistin in a group of subjects characterized for exposure to childhood trauma or to severe stressful life events occurred recently in life. We also explore whether BMI

and smoking may mediate the association between stress exposures and alterations in the levels of these biomarkers related to glucose metabolism.

Methods

Participants and Clinical Assessments

Healthy subjects, aged between 19 and 54 years, were recruited through notices posted at the Verona University Hospital, Verona (Italy). Individuals presenting a history of any physical or psychiatric diseases, prior traumatic brain injury, or intellectual disability (IQ<70) were excluded from the study. Additionally, being pregnant or breastfeeding represented exclusion criteria. The absence of any psychiatric disorder was ascertained via two schedules: the Mini International Neuropsychiatric Interview (M.I.N.I. Plus, Sheehan et al., 1998), to exclude any psychiatric disorder in Axis I; and the Structured Clinical Interview for DSM disorders (SCID-II, Spitzer et al., 1992, 1993) to exclude any psychiatric disorder in Axis II. Moreover, depressive symptoms were assessed by the Hamilton Rating Scale for Depression (HAMD, Hamilton, 1960), and symptoms of mania by the Bech-Rafaelsen Mania Rating Scale (BRMRS, Bech et al., 1978). Subjects were asked regarding present physical illness including tobacco use. Weight was measured in Kg, wearing minimal clothing, with a floor scale; height was measured in m, without shoes and using a measuring tape. Then, BMI was calculated as body weight (Kg)/square of their height (m2). Written informed consent was obtained by participants after receiving a complete description of the study, which has been approved by the Ethic Committee of the Verona University Hospital (Prog. N. 1995 approved on 31/08/2011; Prog. N. 1816 approved on 29/05/2013).

The presence of childhood trauma was assessed by the CECA-Q scale (Bifulco et al., 2005). It elicits information concerning experiences of childhood adversity before age 17, including physical and sexual abuse, separation from at least one of the parental figures longer than 6 months, and death of a parent. Based on previous literature (Tomassi et al., 2017, Bifulco et al., 2005), the severity of each maltreatment variable was dichotomized into severe and non-severe categories. Physical abuse was defined as repeated exposure to physical violence from parental

figures. To be considered “severe”, these incidents had to meet at least two of the following criteria: the abuse consisted of being hit with a belt or stick or being punched or kicked; the abuse resulted in an injury, including broken limbs, black eyes or bruising; the perpetrator was considered to be out of control. Sexual abuse was considered among unwanted sexual experiences. It was defined as “severe” if at least two of the following criteria were met: the individual knew the perpetrator; the perpetrator was a relative; the perpetrator lived in the same household; the unwanted sexual experience occurred more than once; the perpetrator touched child’s genitals; the perpetrator forced the child to touch his/her genitals; the abuse involved intercourse. Separation was defined as a detachment from at least one of the parental figures longer than 6 months; whereas, loss was determined whether one or both parents died during subjects’ childhood. Childhood trauma was defined as having experienced at least one among severe physical abuse, severe sexual abuse and loss/separation. The presence of stressful life events (SLEs) was collected using a modified version of the Life Events Scale (Paykel et al., 1971), which lists 56 commonly encountered life stressors. Subjects were asked to indicate and date events that had occurred in the 6 months preceding the assessment. Based on a previous work (Lopizzo et al., 2017; Ira et al., 2014), only "severe life events" (i.e. death of a family member, sexual or physical abuse, being accused of having committed a crime, sentence of imprisonment, being exposed to war or natural catastrophes, family breakdown, being removed from home, sentimental breakdown, severe physical illness) were taken into account.

Finally, the variable “childhood trauma and severe life events” was calculated for subjects who had experienced both childhood trauma and severe life events in the last 6 months.

Metabolism biomarkers plasma levels

Fasting blood samples were collected in the morning from each subject involved in the study. The tubes were kept at room temperature for 2 hours, then processed for plasma separation by centrifugation (1.620 x g for 15min). Plasma samples were then stored at -80 °C until they were processed for biochemical analyses. The concentrations of C-peptide, Ghrelin, GIP, GLP-1, Glucagon, Insulin, Leptin, PAI-

1, Resistin and Visfatin were determined using Bio-PlexPro™ Human Diabetes Assays on a Bio-Plex 200 System array reader (Bio-Rad, CA, USA), following the manufacturer's instructions. Each sample was diluted 1:4, by using a kit sample diluent, and incubated in a pre-wet filter plate with biotinylated detection antibody. Each captured analyte was detected by the addition of streptavidin–phycoerythrin and quantified using the BioPlex array reader. Standard curves were generated using the reference standard specimens supplied by the manufacturer. The different analytes concentrations were calculated using the Bio-Plex Manager software. All the measurements were performed in duplicates and all the samples with different clinical features and negative control samples were run together in the same plates.

Statistical analysis

Continuous variables were described by using the means (standard deviations) and categorical variables were expressed as n (%). Normality of biomarkers plasma levels was confirmed by Kolmogorov-Smirnov tests. In case of non-normal distributions, a secondary data analysis was performed after applying Box-Cox transformations.

Univariate linear regression models were estimated to test the association of each trauma exposure [childhood trauma, physical abuse, severe physical abuse, sexual abuse, severe sexual abuse, loss/separation, severe stressful events (last 6 months), childhood trauma and severe stressful life events (last 6 months)] with each of the metabolism related biomarkers. Multivariate linear regression analyses were performed to test these associations, adjusting for sex and age. Separate mediation effect of BMI and smoking on the association of trauma exposure with each biomarker was tested by a series of linear regression models after controlling for the aforementioned confounders. Although several design frameworks for mediation effect have been proposed to date, the classic work by Baron and Kenny (1986) is still the most prevalent approach. Mediation is demonstrated when the following steps are met: (1) the main independent variable (i.e. trauma) is significantly associated with the main dependent variable (i.e. biomarker). This step establishes that there is an overall direct effect that may be mediated; (2) the independent variable (i.e. trauma) is significantly related with the mediating

variable (i.e. BMI and smoking); (3) the mediating variable (i.e. BMI and smoking) is significantly associated with the dependent variable (i.e. biomarker) when the independent variable (i.e. trauma) is controlled for. Note that it is not sufficient just to regress the mediator only on the biomarker; the mediator and the biomarker can be related because they are both caused by the trauma/life events. So, the trauma/life events must be controlled in establishing the effect of the mediator on the biomarker. Combined mediation analysis through a structural equation model including all biomarkers has not been conducted due to the fact that the number of parameters to be estimated was too high with respect to the available sample size. The procedure was repeated after applying the Box-Cox transformation (Fox, 1997) to the biomarkers plasma levels in order to check if conclusions change. All p values were two-tailed with an accepted significance level of 0.05. No multiple testing correction was applied due to the exploratory nature of the study. Analyses were performed by SPSS 22.0 for Windows.

Results

Clinical and biological characteristics of the sample

We approached 91 subjects: overall, 72 (79.1%) people were included in the study. Of the other 19 subjects, 7 (36.8%) were excluded because under antidepressant treatment and 12 (63.2%) because affected by physical chronic diseases. The sample characteristics and the presence of childhood trauma and severe life events in the last 6 months are shown in Table 22.

In details, 22.2% of participants reported childhood trauma and 29.2% reported severe stressful life events in the last 6 months. By considering childhood trauma and severe stressful life events in the last 6 months, 8.3% declared to have experienced both events.

Table 22: Socio-demographics, life style characteristics, childhood trauma and stressful life events (last 6 months) (n=72)

Variable	Mean (sd) or N (%)
Age (years)	37.9 (12.4)
Gender	
Male	33 (45.8%)
Race	
Caucasian	72 (100.0%)
Nationality	
Italian	72 (100.0%)
Tobacco Use	
Yes	25 (34.7%)
Body Mass Index (BMI)	24.3 (4.3)
Underweight (<18.5)	2 (2.8%)
Normal (18.5-24.9)	39 (54.2%)
Overweight (25.0-29.9)	24 (33.3%)
Obesity (Class I 30.0-34.9)	7 (9.7%)
Obesity (Class II 35.0-39.9)	0 (0.0%)
Obesity (Class III >40.0)	0 (0.0%)
Bech-Rafaelsen Mania Rating Scale (BRMRS)	
No mania (≤6)	72 (100.0%)
Hamilton Rating Scale for Depression (HAM-D)	
Absence of depression (≤7)	72 (100.0%)
Organic Disease	
No disease	72 (100.0%)
Medication Use	
No medication	72 (100.0%)
Adversity	
Childhood Trauma*	
Yes	16 (22.2%)
Physical Abuse	
Yes	14 (19.4%)
Severe Physical Abuse	
Yes	7 (9.7%)
Sexual Abuse	
Yes	5 (6.9%)
Severe Sexual Abuse	
Yes	3 (4.2%)
Loss/Separation	
Yes	10 (13.9%)
Severe Life Events° (last 6 months)	
Yes	21 (29.2%)
Childhood Trauma and Severe Life Events (last 6 months)	
Yes	6 (8.3%)

* at least one among loss/separation, severe physical abuse and/or severe sexual abuse

° death of a family member, sexual or physical abuse, being accused of having committed a crime, sentence of imprisonment, being exposed to war or natural catastrophes, family breakdown, being removed from home, sentimental breakdown, severe physical illness

Effects of childhood trauma and/or severe stressful life events on metabolism biomarkers

First, we wanted to investigate any effect of childhood trauma and/or severe recent stressful life events on the panel of biomarkers that we measured. Univariate linear regression models showed that stressful life events are influencing only the levels of GIP; we also found that specific childhood trauma exposures and childhood trauma plus severe recent stressful life events were associated with the levels of Insulin, Visfatin, GIP, PAI-1, C-Peptide and GLP1 (see Table 23), thus suggesting that there was an overall direct effect that could be mediated (first step for mediation). Only Ghrelin, Glucagon, Leptin and Resistin levels were not found significantly associated with trauma (data not showed). Also, when we took into account gender and age as cofounders, multivariate linear regression analyses confirmed the associations found between exposure to trauma and the above-mentioned biomarkers (data not showed).

Mediation of BMI and smoking on the association between childhood trauma and/or severe stressful life events on metabolism biomarkers

Subsequently, in order to evaluate possible mediation effects of BMI and smoking on the association between childhood trauma and/or severe stressful life events on metabolism biomarkers, multivariate linear regression models were performed and each biomarker was used as the dependent variable. When found significantly associated in the univariate models, we considered age, gender, BMI and smoking as independent variables (GIP and GLP1 were associated with only trauma exposure, so multivariate models were not estimated) (see Table 24).

Table 23: Univariate linear regression models for metabolism biomarkers (n=72)

Independent variables[#]	**β Coefficient (p-value; adj-R^2%)**					
TRAUMA EXPOSURE	**Insulin**	**Visfatin**	**GIP**	**PAI-1**	**C-Peptide**	**GLP1**
Childhood Trauma (Ref. Yes)	0.067 (0.578; 0.4%)	0.149 (0.224; 0.8%)	0.065 (0.585; 0.4%)	0.079 (0.511; 0.6%)	0.106 (0.374; 1.1%)	0.069 (0.567; 0.0%)
Physical Abuse (Ref. Yes)	**0.290 (0.013; 7.1%)**	**0.408 (0.001; 15.4%)**	**0.276 (0.019; 6.3%)**	0.097 (0.415; 1.0%)	**0.367 (0.002; 12.3%)**	**0.237 (0.045; 4.3%)**
Severe Physical Abuse (Ref. Yes)	0.098 (0.411; 1.0%)	**0.266 (0.029; 5.7%)**	0.193 (0.104; 2.4%)	-0.18 (0.878; 0.0%)	0.229 (0.053; 3.9%)	0.226 (0.056; 0.1%)
Sexual Abuse (Ref. Yes)	0.101 (0.398; 1.0%)	-0.044 (0.723; 0.2%)	0.001 (0.997; 0.0%)	-0.064 (0.594; 0.4%)	0.195 (0.100; 2.4%)	-0.035 (0.769; 0.0%)
Severe Sexual Abuse (Ref. Yes)	0.085 (0.480; 0.7%)	-0.049 (0.690; 0.2%)	0.050 (0.675; 0.3%)	-0.083 (0.486; 0.7%)	**0.278 (0.018; 6.4%)**	0.029 (0.806; 0.0%)
Loss/Separation (Ref. Yes)	0.044 (0.714; 0.2%)	-0.047 (0.703; 0.2%)	-0.096 (0.424; 0.9%)	0.100 (0.405; 1.0%)	-0.046 (0.703; 0.2%)	-0.114 (0.339; 0.0%)
Severe Life Events (last 6 months) (Ref. Yes)	0.078	0.034	**0.259**	0.094	0.119	0.103

	(0.517; 0.6%)	(0.781; 0.1%)	**(0.028; 5.4%)**	(0.432; 0.9%)	(0.321; 1.4%)	(0.388; 0.0%)
Childhood Trauma and Severe Life Events (last 6 months) (Ref. Yes)	**0.334** **(0.004; 9.9%)**	**0.239** **(0.049; 4.3%)**	**0.265** **(0.024; 5.7%)**	**0.325** **(0.005; 9.3%)**	**0.363** **(0.002; 11.9%)**	0.084 (0.481; 0.0%)
CONFOUNDERS						
Age (years)	0.151 (0.206; 0.9%)	**0.243** **(0.046; 4.5%)**	0.190 (0.110; 2.2%)	0.189 (0.112; 2.2%)	0.142 (0.234; 0.6%)	0.067 (0.575; 0.0%)
Sex (Ref. Male)	0.038 (0.753; 0.1%)	0.026 (0.836; 0.1%)	-0.025 (0.834; 0.1%)	**0.260** **(0.028; 5.4%)**	0.012 (0.922; 0.0%)	-0.026 (0.830; 0.0%)
MEDIATORS						
Tobacco Use (Ref. Yes)	0.055 (0.646; 0.3%)	0.070 (0.572; 0.5%)	-0.007 (0.950; 0.5%)	-0.003 (0.977; 0.0%)	0.100 (0.402; 1.0%)	-0.003 (0.980; 0.0%)
BMI	**0.432** **(0.000; 17.5%)**	0.211 (0.086; 3.0%)	0.018 (0.881; 0.0%)	**0.435** **(0.000; 17.7%)**	**0.310** **(0.009; 8.3%)**	0.089 (0.461; 0.0%)

[#] No significant association for Ghrelin, Glucagon, Leptin and Resistin

Table 24: Multivariate linear regression models for metabolism biomarkers (only biomarkers significantly associated with at least one trauma exposure variable and one confounder and/or mediator) (n=72)

	β Coefficient (p-value; adj-R^2%)								
Independent variables[#]	**Insulin**		**C-Peptide**			**Visfatin**			**PAI-1**
Physical Abuse (Ref. Yes)	0.196 (0.079; 6.9%)	-	**0.313 (0.007; 12.3%)**	-	-	**0.381 (0.001; 15.4%)**	-	-	-
Severe Physical Abuse (Ref. Yes)	-	-	-	-	-	-	0.230 (0.058; 5.7%)	-	-
Severe Sexual Abuse (Ref. Yes)	-	-	-	**0.279 (0.014; 3.9%)**	-	-	-	-	-
Severe Life Events (last 6 months) (Ref. Yes)	-	-	-	-	-	-	-	0.212 (0.078; 4.3%)	-
Childhood Trauma and Severe Life Events (last 6 months) (Ref. Yes)	-	**0.279 (0.010; 9.8%)**	-	-	**0.328 (0.004; 12.0%)**	-	-	-	**0.267 (0.011; 9.2%)**
CONFOUNDERS									

Age (years)	-	-	-	-	-	0.186 (0.101; 2.2%)	0.202 (0.094; 2.6%)	0.216 (0.073; 3.3%)	-
Sex (Ref. Male)	-	-	-	-	-	-	-	-	**0.214** **(0.041; 5.7%)**
MEDIATORS									
BMI	**0.386** **(0.001; 13.1%)**	**0.395** **(0.000; 14.4%)**	**0.237** **(0.039; 4.2%)**	**0.311** **(0.008; 6.7%)**	**0.266** **(0.018; 5.8%)**	-	-	-	**0.371** **(0.001; 12.6%)**

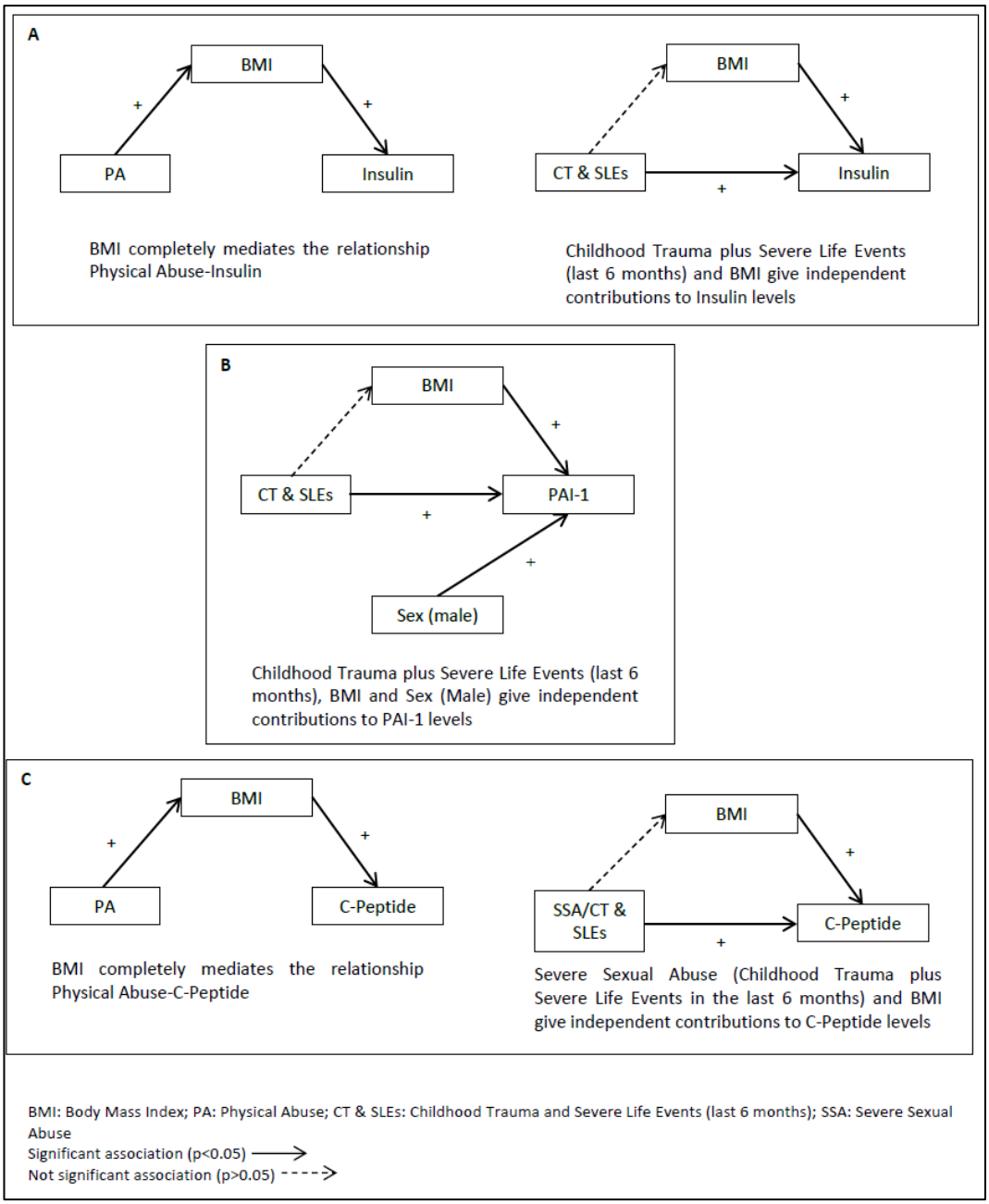
[#] Independent variables: only those ones which were found significantly associated in the univariate models

We found that participants who showed higher Insulin levels were those who: (1) experienced at least one childhood trauma and one severe life event in the last 6 months; (2) had higher BMI and these variables accounted for 24.2% of the variance. Subjects who showed higher PAI-1 levels were those who: (1) reported at least one childhood trauma and one severe life event in the last 6 months; (2) were males; (3) had higher BMI with an overall effect of the 27.5% of the variance.

Subjects who experienced higher C-Peptide levels were those who: (1) declared childhood physical abuse or severe sexual abuse or at least one childhood trauma and one severe life event in the last 6 months; (2) had higher BMI. Overall, these models accounted for 16.5%, 10.6% and 17.8% of the variance, respectively. Finally, For Visfatin, participants who experienced higher levels were those who declared physical abuse during childhood with an effect that accounted the 15.4% of the variance.

The different pathways that could explain the effect of trauma on Insulin, PAI-1 and C -Peptide were explored by mediation models (see the path diagrams of Figure 15). BMI was found significantly associated with only physical abuse (β=0.235, p=0.049), while severe sexual abuse (β=-0.004, p=0.972) and childhood trauma plus severe life events (β=0.135, p=0.261) were not. Smoking was not associated with trauma exposures (data not showed).

Figure 15: Mediation models: A) Insulin; B) PAI-1; C) C-Peptide (Kenny approach) (n=72) (only models involving childhood trauma and/or severe stressful life events in the last 6 months)



When we considered Insulin (diagram paths A), we found that BMI completely mediated the association with physical abuse during childhood, while childhood trauma plus severe life events in the last 6 months and BMI gave independent contributions. For PAI-1 (path diagram B), childhood trauma plus severe life events in the last 6 months, gender (being male) and BMI gave independent contributions. Finally, when we considered C-Peptide (path diagrams C), we found that BMI completely mediated the association with physical abuse, while severe sexual abuse or childhood trauma plus severe life events in the last 6 months and BMI independently contributed to C-Peptide levels.

The distribution of biomarkers showed a departure from normality for all of them (Kolmogorov-Smirnov test), with the exception of C-Peptide ($p=0.071$), Glucagon ($p=0.200$) and Resistin ($p=0.095$), thus, a secondary data analysis was performed after applying Box-Cox transformations, however, the results and the significance remained unchanged (data not showed).

Discussion

This is the first study reporting that stress occurring in different periods of life can exert distinct effects on markers related to metabolism. In particular, here we explored, in a pilot study, the impact of childhood trauma and/or recent severe life events on several biomarkers, related to glucose metabolism, measured in plasma samples of 72 healthy subjects drawn from the Italian general population. Specifically, we have tried to dissect the pathways that could explain the effect of childhood trauma and/or recent severe life events on a panel of biomarkers related to glucose metabolism (Insulin, C-peptide, GIP, PAI-1, Visfatin, GLP-1, Ghrelin, Glucagon, Leptin, and Resistin) by exploring indirect effects given by specific mediators (sex, age, BMI and tobacco).

We found that subjects that have been exposed to childhood physical abuse or childhood trauma in addition to experience recent severe life events showed higher plasma levels of Insulin. Moreover, we found that BMI completely mediates the association between insulin levels and physical abuse, while childhood trauma plus recent severe life events and BMI gave independent contributions.

It is well known that stress influences our body functions and several evidences have indeed showed that subjects exposed to stress early in life, such as childhood trauma, are more vulnerable to develop obesity in adulthood (Harding et al., 2014; Block et al., 2009) as well metabolic dysfunctions such as insulin-resistance and diabetes (Huffhines et al., 2016). In line with this, we found that BMI completely mediates the association between physical abuse and insulin levels, suggesting that physical abuse can cause an increase in BMI, which later can, in turn, entail a rise in insulin levels. Our finding is also in line with previous works that showed a significant association between physical abuse and obesity (Rehkopf et al., 2016; Danese & Tan, 2014). This effect may occur through the stimulation of different biological mechanisms and among them, there is the activation, due to the stress, of the neuroendocrine and inflammatory pathways, which are known to directly increase fat accumulation (Davis et al., 2014), promote visceral adiposity (Wardle et al., 2011) and the release of appetite hormones with increased food consumption (Bjorntorp, 2001). Conversely, we found that childhood trauma plus recent severe life events and BMI give independent contributions to insulin levels, indicating that the association between childhood trauma and at least one recent severe life events and higher levels of insulin is, in our sample, independent from the BMI. The occurrence of stressful life events later in life could represent a sort of "second hit", which may lead to an even more dysfunctional response by the stress response system. Insulin has been proven to exert an inhibitory activity on HPA axis (Takao et al., 2000), thus, increased levels of insulin, observed in association with childhood trauma and severe life events, could then imply an attempt to reduce HPA activity, denoting a compensatory, albeit dysfunctional, mechanism.

We also found that physical abuse was associated with higher levels of C-Peptide, but BMI completely mediates this association; conversely, severe sexual abuse or childhood trauma plus recent severe life events and BMI independently contribute to C-Peptide levels. C-peptide is a biologically active peptide, stored with insulin in the secretory vesicles until stimulation of the pancreatic β-cells by elevations in extracellular glucose concentration. C-peptide does not appear to directly alter glucose metabolism (Wahren & Larsson, 2015) even although there is evidence that C-peptide and insulin-initiated signalling cascades interact each other; thus, this

interaction is important for the normal functioning of both peptides, particularly in erythrocytes (Richards et al., 2013). No physiological or pathophysiological situation exists in which cells in vivo are exposed to C-peptide in the absence of insulin, and thus the precise role of C-peptide in physiological condition is difficult to ascertain. C-peptide likely exerts anti-inflammatory (Haidet et al., 2012) and vasodilatory (Wallerath et al., 2003) effects in the vascular endothelium through both direct actions on the endothelial cells and indirectly through interaction with erythrocytes and immune cells. As suggested (Yosten and Kolar, 2015), C-peptide plays a physiologically relevant role in the tuning of insulin signaling and in endothelial physiology, that could account for the microvascular dysfunction observed in diabetes. To date there are no evidence reporting altered C-peptide levels in adult subjects exposed to childhood trauma, although there are data in an animal model of stress early in life, the prenatal stress model (PNS). In particular, higher levels of C-peptide were observed in adult animals exposed to PNS, although this increase in C-peptide was not accompanied by alterations in caloric intake, body weight gain or fat mass (Balasubramanian et al., 2015). Our findings are partially in line with these preclinical data (Balasubramanian et al., 2015). Indeed, in our sample, the association between physical abuse and C-peptide is completely mediated by BMI, whereas the association between severe sexual abuse or childhood trauma plus severe life events and C-peptide is BMI-independent. This could suggest different and specific roles for physical and severe sexual abuse on the C-peptide levels. Results concerning C-peptide basically overlap with those in reference to insulin. Given the strong link between C-peptide and insulin plasma levels, the formers could be seen as a consequence of the latter. It stands then possible the "second hit" hypothesis with reference to the association between childhood trauma plus severe life events and C-peptide levels.

In our sample, we also found that childhood trauma plus recent severe life events, gender (being male) and BMI give independent contributions to PAI-1 levels. PAI-1 prevents plasmin generation and is considered a primary inhibitor of fibrinolysis, playing a primary role in balancing coagulation and fibrinolysis (Savoy et al., 2017). PAI-1 represents also an important contributor to obesity and it has been found associated with the Metabolic Syndrome in several studies (Kraja et al., 2007;

Hanley et al., 2004). Our result of the contribution of BMI to PAI-1 levels is in line with the fact that PAI-1 is lowered by weight loss and drugs that improve insulin sensitivity (Kursawe & Santoro, 2014). Moreover, PAI deficient mice were found resistant to diet-induced obesity, explained by increased energy expenditure (Liang et al, 2006). Furthermore, PAI-1 has been proven potentially implicated in emotion, stress appraisal and response (Savoy et al., 2017). PAI-1 gene expression is mediated by pro-inflammatory cytokines, such as TNF-a and IL-1, and both PAI-1 expression and protein synthesis are increased by acute systemic inflammation (Ekström et al., 2012). In turn, systemic PAI-1 may cross the brain-blood barrier and, once in the medial and central amygdala, can regulate stress-related neural modelling (Pawlak et al., 2003). Because this brain region strongly projects onto the hypothalamus, PAI-1 potentially play a role in regulating the physiological response to external stress via the HPA axis. These findings could suggest a bridge between the childhood trauma/life events-induced inflammation and elevated PAI-1 concentration.

The present study has several strengths. First, reliable, internationally validated instruments and adopted conservative cut-off points, previously applied, to identify also the most severe forms of abuse were used. Second, since childhood maltreatment is an important predictor of depression over the life-course (Nanni et al., 2011) and depression is prospectively associated with obesity (Luppino et al, 2010), we have recruited subjects without a positive psychiatric history and without current diagnosis of depression, to exclude a factor mediating the effect of childhood maltreatment on obesity.

Some limitations also need to be acknowledged in our study. First, although our is the largest sample of healthy subjects in which the relationship between childhood trauma, severe stressful life events and metabolic markers has been investigated, the number of subjects with childhood trauma history was relatively small in size, and thus our findings need to be replicated in larger samples. Second, a substantial number of tests were performed, which means that any Bonferroni correction for multiple testing would be large, thus any significant p-value should be regarded as nominal. Third, as for the majority of the studies on childhood trauma, abuse data rely on retrospective reporting. This limitation has been mitigated by the use of a

standardized interview as CECA-Q which has good reliability and validity both in general (Smith et al., 2002) and clinical populations (Bifulco et al., 2005). Fourth, although we selected a group of healthy subjects with negative physical and psychiatric history, none of these participants had evidence of metabolic disease other than obesity and none is under any systemic disease or medication use, we do not have information about their diet style. Moreover, blood samples were not taken to verify the presence/absence of diabetes by an oral glucose tolerance test (OGTT) according to criteria from the American Diabetes Association. Finally, there are the possibility that the association between childhood trauma and/or recent severe stressful life events with plasma levels of glucose related biomarkers could be mediated not only by BMI and smoking, but also by other factors, such as psychosocial elements including low socio-economic levels (Dich et al, 2015).

Conclusions

In conclusion, our results suggest that a history of childhood trauma and/or recent severe stressful life events increase the plasma levels of a panel of biomarkers related to glucose metabolism, and this could underlie the possible enhanced risk to develop poorer health outcomes. These findings suggest that children with childhood trauma history and/or adult with recent severe stressful life events may benefit from specific interventions. For example, the stability and permanency in safe, in some cases also foster, care place could help maltreated children in developing secure attachment and recovering from the psychological effects of abuse. In these interventions, attention should be paid also to the metabolic effects of the abuse, thus, teaching them a correct life style and dietary regime in order to prevent the later development of metabolic disorders. Up to now, only few studies (Isasi et al., 2015, Davis et al., 2014, Sinha et al., 2013) have investigated possible differential effects exerted on obesity and metabolism by stressful events occurring more recent in life as compared to events occurred during childhood. More recent in life and short-lasting stress exposure, may not entail gain of weight, while early traumatic experiences, especially if occurring for a prolonged amount of time during development, may lead to the activation of biological and behavioural patterns that lead to obesity and other pathologies related to metabolism (Isasi et

al., 2015). Our study provides important insights on the extent to which effects of childhood traumatic experiences persist over time influencing important markers associated with physical health three decades later.

CONCLUSION

Findings of this PhD thesis confirmed the importance of early life experiences in influencing adult health outcomes.

On one side, they corroborated the detrimental effect of childhood traumatic experiences. To begin with, First Episode Psychosis patients exposed to childhood trauma appeared to constitute a distinctive subgroup characterized by diverse features in terms of nosology and drug use. Our study has elucidated, albeit partially, to what extent the presence of childhood traumatic experiences can affect early psychosis features. Findings therefore provide some important hints for specific therapeutic and/or preventive interventions, which might carry within themselves an enhanced impact on illness course, outcomes, and prognosis. Moreover, our results suggest a significant association between childhood traumatic experiences, stressful life events, and metabolic abnormalities in the first phase of psychosis. Childhood trauma appears to represent an additional risk factor for the development of metabolic disorders in patients affected by psychosis. It is therefore recommended to conduct an accurate and meticulous medical history, which would allow settling an early and intensive program of food and nutrition education, and would guide the choice of medication.

Second, childhood traumatic experiences were found significantly associated in Hepatitis C patients with greater emotional distress and higher levels of depression, anxiety, and fatigue. It appears then of primary importance to systematically investigate the presence of a history of childhood traumatic events in these patients. It could help the clinician to identify, along with the routine medical caring, the most appropriate therapeutic interventions, which can be both pharmacological and psychosocial, to offer to the patient (Larkin & Read, 2008). Third, early adverse experiences were found associated, even in a non-clinical sample made by healthy adult subjects, with higher lifetime frequency of positive psychotic-like experiences. Finally, a significant difference was found between traumatized/stressed and non-exposed healthy subjects in terms of glucose metabolism. These findings suggested that children with childhood trauma history

and/or adult with recent severe stressful life events could benefit from specific interventions regarding general health. In these interventions, attention should be paid also to the metabolic effects of the abuse, thus, teaching them a correct life style and dietary regime in order to prevent the later development of metabolic disorders.

Well-designed, population-based, prospective longitudinal studies are desirable to disentangle the lifelong multi-factors (biological, psychological, and environmental) bundle, which most likely mediates the association between childhood traumatic experiences and health outcomes. Optimistically, studies should follow traumatized children, in comparison with a group of control, collect information about potentially involved mediators, and explore risk and resilience factors for health outcomes.

On the other side, we found that healthy subjects with a history of maternal antipathy were less likely to experience negative symptoms lifetime and reported lower levels of distress in association to depressive symptomatology. I would like therefore to close my thesis, stressing the knowledge that, even though the negative impact of early adverse experiences on mental health is well-established (Varese et al., 2012a), traumatized children do not develop a clinically significant disorder in the majority of cases (Matheson et al., 2013). Our findings, evoking a potential increased resilience in subjects with a history of childhood traumatic experiences, suggest an alternative starting point for future research on childhood adversities, which should be more positively resilience-oriented.

REFERENCES

Aas M, Andreassen OA, Aminoff SR, et al. (2016). A history of childhood trauma is associated with slower improvement rates: Findings from a one-year follow-up study of patients with a first-episode psychosis. BMC Psychiatry; 16:126.

Aas M, Navari S, Gibbs A, et al. (2012). Is there a link between childhood trauma, cognition, and amygdala and hippocampus volume in first-episode psychosis? Schizophr Res; 137(1-3):73-79.

Adam EK & Kumar M. (2009). Assessing salivary cortisol in large-scale, epidemiological research. Psychoneuroendocrinology; 34(10):1423–36.

Alexander N, Wankerl M, Hennig J, et al. (2014). DNA methylation profiles within the serotonin transporter gene moderate the association of 5-HTTLPR and cortisol stress reactivity. Transl Psychiatry; 4: e443.

Allen AJ, Griss ME, Folley BS, et al. (2009). Endophenotypes in Schizophrenia: A selective review. Schizophr Res; 109(1-3):24-37.

Allen J, Balfour R, Bell R, et al. (2014). Social determinants of mental health. Int Rev Psychiatry; 26(4):392-407.

Allen JG, Coyne L, & Console DA. (1997). Dissociative detachment relates to psychotic symptoms and personality decompensation. Comprehensive Psychiatry; 38:327-334.

Alvarez MJ, Roura P, Osés A, et al. (2011). Prevalence and clinical impact of childhood trauma in patients with severe mental disorders. J Nerv Ment Dis; 199(3):156-61.

American Psychiatric Association. (2000). Diagnostic and Statistical Manual of Mental Health Disorders, 4th ed. (DSM-IV). Washington DC.

American Psychiatric Association. (1994). Diagnostic and Statistical Manual of Mental Disorder (DSM-IV). Washington DC.

Anderson EL, Fraser A, Caleyachetty R, et al. (2018). Associations of adversity in childhood and risk factors for cardiovascular disease in mid-adulthood. Child Abuse Negl; 76:138-148 (2018).

Arranz B, Rosel P, Ramírez N, et al. (2004). Insulin resistance and increased leptin concentrations in noncompliant schizophrenia patients but not in antipsychotic-naive first-episode schizophrenia patients. J Clin Psychiatry; 65(10):1335-42.

Babor TF, De La Fuente JR, Saunders J, et al. (1992). The alcohol use disorder identification test. Guidelines for use in primary health care. Geneva, Switzerland. World Health Organization WHO.

Balasubramanian P, Varde PA, Abdallah SL, et al. (2015) Differential effects of prenatal stress on metabolic programming in diet-induced obese and dietary-resistant rats. Am J Physiol Endocrinol Metab; 309(6): E582-8.

Banducci AN, Hoffman E, Lejuez CW, et al. (2014). The relationship between child abuse and negative outcomes among substance users: psychopathology, health, and comorbidities. Addict Behav; 39(10):1522-7.

Barbosa IG, Rocha NP, de Miranda AS, et al. (2012). Increased adipokines in bipolar disorder. J Psychiatr Res; 46:386–393.

Barkus EJ, Stirling J, Hopkins RS, et al. (2006). Cannabis-Induced Psychosis-Like Experiences Are Associated with High Schizotypy. Psychopathology; 39(4):175–8.

Baron RM & Kenny DA. (1986). The moderator-mediator variable distinction in social psychological research: conceptual, strategic, and statistical considerations. J. Personal. Soc. Psychol; 51:1173–1182.

Bartholomew K & Horowitz LM. (1991). Attachment styles among young adults: a test of a four-category model. Journal of Personality and Social Psychology; 61:226–244.

Baumeister D, Akhtar R, Ciufolini S, et al. (2016). Childhood trauma and adulthood inflammation: a meta-analysis of peripheral C-reactive protein, interleukin-6 and tumour necrosis factor-α. Mol Psychiatry; 21(5):642–649.

Beach SR, Brody GH, Todorov AA, et al. (2010). Methylation at SLC6A4 is linked to family history of child abuse: an examination of the Iowa Adoptee sample. Am J Med Genet B Neuropsychiatr Genet; 153(2):710-713.

Beach SR, Brody GH, Todorov AA, et al. (2011). Methylation at 5HTT mediates the impact of child sex abuse on women's antisocial behavior: an examination of the Iowa adoptee sample. Psychosom Med; 73(1):83-87.

Beards S, Gayer-Anderson C, Borges S, et al. (2013) Life events and Psychosis: A review and meta-analysis. Schizophr Bull; 39:740-747.

Beauchaine TP. (2009). The role of biomarkers and endophenotypes in prevention and treatment of psychopathological disorders. Biomarkers in Medicine; 3:1–3.

Bebbington PE, Bhugra D, Brugha T, et al. (2004) Psychosis, victimisation and childhood disadvantage: evidence from the second British National Survey of Psychiatric Morbidity. Br J Psychiatry; 185:220-6.

Bech P, Rafaelsen OJ, Kramp P, et al. (1978) The Mania Rating Scale: scale construction and inter-observer agreement. Neuropharmacology; 6:420–431

Bentall RP, Wickham S, Shevlin M, et al. (2012). Do specific early-life adversities lead to specific symptoms of psychosis? A study from the 2007 the adult psychiatric morbidity survey. Schizophrenia Bulletin; 38(4):734–740.

Bentall RP & Fernyhough C. (2008). Social predictors of psychotic experiences: Specificity and psychological mechanisms. Schizophrenia Bulletin; 34:1012–1020.

Bernstein DP, Stein JA, Newcomb MD, et al. (2003). Development and validation of a brief screening version of the Childhood Trauma Questionnaire. Child Abuse Negl; 27(2): 169-190.

Bernstein DP & Fink L. (1998): Childhood Trauma Questionnaire: a Retrospective Self-report Manual, San Antonio, TX: The Psychological Corporation.

Bernstein DP, Fink L, Handelsman L, et al. (1994). Initial reliability and validity of a new retrospective measure of child abuse and neglect. Am J Psychiatry; 151(8):1132-6.

Bertani M, Lasalvia A, Bonetto C, et al. (2012). The influence of gender on clinical and social characteristics of patients at psychosis onset: A report from the Psychosis Incident Cohort Outcome Study (PICOS). Psychol Med; 42(4): 769-80.

Beshai S, Dobson KS, Bockting CLH, et al. (2011). Relapse and recurrence prevention in depression: current research and future prospects. Clinical Psychology Review; 31(8):1349-60.

Bick J, Naumova O, Hunter S, et al. (2012). Childhood adversity and DNA methylation of genes involved in the hypothalamus-pituitary-adrenal axis and immune system: whole-genome and candidate-gene associations. Dev Psychopathol; 24(4):1417-1425.

Bifulco A, Bernazzani O, Moran PM, et al. (2005). The Childhood Experience of Care and Abuse Questionnaire (CECA.Q) - Validation in a community series. Br J Clin Psychol; 44(4):1-20.

Bifulco A, Moran PM, Baines R, Bunn A, Stanford K. (2002). Exploring psychological abuse in childhood: II. Association with other abuse and adult clinical depression. Bull Menninger Clin; 66:241–58.

Binder EB. (2009). The role of FKBP5, a co-chaperone of the glucocorticoid receptor in the pathogenesis and therapy of affective and anxiety disorders. Psychoneuroendocrinology; 34(suppl. 1): S186-S195.

Björntorp P. (2001). Heart and soul: stress and the metabolic syndrome. Scand Cardiovasc J; 35(3):172-7.

Block JP, He Y, Zaslavsky AM, et al. (2009). Psychosocial stress and change in weight among US adults. Am J Epidemiol; 170(2):181-192.

Bocchio-Chiavetto L, Zanardini R, Tosato S, et al. (2018). Immune and metabolic alterations in first episode psychosis (FEP) patients. BrainBehav Immun; 70:315-24.

Bonoldi I, Simeone E, Rocchetti M, et al. (2013). Prevalence of self-reported childhood abuse in psychosis: a meta-analysis of retrospective studies. Psychiatry Res; 210:8-15.

Borges S, Gayer-Anderson C, & Mondelli V. (2013). A systematic review of the activity of the hypothalamic-pituitary-adrenal axis in first episode psychosis. Psychoneuroendocrinology; 38(5):603–611.

Bowlby J. (1973). Separation: Anxiety and Anger. Hogarth Press, London.

Bremner JD, Vermetten E, & Mazure CM. (2000). Development and preliminary psychometric properties of an instrument for the measurement of childhood trauma: the Early Trauma Inventory. Depress Anxiety; 12(1):1-12.

Brown GW, Sklair F, Harris TO, et al. (1973). Life-events and psychiatric disorders. 1. Some methodological issues. Psychol Med; 3(1):74-87.

Bywaters P, Bunting L, Davidson G, et al. (2016). JFR Joseph Rowntree Foundation - The relationship between poverty, child abuse and neglect: an evidence review. Report. www.jrf.org.uk

Buck T. (2014). International Child Law (Routledge) page 89.

Buckman JEJ, Underwood A, Clarke K, et al. (2018). Risk factors for relapse and recurrence of depression in adults and how they operate: A four-phase systematic review and meta-synthesis. Clin Psychol Rev; 64:13-38

Campbell J, Assanand S, & Di Paula A. (2003). The structure of the self-concept and its relation to psychological adjustment. Journal of Personality; 71:115-140.

Campbell J, Trapnell P, Heine S, et al. (1996). Self-concept clarity: Measurement, personality correlates, and cultural boundaries. Journal of Personality and Social Psychology; 70:141-156.

Campion J, Bhugra D, Bailey S, et al. (2013). Inequality and mental disorders: opportunities for action. Lancet; 382(9888):183 – 184.

Cannon M, Jones PB, & Murray RM. (2002). Obstetric complications and schizophrenia: historical and meta-analytic review. Am J Psychiatry; 159:1080–1092.

Cannon TD & Keller MC. (2006). Endophenotypes in the genetic analyses of mental disorders. Annu Rv Clin Psychol; 2:267-90.

Cantor-Graae E & Selten JP. (2005). Schizophrenia and migration: a meta-analysis and review. Am J Psychiatry; 162:12–24.

Capuron L. & Miller A.H. (2011). Immune system to brain signaling: neuropsychopharmacological implications. Pharmacology & therapeutics; 130:226–38.

Capuron L, Gumnick JF, Musselman DL, et al. (2002a). Neurobehavioral effects of interferon-alpha in cancer patients: phenomenology and paroxetine responsiveness of symptom dimensions. Neuropsychopharmacology; 26(5):643-52.

Capuron L, Ravaud A, Neveu PJ, et al. (2002b). Association between decreased serum tryptophan concentrations and depressive symptoms in cancer patients undergoing cytokine therapy. Molecular Psychiatry; 7:468−473.

Carpenter LL, Carvalho JP, Tyrka AR, et al. (2007). Decreased ACTH and Cortisol Responses to Stress in Healthy Adults Reporting Significant Childhood Maltreatment. Biol Psychiatry; 62(10): 1080-7.

Carvalho LA, Garner BA, Dew T, et al. (2010). Antidepressants, but not antipsychotics, modulate GR function in human whole blood: an insight into molecular mechanisms. Eur Neuropsychopharmacol; 20(6):379–387.

Carver SC, Scheier MF, & Weintraub. (1989). Assessing coping strategies: a theoretically based approach. Journal of Personality and Social Psychology; 56:267-283.

Cecil CA, Smith RG, Walton E, et al. (2016). Epigenetic signatures of childhood abuse and neglect: Implications for psychiatric vulnerability. J Psychiatr Res; 83:184-194.

Chalder T, Berelowitz G, Pawlikowska T, et al. (1993). Development of a fatigue scale. J Psychosom Res; 37(2):147-53.

Chang YH, Chang DM, Lin KC, et al. (2011). Visfatin in overweight/obesity, type 2 diabetes mellitus, insulin resistance, metabolic syndrome and cardiovascular diseases: a meta-analysis and systemic review. Diabetes Metab Res Rev; 27(6):515-27.

Chapman DP, Whitfield CL, Felitti VJ, et al. (2004). Adverse childhood experiences and the risk of depressive disorders in adulthood. J Affect Disord; 82:217–25.

Charmandari E, Tsigos C, & Chrousos G. (2005). Endocrinology of the stress response. Annu Rev Physiol; 67:259-284.

Chen S, Broqueres-You D, Yang G, et al. (2013). Relationship between insulin resistance, dyslipidaemia and positive symptom in Chinese antipsychotic-naive first-episode patients with schizophrenia. Psychiatry Res; 210(3):825-9.

Chong AC, Vogt MC, Hill AS, et al. (2014). Central insulin signaling modulates hypothalamus-pituitary-adrenal axis responsiveness. Mol Metab; 4(2):83-92.

Chu JA & Dill DL. (1990). Dissociative symptoms in relation to childhood physical and sexual abuse. American Journal of Psychiatry;147:887–892.

Chuang JC & Jones PA. (2007). Epigenetics and microRNAs. Pediatr Res; 61:24R-29R.

CISMAI-Terre des Hommes. (2015). Maltrattamento sui bambini: quante le vittime in Italia? Prima Indagine nazionale quali–quantitativa sul maltrattamento a danno di bambini.

Cohen S, Kamarck T, & Mermelstein. (1983). A global measure of perceived stress. Journal of Health and Social Behavior; 24 (4): 385–396.

Cole JD & Kazarian SS. (1988). The Level of Expressed Emotion Scale: a new measure of expressed emotion. J Clin Psychol; 44(3):392-7.

Collip D, Myin-Germeys I, & Van Os J. (2008). Does the concept of "sensitization" provide a plausible mechanism for the putative link between the environment and schizophrenia? Schizophr Bull; 34(2):220-5.

Colombo L, Sartori G, & Brivio C. (2002) Stima del Quoziente Intellettivo tramite l'applicazione del TIB (Test Breve di Intelligenza) Giornale Italiano di Psicologia; 29:613–637.

Conradi HJ, Da Jonge P. & Ormel J. (2008). Prediction of the three-year course of recurrent depression in primary care patients: different risk factors for different outcomes. Journal of Affective Disorders; 105(1):267-71.

Conus P, Cotton S, Schimmelmann BG, et al. (2010). Pretreatment and outcome correlates of past sexual and physical trauma in 118 bipolar I disorder patients with a first episode of psychotic mania. Bipolar Disord; 12(3): 244-52.

Danese A & Tan M. (2014). Childhood maltreatment and obesity: systematic review and meta-analysis. Mol Psychiatry; 19(5):544-54.

Danese A, Moffitt TE, Harrington H, et al. (2009). Adverse childhood experiences and adult risk factors for age-related disease: depression, inflammation, and clustering of metabolic risk markers. Arch Pediatr Adolesc Med; 163(12):1135–1143.

Danese A, Moffitt TE, Pariante CM, et al. (2008). Elevated inflammation levels in depressed adults with a history of childhood maltreatment. Arch Gen Psychiatry; 65(4):409–415.

Danese A, Pariante CM, Caspi A, et al. (2007). Childhood maltreatment predicts adult inflammation in a lifecourse study. Proc Natl Acad Sci USA; 104 (4):1319–1324.

Dantzer R, O'Connor JC, Freund GG, et al. (2008). From inflammation to sickness and depression: when the immune system subjugates the brain. Nat Rev Neuroscience; 9:46−56.

Daruy-Filho L, Brietzke E, Lafer B, et al. (2011). Childhood maltreatment and clinical outcomes of bipolar disorder. Acta Psychiatr Scand; 124: 427-34.

David AS, Buchanan A, Reed A, et al. (1992). The assessment of insight in psychosis. Br J Psychiatry; 161:599–602.

Davis CR, Dearing E, Usher N, et al. (2014). Detailed assessments of childhood adversity enhance prediction of central obesity independent of gender, race, adult psychosocial risk and health behaviors. Metabolism; 63(2):199-206.

De Franceschi L, Fattovich G, Turrini F, et al. (2000). Hemolytic anemia induced by ribavirin therapy in patients with chronic hepatitis C virus infection: role of membrane oxidative damage. Hepatology; 31: 997-1004

De Hert M, Correll CU, Bobes J, et al S. (2011). Physical illness in patients with severe mental disorders. I. Prevalence, impact of medications and disparities in health care. World Psychiatry; 10:52–77.

Deppermann S, Storchak H, Fallgatter AJ, et al. (2014). Stress-induced neuroplasticity: (mal)adaptation to adverse life events in patients with PTSD--a critical overview. Neuroscience; 283:166-177.

Di Nicola M, Cattaneo A, Hepgul N, et al. (2013). Serum and gene expression profile of cytokines in first-episode psychosis. Brain Behav Immun; 31: 90-95.

Dich N, Hansen ÅM, Avlund K, et al. (2015). Early life adversity potentiates the effects of later life stress on cumulative physiological dysregulation. Anxiety Stress Coping; 28(4): 372-90.

Dickens C, McGowan L, Clark-Carter D, et al. (2002). Depression in rheumatoid arthritis: a systematic review of the literature with meta-analysis. Psychosomatic Medicine; 64:52-60.

Dinwiddie SH, Shicker L, & Newman T. (2003). Prevalence of hepatitis C among psychiatric patients in the public sector. American Journal of Psychiatry; 160: 172–174.

D'Mello C, Le T, & Swain T. (2009).cerebral microglia recruit monocytes into the brain in response to tumor necrosis factor alpha singaling during peripheral organ inflammation. J Neurosci; 29(7):2089-102.

DMS 5, APA 2013

Douglas KR, Chan G, Gelernter J, et al. (2010). Adverse childhood events as risk factors for substance dependence: Partial mediation by mood and anxiety disorders. Addict Behav; 35(1): 7−13.

Dowlati Y, Herrmann N, Swardfager W, et al. (2010). A meta-analysis of cytokines in major depression. Biol Psychiatry; 67(5):446-57.

Dube SR, Williamson DF, Thompson T, Felitti VJ, Anda RF. (2004). Assessing the reliability of retrospective reports of adverse childhood experiences among adult HMO members attending a primary care clinic. Child Abuse Negl; 28:729–37.

Duhig M, Patterson S, Connell M, et al. (2015). The prevalence and correlates of childhood trauma in patients with early psychosis. Aust N Z J Psychiatry; 49(7): 651-9.

Duman EA & Canli T. (2015). Influence of life stress, 5-HTTLPR genotype, and SLC6A4 methylation on gene expression and stress response in healthy Caucasian males. Biol Mood Anxiety Disord; 5: 2.

Duman RS & Monteggia LM. (2006). A neurotrophic model for stress-related mood disorders. Biol Psychiatry; 59:1116−1127.

Dvir Y, Denietolis B, & Frazier JA. (2013). Childhood Trauma and Psychosis. Child and Adolescent Psychiatric Clinics of North America; 22(4):629–641.

Ek M, Wicks S, Svensson AC, et al. (2015). Advancing paternal age and schizophrenia: the impact of delayed fatherhood. Schizophr Bull; 41(3):708-14.

Ekström M, Liska J, Eriksson P, et al (2012). Stimulated in vivo synthesis of plasminogen activator inhibitor-1 in human adipose tissue. Thromb Haemost; 108(3):485-492.

El Serag HB, Kunik M, Richardson P, et al. (2002). Psychiatric disorders among veterans with hepatitis C infection. Gastroenterology; 123:476–482.

El-Mesallamy HO, Kassem DH, El-Demerdash E, et al. (2011). Vaspin and visfatin/Nampt are interesting interrelated adipokines playing a role in the pathogenesis of type 2 diabetes mellitus. Metabolism; 60(1):63-70.

Enns MW, Cox BJ, & Clara I. (2002). Parental bonding and adult psychopathology: results from the US National Comorbidity Survey. Psychological Medicine; 32:997-1008.

Essex MJ, Shirtcliff EA, Burk LR, et al. (2011). Influence of early life stress on later hypothalamic-pituitary-adrenal axis functioning and its covariation with mental health symptoms: a study of the allostatic process from childhood into adolescence. Dev Psychopathol; 23(4):1039-58

Etain B, Mathieu F, Henry C, et al. (2010) Preferential association between childhood emotional abuse and bipolar disorder. J Trauma Stress. Jun;23(3):376-83.

Fábregas BC, Vitorino FD, Rocha DM, et al. (2012). Screening inventories to detect depression in chronic hepatitis C patients. General Hospital Psychiatry. 34(1): 40–45

Fagiolini A, Frank E, Turkin S, et al. (2008) Metabolic syndrome in patients with bipolar disorder. J Clin Psychiatry. Apr;69(4):678-9.

Fagiolini A & Goracci A. (2009). The effects of undertreated chronic medical illnesses in patients with severe mental disorders. J Clin Psychiatry. 70 Suppl 3:22-9.

Faravelli V & Ambonetti A. Assessment of life events in depressive disorders. A comparison of three methods. Soc Psychiatry; 18(2): 51-6.

Fergusson DM, Horwood LJ, Lynskey MT. (1996). Childhood sexual abuse and psychiatric disorder in young adulthood: II. Psychiatric outcomes of childhood sexual abuse. J Am Acad Child Adolesc Psychiatry; 35:1365–74.

Fergusson DM, McLeod GF, Horwood LJ. (2013) Childhood sexual abuse and adult developmental outcomes: findings from a 30-year longitudinal study in New Zealand. Child Abuse Negl; 37(9):664-74.

First MB, Gibbon M, Spitzer RL, et al. (1997). Structured Clinical Interview for DSM-IV Axis II Personality Disorders, (SCID-II). Washington, D.C.: American Psychiatric Press, Inc.

Fisher HL & Craig T. (2008). Childhood adversity and psychosis. Society and Psychosis (ed. C Morgan, K McKenzie and P Fearon). Cambridge University Press: 95–111.

Fisher HL, Craig TK, Fearon P, et al. (2011). Reliability and comparability of psychosis patients' retrospective reports of childhood abuse. Schizophr Bull; 37(3): 546-53.

Fisher HL, Jones PB, Fearon P, et al. (2010). The varying impact of type, timing and frequency of exposure to childhood adversity on its association with adult psychotic disorder. Psychol Med; 40(12):1967-78.

Fisher HL, McGuffin P, Boydell J, et al. (2014) Interplay between childhood physical abuse and familial risk in the onset of psychotic disorders. Schizophr Bull; 40(6): 1443-51.

Fisher HL, Morgan C, Dazzan P, et al. (2009). Gender differences in the association between childhood abuse and psychosis. Br J Psychiatry; 194(4): 319-25.

Foerster A, Lewis S, Owen M, et al. (1991) Pre-morbid adjustment and personality in psychosis: effects of sex and diagnosis. Br J Psychiatry;158:171–176.

Fontana RJ, Hussain KB, Schwartz SM, et al. (2002). Emotional distress in chronic hepatitis C patients not receiving antiviral therapy. J Hepatol; 36:401-407.

Forst T, Rave K, Pfuetzner A, et al. (2002) Effect of C-peptide on glucose metabolism in patients with type 1 diabetes. Diabetes Care; 25(6):1096-7.

Fox J. (1997). Applied Regression, Linear Models, and Related Methods. Sage Publications, Inc., Thousand Oaks, CA, US.

Friebe D, Neef M, Kratzsch J, et al. (2011) Leucocytes are a major source of circulating nicotinamide phosphoribosyltransferase (NAMPT)/pre-B cell colony (PBEF)/visfatin linking obesity and inflammation in humans. Diabetologia; 54(5):1200-11.

Frodl T & O'Keane V. (2013). How does the brain deal with cumulative stress? A review with focus on developmental stress, HPA axis function and hippocampal structure in humans. Neurobiology of Disease; 52: 24–37.

Fujigaki H, Saito K, Fujigaki S, et al. (2006). The signal transducer and activator of transcription 1alpha and interferon regulatory factor 1 are not essential for the induction of indoleamine 2,3-dioxygenase by lipopolysaccharide: involvement of p38 mitogen-activated protein kinase and nuclear factor-kappaB pathways, and synergistic effect of several proinflammatory cytokines. J Biochem; 139:655−662.

Fusté M, Pinacho R, Meléndez-Pérez I, et al. (2013). Reduced expression of SP1 and SP4 transcription factors in peripheral blood mononuclear cells in first-episode psychosis. J Psychiatr Res; 47(11): 1608-1614.

Gamble SA & Roberts JE. (2005). Adolescents' perceptions of primary caregivers and cognitive style: the roles of attachment security and gender. Cognitive Therapy and Research; 29:123–141.

Garety PA, Bebbington P, Fowler D, et al. (2007). Implications for neurobiological research of cognitive models of psychosis: a theoretical paper. Psychol Med; 37(10):1377-91.

Garety PA, Kuipers E, Fowler D, et al. (2001). A cognitive model of positive symptoms of psychosis. Psychological Medicine; 31:189–195.

Garno JL, Goldberg JF, Ramirez PM, et al. (2005). Impact of childhood abuse on the clinical course of bipolar disorder. Br J Psychiatry; 186:121–125

Garten A, Schuster S, Penke M, et al. (2015) Physiological and pathophysiological roles of NAMPT and NAD metabolism. Nat Rev Endocrinol; 11(9):535-46.

Gaudiano BA & Zimmerman M. (2010). The relationship between childhood trauma history and the psychotic subtype of major depression. ActaPsychiatrScand; 121: 462–70.

Gershon ES & Goldin LR. (1986). Clinical methods in psychiatric genetics, I: robustness of genetic marker investigative strategies. Acta Psychiatr Scand; 74 (2): 113–118.

Gershuny B, Najavits L, Wood P, et al. (2004). Relation between trauma and psychopathology: Mediating roles of dissociation and fears about death and control. Journal of Trauma & Dissociation; 5:101-117.

Giebink AW, Vogel PA, Medawala W, et al. (2013) C-peptide-stimulated nitric oxide production in a cultured pulmonary artery endothelium is erythrocyte mediated and requires Zn(2+). Diabetes Metab Res Rev; 29(1):44-52.

Gilbert R, Widom C, Browne K, et al. (2009). Burden and consequences of child maltreatment in high-income countries. Lancet; 373(9657): 68-81.

Goldberg D & Williams P. (1988) A user's guide to the General Health Questionnaire. NFER-Nelson, Windsor.

Gottesman I & Gould T. (2003). "The Endophenotype Concept in Psychiatry: Etymology and Strategic Intentions". The American Journal of Psychiatry; 160 (4): 636–45.

Gouvea ES, Ota VK, Noto C, et al. (2016). Gene expression alterations related to mania and psychosis in peripheral blood of patients with a first episode of psychosis. Transl Psychiatry; 6(10):e908.

Gracie A, Freeman D, Green S, et al. (2007). The association between traumatic experience, paranoia and hallucinations: a test of the predictions of psychological models. Acta Psychiatr Scand; 116(4):280-9.

Green MJ, Girshkin L, Teroganova N, et al. (2014) Stress, Schizophrenia and Bipolar Disorder. Curr Topics Behav Neurosci. 18: 217-35.

Guest PC, Schwarz E, Krishnamurthy D, et al. (2011). Altered levels of circulationg insulin and other neuroendocrine hormones associated with the onset of schizophrenia. Psychoneuroendocrinology; 36(7):1092-6.

Guest PC, Wang L, Harris LW, et al. (2010). Increased levels of circulationg insulin-related peptides in first onset, antipsychotic naïve schizophrenia patients. Mol Psychiatry; 15(2): 118-9.

Guillemin C, Provençal N, Suderman M, et al. (2014). DNA methylation signature of childhood chronic physical aggression in T cells of both men and women. PLoS One; 9(1): e86822.

Haidet J, Cifarelli V, Trucco M, et al. (2012). C-peptide reduces pro-inflammatory cytokine secretion in LPS-stimulated U937 monocytes in condition of hyperglycemia. Inflamm Res; 61(1):27-35.

Hamilton M. (1960). A rating scale for depression. J Neurol Neurosurg Psychiatry. 23:56–62.

Hammersley P, Dias A, Todd G, et al (2003). Childhood trauma and hallucinations in bipolar affective disorder: preliminary investigation. Br J Psychiatry; 182: 543-7.

Hanley AJ, Festa A, D'Agostion RB Jr, et al. (2004). Metabolic and inflammation variable clusters and prediction of type 2 diabetes: factor analysis using directly measured insulin sensitivity. Diabetes; 53: 1773–1781.

Hardeveld F, Spijker J, De Graaf R, et al. (2010). Prevalence and predictors of recurrence of major depressive disorder in the adult population. Acta Psychiatrica Scandinavica; 122(3):184-91.

Harding JL, Backholer K, Williams ED, et al. (2014). Psychosocial stress is positively associated with body mass index gain over 5 years: evidence from the longitudinal AusDiab study. Obesity; 22(1): 277-286.

Hardt J & Rutter M. (2004). Validity of adult retrospective reports of adverse childhood experiences: review of the evidence. J Child Psychol Psychiatry; 45(2): 260-73.

Hart BL. (1988). Biological basis of the behaviour of sick animals. Neurosci Biobehav Rev; 12:123−137.

Haug E, Øie M, Andreassen OA, et al. (2015). Anomalous self-experience and childhood trauma in first-episode schizophrenia. Compr Psychiatry; 56: 35-41.

Haung E, Øie M, Andreassen OA, et al. (2015) Anomalous self-experience and childhood trauma in first-episode schizophrenia. Compr Psychiatry; 56: 35-41.

Hazan C & Shaver P. (1987). Romantic love conceptualized as an attachment process. Journal of Personality and Social Psychology; 52: 511–524.

Heatherton TF, Kozlowski LT, Freker RC, et al. (1991). The Fagerstrom test for nicotine dependence: a revision of the Fagerstrom tolerance questionnaire. Br J Addict; 86(9):1119-27.

Heim C & Binder EB. (2012). Current research trends in early life stress and depression: review of human studies on sensitive periods, gene-environment interactions, and epigenetics. Exp Neurol;233:102–11.

Heim C & Nemeroff CB. (2001). The role of childhood trauma in the neurobiology of mood and anxiety disorders: preclinical and clinical studies. Biol Psychiatry; 49: 1023–1039.

Heim C, Newport DJ, Heit S, et al. (2000). Pituitary-adrenal and autonomic responses to stress in women after sexual and physical abuse in childhood. JAMA; 284: 592–7.

Henquet C, Krabbendam L, Spauwen J, et al (2005). Prospective cohort study of cannabis use, predisposition for psychosis, and psychotic symptoms in young people. BMJ; 330 (7481):11.

Horne R, Weinman J, & Hankins M. (1999). The beliefs about medicines questionnaire: the development and evaluation of a new method for assessing the cognitive representation of medication. Psychology and Health 14(1):1-24.

Houston JE, Murphy J, Shevlin M, et al. (2011). Cannabis use and psychosis: re-visiting the role of childhood trauma. Psychol Med; 41(11): 2339-48.

Houtepen LC, Vinkers CH, Carrillo-Roa T, et al. (2016). Genome-wide DNA methylation levels and altered cortisol stress reactivity following childhood trauma in humans. Nat Commun; 7: 10967.

Howard LM. (1993). Allegations of abuse in psychotic patients. Am J Psychiatry; 150:839–840.

Huang H, Yan P, & Shan Z. (2015). Adverse childhood experiences and risk of type 2 diabetes: a systematic review and meta-analysis. Metabolism Clinical and Experimental; 64: 1408-1418.

Huffhines L, Noser A, & Patton SR. (2016). The Link Between Adverse Childhood Experiences and Diabetes. Curr Diab Rep; 16(6): 54.

Ira E, De Santi K, Lasalvia A, et al. (2014). Positive symptoms in first-episode psychosis patients experiencing low maternal care and stressful life events: a pilot study to explore the role of the COMT gene. Stress; 17(5): 410-415.

Isasi CR, Parrinello CM, Jung MM, et al. (2015). Psychosocial stress is associated with obesity and diet quality in Hispanic/Latino adults. Ann Epidemiol; 25(2): 84-89.

Ittermann T, Volzke H, Baumeister SE, et al. (2015). Diagnosed thyroid disorders are associated with depression and anxiety. Soc. Psy Epidemiol; 50(9): 1417-25.

Jablensky A, Sartorius N, Ernberg G, et al. (1992). Schizophrenia: manifestations, incidence and course in different cultures. A World Health Organization ten-country study. Psychol Med Monogr Suppl; 20:1–97.

Janusek LW, Tell D, Gaylord-Harden N, et al. (2016). Relationship of childhood adversity and neighborhood violence to a proinflammatory phenotype in emerging adult African American men: An epigenetic link. Brain Behav Immun; 60: 126-135.

Jorm AF, Henderson AS. (1992). Memory bias in depression: implications for risk factor studies relying on self-reports of exposure. Int J Methods Psychiatr Res; 2:312–38.

Juster RP, McEwen BS, & Lupien SJ. (2010). Allostatic load biomarkers of chronic stress and impact on health and cognition. Neurosci Biobehav Rev; 35(1), 2-16.

Kaptoge S, Seshasai SR, Gao P, et al. (2014) Inflammatory cytokines and risk of coronary heart disease: new prospective study and updated meta-analysis. Eur Heart J; 35(9):578-89.

Kaufman J, Plotsky PM, Nemeroff CB, et al. (2000). Effects of early adverse experiences on brain structure and function: Clinical implications. Biol Psychiatry; 48(8): 778-90.

Kay SR, Fiszbein A, & Opler LA. (1987). The Positive and Negative Syndrome Scale for schizophrenia. Schizophr Bull; 13:261–276.

Kelly BD, O'Callaghan E, Waddington JL, et al. (2010) .Schizophrenia and the city: a review of literature and prospective study of psychosis and urbanicity in Ireland. Schizophr. Res; 116: 75–89

Kendler KS, Kuhn JW, & Prescott CA. (2004). Childhood sexual abuse, stressful life events and risk for major depression in women. Psychol Med; 34:1475–82.

Kessler RC, Chiu WT, Demler O, et al. (2010). Prevalence, Severity, and Comorbidity of Twelve-month DSM-IV Disorders in the National Comorbidity Survey Replication (NCS-R).

Khandaker GM, Zimbron J, Lewis G, et al. (2013). Prenatal maternal infection, neurodevelopment and adult schizophrenia: a systematic review of population based studies. Psychol Med; 43(2):239-57.

Khulan B, Manning JR, Dunbar DR, et al. (2014). Epigenomic profiling of men exposed to early-life stress reveals DNA methylation differences in association with current mental state. Transl Psychiatry; 4: e448.

Kilcommons AM & Morrison AP. (2005). Relationships between trauma and psychosis: an exploration of cognitive and dissociative factors. Acta Psychiatrica Scandinavica; 112: 351-359.

Kim HS, Han SY, Sung HY, et al. (2014) Blockade of visfatin induction by oleanolic acid via disturbing IL-6-TRAF6-NF-κB signaling of adipocytes. Exp Biol Med (Maywood); 239(3):284-92.

Kitagami T, Yamada K, Miura H, et al. (2003). Mechanism of systemically injected interferon-alpha impeding monoamine biosynthesis in rats: role of nitric oxide as a signal crossing the blood-brain barrier. Brain Res; 978:104−114.

Klaassens ER, van Noorden MS, Giltay EJ, et al. (2009). Effects of childhood trauma on HPA-axis reactivity in women free of lifetime psychopathology. Progress in Neuro-Psychopharmacology and Biological Psychiatry; 33(5): 889–894.

Klengel T, Mehta D, Anacker C, et al. (2013). Allele-specific FKBP5 DNA demethylation mediates gene-childhood trauma interactions. Nat Neurosci; 16(1): 33-41.

Konings M, Stefanis N, Kuepper R, et al. (2011). Replication in two independent population-based samples that childhood maltreatment and cannabis use synergistically impact on psychosis risk. Psychol Med; 42(1): 149–59.

Konsman JP, Parnet P, & Dantzer R. (2002). Cytokine-induced sickness behaviour: mechanisms and implications. Trends Neurosci; 25:154−159.

Korosi A, Naninck EF, Oomen CA, et al. (2012). Early-life stress mediated modulation of adult neurogenesis and behavior. Behav Brain Res; 227(2): 400-409.

Kozielewicz D & Halota W. (2012). Interferon-induced thyroiditis during treatment of chronic hepatitis C. Endokrynol Pol; 63(1):66-70.

Kraja AT, Province MA, Arnett D, et al. (2007). Do inflammation and procoagulation biomarkers contribute to the metabolic syndrome cluster? Nutr. Metab; 4:28.

Krishnadas R & Cavanagh J. (2012). Depression: an inflammatory illness? Journal of neurology, neurosurgery, and psychiatry; 83:495–502.

Kumsta R, Marzi SJ, Viana J, et al. (2016). Severe psychosocial deprivation in early childhood is associated with increased DNA methylation across a region spanning the transcription start site of CYP2E1. Transl Psychiatry; 6(6): e830.

Kursawe R & Santoro N. (2014). Metabolic syndrome in pediatrics. Adv Clin Chem; 65: 91-142.

Kuzman MR, Medved V, Terzic J, et al. (2009). Genome-wide expression analysis of peripheral blood identifies candidate biomarkers for schizophrenia. J Psychiatr Res; 43(13): 1073-1077.

Kwong-Ming Kee, Chuan-Mo Lee, & Jing-Houng Wang. (2006). Thyroid dysfunction in patients with chronic hepatitis C receiving a combined therapy of interferon and ribavirin: incidence, associated factors and prognosis. J of Gastroenterology and Hepatology; 21:319–326

Langner TS & Michael ST. (1963). Life Stress and Mental Health. London: Collier-Macmillan.

Larkin W & Read J. (2008). Childhood trauma and psychosis: Evidence, pathways, and implications. J Postgrad Med; 54:287-93

Lasalvia A, Tosato S, Brambilla P, et al. (2012). Psychosis Incident Cohort Outcome Study (PICOS). A multisite study of clinical, social and biological characteristics, patterns of care and predictors of outcome in first-episode psychosis. Background, methodology and overview of the patient sample. Epidemiol Psychiatr Sci; 21(3):281-303.

Lawrence D, Hancock KJ & Kisely S. (2013). The gap in life expectancy from preventable physical illness in psychiatric patients in Western Australia: retrospective analysis of population based registers BMJ; 346 :f25

Leadbeater B, Dodgen D, & Solarz A. (2005). The resilience revolution: A paradigm shift for research and policy. In: Peters, R.D., Leadbeater, B., McMahon, R.J., (Eds.), Resilience in children, families, and communities: Linking context to practice and policy. New York, NY: Kluwer. pp. 47-63.

Leboyer M, Soreca I, Scott J, et al. (2012). Can bipolar disorder be viewed as a multi-system inflammatory disease? J Affect Disord; 141(1):1-10.

Lee J, Goh LK, Chen G, et al. (2012). Analysis of blood-based gene expression signature in first-episode psychosis. Psychiatry Res; 200(1): 52-54.

Lencz T, Smith CW, Auther A, et al. (2004). Nonspecific and attenuated negative symptoms in patients at clinical high-risk for schizophrenia. Schizophrenia Research; 68(1), 37–48.

Lever-van Milligen BA, Vogelzangs N, Smit JH, et al. (2014). Hemoglobin levels in persons with depressive and/or anxiety disorders. J Psychosom Res; 76(4):317-21.

Levine ME, Cole SW, Weir DR, et al. (2015). Childhood and later life stressors and increased inflammatory gene expression at older ages. Soc Sci Med; 130: 16-22.

Lewis SW, Owen MJ, & Murray RM. (1989). Obstetric complications and schizophrenia: methodology and mechanisms. In: Schultz SC, Taminga CA, editors. Schizophrenia: A Scientific Focus. Oxford, UK: Oxford University Press; 1989. pp. 56–68.

Li M, D'Arcy C, & Meng X. (2016). Maltreatment in childhood substantially increases the risk of adult depression and anxiety in prospective cohort studies: systematic review, meta-analysis, and proportional attributable fractions. Psychol Med; 46(4), 717-730.

Liang X, Kanjanabuch T, Mao SL, et al. (2006). Plasminogen activator inhibitor-1 modulates adipocyte differentiation. Am J Physiol Endocrinol Metab; 290(1) :E103–E113.

Linee di indirizzo AISF – HCV, Dicembre 2014

Linscott RJ & Van Os J. (2013). An updated and conservative systematic review and meta-analysis of epidemiological evidence on psychotic experiences in children and adults: On the pathway from proneness to persistence to dimensional expression across mental disorders. Psychological Medicine; 43(6): 1133–1149.

Liu Y, Ho RC-M, & Mak A. (2012). Interleukin (IL)-6, tumour necrosis factor alpha (TNF-α) and soluble interleukin-2 receptors (sIL-2R) are elevated in patients with major depressive disorder: a meta-analysis and meta-regression. Journal of affective disorders; 139:230–9.

Lo Fermo S, Barone R, Patti F, et al. (2010). Outcome of psychiatric symptoms presenting at onset of multiple sclerosis: a retrospective study. Multiple sclerosis (Houndmills, Basingstoke, England); 16:742–8

Lopizzo N, Tosato S, Begni V, et al. (2017). Transcriptomic analyses and leukocyte telomere length measurement in subjects exposed to severe recent stressful life events. Transl Psychiatry; 7(2): e1042 (2017).

Lund C, Breen A, Flisher A, et al. (2010). Poverty and common mental disorders in low and middle income countries: A systematic review. Social Science and Medicine; 71:517 – 528.

Luo X & Kraus WL. (2012). On PAR with PARP: cellular stress signaling through poly(ADP-ribose) and PARP-1. Genes Dev; 26(5):417-32

Lupien SJ, McEwen BS, Gunnar MR, et al. (2009). Effects of stress throughout the lifespan on the brain, behaviour and cognition. Nature Reviews Neuroscience; 10(6):434–445.

Luppino FS, De Wit LM, Bouvy PF, et al. (2010). Overweight, obesity, and depression: a systematic review and meta-analysis of longitudinal studies. Arch Gen Psychiatry; 67(3):220-229.

Luthar SS, Sawyer JA, & Brown PJ (2006). Conceptual issues in studies of resilience: past, present, and future research. Ann N Y Acad Sci; 1094:105-15.

Lutz C & Ross S. (2003). Elaboration versus fragmentation: Distinguishing between self-complexity and self-concept differentiation. Journal of Social and Clinical Psychology; 22: 537-559.

MacLean KI & Littleton JM. (1977). Environmental stress as a factor in the response of rat brain catecholamine metabolism to delta8-tetrahydrocannabinol. Eur J Pharmacol; 41(2): 171–82.

Madruga CS, Laranjeira R, Caetano R, et al. (2011). Early life exposure to violence and substance misuse in adulthood – The first Brazilian national survey. Addict Behav; 36(3): 251-5.

Maksimovic JM, Vlajinac HD, Pejovic BD, et al. (2014). Stressful life events and type 2 diabetes. Acta Clin Belg; 69(4):273-276.

Mandelli L, Petrelli C, & Serretti A. (2015). The role of specific early trauma in adult depression: A meta-analysis of published literature. Childhood trauma and adult depression. Eur Psychiatry; 30(6):665-80.

Mansueto G & Faravelli C. (2017). Recent life events and psychosis: the role of childhood adversities. Psychiatry Res; 256:111-7.

Mariano A, Scalia Tomba G, Tosti ME, et al. (2009). Estimating the incidence, prevalence and clinical burden of hepatitis C over time in Italy. Scand J Infect Dis; 41:689-99.

Mariano A, Tomba G, Tosti ME, et al. (2006) Future burden of hepatitis C virus infection: the case of Italy. In: 41st Annual Meeting of the European Association for the Study of the Liver (EASL). Vienna, Austria, 26-30,

Matheson SL, Shepherd AM, Pinchbeck RM, et al. (2013). Childhood adversity in schizophrenia: A systematic meta-analysis. Psychological Medicine; 43(2):225–238.

May-Chahal C & Cawson P (2005). Measuring child maltreatment in the United Kingdom: a study of the prevalence of child abuse and neglect. Child Abuse and Neglect; 29:969–984.

Mazzoncini R, Zoli M, Tosato S, et al. (2009). Can the role of genetic factors in schizophrenia be enlightened by studies of candidate gene mutant mice behavior? World J Biol Psychiatry; 10:778-97.

McCrone P, Leese M, Thornicroft G, et al. (2000). Reliability of the Camberwell Assessment of Need – European Version. EPSILON Study 6. Br J Psychiatry; 177(Suppl 39):34–40.

McEwen BS. (2003). Early life influences on life-long patterns of behavior and health. Ment Retard Dev Disabil Res Rev; 9(3):149-54.

McGowan PO. (2013). Epigenomic Mechanisms of Early Adversity and HPA Dysfunction: Considerations for PTSD Research. Front Psychiatry; 4: 110.

McIntyre RS, Cha DS, Jerrell JM, et al.(2013). Obesity and mental illness: implications for cognitive functioning. Adv Ther; 30:577–588.

McManus S, Meltzer H, Brugha T, et al. (2007). Adult Psychiatric Morbidity in England, 2007. Leeds: NHS Information Centre for Health and Social Care.

McNally L, Bhagwagar Z, & Hannestad J. (2008). Inflammation, glutamate, and glia in depression: a literature review. CNS Spectr; 13:501−510.

Mechanic MB, Resick PA, Griffin MG. (1998). A comparison of normal forgetting, psychopathology, and information-processing models of reported amnesia for recent sexual trauma. J Consult Clin Psychol; 66:948–57.

Medeiros LP, Kayo M, Medeiros RB, et al. (2014) Interferon-induced depression in patients with hepatitis C: an epidemiologic study. Rev Assoc Med Bras; 60(1):35-9.

Melas PA, Rogdaki M, Osby U, et al. (2012). Epigenetic aberrations in leukocytes of patients with schizophrenia: association of global DNA methylation with antipsychotic drug treatment and disease onset. FASEB J; 26(6): 2712-2718.

Mellor AL & Munn DH. (1999). Tryptophan catabolism and T-cell tolerance: immunosuppression by starvation? Immunol Today; 20(10):469-73.

Melzer D, Fryers T, Jenkins R, et al. (2003). Social position and the common mental disorders with disability: Estimates from the National Psychiatric Survey of Great Britain. Social Psychiatry and Psychiatric Epidemiology; 38:238 – 243 .

Miller AH, Maletic V, & Raison CL. (2009). Inflammation and its discontents: the role of cytokines in the pathophysiology of major depression. Biol Psychiatry; 65:732−741.

Miller GE, Chen E, & Parker KJ. (2011).Psychological Stress in Childhood and Susceptibility to the Chronic Diseases of Aging: Moving Towards a Model of Behavioral and Biological Mechanisms. Psychol Bull; 137(6):959–997.

Minozzi S, Davoli M, Bargagli AM, et al. (2010) An overview of systematic reviews on cannabis and psychosis: discussing apparently conflicting results. Drug Alcohol Rev; 29:304–317.

Misiak B, Szmida E, Karpiński P, et al. (2015). Lower LINE-1 methylation in first-episode schizophrenia patients with the history of childhood trauma. Epigenomics; 7(8): 1275-1285.

Mitchell AJ, Vancampfort D, De Herdt A, et al. (2013a). Is the prevalence of metabolic syndrome and metabolic abnormalities increased in early schizophrenia? A comparative meta-analysis of first episode, untreated and treated patients. Schizophr Bull; 39(2):295-305

Mitchell AJ, Vancampfort D, Sweers K, et al. (2013b). Prevalence of metabolic syndrome and metabolic abnormalities in schizophrenia and related disorders--a systematic review and meta-analysis. Schizophr Bull; 39(2):306-18.

Mondelli V, Cattaneo A, Belvederi Murri M, et al. (2011). Stress and inflammation reduce brain-derived neurotrophic factor expression in first-episode psychosis: a pathway to smaller hippocampal volume. J Clin Psychiatry; 72(12): 1677-1684.

Mondelli V, Dazzan P, Hepgul N, et al. (2010). Abnormal cortisol levels during the day and cortisol awakening response in first-episode psychosis: the role of stress and of antipsychotic treatment. Schizophr Res; 116(2-3):234-42.

Moore THM, Zammit S, Lingford-Hughes A, et al. (2007). Cannabis use and risk of psychotic or affective mental health outcomes: a systematic review. Lancet; 370:319–328.

Morgan C & Fisher H. (2007). Environment and schizophrenia: environmental factors in schizophrenia: childhood trauma – a critical review. Schizoph Bull; 33: 3–10.

Mossakowski KN. (2014). Social Causation and Social Selection. In The Wiley Blackwell Encyclopedia of Health, Illness, Behavior, and Society (eds W. C. Cockerham, R. Dingwall and S. Quah).

Murali V & Oyebode F. (2004). Poverty, social inequality and mental health Advances in Psychiatric Treatment, vol. 10. http://apt.rcpsych.org/

Myin-Germeys I, Krabbendam L, Delespaul PA, et al. (2004). Sex differences in emotional reactivity to daily life stress in psychosis. J Clin Psychiatry; 65(6): 805-9;

Myin-Germeys I & Van Os J. (2007). Stress-reactivity in psychosis: evidence for an affective pathway to psychosis. Clin Psychol Rev; 27(4): 409-24.

Nanni V, Uher R, & Danese A. (2011). Childhood maltreatment predicts unfavorable course of illness and treatment outcome in depression: a meta-analysis. Am J Psychiatry; 169(2):141–51.

Naumova OY, Lee M, Koposov R, et al. (2012). Differential patterns of whole-genome DNA methylation in institutionalized children and children raised by their biological parents. Dev Psychopathol; 24(1):143-155.

Neria Y, Bromet EJ, & Sievers S. (2002). Trauma exposure and post---traumatic stress disorder in psychosis: findings from a first---admission cohort. Journal of Consulting and Clinical Psychology; 70:246–251.

Nishioka M, Bundo M, Koike S, et al. (2013). Comprehensive DNA methylation analysis of peripheral blood cells derived from patients with first-episode schizophrenia. J Hum Genet; 58(2): 91-97.

Noto C, Ota VK, Santoro ML, et al. (2016). Depression, Cytokine, and Cytokine by Treatment Interactions Modulate Gene Expression in Antipsychotic Naïve First Episode Psychosis. Mol Neurobiol; 53(8): 5701-5709.

Novo P, Landin-Romero R, Radua J, et al. (2014) Eye movement desensitization and reprocessing therapy in subsyndromal bipolar patients with a history of traumatic events: A randomized, controlled pilot-study. Psychiatry Res; 19:122-8.

NSPCC annual report (Jütte et al., 2014, pp. 10 and 13)

Nussdorfer GG, Bahçelioglu M, Neri G, et al. (2000). Secretin, glucagon, gastric inhibitory polypeptide, parathyroid hormone, and related peptides in the regulation of the hypothalamus- pituitary-adrenal axis. Peptides; 21(2):309-24.

Nyholm B, Walker M, Gravholt CH, et al. (1999) Twenty-four-hour insulin secretion rates, circulating concentrations of fuel substances and gut incretin hormones in healty offspring of type II diabetic parents: evidence of several aberrations. Diabetologia; 42:1314-1323.

Ota VK, Noto C, Gadelha A, et al. (2014). Changes in gene expression and methylation in the blood of patients with first-episode psychosis. Schizophr Res; 159(2-3): 358-364.

Ota VK, Noto C, Santoro ML, et al. (2015). Increased expression of NDEL1 and MBP genes in the peripheral blood of antipsychotic-naïve patients with first-episode psychosis. Eur Neuropsychopharmacol; 25(12): 2416-2425.

Olfson M, Gerhard T, & Huang C. (2015). Premature mortality among adults with schizophrenia in the United States. JAMA Psychiatry; 72(12):1172-81.

Owens T. (2003). Self and identity, in: Delamater, J. (Ed.), Handbook of Social Psychology. Kluwer Academic/Plenum Publishers, New York.

Pace TW, Hu F, & Miller AH. (2007). Cytokine-effects on glucocorticoid receptor function: relevance to glucocorticoid resistance and the pathophysiology and treatment of major depression. Brain Behav Immun; 21(1):9-19.

Padhy SK, Sarkar S, Davuluri T, ET AL. (2014). Urban living and psychosis – an overview. Asian J Psychiatr; 12:17-22.

Pariante CM & Miller AH. (2001). Glucocorticoid receptors in major depression: relevance to pathophysiology and treatment. Biol Psychiatry; 49:391−404.

Pariante CM & Lightman SL. (2008). The HPA axis in major depression: classical theories and new developments. Trends Neurosci; 31(9):464–468.

Parker G, Tupling H, & Brown LB. (1979). A Parental Bonding Instrument. British Journal of Medical Psychology; 52:1-10.

Parvanidou P & Chrousos GP. (2012). Metabolic consequences of stress during childhood and adolescence. Metabolism. 61(5):611-9.

Patel A. (2013). Review: the role of inflammation in depression. Psychiatr Danub; 25 Suppl 2:S216-23.

Patel V & Kleinman A. (2003). Poverty and common mental disorders in developing countries. Bulletin of the World Health Organization; 81:609 – 615.

Patel V, Lund C, Hatheril S, et al. (2010). Mental disorders: Equity and social determinants. In E. Blas & A.S. Kurup (Eds.) Equity, Social Determinants and Public Health Programmes (pp. 115 – 134). Geneva: World Health Organization.

Pavlová T, Novák J, & Bienertová-Vašků J. (2015). The role of visfatin (PBEF/Nampt) in pregnancy complications. J Reprod Immunol; 112:102-10

Pawlak R, Magarinos AM, Melchor J, et al. (2003). Tissue plasminogen activator in the amygdala is critical for stress-induced anxiety-like behavior. Nat. Neurosci; 6:168–174.

Paykel ES, Prusoff BA, & Uhlenhuth EH. (1971).Scaling of Life Events. Arch Gen Psvchiatry; 25:340-347.

Penner JD & Brown AS. (2007). Prenatal infectious and nutritional factors and risk of adult schizophrenia. Expert RV Neurother; 7(7):797-805.

Perona-Garcelán S, Carrascoso-López F, García-Montes JM, et al. (2012). Dissociative experiences as mediators between childhood trauma and auditory hallucinations. Journal of Traumatic Stress; 25:323–329.

Pervanidou P & Chrosusos GP. (2012). Metabolic consequences of stress during childhood and adolescence. Metabolism; 611-619.

Pfeifer S, Krabbendam L, Myin-Germeys I, et al. (2010). A cognitive intermediate phenotype study confirming possible gene-early adversity interaction in psychosis outcome: a general population twin study. Psychosis; 2: 1-11.

Pickering L, Simpson J, & Bentall RP. (2008). Insecure attachment predicts proneness to paranoia but not hallucinations. Personality and Individual Differences; 44:1212–1224.

Pillinger T, Beck K, Gobilla C, et al. (2017). Impaired glucose homeostasis in first-episode schizophrenia: a systematic review and meta-analysis. JAMA Psychiatry; 74(3): 261-9.

Pilz S, Mangge H, Obermayer-Pietsch B, et al. (2007) Visfatin/pre-B-cell colony-enhancing factor: a protein with various suggested functions. J Endocrinol Invest; 30(2):138-44.

Plomin R & DeFries JC. (1977). Genotype-enviroment interaction and correlation in the analysis of human behaviour. Psycol Bull; 84(2):309-22.

Plotkin SR, Banks WA, & Kastin AJ. (1996). Comparison of saturable transport and extracellular pathways in the passage of interleukin-1 alpha across the blood-brain barrier. J Neuroimmunol; 67:41−47.

Pollack P, Broadbent M, Clarke S, et al. (2001). The personality structure questionnaire (PSQ): A measure of the multiple self-states model of identity disturbance in cognitive analytic therapy. Clinical Psychology and Psychotherapy; 8:29-72.

Portela A & Esteller M. (2010). Epigenetic modifications and human disease. Nat Biotechnol 28(10): 1057-1068.

Poulton R, Moffitt TE, & Silva PA. (2015). The Dunedin Multidisciplinary Health and Development Study: overview of the first 40 years, with an eye to the future. Soc Psychiatry Psychiatr Epidemiol; 50(5): 679-93.

Priebe S, Huxley P, Knight S, et al. (1999). Application and results of the Manchester Short Assessment of Quality of Life (MANSA). Int J Soc Psychiatry; 45(1):7-12.

Provençal N, Suderman MJ, Caramaschi D, et al. (2013). Differential DNA methylation regions in cytokine and transcription factor genomic loci associate with childhood physical aggression. PLoS One; 8(8): e71691.

Provençal N, Suderman MJ, Guillemin C, et al. (2014). Association of childhood chronic physical aggression with a DNA methylation signature in adult human T cells. PLoS One; 9(4): e89839.

Pruessner M, Vracotas N, Joober R, et al. (2013). Blunted cortisol awakening response in men with first episode psychosis: relationship to parental bonding. Psychoneuroendocrinology; 38:229-240.

Pruessner JC, Kirschbaum C, Meinlschmid G, et al. (2003). Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. Psychoneuroendocrinology; 28(7):916–931.

Quan N & Banks WA. (2007). Brain–immune communication pathways. Brain Behav Immun; 21:727−735.

Radtke KM, Schauer M, Gunter HM, et al. (2015). Epigenetic modifications of the glucocorticoid receptor gene are associated with the vulnerability to psychopathology in childhood maltreatment. Transl Psychiatry; 5: e571.

Raison CL, Borison AS, Broadwell SD, et al. (2005). Depression during pegylated interferon-alpha plus ribavirin therapy: Prevalence and predictors. J Clin Psychiatry; 66(1): 41-8.

Raison CL, Borisov AS, Woolwine BJ, et al. (2010a). Interferon-alpha effects on diurnal hypothalamic–pituitary–adrenal axis activity: relationship with proinflammatory cytokines and behavior. Mol Psychiatry; 15:535−547.

Raison CL, Capuron L, & Miller AH. (2006). Cytokines sing the blues: inflammation and the pathogenesis of depression. Trends Immunol; 27:24−31.

Raison CL & Miller AH. (2011). Is depression an inflammatory disorder? Curr Psychiatry Rep; 13(6):467-75.

Raison CL, Dantzer R, Kelley KW, et al. (2010b). CSF concentrations of brain tryptophan and kynurenines during immune stimulation with IFN-alpha: relationship to CNS immune responses and depression. Mol Psychiatry; 15(4):393-403.

Ramsay CE, Flanagan Gantt S, Broussard B, et al. (2011). Clinical correlates of maltreatment and traumatic experiences in childhood and adolescence 27 among predominantly African American, socially disadvantaged, hospitalized, first-episode psychosis patients. Psychiatry Res; 188: 343-9.

Read J, Perry BD, Moskowitz A, et al. (2001). The contribution of early traumatic events to schizophrenia in some patients: a traumagenic neurodevelopmental model. Psychiatry Res; 64(4): 319-45.

Read J, van Os J, Morrison AP, et ala. (2005). Childhood trauma, psychosis and schizophrenia: a literature review with theoretical and clinical implications. Acta Psychiatr Scand; 112:330—350.

Rehkopf DH, Headen I, Hubbard A, et al. (2016). Adverse childhood experiences and later life adult obesity and smoking in the United States. Ann. Epidemiol; 26(7):488-492.

Reichenberg A, CaspiA, Harrington H, et al. (2010). Static and dynamic cognitive deficits in childhood preceding adult schizophrenia: a 30-year study. Am J Psychiatry; 167(2):160-9.

Richards JP, Stephenson AH, Ellsworth ML, et al. (2013) Synergistic effects of C-peptide and insulin on low O2-induced ATP release from human erythrocytes. Am J Physiol Regul Integr Comp Physiol; 305(11):R1331-6.

Richardson MA, Read LL, Taylor Clelland CL, et al. (2005). Evidence for a tetrahydrobiopterin deficit in schizophrenia. Neuropsychobiology; 52(4):190-201.

Riley EH, Wright RJ, Jun HJ, et al. (2010) Hypertension in adult survivors of child abuse: observations from the Nurses' Health Study II. J Epidemiol Community Health; 64(5):413-8

Rivest S, Lacroix S, Vallieres L, et al. (2000). How the blood talks to the brain parenchyma and the paraventricular nucleus of the hypothalamus during systemic inflammatory and infectious stimuli. Proc Soc Exp Biol Med; 223:22−38.

Romens SE, McDonald J, Svaren J, et al. (2015). Associations between early life stress and gene methylation in children. Child Dev; 86(1): 303-309.

Rosenberg M. (1979). Conceiving the Self. Basic Books, New York.

Rössler W, Vetter S, Müller M, et al. (2011). Risk factors at the low end of the psychosis continuum: Much the same as at the upper end? Psychiatry Research; 189(1): 77–81.

Rosso G, Cattaneo AM, Zanardini R, et al. (2015). Glucose metabolism alterations in patients with bipolar disorder.J Affect Disord; 184:293-8.

Ruggeri M, Bonetto C, Lasalvia A, et al. (2012). A multi-element psychosocial intervention for early psychosis (GET UP PIANO TRIAL) conducted in a catchment area of 10 million inhabitants: study protocol for a pragmatic cluster randomized controlled trial. Trials; 13:73.

Ruggeri M, Bonetto C, Lasalvia A, et al. (2015). Feasibility and Effectiveness of a Multi-Element Psychosocial Intervention for First-Episode Psychosis: Results From the Cluster-Randomized Controlled GET UP PIANO Trial in a Catchment Area of 10 Million Inhabitants. Schizophr Bull; 41(5): 1192-203.

Ruggeri M, Lasalvia A, Dall'Agnola R, et al. (2000). Development, internal consistency and reliability of the Verona Service Satisfaction Scale – European Version. EPSILON Study 7. Br J Psychiatry; 177(Suppl 39):41–48.

Rush AJ, Gullion CM, Basco MR, et al. (1996). The inventory of depressive symptomatology (IDS): psychometric properties. Psychol Med; 26(3):477-86.

Rutters F, Pilz S, Koopman AD, et al. (2015). Stressful life events and incident metabolic syndrome: the Hoorn study. Stress; 18(5):507-513.

Ryan MC, Collins P, & Thakore JH. (2003). Impaired fasting glucose tolerance in first-episode, drug-naive patients with schizophrenia. Am J Psychiatry; 160(2):284-9.

Samele C. (2004). Factors leading to poor physical health in people with psychosis. Epidemiol Psichiatr Soc; 13(3): 141-5.

Santoro ML, Gadelha A, Ota VK, et al. (2015). Gene expression analysis in blood of ultra-high risk subjects compared to first-episode of psychosis patients and controls. World J Biol Psychiatry; 16: 441-446.

Savoy C, Van Lieshout RJ, & Steiner M. (2017). Is plasminogen activator inhibitor-1 a physiological bottleneck bridging major depressive disorder and cardiovascular disease? Acta Physiol; 219(4):715-727.

Scammell JG, Denny WB, Valentine DL, et al. (2001). Overexpression of the FK506-binding immunophilin FKBP51 is the common cause of glucocorticoid resistance in three New World primates. Gen Comp Endocrinol; 124: 152–165.

Schaefer M. Capuron L, Friebe A, et al. (2012). Hepatitis C infection, antiviral treatment and mental health: a European expert consensus statement. J Hepatol; 57(6):1379-90.

Schäfer I & Fisher HL. (2011a). Brief report. Childhood trauma and psychosis—what is the evidence? Dialogues in Clinical Neuroscience; 13(3):360–365

Schäfer I & Fisher HL. (2011b). Childhood trauma and posttraumatic stress disorder in patients with psychosis: clinical challenges and emerging treatments. Curr Opin Psychiatry; 24: 514-8.

Schalinski I, Elbert T, Steudte-Schmiedgen S, et al. (2015). The cortisol paradox of trauma-related disorders: Lower phasic responses but higher tonic levels of cortisol are associated with sexual abuse in childhood. PLoS ONE; 10(8):1–18.

Schmitt A, Malchow B, Hasan A, et al. (2014). The impact of environmental factors in severe psychiatric disorders. Front Neurosci; 11: 8-19.

Schübeler D. (2015). Function and information content of DNA methylation. Nature; 517(7534): 321-326.

Schwaiger M, Grinberg M, Moser D, et al. (2016). Altered Stress-Induced Regulation of Genes in Monocytes in Adults with a History of Childhood Adversity. Neuropsychopharmacology; 41(10): 2530-2540.

Schwarcz R & Pellicciari R. (2002). Manipulation of brain kynurenines: glial targets, neuronal effects, and clinical opportunities. J Pharmacol Exp Ther; 303:1−10.

Sheehan DV, Lecrubier Y, Sheehan KH, et al. (1998). The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. J Clin Psychiatry; 59 Suppl 20:22-33;quiz 34-57.

Shields AE, Wise LA, Ruiz-Narvaez EA, et al. (2016). Childhood abuse, promoter methylation of leukocyte NR3C1 and the potential modifying effect of emotional support. Epigenomics; 8(11): 1507-1517.

Shimabukuro M, Sasaki T, Imamura A, et al. (2007). Global hypomethylation of peripheral leukocyte DNA in male patients with schizophrenia: a potential link between epigenetics and schizophrenia. J Psychiatr Res; 41: 1042-1046.

Sideli L, Mule A, La Barbera D, et al. (2012). Do child abuse and maltreatment increase risk of schizophrenia? Psychiatry Investig; 9: 87-99.

Sinha R & Jastreboff AM. (2013). Stress as a common risk factor for obesity and addiction. Biol Psychiatry; 73(9):827-835.

Smearman EL, Almli LM, Conneely KN, et al. (2016). Oxytocin Receptor Genetic and Epigenetic Variations: Association With Child Abuse and Adult Psychiatric Symptoms. Child Dev; 87(1): 122-134.

Smith CL, Bolton A, & Nguyen G (2010). Genomic and epigenomic instability, fragile sites, schizophrenia and autism. Curr. Genomics 11(6): 447-469.

Smith N, Lam D, Bifulco A, et al. (2002). Childhood Experience of Care and Abuse Questionnaire (CECA.Q). Validation of a screening instrument for childhood adversity in clinical populations. Soc Psychiatry Psichiatr Epidemiol; 37(12):572-9.

Stefanis NC, Hanssen M, Smirnis NK, et al. (2002). Evidence that three dimensions of psychosis have a distribution in the general population. Psychol Med; 32(2):347-58.

Sockalingam S, Links PS, & Abbey SE. (2011). Suicide risk in hepatitis C and during interferon-alpha therapy: a review and clinical update. J Viral Hepat; 18:153-60.

Spelman LM, Walsh PI, Shafiri N, et al. (2007). Impaired glucose tolerance in first-episode drug-naïve patients with schizophrenia. Diabet Med; 24(5):481-5.

Spitzer RL, Williams JBW, Gibbon M, et al. (1992). The Structured Clinical Interview for DSM-III-R (SCID). I: History, rationale, and description. Arch Gen Psychiatry; 49(8):624-629 (1992).

Sroufe AL. (2005). Attachment and development: a prospective, longitudinal study from birth to adulthood. Attachment and Human Development; 7:349–367.

Stain HJ, Bronnick K, Hegelstad Wenche TV, et al. (2014). Impact of interpersonal trauma on the social functioning of adults with first-episode psychosis. Schizophr Bull; 40 (06):1491-8.

Stewart R & Hirani V. (2012). Relationship between depressive symptoms, anemia, and iron status in older residents from a national survey population. Psychosom Med; 74:208–13.

Stubbs B, Vancampfort D, De Hert M, et al. (2015). The prevalence and predictors of type two diabetes mellitus in people with schizophrenia: a systematic review and comparative meta-analysis. Acta Psychiatr Scand; 132(2):144-57.

Suderman M, Borghol N, Pappas JJ, et al. (2014). Childhood abuse is associated with methylation of multiple loci in adult DNA. BMC Med Genomics; 7: 13.

Sullivan PF. (2012). Puzzling over schizophrenia: schizophrenia as a pathway disease. Nat Med; 18(2): 210-211.

Sullivan PF, Kendler KS, & Neale MC. (2003). Schizophrenia as a complex trait: evidence from a meta-analysis of twin studies. Arch Gen Psychiatry; 60:1187–1192.

Suplita RL, Eisenstein SA, Neely MH, et al. (2008). Cross-sensitization and cross-tolerance between exogenous cannabinoid antinociception and endocannabinoid mediated stress-induced analgesia. Neuropharmacol; 54(1):161-71.

Takao T, Tojo C, Nishioka T, et al. (2000). Increased adrenocorticotropin responses to acute stress in Otsuka Long-Evans Tokushima Fatty (type 2 diabetic) rats. Brain Res; 852(1):110-5.

Taylor TF. (2015). The influence of shame on post trauma disorders: have we failed to see the obvious? Eur J Psychotraumatol; 6: 28847

Teff KL, Rickels MR, Grudziak J, et al. (2013)Antipsychotic-induced insulin resistance and postprandial hormonal dysregulation independent of weight gain or psychiatric disease. Diabetes; 62(9):3232-40.

Perry BI, McIntosh G, Weich S, et al. (2016). The association between first-episode psychosis and abnormal glycaemic control: systematic review and meta-analysis. Lancet Psychiatry; 3(11):1049-1058.

Thomas C, Hyppönen E, & Power C. (2008). Obesity and type 2 diabetes risk in midadult life: the role of childhood adversity. Pediatrics; 121(5):e1240-9.

Tod M, Farcy-Afif M, Stocco J, et al. (2005). Pharmacokinetic/pharmacodynamic and time-to-event models of ribavirin-induced anaemia in chronic hepatitis C. Clin Pharmacokinet; 44(4):417-28.

Toft-Nielsen MB, Damholt MB, Madsbad S, et al. (2001). Determinants of the impaired secretion of glucagon-like peptide-1 in type 2 diabetic patients. J Clin Endocrinol Metab; 86: 3717-3723.

Tomassi & Tosato. (2017). Epigenetics and gene expression profile in first-episode psychosis: The role of childhood trauma. Neuroscience Biobehavioral Reviews; 83:226-237.

Tomassi S, Tosato S, Mondelli V, et al. (2017). Influence of childhood trauma on diagnosis and substance use in first-episode psychosis. Br J Psychiatry; 211(3):151-156.

Tosato S, Lasalvia A, Bonetto C, et al. (20139. The impact of cannabis use on age of onset and clinical characteristics in first-episode psychotic patients. Data from the Psychosis Incident Cohort Outcome Study (PICOS). J Psychiatr Res; 47(4): 438-44.

Trotta A, Murray RM, & Fisher HL. (2015). The impact of childhood adversity on the persistence of psychotic symptoms: a systematic review and meta-analysis. Psychol Med; 45(12): 2481-2498.

Tunnard C, Rane LJ, Wooderson SC, et al. (2014). The impact of childhood adversity on suicidality and clinical course in treatment-resistant depression. J Affect Disord; 152-154:122-30

Tyrka AR, Price LH, Marsit C, et al. (2012). Childhood adversity and epigenetic modulation of the leukocyte glucocorticoid receptor: preliminary findings in healthy adults. PLoS One; 7(1): e30148.

Ucok A & Bıkmaz S. (2007). The effects of childhood trauma in patients with first-episode schizofrenia. Acta Psychiatr Scand; 116(5): 371-7.

Udina M, Castellví P, Moreno-España J,et al. (2012). Interferon-induced depression in chronic hepatitis C: a systematic review and meta-analysis. J Clin Psychiatry; 73(8):1128-38.

Reininghaus U, Dutta R, Dazzan P, et al. (2015). Mortality in Schizophrenia and Other Psychoses: A 10-Year Follow-up of the ÆSOP First-Episode Cohort, Schizophrenia Bulletin; 41(3): 664–673,

Ungar M. (2013). Resilience, Trauma, Context, and Culture. Trauma, Violence, and Abuse; 14(3):255–266.

Ungar M & Liebenberg L. (2011). Assessing resilience across cultures using mixed methods: Construction of the Child and youth resilience measure. Journal of Mixed Methods Research; 5(2):126–149.

United Nations, Geneva Declaration of the Rights of the Child, 1924.

United Nations General Assembly, Resolution 14/1386. Declaration of the Rights of the Child, 20 November 1959

United Nations General Assembly, Resolution 44/25. Declaration of the Rights of the Child, 20 November 1989.

Upthegrove R, Chard C, Jones L, et al. 820159. Adverse childhood events and psychosis in bipolar affective disorder. Br J Psychiatry; 206(3): 191-7.

Van den Berg DPG & van der Gaag M. (2012). Treating trauma in psychosis with EMDR: A pilot study. J Behav Ther & Exp Psychiat; 43: 664-71.

Van der Knaap LJ, Riese H, Hudziak JJ, et al. (2014). Glucocorticoid receptor gene (NR3C1) methylation following stressful events between birth and adolescence. The TRAILS study. Transl Psychiatry; 4: e381.

Van der Knaap LJ, van Oort FV, Verhulst FC, et al. (2015). Methylation of NR3C1 and SLC6A4 and internalizing problems. The TRAILS study. J Affect Disord; 180: 97-103.

Van Os J, Kenis G, & Rutten BP. (2010).The environment and schizophrenia. Nature; 468(7321):203-12.

Van Vlierberghe H, Delanghe JR, De Vos M, et al. (2001). Factors influencing ribavirin-induced hemolysis. J Hepatol; 34: 911-6

Van Wijngaarden B, Schene AH, Koeter M, et al. (2000) Caregiving in schizophrenia: development, internal consistency and reliability of the Involvement Evaluation Questionnaire – European Version. EPSILON Study 4. Br J Psychiatry; 177(Suppl 39):21–27.

Van Winkel R, Stefanis NC, & Myin-Germeys I (2008). Psychosocial stress and psychosis. A review of the neurobiological mechanisms and the evidence for gene-stress interaction. Schizophr Bull; 34(6): 1095-105.

Vancampfort D, Mitchell AJ, De Hert M, et al. (2015a). Prevalence and predictors of type 2 diabetes mellitus in people with bipolar disorder: a systematic review and meta-analysis. J Clin Psychiatry; 76(11):1490-9.

Vancampfort D, Stubbs B, Mitchell AJ, et al. (2015b). Risk of metabolic syndrome and its components in people with schizophrenia and related psychotic disorders, bipolar disorder and major depressive disorder: a systematic review and meta-analysis. World Psychiatry; 14(3):339-47.

Vancampfort D, Vansteelandt K, Correll CU, et al. (2013). Metabolic syndrome and metabolic abnormalities in bipolar disorder: a meta-analysis of prevalence rates and moderators. Am J Psychiatry; 170(3):265-74.

Varese F, Smeets F, Drukker M, et al. (2012a). Childhood adversities increase the risk of psychosis: a meta-analysis of patient-control, prospective- and cross-sectional cohort studies. Schizophr Bull; 38(4):661–671

Varese F, Barkus E, & Bentall RP. (2012b). Dissociation mediates the relationship between childhood trauma and hallucination-proneness. Psychological Medicine; 42:1025-1036.

Vejbaesya S, Luangtrakool P, Luangtrakool K, et al. (2007). Decreased ACTH and Cortisol Responses to Stress in Healthy Adults Reporting Significant Childhood Maltreatment. Biological Psychiatry; 62(10):1080–1087.

Verma SK, Subramaniam M, Liew A, et al. (2009). Metabolic risk factors in drug-naive patients with first-episode psychosis. J Clin Psychiatry; 70(7):997-1000.

Veru-Lesmes F, Rho A, King S, et al. (2018). Social Determinants of Health and Preclinical Glycemic Control in Newly Diagnosed First-Episode Psychosis Patients. Can J Psychiatry; 1:706743718762097.

Vijayendran M, Beach SR, Plume JM, et al. (2012). Effects of genotype and child abuse on DNA methylation and gene expression at the serotonin transporter. Front Psychiatry; 3: 55.

Voet D & Voet JG. (2011). Biochemistry. 4th Eds. John Wiley and Sons. New Jersey. US.

Wahren J & Larsson C. (2015). C-peptide: new findings and therapeutic possibilities. Diabetes Res Clin Pract; 107(3), 309-319.

Wallerath T, Kunt T, Forst T, et al. (2003). Stimulation of endothelial nitric oxide synthase by proinsulin C-peptide. Nitric Oxide; 9(2):95-102.

Walsh C, MacMillan H, Jamieson E. (2002). The relationship between parental psychiatric disorder and child physical and sexual abuse: findings from the Ontario Health Supplement. Child Abuse Negl; 26(1):11-22.

Wang Y, Wu B, Yang H, et al. (2015) The effect of childhood abuse on the risk of adult obesity. Ann Clin Psychiatry; 27(3):175-84.

Wankerl M, Miller R, Kirschbaum C, et al. (2014). Effects of genetic and early environmental risk factors for depression on serotonin transporter expression and methylation profiles. Transl Psychiatry; 4: e402.

Wardle J, Chida Y, Gibson EL, et al. (2011) Stress and adiposity: a meta-analysis of longitudinal studies.Obesity (Silver Spring); 19(4):771-8

Ware JE Jr & Sherbourne CD. (1992). The MOS short-form healthy survey (SF-36). I. Conceptual framework and item selection. Medical Care; 30:473-483

Watkins LR, Goehler LE, Relton JK, et al. (1995). Blockade of interleukin-1 induced hyperthermia by subdiaphragmatic vagotomy: evidence for vagal mediation of immune-brain communication. Neurosci Lett; 183:27−31.

Weaver IC. (2007). Epigenetic programming by maternal behaviour and pharmacological intervention. Nature versus nurture: let's call the whole thing off. Epigenetics; 2(1): 22-28.

Weder N, Zhang H, Jensen K, et al. (2014). Child abuse, depression, and methylation in genes involved with stress, neural plasticity, and brain circuitry. J Am Acad Child Adolesc Psychiatry; 53(4):417-424.

Weissbecker I, Floyd A, Dedert E, et al. (2006). Childhood trauma and diurnal cortisol disruption in fibromyalgia syndrome. Psychoneuroendocrinology; 31(3):312–324.

Werner EE. (1992). The children of Kauai: resiliency and recovery in adolescence and adulthood. J Adolesc Health; 13(4), 262-8.

Werner EE. (2004). Journeys from childhood to midlife: risk, resilience, and recovery. Pediatrics; 114(2), 492.

Westendorf JM & Schonbrunn A. (1985). Peptide specificity for stimulation of corticotropin secretion: activation of overlapping pathways by the vasoactive intestinal peptide family and corticotropin-releasing factor. Endocrinology; 116:2528 –35

WHO. (1988). Disability Assessment Schedule. Geneva, World Health Organization

WHO. (1992). Schedules for Clinical Assessment in Neuropsychiatry. Geneva, World Health Organization.

WHO. (1999). Report of the Consultation on Child Abuse Prevention. Geneva, World Health Organization.

WHO. (2002). World report on violence and health. Geneva, World Health Organization.

WHO. (2014). Global Status Report on Violence Prevention. Geneva; World Health Organization.

WHO. (2015). Bridging the gap. Geneva; World Health Organization.

WHO. (1992). Life Chart Schedule (LCS). Geneva; World Health Organization.

WHO. (1998). WHOQOL-BREF. Geneva. World Health Organization.

Wichers MC, Kenis G, Koek GH, et al. (2007). Interferon-alpha-induced depressive symptoms are related to changes in the cytokine network but not to cortisol. J Psychosom Res; 62:207−214.

Wicks S, Hjern A, & Dalman C. (2010). Social risk or genetic liability for psychosis? A study of children born in Sweden and reared by adoptive parents. Am J Psychiatry; 167:1240-1246.

Wicks S, Hjern A, Gunnell D, et al. (2005). Social adversity in childhood and the risk of developing psychosis: a national cohort study. Am J Psychiatry; 162(9):1652-7.

Widom CS, DuMont K, Czaja SJ. (2007). A prospective investigation of major depressive disorder and comorbidity in abused and neglected children grown up. Arch Gen Psychiatry; 64:49–56.

Wigman JTW, van Winkel R, Ormel J, et al. (2012) Early trauma and familial risk in the development of the extended psychosis phenotype in adolescence. Acta Psychiatr Scand; 126: 266-273.

Wing JK, Babor T, Brugha T, et al. (1990). SCAN. Schedules for Clinical Assessment in Neuropsychiatry. Arch Gen Psychiatry; 47(6): 589-93.

Wingenfeld K, Spitzer C, Mensebach C, et al. (2010). [The German version of the Childhood Trauma Questionnaire (CTQ): preliminary psychometric properties]. Psychother Psychosom Med Psychol; 60: 442-450. [Article in German]

Wochnik GM, Rüegg J, Abel GA, et al. (2005). FK506-binding proteins 51 and 52 differentially regulate dynein interaction and nuclear translocation of the glucocorticoid receptor in mammalian cells. J Biol Chem; 280: 4609-4616.

World Medical Association, Review, Communication & Principles. (2013). World Medical Association Declaration of Helsinki. Jama; 310(20): 2191.

Wray NR & Gottesman II. (2012) Using summary data from the danish national registers to estimate heritabilities for schizophrenia, bipolar disorder, and major depressive disorder. Front Genet; 3:118.

Wu X, Huang Z, Wu R, et al. (2013). The comparison og glycometabolism parameters and lipid profiles between drug-naïve, first-episode schizophrenia patients and healthy controls. Schizophr Re; 150(1): 157-62.

Yang BZ, Zhang H, Ge W, et al. (2013). Child abuse and epigenetic mechanisms of disease risk. Am J Prev Med; 44(2): 101-107.

Young M, Read J, Barker-Collo S, Harrison R. (2001). Evaluating and overcoming barriers to taking abuse histories. Prof Psychol Res Pr; 32:407–414.

Yosten GL & Kolar GR. (2015). The Physiology of Proinsulin C Peptide: Unanswered Questions and a Proposed Model. Physiology; 30(4):327-332.

Zavaschi ML, Graeff ME, Menegassi MT, et al. (2006). Adult mood disorders and childhood psychological trauma. Rev Bras Psiquiatr; 28:184–90.

Zigmond AS & Snaith RP. (1983). The hospital anxiety and depression scale. Acta Psychiatr Scand; 67(6):361-70.

Zisook S, Lesser IM, Lebowitz B, et al. (2011) Effect of antidepressant medication treatment on suicidal ideation and behavior in a randomized trial: an exploratory report from the combining medications to enhance depression outcomes study. J ClinPsychiatry; 72:1322-32.

Zubin J & Spring B. (1977). Vulnerability-a new view of schizophrenia. J Abnorm Psychol; 86:103-26.