Depression among Adolescents

Measurement and diagnosis, Environmental factors, and Genetics

CATALINA TORRES SOLER
Abstract


Depression is a common disorder and a major cause of disability. The prevalence of depression in adolescents is 12-25%. The use of valid instruments in psychiatric assessment contributes to diagnostic validity and consistency.

This thesis is divided into four parts:

The first and second parts study the psychometric properties of the MADRS-P and CSDS/CSDSP scales. The scales are adapted to measure symptoms and function in adolescents. In the first part, the psychometric properties of the Montgomery-Åsberg Depression Rating Scale Parent used for the assessment of parent-reported depressive symptoms in adolescents are studied. In the second part, the properties of the Swedish Child Sheehan Disability Scales, in the adolescent and parent versions, are studied. These scales are used to assess levels of function in school, relationships with friends and relationships with family. The properties of the scales were studied in a population of adolescents referred to the child and adolescent psychiatric clinic.

The third study analyses the interaction between BDNF rs6265 polymorphism, childhood stress and physical activity in relation to depressive symptoms, showing that the interaction of BDNF rs6265 with childhood stress and physical activity is significant in moderation models. For A allele carriers, physical activity reduces depressive symptoms in youth exposed to childhood stress. In turn, exposure to a higher level of stress increases depressive symptoms in adolescents aged 13 and 15. The minor allele confers plasticity characteristics when interacting with the mentioned environmental factors.

The fourth study shows that the interaction of the three factors PER2 rs56013859, sex and family maltreatment in relation to depressive symptoms is significant in female minor C allele carriers aged 13 and 15 years.

The results obtained in this thesis are applicable in the practice of child and adolescent psychiatry, and in the prevention of depressive disorders in the child and adolescent population.

Keywords: Adolescent, parent, depression, scale, genetics

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Dans la vie, rien n’est à craindre, tout est à comprendre.

—Marie Curie

For Ale
This thesis is based on the following papers, which are referred to in the text by their Roman numerals.


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Abbreviations

ADHD  Attention-deficit/hyperactivity disorder
AUC  Area under the curve
BDI  Beck Depression Inventory
BDNF  Brain-derived neurotrophic factor
BSCT  Biological sensitivity to context theory
CI  Confidence interval
CLOCK  Circadian locomotor output cycles kaput
CM  Childhood maltreatment
CSDS/CSDS-P  Child Sheehan Disability Scale for self-report and parent report
DNA  Deoxyribonucleic acid
DRD2  Dopamine receptor D2
DRD4  Dopamine receptor D4
DSM  *Diagnostic & Statistical Manual of Mental Disorders*
DSM-5  *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition*
DSRS  Depression Self-Rating Scale
DST  Differential susceptibility theory
GAF  Global Assessment of Functioning
GAS  Global Assessment Scale
GWAS  Genome-wide association study
HPA  Hypothalamic–pituitary–adrenal
HTR2A  Serotonin receptor
ICD-11  *International Classification of Diseases, 11th Revision*
K-SADS-PL  Kiddie Schedule for Affective Disorders and Schizophrenia, Present and Life version
KSQ  Karolinska Sleep Questionnaire
LEAD  Longitudinal, Expert, All Data
MDD  Major depressive disorder
MADRS  Montgomery–Åsberg Depression Rating Scale
MADRS-P  Montgomery–Åsberg Depression Rating Scale, Parent
MADRS-S  Montgomery–Åsberg Depression Rating Scale, Self-report
MAF  Minor allele frequency
MAOA  Monoamine oxidase A
MDD Major depressive disorder
mRNA Messenger RNA
LR− Negative likelihood ratio
NPV Negative predictive value
PA Physical activity
PABAK Prevalence- and bias-adjusted κ
PCA Principal component analysis
Per2 Period 2
LR+ Positive likelihood ratio
PPV Positive predictive value
RNA Ribonucleic acid
ROC Receiver operating characteristic
SALVe Survey of Adolescent Life in Västmanland
SCID Structured clinical interview for DSM
SD Standard deviation
SDQ Strengths and Difficulties Questionnaire
SDS Sheehan Disability Scale
SLC6A4 Serotonin transporter
SNP Single-nucleotide polymorphism
SUD Substance use disorders
TCA Tricyclic antidepressants
5HTTP 5-hydroxytryptophan
Introduction

Depression

According to the *Diagnostic & Statistical Manual of Mental Disorders*, Fifth Edition (DSM-5) (1) a major depressive disorder (MDD) is characterised by one episode of at least five of the following symptoms: depressed mood, irritable mood, or reduced interest or pleasure in most activities (anhedonia), appetite and/or weight changes, sleep pattern changes, low energy or fatigue, psychomotor changes, reduced ability to think or concentrate, feelings of worthlessness or guilt and thoughts of death or suicide. One of the symptoms must be depressed mood or loss of interest or pleasure. These symptoms are present for at least 2 weeks, practically every day, for most of the day and accompanied by reduced levels of functioning in two or more contexts, such as social or occupational settings. Symptoms are not explained by other mental or somatic disorder. Severity and course differ; impairment ranges vary from mild to complete incapacity and the course can be a single episode or be recurrent.

Depressive symptoms

Although not included in the DSM-5, other symptoms may also be present, such as feeling tense and nervous, or experiencing hopelessness, early morning awakening, mood reactivity, diurnal variation, loss of sexual interest, panic/phobia, gastrointestinal problems, somatic complaints, sympathetic arousal, interpersonal sensitivity and paralysis (2).

Symptom variation over time is related to depression severity (3). Although there is some continuity of symptoms, their relation to stressful events decreases in proportion to the number of depressive episodes (4). The presence of anhedonia without somatic symptoms indicates severe depression, while somatic symptoms are related to moderate depression (5).

Moreover, the early onset of symptoms is associated with longer depressive episodes, existence of atypical symptoms, higher comorbidity and increased risk of suicide (6, 7), and is further associated with increased risk for bipolar disorder or schizophrenia (8, 9).

Symptom appearance differs between adults and adolescents. Adolescents have more irritability and vegetative symptoms, such as insomnia, loss of and
energy and appetite and weight change, than adults, while adults present more anhedonia/loss of interest and concentration problems (10).

The average length of a depressive episode is 4 months in childhood and 2 months during adolescence, with a 70% recurrence rate within the following 5 years (7, 11).

Epidemiology of depression
Depression is a common disease and an important cause of disability (12). The worldwide prevalence of years lived with disability due to depression in people aged 15–49 years is 3.6% (13).

Screening of depressive symptoms in adolescent samples shows a prevalence of 11.7–25% (14-18). The point prevalence of MDD among adolescents is 2.9–12% and lifetime prevalence is 13–23% (19). Depression prevalence in childhood (ages 5–12 years) is 1–2% and increases progressively until adolescence (ages 13–17 years) at 5–8% (6, 14, 19, 20). In Sweden, the 1-year prevalence of depression is 5.8% and lifetime prevalence is 11.4% among 16–17-year-old students (21).

At the age of 13 years, the prevalence of depressive symptoms increases to 25% among female adolescents with the prevalence in male adolescents 10% (22-24). The prevalence increases in male adolescents during late adolescence (25). However, the higher overall rate of depression in female adolescents continues with a ratio of four female adolescents to one male adolescent (21, 26). These frequency differences between sexes are probably related to the differential course of symptoms, help-seeking, risk of experiencing stressful events and vulnerability (24).

Epidemiological data should be reviewed after the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) or (COVID-19) pandemic in 2020, due to global changes in social restrictions, and changes in schooling and socio-economic conditions that affected diverse regions, increasing the numbers of persons suffering anxious and depressive symptoms (27, 28).

In sum, in early childhood, there is no difference in frequencies between sexes (29).

Depression and Comorbidity
The economic burden of comorbid depression is considerable (30). Further, MDD comorbidity is frequent (31), especially with attention-deficit/hyperactivity disorder (ADHD) (32). In an epidemiological study, Kessler et al. (14) found that 59% of their depression cases were comorbid with anxiety, 30% were comorbid with substance abuse and 24% were comorbid with impulse control disorder.

In a clinical study of 88 adolescents interviewed using the Kiddie Schedule for Affective Disorders and Schizophrenia (K-SADS), 8% had depression. Of
these, 3% had comorbid ADHD, 2% had comorbid anorexia nervosa, 2% had comorbid conduct disorders and 1% had comorbidity for each of schizophrenia, generalised anxiety disorder, separation anxiety disorder and adjustment disorder (33).

Comorbid depression and anxiety in youths has several pathways: first, a shared diathesis for anxiety and depression with primary depression and anxiety; second, a diathesis for anxiety with social phobia or separation anxiety followed by depression; and third, a diathesis for depression followed by social anxiety (34).

Around 10% of early adolescents with depression have comorbid substance use disorders (SUD) with alcohol or other substances (35, 36). Among adolescents with SUD, 25–50% have depression (37, 38), and MDD is a predictor for SUD (39). It is possible that SUD was used to handle depressive feelings (40), or alternatively, that MDD and SUD have risk factors in common (41). Depressive symptoms in male adolescents are associated with increased amounts of consumed alcohol per occasion, while in female adolescents are associated with elevated consumption frequency at 18–19 years (42). Although depression has a negative effect on SUD relapse, depressive symptoms increase retention in treatments (42, 43).

Theories of depression development

**Psychology**

The different theories about depression are derived from diverse approaches, where some emphasise environmental factors while others focus on psychological mechanisms.

Freud’s psychodynamic theory explains the conceptual framework of the topographical model of the psyche, with levels of consciousness (i.e. unconscious, the pre-conscious and the conscious), in addition to the structural model of the psychic apparatus with the id, ego and super ego (44, 45). This model was later developed by Klein (46), who theorised about the relationship between the child and the mother in the first year of life, and Fenichel (47) who recapitulated defence mechanisms and their relationship with psychopathology. In *Mourning and melancholia*, Freud explained how in normal mourning, the attachment of the libido to a loved one is recovered and displaced to a new object if the loved person disappears. In cases where the libido is not displaced, identification of the ego with the loss object occurs and the loss of the object/person is experienced as a loss of the ego with a narcissistic identification. This mechanism generates ambivalence, which is a predisposing factor to melancholia.

Clark and Beck’s cognitive theory (48) explained the emotional symptoms of depression as derived from a thinking process characterised by negative automatic thoughts, negative schemas and bias in the information process with
a negative interpretation. These components lead to a predisposition to depression.

Abramson and colleagues (49) proposed hopelessness depression, in which causal attribution develops in response to negative life events and increases the risk of becoming depressed. The attributions have three dimensions; internal/external, stable/instable and global/specific. A chronic exposure to an event that has consequent internal, stable and global consequences enhances the possibility of developing depression (50). Other than cognitive vulnerability, responses to negative life events with rumination prolong depression (51). Moreover, the negative cognitive style could be a mediator of negative life events on the relationship with depressive symptoms (52). On the question of parent–child relations, an aggregate of parental psychopathology enhances the risk of developing depression (53). In addition, overprotection and lack of emotional warmth predicts the development of negative cognition among children (54).

With respect to the interaction of other psychological factors, Hankin (55) adduced that the interaction of a negative inferential style with negative events is a predictor of anhedonic depression. In addition, Nima and colleagues (56) suggested that anxiety partially mediates the effect of self-esteem and stress on depression. In turn, stress mediates the effect of self-esteem on depression with an interaction between positive or negative affects.

In their humanistic approach, Maslow and Lewis introduced the concept of self-actualisation and the theory of hierarchy of needs, including safety, love and belonging, and self-actualisation (57). Maslow’s hierarchy of needs qualifies the love of parents as conditional. In this way, children try to project the image desired by the parents and deny a part of themselves by dividing their real self and limiting their opportunities of developing their relationships or interests.

Behaviourism is a theory of learning. Watson (58) criticised psychological studies based on extrapolations from animal models, raised doubts about the validity of observations of behaviour and questioned the relationships established with emotions in animal models. Behaviourism is based on observations of stimulus–response behaviours and their conditioned interaction with the environment using designed experiments and measurement of responses. Behaviour can be tested, and predictions made. Classical conditioning can be applied to study learned emotions, aversion and phobias (59).

In his social learning theory, Bandura (60) emphasised that behaviour was learned from the environment through observation. Learning is facilitated by identification (i.e. the extent to which the observer relates to a model) and by observing the consequences the model receives for their behaviour. This is known as vicarious reinforcement. Factors such as the status or expertise of the model, and reinforcement or punishment intervene in this process.

Learning is an information process in which thinking about behaviours and consequences—a mediational process—is required. This process has
components of attention, retention, motor reproduction and motivation. Bandura explains his models as a triad of the interaction between environmental factors, personal factors (i.e. cognitive, affective and biological) and behaviours (61).

Social cognitive theory is an expansion of social learning theory to include personal factors, regulation of attention and the schematic processing of experiences. This theory considers that self-efficacy is affected by active choice, goal setting, effort and persistence, as well as learning and achievement (62).

**Biology of depression**

*Anatomy*

The principal neuronal regions of the brain related to depression are the prefrontal and cingulate cortex, in which there is decreased metabolism (63, 64) and the hippocampus, in which there is decreased volume (65). The prefrontal and cingulate cortex and the hippocampus regulate memory impairments, hopelessness, guilt and suicidality (66). The striatum, which includes the nucleus accumbens, and amygdala regulates the emotional memory and anxiety, and mediates anhedonia. In patients with depression, the amygdala has a high active metabolism (67). Finally, the thalamus regulates sleep, appetite, energy and pleasurable activities (66, 68).

*Amines and glutamate*

The monoamine hypothesis of depression proposes a deficiency of norepinephrine and/or serotonin in the brain based on observations in 1950s. Then, it was noted that lysergic acid diethylamide (aka LSD) blocks the peripheral serotonin receptors and has a simultaneous central effect (69). Reserpine downloads the serotonin storage (70). Norepinephrine, a precursor of dihydroxyphenylalanine (aka DOPA), reversed the effects of reserpine (71). This occurs together with the observed effects of tricyclic antidepressants (TCAs) (72). However, the agonist effect of some antidepressants, such as buspirone and TCAs, on serotonin receptor 5-HT1a and the binding to histaminergic and muscarinic receptors, are not explained by this theory (73).

On the one hand, the glutamate hypothesis of depression is based on animal and neurobiological studies that highlight the importance of glutamate as a neurotransmitter (74). The hypothesis is supported by evidence that glutamate neurotransmission correlates with learning and memory (75) and γ-aminobutyric acid levels present abnormalities in mood disorders (76). On the other hand, antidepressive treatment may decrease the levels of glutamate in depressed subjects (77). In addition, higher concentrations of glutamate are found in the frontal cortex of individuals with depression in post-mortem studies (78) and with stress, high concentrations of glutamate cause neuronal degeneration, constituting a possible vulnerability (79).
The glutamatergic system has also been associated with addictions (80) and schizophrenia (81).

**Metabolism and stress**

Chronic stress induces a metabolic syndrome (82); hence, adverse childhood events may lead to depression and metabolic disturbances (83). Stress is related to insulin resistance (84). Moreover, the elevation of the corticotrophin-releasing factor in the corticolumbic regions of the brain activates the sympathetic nervous system (85). One consequence is that noradrenaline and adrenaline activate the $\alpha$- and $\beta$-adrenergic receptors of macrophages and microglia (86, 87). In addition, noradrenaline enhances the production of pro-inflammatory cytokines (88). Further, chronic exposure to glucocorticoids increases visceral fat (89). In turn, fatty acids induce the production of pro-inflammatory cytokines (90), which influence the severity and progression of depressive disorders (91).

In sum, stress induces hormonal, metabolic and immunological changes that influence the symptoms of depressive disorders. Interactions with environment.

**Interactions with environment**

**Stress vulnerability model**

The stress vulnerability model explores the existence of an intrinsic vulnerability in functional organisation, such as a genetic predisposition to mental illness (92). However, vulnerability alone is not enough, and an additional biopsychosocial stressor (such as a life crisis or substance use) is necessary to develop a mental illness (e.g. anxiety, depression, bipolar disorder) (see Figure 1).

![Vulnerability + Psychosocial stress → Mental illness](image)

**Figure 1. The stress vulnerability model (92).**

There are similarities between diverse conditions in the response to stress. The response is via the hypothalamic–pituitary–adrenal (HPA) axis and hypercortisolaeemia, which may result in hippocampal atrophy in the brain, and metabolic syndrome in the body. In depression, this occurs through serotonin, noradrenaline, and secondary messengers modulating brain-derived neurotrophic factor (BDNF) (93). In a new approach, the effects of environmental factors are also considered as epigenetic changes (94).
Diathesis stress model
In psychopathology, the diathesis–stress model recognises a vulnerability to negative effects or adversity (95, 96). A person with a pre-existing vulnerability is more prone to develop a pathology if exposed to a stressor. This conceptual perspective limits the interpretation of ‘something else’ that is missing (see Figure 2).

In depression, the diathesis–stress interaction was proposed to be based on biological factors (97). Stress is related to life experiences and circumstances (98). To use stress as a factor in the model, it is important to understand the associated aspects in stress as duration, dimension and quality, and the relation between these aspects (99). Stress can be considered as acute, intermittent or chronic. Without clarity in definitions, it is difficult to understand the significance of stress for the disease onset (49). Stress could be understood in relation to the severity of events, such as a major life event or minor event, while daily inconveniences or health issues are better understood as vulnerabilities (100, 101).

The creation of stress scores permits an additive approach to stressful experiences (102); however, the assessment of stress is an issue because an individual’s perception could be modified by their depressive symptoms (103) or by how their experiences are recalled, defined or associated, which leads to errors (104). Another aspect to consider is the consequence of the diathesis. For example, whether depressive symptoms, such as fatigue or concentration difficulties, have consequences in the environment, such as problems in work and relations.
It is not clear whether the diathesis–stress interaction is additive, synergic or complex (105). Assuming that the general model is depression = b_0 + b_1 stress + b_2 diathesis + b_3 (stress × diathesis) (see Figure 3).

![Figure 3. The interaction of diathesis stress in depression (95).](image)

In this model, stress and depression are considered to be continuous. Stress and depression are represented by the X- and Y-axes, respectively, and the slope of the line should show the presence and loading of the diathesis (or the absence if the line is parallel to the X-axis). Therefore, any effect of the diathesis is visualised. This model leaves some questions unexplained. For example, what is the relation between exposure to stress and the prevalence of depression in the general population? How is depression transmitted in families? How does the model represent the subtypes of depression? New theories were developed to address these questions.

**Biological sensitivity to context theory**

Natural selection has favoured adaptations in response to ecological conditions. These responses are conditional and adjust the level of biological sensitivity to the context. Biological reactivity is polygenic and high reactivity phenotypes have dual risk effects. Conditional adaptation means that adversity has negative potential, while support conditions have a positive effect.

To obtain a better adaptation, children with high levels of physiological stress reactivity should be present in all types of environments (including positive and negative environments). In a positive environment, the child develops competences that increases their reproductive fitness. In an adverse environment, an individual with high vigilance has more opportunities to survive and reproduce than others (106).
In a graph representing the levels of early psychosocial stress, where the X-axis moves from supportiveness to stressfulness and the Y-axis shows biologic reactivity, the expected line of the relation is a U-shaped curve (see Figure 4).

![Figure 4. The relation of biologic reactivity to early stress and adversity (106).](image)

Reactivity is defined as the variation of the physiological response between an individual and a control when exposed to an environmental stimulus. Phenotypes with high reactivity may be more sensitive to the effects of the context. Some examples are cases of disruptions of attachment relations, maternal depression or sexual abuse, which modifies the function of the corticotropin-releasing hormone system (107).

**The differential susceptibility theory**

Natural selection requires survival and reproduction with direct or indirect transmission of genes. Reproductive fitness is the capacity for the dispersion of one’s genes to coming generations. For natural selection, the existence of variations with different levels of gene plasticity is advantageous. If a person has been negatively affected by a stressful situation and this has limited their reproduction (i.e. direct reproductive fitness), reproduction by their relatives allows the common genes to expand in the new generation. Differential susceptibility has been proved in some gene × environment interactions. In those interactions, the ‘risk allele’ functions better than other alleles in conditions of absence of exposure to negative events.

Some examples are low activity alleles in monoamine oxidase A (MAOA) polymorphism and the effects of maltreatment in relation to antisocial behaviour (108). Male carriers of the allele with low activity showed the lowest
antisocial behaviour in the absence of abuse (109). Similar findings were obtained with the serotonin transporter-linked polymorphic region in \textit{SLC6A4} (110). The S-allele carriers had reduced expression of the transporter compared with l/l carriers and were more depressed than those who experienced stressful life events in early childhood. Life events moderated the association with suicide ideation. The results have been repeated in other studies.

The model of differential susceptibility has also proved correspond with the function of genes as the serotonin receptor \textit{(HTR2A)} with the C- and T-alleles, with the T-allele being susceptible in relation to depression (111), and with polymorphisms of dopamine receptors \textit{(DRD2)} (112).

When a person is a carrier of multiple susceptible alleles in different genes, these confers a cumulative genetic plasticity making the person more susceptible to one environmental influence (113).

\textit{Evolutionary-neurodevelopmental theory}

A new theory based on an evolutionary–neurodevelopmental perspective integrating the biological sensitivity to context theory (BSCT) and differential susceptibility theory (DST) explains the individual \times environment interactions during development, the neurobiological susceptibility to the environment and its regulation effects on adaptation and development (115).

This theory is based on the following concepts. First, there are individuals with high environmental susceptibility for both negative (risk-promoting) and positive (development-enhancing) environments. Second, individuals with high susceptibility to environment experience continued development changes as a response to the environment. Third, this susceptibility is neurobiological and composed of a genetic susceptibility and neurobiological processes, which are expressed in behaviours. Fourth, polygenic variation and developmental experiences determine individual differences in biological susceptibility. Fifth, this susceptibility varies within individuals across the life span. Sixth, differences between individuals are adaptive in an evolutionary perspective and are conserved through changes in selective pressures. Finally, variation of neurobiological susceptibility regulates human development, moderating the environmental exposure (116).

BSCT and DST share the premise that the variation of neurobiological susceptibility is conserved in natural selection and vary in their predictions about the development and distribution of susceptibilities.

In BSCT, the variation of susceptibility is a conditional adaptation under the pressure of an environmental factor. The adaptation is not random. Characteristics of susceptible phenotypes are expressed in highly protective or highly stressful environments and their fitness is improved in both cases (114).

In DST, differential susceptibility could be maintained during evolution, due to the exposure to unpredictable and fluctuating conditions. Strong trades with specialised phenotypes do not perform well in different conditions. Following combined selection pressures, the selection of single phenotypes is
limited and the selection of lineages across generations diversified (117),
which is how the risk is spread (118). This systematic genetic variation could
be a predictor for the variability of susceptibility in members of a family (119).
Models for adaptative genetic variation are in development (120). Gene
frequencies for high and low susceptibility vary in populations. Their pheno-
types have been called ‘orchid and dandelion’ and the phenotype frequencies
vary depending on the gene frequencies in populations (120). For example,
dandelions and orchids complete with each other, but dandelions thrive in
more stable environments, while orchids are more successful in changing en-
vironments (120).

**Plasticity genes**
Gene–environment interaction studies have problems with replication, which
appear to be linked to the theoretical model used to interpret the results. For
example, the diathesis–stress model does not explain susceptibility ‘for better
and for worse’ (see Figure 5).

![Figure 5. Plasticity of gene polymorphism in gene–environment interaction.](image)

Differential susceptibility was missed in the genetics of psychopathology,
probably because observations only looked at risks in a defined context. How-
ever, genes with plasticity show potential to confer, at same time producing
positive or negative outcomes depending on the resources of the environment.
Examples include MAOA, 5-HTTLPR and DRD4 (121).
Moreover, it has been demonstrated that the combination of two genes,
each of which has a significant gene × environment effect, gives more moder-
ating effects (122).

**Genetics of depression**
In family studies, the odds ratio of increased risk for MDD in first degree rel-
atives is 2.84 (95% confidence interval [CI]: 2.31–3.49) (123). In twin sam-
ple, the estimated heritability of MDD is 38% (124). Sex-specific heritability
was reported by one study to be 40% for women and 30% for men (125), while other studies have failed to demonstrate differences (125, 126).

Genome-wide association studies (GWASs) are used for screening and for replications to examine candidate genes. GWASs focus on variations in genome greater than 5%. The genome-wide significance threshold for 5% frequencies is $5 \times 10^{-8}$ (127). However, significance thresholds vary depending on the number of variants tested.

Allele frequency is reported for the less-frequent allele in diallelic single-nucleotide polymorphism (SNP), which is called minor allele frequency (MAF). SNPs are correlated and their distributions are not random due to recombination patterns that generates groups of linkage disequilibrium (128).

GWASs fail to detect MDD, probably because they are underpowered (129). Furthermore, the relation between sample size and effect size is not linear for frequent variables, and the required sample size depends also on the prevalence. Moreover, GWASs have limitations on the effect sizes for variants contributing to MDD (130). In meta-analyses, 200 genes have been studied for association with depression, showing significant relations with 5HTTP, SLC6A4, SLC6A3 and DRD4.

Candidate gene studies in MDD have poor support (131). SNP heritability for MDD is 21–30% (132, 133), but common variants with small effects contribute to genetic susceptibility with more than 50% of the heritability. In complex traits their polygenic nature with multiple loci of small effect (132).

Symptoms, function, and diagnoses

Adolescence is a phase of life that begins with pubertal maturation and ends with the assumption of adult roles (134). Adolescence is a fundamental stage in development characterised by a transition in which physical and sexual maturity is reached and independence and autonomy are developed (134).

At this stage, important cognitive and moral development occurs, including development of the notion of the identity (135-137). The ability to develop relationships outside the family is of particular importance and adaptation to family, school and cultural environments are fundamental (138).

The increased intensity of emotions in this phase constitutes a risk for the development of mental disorders (134). There is also an increment of self-assessment capacity with expanded capacity for self-evaluation of emotional state (134). In early and middle adolescence, increased levels of negative affect occur, followed by a decrement of daily positive affect levels after middle adolescence (139).

Longitudinal studies allow a better follow-up of the physical, hormonal and neurobiological changes that occur during adolescence (140).
Symptoms and disorders

The observation and assessment of behaviour allows the recognition of symptoms, which are considered as dimensional measurements of continuous characteristics in relation to established functioning, generally for age and sex and context (141). These symptoms can occur simultaneously or evolve over time in disorders (142, 143). Syndromes are defined by the identification of a cluster of symptoms and their related patterns (144).

Medical disciplines must recognise and guide the pathophysiology, course, prognosis and treatment of disorders. Categories must be used to assess and classify the level of functioning or dysfunction and diagnoses. It is important to note that to determine a diagnosis, the presence of a level of dysfunction in more than one context is mandatory (1, 145). Nowadays, the diagnostic manuals in use are the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) (1) and International Classification of Diseases, 11th Revision (ICD-11) (146).

Functioning in disorders

Having a classification of dysfunction is important because it improves communication between patients and health-care professionals, favours patient collaboration in rehabilitation and allows identification of individual functioning (147). The assessment of functioning includes the analysis of the interactions of bodily functions, activities and participation in a context and, with individual conditions, allows us to determine the impairment, the limitations and the restrictions, respectively (148) (see Figure 6).

![Figure 6. Interactions among the components of the International Classification of Functioning, Disability and Health (148).](image)

Functioning refers to bodily functions and activities, while disability refers to limitations and restrictions. In each mental diagnosis, common dysfunction patterns are found, such as internalising versus externalising disorders (149).
Diagnoses

The best estimate of lifetime psychiatric diagnosis (150) and the Longitudinal, Expert, All Data (LEAD) procedure (151-154) are considered the gold standard for the diagnosis process. LEAD requires the collection of all existing information from various sources at consecutive points in time.

Diagnoses should be valid and reliable (155, 156) because misdiagnoses have negative consequences, such as ineffective, unnecessary or risky treatments and failures in risk assessments. The use of validated diagnostic tools contributes to the reliability of data and is regarded as a better approach to the determination of the diagnosis (157, 158).

Information sources are diverse and include parent and adolescent interviews, family history and medical records (150). The information from various sources often has a low level of concordance (159-161). Other issues are that one symptom can be present in several different diagnoses and that comorbidity is common (162-164).

Diagnostic tools

Clinical interviews

The agreement between professionals is low when assessments are conducted without semi-structured interviews. In addition, quality improves when validated interviews are used (165-169).

In psychiatry, semi-structured interviews are associated with the mental diseases classification system. The structured clinical interview for DSM-III-R (SCID) (170, 171) and the structured clinical interview for DSM-5 SCID-5 (172, 173) are diagnostic interviews for adult populations. The Kiddie Schedule for Affective Disorders and Schizophrenia, Present and Life version (K-SADS-PL) is used for children and adolescents (6–17 years), and their parents (174-176).

The K-SADS is used to interview adolescents with their parents or separately, and the rating includes the expert’s assessment of symptoms and functioning to determine whether the diagnoses are registered present or lifetime.

The K-SADS is composed of a screening interview with 23 domains and eight supplements grouped as affective disorders, psychotic disorders, anxiety disorders, behavioural disorders, SUD, eating disorders, tic disorders and autism spectrum disorders, allowing examination of 52 DSM-IV psychiatric diagnoses (176, 177). If a disorder is screened as positive in the introductory interview, then the related supplement is completed. After all required supplements are completed, the expert codes the present diagnoses.

The inter-rater reliability can be measured using $\kappa$ or prevalence-adjusted $\kappa$ (178). The Swedish version was validated against the LEAD in a clinical sample of Swedish adolescents by Jarbin and colleagues (176). The appropriate use of the Swedish version of the K-SADS requires 4 days of theory and
practical training (179). In this thesis, the K-SADS with present diagnose was used as the gold standard for diagnosis.

**Scales of function**

The Global Assessment Scale (GAS) (180), the Global Assessment of Functioning (GAF) (181-183), and the World Health Organisation’s Disability Assessment Schedule (1) have been used to assess functioning in adults. The Disability Assessment Schedule is applicable in general and clinical populations, and across all diseases and disorders. It covers six domains and is related to the International Classification of Functioning Disability and Health. Moreover, Sheehan and Sheehan (145) developed a short self-report, discan scale to assess disability with three items asking about work, social relations and family. This Sheehan Disability Scale (SDS) was translated into Swedish and validated in a population of psychiatric patients (184).

Comparable to scales for adults, the Children Global Assessment Scale (CGAS) and the GAF (185) are used in child and adolescent populations. In addition, Whiteside (186) developed a scale based on SDS to assess child and parent impairment related to childhood anxiety disorders.

**Scales of depression**

The applied use of scales for depression varies depending on whether they are used to identify symptoms for screening purposes or to assess the severity of symptoms (187-189). Validated scales facilitate data collection and screening as a part of the diagnostic process and are recommended by Statens Beredning för Medicinsk och Social Utvärdering (190). There are scales for general use as functioning scales and specific scales to measure symptoms of a defined disorder. Scales can be constructed for professional assessment or self-report (191). Depressive symptoms in adults are frequently measured with the Beck Depression Inventory (BDI) (192, 193), the Hamilton Depression Rating Scale (194, 195) and the Montgomery–Åsberg Depression Rating Scale (MADRS) (189, 196). The MADRS includes 10 items and has been shown to be sensitive to change; therefore, it is useful for following up the effect of treatments. The MADRS-S is a 9-item self-report version that has been validated in adolescents and shows good to excellent diagnostic accuracy properties (197). In this thesis, the parent version, the MADRS-P is presented.

**Scales properties**

To study the properties of scales, the reliability—how well the scale measures the construct every time (198)—and the validity—whether the scale measures what is intended to be measured (199)—are considered.

Reliability refers to the stability of measurement; if the same instrument is used in the same conditions, it should give the same results (200). However, each observed measurement is composed by a true component and a variable error (200). To evaluate reliability, methods such as the estimation of the true
value or the mean of repeated measures are used. The test–retest method is used to determine the variation between the results of measurement at various times. Inter-rater reliability is used to measure the level of agreement between observers when they rate the same event (198). The measure of reliability is expressed as $\kappa$ or Pearson coefficients (198).

The $\kappa$ coefficient is used to measure the proportion of the agreement (201) and it is appropriate for dichotomous–ordinal scales. An overall $\kappa$ can be calculated to estimate the total agreement. If the partial agreement is relevant, then it is better to use a weighted $\kappa$ (202). In any case, the $\kappa$ measurement is affected by the level of observer bias and the prevalence of the observed phenomenon (178). The prevalence- and bias-adjusted $\kappa$ (PABAK) method helps to solve this problem (178, 203, 204).

Internal consistency is quantified as Cronbach’s $\alpha$ (205) as a measure of how homogeneous a scale is and how well its items correlate with the measured variable in a defined population (187, 198, 206). Cronbach’s $\alpha$ is calculated as an average of correlations between all items. Values range from 0 to 1, with the current acceptable value above 0.7 and below 0.9 (207).

Principal component analysis (PCA) (208) searches for the relations between items that allow them to be grouped together and eventually reduces the number of redundant items.

There are several types of validity. A measure or scale has content validity if it is related to the concept to be measured (i.e. face and sampling validities) (198, 200). Criterion or predictive validities indicate how the scale items are related to the measured trait (187). Concurrent validity is tested when an evaluated instrument is compared with a standardised instrument for the measure of a defined variable (187, 209), while construct validity reflects the relationship between the instrumental and theoretical variables (187, 198).

Spearman’s rank correlation coefficient was used to study concurrent validity. This is a non-parametric equivalent to Pearson’s correlation (i.e. Spearman’s $\rho$) (210), which is used to estimate the strength of a linear relation between two continuous variables for its true value when the variables are skewed. The value can range from −1 to 1 and be considered positive or negative. A score of 0.9–1 is considered very high, 0.7–0.9 is high, 0.5–0.7 is moderate, 0.3–0.5 is low and 0.0 to 0.3 is negligible (210).

**Diagnostic accuracy properties**

The convenience of using one test depends on its capacity to distinguish cases from non-cases. The properties of a new test are compared with the gold standard test and used to categorise the presence of a disease. The positive and negative results of both tests are compared in a $2 \times 2$ table. The diagnostic accuracy of a test is studied considering such measures as sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and positive
and negative likelihood ratios (LR+ and LR−, respectively) for different cut-off values (207, 211, 212) (see Table1).

Table 1. Formulas for sensitivity, specificity, PPV, NPV, LR+ and LR−.

<table>
<thead>
<tr>
<th>New test</th>
<th>Gold standard</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
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<td>Positive</td>
<td>a</td>
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<td>Negative</td>
<td>c</td>
</tr>
<tr>
<td>Total</td>
<td>a + c</td>
</tr>
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</table>

Sensitivity is defined as the proportion of those positive result in the test of those with a disease. Its mathematical expression is: sensitivity = a / (a + c).

Specificity is the proportion of the population who have a negative result in the test of those who do not have a disease. The specificity expression is: specificity = d / (b + d).

PPV is the proportion of the population who have the disease among those with a positive test and is expressed as PPV = a / (a + b).

NPV is the proportion of the population who do not have the disease among those with a negative test result and is expressed as NPV = d / (c + d). PPV and NPV depend on the prevalence of the disease and their usefulness is limited (211). The prevalence of the disease is expressed as prevalence = a + c / n (207).

LR+ is equal to the true positive rate divided by the false positive rate = [a / (a + c)] / [b / (b + d)] or sensitivity/(1-specificity). LR− is equal to false negative rate divided by true negative rate (213) or LR− = [c/(a + c)] / [d / (b + d)] or (1-sensitivity)/specificity (207).

The cut-off values in a continuous scale are used to compare the results of the measurement against defined norms or other groups and as predictors of an outcome (198, 211). These are also used to compare the above-described values between populations and to define the diagnostic accuracy of the receiver operating characteristic (ROC) curves used to describe the performance of the scale in a defined population.

The ROC is used to estimate the area under the curve (AUC), which is a graphical representation of a curve that plots each cut-off point of a scale on the X-axis (1-specificity) and the Y-axis (1-sensitivity) (214). These curves permit identification of the cut-off level at which the highest sensitivity and specificity are attained, assessing the diagnostic accuracy of the test. The ROC are used to compare the performance of two tests (198, 215) (see Figure 7).
The AUC indicates the overall performance of the test (216, 217). In the figure, a diagonal line that joins the points (0,0) and (1,1) is traced. This line serves to find the Youden Index point (218), which indicates the optimal cutoff point for a test (218), identifying the point that is located in the curve at the maximum distance of a perpendicular line drawn from the diagonal between the first and the last point of the ROC.

Despite diagnostic tests being designed to identify cases in a population, false negative and false positive errors can happen. Therefore, the Youden Index is defined by the expression $J = \frac{(ad - bc)}{(a + b)(c + d)}$, where ‘a’ is correctly diagnosed, ‘b’ is false negative, ‘(c + d)’ are controls, ‘d’ is correctly reported and ‘c’ is false positive. The Youden Index value ranges from 0 to 1. If the tests report the same proportion of positive results for disease and control groups, the value of the index is 0 and only when there are neither false positives nor false negatives can the result be 1. The means of standard error of the indexes can be used to compare two diagnostic tests (218).

The AUC compiles the discriminative capacity of the test over the complete spectrum of cut-offs. A value of $> 0.9$ is considered high, 0.7 to 0.9 moderate from, 0.5 to 0.7 low and 0.5 as chance (216).
Environmental factors

Stress
Childhood stress has been associated with an increased risk for depression (219). Adverse experiences in childhood increase the risk of later mood disorders by 22.9% (220). Stress factors in childhood include disabilities, chronic illness, dysfunction in family relations, parental substance abuse, poverty and stress in the family and community (221, 222).

Stress sensitivity and the neurobiological changes induced by stress are mediators of the risk for depression (223, 224). Prolonged stress increases the levels of glucocorticoid in the brain, which induces changes in the hippocampal formation (225, 226). Significative reductions of hippocampal white matter have been found in male adolescents with depression exposed to emotional neglect using high-resolution magnetic resonance images (223).

Another significant aspect in vulnerability for depression is the modification of the expression of the genes by deoxyribonucleic acid (DNA) methylation after stressful experiences (227-229). In addition, exposure to stress downturns the expression of BDNF in the limbic region and decreases neurogenesis (230). It is also known that the interaction between BDNF and 5 HT transporter gene is related to depression (231, 232).

Candidate genes related to stress and depression are glucocorticoid receptor (NRC1), BDNF, serotonin transporter (SLC6A4) and monoamine oxidases (MAOA and MAOB) (233, 234).

Maltreatment
Childhood maltreatment (CM) can take different forms, including physical abuse, sexual abuse and neglect. CM does not occur randomly: that is, children who experience one type of CM are more likely to experience any other type (235). In a general population, 30% experienced maltreatment (236). Of those exposed, 49% had suffered more than one type of CM (237). Women with low socio-economic status in the Minnesota Longitudinal Study of Parents and Children reported physical abuse (40%), sexual abuse (27.6%) and neglect (32.8%) with a 22.9% rate of concurrence (237). The evidence is insufficient to identify gender differences in the effects of maltreatment (238). Risk factors include being younger than 4 years or being an older adolescent, having special needs, being unwanted, having parents who have been maltreated and who have SUD or poor impulse control, economic problems or criminal activity (239).

As explained above, early stress and maltreatment produce changes in brain development at neurohumoral levels, especially in the HPA axis, and at varied structural levels (240).

Depending on the type of CM, the exposed person may show diverse behavioural and emotional problems in late adolescence (237). Subjective
experience, severity, type of CM experienced, duration and frequency are relevant to the development of CM symptoms (241).

The association of CM and depressive symptoms differs across ethnic groups (242) and their antecedents influence the course and recurrence of depression (243). One reason is that victims receive negative messages and criticism that undermine their self-esteem (244). Emotional abuse is a moderator in the significant association between depression and maltreatment. Its effect is larger than the effect of physical abuse (245). Psychopathology associated to physical maltreatment declines over time, while that related to sexual abuse remains (246). In addition, early maltreatment has a greater association with suicidality (247).

Information about CM is obtained most from retrospective self-reports (237), although the association between CM and depression is also found in prospective studies (248).

With regard to genetics, Caspi et al. (108) studied the role of the MAOA genotype in violence after maltreatment, and Nilsson et al. (249) studied the association of MAOA variants, poor quality relations in families and alcohol problems.

Sleep
In adolescence, sleep patterns and sleep architecture change (250, 251). Adolescents find it more difficult to wake up in the morning and easier to stay up later at night (252). In general, there is a reduction in the sleep time due to delayed sleep, although sleep time is longer in the weekends (253). Other circumstances that influence sleep hours are school, homework and the use of television and electronics (252, 254).

The circadian rhythm fluctuates over a 24-hour period and the homeostatic process is related to the number of hours sleeping or awake (255). Sufficient sleep is needed for adequate mood functioning (256).

There is a bidirectional relationship between sleep disturbances and mental health. Insufficient sleep is related to hopelessness and suicide ideation (257) and sleep quality is related to mood (258, 259) and depression (260-262).

Moreover, sleep is important in attention regulation, cognitive functioning (263), working memory and long-term memory (263), in addition to the restoration of functioning, learning and memory (266).

Estimates of the sleep hours needed by adolescents are related (e.g. 8.5–10 hours are required at 13 years of age while 8.5 hours are required at 18 years) (253). The prevalence of sleep disturbances is estimated to be 25% among adolescents (267-269).

Candidate genes in sleep disorders are 5-HTTLPR, Period 3 (PER3), circadian locomotor output cycles kaput (CLOCK) and catechol O-methyltransferase (COMT). Per2 (SNP) 10870 (A/G) is related to sleep problems among male adolescents (273).
Physical activity
In large population studies, the estimate of physical activity (PA) is a mean of one hour per day, with at least in moderate intensity for 20 minutes 3 or more times per week. The above found in two thirds of males and one quarter of females in adolescents. Activity level decreases with age most notably in females (274).

Adolescence is a period when risky behaviours can be prevent (275). Directs effects of PA on health are difficult to measure, on the contrary to increase in one hour a day sedentary behaviour increases depression in 8-11% at the age of 18-year-old (276).

In transition to young adults many adolescent change patterns increasing sedentary time. Increasing school physical education and reducing dropouts from sports could be prevented sedentarism (277).

Aerobic exercise reduces anxiety and depression and influences HPA axis regulating the reactivity to stress(278). An additional benefit of sports is to have more opportunities to social interactions (279). By contrast, the levels of PA are diminished in populations with chronic stress (280-282).

Exercise, as is called a structured program of PA, promotes the production of BDNF (283). High BDNF is associated with reduction of anxiety and depression and improvement of cognitive functions (284).

Physical exercise induces BDNF gene expression and BDNF protein influences synaptic plasticity (285). Production of BDNF is increased after PA selectively, however the way is not well understood yet. There are hypotheses related with induction of expression of Fibronectin type III domain containing 5FNDc5, a PGC-1α-dependent myokine (286) or through epigenic changes of BDNF promoter (287, 288).

Genetic
Basic genetics
The Human Genome Project was conducted from 1990 to 2003. One of the goals was to DNA sequence the entire human genome. The Project was a collaboration between researchers from more than 20 universities worldwide. The results of this Project changed the biomedical research literature (289).

DNA is a double stranded helical molecule composed by nucleotides, where the genetic information is coded by the sequence order of nucleotides. DNA nucleotides are adenine A, thymine T, guanine G and cytosine C and these are paired as A-T and C-G in the double strand of DNA (290).

Functional molecules called proteins are the result of gene expression. The process requires transcription and translation steps, where transcription takes place in the cell nucleus. The DNA information is copied to ribonucleic acid (RNA), then the messenger RNA (mRNA) comes out into the cytoplasm.
Once mRNA is in the cytoplasm, the ribosomes (i.e. specialised cellular complexes) copy the mRNA sequence, grouping the information from the combination of three nucleotides or a codon. Each codon contains the information corresponding to one amino acid. Finally, RNA transfer (tRNA) adapts the code to sequence the amino acids that make up the protein (i.e. translation) (291) (see Figure 8).

![Figure 8. DNA transcription and translation.](image)

Nucleotide sequences of a gene contains exons and introns. Exon sequences are included in mature mRNA to be expressed, while intron sequences are taken away during RNA splicing but are considered intervening sequences. Introns can occur in protein coding genes, in tRNA genes and self-splicing introns (292).

A SNP is defined as a substitution of a single nucleotide in a specific position in DNA and occurs in at least 1% of the population. SNP occurs in exons and introns (293).

Laboratory techniques, such as polymerase chain reaction (PCR), permit the copying of one specific segment of DNA, which facilitates the identification of SNP. In this process, a synthetic DNA or primer is used to select the segment to be amplified (294). In DNA replication, a DNA polymerase adds the complementary nucleotides to the single stranded DNA, which is used as template. The application of this technique allows identification of gene variants, some of which have been associated with diseases (295).
BDNF

The *BDNF* gene provides instructions for the BDNF protein and is located on chromosome 11 position 27658369 or 11p14.1, and is composed of 12 exons (296).

The SNP called Val66Met or *rs6265* is a polymorphism with G- and A-alleles, which code the amino acids, methionine and valine, respectively (296, 297) (see Figure 9).

![Chromosome 11 BDNF rs6265 (Val66Met)](image)

Figure 9. BDNF *rs6265*.

The minor allele (A) frequency of *rs6265* varied from 0% in a Maasai group to 27.7% in a Chinese group (296). The A-allele has been considered a risk allele, as A-allele carriers show a limited capacity to process pro-BDNF and have lower levels of neuronal plasticity during their development (298). The A-allele also carries more vulnerability to childhood adversity (299) and is associated with a higher risk of suicide if the individual is depressed (296), in addition to higher levels of cortisol (300). However, the effect of genotype differences decreases with age (301, 302), indicating early sensitive periods (302).

Stress downregulates BDNF (302) while PA increases BDNF levels temporarily (303).

PER2

Data suggest the implication of *CLOCK* genes in the control of the HPA axis (304) and through MAOA, which increases dopamine levels (305).

Also called Spanagel–Albrecht or SNP #10870, PER2 *rs56013859* is a variation in intron 3 that contains potential transcription binding motifs (306) and
is located in chromosome 2:238276865 (272, 307-309) with the T- and C-alleles (see Figure 10).

The minor allele (C) frequency is 1% and rs56013859 has been studied in addictions. PER2 rs56013859 has been studied regarding alcohol use and stressful life events in young adults. In this one, minor allele carriers were less inclined to alcohol use (310). In another study, male AA carriers showed sleep problems and high alcohol consumption (272).

Figure 10. PER2 rs56013859 (309).
Aims

The overall aims of the first two papers were to improve the availability of validated tools for the assessment of depressive symptoms and functioning in adolescents referred for psychiatric assessment. The aims for the final two papers were to increase knowledge about the interaction of environmental factors with candidate genes in the development of depressive symptoms in a cohort of adolescents.

Specific aims

Paper I
To explore the psychometric properties and the diagnostic accuracy of the MADRS-P in adolescent psychiatric outpatients in Sweden.

Paper II
To evaluate the psychometric properties of the Swedish Child Sheehan Disability Scale for self-report (CSDS) and parent report (CSDS-P) in a sample of adolescent psychiatric patients and their parents.

Paper III
To explore the relation between BDNF rs6265 polymorphism, in interaction with childhood stress in relation to depressive symptoms, as well as the possible moderating effect of PA, in the Survey of Adolescent Life in Västmanland (SALVe) cohort.

Paper IV
To analyse the three-way interaction of sex, rs56013859 polymorphism and family maltreatment on depressive symptoms in the adolescents from the SALVe cohort.
Method

This thesis is based on four papers. Paper I and Paper II were included in the licentiate thesis *Assessment of Depressive Symptoms and Functioning in Adolescent psychiatric patients* by the author in 2019.

Study designs and settings

A cross-sectional design was used for Papers I and II. Studies were performed with a consecutive sample of adolescents and their parents who were referred to two child and adolescent outpatient psychiatry units in the county of Västmanland, Sweden between September 2011 and June 2013. The Sala clinic was in an area with 21,568 inhabitants in 2011 and the Västerås clinic was in an area with 130,000 inhabitants. In Papers III and IV, the data were obtained from participants in the SALVe cohort at three time points (i.e. 2012, 2015 and 2018). Västmanland county had 249,974 inhabitants in 2011 and 273,929 in 2018. In Paper III, the analyses were cross-sectional at the three time points, while in Paper IV, the analyses were cross-sectional in 2012 and longitudinal from 2012 to 2015 and from 2012 to 2018.

Participants

The participants in Papers I and II were recruited from patients aged between 13 and 17 years who were referred to the outpatient psychiatry units, regardless of the reason for consultation. Exclusion criteria were intellectual disabilities or the patients’ or parents’ deficiency in Swedish-language skills. Two hundred and two patients were eligible, 125 of whom agreed to participate and gave their written informed consent. In Paper I, 101 patients provided full information in response to the K-SADS and MADRS-P. In Paper II, 107 adolescents provided full information in response to the K-SADS and CSDS, and 104 provided full information on the Strengths and Difficulties Questionnaire (SDQ). Their parents completed the CSDS-P \( n = 107 \) and SDQ \( n = 104 \) (see Figure 11).
The participants in Papers III and IV were sourced from the SALVe cohort study. In 2012, there were 5,233 adolescents born in 1997 or 1999 living in Västmanland county. After excluding individuals who had lived in Sweden for less than 5 years ($n = 358$) and individuals with mental disability or severe illness, 4,712 adolescents were eligible for participation in the studies (see Figure 12).
In Paper IV, 533 of the adolescents born in Västmanland county were excluded because of their language difficulties, illness, not living in Västmanland or did not have the informed consent of their legal guardian if they were younger than 15 years old. An additional 2,866 declined to participate, did not respond or had missing forms. In total, 1,834 adolescents were included in 2012. At the second time point in 2015, 2,334 adolescents were invited to participate, together with 500 of whom were non-responders in 2012. A total of 1,575 were followed up. New participants in 2015 were 69. In total, 1,212 adolescents were included in 2018.
of 1,644 participants were included and 690 excluded at this time point. At the third time point in 2018, 1,920 were invited, including 276 who had participated in 2012 but not in 2015. In summary, 1,212 participants were obtained and 708 were excluded (see Figure 12).

Measures

Symptoms and functioning

The clinical interview Schedule for affective and schizophrenia for school-age children—Present version

The clinical interview Schedule for affective and schizophrenia for school-age children—Present (K-SADS present version, hereinafter referred to as K-SADS), was used as the gold standard in Papers I and II.

The K-SADS is a semi-structured interview designed to assess psychopathology in children and adolescents according to the DSM-III and DSM-IV criteria (174). It is an integrated parent–child interview and diagnoses are made after the clinical evaluation. For the assessment of major depression, agreement between interviewers has reached 91% (176). It has good to excellent reliability in the test–retest assessment for most diagnoses (174). The K-SADS-PL-2009 was has been translated into Swedish and validated in comparison with the LEAD procedure (176). The concurrent validity for any depressive disorder was \( \kappa = 0.91 \) in the Swedish sample (176). The K-SADS consists of a symptoms screening section with 105 symptoms describing 25 diagnoses, and eight supplements that gather information about the disorders that were positively evaluated in the screening interview. Supplements assess affective, psychotic, anxiety, behavioural, SUD, eating, tics and autism spectrum disorders (176). The items in the supplements are rated using a 4-point Likert scale (0 = no information is available, 1 = absence of the symptom, 2 = subthreshold symptom level and 3 = presence of the symptom).

For depressive symptoms, there are eight items (range, 0–24) in the screening interview and 20 items (range, 0–60) in the supplement. The K-SADS-PL MMD severity score (range, 0–84) is calculated as the sum of the ratings of depressive symptoms without functioning items.

The K-SADS symptom summation index (range, 0–1,488) is calculated as the sum of the severity index for each item evaluated in the screening interview and in the supplements.

Interviewers received basic training in theory and practice for the K-SADS over 4 days and were supervised by the child and adolescent psychiatrist who participated in the national programme for K-SADS trainers before the start of the interviews. Inter-rater reliability was calculated, with the reference being the specialist who led the training. The overall \( \kappa \) for all the interviewer
pairs for all diagnoses for the initial five interviews was 0.92 and PABAK was 0.94. The $\kappa$ for a MDD diagnosis was 0.89 and PABAK was 0.90, which is considered excellent (178, 203).

**Montgomery and Åsberg Depression Rating Scale, parent report**

The properties of the MADRS-P were studied in Paper I. The MADRS-P is based on the Montgomery–Åsberg Depression Rating Scale, Self-report (MADRS-S) (189), which was originally designed for application among adult patients. The MADRS-P is analogous to the MADRS-S and is designed to collect parents’ information about their adolescents’ depressive symptoms. For this purpose, the subject in the questions was reworded (i.e. ‘you’ was replaced with ‘your youth’). The MADRS-P is composed of nine assessment items (i.e. 1 = mood; 2 = tension or feelings of unease; 3 = sleep; 4 = appetite; 5 = ability to concentrate; 6 = lassitude; 7 = inability to feel; 8 = pessimism; and 9 = zest for life, each measured on a subscale from 0–6). The total range for the scale is 0–54 (see the Appendix).

**Child Sheehan Disability Scale, self-report and parent report**

The properties of the CSDS and CSDS-P were studied in Paper II. These scales were developed from the SDS (311), which is used to assess functioning in adults with mental illness and is validated in a Swedish population (184). Whiteside (186) developed the CSDS and CSDS-P for use among children and adolescents with anxiety. Both scales have been translated into Swedish. However, one change was made to the text to assess general functioning about ‘troubles and feelings’ instead of ‘fears and worries’ (186). The research group translated and back-translated both scales.

The CSDS for adolescents has three items measuring impairment at school, and in social and family life. Items are scored on a 11-point Likert scale (0 = not at all to 10 = extremely). Each item is rated in words, numbers and visual space with a discan method (i.e. three visual levels for scores with a range of 0–10 for each item). The total score range is 0–30.

The CSDS-P for parents includes two questions in relation to the adolescent’s school and social life and three additional questions about the adolescent’s interference in the parent’s work, social and family life. Each item is rated on an 11-point Likert scale (0 = not at all to 10 = very, very much) using the discan method for all answers. The total score range is 0–50 (see the Appendix).

**Adolescent Strengths and Difficulties Questionnaire**

The adolescent version of the SDQ was used as a reference tool in Paper II. The SDQ (312-314) was developed from the Child Behaviour Check List (141, 315), with versions for children and adolescents (11–17 years) and parents. The two versions consist of 25 items grouped in a subscale that assess pro-social behaviour and another four subscales that assess difficulties related
to emotional symptoms, conduct problems, hyperactivity/inattention and peer problems. Each subscale consists of five items rated on a 3-point Likert scale (0 = not true, 1 = somewhat true and 2 = certainly true), and items 7, 11, 14, 21 and 25 are rated in reverse order.

**Depression Self-Rating Scale**

The Depression Self-Rating Scale (DSRS) was designed using the DSM-IV criteria for MDD (177, 316) and used in Papers III and IV. The symptom criteria (range, 0–9) were based on the DSM-IV criterion for MDD. The DSRS has been used as a diagnostic tool in epidemiological research (316).

In Paper III, the assessment of depressive symptoms at all three time points was in relation to symptoms experienced during the past 2 weeks. The total number of symptom was calculated as a continuous variable, ‘number of depressive symptoms’, and also as a binary variable, ‘showing depressive symptoms’, if at least one of the general criteria and four more criteria symptoms were present, as reported previously (317).

In Paper IV, assessments of depressive symptoms were made at all three time points, with only eight questions selected for the core symptoms depressive index based on the DSRS (318, 319).

The selected questions were: ‘1. …have you felt down, sad, or empty, almost all the time, almost every day? 2. …have you felt annoyed, angry, or upset almost all the time, almost every day? 3. …almost every day, have you felt disinterested in most things or found it difficult to enjoy the things you normally enjoy? 4. …have you been feeling weak, tired, or low on energy? 5. …has your self-confidence been worse than usual? 6. …have you felt guilty or worthless? 7. …have you had difficulty thinking or concentrating? 8. …have you had thoughts about death, or have you thought that it would be better to be dead?’ The self-rated answers (no = 0, yes = 1) were added together in a core depressive symptoms index (range, 0–8). The Cronbach’s $\alpha$ for the scale was 0.82, 0.84 and 0.86 in 2012, 2015 and 2018, respectively. In a PCA, where the factors were extracted based on eigenvalue 1, all three time points showed one component.

**Suicidal ideation**

In Paper IV, two questions were used for the assessment of suicidal ideation. The first question about suicidal ideation was from the DSRS and was included in the core depressive symptoms. The second question was ‘…have you had recurring thoughts of taking your own life?’ Each question was rated as no = 0 or yes = 1. The two questions formed the variable ‘thoughts of death and suicide’. If both questions were answered in the affirmative, the variable was coded as yes = 1, otherwise the variable was coded as no = 0.
Sleep assessment

In Paper IV, self-reports on sleep-related issues during the past 3 months were collected. The questions were based on the Karolinska Sleep Questionnaire (KSQ) (320). The number of included questions varied at each time point, with 18, 19 and 16 questions in 2012, 2015, and 2018, respectively. The responses to each question were made on the original 6-point Likert scale (0 = never, 5 = almost always/5 times per week).

To evaluate the consistency of the KSQ factor structure over time among this adolescent population, we selected the items available at all three time points: ‘Have you been bothered by the following complaints during the past 3 months; 1. ... difficulties falling asleep. 2. ...difficulties waking up. 3. ...repeated awakenings with difficulties falling asleep again. 4. ...not well-rested on awakening. 5. ...premature (final) awakenings. 6. ...disturbed/restless sleep. 7. ...feelings of being exhausted at awakening. 8. ...sleepy during school/work. 9. ...sleepy during leisure time. 10. ...involuntary sleep episodes during school/work. 11. ...involuntary sleep episodes during leisure time. 12. ...need to fight off sleep to stay awake’. A reliability test of the 12 items revealed Cronbach’s $\alpha$ of 0.89, 0.87 and 0.88 in 2012, 2015, and 2018, respectively.

Moreover, PCAs were completed with factors extracted based on eigenvalue 1 using varimax rotation. The PCA revealed two factors with an initial eigenvalue of 5.1/42.4% of the variance for factor 1 and 1.3/10.8% for factor 2 in 2012, three components (i.e. 5.5/46.0%, 1.5/12.1%, 1.1/9.1%) in 2015 and three components (i.e. 5.4/44.6%, 1.5/12.8%, 1.1/9.4%) in 2018. Because of the discrepancy between the factor analyses and the proposed indexes, all items were instead included in a sleep complaints index calculated as the total of the sum of the points obtained for the 12 items, measured once for every time point.

Environmental factors

Childhood stress

In Paper III, childhood stress was assessed using a part of the Neuropattern translational tool, which was designed to detect and treat stress pathology (NPQ–PSQ) (321, 322). This tool was translated into a Swedish version by the SALVe research group following recommended procedures (323, 324) and adapted by adding more age levels. For example, parents indicated whether their adolescent had experienced some of 19 different stressful events, such as accidents, physical or sexual abuse, time in hospital, emotional neglect and time in foster care during their childhood. The response options were ‘no’ or ‘yes’. When the response was ‘yes’, then the parents indicated the age at which the event occurred (i.e. 0–1, 2–5, 6–10 and 11–15 or unknown).

In an additional question, the parents reported their thoughts about all these stressful events by indicating the overall level of stress during childhood on a
continuous variable scale (range, 1–10), where 1 = no stress and 10 = high stress (321). The parents’ information about childhood stress was collected in 2015 and only a 10-point Likert scale was used.

**Assessment of family maltreatment**
For Paper IV, information about family maltreatment was collected in 2012 based on the adolescents’ self-reports on the four following questions about violence in the family at any time (325): ‘1. Have there been difficult and upsetting arguments between your parents? 2. Has it happened that one of your parents pushed, hit, or used other violence against the other? 3. Have you ever been mentally abused (e.g., mocked, insulted) by one of your parents? 4. Has it happened that one of your parents pushed, hit, or used other violence against you?’ Each question had six possible responses (0 = no or has not occurred, 1 = yes, less than once a year, 2 = yes, once a year, 3 = yes, once a month, 4 = yes, once a week and 5 = yes, every or almost every day). The total sum of the answers was calculated into the family maltreatment index (range, 0–20). The Cronbach’s $\alpha$ was 0.57 and the PCA showed one component, with an eigenvalue 2.1/51.2% of the explained variance.

**Physical activity**
In Paper III, the participants self-reported PA during leisure time lasting >30 minutes associated with increased breathing/sweating. The seven options were: every day, 4–6 times a week, 2–3 times a week, once a week, 1–3 times a month, less than once a month and never. The answers were coded into a six-point continuous variable scale (0 = never, 6 = every day). The PA self-reports were completed using the same tool at all three time points.

**Genetic**

**Genotyping**
Saliva samples were obtained through a self-collection kit (Oragene DNA, DNA Genotek Ontario, Canada) in 2012. The genotyping rate for all eligible study participants was 86.5%. DNA extraction was performed using a silica-based method (Kleargene™, LGC Biosearch Technologies, Hoddesdon, UK) from 200 $\mu$L of saliva. Genotype variants were achieved using KASP™ (LGC Biosearch Technologies).

**BDNF rs6265**
In Paper III, BDNF rs6265 (Val66met) (G/A) polymorphism was described as GG = 0, GA = 1 and AA = 2.
**PER2 rs56013859**

In Paper IV, the rs56013859 C/T polymorphism was described as CC, CT and TT. For the statistical analyses and due to the low frequency in the population of the homozygote polymorphism CC \( n = 25, 1.4% \), it was added to CT \( n = 364, 19.8% \) and the variable was dichotomised as CC/ CT and TT.

**Statistical Analysis**

The statistical analyses were conducted using IBM SPSS software (v. 24, IBM SPSS, Armonk, NY) running on Windows 7 and 10 (Microsoft, Redmond, WA).

**Descriptive statistic**

In Papers I–IV, the descriptive statistics included sample size \( n \), categorical variables that were measured with frequencies and percentages, and continuous variables with central tendency (mean) and dispersion (standard deviation, SD) (208).

The chi-square test was used to study the association between two categorical variables. The null hypothesis is that there is no association between the variables in the studied population. In the test, the expected frequencies are calculated as if there is no association, and expected frequencies are compared with the observed frequencies. The probability was based on the chi-square distribution with \( p \)-values (207).

A \( t \)-test was used to compare the means of two samples because it permits the calculation of the CI differences and requires normally distributed data (207).

The Mann–Whitney \( U \) test is a non-parametric rank test that compares two means (207).

**Measurement reliability and validity**

**Reliability**

In Papers I and II, PABAK was used to measure inter-rater reliability between K-SADS interviewers. The Cronbach’s \( \alpha \) (205) was used in Papers I, II and IV. PCA was used to explore the properties of the following scales: MADRS-P in Paper I, CSDS/CSDS-P in Paper II and the core depressive symptoms and sleep complaints indices in Paper IV.

**Concurrent validity**

To explore the correlations, Spearman’s \( \rho \) was used in Papers I and II.
Diagnostic accuracy
In Paper I, the sensitivity, specificity, PPV, NPV, ROC and AUC, divided by sex and the full population, were studied. The LR+ and LR− and the LR nomograms for MADRS-P at scores 10 and 15 were obtained for the whole sample.

Regressions
Linear regressions study the relationship between two numerical variables of each subject in a sample. The simple linear regression is defined by the equation $Y = a + bx$, where $x$ is the independent variable, $Y$ is the dependent variable, $a$ is the estimated value in $y$ when $x = 0$ and $b$ is the slope of the estimated line, which is called the regression coefficient (326).

Multiple linear regressions were used to study diverse explanatory variables on a response variable. The equation is $Y = a + b_1x_1 + b_2x_2 + \ldots + b_kx_k$, where $x$ is an explanatory variable ($i = 1, 2, 3 \ldots$), $Y$ is the mean of the expected predicted variable, and $b_i$ is the partial estimated coefficient that represents the value increased in $Y$, if $X_i$ increases in one unit and other $X$s are constant. Multiple linear regression permits identification of explanatory variables, considers the extend of the explanatory variables and predicts the value of the dependent variable (326). If an explanatory variable is binary, we increase $X$ by one unit from the reference and estimate the mean values of the difference of $Y$ after adjusting for other predictor variables.

Analysis of variance looks at the variation on $Y$ due to the variation explained by the regression and the remaining variability—the residual error. This gives the proportion of the variation explained by the $R^2$ regression (326).

Logistic regressions are used to study the relation of predictors to a binary outcome, which measures the probability that an individual who has a covariate pattern of predictors presents the studied outcome. The probability has a cut-off (generally 0.5). A logit transformation is conducted, and the logistic regression equation is $\text{logit}(P) = a + b_1x_1 + b_2x_2 + \ldots + b_kx_k$, where $X$ is an explanatory variable, $P$ is the estimated value of the true probability that an individual presents the outcome with a particular set of $X$ values, $b_1, b_2, b_3, \ldots$ and so forth. The exponential $e^{b_1}$ is an odds ratio estimate when it is adjusted to all $X$s in the equation and is called an adjusted odds ratio. For each variable, the logistic regression coefficient with standard error, the estimated odds ratio (the exponential of the coefficient) and a CI of the true value were estimated. The Wald test was used to test the null hypothesis (326).

Moderation models
Hayes and Rockwood (327) proposed the use of the moderation analysis to establish ‘when and for whom’ the effect of the studied variable is present. A variable that modifies the effect of another variable in a third variable is
considered a moderator. The linear moderation is the most used method and is represented by the equation; \[ Y = \beta_0 + \beta_1 X + \beta_2 W + \beta_3 XW, \]
where the Xs’ effect on Y is linearly moderated by W.

The conditional effect of X on Y is the weight for X in the equation \((\beta_1 + \beta_3 W)\), which estimates the difference in the Y values between two cases that differ by one unit in their X values at a given value of W (327).

In a moderation model, the Johnson–Neyman method is used to find the region of significance of the conditional effect of X on Y, if the value of a moderator W (continuum) is such that the ratio of the conditional effect to its standard error is equal to the critical \(t\) value. The critical \(t\) value is defined using the \(t\) distribution and is associated with the \(p\)-value = \(\alpha\), the value chosen for the level of significance (328).

In Paper III, binary logistic regression was used to explore the one-, two- and three-way interactions of BDNF rs6265, PA and childhood stress, on showing depressive symptoms. Moderation models 1, 2 and 3 were used to explore the interactions of BDNF rs6265, PA and childhood stress, respectively, on the number of depressive symptoms at all three time points as cross-sectional analyses (see Figures 13–15).

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**Figure 13.** Diagram of a simple moderation (327).

**Figure 14.** Moderation model 2 (327).
In Paper IV, binary logistic regression was used to explore the relation of the three-way interaction of sex, \textit{rs56013859} polymorphism, and family maltreatment on thoughts of death and suicide. In addition, multiple linear regression, analysis of variance and Moderation model 3 were used to explore the relations in the three-way interaction on the core depressive symptoms and sleep complaints indices with the cross-sectional analyses in 2012 and longitudinal analyses as described previously. For easier understanding of the nature of the interactions, visualisation of the moderation model results was used. These models have the strength to permit the quantification of the conditional effect of X on Y for the values of the moderator W. In the visualisation, the Y variable is represented on the Y-axis, the moderator W is represented on the X-axis, and the effect of the values of X are shown by the regression line (328).
Table 2. Synopsis of the methods.

<table>
<thead>
<tr>
<th>Design</th>
<th>Paper I</th>
<th>Paper II</th>
<th>Paper III</th>
<th>Paper IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participants</td>
<td>129 participants, aged 12-18 years and their parents.</td>
<td>125 participants, aged 12-18 years and their parents.</td>
<td>1,337 participants aged 13 and 15 years in 2012, 1,260 participants, aged 16 and 18 years in 2015, and 890 participants aged 19 and 22 in 2018.</td>
<td>1,834 participants aged 13 and 15 years in 2012, 1,644 participants aged 16 and 18 years in 2015, and 1,212 participants aged 19 and 22 in 2018.</td>
</tr>
<tr>
<td>Data</td>
<td>Major depression was assessed by the K-SADS diagnostic interview as reference. The screening tool to be validated was MADRS -P.</td>
<td>Symptoms and function of psychiatric diagnoses were assessed with K-SADS. and SDQ adolescent and parent as references. The screening tools to be validated were CSDS and CSDS-P.</td>
<td>Genotyping of BDNF rs6265 polymorphism (G/A) variants was achieved using KASP™. The DSRS for the assessment of depressive symptoms. NPQ-PSQ for the assessment of childhood stress. Adolescent’s self-reported physical activity.</td>
<td>Genotyping of rs56013859 (C/T) polymorphism was achieved using KASP™. The DSRS for the assessment of core depressive symptoms index. KSQ for the assessment of sleep complaints index. Adolescent self-report for family maltreatment.</td>
</tr>
<tr>
<td>Statistical analyses</td>
<td>t test and Mann-Whitney- U test Chi-squared tests PABAK Reliability Cronbach’s α Principal component analysis Concurrent validity Spearman’s rho Diagnostic accuracy ROC and AUC Sensitivity, specificity positive, and negative predictive values.</td>
<td>Chi-square tests Mann-Whitney- U test Principal component analysis of CSDS and CSDS-P Cronbach’s α Spearman’s rho.</td>
<td>Chi-square tests Mann-Whitney- U test Moderation models 1, 2 and 3 Binary logistic regression models Linear regression</td>
<td>Chi-square tests Mann-Whitney- U test Cronbach’s α Principal component analysis Binary logistic regression models GLM Linear regression (analysis of variance) Moderation model 3.</td>
</tr>
</tbody>
</table>
Ethical Considerations

The autonomy of participants was preserved insomuch as participation was voluntary in all studies. All participants completed the informed consent form and the parents or legal representatives of those under 15 years of age also signed the respective forms. Data handling was confidential.

Using structured diagnostics methods, Papers I and II were expected to show an improvement of diagnostic quality for a more specific treatment. The benefits outweighed the risk because to fill in the forms was considered a low-risk process. The results allowed the validation of diagnostic instruments for a potential application in a broader group of adolescent patients.

Papers III and IV were community studies with forms that were sent to the potential participants. Additional information about the studies were available from the Centre for Clinical Research website where a phone number for the principal investigator was provided. The collection of saliva samples did not imply biological risks for the donor. The storage and sample-processing results were also handled confidentially. The results provided epidemiological data that could be used for subsequent morbidity prevention.

All studies were conducted in accordance with the 2013 Declaration of Helsinki (329).

Papers I and II
The Regional Ethics Committee of Uppsala approved the studies (Dnr 2008/214).

Papers III and IV
The Regional Ethics Committee of Uppsala approved the studies (Dnr 2012/187).
Results

Paper I

The study of the psychometric properties and diagnostic accuracy of the MADRS-P in the 101 adolescent psychiatric outpatients included in the analyses showed the following results. The inter-rater reliability of the K-SADS measured with Cohen’s $\kappa$ was 0.89 and measured with PABAK was 0.93 for the MDD diagnosis. The internal consistency of the MADRS-P measured with Cronbach’s $\alpha$ was 0.85 for all participants. The factor analysis showed two components with eigenvalues above 1 and the explained variances were 4.317/47.97% and 1.374/15.27%, respectively. The first component loaded included feelings of unease, sleep, appetite, initiative, emotional involvement, pessimism and zest for life. The second component included the reduced ability to concentrate, lassitude and sleep. The concurrent validity of MADRS-P in relation to K-SADS MDD severity score was Spearman’s $\rho = 0.580$ for all participants. A ROC analysis showed an AUC of 0.786 for all participants (95% CI: 0.694–0.877; $p < 0.001$). No significant differences between the two groups were found (see Figure 7). Table 3 presents the results for sensitivity, specificity, PPV, NPV, LR+ and LR−. The MADRS-P showed a cut-off of 15 for best overall sensitivity, specificity, PPV and NPV for all patients (see Table 3).
Table 3. Cut-off score, and sensitivity, specificity, PPV\textsuperscript{c}, NPV\textsuperscript{d}, LR\textsuperscript{+e} and LR\textsuperscript{−f} in MADRS-P for all participants, males and females.

<table>
<thead>
<tr>
<th>MADRS-P Cut-off score</th>
<th>Sens.\textsuperscript{a}</th>
<th>Spec.\textsuperscript{b}</th>
<th>PPV\textsuperscript{c}</th>
<th>NPV\textsuperscript{d}</th>
<th>LR\textsuperscript{+e}</th>
<th>LR\textsuperscript{−f}</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>8</td>
<td>89%</td>
<td>37%</td>
<td>52%</td>
<td>80%</td>
<td>1.41</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>86%</td>
<td>54%</td>
<td>59%</td>
<td>84%</td>
<td>1.87</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>75%</td>
<td>75%</td>
<td>70%</td>
<td>80%</td>
<td>3</td>
</tr>
<tr>
<td>Males</td>
<td>8</td>
<td>87%</td>
<td>43%</td>
<td>43%</td>
<td>87%</td>
<td>1.53</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>86%</td>
<td>63%</td>
<td>54%</td>
<td>90%</td>
<td>2.35</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>80%</td>
<td>80%</td>
<td>80%</td>
<td>89%</td>
<td>4</td>
</tr>
<tr>
<td>Females</td>
<td>8</td>
<td>90%</td>
<td>30%</td>
<td>58%</td>
<td>73%</td>
<td>1.29</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>86%</td>
<td>44%</td>
<td>63%</td>
<td>75%</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>72%</td>
<td>70%</td>
<td>72%</td>
<td>70%</td>
<td>2.4</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Sensitivity  
\textsuperscript{b}Specificity  
\textsuperscript{c}Positive predictive value  
\textsuperscript{d}Negative predictive value  
\textsuperscript{e}Positive likelihood ratio  
\textsuperscript{f}Negative likelihood ratio

The following figures present the LR\textsuperscript{+} at cut-offs of 10 and 15 in this population. There was an almost threefold increase at LR\textsuperscript{+} when the cut-off increased from 10 to 15 (see Figure 16 and Figure 17).

Figure 16. Positive likelihood ratio nomogram of MADRS-P at cut-off score 10 with a MDD prevalence of 45% in the sample (330).
The MADRS-P, a tool adapted for parent report of depressive symptoms, was validated against the K-SADS with an internal consistency similar to that obtained in adult populations (331-333). The concurrent validity was moderate (210). The ROC showed a moderate accuracy (216). The psychometric properties were acceptable and measurement of the LR > 1 indicates usefulness of the test even though it was small (334). Considering that the MADRS-P collects information from parents and is simple to apply in a short time, we considered its use in clinical practice as being favourable.

**Paper II**

To evaluate the psychometric properties of the CSDS/CSDS-P in adolescent psychiatric patients, their internal consistency and factor structure, and concurrent validity against K-SADS and SDQ were studied.

The inter-rater reliability between the K-SADS interviewers was good to excellent. The overall $\kappa$ was 0.84 (range, 0.54–1.00), while the mean $\kappa$ was 0.89 for depressive disorders, 0.79 for ADHD and 0.64–1.00 for anxiety. The diagnoses were grouped as ADHD (55.1%), MDD (42.1%), social anxiety disorder (SAD) (31%), any other diagnosis (4.6%) and without any diagnosis (6.5%). The studied patients showed a mean number of 2.6 disorders (SD = 2.1), which was higher in female adolescents (3.1, SD = 2.1) than in male adolescents (2.6, SD 2.1).
The cut-off score of 5 was chosen for the CSDS based on previous studies (145, 186, 335) (see Figure 18). The cut-off score of 17 was recommended for the CSDS-P, based on Whiteside (186), and the score showed 80% sensitivity and 50% specificity for identifying functioning disability in adolescents with at least one diagnosis.

Figure 18. Impaired functioning measured by a cut-off of 5 on the CSDS and DSM-IV diagnostic groups: any ADHD, any anxiety and any depression (according to the K-SADS). Note: Due to a graphics software limitation, one participant with depression and a CSDS score < 5 is not represented.

Figure 18 shows there were 87 individuals who had one or more diagnoses and who estimated their functioning to be impaired. Five individuals did not have any diagnosis but believed they had impaired functioning. The 13 remaining individuals did not report any impaired functioning, but the interviewer diagnosed them with impaired functioning. Among the patients with ADHD, anxiety and/or depression \((n = 95)\), 82 reported a total CSDS score of 5 points.

With respect to factor structure, the CSDS showed one component with an eigenvalue and explained variance of 2.19/72.9%. The CSDS-P also had one component with an eigenvalue and an explained variance of 3.09/68.8%. Regarding internal consistency, the Cronbach’s \(\alpha\) was 0.81 and 0.84 for the CSDS and CSDS-P, respectively. The Spearman’s \(\rho\) correlation between the K-SADS symptom summation index and the CSDS was low, while the correlations between the CSDS and the summation index for each diagnosis were moderate for MDD. The correlation between the CSDS-P and K-SADS symptom summation index was insignificant. The correlations between K-SADS function index and CSDS/CSDS-P were low or negligible, except for the correlations with manic and psychotic episodes, which were high. The
correlations between the SDQ and CSDS/CSDS-P were low for the total difficulties score and the emotional problems subscale.

The obtained Cronbach’s $\alpha$ values for CSDS/CSDS-P were similar to the adult SDS (186). The CSDS/CSDS-P showed one component, as found by Whiteside (186). Explanations for the low correlation with the K-SADS symptom summation index include that there are non-linear relations between functioning and each diagnosis, there is a general high comorbidity in the sample, that not all cases with impairment have a diagnosis (336) and variation in the impairment self-reports. In the few cases with manic or psychotic episodes, the scale registered these serious functioning impairments (337, 338). The higher correlation observed for the emotional subscale of the SDQ was probably related to the adolescents’ awareness of emotional symptoms (339).

Hence, the CSDS/CSDS-P are useful for the collection of relevant information from parents and adolescents about adolescent functioning within diverse disorders.

Paper III

This study explores the relationship between BDNF $rs6265$ polymorphism, childhood stress and PA in relation to depressive symptoms.

In the crude analyses of sex differences, there were no significant differences for age. The number of depressive symptoms and proportions of participants with depression were higher in female adolescents at all three time points. Childhood stress was higher in female adolescents than in male adolescents. Furthermore, there were differences in PA at all three time points (Waves 1, 2 and 3 in 2012, 2015, and 2018, respectively) with male adolescents showing more PA than female adolescents in Waves 2 and 3. In addition, allele frequencies of BDNF $rs6265$ did not show differences between the groups.

One-way interactions

The interactions between BDNF $rs6265$ and PA and BDNF $rs6265$ and childhood stress showing depressive symptoms were explored using binary logistic regressions in all three waves.

Two models were conducted in Wave 1, for BDNF $rs6265$ and PA; the first model explored the main effects while the second model explored the main effects and the effect of the interaction. Both models were significant and the significant interaction between BDNF $rs6265$ GA and PA was demonstrated.

Two models were conducted in Wave 2 for BDNF $rs6265$ and childhood stress. Again, the first model explored the main effects, while the second model explored the main effects and the effect of the interaction. Both models
were significant, while the second model showed a statistically significant inter-

raction between BDNF rs6265 AA and childhood stress.

Moreover, moderation analyses with Moderation model 1 (see Figure 13) were used to investigate the PA as a simple moderator with the predictor BDNF rs6265 (i.e. GG, GA and AA). The results showed that the number of depressive symptoms had a significant effect ($R^2 = 0.076$, $p < 0.001$) with a significant change ($R^2 = 0.006$, $p = 0.013$). The interaction for carriers of BDNF rs6265 GA was significant at a low moderator value (PA 2) with a conditional effect of PA, and was also significant at a middle moderator value (PA 4). The Johnson–Neyman region of significance for the conditional effect of BDNF rs6265 on the number of depressive symptoms at different levels of PA was found to be below 1.259 ($p = 0.05$ for 11.97%).

In Wave 2, Moderation model 1 was used to investigate childhood stress as a simple moderator with the predictor BDNF rs6265 (GG, GA and AA) as the outcome and the number of depressive symptoms, with sex and age, as covariates, was significant ($R^2$ of 0.164, $p < 0.001$) and remained significant with the addition of interaction with a significant change ($R^2 = 0.008$, $p = 0.002$). Statistically significant interactions were found between BDNF rs6265 AA and childhood stress, and between rs6265 GA and childhood stress.

The increase in the number of depressive symptoms with childhood stress was less pronounced in rs6265 GA and rs6265 GG carriers, indicating susceptibility to childhood stress in individuals with the rs6265 AA polymorphism.

The Johnson–Neyman regions of significance for the conditional effect of BDNF rs6265 on depressive symptoms at various levels of childhood stress were below 1.561 with 26.71% and above 4.515 with 18.20%.

Two-way interactions

In Wave 2, a binary logistic regression of the interaction between childhood stress and PA with BDNF rs6265 was significant. The overall correct prediction was 76% with statistically significant predictors of sex, PA and childhood stress, as well as the interactions between BDNF rs6265 AA and childhood stress.

Moderation model 2 using the number of depressive symptoms as the outcome was also performed for Wave 2, with BDNF rs6265 as the predictor and childhood stress (W) and PA (Z) as moderators (see Figure 14).

The model summary ($R^2 = 0.182$, $p < 0.001$) and the addition for the interaction between BDNF rs6265 and childhood stress showed a statistically significant change ($R^2 = 0.008$, $p = 0.003$). The combined interactions between BDNF rs6265 and childhood stress and between BDNF rs6265 and PA resulted in $R^2 = 0.009$, $p = 0.006$. The interaction effect for BDNF rs6265 GA and childhood stress and the interaction effect for BDNF rs6265 AA and childhood stress were significant.
The conditional effect of the focal predictor BDNF rs6265 AA was 1.996 (95% CI: 0.399–3.593; p = 0.014) at moderator levels of 5 for childhood stress and 2 for PA, and 1.761 (95% CI: 0.298–3.224; p = 0.018) at moderator levels of 5 for childhood stress and 4 for PA.

Three-way interactions

In Wave 1, moderation model 3 (see Figure 15) with number of depressive symptoms as the outcome variable, the predictor variable BDNF rs6265, and childhood stress (W) and PA in Wave 1 (Z) as moderators showed a significant result ($R^2 = 0.348$, $p < 0.001$). The addition of the interaction had a significant effect ($R^2 = 0.004$, $p = 0.038$). In addition, the interaction between BDNF rs6265 AA, childhood stress and PA in Wave 1 was found to be significant ($\beta = -0.323$, 95% CI: $-0.594$–$-0.051$; $p = 0.011$).

![Figure 19. Interaction between BDNF rs6265 AA, physical activity, and childhood stress at different levels of childhood stress in Wave 1.](image)

In brief, the following significant results were found in Paper III: 1. The interaction of PA and BDNF rs6265 GA in Wave 1; 2. The interaction of childhood stress and BDNF rs6265 AA in Wave 2, and in Waves 1, and 3. The three-way interaction of BDNF rs6265 AA, childhood stress and PA. The sum of the findings suggests that BDNF rs6265 A shows characteristics of plasticity in its interaction with environmental factors. This property is highlighted by the interaction of rs6265 A with a positive environmental factor (PA, which has low intensity protection against depressive symptoms) and the interaction with a negative environmental factor, such as childhood stress, which increases the risk of presenting depressive symptoms to the extent that the exposure to childhood stress increases.
Paper IV

The study explored the relation of the three-way interaction of sex, PER2 rs56013859 and family maltreatment to depressive symptoms. Crude analyses of sex differences did not show differences in age, family maltreatment index, or PER2 rs56013859 allele frequencies at any time. In 2012, female adolescents showed higher frequency of thoughts of death and suicide than male adolescents, while in 2018 no differences were found. Female adolescents showed more sleep complaints and core depressive symptoms than male adolescents at the three timepoints of measurement.

Cross-sectional analyses

In 2012, the linear regression of the relation of the three-way interaction of sex, PER2 rs56013859 and family maltreatment to core depressive symptoms was significant ($\chi^2 (7, 1,634) = 231,685; p < 0.001$). The main effects of sex and family maltreatment were significant, and the three-way interaction in female adolescents with allele TC/CC was significant.

Moderation model 3 (see Figure 15) with outcome core depressive symptoms resulted in $R^2 = 0.132, p \leq 0.001$. The addition of the interaction showed a significant change ($R^2 = 0.003, p = 0.032$). The interaction of rs56013859, sex and family maltreatment was significant with ($t = 2.146; 95\% \text{ CI: } 0.032–0.713; p = 0.032$). Using the Johnson–Neyman method, the conditional effect of sex by rs56013859 on depressive symptoms transitioned from non-significant to significant at 1.110 level of family maltreatment with 19.28% of the sample reporting family maltreatment at or above this level. An analogous model used to study the relation of the interaction to sleep complaints index and a binary regression model to study the relation to thoughts of death and suicide were performed without finding a significant relation.

Longitudinal analyses

In 2018, the result of the linear regression of the three-way interaction in relation to core depressive symptoms was ($\chi^2 (7, 1,061) = 70.428; p < 0.001$), that is, $\beta = 0.592$ (95\% CI: 0.355–0.986; $p = 0.044$), a relation with male adolescents who were carriers of allele TC/CC. Analysis with Moderation model 3 gave $R^2 = 0.253, p \leq 0.001$. There was no significant change when the interaction was added ($R^2 = 0.003, p = 0.06$) and no transition points were found using the Johnson–Neyman method. No other longitudinal analyses showed significant results.

Paper IV was the first study to analyse the relationship between PER2 rs56013859 polymorphism and depressive symptoms. Previous studies looked at relationships between the polymorphism and alcohol use patterns. Different sex and genotype patterns were identified in these studies. Our
analyses indicated that there is a three-way interaction of sex, PER2 rs56013859 and family maltreatment in relation to core depressive symptoms in 13–15-year-old female carriers of the minor allele (TC/CC). No significant finding was made in relation to the sleep complaints index or thoughts of death and suicide. The effect of this interaction loses relevance for older groups, possibly by the addition of other life experiences.
Table 4. Summary of the studies in the thesis: Aims, findings, and conclusions

<table>
<thead>
<tr>
<th>Aims</th>
<th>Main findings</th>
<th>Conclusions</th>
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<tr>
<td>I To explore the psychometric properties and diagnostic accuracy of the MADRS-P in general child and adolescent psychiatric outpatient services in Sweden.</td>
<td>The MADRS-P Cronbach’s alpha was 0.846. The correlation between the K-SADS MDD symptom severity score and the MADRS-P score using Spearman’s rho was 0.580. The AUC in a ROC analysis for all participants was 0.786 (95% CI: 0.694–0.877, ( p &lt; .001 )). At cut-off 15 sensitivity was 0.75, specificity was 0.75, positive predictive value was 0.70 and negative predictive value was 0.80.</td>
<td>The MADRS-P has acceptable diagnostic accuracy for screening for MDD in adolescents in a general psychiatric setting.</td>
</tr>
<tr>
<td>II To explore the psychometric properties of the Swedish CSDS and the CSDS-P among adolescent psychiatric patients.</td>
<td>Cronbach’s alpha of the CSDS was 0.813 and of the CSDS-P was 0.842. Principal component analyses for both scales showed one component. The correlations between the total scores of the CSDS and CSDS-P in relation to a general K-SADS symptom summation index were Spearman’s rho 0.332, ( p &lt; 0.001 ) and 0.237, ( p = 0.014 ), respectively. Correlations with the total K-SADS function summation index were 0.300 for both. The correlation between the CSDS and the total difficulties score on the SDQ was 0.433, ( p &lt; 0.001 ).</td>
<td>The Swedish translations of the CSDS and CSDS-P had similar psychometric properties to Whiteside’s CSDS and the Adult Sheehan Disability Scale. Concurrent validity and correlation between the CSDS and CSDS-P were weak.</td>
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<tr>
<td>III To explore the relation between BDNF ( rs6265 ) polymorphism and childhood stress, as well as the moderating effect of physical activity in relation to depressive symptoms at three time points from the Survey of Adolescent Life in Västmanland cohort study (SALVe).</td>
<td>Both childhood stress and physical activity had moderating effects: – Physical activity in wave 1 with an R(^2) change = 0.006, ( p = 0.013 ), and the Johnson–Neyman regions of significance (RoS) below 1.259, ( p = 0.05 ) for 11.97%. – Childhood stress in wave 2 with the R(^2) change = 0.008, ( p = 0.002 ), and RoS ( \leq 1.561 ) with 26.71% and &gt;4.515 with 18.20%. – A three-way interaction in wave 1 in genotype AA carriers.</td>
<td>These results suggest that allele A is susceptible to physical activity (positive environment) and childhood stress (negative environment) with plasticity properties.</td>
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<tr>
<td>IV To study the relation of the three-way interaction of sex, PER2 ( rs56013859 ) and family maltreatment on core depressive symptoms, sleep complaints, and thoughts of death and suicide in self-reports from a cohort of Swedish adolescents in 2012, 2015 and 2018.</td>
<td>The three-way interaction of sex, ( rs56013859 ) and family maltreatment in relation to core depressive symptoms in 2012 was significant. Moderation model 3, with ( n = 1,634 ), R(^2) = 0.132, ( p = &lt; 0.001 ), the addition of the interaction showed a significant change to R(^2) = 0.003, ( p = 0.032 ). The Johnson–Neyman method identified the level of family maltreatment at 1.110 with 19.28% of the sample reporting family maltreatment at or above this level.</td>
<td>This suggests a susceptibility to depressive symptoms in female carriers of PER2 ( rs56013859 ) C who are exposed to family maltreatment.</td>
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General Discussion

The first main finding from the scale validation studies was a high comorbidity in this clinical population, which is more frequent in female adolescents with depression.

Second, the MADRS-P can be used to collect parent information for the assessment of adolescents in psychiatric populations. A cut-off score of 10 has enough screening properties for major depression and a cut-off score 15 has the best pooled values for sensitivity and specificity with a LR of 3 for MDD diagnosis compared with K-SADS.

In turn, cut-off scores of 5 and 17 for the CSDS and CSDS-P, respectively, allowed the identification of low functioning in any diagnoses, especially those with a greater severity.

Third, the scales studied have shown their usefulness in the assessment of adolescents for depressive symptoms (MADRS-P) and for the assessment of functioning in general (CSDS/CSDS-P).

In the gene × environment interaction studies, a moderation effect was found between BDNF rs6265 polymorphism and PA in relation to the number of depressive symptoms. PA helps to reduce the number of depressive symptoms that present in adolescents, possibly via transient elevation of the neurotrophin BDNF. This effect is most noticeable in carriers of BDNF rs6265 GA, while adolescent carriers of the A:A genotype were more likely to show more depressive symptoms when exposed to higher childhood stress. When studying the three-way interactions of the moderating effect of PA on childhood stress and BDNF rs6265 genotype, carriers of the A:A genotype showed a reduction of depressive symptoms with PA if they were exposed to higher levels of childhood stress. This suggests that the A-allele results in a differential susceptibility on childhood stress and PA in early adolescents.

In the three-way interaction of PER2 rs56013859, sex and family maltreatment in adolescents aged 13–15 years, the interaction of female carriers of CC/CT genotype exposed to higher levels of family maltreatment showed higher levels of depressive symptoms than female carriers of TT genotype and males. At the same time, the visualisation of the lines of the regression model showed parallel lines for female and male carriers of TT genotype. This indicates an increased susceptibility to presenting core depressive symptoms in relation to family maltreatment in female carriers of the PER2 rs56013859 CC/CT genotype.
Complementary preclinical and clinical studies are needed to understand the physiological basis of the interaction and the possible associations of gene × gene × environment and epigenetic mechanisms.

Scales validation studies

Psychiatric assessment is complicated and requires the study of signs and symptoms and comorbidities (340). Assessment of the level of functioning is also recommended (341). Rating scales facilitate the diagnostic assessment allowing information to be gathered from a variety of sources in a way that is fast, inexpensive and easy to interpret (209). Scales should be reliable, valid and standardised (209).

The LEAD procedure has been proposed as a criterion to estimate the validity of diagnostic instruments (342). In a Swedish study of the predictive validity of the K-SADS-PL 2009 in adolescent psychiatric outpatients, the $\kappa$ value for depressive disorder was 0.91 and excellent validity for the major psychiatric diagnoses was shown (176). Due to the importance of the validity of the diagnosis, we used the K-SADS as the gold standard to estimate the psychometric properties of the studied scales—the MADRS-P in Paper I and CSDS/CSDS-P in Paper II.

The interviewer training was conducted in accordance with the Swedish standard (343) and the interviewers were blinded to the results of the scales. The participants in these studies were referred outpatient adolescents who were consecutively recruited and included in only one of the two studies. The external drop-out rate was the same (38.1%), while internal exclusion was 19.2% and 14.4%, respectively. Consecutive recruitment reduces the risk of bias, although the small sample size (< 150) and the external drop-out rate reduce the power in the sample (131). Eligible patients with more severe symptoms who required urgent attention did not participate in the K-SADS.

Based on the PCA, a one-factor solution was considered in Paper I because the two items related to attention loaded similarly on the first and second components and the eigenvalue for the second component was less than the recommended value to be considered separately (208, 344).

The Cronbach’s $\alpha$ for the MADRS-P was acceptable consistent with that reported in the MADRS-S literature (331, 332). Sensitivity, specificity, PPV and NPV for the MADRS-P cut-off values 7–20 were calculated. The best pooled values were found at cut-off 15 (52) and the LR+ was 3, which shows the usefulness of the scale. This is the first study to compare the MADRS-P with the K-SADS. The sample was relatively small and the prevalence of MDD was less than 50%, so there is a risk that sensitivity and specificity were overestimated (345). Another limitation is that all parents completed the MADRS-P after the K-SADS,
which could have influenced their awareness about their adolescent’s depressive symptoms, increasing the number of cases identified using the scale. The two scales were completed in the same day, avoiding symptom variation over time.

Some symptoms of depression in adolescents are not included in the MADRS-P, including irritability, increased sleep and increased appetite. In addition, the comorbidity in the sample could have modified the constellation of the present symptoms, as in the case of ADHD and the attentional component of the scale. Despite this, the AUC of the scale showed a moderate capacity to discriminate between cases and no cases, when compared with the K-SADS. In any case, the scale should be used with other instruments for diagnostic purposes.

In Paper II, the Cronbach’s α for both scales was acceptable, similar to SDS (184). However, Cronbach’s α measurements are weakened in scales with few items (205).

With cut-off scores of 5 for CSDS and 17 for CSDS-P, both scales identified more than 80% of cases with at least one diagnosis, considering that the criteria of functional impairment at work/school or home, or interference with social activities must be met to determine a diagnosis (1). The cut-off of 5 for the CSDS is consistent with that proposed for the SDS by Leon (335) and Sheehan (346).

It was not possible to identify a functioning profile for each diagnosis using the scales due to overlap of symptoms in the highly comorbid sample, which also indicates an outcome intersection problem (347). In addition, the relationship between symptoms and function may vary depending on the demands of the environment (348), and a non-linear relationship may exist, which could explain the low Spearman’s ρ correlations (349) found between the K-SADS summation indexes and the CSDS/CSDS-P. Another limitation of the study was the lack of comparison with another functioning scale.

Notwithstanding all the above, the CSDS/CSDS-P allows a useful, general, and agile assessment of the functioning of young people, and the joint use of the two scales contributes to the clinical assessment of depressive symptoms and functioning level in clinical practice.

Gene x environment interaction studies

In Papers III and IV, the two SNPs, BDNF rs6265 and PER2 rs56013859, and their interaction with environmental factors in relation to depressive symptoms were explored.
Genetics

It is worth highlighting that the study of the genetics of depressive disorders is complicated, as one gene can have multiple effects as a consequence of environmental or other genetic factors. Previous studies in families and twins have demonstrated a higher risk for depressive symptoms between relatives than in a general population, and the risk increases in relation to the number of shared genes (350). MDD is a complex disorder and its point estimate heritability of liability is 31–42% (123). Relatively little is known about the MDD hidden genetic composition (which gene and how the interplay with environmental factors occurs). When there are few individuals with an increased susceptibility to one defined environmental factor, the gene × environment studies have shown greater power than environmental studies alone to identify environmental effects (351). Knowledge about the main effects and measurement quantities of the effects is necessary for the application in public health (352). In the studies, the inclusion of gene × gene × environment interactions or epigenetic mechanisms was not possible (353-355).

Gene selection

Regarding gene selection in Paper III, there is an extensive literature that supports the importance of the BDNF in neuronal survival and the regulation of the stress in mood disorders (356-358).

In Paper IV, the biology of the gene studied differs because PER2 rs56013859 is located in an intron region (307) and it has the potential to regulate other genes directly or indirectly (359). Previous studies are scarce and relate to alcohol use and stress, so we consider it interesting to study its relationship with depressive symptoms. The hypothesis concerning the biochemical pathway by which the effect is produced cannot be elucidated in this study; therefore, preclinical studies are recommended.

Methods

Population

Sample size in both studies was relatively small for genetic studies, with around 1,000 participants (360). Nonetheless, there was sufficient theoretical power to detect a risk genotype, which was calculated for a two-way interaction model in Paper IV (361). The number of participants in the first measurement was larger for the two studies, increasing the power, which probably influenced the results obtained in the two studies.

Design

In Paper III, the cohort design allowed us to follow the effect of the variation of the PA over time (362). Moreover, the study of the gene × environment
interaction, including a protective factor and a risk factor, allowed observation of the crossover shape of the interaction, indicating a differential susceptibility of the minor allele (121).

In Paper IV, the interaction of the gene with family maltreatment and sex was considered because of the higher frequency of depressive symptoms in female adolescents and a differential susceptibility related with sex showed in previous alcohol use studies (272). The definition of family maltreatment as a risk factor, leaving protective factors unexplored, guided the design to the exploration of the diathesis–stress model and suggests that further studies should explore the differential susceptibility model. In this way, the low level family maltreatment of this population and the studied interaction in relation to depressive symptoms shows different slopes for minor alleles but any crossing point between lines is visible in the figure of the interaction in 2012 (363). Another difficulty is following relatively small effects over time when the external factor studied loses relevance compared with other aggregated events (362).

Variables
Concerning the coding of the genotype in both studies, due to its low frequency in the population, the minor allele was collapsed to enhance the power, even though it could enhance the type I error and limits a possible generalisation to populations with other underlying stratification (364, 365). In each study, depressive symptoms were analysed as the continuous trait of a complex disease in relation to the gene polymorphisms that are in somatic chromosomes without a clear Mendelian inheritance.

Another issue to consider is the use of arbitrary scales to measure continuous traits and the categorisation of the genotype. Genotypes were coded as binary and the slope difference between the two categories in relation to the outcome were compared (366, 367), even though the difference between one point and another on the symptom scales does not correspond to a ratio. The adverse childhood events self-assessment scales seemed to be stable over time and added reliable information in relation to mental health outcomes (368).

A limitation was the absence of measurements of other biomarkers to facilitate the confirmation of biological underlying systems (73, 369-373).

Statistical models
Due to the explorative nature of the studies, multiple statistical methods to evaluate the effect were used and matches were found between the results in the level of interaction explored. The use of the Bonferroni correction was not considered necessary due to the explorative nature of the studies in addition to the risk of type II error (374-376).

In Papers III and IV, binary logistic regression for dichotomous outcomes, and linear regression and moderation models for continuous outcomes, were
considered as the best statistical methods given the characteristics of the variables and their distribution in the population.

In Paper IV, a generalised linear model with robust estimator was best suited to explain the effect of the interaction (377). After all, analyses of variance were applied as a complementary method and the results obtained were congruent with the previous ones. To rule out the presence of spurious interaction effects, additional analyses substituting PER2 rs56013859 with another unrelated gene, AUSTS2 rs69343555 (378), were performed in a similar interaction model without statistically significant results.

For easier understanding of the nature of the interactions, visualisation of the moderation model results was used. These models have the strength to permit the quantification of the conditional effect of X on Y for the values of the moderator W. In the visualisation, the Y variable is represented on the Y-axis, the moderator W is represented on the X-axis, and the effect of the values of X are shown by the regression line (328).
Conclusions

The psychometric properties and diagnostic accuracy of the MADRS-P in adolescent outpatients are acceptable and the scale is useful in clinical settings to gather information about adolescent depressive symptoms from parents. The psychometric properties of the CSDS and CSDS-P are similar to the adult scale for assessing patient functioning, have transdiagnostic applicability and, additionally, the parent scale collects information on parent functioning. Both scales constitute a contribution to clinical practice.

In the second part, the plasticity properties of BDNF rs6265 A-allele are demonstrated in its interaction with PA and childhood stress in relation to depressive symptoms in the SALVe cohort. The results show a benefit of PA in early adolescence reducing depressive symptoms, especially in A-allele carriers who have been exposed to high levels of childhood stress.

On the question of the three-way interaction of sex, rs56013859 polymorphism, and family maltreatment on depressive symptoms in the adolescents from the SALVe cohort, the study of rs56013859 polymorphism associated with depressive symptoms is novel and focuses attention on possible bases for the widely described relationship between adverse experiences such as family maltreatment and depressive symptoms.

In sum, the results of this thesis provide two validated instruments for use in clinical practice for the evaluation of adolescents, the first in the assessment of depressive symptoms and the second in the assessment of functioning level. The instruments studied give importance to parent information, and it is hoped that they will facilitate the clinical diagnosis and follow-up for depression in clinical adolescents.

The finding of the BDNF gene study shows how those who are at higher risk of stress obtain greater benefit from preventive actions, such as the promotion of PA and sports in early adolescence.

The second study, about the PER2 gene, contributes to the understanding of why female adolescents are at greater risk of suffering depressive symptoms than male adolescents from adolescence and suggests the importance of the glutamatergic system in stress and depression.

Several questions remain unanswered and future investigations are recommended.
Svensk sammanfattning


Utvecklingen inom genetiken har underlättat att identifiera genotyper och bidragit till studier av enstaka nukleotidpolymorfismer samt helgenomsstudier (360). Exempelvis brain-derived neurotrophic factor (BDNF) är en kandidatgen som visats associerad till depressiva symtom (379, 380). Negativa händelser under barndomen och familjesvårigheter är riskfaktorer för depression (381). Fysisk aktivitet har i sin tur visat sig ha ett samband med nivåerna av BDNF och ha en skyddande effekt mot stress (382). I delarbete III undersökt det modererande sambandet mellan BDNF rs6265 genotyp och stress i barnomen, samt fysisk aktivitet i förhållande till depressiva symtom i ett urval av svenska ungdomar (SALVe Kohort).

PER2 har studerats i relation till sömn, anpassning till stress och sårbarhet för depression (383, 384). PER2 rs56013859 är en enkel nukleotidmutation kallad Spanagel (309). Den har studerats i samband med alkoholkonsumtion och sömnstörningar hos ungdomar (272). Såvitt vi vet, hade PER2 rs56013859 inte studerats i samband med depressiva symtom tidigare. På grund av dess samband med sömnbesvär och stress, och de differentierade effekternas mellan könen, studerades interaktionen mellan PER2 rs56013859, kön, och misshandel i familjen i förhållande till depressiva symtom som depressiva kärnsymtom, sömnbesvär och tankar på död och självmord i delarbete IV.
Delarbete I

De statistiska analyser som användes var t-test och Mann/Whitney-U-test, Chi-två-test, PABAK (Kappa), Cronbachs α, Principiell komponentanalys (PCA), validitet Spearmans ρ, ROC och AUC, sensitivitet, specificitet positivt prediktivt värde samt negativt prediktivt värde.

Huvudresultatet visade att MADRS-P Cronbachs α var 0,846. Jämförelse mellan K-SADS MDD-symtom och MDRS-P visade följande resultat; Vid cut-off 10 var sensitiviteten 0,86, specificiteten 0,54, det positiva prediktiva värdet 0,59 och det negativa prediktiva värdet 0,84. Vid en cut-off på 15 var sensitiviteten 0,75, specificiteten 0,75, det positiva prediktiva värdet 0,70 och det negativa prediktiva värdet 0,80. MADRS-P-poängen med Spearmans ρ var 0,580. AUC i en ROC-analys för alla deltagare var 0,786 (95% konfidensintervall 0,694 - 0,877, p <0,001).

Slutsats; MADRS-P kan användas för att samla in föräldrainformation för bedömning av ungdomar i psykiatriska populationer. MADRS-P vid gränsvärdet 10 har tillräckliga screeningegenskaper för egentlig depression och gränsvärdet 15 har de bästa värdena för sensitivitet och specificitet med en sannolikhetskvot på 3 för MBD-diagnos jämfört med K-SADS.

Delarbete II
Syftet var att undersöka de psykometriska egenskaperna hos de svenska formulären (CSDS och CSDS-P) avsedda för att mäta funktion bland psykiatriska tonårspatienter. Studiepopulationen var densamma som i delarbete I men nu med 125 deltagare i åldern 12–18 år och deras föräldrar. Symtom och funktion av psykiatriska diagnoser bedömdes med den diagnostiska intervjun K-SADS. Därefter användes dels en K-SADS summering av de aktuella symtomen, dels ett K-SADS index för summering av funktion och SDQ för ungdomar samt föräldrarscreeningverktyg för att validera CSDS och CSDS-P.

De statistiska analyser som användes var Chi-två-test, Mann-Whitney-U-test, Principalkomponentanalys av CSDS och CSDS-P, Cronbachs α och Spearmans ρ.

Huvudresultatet visade att Cronbachs α för CSDS var 0,813 och att Cronbachs α för CSDS-P var 0,842. Principalkomponentanalyser visade en
komponent för båda skalorna. Korrelationerna mellan totalpoängen för CSDS och CSDS-P i förhållande till ett allmänt K-SADS-PL symtomsummeringsindex var Spearman’s $\rho = 0,332$, $p < 0,001$ respektive $0,237$, $p = 0,014$. Korrelationerna med det totala K-SADS-funktionsummeringsindexet var $0,300$ för båda. Korrelationen mellan CSDS och den totala svårighetspoängen på SDQ var $0,433$, $p < 0,001$.

Slutsats: CSDS/CSDS-P vid cut-off-poäng på 5 respektive 17 har egenskaperna att identifiera låg funktionsnivå i alla diagnoser, särskilt de med en större svårighetsgrad.

De studerade skalorna har visat sig vara användbara vid bedömning av funktion bland ungdomar med depressiva symtom samt för bedömning av funktionsförmåga i allmänna psykiatiska diagnoser hos tonåringar.

Delarbete III

Syftet var att undersöka interaktionen mellan BDNF rs6265 och stress i barndomen, BDNF rs6265 och fysisk aktivitet, samt den modererande effekten av fysisk aktivitet, BDNF rs6265 och stress i barnomen i förhållande till depressiva symtom, vid tre longitudinala tidpunkter the Survey of Adolescent Life in Västmanland (SALVe-kohorten). Den var en longitudinal studie, hos den allmänna ungdomsbefolkningen med upprepade datainsamlingar år 2012, 2015 och 2018.

Genotypning av BDNF rs6265 (val66met) polymorfismen (G/A) och dess varianter genomfördes med hjälp av KASP™. Vidare användes Depression Self-Rating Scale (DSRS) för bedömning av depressiva symtom, The Neuropattern Pre-/postnatal Stress Questionnaire (NPQ-PSQ) för bedömning av stress i barnomen samt självrapporterad fysisk aktivitet.

De statistiska analyser som användes var Chi-två-test, Mann-Whitney-U-test, Moderationsmodeller 1, 2 och 3, binära logistiska regressionsmodeller och linjära regressorer.

Huvudresultatet visade att både stress i barnomen och fysisk aktivitet hade en modererande effekt. Fysisk aktivitet i våg 1 med en $R^2$-change = 0,006, $p = 0,013$, och Johnson-Neyman signifikansregioner (RoS) under 1,259, $p = 0,05$ för 11,97 %. Stress i barnomen i våg 2 med $R^2$-change = 0,008, $p = 0,002$, och RoS under 1,561 hos 26,71 % och >4,515 hos 18,20 %. Det förelåg en trevägsinteraktion (GxE) i våg 1 hos bärare av genotyp AA.

Slutsats: det finns en modererande effekt mellan BDNF rs6265-polymorfism och fysisk aktivitet i förhållande till antalet depressiva symtom. Fysisk aktivitet bidrar till att minska antalet depressiva symtom hos ungdomar, möjligtvis via en övergående förhöjning av BDNF. Den effekt är mest märkbar hos bärare av BDNF rs6265 GA. Utöver det observerades att bärare av genotypen AA var mer benägna att uppvisa fler depressiva symtom när de utsattes för högre stress under barnomen. När man studerade den
trevägsinteraktionen, dvs den modererande effekten av fysisk aktivitet på stress i barndomen och BDNF rs6265-genotyp, visade bärare av AA-genotyp en minskning av depressiva symtom med fysisk aktivitet om de utsattes för högre nivåer av stress i barndomen. Detta tyder på att A-allelen bidrar till en differentierad känslighet för stress i barndomen men att fysisk aktivitet kan påverka denna känslighet i relation depressiva symtom hos ungdomar.

Delarbete IV

Syftet var att studera sambandet av en trevägsinteraktion mellan; PER2 rs56013859, kön och misshandel i familjen i relation till; depressiva kärnsymtom, sömnbesvär och tankar på döden och självmord, i självrapporter från en kohort av svenska ungdomar. Design var både en tvärnittsstudie och longitudinell studie hos the Survey of Adolescent Life in Västmanland (SALVe-kohorten), med datainsamlingar 2012, 2015 och 2018. Genotypning PER2, rs56013859 (C/T) polymorfism genomfördes med KASP™. Dessutom användes Depression Self-Rating Scale (DSRS) för bedömning av kärnsymтом-index för depressiva symtom, Karolinska Sleep Questionnaire index (KSQ) för bedömning av för sömnbesvär, samt ungdomarnas självrapportering av misshandel i familjen.

De statistiska analyser som användes var Chi-två-test, Mann-Whitney-U-test, Cronbachs α, Principiell komponentanalys, binära logistiska regressionsmodeller, GLM, linjära regressioner (variansanalyser), samt process Modering Modell 3.

Huvudresultatet visade att trevägsinteraktionen mellan kön, rs56013859 och misshandel i familjen i förhållande till depressiva kärnsymtom i var signifikant ($\chi^2(7, 1,634) = 231,685, p <0,001$) vid undersökningstillfället år 2012.

Moderationsmodell 3 Med $n = 1,634$, $R^2 = 0,132$, $p = <0,001$, visade tillägget av interaktionen en signifikant förändring till $R^2 = 0,003$, $p = 0,032$.

Johnson-Neyman-metoden identifierade signifikanta effekter vid nivån av misshandel i familjen till 1,110 med 19,28 % av urvalet som rapporterade misshandel i familjen på eller över denna nivå.

Slutsats; det finns en trevägsinteraktion mellan PER2 rs56013859, kön och misshandel i familjen i relation till med depressiva kärnsymptom hos ungdomar i åldern 13–15 år. Kvinnliga bärare av CC/ CT genotypen som utsatts för högre nivåer av misshandel i familjen rapporterade högre nivåer av depressiva kärnsymtom än kvinnliga bärare av TT-genotypen och män. En visualisering av linjerna i regressionsmodellen visade parallella linjer för kvinnliga och manliga bärare av TT-genotypen. Detta indikerar en ökad känslighet för depressiva symtom i samband med misshandel i familjen hos kvinnliga bärare av genotypen PER2 rs56013859 CC/ CT.
Kompletterande prekliniska och kliniska studier behövs för att förstå den fysiologiska grunden för interaktionen och eventuella associerade GxGxE och epigenetiska mekanismer.
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To my co-authors: Mia Ramklint, thank you for your careful supervision, for prompting our co-authored manuscripts, giving relevant contributions to clinical and methodological questions, and for your cooperation; Susanne Olöfsdotter, thank you for co-authoring all four papers included in this thesis, and for giving me the important keys to scientific thought, methodology and statistics, which opened doors to new knowledge in every meeting; and Sofia Vadlin, thank you for co-authoring almost all papers and for your motivation and time shared with me.

To Mattias Rehn, thank you for patiently collaborating with data management, statistical software and images in each paper, always giving me your time when it was necessary and sharing your mathematical knowledge.

To Philippe Wagner, thank you for your effective statistical advice, personal availability and the time spent on our analyses.

To Lotta Nilsson, my office mate, thank you for your kind support, patient listening and cooperation.

Gratitude also goes to the supervisors and professors from other areas who contributed their teaching in our scientific education, to previous and contemporary PhD colleagues, for study and break moments, seminars and conferences shared, and to Maria Dell Uva, Mariana Ehn and Maria Pettersson for their administrative support.
I am grateful to Uppsala University and Västmanland Region Psychiatry Clinic for having made my doctoral studies possible, to the chiefs and colleagues in the clinic for their understanding and encouragement, and to all the adolescents and parents who participated in the studies.

Thank you to my family: Ale, you always show me the way and give examples; Juan Carlos, for your company and unconditional support; and to my parents, Constanza and Francisco, for their energy and faith.

Finally, I wish to thank all those who have collaborated in this project and who, although not named, have been important to me.
References


47. Fenichel O. The Psychoanalytic Theory Of Neurosis London: Routledge; 1946 [cited 2023 October 03]. Available from:


disorders with adult-onset chronic physical conditions. Arch Gen Psychiatry. 2011;68(8):838-44.


198. Streiner DL, Norman GR, Cairney J. Health measurement scales: a practical guide to their development and use: Oxford University Press, USA; 2015.


264. Merikanto I, Partonen T, Paunio T, Castaneda AE, Marttunen M, Urrila AS. Advanced phases and reduced amplitudes are suggested to characterize the daily rest-activity cycles in depressed adolescent boys. Chronobiol Int. 2017;34(7):967-76.


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383. (OMIN) OMIM. # 604348 Advanced Sleep Phase Syndrome, Familial, 1; FASPS1: Johns Hopkins University; 2018 [Available from: https://omim.org/entry/604348.
Appendix

- The MADRS-P
- The CSDS
- The CSDSP
**MADRS-F: FÖRÄLDERS SKATTNING AV SIN UNGDOMS MÅENDE**

**Ungdomens namn**  **Ungdomens personnummer**  **Datum**

Skattare/Uppgiftslämnare: Mor ☐  Far ☐  Annan?: ☐


Frågorna innehåller en rad olika påståenden om hur man kan mä på olika avseenden. Påståendena uttrycker på en skala 0-6 olika grader av psykiska besvär, alltifrån frånvaro av besvär (0 poäng) till maximalt uttalade besvär (6 poäng). Sätt en ring runt siffran som du tycker bäst stämmer överens med hur du uppfattat att din ungdom mätt de senaste tre dagarna. Tänk inte allt för länge, utan försök att svara spontant.

**KOM IHÅG, att bedömningen endast gäller de senaste tre dagarna.**

### 1. Sinnesstämning

Här ber vi dig beskriva din ungdoms sinnesstämning, om han/hon känner sig ledsen, tungsint eller dyster till mods. Tänk efter hur du uppfattat att din ungdom har känt sig de senaste tre dagarna, om han/hon har skiftat i humöret eller om det har varit i stort sett detsamma hela tiden, och försök särskilt komma ihåg om du uppfattat att han/hon verkar ha känt sig lättare till sinnes om det har hänt något positivt.

<table>
<thead>
<tr>
<th>0</th>
<th>Min ungdom kan känna sig glad eller ledsen, allt efter omständigheterna.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Min ungdom känner sig mestadels lugn.</td>
</tr>
<tr>
<td>2</td>
<td>Ibland har min ungdom obehagliga känslor av inre spänning, olust</td>
</tr>
<tr>
<td>3</td>
<td>Min ungdom har ofta en känsla av inre oro som ibland kan bli mycket stor, och han/hon måste anstränga sig för att bemästra.</td>
</tr>
<tr>
<td>4</td>
<td>Min ungdom har fruktansvärda, långvariga eller outhärdliga ångestkänslor.</td>
</tr>
</tbody>
</table>

### 2. Oroskänslor

Här ber vi dig markera i vilken utsträckning du uppfattat att din ungdom har haft känslor av inre spänning, olust och ångest eller odefinierad rädsla under de senaste tre dagarna. Tänk särskilt på hur intensiva du uppfattar att känslorna varit, och om de kommit och gått eller funnits hela tiden.

<table>
<thead>
<tr>
<th>0</th>
<th>Min ungdom kan känna sig mestadels lugn.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ibland har min ungdom obehagliga känslor av inre oro.</td>
</tr>
<tr>
<td>2</td>
<td>Min ungdom har ofta en känsla av inre oro som ibland kan bli mycket stor, och han/hon måste anstränga sig för att bemästra.</td>
</tr>
<tr>
<td>3</td>
<td>Min ungdom har fruktansvärda, långvariga eller outhärdliga ångestkänslor.</td>
</tr>
</tbody>
</table>

### 3. Sömn

Här ber vi dig beskriva hur bra du uppfattar att din ungdom sover. Tänk efter hur länge din ungdom sovit och hur god sömnen varit under de senaste tre nätterna. Bedömningen skall avse honom/henne faktiskt sovitt, oavsett om han/hon tagit sömnmedel eller ej. Om din ungdom sover mer än vanligt, markera då det på 0.

<table>
<thead>
<tr>
<th>0</th>
<th>Min ungdom sover lugnt och bra och tillräckligt länge för sina behov.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Min ungdom har vissa sömnsvårigheter.</td>
</tr>
<tr>
<td>2</td>
<td>Min ungdom har vissa sömnsvårigheter.</td>
</tr>
<tr>
<td>3</td>
<td>Min ungdom har vissa sömnsvårigheter.</td>
</tr>
<tr>
<td>4</td>
<td>Min ungdom sover mycket dåligt, inte mer än 2-3 timmar per natt.</td>
</tr>
</tbody>
</table>

### 4. Matlust

Här ber vi dig ta ställning till hur din ungdoms aptit är, och tänka efter om den på något sätt skilt sig från vad som är normalt för honom/henne. Om din ungdom skulle ha bättre aptit än normalt, markera då det på 0.

<table>
<thead>
<tr>
<th>0</th>
<th>Min ungdoms aptit är som den brukar vara.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Min ungdoms aptit är sämre än vanligt.</td>
</tr>
<tr>
<td>2</td>
<td>Min ungdoms aptit är sämre än vanligt.</td>
</tr>
<tr>
<td>3</td>
<td>Min ungdoms aptit har nästan helt försvunnit.</td>
</tr>
<tr>
<td>5</td>
<td>Min ungdom vill inte ha någon mat. Om min ungdom skall få någonting i sig, måste min ungdom övertalas att äta.</td>
</tr>
</tbody>
</table>

**Summa denna sida:** 100
5. Koncentrationssvårigheter
Här ber vi dig ta ställning till din ungdoms förmåga att hålla tankarna samlade och koncentrera sig på olika aktiviteter. Tänk igenom hur din ungdom fungerar vid olika sysslor som kräver olika grad av koncentrationssvårigheter, t ex läsning av komplicerad text, lätt tidningstext och TV-tittande.

<table>
<thead>
<tr>
<th>0</th>
<th>Min ungdom har inga koncentrationssvårigheter</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Min ungdom har tillfällig svårt att hålla tankarna samlade på sådant som normalt skulle fånga min ungdoms uppmärksamhet (t ex läsning eller TV-tittande)</td>
</tr>
<tr>
<td>3</td>
<td>Min ungdom har påtagligt svårt att koncentrera sig på sådant som normalt inte kräver någon ansträngning från min ungdoms sida (t ex läsning eller samtal med andra människor)</td>
</tr>
<tr>
<td>5</td>
<td>Min ungdom kan överhuvudtaget inte koncentrera sig på något</td>
</tr>
</tbody>
</table>

6. Initiativförmåga
Här ber vi dig försöka värdera din ungdoms handlingskraft. Frågan gäller om din ungdom har lätt eller svårt för att komma igång med sådant som du tycker han/hon bör göra, och i vilken utsträckning din ungdom måste övervinna ett inre motstånd när han/hon skall ta itu med något.

| 0 | Min ungdom har inga svårigheter med att ta itu med nya uppgifter |
| 1 | När min ungdom skall ta itu med något, tar det emot på ett sätt som inte är normalt för homon/henne |
| 3 | Det krävs en stor ansträngning för min ungdom att ens komma igång med enkla uppgifter som han/hon vanligtvis utför mer eller mindre rutinemässigt |
| 5 | Min ungdom kan inte förmå sig att ta itu med de enklaste vardagsuppgifter |

7. Känslomässigt engagemang
Här ber vi dig ta ställning till hur du upplever din ungdoms intresse för omvärlden och för andra människor, och för sådana aktiviteter som brukar bereda homon/henne nöje och glädje.

| 0 | Min ungdom är intresserad av omvärlden och engagerar sig i den, och det bereder homon/henne både nöje och glädje |
| 1 | Min ungdom känner mindre starkt för sådant som brukar engagera homon/henne. Min ungdom har svårare än vanligt att bli glad eller svårare att bli arg när det är befogat |
| 3 | Min ungdom kan inte känn så något intresse för omvärlden, inte ens för vänner och bekanta |
| 5 | Min ungdom har slutat uppleva några känslor. Min ungdom känner sig snart samtid likgiltig även för sina närmaste |

8. Pessimism
Frågan gäller hur du uppfattar att din ungdom ser på sin egen framtid och sitt eget värde. Tänk efter i vilken utsträckning du uppfattat att din ungdom gör sig självförebråelser, att din ungdom pågår av skuldåterfall, och om din ungdom oroat sig ofta än vanligt för t ex sin ekonomi eller sin hälsa.

| 0 | Min ungdom ser på framtid med tillförsikt. Min ungdom är på det hela taget ganska nöjd med sig själv |
| 2 | Ibland klandrar min ungdom sig själv och tycker att han/hon är mindre värd än andra |
| 4 | Min ungdom gruberar ofta över sina misslyckanden och känner sig mindervärdig eller dålig, även om andra tycker annorlunda |
| 6 | Min ungdom ser allting i svart och kan inte se någon ljustring. Min ungdom uppfattar sig själv som en alltigenom dålig människa, som aldrig skulle kunna få någon förlåtelse för det hemska han/hon gjort |

9. Livslust
Frågan gäller din ungdoms livslust, och om han/hon känt livsleda. Har du uppfattat att din ungdom har tankar på självmord, och i så fall, i vilken utsträckning upplever du att din ungdom ser detta som en verklig utväg?

| 0 | Min ungdom har normal aptit på livet |
| 2 | Min ungdom tycker inte att livet känns särskilt meningsfullt men han/hon önskar ändå inte att han/hon vore död |
| 4 | Min ungdom tycker ofta det vore bättre att vara död, och trots att han/hon egentligen inte önskar det, kan självmord ibland kännas som en möjlig utväg |
| 6 | Min ungdom är egentligen övertygad om att hans/ hennes ende utväg är att dö, och han/hon tänker mycket på hur han/hon bäst skall gå tillväga för att ta sitt eget liv |

NEDANSTÅENDE FYLLS I AV VÅRDPERSONAL

Summa denna sida: [ ]
Summa föregående sida: [ ]
Totalpoäng: [ ]
CSDS

Nu har du berättat för oss om olika bekymmer och känslor. Vi skulle också vilja veta hur mycket de här bekymren och känslorna förstör för dig. Alltså, hur mycket de hindrar dig från att göra saker som du vill göra.

Ställer bekymren och känslorna till problem för dig när du är i skolan och när du gör läxor?

Ställer bekymren och känslorna till problem för dig när du är tillsammans med kompisar?

Ställer bekymren och känslorna till problem för dig hemma?
CSDS-P

Markera den siffra som motsvarar i hur stor utsträckning ditt barns symtom påverkar olika områden i livet, just nu:

**Symtomen påverkar ditt barns skolarbete negativt:**

<table>
<thead>
<tr>
<th>Inte alls</th>
<th>Lite grann</th>
<th>Ganska mycket</th>
<th>Mycket</th>
<th>Jättemycket</th>
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<tr>
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</table>

**Symtomen påverkar ditt barns sociala liv negativt:**

<table>
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<tr>
<th>Inte alls</th>
<th>Lite grann</th>
<th>Ganska mycket</th>
<th>Mycket</th>
<th>Jättemycket</th>
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**Ditt barns symtom påverkar dig negativt i ditt arbete:**

<table>
<thead>
<tr>
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<th>Ganska mycket</th>
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**Ditt barns symtom påverkar ditt sociala umgångar negativt:**

<table>
<thead>
<tr>
<th>Inte alls</th>
<th>Lite grann</th>
<th>Ganska mycket</th>
<th>Mycket</th>
<th>Jättemycket</th>
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**Ditt barns symtom påverkar ert gemensamma familjeliv negativt:**

<table>
<thead>
<tr>
<th>Inte alls</th>
<th>Lite grann</th>
<th>Ganska mycket</th>
<th>Mycket</th>
<th>Jättemycket</th>
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</thead>
<tbody>
<tr>
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Acta Universitatis Upsaliensis

*Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine 1987*

Editor: The Dean of the Faculty of Medicine

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