Depression and Antisocial Behaviour in Adolescents

Influence of Social Status, Shaming, and Gene-Environment Interaction

CECILIA ÅSLUND
Dissertation presented at Uppsala University to be publicly examined in Samlingssalen Psikiatricentrum, Ing 29, Centrallasarettet, Västerås, Thursday, December 10, 2009 at 13:15 for the degree of Doctor of Philosophy in Medicine. The examination will be conducted in Swedish.

**Abstract**


This thesis investigated (1) social status and shaming experiences in relation to aggressive behaviour and depression, and (2) gene-environment interactions between two genetic polymorphisms related to the serotonergic system – MAOA-VNTR and 5HTTLPR – and experiences of maltreatment in relation to delinquent behaviour and depression among adolescents.

The four included studies are based on questionnaire data from the Survey of Adolescent Life in Västmanland 2006 (SALVe-2006). A total of 5396 students in 9th (15-16 years old) grade of elementary school and 2nd (17-18 years old) grade of high school comprised the target population. The students in 2nd grade of high school also provided a saliva sample for gene extraction.

There were strong associations between shaming experiences and both aggressive behaviour and depression. In addition, individuals who reported many shaming experiences and had either low or high social status had increased risks of physical aggression or depression, whereas medium social status seemed to have a protective effect.

Gene-environment interactions were found between experiences of maltreatment and the MAOA-VNTR in relation to delinquent behaviour. Moreover, the direction of the gene-environment interaction differed depending on sex: boys with the short (S) variant of the MAOA-VNTR, in contrast to girls with the long (L) variant, had the highest risk of delinquency in combination with maltreatment.

Gene-environment interactions were also found between experiences of maltreatment and the 5HTTLPR in relation to depression among girls. The girls that were homozygous for the S allele (SS) had the highest risk of depression in combination with maltreatment. Among boys however, no gene-environment interaction was found between the 5HTTLPR and maltreatment in relation to depression.

In conclusion, it is important to consider both genetic effects, and psychosocial factors such as social status, shaming experiences, and experiences of maltreatment when investigating different aspects of health and behaviour among adolescents.

**Keywords:** adolescent, antisocial behaviour, depression, gene-environment interaction, maltreatment, monoamine oxidase, serotonin, shame, social status

*Cecilia Åslund, Centre for Clinical Research, County of Västmanland, Centrallasarettet, Uppsala University, SE-72189 Västerås, Sweden*

© Cecilia Åslund 2009

ISSN 1651-6206
ISBN 978-91-554-7649-6
urn:nbn:se:uu:diva-109851 (http://urn.kb.se/resolve?urn=nbn:se:uu:diva-109851)
List of papers

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


Reprints were made with permission from the respective publishers.
Abbreviations

5-HIAA 5-hydroxyindole acetic acid
5-HT 5-hydroxytryptamine/serotonin
5HTT Serotonin transporter
5HTTLPR Serotonin transporter gene-linked polymorphic region
A Adenine
C Cytosine
CI Confidence interval
DMSO Dimethyl sulfoxide
DNA Deoxyribonucelie acid
dNTPs Deoxyribonucleotide triphosphates
DSM-IV Diagnostic and Statistical Manual of Mental Disorders, Fourth edition
DSRS Depression Self-Rating Scale
fMRI Functional magnetic resonance imaging
G Guanine
GxE Gene-environment
GLM General linear model
HPA Hypothalamic-pituitary-adrenal
L Long
LL Long-long
LS Long-short
MAO Monoamine oxidase
MgCl₂ Magnesium chloride
mRNA Messenger ribonucleic acid
OR Odds ratio
PCR Polymerase chain reaction
rh5-HTLPR Macaque serotonin transporter polymorphism
RNA Ribonucleic acid
S Short
SALVe Survey of Adolescent Life in Vestmanland
SD Standard deviation
SES Socioeconomic status
SNP Single nucleotide polymorphism
SS Short-short
SSRI Selective serotonin reuptake inhibitor
<table>
<thead>
<tr>
<th>T</th>
<th>Thymine</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEB</td>
<td>Tris-EDTA Buffer</td>
</tr>
<tr>
<td>tRNA</td>
<td>Transfer ribonucleic acid</td>
</tr>
<tr>
<td>VNTR</td>
<td>Variable number of tandem repeats</td>
</tr>
<tr>
<td>WHO</td>
<td>World health organization</td>
</tr>
</tbody>
</table>
The mysteries of the human mind have absorbed scientists for centuries, and yet its nature and properties remain obscure. Even more mysterious are the properties of the communication between the brain and the human mind, namely, how thoughts, feelings, and memories can arise from electrical signals and small physical entities such as proteins at specific positions in the brain. Research on the function of the brain and its elements is however rapidly moving forward and new findings are presented every day.

In western societies, medical science has long been built upon physical, measurable and distinctly observable factors. Over recent years however, the importance of emotion and cognition in relation to health has been increasingly acknowledged. Michael Marmot’s book *The Status Syndrome* from 2004 received much attention with its evidence that one’s social position in a society or a social hierarchy influences health and mortality, independently of known health-related factors such as diet, smoking, exercise and other life-style related habits. Unequal societies create enormous psychological stresses for the inhabitants that mainly fall upon the individuals at the bottom of the social ladder. The more inequality in a society, the steeper is the gradient of health, and the larger the differences in health and mortality between social groups or classes.

Another phenomenon that has received much attention, specifically within psychiatric research over the last decades, is the feeling of shame. Shame has been proposed as one of the fundamental emotions that make human civilisation possible, as it regulates undesired behaviours in a society. Humankind has a basic urge to belong to and be part of fellowships and social communities, and the feeling of shame is a signal that we are at risk of being excluded from a social group if we do not change our behaviour. Both social status and feelings of shame are, in other words, closely connected to social stress and negative emotion that may influence health and behaviour.

Emotion and stress are interlaced with chemical processes in the brain involving many different neurotransmitters and hormones. The neurotransmitter serotonin is closely related to emotion and regulates mood and anxiety as well as a number of important bodily functions. Researchers have long suspected that genetic variations which influence the functionality of the serotonergic system may be of importance for the risk of developing depression or deviant behaviours. In the year 2002, an important
breakthrough was presented in a scientific paper by the British researchers Avshalom Caspi and Terrie Moffitt. They found that a variation of a gene related to monoamine oxidase A, which is involved in the regulation of serotonin levels during neurotransmission in the brain, influenced antisocial behaviour, but only in interaction with environmental stress factors. In the following year, the same research group presented another study where a gene related to the serotonin transporter, which also is involved in the regulation of levels of serotonin during neurotransmission, was found to influence risk of depression, but again only in interaction with environmental stress. They proposed that specific genetic variations associated with a somewhat less effective function of the serotonergic system may have an impact on how environmental stress affects health and behaviour. Such gene-environment interaction effects may be one possible explanation for the phenomenon of so called “dandelion children” (“maskrosbarn” in Swedish). That is, why some children who grow up under stressful and difficult conditions seem to turn out fine without any social or psychological problems, whereas other children from the same environmental conditions develop deviant behaviours or depression.

This thesis focuses on the possible associations and mechanisms behind antisocial behaviour and depression among adolescents. In papers I and II, the focus is on associations between social status and shaming experiences in relation to antisocial behaviour and depression. In papers III and IV, the focus is on whether genetic variations associated with the functionality of the serotonergic system may interact with a stressful environment in relation to antisocial behaviour and depression.

**Depression**

Depression is the leading cause of years lost due to disability and causes 6% of the burden of all diseases in Europe. The lifetime prevalence of major depressive disorder is at least 10%, with the risk in women being twice that in men. At least 21 million people are affected in Europe, and the annual cost due to treatment, loss of production, sick-leave and early retirement is estimated to be 118 billion Euros which makes depression the most costly brain disorder accounting for 33% of the total cost and 1% of Europe’s total national income. Depressive symptomatology increases dramatically in adolescence and often continues into later ages. It is widely recognised that both environmental and genetic factors are of importance. Childhood maltreatment and stressful life events are known to be strong risk factors for depression and twin studies have suggested a heritability of about 40-50%. However, the causal mechanisms behind depression are still to a large extent unknown, and there is a need to further understand how
psychological, environmental, and genetic factors, as well as interactions between heredity and environment, may be of importance.

**Antisocial behaviour**

Delinquency and conduct problems are examples of antisocial behaviour that often have an early onset in life. Childhood and adolescent antisocial behaviour is associated with life-long and pervasive mental, physical, interpersonal, and economic problems that create an enormous societal burden. Perpetrators of family violence and other violent crimes account for a massive burden of mortality, disease, disability, and compromised well-being in society. About 1.6 million people die from violence around the world every year, and it is the leading cause of death for people aged 15–44 years, accounting for 14% of deaths among men and 7% among women. For every person that dies due to violence, many more are physically or psychologically injured or damaged. In Sweden, 1.378 million crimes were reported during 2008 which is equivalent to a 5% increase since 2007, and follows a trend of increasing criminality in Swedish society. A majority of all crime is committed by young men. There is a need to achieve a more complete understanding of the causes of antisocial behaviour and ways to identify youth at risk in order to provide an evidence base for effectively preventing and controlling it.

**Definition of concepts**

The present thesis will investigate possible associations and mechanisms behind depression and antisocial behaviour. I would like to start by defining these two concepts.

The concept of depression may have social as well as medical meanings. Depression may, for example, refer to a period of great economic recession such as occurred at the beginning of the 1930s. The concept is also often used for describing a temporary low mood which is a normal reaction to many of life’s stresses. Depression is considered abnormal only when it is out of proportion to the event that caused the sadness, and continues past the point at which most people begin to recover. In that case, it is medically defined as a psychological disease associated with the wide spectrum of affective syndromes. Unipolar depression involves feeling sad, lethargic and uninterested in any activities. It is also commonly referred to as major depression. Bipolar depression also involves episodes of mania and will not be investigated in this thesis. The Diagnostic and Statistical Manual of Mental Disorders, Fourth edition (DSM-IV) is a widely recognised diagnostic tool within psychiatric care. The Depression Self-Rating Scale...
(DSRS) is a survey based on the DSM-IV criteria for major depression. It was used in the papers of the present thesis to determine the prevalence of depressive symptoms in the populations of the studies. However, although the participants answered the questions of the DSRS and may have fulfilled the criteria for major depression, they did not see a physician or psychiatrist and we can therefore not define any clinical diagnoses. Consequently, in this thesis, the concept of depression refers to the number of depressive symptoms that is requested for the clinical diagnosis of depression according to the DSRS. This is, however, not a valid clinical diagnosis as the symptoms were self-reported and no physician or psychiatrist was involved.

Antisocial behaviour may be defined in many different ways, but often refers to public behaviour that lacks judgement and consideration for others and may cause damage to them or their property. In common parlance, “antisocial” often refers to a person lacking social skills or being excessively introverted. However, actions that would be seen as extremely antisocial by most people, such as attacking or killing another person, may be perceived as quite normal and even expected under certain circumstances, such as in a boxing match or during a war. Consequently, an important part of the definition of antisocial behaviour involves norm-breaking acts. In this thesis, the concept of antisocial behaviour is defined as a norm-breaking, acting out and aggressive behaviour. This will be measured by, for example, physical or verbal harassment of classmates at school, or committing acts that are illegal under Swedish law.
Social status and shame - influence on health and behaviour

Background

Social status gets under your skin. In 2004, Michael Marmot described the concept of the status gradient of health as the strong association between low social status and elevated risk for disease and premature death in western economies. Large income inequalities within a society have been associated with high rates of ill health and crime. When comparing different levels of income inequality between countries or societies, there is strong evidence for associations between wide income disparities and high mortality rates and disease. These associations remain after controlling for other risk factors related to low socioeconomic status, for example pollution and unhealthy lifestyle habits such as smoking, alcohol abuse etc. The relationship between inequality and ill health has been explained by the fact that social environments marked by wide income disparities generate invidious social comparisons, which create a sense of exclusion and alienation among vulnerable individuals. Such environments generate experiences of shame, inferiority, subordination, individuals being “put down” and feeling disrespected etc, which are common sources of anxiety and psychological stress. The experience of feeling shamed or disrespected is, moreover, the most frequent trigger of violent crime. These emotions may, through neuroendocrine pathways, be translated into physical manifestations of ill health.

Social status

Social status may be defined as one’s social position in relation to other individuals in a society, hierarchy or social context. It refers to the power, prestige, authority or honour attached to a social position. Socioeconomic status (SES) is the most common measure of social status, and refers to income, education and occupational prestige. Low socioeconomic status has been associated with an increased risk of depression, obesity, coronary heart disease, early mortality and criminality. High socioeconomic status has, on the other hand, repeatedly been shown to be a protective factor.
against ill health and mortality. Humans are social beings with a well-developed capacity to evaluate the quality of their relationships to others, and what other people think of them. The human striving for social acceptance may be an evolutionary adaptation that promoted survival and reproduction. Likewise, low social status is, from an evolutionary perspective, a potential threat to survival, as it could mean less access to resources, less opportunity to cooperate with others, and less opportunity to mate. In the modern world, however, low social status may be more connected to symbolic threats to the self, such as less control and options in life and higher exposure to pollution, crime and unhealthy environments, thus creating high rates of chronic stress. The inequality of health depending on social position is commonly referred to as the status syndrome, or the social gradient of health.

Shame

Shame has been defined as a class name for a large family of emotions, ranging from social discomfort and embarrassment – characterised by weak intensity and transitory duration, to humiliation – characterised by strong intensity and long duration. Self-evaluation and social comparison are important parts of our social interaction with others in order to evaluate our social position and the status of our relationships. They also function as a monitor to our behaviour. A common form of self-evaluation is the feeling of shame, which arises when our social bonds to other people are threatened, we lose status in a social context, or a core aspect of the self is judged as defective, inferior or inadequate. Shame signals a need for a behavioural change in order to mend our damaged social bonds to people around us. Feelings of shame and pride have thus served a purpose of social control and behaviour regulation in all known civilisations throughout human history. A problem arises, however, when people are subjected repeatedly to humiliation, ridicule, and social exclusion without the possibility to change their behaviour and mend the threat to their social bonds. This results in stigmatisation and “toxic shame” which may form a basis for psychological and physical pathological reactions.

Social capital theory

One proposed explanation for the associations between social status, ill health, and criminality is the concept of social capital. Social capital is often referred to as how social organisations, networks and relations influence collective action and cooperation for mutual benefit in a society. Solidarity, fellowship, sympathy and cooperation are factors that build
social capital in a society. Unequal societies have lower levels of social capital, i.e. people tend to trust each other less, are less likely to be involved in community life and there are higher levels of hostility between citizens. Low levels of social cohesion have been associated with chronic stress and a high risk of ill health.

Social rank theory
Theories of social rank originate from evolutionary perspectives, suggesting that humans and other species through evolutionary selection have acquired behavioural strategies for contesting and safeguarding resources. As a consequence, hierarchical organisations have developed where an individual’s order of rank corresponds to that individual’s priority and access to resources. In order to maintain hierarchical positions, threats from a subordinate may elicit down-hierarchy aggression to restrain resource access, whereas threats from a superior may elicit up-hierarchy appeasement efforts to repair cooperation alliances and reduce the risk of further aggression. Such behavioural strategies have been observed both among animals and in hierarchical relationships among humans. However, as social rank among humans is much more dynamic than the rank systems of animals this theory may be difficult to apply in relation to human health and behaviour. In animals, the rank system is fixed, whereas humans function in a number of different hierarchical or social positions depending on the social context they are in at a given moment. An individual may, for example, have one social position at work, another at home, a third among relatives, a fourth in the closest group of friends, a fifth in the local community soccer team, and so on. Consequently, an individual’s social status may vary between different social contexts. Low social position in one context might be compensated by high status in another, or the other way around. Large inconsistencies in expected contra factual status are, however, related to an increased risk of ill health.

Social status and sensitivity to shame
Individuals with low social status have been suggested to be particularly vulnerable to acts of humiliation and disrespect from others. This has been proposed as an explanation for the relationship between large income inequalities and high rates of violent crime in societies. Violent behaviour may be seen as a quest for respect from others in a social environment marked by wide income disparities, social comparison and social exclusion. Even small humiliations may be unbearable for individuals already marked by shame, lack of self-esteem, and low control over their lives due to
marginalised positions in society. Common reactions to feelings of entrapment and repeated humiliation are aggression and depression. Hochschild introduced the concept of “status shields” in her book *The Managed Heart*. She suggested that high status individuals have greater access to emotional and social capital which may serve as a protective shield against insults. Individuals with low social status lack such a status shield against poor treatment of their feelings, displaced feelings from others such as anger and rudeness, and humiliation. The weaker the status shield, the more devastating is the psychological effect of humiliating experiences and shame. It has moreover been suggested that lower classes suffer from status-related shame, as they adapt to the norms and values of the dominating classes in society, thereby accepting injustice and seeing themselves through the eyes of disdainful others.

**Social status and shame in adolescence**

Adolescents often show a strong sensitivity to feelings of shame as a consequence of cognitive and physical maturational changes, increased sensitivity to peer evaluation, and intensified concern about gender roles. Social status in the peer group and school environment has been suggested to be a strong influence in the development of self-esteem and self-image among young people. Moreover, adolescence is often characterised by a strong urge to conform to the peer group, and not stand out or differ from others. Conformity increases when individuals value their membership in a group but feel insecure in that membership. Conformity is often high at the middle and low at either end of a status hierarchy. High status individuals who feel confident in their social acceptance are emboldened to deviate from conventional norms, whereas low status individuals may defy the same norms since these individuals will be excluded regardless of their actions.

**Subjective or objective measures of social status?**

Most prior research on relations between social status, health and behaviour has used measurements of objective social status, such as registered income, education and occupation. However, when regarding social status as a fundamental stress factor related to hierarchical structures and comparison of oneself to others, it is likely that subjective socioeconomic status as well as personal beliefs of one’s status in social contexts will have at least as strong an influence as objective socioeconomic status. This theory has been confirmed in several studies, where an individual’s subjective beliefs about their social position had strong implications for their health. It has,
moreover, been suggested that adolescent subjective social status is most effectively measured by status in peer group and school, since conventional measures of socioeconomic status are more related to the social status of the parents. Additionally, objective measures of social status can be considered to be problematic as they tend to change over time. Education levels have, for example, increased steadily in industrialised countries during the 20th century.

Stress

The human body has evolved to respond automatically to the challenge of external, potentially lethal, short-term threats. This stress response activates the nervous system and hormones in order to prepare the individual for “fight or flight”. The response is highly adaptive and may be life-saving in the short term. But if the biological stress response is activated too often or for too long, there may be severe health costs. The “fight or flight” response functions similarly in all mammals. In humans, it activates the hypothalamic-pituitary-adrenal (HPA) axis which results in cortisol release into the blood stream, and the sympathetic nervous system which releases noradrenaline and adrenaline, evoking responses throughout the body. The fight-flight response involves instant psychological arousal and energy mobilisation, and inhibits functions that are irrelevant for immediate survival, such as digestion, growth, immune defence and healing. Instant effects involve accelerated heart rate, increased metabolic rate and blood pressure, constriction of blood vessels in the skin and gut, raised blood glucose, and promotion of fatty acid release from fat tissues. It has been suggested that elevated cortisol levels, possibly caused by stressful life events, may lower brain serotonin function and be a cause of depression.

Although the social environment for humans has changed dramatically over the past 10,000 years when agriculture began, and in the last 200 years with waves of industrial development altering our living conditions, our underlying biology remains essentially the same. Physical and biological life-threatening emergencies are rare for most individuals, but modern life is nevertheless filled with psychological demands and challenges that may activate the fight-flight response too often or too hard. Among groups of low socioeconomic status, financial strain, lack of social support and low control over life and work situations may produce a low level of psychosocial stress as a feature of daily life. Stress that persists for a long time, either because it occurs repeatedly, episodically, continuously, or involves severe threats that are not easily adapted to, may be referred to as “chronic stress”. This may be background stress due to more or less constant stressors associated with living or working environments, or long-lasting stressors from acute incidents that have effects that persist far beyond the initiating event. It has
been suggested that one pathway by which socioeconomic status affects health is by establishing a chronic level of stress. Animal studies of social status and stress

Depression has been proposed to be an evolved mechanism enhancing survival in a threatening situation and is closely related to the “flight” part of the “fight-flight” response. It involves yielding in a situation of social competition where the individual recognises that defeat is inevitable. This has been proposed as a genetically programmed reaction that reduces the risk for serious injury or death to combatants by triggering either submission or flight as well as acceptance of a new status quo, and thus providing an effective losing strategy. Such behavioural strategies are well known among mammals, and involuntary submission is strongly related to severe stress, increased cortisol levels and withdrawal. A study involving the manipulation of social status in a group of monkeys, showed that involuntary subordination was related to dysfunction of the HPA-axis and depressive behaviour. A study of common marmosets showed that socially stressful conditions such as isolation or peer group formations were associated with increased HPA-axis function and behavioural arousal. Moreover, male mice that lost a social competition against an intruder and were forced into submission showed immunoenocrine alterations and metabolic disorders. Social status threat and subordination have, moreover, been associated with atherosclerosis in monkeys.

Threats to the social self

Threats to the social self are situations that involve the potential loss of social status, self-esteem or social acceptance and are characterised by the potential risk of social rejection. Typical forms of threats to the social self are situations where an important aspect of the self-identity is, or could be, negatively judged by others. A key emotional response to social threats to the self is the feeling of shame. It has been suggested that human beings possess specialised psychological systems that monitor and regulate the quest for social acceptance. Social rejection leads to efforts to increase the acceptability to others and/or restore one’s social status by a change in behaviour. It has been proposed that threats to the social self are accompanied by specific psychological and physiological responses, and feelings of shame may be the key affective component in such psychobiological reactions. Stress responses to threats to the social self activate the HPA-axis, accompanied by increased pro-inflammatory cytokine activity and cortisol levels. Thus, chronic or repeated experiences...
of social threats to the self or other negative self-related cognitions may cause long-term immunological and health effects. As social threats to the self activate the fight-flight response, consequently two common reactions to feelings of social rejection and shame are aggression (fight) and depression (flight).

Social status, shame and aggression

Gilligan, who spent many years as a prison psychiatrist, suggests that violence is a defence of status among those who have few sources of status and self-esteem. He says: “I have yet to see a serious act of violence that was not provoked by the experience of feeling shamed and humiliated, disrespected and ridiculed, and that did not represent an attempt to prevent or undo this ‘loss of face’ – no matter how severe the punishment”. Violent behaviour may originate from an extreme sensitivity to issues of personal social status among individuals who are excluded from many of the usual sources of social status. Leary et al have identified different dimensions to the relation between social rejection and aggression. Although the rejection-aggression effect may seem counter-productive and paradoxical, as aggressive and angry behaviour would increase the risk of further rejection rather than bringing people closer, aggression may have hidden benefits such as influencing others, releasing frustration, and establishing control. A study by Eisenberger et al used an fMRI brain scan on subjects playing an ostracism game of Cyberball. Ostracised participants showed activation in brain regions that are also active when experiencing physical pain. The authors concluded that social pain is analogous in its neurocognitive function to physical pain, signalling a sustained injury to our social relations. Considering the connection between physical pain and aggression, this may be a pathway for the relation between social rejection and aggression. The feeling of shame is painful and associations between shame and aggression are well established.

Social status, shame and depression

Although violence is a common response to humiliation and the feelings of shame and disrespect, not all people react with aggression when exposed to threats to the self. Among humans, depression has been proposed as an evolutionary evolved mechanism that serves as an unconscious, involuntary losing strategy in relation to social competition. Depression inhibits investment in challenging or risky activities with a low probability of success, and perceptions of hopelessness, pessimism and behavioural inactivity may be seen as manifestations of this mechanism. Social
competition and chronic stress has thus been proposed as one explanation for the relations between low social status and depression \(^{101}\). Social defeat has, in general, been identified as a major stressor for most species, and involuntary subordination has been related to depression among humans \(^{102}\). Animal models of social defeat are often paralleled to bullying in school and workplaces which involve severe, often chronic stress for the victim with increased risk of depression \(^{103}\). Studies show that shame and humiliation are important factors for the onset of depression \(^{104,105}\). Shaming has been shown to co-vary with mental ill health among social benefit recipients \(^{106}\) and the unemployed \(^{107,108}\). Moreover, shaming has been proposed as a co-varying factor in the relation between sexual abuse experiences and depression \(^{109}\), as well as between obesity and depression \(^{110}\) among adolescents.

The status-shaming model

A status-shaming model (Figure 1) is proposed where social status and shaming experiences may influence the risk of aggression and depression. Moreover, an individual’s social status position may influence the effects of shaming experiences on the risk of aggression and depression.

Figure 1. The status-shaming model
Gene-environment interaction

Background
The nature versus nurture debate has been ongoing since the times of ancient Greece. Today, however, it is generally accepted that both genetic and environmental factors contribute to personality, behaviour and health, and the theory of the “blank slate” has been severely criticised by scientists in the research field of psychology and medicine. In 1998, Eric Kandel presented a new intellectual framework for psychiatry, highlighting the importance of understanding the biological link between the mind and the brain when treating psychiatric disorders. Research on how genes and environment interact is, however, fairly new. Twin studies have been used as an effective way of determining influence from genes and environment. Dizygotic twins share about 50% of their genetic make-up, whereas monozygotic twins share 100% of theirs, when excluding individual mutations. Therefore, if nothing but genes influenced, for example, antisocial behaviour, the behaviour of monozygotic twins ought to be about twice as similar as that of dizygotic twins. If that is not the case, it can be assumed that something environmental has influenced the behaviour and similarity of the twins. Adoption studies are another effective way of determining influences from genes and environment. This is the case, for example, in The Swedish Adoption/Twin Study of Aging that studied monozygotic and dizygotic twins reared together or apart. Although twin studies are effective in determining influence from genes or environment, genes may also influence their environment, and the environment may influence the genetic expression. Individuals may, for example, vary in their ability to cope with stressful environments and experiences depending on their genetic make-up. This phenomenon is commonly referred to as gene-environment (GxE) interaction.

Molecular genetics
Genetic information is stored in a macromolecule called deoxyribonucleic acid (DNA). The DNA molecule consists of two complementary nucleotide chains held together by hydrogen bondings in a double-helix. The information in the molecule consists of pairs of four chemical nucleotides or
bases: adenine (A), guanine (G), cytosine (C) and thymine (T). The base A is always paired with the base T, and the base C with the base G. DNA replicates itself by unwinding the double helix, whereby each strand serves as a template for the synthesis of a complementary sequence of bases. In this way, two double helix molecules are generated, each helix containing one original strand and one complementary strand (Figure 2). DNA replication is how cell division, or mitosis, occurs. This is the reason why the single cell, that each one of us consists of at conception, has turned into $10^{14}$ cells at adulthood.  

Figure 2. DNA and self-replication

https://creationwiki.org/DNA_replication

Another main task of DNA is to synthesise proteins according to the genetic information that is coded in the sequence of the bases. DNA is replicated and copied into messenger RNA (mRNA) through transcription. A number of transcription factors bind to the strand of DNA called the promoter region and show where the transcription will begin. The mRNA then moves out of the nucleus into the cytoplasm of the cell where it connects with the ribosomes. The ribosomes convert the nucleotide sequences of the mRNA into the amino acid sequence that forms the protein (Figure 3). A group of
three bases serially codes for each amino acid and is called a codon. Another RNA molecule, called transfer RNA (tRNA) assembles the protein by matching each codon with the right sequence of bases for each amino acid. The process of translating the base coding of the mRNA to proteins is called translation.\textsuperscript{114}

Figure 3. Translation of mRNA into amino acids

The DNA is packaged into highly compact structures called chromosomes which consist of DNA that is tightly wrapped around proteins for structural support. Humans have 46 chromosomes organised in 23 duplicate pairs, with one chromosome inherited from each parent. The exception is the sex chromosomes where females carry two copies of the X chromosome while males carry one X and one Y. The sex chromosomes determine the sex of the foetus, with possession of a Y chromosome resulting in male development.\textsuperscript{114}

The greater part of the genome is identical in all humans and less than one percent of the total DNA differs slightly between individuals. Occasionally, mutations occur which change the DNA sequence and cause genetic variation. Without this variation, every human would begin life as a clone, each with identical genetic information. These variations contribute to differences in physical appearance, behaviour, elevated risk for hereditary diseases etc. A DNA sequence that is situated at a defined position on a chromosome is called a locus, and if a given locus shows variation in the population, that locus is said to show allelic variation where each variant is called an allele. When a locus has at least two allelic variants that are found
in at least one percent of the population, this is called a polymorphism. If both alleles at a locus are identical, the individual is referred to as homozygous, and if the individual has two different allelic forms the individual is referred to as heterozygous for that particular locus. The most common forms of mutation are single base substitutions, insertions and deletions. These are termed single nucleotide polymorphisms, or SNPs. Such changes are very common and occur on average around once every 1500 bases in the human genome. 114

Different polymorphisms in the promoter sequence of the gene may differ in efficacy of gene transcription. The exact mechanisms of gene expression are not known, although a number of factors are supposed to be involved including polymorphic variations, transcription factors and interaction between different sequences 114. Gene expression is usually defined as the result of a certain gene allele’s production of mRNA or protein. If an allelic variant results in a difference in gene expression that allelic variant is said to be functional 114. Thus, for example, the short allele of a polymorphism consisting of an insertion of a variable length might be associated with a lower expression of the corresponding protein than the long allele. The measurements of gene expression have met with varying results, as transcription and translation of genes in the human brain are difficult to measure. The proposed functionalities of the genes of this thesis are based on results with specific cell cultures and are not yet completely confirmed in other tissues 115-118.

Modern molecular genetics technology employs the polymerase chain reaction (PCR) to identify small fragments of DNA that can be manipulated in laboratory. The process involves replication of the DNA by the use of primers - single stranded DNA molecules identical to the DNA sequence that is to be copied. While heating the DNA molecule the double helix unwinds, and when the mixture is cooling the primers bind to the single stranded genomic DNA by base pairing. The process is repeated multiple times until the concentration of DNA has been increased 10^5 to 10^6 times and polymorphisms can easily be detected by different genotyping techniques. 114

**Neurotransmission**

The brain consists of more than 100 billion neurons that communicate with each other by receiving, processing and sending electro-chemical signals. Each neuron (cell body) has thin branched projections called dendrites that receive information from other neurons. The area that connects two neurons that are communicating by neurotransmission is called a synapse. This consists of one sending part called the presynapse and one receiving part called the postsynapse. The small space (about 20 nm) between the
presynapse and postsynapse is called the synaptic cleft. A neuron transmits an electric impulse by the axon, which splits into branches of presynaptic terminals (Figure 4). Upon the electric impulse from the axon, small vesicles in the presynaptic terminal release transmitters into the synaptic cleft, which bind to receptors at the postsynapse. These may in turn cause a reaction at the new neuron, generating an electric impulse which is then transmitted to other neurons. Each neuron can have up to 10 000 synapses which react to different types of neurotransmitters. The monoamines serotonin, dopamine, and noradrenaline are examples of neurotransmitters that have long and complex systems of axons throughout the brain and spinal marrow.

Figure 4. Neuron

https://creationwiki.org/Neuron

The serotonergic system

Serotonin (5-hydroxytryptamine, 5-HT) is one of the most widely distributed neurotransmitters in the brain, and plays a central role in mood regulation, emotion and cognition 119. It influences a variety of behavioural and neuroendocrine functions including the sleep-wake cycle, appetite, aggression, sexual behaviour, pain sensitivity, sensorimotor reactivity and learning 120 121. Most serotonergic neurons are located along the midline of the brain stem in the raphe nuclei, sending axons to almost all parts of the central nervous system 121. Important terminal regions are among others
found in the frontal cortex, striatum, hypothalamus, hippocampus, and amygdala. Serotonin has also been suggested to act as a modulator of the brain’s structure both during development and adulthood, influencing cell proliferation, differentiation, and synaptogenesis.

Post-synaptic receptors regulate many of the effects of serotonin. There are also a large number of auto-receptors located at the cell body (dendrites) and in the terminal region of the serotonergic neurons. At least 14 serotonergic receptors have been identified, although the exact function of some of these are yet to be determined. Serotonergic receptors will not be described further as they have not been investigated in this thesis.

Levels of serotonin in the synaptic cleft are regulated by a reuptake mechanism in the form of the 5-HT transporter (5HTT), which removes serotonin from the synaptic cleft and transports it back into the presynaptic neuron (Figure 5). Thus, the 5HTT regulates the magnitude and duration of postsynaptic receptor-mediated signalling and plays an important role in the fine-tuning of serotonergic neurotransmission. The antidepressant SSRI (selective serotonin reuptake inhibitor) increases levels of serotonin in the synaptic cleft by inhibiting the reuptake of remaining serotonin into the presynaptic terminal.

The degradation of serotonin to its main metabolite, 5-hydroxyindole acetic acid (5-HIAA), is catalysed by the enzyme monoamine oxidase A (MAOA). It is localised to the outer membrane of the mitochondria in the presynaptic terminal (Figure 5).

The 5HTT gene

Recent neurobiological research has mainly focused on functional candidate genes and their importance for the development of, for example, a specific disease. By screening for sequence variation and testing populations affected and non-affected by a disease, the aim is to find variants in the candidate gene that are more commonly associated with the affected status. One candidate gene that has aroused a great deal of interest is the 5HTT gene. It is located at approximately chromosome 17q12. Transcription of the gene is modulated by the polymorphism 5HTTLPR which is located in the upstream regulatory promoter region of the 5HTT gene and consists of different lengths of a repetitive sequence containing 20-23-bp-long repeat elements. Insertion or deletion of the 5HTTLPR has at some point in evolution about 40 million years ago resulted in a short (S) 14-repeat and a long (L) 16-repeat allele where the short variant has been associated with lower transcriptional efficiency. A number of alleles with 15, 18-20, or 22 repeat copies and variants with single-base insertions/deletions or substitutions within individual repeats are rare. The serotonin transporter is present and shows polymorphic variation in great apes and
monkeys as well as in lower mammals not, however, necessarily similar to the variations in humans \(^\text{142}\). The short variant of the 5HTTLPR has been associated with anxiety-related personality traits \(^\text{135\ 143\ 144}\), susceptibility to depression \(^\text{145}\), sub-threshold depression \(^\text{146}\), amygdala hyperreactivity in relation to emotion-related tasks or environmental threat \(^\text{147-149}\) and elevated levels of amygdala activation at rest \(^\text{119}\).

Figure 5. The synaptic cleft with 5HTT and MAOA
The MAOA gene

The MAOA enzyme metabolises serotonin, norepinephrine and dopamine, which are involved in multiple brain functions associated with stress regulation \(^{115}\) \(^{129}\). Humans and most other mammals produce two MAO enzymes, MAOA and MAOB \(^{150}\). They are encoded by two genes that are oriented tail-to-tail on the X-chromosome between bands Xp11.23 and Xp11.4 \(^{115}\) \(^{129}\). As the MAOB is not investigated in this thesis, only the MAOA will be discussed here.

Males have one X-chromosome (X inherited from the mother and Y from the father) whereas females have two X-chromosomes (one from each parent). This means that males have one copy of the gene encoding for the MAOA, in contrast to females who have two. Females may thus be either homozygous (two similar variants) or heterozygous (two different variants) for the MAOA gene.

A nonsense mutation in the MAOA gene resulting in MAOA deficiency has been found in one single human family in Holland, and is associated with a syndrome of mild retardation and impulsive aggressive behaviour among affected males \(^{151}\) \(^{152}\). MAOA knockout mice have elevated levels of serotonin, norepinephrine and dopamine, and exhibit abnormal aggression \(^{129}\) \(^{153}\). Across species, the absence of MAOA seems to produce aggressive phenotypes. This is further supported by a recent finding that low brain MAOA activity is correlated with the trait of aggression in human males \(^{154}\).

A variable number of tandem repeats (VNTR) of the MAOA gene is located 1.2 kb upstream of the MAOA coding sequences and consists of a 30 base-pair repeated sequence that can have 2, 3, 3.5, 4, or 5 copies \(^{115-117}\) \(^{155}\). By analysing enzyme activity in human skin fibroblast cultures, Sabol et al found a lower transcriptional activity of the 3 and 5 repeat alleles compared with the 3.5 and 4-repeat alleles \(^{115}\), whereas Deckert et al found that the 3.5, 4, and 5-repeat alleles had higher transcriptional activity than the 3-repeat allele \(^{117}\). Denney et al questioned the whole method of defining MAOA transcriptional activity in this way, when they showed that the proportion of MAOA cells in the cultures, rather than true difference in expression, was the primary factor responsible for variation in MAOA activity in skin fibroblast homogenates \(^{156}\). The same research group later found that MAOA expression was lower in the 3-repeat allele than in the 4-repeat allele by controlling for the number of cells in each fibroblast culture \(^{116}\). Regarding the rare 2-repeat allele, it was found to have lower transcriptional activity than the 3-repeat allele, which in turn had lower than the 4-repeat allele \(^{157}\).
Sex differences and the serotonergic system

The serotonergic system is thought to behave differently in males and females. The density of the 5HT2 serotonin receptors is lower in the brains of women \(^\text{158}\), and receptor binding of the 5HT1A serotonin receptor decreases with age in women but not men \(^\text{159}\). The S allele of the 5HTTLPR is associated with higher cerebrospinal fluid levels of the major serotonin metabolite 5-HIAA in women but lower levels in men \(^\text{160}\). SSRIs are known to be more effective in women compared with men \(^\text{161}\). Tryptophan is a precursor of serotonin and may be artificially enhanced or reduced in order to study serotonin function. Tryptophan enhancement is associated with lower levels of neuroticism among women, but the opposite among men \(^\text{162}\). Another study using tryptophan depletion found that men became more impulsive whereas women reported mood reduction and showed symptoms of depression \(^\text{163}\). In a genotype x gender study, men with the LL variant of 5HTTLPR and women with the SS variant showed increases in negative affect during tryptophan infusion \(^\text{164}\). Moreover, sex differences in brain functions associated with aggression have been found in relation to the short variant of the MAOA allele, suggesting that MAOA allelic variation affects males and females differently \(^\text{165}\).

Maltreatment

Childhood maltreatment such as physical, emotional and sexual abuse and family adversity such as inter-parental violence are universal risk factors for a wide variety of mental health problems \(^\text{166-169}\). Among many other things, childhood maltreatment has been associated with an increased risk of antisocial behaviour \(^\text{170-173}\) and depression \(^\text{23 171 174 175}\). Maltreatment is a strong chronic inducer of stress \(^\text{113}\) and has been found to promote adaptations in brain structures as well as elevated neurotransmitter levels that persist into adulthood \(^\text{24 176 177}\). Early life stress increases cortisol levels which in turn has been suggested to down-regulate 5HT1A receptors, resulting in a lower threshold for anxiogenic stressful life events \(^\text{178}\). The experience of maltreatment may thus involve a whole lifetime of consequences for exposed individuals. However, not all children who have been abused develop psychological adversities \(^\text{169 179}\). A number of potential explanations have been proposed including differences in severity and frequency of maltreatment and whether it occurred during childhood or adolescence \(^\text{167}\). Recently, however, the differences in underlying genetic risk factors have received a great deal of attention. A biological vulnerability to stress may predispose certain individuals exposed to maltreatment to an increased risk of psychological adversity, through gene-environment interactions \(^\text{30 180}\).
Psychobiological pathways of antisocial behaviour

Sociopsychological research often underscores the importance of cognition and emotion in relation to aggressive behaviour. Negative affects such as anxiety and fear often precipitate, accentuate and modulate aggression. It seems reasonable to suppose that neural circuitries affecting emotion, such as the central serotonergic system, may also influence a predisposition towards aggression. Concerning the MAOA, the low expressing 3-repeat allele has been associated with differences in limbic circuitry for emotion regulation and cognitive control that may be connected to impulsive aggression. The same allele has been associated with higher behavioural aggression following provocation in males. MAOA has been suggested to have a direct link with stress reactivity and sensitivity. Doyle et al showed that stress is correlated with an increase in salivary MAOA and MAOA deficient mice had an increased reactivity to stress in a forced swim test.

MAOA is the key metaboliser of serotonin, and serotonin has, among other things, been associated with aggression. For example, a study analysing whole blood serotonin in relation to self-reported violence and court conviction reports of violent crime showed that violent men had higher serotonin levels than the normal male population.

In 2002, Caspi et al reported on a gene-environment interaction effect where maltreated boys who carried the short polymorphism of the MAOA gene had a higher risk of developing antisocial problems. Since then, at least four studies have replicated these findings. However, at least three other studies have not found any significant gene-environment interaction effects, and one study only found a non-significant trend in the same direction as the Caspi study. It has been suggested, moreover, that these gene-environment interaction effects may have different directions depending on sex, as one study found that girls carrying the long MAOA allele had the highest risk of criminality in the presence of psychosocial risk. Similar findings, although with weak effects, were recently reported in a study where girls carrying the long allele had higher risk of conduct disorder in combination with childhood adversity. A study by Deckert et al found that the longer, high activity MAOA alleles were more frequent in female panic disorder patients, further suggesting that MAOA may differ in expression depending on sex. However, another recent study that excluded heterozygous girls did not find any sex differences in the interaction between MAOA allelic variation and childhood trauma in relation to aggressive behaviour.
Psychobiological pathways of depression

Major depressive disorder is associated with hyperactivity of the HPA-axis. A number of studies have shown that early stressors and trauma such as child sexual abuse or experience of life-threatening events may create long-term dysregulation of the HPA-axis similar to that seen in depressed individuals. Such neurophysiological changes have been found in animals as well as humans exposed to early stress in the form of maternal deprivation or childhood maltreatment. Neurotoxic effects such as excessive corticotropin activity or the inflammatory effects of cytokines are thought to damage or kill hippocampal cells, which in turn mediate many depressive symptoms.

In 2003, Caspi et al identified a genetic variation of the polymorphism 5HTTLPR at the 5HTT-gene as a possible moderator for the development of depressive disorders following stressful life-events, where individuals with at least one copy of the S allele had a higher risk of depression. Attempts at replicating this study have met with varying results. Several studies have found that individuals homozygous for the S allele have an increased risk of depression when exposed to lifetime stress or adversity. Some studies have, however, only found such patterns among women. One study found an interaction between the S allele and stressful life events, but not with maltreatment, in the risk of depression. Some studies have not found any significant GxE effects at all, whereas one study found an increased risk of depression among adolescents homozygous for the L allele who reported childhood family adversity. It has also been suggested that the 5HTTLPR may have opposite effects on behavioural characteristics depending on sex. A previous study by our research group found that girls with the S allele and boys with the L allele had the highest risk of depression when exposed to psychosocial risk. Moreover, the study suggested that varying forms of psychosocial risk may affect boys and girls differently. Brummett et al recently reported similar findings of an interaction between 5HTTLPR, psychosocial stressors, and sex in the prediction of depression, where females with the S allele and males with the L allele who were exposed to chronic environmental stress had the highest risk of depression. Similar findings of sex differences have been reported in macaques, where the orthologous LS or LL variant of the monkey serotonin transporter polymorphism rh5-HTLPR interacted with adversities to predict higher adrenocorticotropic hormone responses to separation among males, whereas the same was true for females with the SS variant. Uher and McGuffin have suggested that the varying results from the attempts to replicate the Caspi et al study may be explained by some of the studies using selected study samples and unreliable measures of environment. They emphasise the importance of future studies using random representative samples and separating the analyses by sex.
Putting the picture together

This thesis is inspired by the notion that stress that evolves at a macro level of society through individual experience may influence the micro level biological systems in the brain and body through cognition, emotion and neurobiology, thereby causing depression and antisocial behaviour through neuroendocrine pathways. An attempt to illustrate this is presented in the map in Figure 6, inspired by several scientists working within the research field of stress and health 37 41 54 220 221.

From a macro perspective, beginning at the top of the framework, structures at societal level involving grades of inequality, relative deprivation and cultural conditions influence three pathways down to the individual level: (1) The strength of the social bonds and relations within society are associated with trust and mistrust between individuals. Societies characterised by large income disparities generate individual comparisons which, in turn, create conflicts and resentment undermining social cohesion and trust 41. When immense riches are matched against economic hardship, solidarity and cohesion within a society are rendered impossible. (2) Lack of social cohesion invites societal problems such as criminality and isolation. Economically inferior neighbourhoods are often marked by unhealthy environments due to pollution, crime and other hazards, as well as low levels of social support. (3) There is a higher risk of experiencing stressful life events, such as being a victim of crime or child abuse, in socially deprived areas with low social support between citizens and high rates of criminality and other social problems.

Moving further down in the frame-work to the micro level of the individual, the social cohesion in society, societal hazards and stressful life-events trigger emotion, cognition and neurobiological stress responses (4). These mechanisms generate stress, which in turn regenerates emotion, cognition and biological stress responses in a looping circle of reactions (5). Individuals may, moreover, vary in their sensitivity to stress depending on their genetic makeup 113.

High levels of stress may cause pathophysiological changes (6) related to ill-health and antisocial behaviour (7). However, the cycle of stress and pathological pathways is modified by individual coping and health behaviours which also may be dependent on genetic makeup (8) 69. Although the risk for developing pathophysiological reactions to stress is thought to be modified by heredity, the expression of genes may also be influenced by
environmental factors and pathophysiology through the mechanisms of epigenetics (9).

The framework shows a generalised illustration of pathways by which societal structures may influence individual experiences which in turn, by emotional, cognitional and neurobiological reactions, may cause pathological pathways of stress associated with depression and antisocial behaviour.

The map in Figure 6 may be seen from a social structuralistic perspective as presented above; where society and societal structures influence the biology of an individual. However, the map may also be interpreted in the other direction, by the use of a more biologically individual perspective, where the biological organisation of an individual (5) influences coping ability (8) in the confrontation with different life situations and environments such as social cohesion (1), social problems (2) and stressful life events (3).
Figure 6. Theoretical pathway model of stress and influence on health and behaviour

1. Social cohesion: Social bonds, relations, individual comparisons
2. Social problems: Environmental hazards, crime, lack of social support, etc
3. Stressful life-events: Victim of crime, child abuse etc
4. Structures of society: Inequality, culture, relative deprivation

Emotion
Cognition

5. Genetics

Neurobiology

6. Pathophysiological changes

7. Ill health
Antisocial behaviour

8. Coping/ Health behaviours
9. Epigenetics
Aims

The aim of this thesis was to study the factors behind depression and antisocial behaviour among adolescents with a focus on psychosocial and genetic factors.

The aims of the following papers have been:

I. To study the influence of social status and shaming experiences in relation to adolescent antisocial behaviour at school.

II. To study the influence of social status and shaming experiences in relation to adolescent depression.

III. To study the influence of the MAOA-VNTR, maltreatment, and interaction between these factors in relation to delinquent behaviour among adolescents.

IV. To study the influence of the 5HTTLPR, maltreatment, and interaction between these factors in relation to depression among adolescents.
Methods

Study population

The Survey of Adolescent Life in Vestmanland (SALVe) - Liv & Hälsa ung - is a survey distributed biannually by the County Council of Västmanland in Sweden in order to monitor the life situation, life habits and health of the adolescent population of the county. All students in 7th (13-14 years old), and 9th (15-16 years old) grade of elementary school and 2nd (17-18 years old) grade of high school comprise the target population. The present thesis is based on data from students in 9th and 2nd grade participating in SALVe 2006. The students answered the questionnaire during class hours and participation was voluntary and anonymous. In addition, all students in the 2nd grade of high school were asked to provide a saliva sample for DNA extraction. For studies I and II, the overall response rate was 80.3 % with an internal (form) response rate of 97.7 %. Fifty-five of the participants did not state their sex and were excluded from the analyses leaving 3157 participants (1608 boys and 1549 girls) in 9th grade, and 2239 participants (1200 boys and 1039 girls) in the 2nd grade of high school. As studies III and IV only involved participants in the 2nd grade of high school the response rate was somewhat lower due to a higher prevalence of truancy and dropouts in this age group, showing a total response rate of 77.4 % of the target population. Studies III and IV moreover lost participants due to failure to provide the saliva sample, failure in the genome isolation or gene analysis, or because the participants did not respond to the questions of interest for each of the studies, which left a total of 1825 participants (943 boys and 882 girls) in study III and 1482 participants (765 boys and 717 girls) in study IV. Flowcharts of the study populations are presented in Figures 7 and 8.
Figure 7. Flowchart of the study population, papers I and II. Administrative non-respondents refers to classes or schools that did not participate in SALVe 2006. External non-respondents refers to students that were absent on the day of the investigation, and did not return their questionnaire by mail.

- **All 15 and 17 year old adolescents in Västmanland N = 7609**
  - Moved/dropouts n = 414
  - Special school n = 53

- **All students n = 7142**
  - Administrative non-respondents: n = 353

- **Students available for participation: n = 6789**
  - External non-respondents: n = 1338

- **Completed questionnaire n = 5451 (80.3 %)**
  - Internal non-respondents: missing sex n = 55

- **Boys n = 2808**
- **Girls n = 2588**
Administrative non-respondents refers to classes or schools that did not participate in SALVe 2006. External non-respondents refers to students that were absent on the day of the investigation, and did not return their questionnaire by mail.

Genomic isolation

All participants in the 2nd grade of high school were asked to provide a saliva sample for DNA extraction by rinsing their mouth with a 0.9 % isotonic sodium chloride solution for 30 seconds.

5HTTLPR

The 5HTTLPR was amplified in a 10 μl reaction mixture containing 30 ng genomic DNA, 1mM PCR Buffer10x with 1.5mM MgCl₂, 0.2 μM dNTPs, 0.8 μM of two primers, and 0.5 U FastStart Taq DNA polymerase (Roche Diagnostics GmbH, Mannheim, Germany). The primer sequences were: forward 5´-AAC ATG CTC ATT TAA GAA GTG GAA C-3´ and reverse 5´-XCT AGA GGG ACT GAG CTG GAC AAC -3´. The reverse primer was labelled with the fluorescent dye 5´-hex. PCR reactions were performed on a GeneAmp 9700 (Applied Biosystems Inc., Foster City, California, USA) starting at 94°C for 4 min, followed by 35 cycles of denaturation at 94°C for 45 s, annealing at 61°C for 1 min and elongation at 72°C for 90 s, with a final extension at 72°C for 7 min.

MAOA-VNTR

The MAOA-VNTR polymorphism was amplified in a 10 μl reaction mixture containing 30 ng DNA, 1mM PCR Buffer10x with MgCl₂, 7 % DMSO, 0.2 μM dNTPs, 0.8 μM of two primers, and 0.5 U Fast Start Taq DNA polymerase (Roche Diagnostics, GmbH, Mannheim, Germany). The primer sequences were: forward 5´-ACA GCC TGA CCG TGG AGA AG-3´ and reverse 5´-GAA CGG ACG CTC CAT TCG GA-3´. The forward primer was labelled with the fluorescent dye 5´-HEX. The PCR reactions were performed on a GeneAmp 9700 (Applied Biosystems Inc., Foster City, California, USA) starting at 95°C for 4 min, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 61°C for 30 s, and elongation at 72°C for 30 s, with a final extension at 72°C for 7 min.

PCR analysis

The PCR products were analysed by capillary electrophoresis using an ABI PRISM®3700 DNA Analyzer (Applied Biosystems, Inc., Foster City, California, USA) and allele sizes were determined manually on chromatograms using Gene Marker® 1.5 AFLP/Genotyping software (SoftGenetics LLC, State College, PA, USA).

As a control method in the case of inconsistencies, the genotype analysis was carried out a second time and the PCR products were analysed by electrophoresis on a 2 % agarose gel. The gel was run for one hour at 120 V
and visualised under UV light. Running buffer was 0.5 x Tris-EDTA-Buffer (TEB), and sizes were determined by comparison with a 100 bp DNA sequencing ladder.

**Classification of genotypes**

For the MAOA-VNTR analysis, individuals were divided into three groups depending on allelic variation: short alleles with 2 or 3 copies (S/SS), long alleles with 3.5, 4, or 5 copies (L/LL), and the heterozygous female variant of one long and one short allele (LS).

For the 5HTTLPR analysis, individuals were also divided into three groups depending on the allelic variation: those homozygous for the short allele (SS), those heterozygous for the short and long allele (LS) and those homozygous for the long allele (LL).

**Measures**

**Social status**

Socioeconomic status as a measure of income, education and occupation is often used in research on health and behaviour in order to identify a person’s social position in society. When regarding adolescent populations however, such measures apply to the parent’s social position rather than the adolescent’s own. It has therefore been suggested that social status in the school community might be a better measure of social position among youth, particularly when considering that peer norms, perceptions and behaviours are such strong determinants of adolescent life. In papers I and II, a modified version of a scale developed by Goodman et al was used in order to measure different aspects of social status among adolescents. Moreover, the status measure was further developed by defining two different forms of adolescent social status. The family’s socioeconomic status and societal position were defined as **attributed status**. This is the status that the adolescent person is born into and has little or no means to influence as it depends on the parents’ income, education and occupation, not the adolescent’s own. Social status in the peer group and school environment were defined as **acquired status**. The adolescent person has acquired this status by him/herself through social interaction with peers and the status is dependent on how successful the social interaction has been. The following questions on a 7-point Likert scale were used in the questionnaire regarding social status.
Attributed status consisted of:

Family socioeconomic status. Imagine society as being like a ladder. At the bottom are those with the least money, at the top are those with the most. If you think about how wealthy your own family is compared to the rest of society, where would you place your family on this scale?

Family societal standing. Imagine society as being like a ladder. At the bottom are those with the lowest standing, at the top are those with the highest. If you think of the standing/position of your family compared to the rest of society, where would you place your family on this scale?

Acquired status consisted of:

Peer group status. Think about your standing/position amongst your group of friends. At the bottom of the scale are those with the lowest standing in the group, at the top are those with the highest. Where would you place yourself on this scale?

Social status at school. Think about your social standing/position amongst your schoolmates. At the bottom of the scale are those with the lowest standing, at the top are those with the highest standing at school. Where would you place yourself on this scale?

In paper I, the four status questions were used separately in the analyses in order to distinguish the influence from each status factor. In paper II, summation indices of the two attributed status questions, as well as the two acquired status questions, were created with a range of 2-14 points.

Shaming experiences

Two questions were used to determine whether the participants had a recent history of shaming experiences: (1) Have you during the last three months experienced that someone has made you an object of ridicule in front of others? (2) Have you during the last three months experienced that someone has insulted your dignity? The answer alternatives were: No, never; Rather seldom; Sometimes; Rather often; Almost always. A summation index of the two questions was created with a range of 0-8 points. It was then dichotomised into two groups depending on the frequency of shaming experiences among the participants: Few/less shaming experiences (0-2 points) and many/more shaming experiences (3-8 points).

Maltreatment and abuse

Several questions were asked regarding different aspects of childhood maltreatment. Some were outright questions of prevalence of maltreatment, others indicators of bad conditions and possible maltreatment in the family environment. The following questions were asked: (1) Have you ever run away from home? No/Yes. (2) Does anyone in your family have a problem with alcohol or narcotics? No/Yes. (3) Have there ever been severe, heart-
rending quarrels between your parents? Never or less than once every month/Yes, at least once every month. (4) Has either of your parents ever pushed, beaten, or used any other kind of violence against the other? Never or seldom/Yes, at least once a year. (5) Has either of your parents ever pushed or beaten you, or used any other kind of violence against you? Never or seldom/Yes, at least once a year. (6) Have you ever been treated badly psychologically (for example taunted or scorned) by either of your parents? Never or seldom/Yes, at least once a year. (7) Has anyone ever touched you or forced you to touch them sexually against your will? No, never/Yes, at least once. (8) Has anyone ever attempted to have intercourse with you (oral, vaginal, anal) against your will? No, never/Yes, at least once. (9) Has anyone ever had intercourse with you (oral, vaginal, anal) against your will? No, never/Yes, at least once.

For paper III, a maltreatment summation index of the nine questions was created, where each reported incident counted as one point with a total index range of 0-9 points. A dichotomised variable was created by dividing the participants into those with 0-1 maltreatment events and those with at least 2 maltreatment events.

For paper IV, a second maltreatment summation index of questions 3, 4, 5, and 6 was created. This index had a range of 0-4 points. A dichotomised variable was created by dividing the participants into those with no maltreatment (0 points) and those with reported maltreatment (at least 1 point).

Antisocial behaviour

For paper I, antisocial behaviour was defined by asking whether the participant had been involved in either physical or verbal aggression at school. If the participant had hit, kicked or in any other way inflicted physical violence upon another student at school at least once during the last school year, this counted for having been involved in physical aggression. If the participant had seriously teased another student at least once during the last school year, this counted for having been involved in verbal aggression.

For paper III, antisocial behaviour was measured by asking the participants how often they had been involved in different criminal activities: (1)...taken goods in a store, shop, or kiosk without paying? (2)...deliberately smashed or wrecked windows, streetlights, benches, or other public objects? (3)...taken money at home that did not belong to you? (4)...without permission, painted graffiti or scrawled on, for example, a public wall? (5)...sold or bought something you knew was stolen? (6)...stolen a bike? (7)...dodged payment at the cinema or a café, on a bus, train, or similar? (8)...driven a moped, motorbike, or car while being drunk? (9)...broken into a house, shop, store, kiosk, or other building with the intention of stealing? (10)...stolen anything from another person’s pocket or
bag? (11)...threatened or coerced someone in order to make them give you money, cigarettes, or anything else? (12)...stolen a car? (13)...stolen a moped, motorbike, or motor scooter? (14)...been involved in breaking into and stealing something from a car? (15)...by yourself broken into and stolen something from a car? (16)...been involved in a fight during your leisure time (not at school)? (17)...been involved in threatening another person to make him/her do something he/she did not want to? (18)...by yourself threatened another person to make him/her do something he/she did not want to? (19)...carried a weapon (knuckle-duster, baseball bat, knife, switchblade, or similar) at school or during your leisure time? (20)...hit/kicked someone so hard he/she needed medical attention? (21)...deliberately hurt someone with a knife, switchblade, knuckle-duster, or similar? The response alternatives were: Never, Once, 2-4 times, 5-10 times, more than 10 times. A total delinquency index was created as a summation of the 21 questions. A factor analysis revealed four factors with an eigenvalue over one. However, as the fourth factor among girls had only two items (numbers 12 and 14) with high correlation to the second factor, a new factor analysis was made with a fixed three factor solution. Following this, a vandalism index was created as a summation of questions 1-8, a stealing index as a summation of questions 9-15, and a violence index as a summation of questions 16-21.

**Depressive symptomatology**

In papers II and IV, the Depression Self-Rating Scale (DSRS) of the Diagnostic and Statistical Manual of Mental Disorders, Fourth edition (DSM-IV) 32 was used for defining major depression. In DSM-IV, the A-criterion for major depression is defined as two weeks of either dysphoric mood, loss of interest or pleasure in most activities, or in children and adolescents irritated mood, accompanied by at least four other symptoms. These could include sleep disturbances, weight loss or gain/appetite disturbances, psychomotor agitation or retardation, fatigue or loss of energy, feelings of worthlessness or guilt, concentration disturbances, and thoughts of suicide. For the C-criterion, the participant must in addition report significant suffering as a consequence of the symptoms. The A-criterion has a reported sensitivity of 96.1 % and a specificity of 59.4 %, whereas the C-criterion has a reported sensitivity of 86.3 % and a specificity of 75.0 % for major depression 32 33. In paper II the A-criterion of the DSRS was used, and in paper IV the A+C criterion was used.

**Statistical analyses**

Non-parametric $\chi^2$ analyses were used in order to analyse sex differences between the investigated variables in papers I, II, and IV. In papers III and
IV, independent samples t-tests were used for investigating sex differences in the prevalence of maltreatment, delinquency and depressive symptoms. The t-test measures whether the variance between two groups differs from the variance within each group.

Principal components factor analysis with varimax rotation, employing Kaiser normalisation, was used in order to investigate the internal relations between the different status measures in paper II. The same kind of factor analysis was used in paper III in order to evaluate relations between the questions of the total delinquency index and create the specific indices of vandalism, violence and stealing. A factor analysis analyses the internal structure of a set of variables to identify any underlying structures. Although each variable in a group of variables measures something different, subsets of these variables may also be measuring a more general principle. The statistical program uses a repetitive process of orienting and reorienting the factors until it can no longer improve the orthogonality of the loadings of the factors. There are several rotation methods, but the two most frequently used are orthogonal rotation – which means that the factors will remain uncorrelated to each other, and oblique rotation – which allows the factors to be correlated. In papers II and III, the orthogonal rotation was used in order to extract as disparate factors as possible.

Somer’s D was used to investigate correlations between the factors of the models in paper I. Somer’s D is a measure of association between two ordinal variables and ranges between -1 to 1. It is an asymmetric extension of gamma that differs by the inclusion of the number of pairs not tied on the independent variable.

Multiple regression analysis, or general linear model (GLM), was used for investigating the main and interaction effects of maltreatment and genotype in relation to delinquency (paper III) and depression (paper IV). Moreover, the GLM was used for investigation of the associations between social status and shaming experiences in relation to aggression (paper I).

The Mann-Whitney test is a non-parametric test equivalent to the t-test. This was used in papers III and IV in order to compare the late-respondent group with the total population regarding prevalence of maltreatment, delinquency, and depression. In paper IV, the Mann-Whitney test was also used to investigate whether the four maltreatment measures differed in relation to depressive symptoms.

The Kruskal-Wallis test is a non-parametric test that measures whether the variance between three or more groups differs from the variance within each group. It was used in papers III and IV in order to investigate whether the different subgroups of genotype and maltreatment differed in relation to delinquency and depressive symptoms.

Binary logistic regression was used in papers I, II, and IV. This analysis results in an odds ratio, which may be described as a ratio between two odds
that expresses a relative risk that an event or a state will occur. The reference value is 1.0.

In paper III, the distributions of the ordinally scaled, dependent variables were skewed. Therefore, the findings of GxE interaction effects were further investigated by the use of Öhrvik’s non-parametric test. This is based on the joint ranking of the Hodges-Lehmann estimation \(^{224}\) of all observations after removing the individual effects of each factor. If suitably normalised, the weighted sum of the squared differences between the mean rank of each subcategory and the total mean rank will be approximately F-distributed \(^{225}\).

In short, the Öhrvik non-parametric test reveals the interaction effects in an analysis after removing the effects of each single factor. This test has been applied in simulation studies \(^{226}\) using multivariate normal and gamma distributions with substantially higher power than a modified F-test, and has also been evaluated in a review \(^{227}\).

**Ethical considerations**

Participation was voluntary and anonymous. The participants were given written as well as verbal information about the study and an assurance that they could decline to participate at anytime without providing an explanation. A research assistant was present in the classroom during the investigation in order to answer questions from the participants and collect the questionnaires and saliva samples. The Salve 2006 investigation was approved by the regional human ethical research board of Uppsala University.
Results

The status-shaming model in aggression and depression (papers I and II)

In this population based sample of adolescents aged 15-18, 19 % \((n = 509)\) of the boys and 5 % \((n = 126)\) of the girls had been involved in physical aggression at school, and 15.6 % \((n = 418)\) of the boys and 8.7 % \((n = 221)\) of the girls had been involved in verbal aggression at school. A total of 14.6 % \((n = 410)\) of the boys and 28.1 % \((n = 728)\) of the girls were depressed according to the DSRS A-criterion. Many shaming experiences were reported by 14.6 % \((n = 403)\) of the boys, and 17.9 % \((n = 457)\) of the girls. In study I, four separate measures of social status were used: family SES, family societal standing, peer-group status and status at school. In study II, family SES and family societal standing were merged into the summation index of attributed status, and peer-group status and status at school into the summation index of acquired status. The distribution of self-reported social status in the population is shown in Table 1.

Influence of social status on aggressive behaviour and depression

Among boys, both individuals with low and high attributed and acquired status had more often been involved in physical and verbal aggression at school compared with those with medium status. Among girls, individuals with low attributed and acquired status had most often been involved in physical aggression, whereas individuals with high acquired status had most often been involved in verbal aggression, as shown in Table 2.

Low status was associated with an increased risk of depression. Among boys, 23.8 % of those with low, 12.5 % of those with medium, and 12.9 % of those with high attributed status were depressed according to the DSRS A-criterion. Among girls, 41.2 % of those with low, 24.3 % of those with medium, and 24.4 % of those with high attributed status were depressed. Regarding acquired status, among boys 26.0 % of those with low, 12.4 % of those with medium, and 14.6 % of those with high acquired status were depressed. Among girls, 40.8 % of those with low, 25.5 % of those with medium, and 26.5 % of those with high acquired status were depressed. Boys with low attributed status had increased odds of depression of 1.82 \((95\% CI: 1.38-2.40)\) and girls increased odds of 1.84 \((95\% CI: 1.48-2.31)\)
compared with the reference group of medium attributed status. Boys with low acquired status had increased odds of depression of 2.48 (95% CI: 1.83-3.35) and girls increased odds of 2.08 (95% CI: 1.65-2.63) compared with the reference group of medium acquired status.

Table 1. Distribution of self-reported social status among boys and girls, 15-18 years old.

<table>
<thead>
<tr>
<th>Paper I</th>
<th>Boys, n (%)</th>
<th>Girls, n (%)</th>
<th>Total, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Attributed status</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family SES</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>450 (16.2)</td>
<td>599 (19.7)</td>
<td>949 (17.9)</td>
</tr>
<tr>
<td>Medium</td>
<td>1875 (67.7)</td>
<td>1765 (69.5)</td>
<td>3640 (68.6)</td>
</tr>
<tr>
<td>High</td>
<td>446 (16.1)</td>
<td>274 (10.8)</td>
<td>720 (13.6)</td>
</tr>
<tr>
<td>Family societal standing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>341 (12.5)</td>
<td>379 (15.2)</td>
<td>720 (13.8)</td>
</tr>
<tr>
<td>Medium</td>
<td>1950 (71.5)</td>
<td>1806 (72.5)</td>
<td>3756 (72.0)</td>
</tr>
<tr>
<td>High</td>
<td>437 (16.0)</td>
<td>305 (12.2)</td>
<td>742 (14.2)</td>
</tr>
<tr>
<td><strong>Acquired status</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peer-group status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>237 (8.6)</td>
<td>264 (10.4)</td>
<td>501 (9.4)</td>
</tr>
<tr>
<td>Medium</td>
<td>1479 (53.5)</td>
<td>1398 (54.8)</td>
<td>2877 (54.1)</td>
</tr>
<tr>
<td>High</td>
<td>1049 (37.9)</td>
<td>888 (34.8)</td>
<td>1937 (36.4)</td>
</tr>
<tr>
<td>Status at school</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>343 (12.7)</td>
<td>466 (18.4)</td>
<td>809 (15.5)</td>
</tr>
<tr>
<td>Medium</td>
<td>1678 (62.0)</td>
<td>1565 (61.9)</td>
<td>3243 (62.0)</td>
</tr>
<tr>
<td>High</td>
<td>684 (25.3)</td>
<td>498 (19.7)</td>
<td>1182 (22.6)</td>
</tr>
<tr>
<td><strong>Paper II</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Attributed status</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>496 (18.3)</td>
<td>546 (22.1)</td>
<td>1042 (20.1)</td>
</tr>
<tr>
<td>Medium</td>
<td>1695 (62.5)</td>
<td>1572 (63.7)</td>
<td>3267 (63.1)</td>
</tr>
<tr>
<td>High</td>
<td>519 (19.2)</td>
<td>349 (14.1)</td>
<td>868 (16.8)</td>
</tr>
<tr>
<td><strong>Acquired status</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>323 (12.1)</td>
<td>424 (16.9)</td>
<td>747 (14.4)</td>
</tr>
<tr>
<td>Medium</td>
<td>1777 (66.5)</td>
<td>1648 (65.8)</td>
<td>3245 (66.2)</td>
</tr>
<tr>
<td>High</td>
<td>574 (21.5)</td>
<td>421 (17.2)</td>
<td>1005 (19.4)</td>
</tr>
</tbody>
</table>

Influence of shaming experiences on aggressive behaviour and depression

Individuals who reported many shaming experiences had a 2-4-fold increased risk of being involved in physical and verbal aggression at school (Table 2). Shaming experiences, moreover, influenced risk of depression. Among boys, 34.2 % of those who reported many shaming experiences were
depressed compared with 11.3 % of those who reported few shaming experiences. Among girls, 55.1 % of those who reported many shaming experiences were depressed compared with 22.4 % of those who reported few shaming experiences. Individuals with many shaming experiences had an approximately 4-fold risk of being depressed (Boys: $OR = 3.94$, $95\% CI = 3.05$-5.09, Girls: $OR = 4.16$, $95\% CI = 3.33$-5.18).

The status-shaming model in relation to aggressive behaviour

When combining social status and shaming experiences into the status-shaming model, the results formed a U-shaped curve for physical aggression. Individuals with high or low status who reported many shaming experiences had the highest risk of aggression, whereas medium status seemed to have a protective function (Figures 9 and 10). Furthermore, gender differences were found where boys with low attributed status and girls with high attributed status who reported many shaming experiences had most often been involved in physical aggression.

Regarding verbal aggression, individuals with high social status, regardless of status category, who reported many shaming experiences, had the highest risk of being involved in verbal aggressive behaviour at school. Boys with high family SES and many shaming experiences had increased odds of 5.68 ($95\% CI = 3.17$-10.18) and girls increased odds of 5.49 ($95\% CI = 2.41$-12.51) for verbal aggression compared with the reference group of few shaming experiences and medium status. Boys with high status at school and many shaming experiences had increased odds of 5.19 ($95\% CI = 3.18$-8.48) and girls increased odds of 4.34 ($95\% CI = 2.31$-8.15) for verbal aggression compared with the reference group.
Table 2. Aggressive behaviour among study participants. Separate analysis of binary logistic regressions, with percentages and odds ratios, of family SES, family societal standing, peer group status, status at school and shaming experiences in relation to physical and verbal aggression among adolescents (paper I).

<table>
<thead>
<tr>
<th></th>
<th>Physical aggression</th>
<th>Verbal aggression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Boys %</td>
<td>OR</td>
</tr>
<tr>
<td><strong>Family SES</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medium</td>
<td>15.7</td>
<td>1</td>
</tr>
<tr>
<td>Low</td>
<td>29.5</td>
<td>2.24***</td>
</tr>
<tr>
<td>High</td>
<td>21.8</td>
<td>1.49***</td>
</tr>
<tr>
<td><strong>Family soc standing</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medium</td>
<td>16.2</td>
<td>1</td>
</tr>
<tr>
<td>Low</td>
<td>29.2</td>
<td>2.13***</td>
</tr>
<tr>
<td>High</td>
<td>21.9</td>
<td>1.45**</td>
</tr>
<tr>
<td><strong>Peer group status</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medium</td>
<td>14.7</td>
<td>1</td>
</tr>
<tr>
<td>Low</td>
<td>21.3</td>
<td>1.57*</td>
</tr>
<tr>
<td>High</td>
<td>24.3</td>
<td>1.85***</td>
</tr>
<tr>
<td><strong>Status at school</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medium</td>
<td>14.9</td>
<td>1</td>
</tr>
<tr>
<td>Low</td>
<td>25.4</td>
<td>1.95***</td>
</tr>
<tr>
<td>High</td>
<td>25.7</td>
<td>1.98***</td>
</tr>
<tr>
<td><strong>Shaming experiences</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less</td>
<td>16.2</td>
<td>1</td>
</tr>
<tr>
<td>More</td>
<td>34.5</td>
<td>2.73***</td>
</tr>
</tbody>
</table>

*** p <= .001, ** p <=.01 * p <=.05, ns = non-significant
Figure 9. Proportion of boys who had been involved in physical aggression at school depending on shaming experiences and family SES (a) and status at school (b) (paper I).

Figure 10. Proportion of girls who had been involved in physical aggression at school depending on shaming experiences and family SES (a) and status at school (b) (paper I).
The status-shaming model in relation to depression

Participants who reported low acquired status and many shaming experiences had the highest odds of being depressed with a 6-8-fold increased risk (Table 3). Regarding attributed status, the results presented a similar U-shaped curve as when considering the status-shaming model in relation to aggression. Individuals with low or high attributed status who reported many shaming experiences had the highest risk of depression, whereas medium status seemed to present a protective function in the relation between shaming experiences and depression (Table 3).

Table 3. Binary logistic regression of the status-shaming model and depression. Associations between attributed status, acquired status and shaming experiences in relation to adolescent depression, and percentage of each group who were depressed according to the DSRS. The reference groups for the analyses were medium status – few shaming experiences (paper II).

<table>
<thead>
<tr>
<th>Attributed status</th>
<th>Shaming experiences</th>
<th>Boys %</th>
<th>OR¹ (95% CI)</th>
<th>Girls %</th>
<th>OR¹ (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medium</td>
<td>Few</td>
<td>10.0</td>
<td>1</td>
<td>20.0</td>
<td>1</td>
</tr>
<tr>
<td>High</td>
<td>Few</td>
<td>9.3</td>
<td>.97 (.66-1.42)</td>
<td>18.5</td>
<td>.90 (.65-1.26)</td>
</tr>
<tr>
<td>Low</td>
<td>Few</td>
<td>18.8</td>
<td>1.67 (1.20-2.34)</td>
<td>33.2</td>
<td>1.67 (1.27-2.19)</td>
</tr>
<tr>
<td>Medium</td>
<td>Many</td>
<td>29.9</td>
<td>3.61 (2.51-5.19)</td>
<td>49.1</td>
<td>3.88 (2.87-5.25)</td>
</tr>
<tr>
<td>High</td>
<td>Many</td>
<td>35.2</td>
<td>5.41 (3.15-9.27)</td>
<td>64.4</td>
<td>6.92 (3.61-13.28)</td>
</tr>
<tr>
<td>Low</td>
<td>Many</td>
<td>42.2</td>
<td>5.47 (3.53-8.48)</td>
<td>60.6</td>
<td>5.41 (3.76-7.78)</td>
</tr>
<tr>
<td>Acquired status</td>
<td>Shaming experiences</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medium</td>
<td>Few</td>
<td>9.9</td>
<td>1</td>
<td>21.3</td>
<td>1</td>
</tr>
<tr>
<td>High</td>
<td>Few</td>
<td>12.3</td>
<td>1.34 (.97-1.85)</td>
<td>22.1</td>
<td>.99 (.75-1.33)</td>
</tr>
<tr>
<td>Low</td>
<td>Few</td>
<td>17.0</td>
<td>1.79 (1.19-2.70)</td>
<td>29.3</td>
<td>1.58 (1.17-2.14)</td>
</tr>
<tr>
<td>Medium</td>
<td>Many</td>
<td>29.7</td>
<td>3.78 (2.67-5.34)</td>
<td>50.0</td>
<td>3.46 (2.58-4.64)</td>
</tr>
<tr>
<td>High</td>
<td>Many</td>
<td>31.9</td>
<td>3.58 (2.02-6.35)</td>
<td>56.4</td>
<td>4.46 (2.51-7.92)</td>
</tr>
<tr>
<td>Low</td>
<td>Many</td>
<td>47.1</td>
<td>8.56 (5.29-13.84)</td>
<td>64.5</td>
<td>6.67 (4.50-9.88)</td>
</tr>
</tbody>
</table>

¹ = Adjusted for type of housing, separated parents, unemployed parents, ethnicity.
Gene-environment interaction in relation to delinquency and depression (papers III and IV)

The index of criminal behaviour was extensive, and a total of 77.6 % of the boys and 63.9 % of the girls had committed a criminal act at least once during their lifetime. Experience of at least two maltreatment events, which was used as the cut off point in the graphs of paper III, was reported by 14.1 % of the boys and 30.5 % of the girls. In paper IV, we used a more limited maltreatment index involving only maltreatment within the family. A total of 12.8 % (n = 98) of the boys and 20.1 % (n = 144) of the girls reported regular quarrels between parents, 2.7 % (n = 21) of the boys and 5.4 % (n = 39) of the girls reported violence between the parents, 3.7 % (n = 28) of the boys and 5.4 % (n = 39) of the girls reported physical maltreatment, and 7.2 % (n = 55) of the boys and 11.4 % (n = 82) of the girls reported psychological maltreatment. A total of 7.5 % (n = 57) of the boys and 18.3 % (n = 131) of the girls were classified as depressed according to the DSRS A+C criterion.

Genotype frequencies

The distributions of the MAOA-VNTR and 5HTTLPR genotypes are shown in Table 4. Neither the frequencies of MAOA-VNTR or 5HTTLPR differed from what could be expected according to the Hardy Weinberg equilibrium.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Boys</th>
<th>Girls</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAOA-VNTR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S/SS</td>
<td>350  (37.2)</td>
<td>126  (14.3)</td>
</tr>
<tr>
<td>LS</td>
<td>-</td>
<td>398  (45.1)</td>
</tr>
<tr>
<td>L/LL</td>
<td>593  (62.8)</td>
<td>358  (40.6)</td>
</tr>
<tr>
<td>5HTTLPR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SS</td>
<td>156  (19.8)</td>
<td>165  (22.0)</td>
</tr>
<tr>
<td>LS</td>
<td>392  (49.7)</td>
<td>346  (46.1)</td>
</tr>
<tr>
<td>LL</td>
<td>240  (30.5)</td>
<td>239  (31.9)</td>
</tr>
</tbody>
</table>

Maltreatment and gene effects

Maltreatment was strongly associated with delinquent behaviour in all four models of delinquency: Total delinquency: $F = 229.60, p < 0.001, df = 1$; Vandalism: $F = 200.25, p < 0.001, df = 1$; Stealing: $F = 139.88, p < 0.001, df = 1$; Violence: $F = 150.83, p < 0.001, df = 1$ (paper III). There was also a strong relation between maltreatment and number of depressive symptoms:
There was a small main effect of the MAOA-VNTR on delinquency, when controlled for maltreatment, in the total delinquency index ($F = 3.78, p = 0.023, df = 2$), stealing index ($F = 5.40, p = 0.005, df = 2$), and violence index ($F = 4.68, p = 0.009, df = 2$) (paper III). No main effect of the 5HTTLPR on depressive symptoms was found ($F = 0.191, p = 0.826, df = 2$) (paper IV).

Gene-environment interaction in relation to delinquency

By GLM analysis controlled for sex, all four models of delinquency showed significant interactions between the MAOA-VNTR and maltreatment: Total delinquency index: $F = 14.56, p = < 0.001, df = 2$; Vandalism index: $F = 4.97, p = 0.007, df = 2$; Stealing index: $F = 19.66, p = < 0.001, df = 2$; Violence index: $F = 21.27, p = < 0.001, df = 2$ (paper III). The results were confirmed by the non-parametric Öhrvik test. In a further exploration of the interaction effects between maltreatment and the different genotypes, the patterns differed depending on sex. Given a history of maltreatment, boys with the short allele (S) had the highest risk of being involved in delinquent behaviour whereas girls with at least one long allele (LS and LL) had the highest risk (Figure 11).

Gene-environment interaction in relation to depression

There was a gene-environment interaction effect of the 5HTTLPR genotype and maltreatment on depression ($F = 4.175, p = 0.016, df = 2$). When analysed separately for sex, the gene-environment effect among girls remained ($F = 3.182, p = 0.042, df = 2$) but this was not the case among boys ($F = 1.370, p = 0.255, df = 2$). Girls who were homozygous for the S allele had the highest risk of depression in the presence of maltreatment with an odds ratio of 3.73 (95% CI = 1.16-12.07).
Figure 11. Gene-environment interaction of the MAOA-VNTR genotype and maltreatment in relation to adolescent delinquent behaviour of violence and stealing (paper III).
Discussion

This thesis presents the findings of four studies investigating a status-shaming model in relation to depression and antisocial behaviour, and gene-environment interaction between maltreatment and two different genetic polymorphisms related to the serotonergic system – MAOA-VNTR and 5HTTLPR – in relation to delinquent behaviour and depression among adolescents.

General limitations

Before discussing the results of the papers in this thesis, a number of general limitations should be mentioned.

Firstly, Salve 2006 is based solely on self-report questionnaires which involve a risk of information bias due to false or inaccurate responses or recollections from the participants. Underreporting is common regarding undesired behaviours outside of societal norms such as criminality, aggression, or maltreatment. There is also a risk of overestimation of positive traits such as social status. Although self-reports, especially in younger populations, must be interpreted carefully, it has been shown that the accuracy of self-reports increases with age, and among adolescents they are proposed to be more reliable. The alternative use of surrogate variables such as parents’ reports, official records etc also include serious risks of bias and underreporting. Official records of criminality and maltreatment greatly underestimate the true number of offences committed, and are furthermore limited by bias in police and court processing. Such measurements might perform even poorer in studies designed to investigate adolescent phenotypic expression. Moreover, subjective beliefs of life situation and experience have been suggested to be more important than objective estimations when concerning, for example, influence from psychosocial stress on health.

Secondly, the DSRS has high sensitivity and low specificity which involves a risk of false-positive classifications. The measures of maltreatment and criminality are, moreover, not thoroughly validated. However, these measures have been evaluated and developed following previous qualitative interview studies by our research group. Thus, we believe that these items represent good measures of subjective
experiences of maltreatment and delinquent behaviour. As we were interested in psychosocial risk as a psychological stress factor, subjective experience was deemed to be more suitable for our studies than objective measures.

Another limitation that concerns all four papers is the question of cause and effect. A depressed person is, for example, more likely to interpret life events and life situation such as social status, shaming experiences and history of maltreatment more negatively than a non-depressed person. Moreover, aggressive behaviour may by itself cause a certain status position, and high status and dominance may in some contexts be a social reward for aggressive behaviour. Aggressive behaviour may thus elicit others to be aggressive in turn which could result in subjective shaming experiences. Regarding delinquency and maltreatment, youth involved in criminal activity might have suffered reprimands from parents due to their behaviour, and such reprimands might be reported as maltreatment. Because of the design of the Salve 2006 study, it is not possible to distinguish between cause and effect in our results.

Moreover, technical problems with the DNA extraction and PCR analysis resulted in an unfortunately large number of excluded participants, particularly in paper IV, which will be discussed later in this chapter.

However, a major strength of the Salve 2006 material is the large, population-based design. As we reached all students in the targeted grades who were at school on the day of the investigation, as well as a number of late-responding students who were absent from school but returned their questionnaires later by mail, there is a possibility to generalise the results to other adolescent populations as well. The large study population involves a high statistical power with a low risk that the results should be random findings. There is, however, a non-negligible bias due to the school population itself. Namely, the students with most behavioural or psychological problems are more likely to be absent from school any day of the year, including the day of the investigation. Although the analyses of the late-respondents in papers III and IV did not reveal any differences regarding maltreatment or depression, there was a significant difference in delinquent behaviour. It is possible that this information bias might have influenced the results.

The status-shaming model in relation to aggression and depression

In papers I and II we investigated the status-shaming model in relation to depression and aggressive behaviour. We found that shaming experiences
and social status were related and formed a U-shaped pattern, where individuals with high or low status who reported many shaming experiences were at the highest risk of aggressive behaviour at school as well as depression. Medium status seemed to have a protective function in the association between shaming experiences and both aggression and depression. The U-shaped pattern was however not found in the relation between acquired status and depression, where low status in combination with many shaming experiences was associated with the highest risk. Regarding attributed social status and aggression, we found a small difference between the sexes, where girls with high status and boys with low status who reported many shaming experiences were most inclined to aggressive behaviour at school. The results confirmed the suggestion by Goodman et al 71 that social status in peer group and school are important for adolescent health and behaviour and should be considered when investigating conditions related to social status in young populations. The U-shaped patterns may have several explanations. One possible underlying cause may be cultural differences depending on social class, where adolescents from different levels of the socioeconomic scale may differ in upbringing and inherited values. A family’s high SES may, for example, involve placing demands to achieve success upon the children in the family, and the psychological effect of humiliating experiences or failure in social interaction with peers may be stronger and more damaging. Moreover, it is possible that the socialisation of boys and girls may differ not only depending on cultural gender roles 238 but also social class. Children with low SES may grow up in a culture more coloured by traditional gender role attitudes. Boys might, for example, be expected to defend themselves with physical strength if needed, whereas fighting as a response to insult might not be equally accepted in girls. In contrast, higher SES and education in the family are often associated with more egalitarian gender role attitudes 239 240. Girls from families with high SES may thus be encouraged to defend themselves if subjected to humiliation or threats, whereas boys with high SES may be more restricted in the use of physical violence compared with other boys.

The U-shaped results suggest that finding oneself at the top or bottom of a social status order may make an adolescent individual more vulnerable to humiliation and shame compared with the large middle-status group. Conformity, which is the adaptation of the same values, attitudes and behaviours as “everyone else”, and the striving for and needing to “belong”, peaks during adolescence and then decreases with age 72. To have the same status as everyone else in the large middle-status group may thus be a protective factor in this age group. Hochschild has suggested that individuals with high social status have more effective “status shields” in the form of social or emotional capital which make them less sensitive to insults 68. However, the studies by Hochschild only regard adult populations. It may be
that such status shields differ between age groups, and that medium status entails the most effective status shield among adolescents. It has been suggested that conformity is high at the middle and low at either end of a status order, creating a U-shaped curve similar to the results found in papers I and II. This may be one explanation for the results regarding aggressive behaviour. Perhaps individuals with low, medium, or high social status are equally aggressive but use different forms of aggression depending on the norms of society. Relational aggression may, for example, be a more common form of aggression in a group where conformity is high and individuals are eager not to stand out from the group. Moreover, it is possible that overt aggression, which was the only type of aggression analysed in paper I, is more or less accepted depending on the social status of the aggressive person. Several studies have, for example, found associations between peer popularity and aggressive behaviour. Adolescents with high social status may find it easier to “get away” with aggressive behaviour and use aggression as a means of expressing anger and frustration. Aggression may involve social benefits in some peer groups, but be deemed inappropriate behaviour and therefore censored in others. Moreover, aggression may be an equally effective tool for both defending a high status position and improving a low status position.

The results of papers I and II may also be related to personality traits as it has been suggested that individuals with high narcissism and low self-concept are more inclined to react with aggression following ego threat, whereas individuals with low narcissism and high self-concept are more likely to react with depression. Furthermore, personality traits related to social support and social integration are to a large extent hereditary.

The status measures differed in design in papers I and II. In paper I, we wanted to examine the importance of each specific attributed and acquired status variable in relation to shaming experiences, thus investigating family SES, family social standing, status at school and status in peer group separately. In paper II, we were interested in investigating the more general principle of attributed and acquired status, and therefore chose to create indices of these measures.

The status-shaming model renewed

Since the submission of paper I, the status-shaming model of this thesis has been further developed. The original version of the model was presented in the introduction chapter. However, as status and shaming are closely related concepts that may cause, as well as be caused by, certain behaviours such as aggression or depression, a more covering model is presented in Figure 12.

(1) Regarding the relations between status and shaming, a person’s status position is closely related to feelings of shame and pride. Low social status
may, for example, be connected to a more or less chronic feeling of shame. A sudden loss of status also generates feelings of shame. Shame furthermore functions as a warning that our social bonds are damaged or threatened and signals a need for a behavioural change or submission in a social context.

(2) Social status is related to aggressive behaviour and depression as was shown in papers I and II. However, aggression may also cause a specific status position, either increasing or decreasing one’s status depending on the norms of the social context. Depression is also related to social status, as a depressed person is more likely to decrease in status, and a lowered status position in turn is likely to be a risk factor of depression.

(3) The relations between shaming and depression and aggression involve complex psychological mechanisms. It has been suggested that women are more prone to so called “shame-shame loops” - feeling shame over being ashamed - which may result in withdrawal or depression, while men are more prone to so called “shame-anger loops” - being angry that one is ashamed and ashamed that one is angry - which may result in rage and aggression 98.

Figure 12. The status-shaming model renewed.

Specific limitations of papers I and II
The self-report design is specifically problematic in papers I and II because of the questions about emotions and relationships. The participants may be either unaware of this area, or think they are aware but have erroneous views. This is particularly problematic regarding the emotion of shame, because most shame occurs out of awareness and such unacknowledged shame may be a cause of a range of psychological and health-related
problems⁹ ⁵⁶ ⁹⁷. Shame is the key response when experiencing threats to the social self. How an individual reacts when subjected to shame may however be dependent upon personality traits. Regarding paper I, both social rejection and aggression may be related to some common underlying factor such as a personality disorder or psychological difficulties⁹². A person with antisocial or narcissistic personality disorder would typically rate him/herself as high in status, be extremely sensitive to social put-down and also possibly have a tendency towards aggressive behaviour. Moreover, the types of overt, or direct, aggression measured in paper I are more common among boys, whereas girls more often may use indirect or relational aggression which involves using one’s relationships to hurt others ²²⁹ ²⁴¹ ²⁴² ²⁴⁵. Some studies have, however, shown that boys perpetrate the majority of both overt and indirect aggression ²³¹. The overt aggression is, in general, more norm-breaking than indirect aggression, which is more “invisible” in its nature. As we were interested in deviant aggressive behaviour in relation to status and shaming, overt aggression was therefore deemed the most appropriate measurement. Moreover, indirect aggression is often particularly underestimated in self-reports ²²⁹. It is furthermore difficult to differ between subjective social status and shaming in relation to self-esteem, as a person with low self-esteem would be more inclined to interpret social signals from others as degrading and negative, whereas a person with high self-esteem would be more likely to interpret his/her own social status as high.

**Gene-environment interaction effects**

In papers III and IV, gene-environment interaction between maltreatment and MAOA-VNTR as well as 5HTTLPR in relation to delinquent behaviour and depression was investigated. Maltreatment was an independent risk factor for both depression and delinquency. There were also small but significant main effects of the MAOA-VNTR in relation to delinquency, as well as the S-allele of the 5HTTLPR in relation to depression, although the later was only found among girls. Moreover, gene-environment interaction effects of the MAOA-VNTR in relation to delinquency, as well as of the 5HTTLPR in relation to depression were found. There were differences between the sexes regarding direction and effect of gene-environment interaction. Boys with the short variant and girls with the long variant of the MAOA-VNTR polymorphism had a higher risk of delinquency when exposed to maltreatment. Regarding 5HTTLPR, no significant GxE effects were found among boys, whereas girls homozygous for the short allele had a higher risk of depression when exposed to maltreatment.
MAOA-VNTR, maltreatment, and delinquency

The results from paper III replicated previous findings of an interaction between the MAOA-VNTR and maltreatment on both male 12 185-187 and female 193 194 delinquency. The reliability of reported main effects of the MAOA-VNTR on delinquency has been much debated as these have been found in some studies but not others. Kim-Cohen et al described main effects of the long allele in a meta-analysis 188, and similar findings among girls have been reported previously by our research group 193. Foley et al, on the other hand, found reduced risk for conduct disorders among short-allele carriers 186. However, several studies have not been able to confirm any main effects of the MAOA-VNTR 185 187 189 191 192. As the reported main effects in paper III in addition are small, the importance of the findings may be questioned. As there are about 35 000 protein-coding genes in the human genome 246 247 influencing functionality and development as well as interacting with each other, the possible influence from one single polymorphism on delinquency, without taking environmental influences into account, is probably fairly negligible when considering the rather small and inconsistent main effects reported here and otherwise.

Concerning the gene-environment interaction effects, one possible interpretation of the findings may be drawn from Boyce and Ellis 248 who proposed that some children may be biologically predisposed to being sensitive to their environments whereas others may be more insensitive. Sensitive children, by Boyce and Ellis called “orchid” children, flourish in contexts with relatively low levels of stress but may fare poorly in more unpredictable and stressful environments. Conversely, children with more nonreactive tendencies, so called “dandelion” children, may not prosper as much as orchid children in optimal contexts, but are more resilient under stress. This theory may be supported by recent findings that individuals with the short variant of the MAOA-VNTR polymorphism are more sensitive to social exclusion, showing higher trait aggression and higher trait interpersonal hypersensitivity during a social exclusion task 249. Moreover, the short variant of the MAOA-VNTR has been suggested to cause an ontogenic excess of 5-hydroxytryptamine, which labilises critical neural circuitry for social evaluation and emotion regulation and thus amplifies the effects of adverse early-life experiences and life-stress 250. A further connection between MAOA and antisocial behaviour has recently been suggested in a study where the short allele variant was associated with increased susceptibility to antisocial traits through alterations to the neural systems for processing threat-related emotion 251.

There has been no evidence of a correlation between MAOA-VNTR genotype and MAOA enzyme activity or expression in the brain, neither in studies on post-mortem brain tissue of MAOA enzyme activity or mRNA expression 252, MAOA mRNA 253 254, nor the use of PET in an imaging study.
However, the findings that absence of MAOA produces aggressive phenotypes across species [129 151-153], as well as human GxE interaction studies [12 185 256] indicate that MAOA should have been of importance at some stage of development. MAOA is the main metaboliser of serotonin, and serotonin is an important inhibitory growth factor for foetal development of CNS [257-259]. The serotonergic importance of MAOA is further accentuated by the finding that MAOA knockout mice have a weaker serotonergic innervation in the brain [260]. It may be that MAOA-VNTR mainly plays a functional role during foetal development [255 261], influencing the development of the serotonergic system, and that the association between MAOA-VNTR and MAOA enzyme expression diminishes with age. Possible factors that may contribute to this decreased association could be hormones [262 263] and epigenetic mechanisms [253]. As both these factors differ between the sexes [264 265], this might be part of the explanation of the sex differences in GxE interaction found in paper III.

5HTTLPR, maltreatment, and depression

The results from paper IV of a GxE interaction effect among girls, where the short variant of the 5HTTLPR interacted with maltreatment in relation to depression, replicated previous findings [209 210 215 218]. However, no GxE effects were found among boys, which went against the hypothesis that the LL allele would be associated with an elevated risk of depression in interaction with maltreatment [164 215 218]. Depression as defined by the DSRS is a symptomatic condition which might be biased for typical female symptoms. It has been suggested that men suffering from depressive disease may show a whole other range of symptoms such as aggression and acting-out behaviour [266-268]. However, among children and adolescents, irritated mood is included in the general criteria of DSM IV [32], and therefore this was also included in the DSRS scale used in SALVe.

Moreover, the SS allele was associated with reduced risk of depression in girls when adjusted for maltreatment and interaction effects. Previous studies have reported associations between the short allele and neuroticism [135 143 269], pointing in the opposite direction from our finding of a relation between the short allele and reduced risk of depression in girls. Although neuroticism and depression are not automatically correlated and quite different from each other, neuroticism might be related to depression in some individuals. However, it is important to note that the personality traits studied by, for example, Lesch et al [135] are not pathological traits, but variations of neurotic personality traits in a random population. Moreover, in an analysis investigating the effect of the 5HTTLPR without adjusting for environmental factors, no significant relation between the SS allele and depression was found. The same results have been reported in a large population-based
study comprising more than 88,000 individuals, where the relation between 5HTTLPR and both depression and neuroticism without adjusting for environmental effects were investigated, and no significant effects were found. Our results thus further pinpoint the importance of adjusting for environmental effects when studying relations between genotype and phenotype. It is, however, essential to emphasise that depression is a multifactorial, complex disorder where a large variety of other factors than 5HTT are of importance. This fact is even more highlighted when considering the small explained variance in our statistical models.

Sex differences in gene-environment interaction

The differences in findings between the sexes may be seen in the light of studies that show that the serotonergic system functions differently in men and women in several important ways. Thus, differences in the functionality of the serotonergic system might be an explanation of the sex differences found in both papers III and IV. Both MAOA and 5HTT are involved in the determination of serotonergic levels in the human brain, both during foetal life and thereafter. The relation between 5HTTLPR, personality traits and psychological well-being has also been shown to differ depending on sex. Previous studies of GxE interaction effects of the MAOA-VNTR and 5HTTLPR have often presented results on whole populations without dividing by sex, or even excluded one of the sexes from analysis. Thus, the sex differences found in papers III and IV are not uncontroversial. Moreover, biological imbalances in the neurocircuits involved in stress reaction, including the serotonergic system, caused by traumatic experiences such as maltreatment may be expressed in many different ways. It is possible that some typical symptoms of such biological imbalance may range under the diagnosis of, for example, attention deficit disorders, hyperactivity or conduct disorder, which are all more common among men.

Specific limitations of papers III and IV

Regarding papers III and IV, a major limitation is that we only reached 77.4% of the target population. Nearly one fourth of the students were absent from school on the day we carried out the investigation in their class. We gave questionnaires to the teachers for distribution to the students not attending class, and 183 students returned their questionnaires and saliva samples later by mail. These late-respondents were included in the general data analyses, but also used for drop out analyses, where they were compared with the rest of the study population regarding the investigated variables. The late-respondents reported slightly higher rates of delinquent
behaviour, but no differences in maltreatment or depressive symptoms. Late-
respondents have been suggested to be similar to non-respondents in survey
studies\textsuperscript{274}, which means that our results in study III are probably somewhat
weaker than what would have been the case if we had reached the whole
target population. We might have missed the most delinquent students who,
possibly as a consequence of general behavioural problems, were not at
school on the day of the investigation. The use of questionnaire contra
interviews has been proposed as one possible explanation for the
inconsistent results of GxE studies on the 5HTTLPR genotype in relation to
depression\textsuperscript{275}. Studies that use structured or stratified interviews in general
find stronger interaction effects compared with large population-based
studies using self-report questionnaires. For example, the results by the
Nilsson et al interview studies were found after selecting an oversized
number of participants that scored at either end of a deviant behaviour risk
index from the total population\textsuperscript{185, 193, 233, 276}. The methodological issues
mentioned by Uher and McGuffin\textsuperscript{275} would apply to all four papers of this
thesis.

It is important to replicate the present findings in other populations of
different culture and ethnicity before drawing any definite conclusions from
the results. For example, Ducci et al recently found that females of native
American ethnicity with a history of sexual abuse that carried the short
MAOA-VNTR allele had the highest risk of alcoholism\textsuperscript{277}, whereas Nilsson
et al found that females of Scandinavian ethnicity with a history of
maltreatment and abuse that carried the long MAOA-VNTR allele had the
highest risk of alcohol-related problem behaviours\textsuperscript{234}.

The maltreatment indices were, moreover, constructed differently in
papers III and IV. In paper III, we wanted a wide and sensitive measure of
maltreatment in order to identify associations with delinquency in relation to
genotype. In paper IV, however, we chose to include the factors that we
deemed to be the most effective measures of maltreatment.

A specific problem with paper III is that the MAOA gene is located on the
X-chromosome, which involves difficulties in the interpretation of the
results among females. In a female cell line, only one of either paternally or
maternally derived X-chromosomes is normally active in each cell\textsuperscript{278}.
Among the heterozygous girls in paper III, we do not know whether the S or
the L allele is inactivated. Other research groups have often chosen to
exclude heterozygous girls from analysis. However, some X-linked genes,
including the MAOA gene, have been suggested to escape inactivation and
the activation level can also vary, expressing different extents of inactive X-
chromosomes\textsuperscript{279}. If MAOA partially escapes X-inactivation, the role of the
MAOA genotype would be more unpredictable for the female than the male
phenotype. There might, moreover, be a MAOA dosage compensation
mechanism that does not correlate with X-inactivation\textsuperscript{253}. An escape of the
X-linked inactivation of the female MAOA gene is, however, not an
undisputed fact. We chose to report the results in the heterozygous girls despite the uncertainty of the activation of the MAOA-VNTR alleles. These results must therefore be interpreted with caution.

A major weakness in paper IV is the large number of excluded participants due to problems with the 5HTTLPR genotype analyses. A total of 586 analyses failed, and it is possible that the method of DNA extraction by rinsing of the mouth with 0.9% sodium chloride solution followed by the primer sequences, PCR and DNA analyser that were used, was not optimal for the definition of the 5HTTLPR genotype. As the design was anonymous, it was not possible to repeat the DNA collection in the failed samples. However, no significant differences in prevalence of depression or maltreatment were found among those with available 5HTTLPR when compared with those where the genotyping had failed.

Another important issue regards the supposed triallelic nature of the 5HTTLPR, as an A>G substitution at nucleotide 6 of the first of two extra 22 base-pair repeats that define the 16-repeat L allele has been identified. This L_G variant has been suggested to be similar in expression to the S allele. The allelic frequency of L_G is suggested to be approximately 0.09-0.14 in Caucasians and 0.24 in African-Americans. However, the functionality studies have been made by analysing mRNA expression in lymphoblast cell lines with rather skewed and small groups. No study has yet been able to show any specific associations between the L_G variant and phenotypic expression. One recent study analysing the L_A and L_G variants separately found marginal evidence for GxE effects on emotional symptoms among 7-year-old boys both in the high and low 5HTTLPR expressing groups. The authors explained their result as an effect of multiple testing. Moreover, another A>G substitution has recently been found in the S allele and the functionality of this allele variant is as yet unclear. All in all, there is a need for further functionality studies of the less common allelic variants of the 5HTTLPR. As the functionality of the 5HTTLPR, especially regarding the A>G substitutions of the L and S allele, is still uncertain, we chose not to investigate the A>G substitutions of the L and S alleles in paper IV.

Statistical limitations

In papers I and II, multiple statistical analyses were used and adjustments by Bonferroni corrections might have been considered in order to avoid Type I errors. On the other hand, such analyses greatly increase the risk of Type II errors, and as the data resulted in positive outcomes of distinct hypotheses, indirectly replicating previous research, it may be argued that results should be presented without Bonferroni corrections.
In papers III and IV the GLM was used for investigating interactions between maltreatment and genotype in relation to delinquency and depression. The choice of statistical methods in a study of gene-environment interaction, with a skewed ordinal or interval scale as outcome, is always a delicate matter. On the one hand, interaction effects are efficiently measured by GLMs and according to the Central Limit Theorem the sampling distribution will be approximately normally distributed as N increases even if the population is not normally distributed. Therefore, GLM or other linear regression models could be used to investigate main and interaction effects in GxE models, interpreted, however, with caution. On the other hand, there are non-parametric interaction tests such as Öhrvik's test. However, this test does not measure the main effect of each variable. Another frequently used test in GxE studies is the Kruskall-Wallis test or the parametric equivalent, ANOVA. These tests do not, however, distinguish between main and interaction effects and must also be interpreted with caution. If the outcome variable can be translated into a dichotomous variable, a multivariate logistic regression can be used to measure main and interaction effects, as in paper IV. To conclude, complementary statistical approaches may be applied to eliminate scaling artefacts, which are one of the most ubiquitous sources of artefacts in interaction research.

Implications and future studies

The finding of relations between the status-shaming model and aggression and depression contribute to the important research on how social status and shame may influence health and behaviour. This may have important implications for the understanding, prevention, and treatment of antisocial behaviour and depression among adolescents. Schools and institutions working to prevent and detect antisocial behaviour and depression in young people might benefit from an awareness of how social status and shame may influence these factors. The results, moreover, highlight the importance of measuring different kinds of social status, i.e. socioeconomic status and social status at school, in studies of the associations between social status, health and behaviour among adolescents. The U-shaped results furthermore emphasise the importance of identifying students at risk at both ends of the socioeconomic scale. It is important to focus on and support socioeconomically disadvantaged students, but one should not forget students at the other end of the socioeconomic scale who might be at a similar increased risk of psychological ill health when exposed to shaming experiences.

There is a clear but complex association between response to stress and risk of an aggressive or depressive outcome. A specific status position in a social context, experiences of humiliation or degradation, as well as
experiences of maltreatment are all examples of psychosocial stress that influence the sensitive biological system in the brain. MAOA-VNTR and 5HTTLPR may be important players in the complex pathways of overlapping and interacting transmitters and hormones related to stress in the human brain. The findings of gene-environment interaction effects in depression and delinquent behaviour should however be viewed as basic research contributing to a small part of the mapping of the human brain and its functions. We do not yet know enough about the functionality of the 5HTTLPR and MAOA-VNTR in relation to other polymorphisms and genotypes to make any general assumptions on how allelic variation actually influences neurotransmission in the serotonergic system. Although there is a growing literature indicating sex differences in how the 5HTTLPR and MAOA-VNTR polymorphisms function in relation to environmental influence, which the papers of this thesis further extend, we do not know how or why these differences occur. Further investigations of the functionality of these genes, how they interact with other genes, identification of key stress factors, and influence from epigenetic mechanisms are needed before any general conclusions of how the serotonergic system influences the human brain can be drawn.

Furthermore, a very interesting approach in research on how social status and social stress are related to health and behaviour concerns the serotonergic system. For example, studies on a number of species have shown associations between chronic stress due to repeated social defeat in subordinated animals and hyperactivity of the HPA-axis, alterations in serotonin neurotransmission and signs of depression. The possibility that depression may in some way be linked to evolutionary developed behavioural systems dealing with conflicts connected to social status could help to explain the associations between experiences of humiliation and entrapment and depressive disorders in humans. Several studies of different species have, moreover, found correlations between social status and serotonin levels. Among humans, associations have been found between SES, as well as interactions between SES and 5HTTLPR, in relation to brain serotonergic responsiveness. Moreover, a recent study found associations between the short variant of the MAOA allele, child saturation in the neighbourhood, and adolescent aggression.

A further question concerns how gene-environment interaction effects might be understood in relation to the social status gradient of health. A higher status position in society is supposed to bring better status shields and, consequently, less stress. The construction of society, in other words, protects high status individuals from the unhealthy stress that falls upon citizens at the bottom of society’s status order. The effect of certain genes on health and behaviour would then be stronger in groups of low socioeconomic status, as these individuals are subjected to higher levels of everyday stress, i.e. a gene-environment interaction.
The original idea of this thesis was to complement the studies of the status-shaming model in relation to aggression and depression with further analyses of how genotype might be of importance in relation to social status. This was unfortunately not possible, as our study population did not offer enough power to support such analyses. Instead the choice was to investigate how psychological stress in the form of experiences of maltreatment might interact with genotype in relation to aggression and depression. However, further investigations on how stress factors such as social status and shame interact with biological mechanisms in relation to antisocial behaviour and depression would be the logical next step in the future research of this area.
Social status in the peer group and at school, as well as family socioeconomic status, are important for adolescent antisocial behaviour and depression and should be considered when investigating conditions related to social status in young populations. Moreover, finding oneself at the top or bottom of a social status order may make an adolescent individual more vulnerable to shaming experiences compared with the large middle-status group. Medium social status may entail a “status shield” among adolescents, making an individual less sensitive to humiliation or insult.

Psychological stress in the form of experiences of maltreatment may interact with genotype in relation to delinquent behaviour and depression. Both MAOA-VNTR and 5HTTLPR may be important elements in the complex pathways of overlapping and interacting transmitters and hormones related to stress reaction in the human brain. There may, moreover, be sex differences in the functionality of the serotonergic system.

The findings pinpoint the importance of having a comprehensive overall view of human health and behaviour, where genes and environment interact in the development of different pathological conditions. Reaction to stress may partly depend upon genetic vulnerability, but it is important to adjust for environmental effects when studying relations between genotype and phenotype. Further investigations of the functionality of these genes, how they interact with other genes, influence from epigenetic mechanisms, and possible differences in functionality depending on sex are, however, needed. The findings may have important implications for the understanding, prevention, and treatment of antisocial behaviour and depression among adolescents.

för att utveckla antisocialt beteende. Ett flertal studier har därefter replikerat dessa resultat medan andra studier inte har hittat några liknande signifikanta interaktionseffekter. Det har även spekulerats i om genmiljö interaktionen kan se olika ut beroende på kön eftersom en studie funnit att flickor med den långa varianten av MAOA-VNTR hade högst risk för kriminalitet i kombination med svåra uppväxtförhållanden. År 2003 presenterade Caspi et al en ny gen-miljö interaktionsstudie där individer med den korta, mindre funktionella, varianten av polymorfismen 5HTTLPR på 5HTT-genen som upplevt svåra och stressande livshändelser hade högst risk för att utveckla depression. Även här har försök till replikationer redovisat varierande resultat.

I denna avhandling presenteras fyra studier som behandlar olika former av psykosocial stress i relation till antisocialt beteende och depression hos ungdomar. I studie I och II undersöks en status-skam modell i relation till aggressivt beteende i skolan och depressiva symptom. I studie III och IV undersöks gen-miljö interaktionseffekter mellan MAOA-VNTR och svåra uppväxtförhållanden i relation till kriminalitet, samt 5HTTLPR och svåra uppväxtförhållanden i relation till depressiva symptom.

Studierna bygger på material från Liv & Hälsa ung i Västmanland 2006 (SALVe 2006). Det är en enkätundersökning som genomförs varannat år i samtliga skolklasser i åk 7 och 9 i grundskolan samt år 2 i gymnasiet i Västmanland på uppdrag av Landstinget Västmanland. Eleverna besvarade enkätten under lektionstid och deltagande var frivilligt och anonymt. Samtliga elever i gymnasiets år 2 tillfrågades även om att lämna ett salivprov för genanalys. Studie I och II baserades på formulär från 5396 elever i åk 9 och år 2 på gymnasiet. Studie III och IV inkluderade endast elever i år 2 på gymnasiet, och efter isolering och PCR analys av de aktuella kandidatgenerna återstod 1825 deltagare i studie III och 1482 deltagare i studie IV.

I studie I upptäcktes att social status och skam samverkade i ett U-format samband, där de individer med låg respektive hög status som utsatts för skamupplevelser hade högst risk för fysiskt aggressivt beteende i skolan. Medel status verkade ha en skyddande effekt i relationen mellan skamupplevelser och aggressivitet. Detta gällde både för den tillskrivna statusen (föräldrarnas socioekonomi och sociala ställning) och den egenförvärvade statusen (social status i kompisgruppen och i skolan).

I studie II upptäcktes också ett U-format samband mellan tillskriven status, skamupplevelser och depression. Individer med låg respektive hög tillskriven status som utsatts för skamupplevelser hade högst risk för depression. Medel status verkade även här ha en skyddande effekt. Gällande den egenförvärvade statusen hade individer med låg social status i skolan eller kompisgruppen högst risk för depression i kombination med skamupplevelser.
I studie III och IV upptäcktes ett starkt samband mellan svåra uppväxtförhållanden och både kriminellt beteende och depression. Det var även könsskillnader i gen-miljö interaktionseffekter i båda studierna. I studie III hade pojkar med den korta (S) allele-varianten av MAOA-VNTR högst risk för kriminellt beteende i kombination med svåra uppväxtförhållanden, medan flickor med minst en lång (LS och LL) allele-variant av MAOA-VNTR hade högst risk för kriminellt beteende i kombination med svåra uppväxtförhållanden.

I studie IV upptäcktes en gen-miljö interaktionseffekt bland flickor, där individer med den korta allele-varianten (S) av 5HTTLPR genen hade högst risk för depression i kombination med svåra uppväxtförhållanden. Inga signifikanta gen-miljö interaktionseffekter hittades dock bland pojkar.


Det finns ett tydligt men komplext samband mellan respons på stress och risk för en aggressiv eller depressiv reaktion. En specifik statusposition i en social kontext, upplevelser av förnedring eller kränkning, eller svåra uppväxtförhållanden är alla exempel på psykosocial stress som kan påverka det känsliga biologiska systemet i hjärnan. MAOA-VNTR och 5HTTLPR kan båda vara viktiga spelare i de komplexa systemen av överlappande och interagerande transmittorer och hormoner som är relaterade till stress i den mänskliga hjärnan. Fynden av gen-miljö interaktionseffekter i relation till kriminellt beteende och depression i studie III och IV visar betydelsen av att se människan ur ett helhetsperspektiv, där både arv och miljö påverkar hälsa och beteende och samverkar vid uppkomsten av olika patologiska tillstånd. Detta innebär att farmakologiska, sociala och psykologiska behandlingsformer kan tänkas samverka och förstärka effekten av varandra när det gäller
behandlingar av tex antisocialt beteende och depression bland ungdomar. Resultaten bör dock än så länge ses som relativt grundläggande forskning som bidrar till en liten del av kartläggningen av den mänskliga hjärnan och dess funktioner. Funktionaliteten av 5HTTLPR och MAOA-VNTR i relation till andra polymorfismer och gener är än så länge inte tillräckligt kartlagda för att man ska kunna dra några generella slutsatser om hur dessa allele-varianter egentligen påverkar neurotransmission i det serotonerga systemet.
Acknowledgements

I would like to express my sincere gratitude and appreciation to all who have supported and helped me with the work underlying this thesis. In particular I wish to thank:

Kent W Nilsson, my supervisor, for never-ending enthusiasm, encouragement and countless “shouting-through-the-door” micro-supervision sessions. You have been a great source of inspiration and an excellent scientific tutor with your broad scientific knowledge, great pedagogical talent, and never-ending patience. But you are also a much appreciated friend and mentor concerning my life outside of the scientific world. I very much look forward to our future work together!

Jerzy Leppert, my co-supervisor and head of the Centre for clinical research in Västerås, for constant support through every step of the research process. Thank you for always taking time to listen and give excellent advice no matter how small an issue. And thank you for giving me the opportunity to work at CKF and creating such a wonderful positive research atmosphere, allowing for the flow of creativity and the pleasure of work.

Bengt Starrin, my co-supervisor, for always entering my room with a rush of positive energy and creativity, for your constant encouragements and excellent advice and tutorship.

Lars Oreland, my co-supervisor, for providing excellent advice with great knowledge and generosity when I most needed it. Also, thank you for providing your lab for the genetic analyses.

Niklas Nordquist, for the organisation of, and work with, the genetic analyses as well as the interpretation of the biological data.

Erika Comasco, for genotyping and analysing all the saliva samples, and for good times in Camargue.

Tony Wiklund, for help with the collection, scanning and validation of the data from SALVe 2006, as well as computer support, always willing to help.
Eva Nohlert, for help with the collection, scanning, and validation of the data from SALVe 2006.

Roland Axelsson, Ulla Händel, Kerstin Håkansson-Bsenko, and Bo Simonsson for the work with SALVe 2006.

Eva Strand, my much appreciated room-mate at CKF, for countless laughters, sharing of successes and misfortunes, and general “dog-talk”.

Marie-Louise Södersved-Källestedt, for “expertise stress counselling”.

Katarina Ringström, for support during the years with powerpoint, endnote, figures, always willing to help.

Michaela Eriksson, for being always supportive and helpful, instantly solving all problems and requests with a catching smile on your lips.

Maria Dell’Uva Karlsson, for help and support with innumerable things, always taking the time to solve a problem or find an answer to a question.

All PhD students and supervisors at CKF for all inspiring and interesting discussions and seminars and all the fun we have had in Västerås as well as in Camargue.

All the staff at CKF for providing a positive, supportive, and creative research environment.

Kristina Sundquist, for excellent comments on the linguistic formulations of the thesis.

David de Boniface, for careful English revisions of the manuscripts and thesis.

Rickard L. Sjöberg, for opening a door into the world of research and suggesting which path to take.

The staff at the Central Hospital library and especially Gunnel Hellman for all support and help in finding references and literature.

All students who participated in the Liv & Hälsa investigation 2006, without you there would not have been any thesis.

Last but not least, thanks to my family! To Niklas for all love and support and for keeping things running when the work days turned out too long. To
my wonderful parents Indra and Sven-Erik and my brothers Fredrik and Kristian for always being there for me and making me who I am, genes and environment in interaction 😊
References


73. Dittes EJ, Kelley HH. Effects of different conditions of acceptance upon conformity to group norms. *Journal of Abnormal and Social Psychology* 1956;53:100-107.


150. Johnston JP. Some observations upon a new inhibitor of monoamine oxidase in brain tissue. *Biochemical Pharmacology* 1968;17(7):1285-1297.


155. Huang YY, Cate SP, Battistuzzi C, Oquendo MA, Brent D, Mann JJ. An association between a functional polymorphism in the monoamine oxidase a gene promoter, impulsive traits and early abuse experiences. *Neuropsychopharmacology* 2004;29(8):1498-1505.


174. Boney-McCoy S, Finkelhor D. Is youth victimization related to trauma symptoms and depression after controlling for prior symptoms and


220. Dahlgren L. 2009-01-08, Personal communication.


Acta Universitatis Upsaliensis

Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine 494

Editor: The Dean of the Faculty of Medicine

A doctoral dissertation from the Faculty of Medicine, Uppsala University, is usually a summary of a number of papers. A few copies of the complete dissertation are kept at major Swedish research libraries, while the summary alone is distributed internationally through the series Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine. (Prior to January, 2005, the series was published under the title “Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine”.)