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The Monoamine Oxidase A Gene and Antisocial Outcomes

An Examination of Genetic, Epigenetic, and Environmental Factors

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Abstract

Checknita, D. 2021. The Monoamine Oxidase A Gene and Antisocial Outcomes. An Examination of Genetic, Epigenetic, and Environmental Factors. *Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine* 1718. 102 pp. Uppsala: Acta Universitatis Upsaliensis. ISBN 978-91-513-1130-2.

Background. Antisocial behaviour involves violation of the basic rights of others or social norms or rules. Such behaviours are indexed in diagnoses such as conduct disorder (CD) in adolescence and antisocial personality disorder (ASPD) in adulthood, which are typified by comorbidity with mood, anxiety, and substance misuse disorders. Alcohol misuse is strongly associated with antisocial behaviour and persistent aggressive behaviours. How environmental and biological factors interface to modulate risk for these outcomes is not yet understood, however, the interaction of adversity with a variable number tandem repeat (uVNTR) polymorphism of the monoamine oxidase gene A (MAOA) gene associates with antisocial behaviour and mental disorders. Further, DNA methylation in a region of interest (ROI) spanning MAOA's first exonic/intronic junction associates with ASPD in men as well as other mood, anxiety, and substance misuse disorders.

Aim and Methods. We characterized methylation of the MAOA ROI by sex and age and examined how negative and positive environmental factors interact with MAOA genotype and methylation on antisocial phenotypes and mental disorders. Participants included men and women from a clinical population of young adults recruited in adolescence at a substance misuse clinic and a community sample of adolescents.

Findings. (1) Sex but not age was associated with methylation levels such that high methylation levels among women likely represent X-chromosome inactivation, and sexual abuse was associated with hypermethylation of the MAOA first exon, (2) high methylation levels mediated associations between sexual abuse and current depression diagnosis in women, (3) the highest levels of aggressive behaviour were found among maltreat male carriers of the low-expressing MAOA-uVNTR allele and displayed high levels of exonic methylation, while no interactions were shown in women, and (4) among adolescent girls, but not boys, positive parent-child relationship attenuated the interaction of maltreatment and the high-expressing MAOA-uVNTR allele on alcohol consumption, though the interactions were not robust to adjustments for tobacco use, substance misuse, and delinquent behaviours.

Conclusion. The findings presented here advance our understanding of how maltreatment interfaces with genotypic and epigenetic factors, in a sex-dependent manner, to promote aggressive behaviour and mental disorders among susceptible individuals.

Keywords: Epigenetics, Antisocial, Genotype, Maltreatment

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Dedicated to all the mentors, colleagues, friends, and family who have guided me through this journey and to the study participants who have made this work possible.

List of Papers

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

I Checknita, D., Tiihonen, J., Hodgins, S., Nilsson, K.W. Associations of Age, Sex, Sexual Abuse, and Genotype with Monoamine Oxidase A Gene Methylation. *Manuscript in submission to Frontiers in Neuroscience*

II Checknita, D., Ekström, T.J., Comasco, Nilsson, K.W., Tiihonen, J., Hodgins, S. (2018) Associations of Monoamine Oxidase A Gene First Exon Methylation with Sexual Abuse and Current De-pression in Women. *J Neur Trans*, 125:1053-1064
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III Checknita, , D., Bendre, M., Ekström, T.J., Comasco, E., Tiihonen, J., Hodgins, S., Nilsson, K.W. Monoamine Oxidase A Genotype and Methylation Moderate the Association of Maltreatment and Aggressive Behaviour. Behav Brain Res, 382:1-11
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IV Bendre, M., Checknita, , D., Todkar, A., Åslund, C., Co-masco, E, Nilsson, K. W. The Interaction of *MAOA*-uVNTR Genotype, Maltreatment, and Positive Parent–child Relationship Predicts Adolescent Alcohol Drinking in a Sex-Dependent Man-ner. *Manuscript*

Contents

Abbreviations

5-HIAA	Hydroxyindoleacetic acid
5-HT	5-hydroxytryptamine, serotonin
5mC	5-methylcytosine
5caC	5-carboxylcytosine
5fC	5-formylcytosine
5hmC	5-hydroxymethylcytosine
ASPD	Antisocial personality disorder
ATD	Acute tryptophan depletion
AUDIT-C	Alcohol use disorders identification test-concise
CD	Conduct disorder
COVID-19	Coronavirus-19
CpG	Cytosine-guanine dinucleotide
DNMT	DNA methyltransferase
FM	Familial maltreatment
GxE	Gene-environment
MAALIN	MAOA-associated lncRNA
MAO	Monoamine oxidase (enzyme)
MAOA	Monoamine oxidase A (gene)
MAOA-L	*MAOA* long allele
MAOA-S	*MAOA* short allele
MAOB	Monoamine oxidase B (gene)
NA	Non-abused
NFM	Non-familial maltreatment
PA	Physical abuse
PCA	Principal components analysis
ROI	Region of interest
SA	Sexual abuse
SCID-I, -II	Structured clinical interviews for DSM-IV (axis I and II)
SES	Socioeconomic status
SNP	Single nucleotide polymorphism
TET	Ten-eleven translocation (enzyme)
TSS	Transcription start site
uVNTR	Variable nucleotide tandem repeat

INTRODUCTION

Antisocial Behaviour

Antisocial behaviour refers to a pattern of behaviour in which the basic rights of others or major age appropriate social norms or rules are violated, destruction of property, deceitfulness, theft, serious violations of rules, and various types of aggressive behaviours and is formalized into a diagnosis of conduct disorder (CD) which onsets prior to age 15[1]. CD is also thought to be a neurodevelopmental disorder, with studies estimating that between 5.8% and 12.0% of children present CD, including twice as many boys than girls[2]. In most cases, patterns of antisocial behaviour persist into adulthood[3]. Normative peaks of aggression occur in early childhood and adolescence which are attenuated by the ability to inhibit aggression while prosocial behaviours are learned[3]. Among antisocial individuals, the ability to inhibit aggressive behaviour is impaired, contributing to consistently high levels of aggression throughout much of the life-span[3]. As such, antisocial individuals pose a significant burden to society through harm done to others, criminal activity, and extensive use of health and social services[4]. Importantly, antisocial individuals contribute to subsequent generations with similar problems by transmitting susceptibility genotypes, fostering a socially adverse environment, and providing inadequate parenting to their children[4]. Persistent aggressive and antisocial behaviour is strongly influenced by the experience of childhood maltreatments such as physical abuse, sexual abuse, and neglect[5–7]. However, associations between exposure to maltreatment and antisocial aggression may not depend as much on the type of maltreatment experienced[8], but more on the severity and duration of maltreatment since they typically co-occur[9–11]. Further, different types of maltreatment may have a more salient impact on the risk for later aggressive behaviours depending on when they are experienced throughout prenatal and post-natal development[12]. Both CD and its adult sequelae, Antisocial Personality Disorder (ASPD), are also typified by significant comorbidity with mood, anxiety, and substance misuse disorders[13–15].

As such, the life-long trajectory among antisocial individuals often results in a lifestyle that situates them in environments characterized by adverse conditions including low socioeconomic status, antisocial peer affiliations, and criminality[3]. Around 40% of boys with CD and 25% of girls with CD will go on to meet criteria for ASPD[4], though even those who do not meet ASPD

criteria in adulthood are at significantly higher risk for other mental disorders, substance misuse, and aggressive behaviours[4,16,17]. Further, continual substance misuse throughout adolescence and adulthood among antisocial youth is associated poorer mental health outcomes later in life[4,16,17]. As such, the environment and lifestyle arising from an individual's persistent antisocial behaviour also contributes to the perpetuation and maintenance of those behaviours across time[3,18–20].

Aggressive Behaviours and the Brain

Aggression is an individual or collective social behaviour carrying adaptive benefits as well as maladaptive antisocial consequences when enacted excessively or pathologically[12,21]. Aggressive behaviour features prominently among antisocial individuals and can be parsed into two broad subtypes. Reactive, or impulsive, aggression is characterized by emotionally driven and often spontaneous acts with the sole intent to harm others which often occur as a response to perceived environmental or social threats, though it may also be used to instigate or fuel aggressive interpersonal interactions[22]. In antisocial individuals, this form of aggression is characterized by associations with elevated autonomic arousal, low impulse control, and social-cognitive biases towards perceiving threats, even to neutral stimuli, thus leading to disproportionately hostile responses to perceived threats[21]. Aggressive behaviours have notable correlates in the brain.

Neurological correlates of antisocial and aggressive behaviours.

There is an underlying corticolimbic neurocircuit implicated in reactive aggression[23,24]. This circuit includes numerous frontal structures such as the orbital-frontal, dorsolateral and ventromedial prefrontal, and anterior cingulate cortices which are involved in social perception and decision making, as well as subcortical and limbic structures including the periaqueductal grey, hypothalamus, and amygdalae involved in emotional regulation and the execution of aggressive behaviour in response to perceived threats[17,21,23–26]. Among individuals presenting with elevated reactive aggression there is less inhibitory signaling from frontal to subcortical and limbic regions, resulting in a lowered threshold of threat perception and impaired ability to inhibit aggressive responses to perceived threats[23,24]. The loss of frontal inhibitory control over the subcortical and limbic regions within this circuit may be a consequence of both aberrant structural development and connectivity of the corticolimbic circuitry[17,26]. Convergent genetic, stress- and sex-hormone, and environmental factors present in early life and across the lifespan have been proposed to contribute to the abnormal neurodevelopment and impaired function of the corticolimbic circuit in aggression[17,21,23,24,26].

The other type of aggression is instrumental, or indirect, aggression that involves premeditated and deliberate acts of physical or mental harm serving as a means to an end, often including damaging social relations[21]. When severe, instrumental aggression associates closely with delinquency, crime, manipulative behaviours, high callous-unemotional traits, and psychopathy[21]. Instrumental aggression is also associated with a similar circuit of cortical-limbic structures, though the relative activity and contribution of the structures implicated may differ by magnitude and direction[21,24]. For instance, elevated amygdala activity is associated with reactive aggression due to over-reactive responses to facial expressions, whereas lower amygdala activity is associated with instrumental aggression due to difficulty recognizing emotive facial expressions[17]. Recent work has also implicated reward systems in the brain such that abnormal activity of the striatum associates with both forms of aggression, and antisocial individuals may be rewarded by the outcomes of instrumental aggression as well as intrinsic reward from reactive aggressive behaviours themselves, in turn promoting future acts of aggression[17]. A recent review found that, in sum, more severe neurological impairments are associated with reactive aggression[17]. While reactive and instrumental types of aggression can be distinguished by their behavioural presentation, both types are often engaged in by antisocial individuals and result in a heterogenous profile of antisocial aggressive behaviours[21].

Sex-differences in aggressive behaviours.

There are stark sex-differences in type and frequency of aggressive behaviour such that males generally display greater levels of reactive aggression whereas females present with higher levels of instrumental aggression[21]. This sex-difference has been observed in both healthy and clinical populations[21]. Factors underlying these sex-differences are suggested to include a complex interplay of evolutionary and environmental factors, stress- and sex-hormone mediation of neurotransmitter systems, and differential regulation of candidate gene expression[21]. It is further notable that studies of aggression has been primarily focused on male samples, although findings from extant studies in females implicate similar neural circuitry and highlight the influence of sex-hormones as a major contributor to sex-differences in aggression[21].

Testosterone levels are implicated in potentiating aggressive behaviours and may help account for sex-differences in aggressive behaviour since males have higher endogenous levels of testosterone than females[21]. Interestingly, among both males and females, there is an inverted "U" association of testosterone and aggressive behaviours such that moderate levels of testosterone show a stronger link with aggressive behaviour than abnormally low or high levels[27,28]. These moderate levels of testosterone are associated with increased amygdalar reactivity to perceived threats[29,30]. The balance of female sex-hormones, estradiol and progesterone, have also been associated with aggressive behaviours although there is inconsistency in extant literature likely owing a

lack of studies in females and that fluctuations of sex-hormone levels throughout the menstrual cycle, and at different points in the lifespan, make generalized conclusions difficult[21]. As such, antisocial aggression is highly complex and heterogenous at behavioural and neurobiological levels, and a particularly sexually dimorphic behavioural phenotype.

Alcohol use and aggressive behaviours.

Alcohol use and misuse are strongly linked to aggressive behaviours, especially among individuals with underlying predispositions towards antisocial behaviour. The impact of acute alcohol consumption disrupts executive inhibitory processes to perceived provocations, while also narrowing attentional focus on the most provocative cues[31]. This shift towards focusing on perceived provocation comes at the expense of attentional shifts away from less salient social cues and consideration of consequences or alternative reactions which may otherwise mitigate aggressive responses, thus increasing the likelihood of enacting aggressive behaviours[31–33]. As such, alcohol can serve as a facilitating factor for a myriad of violent and non-violent aggressive behaviours among some individuals[31,33,34], particularly reactive aggression[35]. Although research on the association of alcohol use and aggressive behaviour has been heavily biased towards male samples, a recent meta-analysis of studies in females found generally similar, albeit milder, associations[33]. One critical factor facilitating alcohol-driven aggression is the presence of predispositions towards aggressive behaviour[33]. As such, antisocial individuals are of particular interest when considering associations between alcohol use and aggression.

Extant evidence points to an association between antisocial phenotypes and risk for alcohol use disorders[36,37]. The timing of conduct problem onset in childhood and adolescence, and its persistence over time, is also associated with the persistence of alcohol misuse throughout life[38]. Individuals presenting with early-onset persistent conduct problems, including CD, show stronger associations with excessive drinking and self-reported aggression in adulthood relative to those with adolescent onset conduct problems, with weak associations observed between childhood-limited conduct problems and later excessive alcohol use and aggression[38]. As such, extant evidence points to alcohol misuse and aggression sharing a closely associated developmental trajectory among individuals predisposed towards antisocial behaviours.

Another study assessing young men and women first recruited in adolescence and followed for 5 years reported that CD and alcohol misuse disorders and other familial factors, including maternal alcohol use disorders among female participants, were predictive of alcohol use disorders in early-adulthood[16]. These findings may suggest that associations of CD and alcohol misuse are impacted by overlapping environmental and genetic factors. Similar to CD and ASPD, maltreatment in childhood is strongly associated with higher risk of developing alcohol misuse[39–41]. One study showed that negative reactivity in childhood was indirectly associated with both antisocial behaviour

and substance use in adolescence via poor social standing among peers[42]. Another study showed that, among adolescents at risk for antisocial behaviours, perceptions of peer-drinking were linked to alcohol misuse as well as externalizing behaviours[43]. As such, persistent aggressive behaviour and alcohol misuse may share common antecedents within individuals and in their proximal social environment.

Alcohol use also associates with elevated aggression in the brain. Chronic alcohol use, directly through alcohol toxicity and indirectly through oxidative stress processes, associates with damage to prefrontal region, amygdalar, and hippocampal structures and their connectivity, exacerbating aggressive behaviour[34]. As such, chronic alcohol misuse is associated with further impairment of the corticolimbic circuitry in aggression. Among antisocial individuals, underlying trait aggression may increase the likelihood of alcohol misuse, which in turn contributes to the exacerbation and maintenance of persistent aggressive behaviors over time[44,45]. Heritable factors have been implicated.

Heritability of aggressive behaviours.

Estimates of heritability of CD and ASPD have been shown to be as high as .80[46]. Given strong evidence of heritability, extant evidence has pointed to candidate genes associated with increased risk for CD/ASPD. Evidence shows a heritability of 27-42% for reactive aggression and 39-45% for instrumental aggression with significant genetic overlap, though more unique genetic factors are present in reactive aggression[47,48]. However, heritability estimates of proactive and reactive aggression, and sex differences in aggressive behaviours, remain difficult due to lack of consistent measures of aggressive behaviour between studies and vary by self-reported, parental, or teacher ratings[49]. However, higher heritability for instrumental aggression than reactive aggression has been reported[49]. Dysregulation of aminergic systems in the brain are an important biological underpinning of aggression.

Serotonin and Aggressive Behaviour

Although numerous neurotransmitter systems including the dopaminergic, GABAergic, glutamatergic, and norepinephrine systems are all strongly implicated in the regulation of aggressive behaviour, their respective roles have not yet been clarified and may involve complex indirect influences on aggressive behaviour[50,51]. However, antisocial aggression most strongly associates with dysfunction of the serotonergic (5-hydroxytryptamine, 5-HT) system[25,50,51]. Serotonin is a primarily inhibitory monoaminergic neurotransmitter that is synthesized in the dorsal raphe and projects throughout most of the cortex, and also plays a dual role as a neurotrophin during early development[25,50,51]. Dysregulation of the serotonergic system contributes to the disruption of inhibitory signal from frontal to subcortical and limbic regions in

the neurocircuitry underlying aggressive behaviour[25,50]. Decades of research have established the association between serotonergic dysregulation and aggressive behaviours.

Studies of serotonin and aggressive behaviours.

Animal and human studies implicate a system-wide serotonergic dysregulation manifesting in reduced central 5-HT activity in aggressive behaviours[50–52]. Initial evidence implicating serotonergic dysregulation in aggressive behaviours came from rodent studies during the mid-20th century. These studies showed that pharmaceutical agents which deplete 5-HT in the brain were accompanied by increases of some forms of species-typical aggression such as mouse-killing behaviour and fighting, while other forms of aggression were suppressed[53,54]. These studies suggested that serotonergic dysregulation may play a role in not only aggressive behaviour, but also specifying what form of aggressive behaviour is enacted. Additional findings from animal studies showed similar associations. Prefrontal 5-HT concentrations drop over 20% from basal in rats during aggressive interactions[55]. Primate studies found associations of impulsivity and aggression with low urinary levels of 5-HT metabolite hydroxyindoleacetic acid (5-HIAA), reflecting diminished 5-HT function in the brain[56]. Such studies also showed that males presenting highly aggressive behaviour also displayed low levels of 5-HIAA[57]. In similar studies of aggressive males, lower 5-HIAA levels were associated with elevated risk-taking behaviour and poor impulse control[58–60]. Early investigations in human clinical and incarcerated populations revealed similar findings implicating reduced central 5-HT among participants who displayed elevated reactive aggression[61,62]. Another study showed an inverse correlation between central and peripheral 5-HT levels among male offenders whose crimes were characterized by violent impulsive aggression versus those with premeditated crimes[63]. Together, this early work helped specify the link between 5-HT dysregulation and impulsive aggression.

The impact of acutely reduced 5-HT are associated with increased aggression in laboratory settings. The acute tryptophan depletion (ATD) paradigm uses a dietary challenge to deplete tryptophan, the precursor of 5-HT, which in turn temporarily halts synthesis of serotonin and blunts serotonergic neurotransmission[64,65]. Studies using ATD have revealed associations between the blunted serotonergic activity with temporarily elevated aggression, along with corresponding reductions in prefrontal-amygdalar connectivity, which was especially pronounced in participants with personality traits that potentiate aggressive behaviours[65–68]. Among incarcerated individuals prone to impulsive aggression, ATD triggers a temporary elevation of impulsive aggression[69]. Although the ATD challenge may involve significant contributions of altered activity of other neurotransmitter systems[64], studies using the method offer some evidence of a causal connection between reduced central 5-HT and aggressive behaviours. More recent work further indicates that the magnitude

and direction of serotonergic abnormalities may differ throughout the brain, including elevated 5-HT in the prefrontal cortex of aggressive individuals[70], contributing to diminished inhibitory signal from frontal to subcortical and limbic regions[35]. This highlights the importance of considering regional serotonergic dysregulations in the brain.

Although serotonergic dysregulation shows a strong association with aggressive behaviour, other biological functions must be considered, particularly when behaviours that co-occur with aggression are present. For instance, oxidative stress processes associated with chronic alcohol use result in exacerbated dysregulation of the corticolimbic circuitry of aggression and is mediated by elevated dopaminergic activity which acts on brain systems involved in addiction[34]. Dopaminergic activity in the reward systems of antisocial individuals may also be involved in the intrinsically rewarding aspects of performing acts of aggression, in turn potentiating future aggressive behaviours[34].

Serotonin and brain development.

Serotonin also acts a neurotrophic factor during sensitive periods in early neurodevelopment and contributes to the formation of frontal and subcortical limbic circuitry that governs adaptive cognitive and emotional stress responses to environmental factors and helps shape behaviour later in life[50,51,71–73]. Notably, 5-HT does not confer a significant impact on gross morphology and connectivity in corticolimbic brain circuitry during early development, but "fine-tunes" development[51,74]. Dysregulated 5-HT may lead to subtle deficits that then interact with the presence of environmental adversity to exacerbate deficits over time and contributing to stable behavioural phenotypes[51]. It has been posited that environmental influences present during sensitive periods of development contributes to behavioural outcomes[51]. Dysregulation of 5-HT functioning in early life may yield particularly long term behavioural consequences, including the development of aggressive behaviour as well as mood, anxiety, and substance misuse disorders[50,51,73]. As such, 5-HT dysregulation is an important common underlying factor contributing to antisocial aggression, as well as the psychiatric comorbidities that typically accompany CD and ASPD. This association represents one of the most robust and well-supported associations currently known in biological psychiatry. In line with extensive evidence showing strong associations between dysregulation of aminergic systems and antisocial behaviours, numerous candidate genes involved in regulating amine transmitter pathway activity and neurodevelopment have emerged[50,75].

Monoamine Oxidase A and Aggressive Behaviour

Although several candidate genes contributing to dysregulation of 5-HT in aggression have been identified[12,50,76,77], the monoamine oxidase A gene

(*MAOA*) which encodes the monoamine oxidase A (MAO-A) enzyme that metabolizes 5-HT into 5-HIAA following reuptake, has shown a powerful association with the development of persistent antisocial behaviour[11,51,78–81]. The *MAOA* gene is located on the forward strand of the X-chromosome and shares a tail-to-tail orientation with the monoamine oxidase B gene (*MAOB*)[82]. Extant evidence indicates that the *MAOA* gene begins expressing around gestational week 19 in humans, then subsequently increases before reducing to a stable level by 2 years of age[51] . The *MAOA* gene acts in early development to help fine-tune corticolimbic circuitry, rather than contributing to gross structural morphology[51]. Dysregulation of *MAOA* in early development has been suggested to inhibit the outgrowth of the serotonergic system itself[73,74], contributing to behavioural phenotypes through subtle neurodevelopmental abnormalities producing a "smaller" overall serotonergic system[73,74]. The prominence of *MAOA* as the key candidate gene in aggressive behaviour has been established over three decades of study.

MAOA polymorphisms and aggressive behaviours.

Initial evidence implicating the *MAOA* gene in aggressive and antisocial behaviour came from a rare mutation which extinguished *MAOA* expression among males in a Dutch family who displayed an array of impulsive, aggressive, and aggressive behaviours along with reduced urinary levels of 5-HIAA suggesting diminished serotonergic function[83]. A subsequent *maoa/maob* knockout study in mice showed elevated aggressive behaviour accompanied by increased global levels of 5-HT throughout the brain[84]. However, follow-up studies revealed that this was largely attributable to neurodevelopmental abnormalities including serotonergic neurons developing in unusual regions and pooling of extracellular 5-HT in somatosensory regions[85,86]. A subsequent study revealed that *maob* knockout mice did not display any aggressive behavioural phenotypes[87]. Together, this early work revealed an important and specific role of *MAOA* in aggressive behaviours, as well as neurodevelopmental abnormalities.

The MAOA-uVNTR.

The promoter region of *MAOA* also contains a functional variable number tandem repeat (uVNTR), including short low-expressing (MAOA-S) and long high-expressing (MAOA-L) genotypes, respectively[88,89]. An *in-vitro* functional analysis indicated that the 2 and 3 repeat alleles of the MAOA-uVNTR comprise the low-expressing MAOA-S genotype while the 3.5, 4, and 5 repeat alleles comprise the high-expressing MAOA-L genotype[88,89], while some discrepancies in these associations have been noted[11,78,90,91]. While direct associations of *MAOA* genotypes with antisocial and aggressive behaviour are not well-supported and highly inconsistent, the interaction of these genotypes with adversity and maltreatment during childhood and adolescence has been robustly associated with a childhood onset of a life-long pattern of antisocial

behaviour and aggression[78]. This is consistent with current understandings positing that rather than conferring a direct impact on the risk for developing persistent aggressive behaviours, the underlying neurodevelopmental abnormalities conferred by 5-HT dysregulation leave an individual more susceptible to the impact of environmental factors[51,73,74].

A neuroimaging study of *MAOA* genotypes identified associations with structural and functional brain abnormalities that predispose individuals to aggressive behaviour[92]. This study showed that male and female MAOA-L carriers displayed lower amygdalar, insular, hypothalamic, cingulate gyral, and corticolimbic grey matter volume, as well as elevated amygdalar activation while viewing emotionally evocative stimuli[92]. Further, males carrying MAOA-L presented lower dorsal anterior cingulate activation during response inhibition, while showing greater amygdalar and hippocampal activation during an aversive recall task[92]. Together, these findings along with a prior review of the literature[93] indicate that increased amygdalar emotional reactivity and diminished regulation from prefrontal and prelimbic areas among the MAOA-L carriers echo those found in other studies of the corticolimbic circuitry of aggression.

Gene-environment interactions of the MAOA-uVNTR.

The impact of maltreatment occurring later in development may be conditional on increased susceptibility to environmental factors stemming from subtle differences in early neurodevelopment which are conferred, in part, by specific *MAOA* genotypes[73,94,95]. As such, gene-environment (GxE) interaction studies of *MAOA* are the primary means of showing associations with aggressive behaviours.

In males, a pivotal 2002 study indicated that, in males, the MAOA-S genotype interacted with severe abuse in childhood in association with an increased risk for developing antisocial aggression later in life[81]. A more recent study similarly showed that an interaction of child abuse and MAOA-S associated with the development of ASPD[89]. Numerous subsequent studies have replicated similar interactions of childhood adversity and MAOA-S in association with the development of aggressive and antisocial behaviours in males[78]. Although such studies have yielded some inconsistent findings, meta-analyses show a moderate interaction of MAOA-S and adversity on antisocial behaviours, further noting a need for subsequent studies to use more clearly defined risk and outcome variables as well as consider additional relevant factors to reduce methodological inconsistencies[78,80]. By contrast, in females, it is the MAOA-L allele which presents with increased susceptibility to environmental factors in modifying the risk for aggressive and antisocial behaviour[11,78,96–100]. Although the role of MAOA-L in gene-environment interactions among females is currently well-supported by recent studies[78,80] and noted as an important avenue of inquiry[78], some studies have yielded contrary results[78,90].

Extant inconsistencies in findings may be due to a sparse number of GxE interaction studies of *MAOA* in females[78].

Neuroimaging studies offer support to the relevance of *MAOA* genotype, and its interactions with adversity and associations with the corticolimbic circuitry associated with aggression. The *MAOA* genotype has been associated with how the brain responds to emotional stimuli, and the function of corticolimbic circuitry of aggression[44,79,92,101–103]. Interestingly, one recent study showed that MAOA-S carriers also showed associations with the basic functional architecture of the brain beyond the corticolimbic circuitry during resting-state analyses[104]. Sex-differences in MAOA-uVNTR genotypes implicated in environmental susceptibility have also been reported in imaging studies.

One study showed that men carrying MAOA-S, versus male MAOA-L carriers, showed greater amygdalar and hippocampal activation during an emotional fearful/angry face-matching task which was concordant with the level of childhood stress experienced which was additionally associated with reduced anterior cingulate cortex activation during a response-inhibition task and lower inhibitory control[105]. By contrast, women carrying MAOA-LL who had experienced childhood stress showed increased hippocampal and amygdalar activity during the emotional fearful/angry face-matching task[105]. Another study using acute tryptophan depletion found that healthy men with higher trait aggression showed a more pronounced response to the challenge on diminished prefrontal-amygdalar connectivity which resulted in greater virtual violence during a video game task, with the most pronounced effects among MAOA-S carriers[25]. Together, this work supports sex-differences in *MAOA* susceptibility genotypes and suggests acute fluctuations in 5-HT function may potentiate aggression in individuals carrying *MAOA* susceptibility genotypes.

Beyond gene-environment interactions of MAOA

It is notable that the *MAOA* gene-environment literature points to associations of the interaction with a variety of aggressive behavioural phenotypes[11,78,79] beyond what would be expected with 5-HT dysregulation alone which is more particular to reactive aggression[50]. This may be due, in part, to the gene's dual role in degrading other aminergic transmitters[51,82] and interactions with sex and stress-hormones[11]. As such, considering the role of *MAOA* in aggression in the context of 5-HT dysregulation may not capture the more pervasive role the gene plays, as evidenced by GxE studies. Gene-environment interaction studies have offered insight into possible mechanisms underlying sex-differences in aggression.

However, MAOA-uVNTR genotypes do not correlate with monoamine oxidase (MAO) enzymatic activity in the adult brain[106]. Further, the contribution of genotype alone does not offer a mechanistic explanation for how *MAOA*

dysregulation contributes to normative and abnormal developmental fluctuations in aggression[11,51]. It has therefore been hypothesized that there must be other biological factors, particularly epigenetic processes, that also contribute to the maltreatment associated *MAOA* dysregulation observed in behavioural phenotypes[11,51,73]. Accordingly, evidence indicates that maltreatment associates with epigenetic processes, such as DNA methylation, resulting in altered gene expression that in turn also contributes to broader systemic dysregulations and behavioural phenotypes underlying risk for mental disorders[107,108].

Epigenetics

Epigenetics is the study of biological mechanisms that can modify gene expression which are not the result of variations in the underlying genomic DNA sequence itself[107,109–111]. Such mechanisms include histone modifications that alter the structure of chromatin to confer euchromatic (open) and heterochromatic (closed) states which regulate access of transcriptional machinery to DNA, methylation of DNA which can alter the binding of transcriptional machinery and transcription factors to the regulatory loci of genes, and non-coding RNAs that can alter gene expression at transcriptional and post-transcriptional levels[109,112–114]. These mechanisms can work independently, or in tandem, serving as multiple interacting layers that regulate and fine-tune gene expression[109,114–116]. Epigenetic processes play a vital role in cell-differentiation, development, regulation of biological systems, aging processes, and are posited as key factors that interface with environmental factors and other biological factors to modify risk for the development of biomedical and mental disorders[109,110,114,117]. DNA methylation represents the most robustly understood and studied epigenetic mechanism, and over the past decade altered methylation has been observed in numerous candidate genes associated with behaviour and mental disorders[107,114,117,118]. Although causal connections have yet to be established, extant evidence suggests that maltreatment and adversity in early life, in conjunction with other environmental and biological factors, leads to alterations to DNA methylation and contribute to risk for developing mental disorders [110,114,119].

DNA Methylation

DNA methylation is an epigenetic mechanism whereby a methyl group (-CH3) is covalently bound to a cytosine base of a cytosine-guanine dinucleotide (CpG, 5'cytosine-phosphate-guanine 3') through the catalytic action of a family of DNA-methyltransferases (DNMTs) resulting in a methylated cytosine (5-mC, 5-methylcytosine). The methylated cytosine residue may then contribute to altered transcriptional regulation of a gene through modifying

access of transcriptional machinery, transcription factors, methyl-CpG-binding domain proteins, and transcriptional repressor and enhancer complexes to their target binding sites[111,120]. Thus, DNA methylation can contribute to stable alterations of gene expression through directly modifying access of transcriptional machinery and transcription factors to a gene locus and indirectly by altering the chromatin structure of DNA[117]. Enrichment of DNA methylation often occurs at regions of the genome called CpG islands, where CpG sites are present at higher frequencies relative to the rest of the genome and are typically located in promoter regions and proximal to transcription start sites (TSS)[121]. It is important to note, however, that DNA methylation levels in these epigenetic "hot spots" are typically very low initially, which leaves them poised for subsequent alteration by methylation processes[111]. Further, associations between methylation and gene expression differ by genomic location, such that methylation of promoter and exonic regions are typically associated with transcriptional down-regulation, while methylation in gene body and intronic regions typically associate with transcriptional up-regulation[121].

Functions of DNA methylation.

All cells in the body contain the same genome, although every cell-type expresses a different phenotypic profile which reflects in cellular and tissue differentiation[111]. Cellular differentiation and diversity are heavily regulated by epigenetic alterations, conferring a unique expression profile for each cell type[117]. Further, DNA methylation confers heritable alterations to expression. However, "heritable" in the epigenetic context refers to consistency of epigenetic alterations carried through cell-division to daughter cells, rather than the intergenerational transmission of DNA methylation profiles from parent to progeny[112,117,122]. As such, the heritability of alterations to DNA methylation across cellular divisions are key to both establishing and maintaining cell-types across the life-span and plays an important role in other biological functions such as genomic imprinting and X-chromosome inactivation among females[123]. Maintenance methylation is largely carried out through by the DNMT1 class of DNA-methyltransferases which recognizes methylated CpGs on the parent template strand and transfers a methyl group to the corresponding CpG on the daughter strand during cellular division[111,112].

By contrast, *de novo* methylation refers to the addition of methylation to previously unmethylated sites, and is largely carried out by the DNMT3A and DNMT3B classes of DNA-methyltransferases which also interact with DNMT3L which aides in the target specificity of methylation processes in early life[112,122]. Contrary to DNMT1, DNMT3a and DNMT3b do not display an affinity towards hemi-methylated sites and can also act outside of cellular division[124,125]. *De novo* methylation has been shown to occur throughout the lifespan and contribute to the regulation of many functions including synaptic plasticity, emotional behaviour, learning, and memory[126–128]. Importantly, numerous other biological factors and environmental factors interface with *de*

novo methylation processes to promote biological and behavioural phenotypes that modify one's risk for developing biomedical and mental illnesses, although the precise underlying mechanisms governing these processes are not fully elucidated[114,117].

Methylation of DNA and epigenetic susceptibility to environmental factors. Extant evidence describes a sensitive period of development between childhood and adolescence of elevated epigenetic susceptibility to environmental influences that contribute to the dysregulations of biological systems that underlie complex behavioural phenotypes and confer risk for developing mental disorders[107,108,119]. Initial evidence that implicating epigenetic susceptibility came from rodent experiments showing that cross-fostered pups who experienced poor maternal care showed elevated responsiveness to stressors later in life[129]. This finding was later associated with elevated DNA methylation and low hippocampal expression of the glucocorticoid receptor gene, which plays a role in regulating social stress[130]. Further, hypermethylation of the homologous gene in humans was found among suicide completers who had experienced severe childhood maltreatment[131].

Subsequent work has shown that environmental adversity during early life associates with epigenetic alterations at the genome-wide scale, and of candidate genes, among individuals displaying elevated antisocial aggression[132–134]. Further, environmental prenatal stressors may also confer epigenetic alterations associated with mental disorders[107,135], and environmentally associated alterations of methylation may be reversible through pharmacological and other therapeutic interventions in adulthood[107,136,137]. As such, epigenetic lability may not be constrained to childhood or adolescence, and may occur throughout the lifespan to varying degrees[107,119]. However, one recent study found little support for direct associations between early-life victimization and methylation at single gene and genome-wide scales, positing that such associations may depend on other factors such as genotypes that facilitate epigenetic lability, and the presence of other risk and protective factors which then further modulate the direction and extent of methylation alteration[119]. Thus, associations between maltreatment and altered methylation may be specific to individuals who present with many convergent biological and environmental factors that facilitate and promote susceptibility.

Recent work points to some degree of epigenetic governance over neuroendocrine, oxytonergic, and serotonergic pathways that mediate biological responses to environmental adversity and contribute to the dysregulation of biological systems that associate with aggressive behaviours[12]. For instance, adversity-associated alterations to methylation in key candidate genes governing these biological systems have been identified[12]. As such, epigenetic investigation represents an important line of inquiry into how environmental factors may influence the risk for aggressive behaviours[12,51].

Reversibility of DNA Methylation

Methylation of DNA may also be reversible, thus promoting genomic flexibility[122,138]. The process of demethylation is reliant on already methylated cytosines being progressively modified by a family of ten-eleven translocation (TET) enzymes into three other oxidative forms including 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC), and 5-carboxylcytosine (5caC)[122,138,139]. At this point, the modified 5caC cytosine may be actively removed through DNA repair processes, or passively lost and replaced during replication[122,138]. Both active and passive demethylation processes ultimately result in the placement of a new unmodified cytosine[138]. Although the study of DNA demethylation is still in its infancy, 5hmC is the most stable modification and is thus the most currently well-studied[122,138]. In addition to being a potential marker of ongoing demethylation processes, 5hmC may also have unique functional impacts on gene transcription that are distinct from those conferred by methylation, although these functional impacts are not yet fully understood[138].

Functions of DNA 5-hydroxymethylation.

Like methylation, 5hmC is also highly enriched in brain tissue and is posited to play a role in plasticity functions and responsive to numerous environmental influences[140]. Exposure to environmental factors are associated with global enrichments of 5hmC throughout the brain that remained up to a year following exposure, suggesting that 5hmC may serve as a stable regulator of gene activity in some cases, not only a marker of ongoing demethylation processes[140]. Further, 5hmC enrichments were observed to be more localized in regulatory regions of the genome such as promoters, enhancers, and exon/intronic junctions[140]. Current gold-standard methylation analysis techniques cannot distinguish methylation from 5hmC, and extant studies on 5hmC have primarily focused on examining genome-wide patterns with little known about how it impacts expression at the level of single genes[122,140]. As such, further study of 5hmC is critical to understanding the flexibility of the methylome, and to interpreting extant literature on methylation. Although 5hmC was not assessed in this thesis, it is important as a consideration when interpreting our findings and those reported by others.

Sex and DNA methylation

Sex differences in DNA methylation levels and methylation processes are apparent throughout the lifespan. There are two periods during very early development when erasure of methylation across the genome occurs and differs across cell-type and by sex[122]. The first erasure of methylation across the genome in preimplantation gametic cells involves a rapid active demethylation

process in sperm cells, while oocytes undergo a more protracted and gradual process of passive demethylation[122]. Once methylation patterns are established in early development, females typically show higher levels of genome-wide methylation than males throughout the lifespan[141–143], though the magnitude and direction of sex differences can vary by genomic region[144]. Accordingly, sex differences in methylation have been identified in genes regulating biological pathways involved in sex-differentiation, endocrine function, and neurodevelopment, in genes that regulate epigenetic processes and mechanisms themselves, and in genes involved in aging[141–145].

Methylation of DNA and X-chromosome inactivation.

One biological process that contributes to sex differences in DNA methylation levels is X-chromosome inactivation in females. X-inactivation in females involves epigenetic silencing of one X-chromosome, serving as a dosage compensation mechanism so that males and females present harmonized levels of X-linked gene expression levels[146]. X-inactivation is also linked to sexually dimorphic phenotypes[147] ranging from cell structure[148] to disease susceptibility[149] and has been hypothesized as a contributor in sex-differences in behaviour[21]. Greater variability in X-linked gene expression has been observed in women versus men[150]. This variability may be partly attributable to the ~15% of X-linked genes that escape inactivation to varying degrees[147] and variations across tissues[148]. As such, sex differences in methylation, particularly among X-linked genes, are key considerations for understanding the associations with environmental factors, treatment response, fundamental biological processes, and also how the epigenome itself operates[142]. Normative sex-differences in epigenetic programming established in early development may also serve as a predisposing factor in sex-differences in negative health conditions throughout life[142]. As such, sex is a vital consideration in epigenetics.

Aging and methylation

Another important factor to consider in epigenetics is aging. Throughout the aging process, there is a gradual decline of methylation levels whereby cumulative inter-individual differences in methylation across the genome, and associated with individual experiences, are harmonized in a process called epigenetic assimilation[151]. Interestingly, this process associates poorly with gene expression[151], and it is posited that gene regulation is slowly adopted and maintained by other epigenetic processes[151–153]. Environmental factors in early development are associated with altered trajectories of epigenetic aging and are linked to risk for health issues throughout life[151,154]. Despite normative genome-wide reductions in methylation, age-related fluctuations in DNA methylation may remain more variable and susceptible to environmental influences

within specific genomic loci[151,154]. Methylation in regions that span first exonic/intronic boundaries are such regions and are of particular interest in health-related outcomes[151].

Epigenetic aging and health risks.
Disruptions to the normative trajectory of epigenetic aging are implicated in risk for negative health outcomes[151,155,156], associated with environmental factors[151,156], and differ by sex[157]. One study showed that among children from low socioeconomic status (SES) families, experience of positive environmental factors associated with better self-control, less aggressive behavior, fewer depressive symptoms, lower rates of substance use, and fewer externalizing problems, increased academic success, and better psychosocial adjustment in young adulthood[155]. These benefits were not observed among low socioeconomic youth who had not experienced positive environmental factors[155]. However, despite the benefits observed among the youth exposed to a positive environment, they did not differ from their low-SES peers without positive environment on accelerated epigenetic aging, and showed similarly poor cardiometabolic health measures predictive of biomedical illness later in life[155]. The authors posit this as "skin deep" resilience, such that the observed protective associations with outcomes that onset in late-adolescence and early adulthood mask a persistent underlying increased risk for other negative outcomes that onset later in life[155]. The impact of environmental factors present in prenatal and early life on the re-programming of epigenetic aging processes across the lifespan is further supported by twin and animal studies, and is associated with alterations of metabolic functions that underlie a myriad of health conditions that emerge throughout the lifespan[156,158]. Age is therefore implicated as critical factor to consider in epigenetic studies since many mental disorders and biomedical illnesses onset at different points across life, but may be affected by aberrant epigenetic aging processes set in motion by environmental factors present early in life[109,151,155].

Genetic Polymorphisms and DNA Methylation

Although earlier conceptualizations held that alterations to DNA methylation conferred by environmental influences represented the molecular mechanisms underpinning GxE interactions, emerging evidence points to complex and dynamic associations between genetic and epigenetic factors. For instance, single nucleotide polymorphisms (SNPs) may create novel CpG sites that may be subsequently methylated or abolish an existing CpG site thus removing the possibility of being methylated, which in turn alters gene expression[159,160]. Repeat sequence polymorphisms may also alter the epigenetic lability of genes. One classic example is Fragile X Syndrome in which the *FMR1* gene promoter contains many CGG repeat trinucleotides which become methylated and, in

turn, weaken the structural integrity of the X chromosome and confer the syndrome[161]. Genetic polymorphisms may also play a role in how environmental adversity impacts on DNA methylation.

One recent review of several candidate genes in mental disorders found no direct associations between maltreatment and altered methylation[119]. However, the authors posit that gene polymorphisms may gate the capacity of genes to be epigenetically modified[119]. This proposition is supported by evidence from genome-wide analyses of single-nucleotide polymorphisms that show allelic skewing of DNA methylation levels, which were also tissue-specific[162]. Other studies also suggest that promoter sequence variants can alter the binding affinity of methyl groups to DNA in upstream regions, which in turn, alter the susceptibility of genes to be epigenetically modified by environmental factors[163,164]. Further, it has been posited that epigenetic processes may serve as a compensatory mechanism to counterbalance the impact of some genetic polymorphisms, such as the MAOA-uVNTR, on the abnormal development of neurobiological systems in early life[74]. Although our understanding into how genetic and epigenetic factors interface is limited, evidence suggests that the interaction of these factors interact with each other, and with the environment, is far more complex than has been previously appreciated. Building a comprehensive understanding how genetic and epigenetic factors interact is critical to elucidating a gene's role in responding to environmental factors.

DNA Methylation of MAOA

Recent studies suggest that *MAOA* may also fall under epigenetic regulation by DNA methylation. Although *MAOA*-uVNTR genotypes do not associate with MAO enzymatic activity in the adult brain[106], methylation in a region of interest (ROI) spanning the gene's first exonic and partial first intronic junction does[165].One study showed hypermethylation of the ROI among adult male offenders with ASPD relative to healthy men, which was also associated with serotonin levels in male offenders, and with transcriptional down-regulation *in-vitro*[166]. However, it is not known if hypermethylation of *MAOA* was specifically associated with ASPD or associated with maltreatment often experienced by men with ASPD[167]. Conversely, hypomethylation of the *MAOA* ROI has been observed among women with depression[168,169], and those with panic disorder relative to healthy women[137]. Maltreatment is also strongly associated with depression and anxiety[170], perhaps contributing to the methylation levels observed in these studies. Although functional analyses associating *MAOA* intronic methylation was not performed in this study, reduced intronic methylation would be expected to associate with lower gene expression[121]. Together, this work offers compelling evidence that maltreatment associates with meth-

ylation levels of *MAOA* which may contribute to gene-environment interactions of maltreatment and aggressive behaviours, as well as commonly associated mental disorders and echoes prior gene-environment studies implicating downregulated expression in men[11,78,80]. Further, a review of methylation studies of the *MAOA* ROI methylation point to associations of methylation of the ROI, particularly the exonic region, with mental disorders such as depression, panic disorder, post-traumatic stress disorder, alcohol consumption, and ASPD[171]. This review also noted the variability in direction of methylation, hyper- or hypo-, associated with different outcomes[171]. Another study by our group found that men carrying MAOA-S, who also displayed exonic hypermethylation, presented with higher levels of alcohol consumption[172]. As the exonic methylation observed in this study would be expected to confer downregulated gene expression, concordant with the low-expressing MAOA-S genotype among these men, this study offers initial evidence that *MAOA* ROI methylation and genotype may act in tandem to drive down gene expression. Notably, one other study showed that *MAOA* ROI methylation levels were normalized and accompanied by symptom alleviation among women with panic disorder following psychiatric treatment[137]. This finding indicates that methylation of the *MAOA* ROI may be flexible and responsive to positive environmental factors. As such, it has been posited that epigenetic processes such as DNA methylation represents a potential molecular mechanism underlying, or further contributing to, gene-environment interactions involving *MAOA*.

Sex-differences and MAOA methylation.

Several disorders are characterized by alterations of methylation of the *MAOA* gene[171], and the different MAOA-uVNTR genotypes in males and females[173]. As *MAOA* is located on the X-chromosome it may undergo X-chromosome inactivation in women. It is not yet definitively known if *MAOA* is among the genes that escape X-inactivation in women[147]. However, one study showed high *MAOA* first exon methylation levels among women relative to men[169], highly suggestive that one *MAOA* allele is silenced by X-inactivation among women[146,169]. Several other studies reported similarly high levels of *MAOA* first exonic methylation among women[137,168,169,174]. Further, one study indicated that the region where *MAOA* is located along the X-chromosome shows a low likelihood of escaping inactivation[149]. However, *MAOA* is located close to numerous immune-related genes that may escape inactivation in some circumstances, and such instances may yield partial re-activation of nearby genes[149]. However, in sum, extant evidence points to a high likelihood of *MAOA* being subject to X-inactivation.

It is not currently understood how, or if, X-inactivation accounts for associations of *MAOA* with sex-differences in aggressive behaviour. However, given the likelihood of *MAOA* being subjected to X-inactivation in females, a process that also varies by tissue type[149], it then follows that *MAOA* expression

in females would be monoallelic rather than biallelic. As such, X-inactivation may play a role in determining whether the MAOA-L susceptibility or MAOA-S non-susceptibility allele is expressed in heterozygous females.

Further, while direct associations of 5-HT and sex-differences in antisocial aggression are not well-supported[175], associations of 5-HT and aggressive behaviours may be regulated, in part, by sex-steroids[21,176,177]. One study in males showed an interaction between *MAOA* genotype and testosterone levels predictive of antisocial behaviours and aggression, further suggesting that this interaction may have been mediated by *MAOA* transcription[41]. Testosterone levels may thus alter *MAOA* transcription through binding at androgen response elements or near promoter transcription factor binding sites such as Sp1 and R1, which play a key role in transcriptional activation and repression of *MAOA*, respectively[11,178]. Given the role of DNA methylation in X-chromosome inactivation and blockage of transcription factor binding, it is an important epigenetic mark to examine in studies of *MAOA* in antisocial aggression and may also yield important insight into sex-differences in these behaviours.

Theoretical Models

Studies of *MAOA* gene-environment interactions in antisocial aggression and related behavioural phenotypes have typically adhered to the diathesis-stress model whereby averse environmental factors interact with genetic risk alleles to influence the risk of developing antisocial aggression. However, more recent work has indicated that both positive and negative environmental influences may interact with these alleles to confer both protective and risk influences on aggressive behaviours[11,99,179,180]. Such evidence fits well within the differential susceptibility model which posits that certain genetic variants increase biological susceptibility to a wide array of aversive and protective environmental factors, which then modifies risk and resilience for developing mental disorders and maladaptive behaviour[11,181].

Recent gene-environment interaction studies of *MAOA* offer support to this model. One study that included MAOA-uVNTR showed that carriers of genotypes that would be expected to display the highest risk for delinquency in an adverse environment showed the lowest delinquency scores if positive relationship with their parents was reported and high levels of positive child–parent relationships, even among children who experienced adversity, reduced the risk of delinquency[179]. Another recent study of the T941G polymorphism in *MAOA* found that adolescent boys carrying the G alleles were more responsive to high parental acceptance/involvement in association with better effortful control performance than T allele carriers[182]. This finding further supports *MAOA* as a candidate gene that may be responsive to an array of negative and positive environmental influences.

Further, the notion of differential-susceptibility dovetails with recent work suggesting that the extent of alteration to the methylome by maltreatment may be contingent on genotypic facilitation and further influenced by other positive and negative environmental factors[119] further support this conceptual model. As such, the differential susceptibility theoretical framework may help account for some of the inconsistencies noted among earlier gene-environment studies of *MAOA* using a diathesis-stress approach[11,78,80].

It is not known if similar associations involving *MAOA* methylation echo these findings in aggression, although one recent study showed that *MAOA* ROI methylation levels among adult women with panic disorder returned to control levels following cognitive behavioural therapy among treatment responders[137]. Thus, it is possible that methylation of *MAOA* may be similarly responsive to environmental adversity and protective factors and may remain labile to alterations by environmental influences across the lifespan. The convergence of environmental factors with genotypic and epigenetic susceptibility factors involving *MAOA* which then influence the development of antisocial aggression may be much more complex than previously thought. As such, further studies are needed to characterize sex-typical patterns of MAOA methylation and examine the complex interplay of environmental factors with genotypic and epigenetic factors of MAOA in antisocial aggression and related phenotypes.

HYPOTHESIS

This thesis offers a series of preliminary and explorative studies intended to characterize methylation in the *MAOA* ROI, examine how *MAOA* genotype and methylation modifies associations of maltreatment with mental disorders and aggressive behaviours in a clinical population of women and men, and explore the interactions of positive and negative experiences with *MAOA* in association with alcohol consumption in a general population sample. Given extant literature from gene-environment studies and methylation studies of *MAOA* in men and women, the overarching hypotheses of our studies were that;

1. Methylation levels of the *MAOA* would differ between men and women and be associated with age and that those who experienced sexual abuse would show differential *MAOA* ROI methylation compared to those who did not.
2. Maltreatment would be associated with altered levels of *MAOA* methylation, and that these methylation differences would modify the relationship between maltreatment and mental disorder diagnoses and aggressive behaviours.
3. *MAOA* genotype and methylation would modify associations between maltreatment and mental disorders as well as aggressive behaviours, and that the expected impact of these methylation differences on *MAOA* expression would be consistent with sex-differences previously reported in gene-environment interaction literature.
4. Maltreatment would interact with *MAOA* genotypes in association with alcohol consumption among males and females in a general population sample, and that the influence of positive parental factors would contribute to the mitigation of this interaction towards lower alcohol consumption.

AIMS

Paper I

1. To characterize methylation levels of the *MAOA* ROI CpGs by age, sex, and the interaction of age and sex.
2. To determine whether MAOA ROI methylation levels were associated with the interaction of MAOA-Uvntr genotypes, sex, and sexual abuse.

Paper II

1. To examine whether physical and/or sexual abuse were associated with methylation of the MAOA ROI.
2. To determine whether associations between abuse and methylation differed by MAOA genotype.
3. To determine whether methylation of the MAOA ROI mediated associations of abuse with alcohol dependence, drug dependence, depression disorders, anxiety disorders, and conduct disorder.

Paper III

1. To examine moderation of the association of maltreatment and aggressive behaviour by *MAOA* genotypes and determine if this association of maltreatment and aggressive behaviour is further modified by methylation in the *MAOA* ROI.

Paper IV

1. To assess if MAOA genotypes interact with maltreatment and positive parenting to predict alcohol consumption in adoles-cents.

MATERIALS AND METHODS

Participants

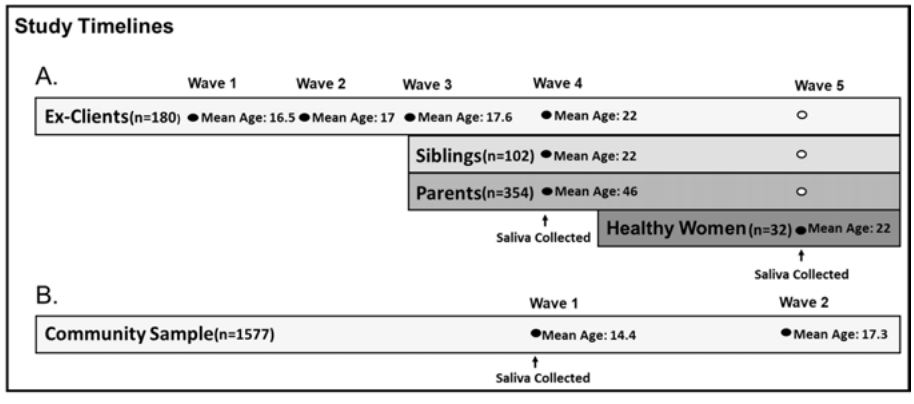

Figure 1. This figure depicts the study timelines. **Panel A** (Papers I-III). At waves 1 through 3, men and women ex-clients initially recruited in adolescence completed interviews and questionnaires. At wave 4, both ex-clients, siblings, and their parents completed interviews and questionnaires and provided saliva samples. At wave 5, healthy women matched on age to the other women completed interviews and questionnaires and provided saliva samples. **Panel B** (Paper IV). At both waves, participants completed questionnaires. Saliva samples were provided at wave 1. In both panels, filled in dots indicate time-points at which data were used in the current studies and empty dots represent time-points at which data were not used in the current studies.

(Papers I,II, and III)

The study design and timeline are illustrated in Figure 1. The overall study sample included a total of 668 participants. Of these individuals, 282 men and women, aged on average 22 years, were recruited in adolescence. A total of 180 of these participants comprised of 81 men and 99 women were recruited at an outpatient clinic for adolescents misusing substances at an average age of 16.5 years. In addition, a total of 102 participants, including 43 of the men and 59 of the women were biological or half- siblings of the clinic attendees. A total of 354 parents were included, comprised of 174 biological or non-

biological fathers and 180 biological or non-biological mothers. Ex-clients completed structured, validated, diagnostic interviews and questionnaires to report on physical abuse (PA) and sexual abuse (SA) at first contact with the clinic and at waves 6, 12, and 60 months later. At the 60 month follow-up, siblings completed the same assessments and parents competed similar assessments. All participants provided saliva samples for DNA extraction at the 60 month follow-up. At a 75 month follow-up, 32 healthy women matched on age to the other women were recruited by announcements placed on company bulletin boards and on the internet and completed assessments similar to those completed by the other participants and provided saliva for DNA extraction.

From this sample, and based on availability of data relevant to the study aims, Paper I included a total of 409 participants, 134 of who we ex-clients, 86 of their siblings, 23 healthy women, and parents of the ex-clients (103 mothers, and 63 fathers). The 166 parents were aged on average 46 years, while the remaining clinic recruits were aged 22 years on average. In total, the sample consisted of 252 women and 157 men.

Paper II included a total of 114 women comprised of 75 ex-clients, 23 healthy women, and 16 women that were biological siblings of clinic attendees not included in this paper and were thus not biologically related to the other participants.

Paper III included 77 men and 117 women. This sample was comprised of 56 men and 75 women who were ex-clients, 21 of the men and 19 of the women who were biological siblings of clinic attendees not participating in the current study, and were thus not biologically related to any other participant, and the 23 healthy women.

(Paper IV)

The community sample study assessed data from adolescents in Sweden in a prospective cohort study called the Survey of Adolescent Life in Västmanland (SALVe cohort) in 2012. The study timeline is depicted in Figure 1. The adolescents were born in 1997 or 1999 and lived in Västmanland that has urban and rural areas that is representative of the Swedish population. The adolescents were initially contacted via regular mail in 2012 (wave 1) and again at 12‑16 years old (mean = 14.4 years) and then followed-up in 2015 (wave-2) at 16‑19 years old (mean = 17.3 years). Invitations were sent to 4,875 adolescents, 1868 participated at wave 1, of which 1577 participated at wave 2, with an attrition rate of 15.59%. The final sample included 1416 adolescents who had complete data on the genetic and environmental variables measured at wave 2.

DNA (Papers I-IV)

Genomic DNA was extracted from saliva samples collected during meeting between the participant and researchers at time points shown in Figure 1, using the Oragene self-collection kit (DNA Genotek Inc. Ottawa, Ontario, Canada) according to manufacturer guidelines. The samples were stored at room temperature in accordance with manufacturer´s guidelines. Genomic DNA was extracted from 200µl of saliva using the silica-based Kleargene DNA extraction method (LGC®, UK: www.lgcgroup.com) and stored at -20^{o}C, then -80^{o}C, prior to genotyping and methylation analyses.

Genotyping of the MAOA-uVNTR Polymorphism (Papers I-IV)

The target 30-bp repeat target-region of *MAOA* (MAOA-uVNTR) was amplified using a well-established primer set'[88]. In accordance with prior *in-vitro* functional assessments of the MAOA-uVNTR[89], t3 repeat variants were denoted as the short (MAOA-S) allele, and 3.5, 4, or 5 repeat variants as the long (MAOA-L) allele. Among females, genotypes were denoted as MAOA-LL, MAOA-SL, and MAOA-LL. However, based on prior work examining sex-differences in MAOA-uVNTR[78,96,99,183], genotypic susceptibility alleles were coded in men (0) MAOA-L and (1) MAOA-S and in women (0) MAOA-SS and (1) MAOA-SL/LL. The pooling of females carrying long-allele genotypes has been used in previous studies of *MAOA*[168] as well as functional studies of MAOA-uVNTR variants[184]. Genotyping procedures were conducted in a blinded manner to mitigate batch effects arising from use of DNA extracted from saliva samples at different time-points. For the samples included in the papers, the MAOA-uVNTR polymorphism was assessed for Hardy-Weinberg Equilibrium using an X^2 test, except for Paper I which included both parents and their children. Hardy-Weinberg Equilibrium for MAOA-uVNTR genotype was confirmed in all studies using an X^2 test among women, though it is not necessary for men since distribution of genotype is the same as the allelic distribution.

Methylation Analysis of the *MAOA* ROI (Papers I- III)

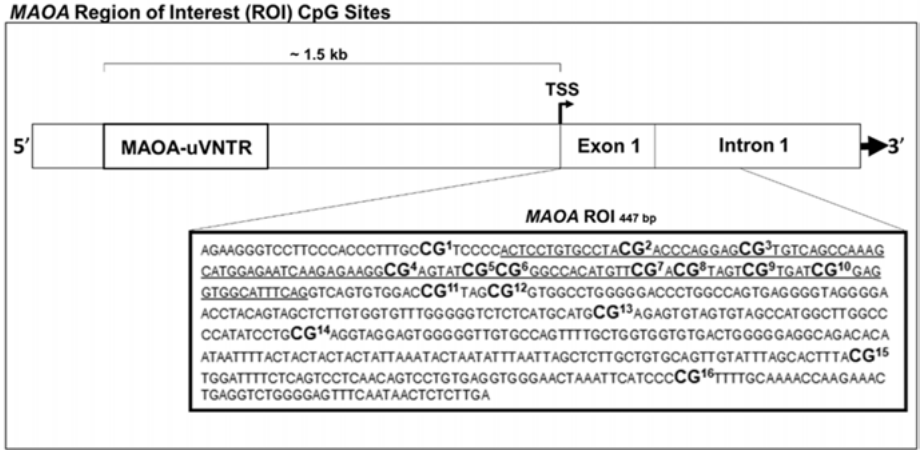

Figure 2. This figure depicts the genomic sequence of the *MAOA* ROI with the 16 constituent CpGs numbered and exonic coding region underlined. The genomic location of the ROI is shown relative to the MAOA-uVNTR polymorphism. The transcription start site (TSS), and first exonic and intronic regions of the *MAOA* gene are also labelled.

Methylation analysis targeted a 448 bp region of interest (UCSC-hg19, ChrX: 43,515,544 - 43,515,991) within the *MAOA* promoter comprised of 16 CpGs of the first exonic and partial first intronic region[166]. The *MAOA* ROI sequence is shown in Figure 2. DNA extracted from saliva was bisulfite-treated using EZ DNA Methylation™ Kit (Zymo Research Corporation, Irvine, California) then quantified using Agena Bioscience's EpiTYPER at Karolinska University Hospital Mutation Analysis Core Facility. Optimization of technical outcomes was ensured by an amplicon designed on the reverse strand including CpGs 1-13 and an amplicon on the forward strand including CpGs 13-16. Resulting data from 14 of the 16 CpGs returning usable data represented percentage of methylation at each CpG to the nearest 0.5%. CpGs were denoted numerically based on their 5' to 3' forward-strand position.

In studies I-III outlined in this thesis, methylation was measured in DNA extracted from saliva. Extant knowledge of *MAOA* ROI methylation comes from studies sampling both blood[137,166,185] and saliva[168,169] . The concordance of *MAOA* ROI methylation levels across peripheral and brain tissue has not yet been directly assessed. However, in-silico cross-tissue examination of two available CpGs within the *MAOA* ROI using IMAGE-CpG (http://hanlab.org/methylation/ default/imageCpG#)[186] indicates a fairly strong association between blood and saliva methylation levels at two CpGs with rho values of 0.64 and 0.79 respectively, and between brain and saliva methylation with

rho values of 0.76 and 0.69 respectively. As such, evidence supports the use of saliva to measure methylation of the *MAOA* ROI.

Maltreatment

(Papers I-III)

Physical Abuse (PA). Participants completed the revised Conflict Tactics Scales[187] to report on PA by parents. Ex-clients reported on maltreatment at baseline and again at the time of saliva collection, while the siblings and healthy women reported on maltreatment at the time of saliva collection. Responses were dichotomously coded as absent (0) or present (1) if "severe" or "extreme" forms of PA were reported.

Sexual Abuse (SA). Sexual abuse was assessed at all waves of data collection using items from the Sexual Experience Survey[188], the Sexual and Physical Abuse Questionnaire[189], and McArthur Community Violence Instrument[190]. SA was coded as absent (0) or present (1) if any instance of SA was reported.

Maltreatment. For Paper III, a composite maltreatment variable was constructed based on participants having experienced: (0) no maltreatment, (1) PA only or SA only, and (2) both PA and SA.

(Paper IV)

Family Maltreatment (FM). Data on FM was collected at both waves using an in-house questionnaire comprised of four questions about observing physical and/or psychological abuse between parents, and direct experience of physical and/or psychological abuse by the parent(s) towards the participants. A summed total FM score of ascending severity ranging from 0 to 20 was calculated. For descriptive analyses, questions were coded as no (0) or yes (1).

Non-familial maltreatment (NFM). Data on maltreatment by a non-familial member was assessed at both waves using an in-house questionnaire including two questions about physical and/or psychological abuse. A summed score ranging from 0-6 with ascending values indicating greater severity of NFM was calculated. For descriptive analyses, responses were coded as no (0) and yes (1).

Parent-child relationship. The perceived quality of an adolescent's relationship with parents was assessed using the parents as social context questionnaire[191,192] at wave 2 only. This instrument queried three positive (warmth, structure, and autonomy support) and three negative (rejection, chaos, and coercion) dimensions of the parent-child relationship. A summed score ranging 0 to 36 from the positive dimensions with ascending values indicating a higher

quality relationship was computed. This variable was used in the main analyses. For descriptive and illustration purposes, responses were coded into three groups; poor (Scores ≤ Mean -1SD), average (Scores > Mean -1SD but ≤ Mean = 24 to 28), and good (Score ≥ Mean +1SD, = 29 to 36). Negative facets of the parent-child relationship were summed into a score ranging from 0 to 36, with higher scores indicating increasingly negative relationship with parents and was used as a covariate in analyses.

Lifetime and Current Diagnoses of Mental Disorders (Papers I-III)

All participants completed the Structured Clinical Interviews for DSM-IV, (SCID I, SCID II)[193] at baseline and at saliva collection. Lifetime and current (prior six months) diagnoses of alcohol dependence, drug dependence, depression disorder (major depression disorder, dysthymia, depression disorder not-otherwise-specified or substance-induced mood disorder), anxiety disorder (agoraphobia, generalized anxiety disorder, anxiety disorder not-otherwise-specified, obsessive compulsive disorder, panic disorder, post-traumatic stress disorder, social phobia, specific phobia, or substance-induced anxiety disorder), and conduct disorder (CD), prior to age 15, were assessed. Thus, CD and lifetime diagnoses were made prior to saliva collection and at the time of the DNA collection when current diagnoses were assessed. For the 23 healthy women, lifetime and current diagnoses were made at the time of saliva collection. Lifetime and current diagnoses of mental disorders were coded as absent (0) or present (1).

Aggressive Behaviours (Paper III)

Aggressive Behaviour. At the time of saliva collection, participants completed the McArthur Community Violence Instrument[78] to report on aggressive behaviours enacted during the previous six months. The total number of types of aggressive behaviours in which the participant had engaged in, were counted and ranged between 0 and 8 with ascending values reflecting enactment of more types of aggressive behaviours.

Alcohol Consumption (Paper IV)

Alcohol Consumption. At both waves, participants reported on heavy alcohol consumption over the prior year using AUDIT-C, a three-item question-

naire[194,195]. The responses to these items were adapted for adolescents as outlined by Nilsson et al., 2011[39]. A summed score from the values recorded from all three items was computed and ranged from 0-14 with ascending values indicating heavier alcohol consumption. The original AUDIT-C questionnaire scores ranged from 0 to 12[194,195]. For descriptive analyses, cut-offs for risky alcohol consumption defined by Nilsson et al., 2011 (males ≥ 8 and females ≥ 6) were used[39]. Responses above and below the cut-off scores were defined as low- and high-alcohol drinkers.

Covariate Measures (Papers I-IV)

Papers I-III. Covariate measures used throughout these studies included lifetime substance dependence diagnoses, current alcohol use, current drug use, lifetime and current use of psychoactive medications (stimulants, hypnotics, anxiolytics, antidepressants, antipsychotics) in women only, and nicotine use (cigarettes and/or snus) in men only.

Paper IV. Covariate measures included negative parent-child relationship, nicotine use (cigarettes and/or snus), use of illicit drugs (hash/marijuana or any other drugs) reported at wave 1 only, bullying at school reported at both waves, delinquent behaviours (non-violent and violent) reported at both waves, parent's alcohol use reported by parents at wave 1 only, and positive and negative life-events reported at wave 2 only.

Ethical Considerations (Papers I-IV)

All procedures were conducted in accordance with ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments. All waves of data collection were approved by the Regional Ethical Review Board in Stockholm (Papers I-III) and the Regional Board for Research Ethics in Uppsala (Paper IV). At each wave of data collection for the clinical sample, participants signed informed consent except at baseline when consent for those under age 18 was given by their parents. At collection of saliva samples, all participants in Papers I-III were 18 or older and gave consent to providing samples for DNA extraction. For the sample of adolescents in Paper IV all of the adolescents and their parents provided signed informed consent forms. Confidentiality of all information was guaranteed, and at each wave of data collection participants received gift certificates as compensation for their time.

Statistical Analyses (Papers I-IV)

In Papers I, II, and III, chi-square and one-way ANOVAs were computed to compare characteristics of carriers of MAOA-uVNTR genotypes.

Paper I.

Principal Component Analyses (PCA) with Varimax rotation and Kaiser normalization were computed to identify homogenous groups (components) of methylation of the 16 CpGs comprising the *MAOA* ROI. These analyses were first performed among all participants, and then separately in women and men. Pearson's correlations were used to verify associations between component methylation and the mean methylation value of the CpGs included in the component.

Next, a two-way mixed model ANOVA with Bonferroni corrections for multiple comparisons were used to compare participants by age group (parent and youth) on single CpG and overall ROI methylation levels. Independent sample t-tests were used to compare the age groups on mean exonic, mean intronic, and component methylation levels. A similar two-way mixed-model ANOVA and set of independent samples t-test was then used to compare methylation levels by sex.

Moderation modeling using PROCESS 2.16 for SPSS was used to determine if age moderated associations of sex with methylation levels of individual CpGs, across the ROI, intronic and exonic regions, and components. Conditional effects ("simple slopes") analyses using PROCESS 2.16 were used to interpret significant interaction effects. Significant models were also re-run with adjustment for *MAOA* genotype.

We then examined associations between age as a continuous variable and methylation separately by sex. Separate PCA analyses were performed by sex to identify sex-specific methylation components.

Linear regression models were then used to examine associations of age with methylation of individual CpGs, overall ROI, intronic and exonic, and sex-specific components of methylation. Significant regression models were adjusted for *MAOA* genotype. Due to the prominence of substance misuse in the study sample, all linear regression analyses that were computed separately by sex were re-run adjusting for lifetime diagnoses of substance dependence.

General Linear Models were used to assess the interaction of *MAOA* genotype, sex, and sexual abuse on exonic, intronic, and component methylation levels. Parents were omitted from these analyses as they had not reported on their own experiences of sexual abuse. To further mitigate against potential effects of relatedness among participants included in these models, the analyses were also run including ex-clients and healthy participants and excluding siblings.

Paper II.

Two-way mixed model ANOVAs with post-hoc LSD for multiple comparisons were used to test group differences on CpG and overall ROI methylation among; (1) participants who had experienced PA and/or SA to non-abused (NA) participants who experienced neither PA nor SA, (2) PA only versus NA, and (3) SA only versus NA.

One-Way ANCOVAs were used to determine if associations between maltreatment and *MAOA* ROI methylation were robust to adjustments for covariate measures.

General linear regression models were then computed to examine associations of *MAOA* genotype, abuse, and interactions of genotype and abuse with CpG and mean exonic methylation.

Chi-square analyses were used to examine associations of abuse with lifetime and current diagnoses of mental disorders. Step-wise logistic regression models were used to determine whether methylation levels mediated associations of abuse and diagnoses. To confirm that mediation effects of methylation differed significantly from direct associations of abuse and diagnoses, PROCESS for SPSS v2.15 was used with the bootstrapping procedure for indirect effects as outlined by Hayes, A. F.[196].

Paper III.

All statistical analyses were conducted separately by sex. Chi-Square and t-tests were used to compare characteristics of carriers of *MAOA* genotypes.

PCA analyses were used to identify a limited number of homogeneous variables that could be entered into the main statistical models, followed by Pearson's correlations to verify associations between component methylation and methylation of component constituent CpGs. Independent sample t-tests were then used to compare methylation levels by *MAOA* genotype, and ANOVAs to compare participants on maltreatment.

Next, hierarchical linear regression models were used to examine if component methylation levels moderated the association of the interaction of maltreatment and *MAOA* genotype with aggressive behaviours. As suggested by others[197,198], only significant three-way interactions were considered for subsequent analyses.

To interpret significant three-way interactions of genotype, maltreatment, and methylation revealed in the main hierarchical linear regressions, conditional effects ("simple slopes") analyses from PROCESS 2.16 moderated moderation analyses were used. Significant conditional effects were re-run adjusting for covariates, individually, due to the small sample size.

Paper IV.

Descriptive analyses for ordinal or continuous variables was examined using both t-test and Mann-Whitney U analyses. Wilcoxon signed-rank test was

used to compare within-group differences on measures at the two waves. Pearson's chi-square (χ^2) test was used to compare categorical variables. Correlations between alcohol consumption, FM, NFM, maltreatment and positive parent-child relationship were assessed using Spearman correlation.

Association of the three-way interaction between *MAOA* genotype, FM or NFM, and positive parent-child relationship with alcohol consumption were assessed in men and women separately using moderated moderation regression models with SPSS PROCESS 2.16 for SPSS. The moderated moderation analyses were only performed with wave 2 since the data on the positive parent-child relationship was collected only at that time-point. The models assessed main effects, all the possible two-way interaction effects, the three-way interaction between the independent variables, and conditional effects.

Moderated moderation models were then used to assess the three-way interactions of *MAOA* genotype, FM, and positive parent-child relationship, and of *MAOA* genotype, NFM, and positive parent-child relationship, on alcohol consumption at wave 2. In these analyses, if the effect of the three-way interaction term was found to be statistically significant, it was further probed using independent-samples Kruskal-Wallis test to examine for whom an effect presented by performing between-group comparisons.

Post-hoc analyses were performed to explore which of the three facets included in the positive parent-child relationship index contributed the most to significant effects, and to adjust these models for the covariates.

RESULTS

Summary and Brief Discussion of Key Results

Paper I

The *MAOA* ROI has emerged as a locus of interest for DNA methylation studies of numerous mental disorders[171,174], including ASPD[166] and related behaviours such as alcohol consumption[172]. This study first sought to provide a novel and comprehensive characterization of *MAOA* ROI methylation by sex and age in a sample of ex-client young adult women and men initially recruited in adolescence at a substance misuse clinic, their siblings, and their parents. To this end, we characterized the methylation levels of the *MAOA* ROI'S 16 constituent CpG sites, overall ROI levels, exonic and intronic regions, and of two empirically derived components identified by PCA.

Characterization of MAOA ROI methylation by sex and age

Table 1. Correlation coefficients for rotated component matrix from Principle Component Analyses of *MAOA* ROI CpG site methylation in all study participants, women, and men.

Component	CpG Site										
	CpG 2/3	CpG 4	CpG 5/6	CpG 7/8	CpG 10	CpG 11	CpG 12	CpG 13	CpG 14	CpG 15	CpG 16
All Participants											
Component 1	.959	.956	.964	.959	.966	.965	.874	.735	.767	-	-
Component 2	-	-	-	-	-	-	-	-	-	.908	.841
Women											
Component 1	.895	.831	.904	.863	.933	.895	.590	-	-	-	-
Component 2	-	-	-	-	-	-	-	.641	.711	.845	.670
Men											
Component 1	.872	.795	.882	.861	.848	.788	.482	-	-	-	-
Component 2	-	-	-	-	-	-	-	.847	.607	.895	.927

The components identified by PCA in all participants is presented in Table 1. Component methylation levels from all participants were highly correlated with those of their constituent CpGs (Component 1, r=.992, p<.001; Component 2, r=.961, p<.001).

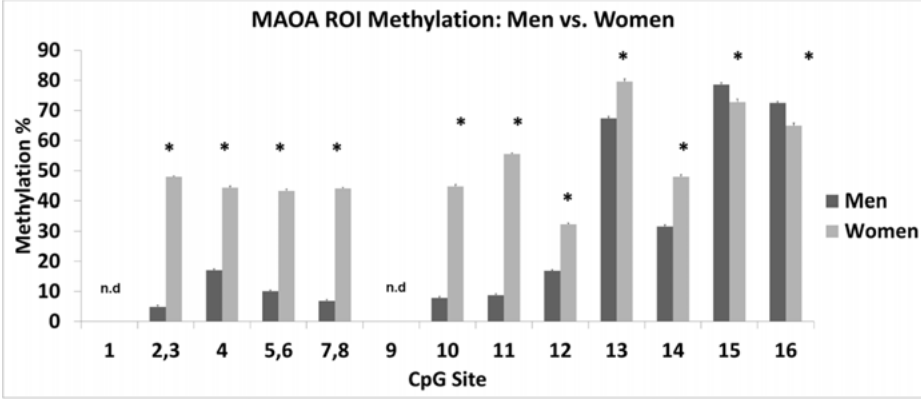

Figure 3. Results of comparisons of women and men on methylation levels across the *MAOA* ROI CpG (*p<.05). (n.d= no data available).

Sex. Analyses by sex using a two-way mixed model ANOVA revealed a significantly higher levels of methylation among women than men on CpGs 2-14 in the *MAOA* ROI. Similarly, women also displayed higher methylation than men on exonic (t(409)=53.21, p<.001), intronic (t(409)=19.95, p<.001), and component 1 (t(409)=47.91, p<.001) regions. By contrast, men showed higher methylation levels of CpGs 15 and 16 (Figure 3), and of component 2 (t(409)=4.21, p<.001). In addition, *MAOA* genotype was only associated with higher CpG14 methylation in males carrying MAOA-L relative to men carrying MAOA-S (p=.024).

Age. A two-way mixed-model ANOVA showed no differences in methylation levels between older and younger participants.

Table 2. Conditional effects of sex and age on *MAOA* ROI methylation among women compared to men.

Age	Unstandardized Simple Slope (beta coefficient)	95% Confidence Interval	p=
	Component 2 Methylation (CpGs 15 and 16)		
1 SD below mean[a]	-0.2269	-0.51– 0.06	.120
Mean [b]	-0.4276	-0.63 – -0.22	<.001
1 SD above mean [c]	0.5997	-0.91 – -0.35	<.001
	CpG 15 Methylation		
1 SD below mean [a]	-0.0311	-0.06 – 0	.051
Mean [b]	-0.0556	-0.08 – -0.03	<.001
1 SD above mean [c]	-0.0801	-0.11 – -0.05	<.001

[a] 1 SD below mean (age=19), [b] Mean (age=32), [c] 1 SD above mean (age=45)

Are the associations of sex with methylation levels modified by age?

In the full sample, moderation analyses with subsequent conditional effects examination of significant interactions showed that age modified few associations of sex with methylation. As presented on in Table 2, women at mean (32 years old) and mean+1 SD (45 years old) had lower methylation levels of CpG15 than men, and women at mean and mean+1SD ages showed lower methylation levels of component 2 than men. However, following adjustments for *MAOA* genotype, only the interaction of sex and age with CpG15 methylation levels remained significant. Sex was the only factor associated with all other methylation measures, with women presenting higher methylation levels than men

Separate linear regression analyses of age and methylation were then performed and included sex-specific components of methylation identified by PCA. The sex-specific components are shown on Table 1. As with the full sample, sex-specific components were highly correlated with their constituent CpGs in women (Component 1, r=.996, p<.001; Component 2, r=.991, p<.001) and in men (Component 1, r=.985, p<.001; Component 2, r=.994, p<.001). Sex-specific analyses revealed few associations of age on methylation levels. Results of the full-sample and sex-specific analyses are summarized in Table 3. Notably, all results reported among the women were robust to adjustment for substance dependence disorders. In men, substance dependence, but not age, was associated with exonic methylation (B=2.876, p=.011). Together, these results also echo findings of investigations of *MAOA* ROI methylation by others showing similarly high methylation levels in females alone[171], and as compared to men[168].

Table 3. Associations of age and sex with methylation levels of the *MAOA* ROI: A summary of results.

	CpGs	Across ROI	Intronic	Exonic	Component 1	Component 2
			All Participants			
Age-group	x	x	x	x	x	x
Sex	↑ women CpGs 2-14 ↓ women CpGs 15 and 16	↑ women	↑ women	↑ women	↑ women	↓ women
Sex modified by age	In women, at CpG 15[B]					In women[A]
Only sex		↑ women	↑ women	↑ women		
			Women Only			
Age	Negative association at CpG13[B]	x	x	x	x	Negative association[A]
			Men Only			
Age	Positive association at CpG15[B]	x	x	x	x	x

A. Not robust to covariation with *MAOA* genotype, B. Robust to adjustment for MAOA-uVNTR genotype. X. No Association

Is higher methylation among women associated with genotype, sex, sexual abuse (SA), and their interactions?

Table 4. General Linear Models of factors associated with MAOA ROI methylation in exonic and intronic regions and components 1 and 2

Predictor	Methylation											
	Mean Exonic			Mean Intronic			Component 1			Component 2		
	df	F	p=	df	F	p=	df	F	p=	df	F	p=
MAOA-uVNTR	1	0.302	.583	1	0.143	.705	1	1.501	.221	1	0.678	.410
Sexual abuse	1	1.510	.219	1	1.050	.305	1	3.688	.055	1	1.363	.243
Sex	1	1747.654	<.001	1	215.215	<.001	1	1294.844	<.001	1	2.414	.120
Sexual abuse x Sex		4.161	.041		1.088	.297		4.973	.026		0.931	.335
MAOA-uVNTR x Sexual Abuse		1.045	.307		2.390	.122		3.092	.079		1.523	.217
MAOA-uVNTR x Sex		0.559	.455		0.036	.850		1.281	.258		0.359	.549
MAOA-uVNTR x Sexual Abuse x Sex		1.223	.269		3.152	.076		3.590	.058		2.269	.132

In the young participants only, a set general linear model analyses examined the three-way interaction of *MAOA* genotype, sex, and SA on exonic, intronic, and component methylation levels. As outlined on Table 4, no significant three-way interactions were identified. However, interactions of sex and SA were identified such that exonic and component 1 methylation levels were higher among women who experienced SA than women who did not. This association was not observed in the men. Results also affirmed higher methylation among women than men on all measures except component 2. Analyses were re-run without siblings to mitigate concerns of their relatedness to ex-clients indicating that, among women only, SA associated with higher exonic methylation but not component 1.

Paper 1 summation. Together, the results of Paper 1 show a stark sex-difference in *MAOA* ROI methylation. Women showed higher exonic methylation levels than men, which was largely consistent across all analyses and was present even after other factors including age, genotype, SA, and substance dependence were considered. This finding is strongly indicative of X-chromosome inactivation in females, rather than a sex-difference in methylation levels on the active X-chromosome. Here, we also show that, among women only, SA was associated with higher exonic methylation, offering further evidence that *MAOA* ROI methylation may be altered by maltreatment. The associations of SA with *MAOA* ROI methylation levels was robust to adjustment for substance dependence, a factor typifying this sample, suggesting that this finding is generalizable to the population.

Paper II

The second study, in women only, focused on examining specific associations of maltreatment type (PA and SA) with *MAOA* ROI methylation, and if ROI methylation mediated associations between maltreatment and mental disorders. Mental disorders examined included lifetime and current diagnoses of alcohol dependence, drug dependence, depressive disorders, anxiety disorders, and CD prior to age 15.

Sample description

No differences were observed between *MAOA* genotypes on the proportions of women with PA and/or SA, PA, CD before age 15, or lifetime diagnoses of mental disorders. However, carriers of MAOA-SL displayed more current anxiety disorders than SS or LL carriers (p=.004).

Does physical abuse and sexual abuse associate with MAOA ROI methylation levels?

PA and/or SA. Analysis using a two-way mixed-model ANOVA showed that women who experienced PA and/or SA exhibited higher *MAOA* ROI methylation levels than women who did not experience either types of abuse (non-abused, NA) ($F(1,204)=13.622$, $p<.001$), with post-hoc LSD showing similar

associations on CpGs 2-11 methylation, while the PA and/or SA group showed lower CpG 15 methylation levels. Subsequent analyses showed that these results were robust to adjustment for psychoactive medication use, alcohol and drug dependence, and current substance use, all of which are factors associated with *MAOA* ROI methylation and/or genome wide alterations of methylation levels[51,137,185,199,200]. With an association of maltreatment and *MAOA* ROI methylation levels established, subsequent analyses then focused on further examining association of the specific types of abuse.

PA only. A two-way mixed-model ANOVA indicated that the PA only group showed higher methylation levels than NA (F(1,115)=4.951, p=.028) with post-hoc LSD indicating higher CpG7/8 levels among PA relative to NA. Adjustments for covariates were not performed due to only nine participants having experienced PA only.

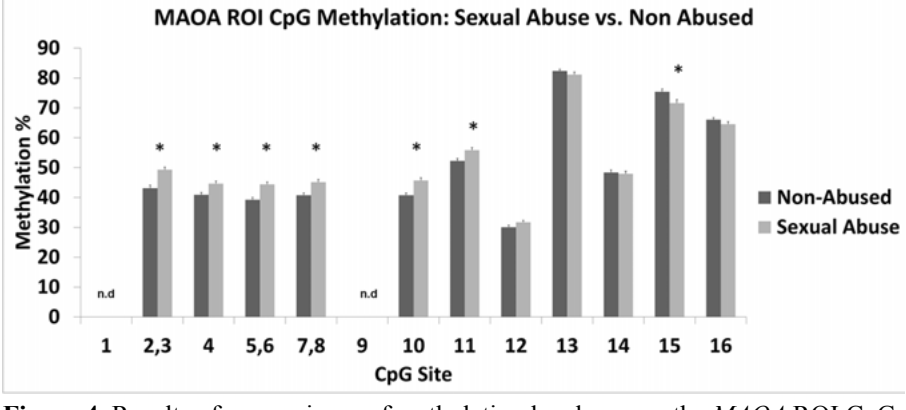

Figure 4. Results of comparisons of methylation levels across the *MAOA* ROI CpGs observed among women who had and who had not experienced sexual abuse (*p<.05) (n.d= no data available).

SA only. A two-way mixed-model ANOVA indicated that the SA only group showed higher *MAOA* ROI methylation levels than NA (F(1,187)=12.693, p<.001). Post-hoc LSD revealed similar directions of associations on CpGs 2-8, and 11 methylation, while lower methylation levels among those who experienced SA only compared to NA were found at CpG15. Results are illustrated on Figure 4. These results were robust to adjustments for all covariates. Notably as SA was associated with higher methylation of primarily exonic CpGs, these findings are also consistent with those reported in Paper I. Subsequent analyses focused on SA only.

Do MAOA genotype and sexual abuse interact to predict MAOA first exon methylation levels?

General linear regression models showed that genotype was associated with methylation levels of CpGs 2/3, 4, 10, and of mean exonic methylation. SA

was associated with methylation levels at CpGs 2-10, and of exonic methylation. No interactions of *MAOA* genotype and SA were predictive of methylation levels.

Do MAOA first exon methylation levels mediate associations of sexual abuse and mental disorders?

Chi-square analyses showed that a larger proportion of women who had experienced SA presented with lifetime and current diagnoses of alcohol and drug dependence, anxiety disorders, and CD prior to age 15. These women also presented more lifetime depression diagnoses, while SA and NA groups did not differ on current depression diagnoses.

Do MAOA first exon methylation levels mediate associations of sexual abuse and mental disorders?

Table 5. Factors associated with current and life-time disorders

	Step 1		Step 2		Step 3		Mediation Analysis[a]
Predictor	**Odds Ratio**	**95% Confidence Interval**	**Odds Ratio**	**95% Confidence Interval**	**Odds Ratio**	**95% Confidence Interval**	**Bootstrapped 95% Confidence Interval**
Current Depression Disorders							
Sexual Abuse	5.60	1.95-16.04			1.20	0.38-3.80	
CpG2/3			1.11	1.03-1.20	1,11	1.02-1.20	
					$X^2(1,N=75)=8.77$, p=.012		0.13-1.33
Sexual Abuse	5.60	1.95-16.04			1.38	0.44-4.26	
CpG4			1.11	1.02-1.21	1.10	1.00-1.21	
					$X^2(1,N=75)=6.05$, p=.049		1.33-1.42
Sexual Abuse	5.60	1.95-16.04			1.01	0.30-3.35	
CpG5/6			1.14	1.04-1.25	1.14	1.04-1.26	
					$X^2(1,N=75)=9.96$, p=.007		0.16-2.01
Sexual Abuse	5.60	1.95-16.04			1.40	0.46-4.29	
CpG7/8			1.11	1.02-1.20	1.10	1.01-1.19	
					$X^2(1,N=75)=7.10$, p=.029		0.04-1.22
Sexual Abuse	5.60	1.95-16.04			1.02	0.31-3.39	
Exon 1			1.16	1.05-1.28	1.16	1.04-1.29	
					$X^2(1,N=75)=9.74$, p=.008		0.17-1.22
Lifetime Depression Disorders							
Sexual Abuse	5.60	1.95-16.04			3.37	1.08-10.54	
CpG5/6			1.14	1.05-1.24	1.10	1.01-1.21	-
					$X^2(1,N=75)=9.74$, p=.008		

Step-wise logistic regression analyses was used to detect mediation effects, and subsequent confirmation of mediation effects was performed with the bootstrapping procedures for indirect effects as outlined by Hayes, A. F.[196] using PROCESS for SPSS v2.15. Results showed that methylation levels of the *MAOA* first exon mediated fully mediated the association of SA with current depression diagnosis, and both sexual abuse and methylation levels were independently predictive of life-time depression.

Paper II Summation. Although this study was conducted prior to the study outlined in Paper I, these results re-affirm the association of SA with *MAOA*

first exon methylation in women. Further, this study expands on that association by also offering a comprehensive examination of how *MAOA* first exonic methylation levels mediate associations between SA and numerous mental disorders. These findings also contribute to a body of evidence showing that adversity associates with epigenetic alterations of genes involved in mood and behaviour to increase risk for depressive disorders[107,108,201]. To our knowledge, this study offered the first evidence showing associations between maltreatment, *MAOA* methylation, and mental health outcomes.

Paper III

The third study sought to determine if the interactions of *MAOA* susceptibility alleles in men (MAOA-S) and in women (MAOA-L) with maltreatment on aggressive behaviours was further moderated by empirically derived components of methylation. Emergent evidence shows that abused individuals typically experience multiple forms of abuse[8], and that interactions of *MAOA* SNPs and maltreatment on antisocial outcomes may be dependent on the severity of maltreatment experienced[202]. Given the high co-occurrence of PA and SA in our sample, this study used a composite maltreatment variable including both types of abuse. Further, as antisocial men and women often exhibit a diverse range of aggressive behaviours, the number of types of aggressive behaviours participants reported was used as the outcome measure. Analyses were performed separately by sex.

Participant Characteristics

No differences by genotype were shown for maltreatment, aggressive behaviours, or covariates, except for men carrying MAOA-L who presented more diagnoses of current drug dependence than MAOA-S carriers (p=.031).

Principal component analyses of MAOA ROI methylation levels and correlations

Table 6. Correlation coefficients for rotated component matrix from Principle Component Analysis of MAOA ROI CpG site methylation in men and women

Component	CpG Site										
	CpG 2/3	CpG 4	CpG 5/6	CpG 7/8	CpG 10	CpG 11	CpG 12	CpG 13	CpG 14	CpG 15	CpG 16
					Men						
Component 1	-	-	-	-	-	-	-	.810	.660	.922	.939
Component 2	.739	.797	.755	-	-	-	-	-	-	-	-
Component 3	-	-	-	.799	.693	.696	.513	-	-	-	-
					Women						
Component 1	.891	.810	.882	.801	.903	.845	.608	-	-	-	-
Component 2	-	-	-	-	-	-	-	.838	.604	.800	.846

The results of PCA analyses are outlined in Table 6. Briefly, three components of methylation were identified in men, while two components were identified in women. Component methylation levels were highly correlated with those

of their constituent CpGs in men (Component 1, r=.984, p<.001; Component 2, r=.939, p<.001; Component 3, r=.950, p<.001) and in women (Component 1; r=.999, p<.001; Component 2; r=.995, p<.001).

Does MAOA ROI methylation further modify the association of the interaction of MAOA genotype and maltreatment on aggressive behaviours?

Table 7. Results of conditional effects analyses of methylation levels and *MAOA* risk genotype, on the association of maltreatment and number of aggressive behaviors among men.

Component Methylation Level	*MAOA* Genotype	Unstandardized Simple Slope (beta coefficient)	95% Confidence Interval
	Men Component 1 Methylation (CpGs 13-16)		
1 SD below mean[a]	MAOA-L	0.2858	-1.12 – 1.69
	MAOA-S	**1.7828**	**0.44 – 3.12**
Mean [b]	MAOA-L	0.4427	-0.41 -1.29
	MAOA-S	0.5737	-0.35 – 1.50
1 SD above mean [a]	MAOA-L	0.5997	-0.43 – 1.63
	MAOA-S	-0.6355	-2.02 – 0.75
	Men Component 3 Methylation (CpGs 7-12)		
1 SD below mean [b]	MAOA-L	-0.7102	-2.11 – 0.69
	MAOA-S	0.1501	-0.90 – 1.20
Mean [c]	MAOA-L	0.3157	-0.49 – 1.12
	MAOA-S	1.3484	0.31 – 2.39
1 SD above mean [b]	MAOA-L	1.3415	0.16 – 2.52
	MAOA-S	**2.5468**	**0.94 – 4.15**

[a] Mean component 1 methylation in men (-.0027), 1 SD below mean (-0.9858), 1 SD above mean (.9805)
[b] Mean component 3 methylation in men (-.0329), 1 SD below mean (-1.0267), 1 SD above mean (.9601)
* Results were robust to subsequent adjustments for all covariates

Men. Hierarchical linear regression analyses followed by conditional effects analyses were used to identify and explore significant three-way interactions of maltreatment, *MAOA* genotypes, and component methylation on aggressive behaviours. Results of the conditional effects analyses are shown in Table 7.

The final model including component 1 (CpGs 13-16) was significant ($F(5,72)=2.79$, $p=.024$) as was the three-way interaction term ($B=-1.37$, $p=.003$, $r^2= 11.2\%$). Conditional effects analyses showed that maltreated MAOA-S carriers with low levels of methylation (mean-1SD) of intronic CpGs 13-16, reported more aggressive behaviour than MAOA-S carriers with high levels of methylation at these sites and the MAOA-L carriers. However, this finding was not significant after adjustment for current alcohol use.

The final model including component 3 (comprised of CpGs 7-12) was not significant ($F(5,72)=2.13$, $p=.072$, $r^2=7.4\%$), though the three-way interaction term was ($B=1.03$, $p=.018$). Conditional effects analyses showed three significant conditional effects. Aggressive behaviours were higher among maltreated men carrying MAOA-S that also had high methylation levels (mean+1SD) than men carrying MAOA-L, a finding that was robust to adjustment for all covariates.

The conditional effects among maltreated men carrying MAOA-S with mean levels of CpG 7-12 methylation was not significant following adjustments for lifetime and current alcohol dependence, lifetime drug dependence, and current alcohol and drug use, nor was the conditional effect among maltreated men carrying MAOA-L with high methylation after adjusting for current alcohol dependence, lifetime and current drug dependence, and current alcohol and drug use.

For the final model that included component 2 (CpGs 2-6), neither the model nor three-way interaction term were significant, and thus subsequent conditional-effects analyses were not performed.

Women. No significant interactions of genotype, maltreatment, and methylation of components 1 or 2 were shown.

Paper III summation. The results of this study indicated that, in men, hypermethylation of primarily exonic CpGs 7-12 further modified the association of the interaction of MAOA-S and maltreatment on higher aggressive behaviours. This finding remained following adjustment for covariates. As evidenced by prior functional analysis of *MAOA* first exon methylation, hypermethylation in this region would be expected to drive down gene-expression[166], concordant with the low-expressing MAOA-S genotype. As such, the finding point to epigenetic and genotypic factors both conferring reduced *MAOA* expression among maltreated men exhibiting aggressive behaviours.

Paper IV

The fourth study extended on the findings from Papers I-III in a larger sample of healthy adolescent boys and girls through the differential-susceptibility theoretical framework. Through this perspective, we examined if interactions of *MAOA* genotypes and negative familial and/or non-familial environment on alcohol were further moderated by positive family environment.

Descriptive summary of main variables

High- and low-drinking boys did not differ on *MAOA* genotypes, quality of parent-child relationship, or FM or NFM except for high-drinking boys reporting more NFM than low-drinking boys at wave 2 ($p=.004$). High-drinking girls reported more FM at waves 1 ($p<.001$) and wave 2 ($p<.001$), and more NFM only at wave 2 ($p<.001$) than low-drinking girls. Low-drinking girls reported a higher parent-child relationship quality than high-drinking girls ($p<.001$).

Does MAOA genotype, family maltreatment, and positive parent-child relationship interact to predict alcohol consumption?

Table 8. Summary of the moderated moderation model for family maltreatment, MAOA genotype, and positive parent-child relationship

	b	*se*	*t*	95% Confidence Interval	*p*=
FM	0.207	0.451	0.458	1.09-0.68	.647
***MAOA*-uVNTR**	1.812	2.625	-0.69	-6.96-3.34	.490
Positive parent-child relationship	0.095	0.082	1.154	-0.26-0.07	.249
FM × *MAOA*-uVNTR	1.048	0.504	2.078	0.06-2.04	.038
FM × Positive parent-child relationship	0.018	0.018	0.995	-0.02-0.05	.320
***MAOA*-uVNTR × Positive parent-child relationship**	0.082	0.088	0.936	-0.09-0.25	.349
FM ×*MAOA*-uVNTR × Positive parent-child relationship	0.043	0.02	2.169	-0.08--0.004	.030

The final moderated moderation model assessing the interaction of *MAOA* genotype, FM, and positive parent-child relationship on alcohol consumption was significant ($F(7,840) = 6.613$, $p < 0.001$). As outlined on Table 8, this three-way interaction was associated with alcohol consumption in girls, accounting for 0.5% of the variance in alcohol consumption. No significant interactions were observed among the boys, who were thus excluded from subsequent analyses.

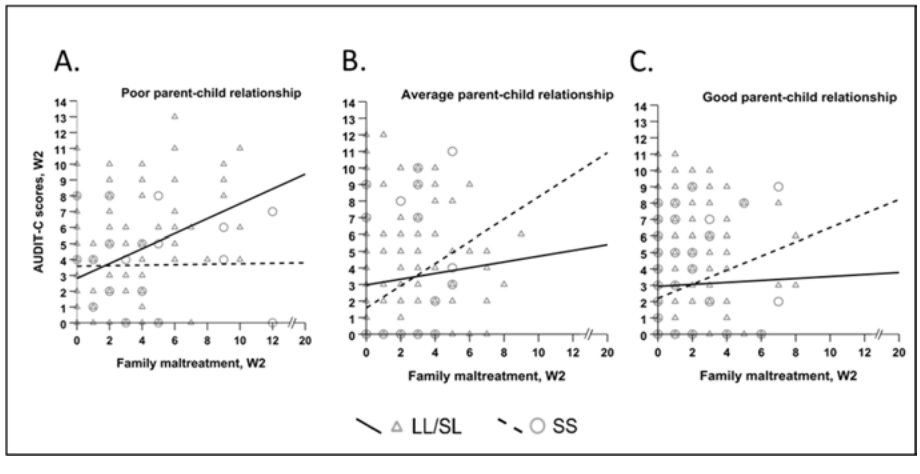

Figure 5. Graphical representation of the three-way interaction effect of MAOA-uVNTR genotype × family maltreatment × positive parent-child relationship on alcohol consumption in the girls relative to poor (**Panel A**), Average (**Panel B**), and Good (**Panel C**) parent-child relationship using scatter plots

The significant three-way interaction of *MAOA* genotype, FM, and positive parent-child relationship on alcohol consumption is depicted in Figure 5.

Among girls carrying MAOA-SL/LL who experienced a poor parent-child relationship, alcohol consumption increased concordantly with higher FM scores (Figure 5, Panel A). By contrast, girls who experienced average or good parent-child relationship displayed no significant increase in alcohol consumption with higher FM scores (Figure 5, Panels B and C). The girls carrying MAOA-SS presented with no significant change in alcohol consumption along with an increase in FM scores among those who had either poor (Figure 5, Panel A), or good parent-child relationship (Figure 5, Panel C).

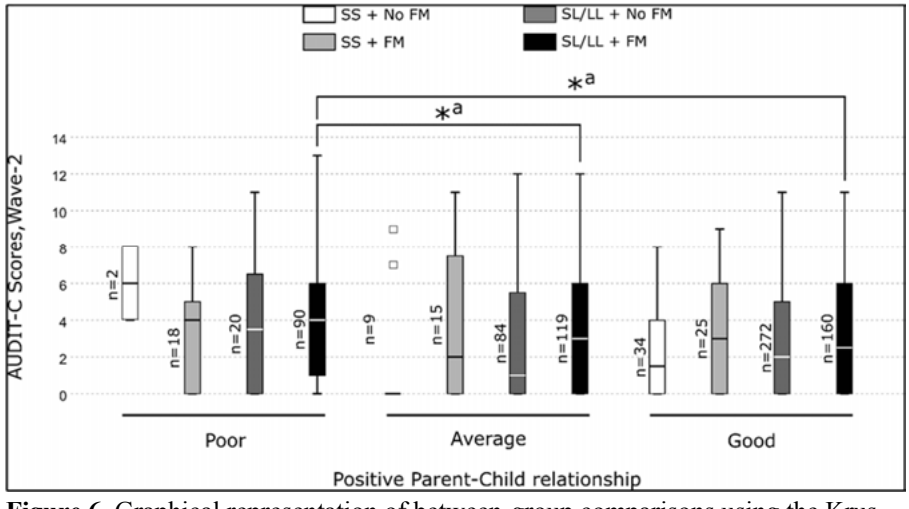

Figure 6. Graphical representation of between-group comparisons using the Kruskal-Wallis test to unpack the three-way interaction effect of MAOA-uVNTR genotype × family maltreatment × positive parent-child relationship on alcohol consumption in the girls. (*p<.05) (a Not significant following Bonferroni correction)

The group differences were confirmed ($H_{(11)} = 24{,}842$, $p = 0.010$) and the three-way interactions were examined using a Kruskal-Wallis test. The test's pairwise comparisons revealed that the risk MAOA-SL/LL genotype carriers who experienced FM and had a poor parent-child relationship presented with higher alcohol consumption scores relative to those with an average or a good parent-child relationship (Figure 6). However, girls carrying MAOA-SL/LL who did not experience FM, no significant differences in alcohol consumption were observed between those who had poor, average or good parent-child relationship. Similarly, among the MAOA-SS carriers with or without FM, no significant differences in alcohol consumption were observed between those who had poor, average or good parent-child relationship (Figure 6).

Post-hoc analyses. The association of the three-way interaction with AUDIT-C was no longer significant in after adjustments for FM (wave 1), nicotine use (wave 2), use of illicit substances in life, positive life events, negative life events, or violent delinquencies (wave 2), and non-violent delinquencies

(waves 1 and 2). Further, corrections rendered all pairwise-comparisons non-significant.

Does MAOA genotype, non-familial maltreatment, positive parent-child relationship interact to predict alcohol consumption?

Table 9. Summary of the moderated moderation model non-family maltreatment, MAOA genotype, and positive parent-child relationship

	b	***se***	***t***	**95% Confidence Interval**	***p*=**
NFM	1.600	1.019	1.569	-3.60-0.40	.117
MAOA-uVNTR	1.942	2.164	0.897	-6.19-2.30	.370
Positive parent-child relationship	0.145	0.070	2.077	-0.28--0.01	.038
NFM × *MAOA*-uVNTR	3.182	1.134	2.807	0.96-5.41	.005
NFM × Positive parent-child relationship	0.091	0.040	2.290	0.01-0.17	.022
MAOA-uVNTR × Positive parent-child relationship	0.089	0.074	1.197	-0.06-0.23	.232
NFM ×*MAOA*-uVNTR × Positive parent-child relationship	**0.131**	**0.044**	**2.996**	**-0.22—0.04**	**.003**

The moderated moderation model assessing the interaction of *MAOA* genotype, NFM, and positive parent-child relationship on alcohol consumption was significant ($F(7,840) = 8.786$, $p < 0.001$). As shown on Table 9, this three-way interaction was only significant among the girls, accounting for 1% of the variance in their alcohol consumption.

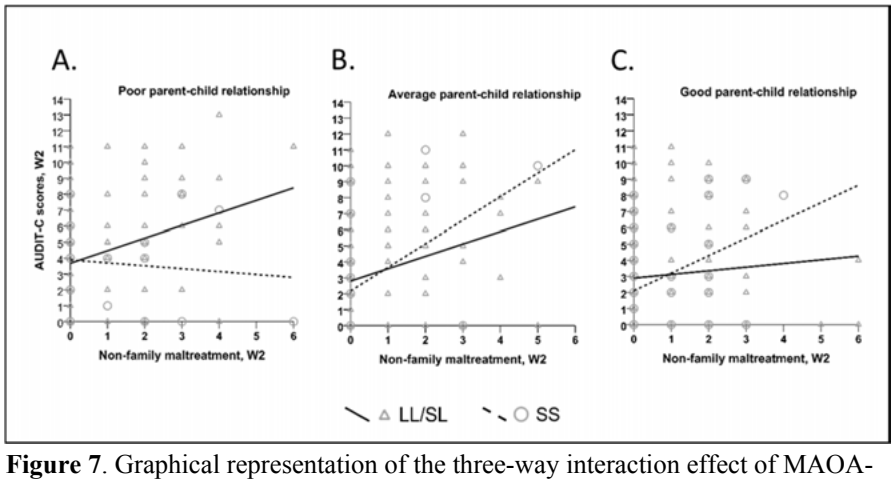

Figure 7. Graphical representation of the three-way interaction effect of MAOA-uVNTR genotype × non-family maltreatment × positive parent-child relationship on alcohol consumption in the girls relative to poor (**Panel A**), Average (**Panel B**), and Good (**Panel C**) parent-child relationship using scatter plots

Graphical representation of the three-way interaction shown in Figure 7. Girls carrying MAOA-SL/LL displayed an increase in alcohol consumption concordant with higher NFM scores among those who had poor or average parent-child relationship (Figure 7, Panel A) and B). However, MAOA-SL/LL carriers who experienced a good parent-child relationship presented no increase in alcohol consumption with higher NFM scores (Figure 7 Panel C). Girls carrying MAOA-SS showed no change in alcohol consumption with increased NFM scores among those who experienced a poor parent-child relationship (Figure 7, Panel A). However, alcohol consumption increased concordantly with NFM scores among those who experienced average or good parent-child relationships (Figure 7, Panels B and C).

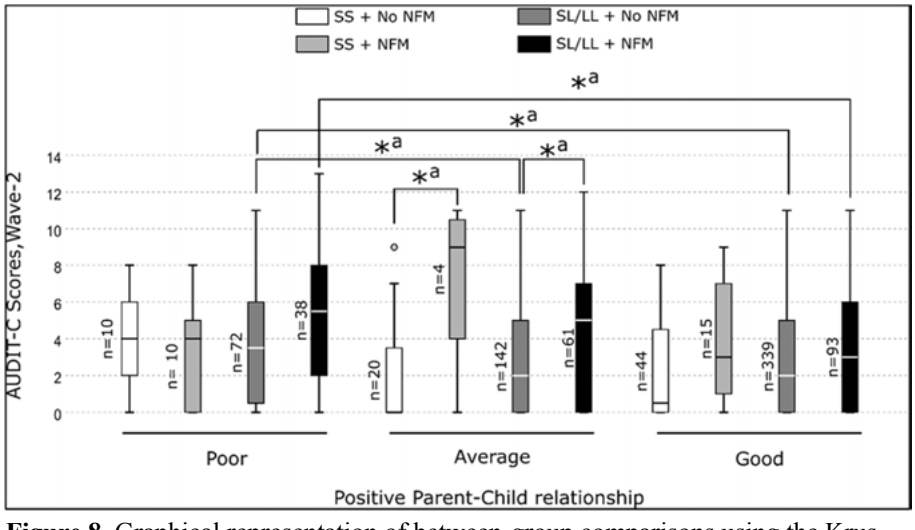

Figure 8. Graphical representation of between-group comparisons using the Kruskal-Wallis test to unpack the three-way interaction effect of MAOA-uVNTR genotype × non-family maltreatment × positive parent-child relationship on alcohol consumption in the females. Significant differences are marked in bold. (*p<.05) (a Not significant following Bonferroni correction)

The group-difference was verified using the Kruskal-Wallis test (H (11) = 41.97, p < 0.001) and the three-way interactions were further examined. Pairwise comparisons suggested differential-susceptibility such that MAOA-SL/LL carriers who experienced both NFM and a poor parent-child relationship presented with significantly higher AUDIT-C scores versus those who experienced a good parent-child relationship. Girls carrying MAOA-SL/LL who did not report NFM but experienced a poor parent-child relationship exhibited higher alcohol consumption versus those who experienced an average or a good positive parent-child relationship. In contrast, among MAOA-SS carriers with or without NFM, no significant differences in AUDIT-C scores were observed between those who had poor, average or good parent-child relationship (Figure 8).

Post-hoc analyses. The association of the three-way interaction with alcohol consumption was not significant following adjustment for nicotine use (Wave 2 only) but was robust to the effects of other covariates including all delinquent behaviours at both waves. No pair-wise comparisons were significant following Bonferroni corrections.

Paper IV summation. The results of this study offer some support for the differential-susceptibility framework such that maltreated girls carrying the susceptibility MAOA-L that also had a good parent-child relationship, reported less alcohol consumption than female peers with average or poor parent-child relationship. This association was not observed among maltreated girls carrying MAOA-SS who presented higher alcohol consumption than MAOA-SL/LL carriers regardless of parent-child relationship quality. However, this association was attributable a much greater benefit reaped by positive-parent child relationship among MAOA- SL/LL carriers than MAOA-SS carriers. Together, these findings suggest that positive parenting may have helped attenuate the association of maltreatment and *MAOA* genotype on alcohol consumption only in girls carrying the MAOA- SL/LL susceptibility genotype. Notably, the models including FM were robust to adjustment for violent delinquency at wave 2, but not nicotine use. The models including NFM were robust to violent and non-violent delinquency at both waves. These findings highlight the close association of alcohol use and antisocial behaviours and importance of considering these behaviours together. These results also echo those of prior studies indicating responsiveness of *MAOA* genotypes to negative and positive environmental factors[97,99].

DISCUSSION

Summary of Thesis Contribution

The four studies comprising this thesis offer a crucial first attempt to examine how maltreatment interacts with both genotypic and epigenetic factors to modify risk for antisocial aggression, alcohol use, and mental disorders. This work focused on the *MAOA* gene, a well-established candidate gene implicated in how environmental and biological factors interface in association with a variety of behaviours, particularly aggression[78,80]. Further, this work also examined the differential-susceptibility framework through assessing how both negative and positive environmental factors interact with *MAOA* genotypes, thus illustrating the importance of considering attenuating factors typically overlooked in GxE research. To this end, our work has produced a novel and comprehensive characterization of DNA methylation in a region spanning the *MAOA* first exonic/intronic region and the associations with age and sex, a description of how *MAOA* genotype and DNA methylation in this region modifies associations of maltreatment with mental disorders and aggressive behaviours in a clinical population of women and men. Finally, we examined how negative and positive factors interact with *MAOA* in association with alcohol consumption in a general population sample of adolescent boys and girls. This work advances our knowledge of *MAOA* and of antisocial behaviours, while offering a foundation of evidence and methodological framework upon which similar studies can be further conducted in *MAOA* and in other candidate genes.

Limitations and Strengths

Limitations

Low sample size.

As the studies presented in this thesis were preliminary and exploratory studies using relatively small samples, we lacked sufficient statistical power to perform multiple corrections for all of the statistical models used[109,203]. Sample size concerns regarding sufficient power to perform multiple corrections were salient in Papers I-III. In these papers we lacked sufficient statistical power to

perform multiple corrections for all statistical models, particularly in the models that included three-way interactions to determine associations with exonic, intronic, and component methylation levels. However, based on previous evidence[121,123] we considered these regions as distinct functional units, greater than their constituent CpG sites. Thus, corrections for multiple testing were not necessary for these models. Further, the large sample sizes recommended to examine gene-by-environment interactions stem from critiques positing that effect sizes of such interactions should exceed those for candidate genes detected by genome-wide association studies[204]. However, it has been argued that there is likely no main effect of *MAOA* genotypes independent of influence from environmental factors[11,197]. Given evidence indicating associations between gene polymorphisms and methylation[161–163], epigenetic factors may also figure into such interactions. Notably, if an interaction term is associated with a dependent variable, the main effect would no longer be interpretable[179], as main effects would continuously change with each additional variable added to the model[11]. Some authors have suggested that studies of gene-environment interactions in smaller samples may be preferable, as dependent variables are more likely to be assessed using in-person interviews with better reliability and validity than the telephone, post, or internet based assessments often used in large sample studies[11,205–207].

Group comparisons of methylation levels by age and by sex in Paper I, and of maltreated and non-maltreated women in Paper II, were made using Two-Way Mixed-Model ANOVA which included CpG as a repeated measure. This statistical approach examined group differences in overall methylation and differences at each CpG using stringent Bonferroni corrections for multiple comparisons. As such, this approach offered a means to help reduce issues with multiple testing and has been similarly used in prior epigenetic studies[208,209].

In Paper III, the small sample-size also limited the power of some statistical models. The three-way interaction term identified in the hierarchical model among women was not significant (p=.063). However, given that the significant conditional effects of that interaction were robust to adjustment of multiple factors, and consistent with findings from previous studies[78,96,98,99,210], and our *a priori* hypothesis, follow-up analyses in larger samples of women is warranted. Similar to Paper I, the use of PCA-derived component methylation variables in Paper III also helped minimize the number of outcome variables used, and the study benefitted from use of single models to test the hypothesis. In addition, post-hoc power calculations for significant regression models in Paper III indicated moderate power (women component 1 model, effect size f2= 0.030, power= 0.457; men component 1 model, effect size f2= 0.140, power= 0.903; men component 3 model, effect size f2= 0.083, power= 0.710). However, these power estimates do not consider the joint distribution of interaction term variables, as they are computed based on R^2 increase for a fixed model rather than a random model[211], though the observed power is inversely

proportional to the p-value and offers similar information as confidence intervals[212].

However, the study presented in Paper IV had a relatively large sample size and benefited from having adequate power to detect a significant effect of moderate to large size as suggested by others[204]

: *MAOA* x FM: R^2 increase = 0.9%, power = 0.778; *MAOA* x NFM: R^2 increase = 0.6%, power = 0.607; *MAOA* x FM x positive parent-child relationship: R^2 increase = 0.5%, power = 0.541; and *MAOA* x NFM x positive parent-child relationship: R^2 increase = 1%, power = 0.832.

Timing of altered methylation.

The study design for the sample used in Papers I-III did not allow us to infer when alterations to methylation may have occurred, nor if they were a direct consequence of maltreatment. However, the associations between maltreatment and methylation reported in these studies are in line with a large body of evidence associating maltreatment with altered methylation, which has been hypothesized to be facilitated by susceptibility genotypes and subsequently modulated by a host of risk and protective factors[107,108,119]. Type, severity, and timing of the maltreatment likely influence both the association and timing of changes to methylation[107,108,119].

Relatedness.

The clinical sample used in Papers I-III included ex-clients, their siblings, and their parents. Relatedness of participants in studies examining genetic and epigenetic factors may skew findings. However, as the sample outlined in Paper I included both parents and their progeny, concerns regarding relatedness of the participants were largely mitigated in analyses of interactions of sex, sexual abuse, and age as the parents were not included in the analyses. Subsequent analyses excluding siblings and including only ex-clients and healthy controls revealed similar results. Further, the characterization of methylation by sex and by age addressed by the first aim of the study outlined in Paper I were robust to corrections for genotype. Studies II and III avoided the inclusion of related ex-clients and siblings. As such, we were able to mitigate concerns of relatedness in our studies, although relatedness among participants in study IV were not adjusted for.

Polyvictimization.

As reported in Paper II, analyses showed that women who had experienced one or both types of abuse exhibited elevated methylation levels of CpGs 1-11, relative to those who experienced no abuse. Methylation levels at these same sites were similar among women who had experienced one or both types of abuse. No group differences in methylation for CpGs 12-16 were observed in this study. These findings emphasize the importance of considering severity of abuse, particularly given that child victims often experience multiple types

of abuse[8,10]. Approximately 89% of individuals who experience sexual abuse are also exposed to physical abuse, while around 9% of those who experience physical abuse are also exposed to sexual abuse[8]. In our clinical sample, few participants experienced physical abuse exclusively, thus limiting confidence in our findings associating physical abuse only with methylation in Paper II. Further, the comparatively low number of men in our study sample led to their exclusion from most analyses in Paper II, as the resulting analyses of specific abuse types would have included too few participants. As such, Paper III benefited from the use of a more inclusive composite maltreatment variable that included physical abuse and sexual abuse, thus better reflecting the typical experience of maltreatment among the participants in our sample and, to some extent, severity of maltreatment. The use of a composite maltreatment variable also allowed us to include men in our analyses. With considerable overlap of sexual abuse and physical abuse noted in the literature[8] and observed in our sample in Studies II and III, only sexual abuse was assessed in Paper I as it would also be expected to indirectly index physical abuse as well. Future studies using prospective investigations with repeated measurements of maltreatment and methylation will be needed to determine causal associations of maltreatment and altered *MAOA* ROI methylation.

Self-reported measures.

Retrospective self-reported measures of maltreatment and aggressive behaviours were used in all the studies described in this thesis. While self-reported measures raise the potential for biases extending from psychological and motivational factors present among the participants at the time of assessment[213], reporting by other sources may be biased by underreporting[214]. In the current study, among most participants maltreatment was assessed in mid-adolescence and then again in adulthood, as some participants who were initially reluctant to report earlier instances of maltreatment only did so during assessments in adulthood. As such, our study benefits from use of validated instruments and follow-up assessments which captured instances of maltreatment and aggression that may have otherwise gone unreported.

It is further notable that sexual abuse was not examined in Paper IV. This was, in large part, because the adolescent participants most often completed the self-report questionnaires at home likely under parental guidance which raises the possibility of underreporting incidents of sexual abuse. The participants in the study outlined in Paper IV also presented with less antisocial behaviours, less maltreatment, and reported less substance use than reported in previous studies of *MAOA* and alcohol use[39–41] which may have also led to too few high-drinking boys to detect similar interaction effects observed in girls. The previous studies were also school-based, whereas the present study was a family-based postal questionnaire. As such, these severity of risk factors and outcomes noted in prior studies were likely not reflected in this study and are in-line with previous work positing that *MAOA* studies conducted in samples

exhibiting less severe predictors and outcomes may yield weaker GxE interactions than have typically been shown in more extreme samples[11,179]. Thus, higher alcohol consumption among the individuals carrying non-susceptibility alleles at a certain point of environmental estimate could be due to sampling effect. Nevertheless, these findings offer an important characterization of this sample and insight into how the factors examined may interact differently in healthy samples.

Use of saliva samples in methylation analysis.

Samples of DNA were extracted from saliva which contains a diverse range of peripheral cell-types, thus limiting the interpretation of our results in relation to central nervous system processes. However, a previous study identified an inverse correlation between *MAOA* promoter methylation in whole blood DNA and MAO enzymatic activity in the brain[165]. In addition, methylation patterns observed in our study are consistent with methylation patterns in the *MAOA* ROI reported elsewhere in whole blood samples in men[166] and in women[137,169]. Thus, measurements of *MAOA* methylation in peripheral tissues may be viable proxies for central processes. Further, in silico analyses using IMAGE-CpG (http://han-lab.org/methylation/default/imageCpG#)[186] revealed a relatively high concordance of methylation levels in brain, blood, salivary, and buccal tissues at the two *MAOA* ROI CpG sites available. Future work is needed to confirm consistency of cross-tissue methylation patterns in the *MAOA* ROI. In addition, methylation levels observed in the male and female participants in our study are similar to methylation levels in the *MAOA* ROI reported elsewhere in whole blood samples from men[166] and women[137,174,215], as well as studies using salivary methylation of the ROI[168,169]. Recent reviews of behavioural epigenetics further emphasize that the value of investigations of methylation in peripheral tissues is critical for the study of mental and behavioural disorders and responses to trauma[114,216]. The extensive characterization of methylation of the *MAOA* ROI provided in this thesis contributes to this effort.

Limitations of the EpiTYPER method.

As EpiTYPER relies on assessing methylation of sequence-based amplicons, the method cannot distinguish between the two X-chromosomes in females, and thus samples randomly from both x-chromosomes in females. As such, the data among women represents an averaged methylation value from both chromosomes[146]. The large sex-difference in methylation levels described in this thesis likely indicates near total methylation from the inactivated chromosome and low methylation from the active chromosome, accounting for why exonic methylation levels in women were in the ~40-50% range. Similar levels of *MAOA* first exon methylation in women have been assessed, reported, and interpreted similarly by others examining methylation in this re-

gion[137,168,169]. As the sex-difference in methylation in the *MAOA* first exon remained after other factors were considered in Paper I, these findings offer further evidence that the *MAOA* first exon may be an X-inactivation site in women. Given that methylation of first exonic regions of genes is associated with transcriptional silencing, rather than downregulation[217], this finding further evidences that *MAOA* does not escape inactivation[147].

The EpiTYPER method is also unable to distinguish between DNA methylation and hydroxymethylation. Recent studies suggest that methylation of the *MAOA* ROI may be dynamic in association with numerous environmental factors, and both hypo- and hypermethylation of this region have been reported[137,165,166,169,172], and thus may be labile to methylation and demethylation. In addition, 5hmC is enriched in genomic regions spanning exon-intron boundaries[151], such as the *MAOA* ROI. Although genome-wide reductions in methylation levels associated with age was not observed in the *MAOA* ROI in Paper I, examination of 5hmC may offer insight into whether the region was undergoing demethylation processes, even if the methylation levels themselves had yet not begun to lower. As such, it is vital for future studies of the *MAOA* ROI to characterize and examine the ratio of DNA methylation to hydroxymethylation across the region, its association with age, and its respective functional contributions to *MAOA* transcriptional activity.

Extant evidence suggests that methylation, including methylation of the *MAOA* ROI, may be sensitive to rapid fluctuations in association with environmental factors and individual experiences[137,174]. Further, one study showed rapid genome-wide demethylation during two weeks of alcohol detoxification which not accounted for by withdrawal severity, detoxification medication dose, or amount of alcohol consumption prior to detoxification[218]. As such, numerous unknown factors that may have been experienced acutely may influence methylation results in our clinical sample. Current 5hmC analyses methods utilize similar methodological principles as EpiTYPER and pyrosequencing and generate produce compatible data[219]. Thus, these findings of the studies in this thesis offer a strong foundation for subsequent studies and meta-analyses to further examine the dynamics of methylation in the *MAOA* ROI.

Lack of functional analyses.

RNA samples were not available from the participants in our studies, thus limiting conclusive interpretations of how methylation results reported in our studies associate with *MAOA* transcriptional activity. However, it has been shown that hypermethylation of the *MAOA* ROI associates with a large reduction in reporter gene activity *in vitro*[166], and methylation in exonic and intronic regions confer decreased and increased gene transcription, respectively[121,123]. Functional analyses of associations between *MAOA* methylation and *MAOA* transcriptional activity are needed in future studies to verify our interpretations, although confidence in the interpretation of reported findings is warranted.

Strengths

Use of extensive data.

The studies in this thesis all included extensive data on mental disorders, antisocial behaviour, alcohol consumption, and maltreatment that were assessed with validated instruments. These rich data allowed us to determine if our main findings were robust to adjustments for numerous important covariates. Such data also allowed us to examine differential susceptibility approach in Paper IV which incorporates a larger range of relevant risk and protective environmental factors, in contrast to studies employing a diathesis-stress framework which may overlook factors that attenuate risk.

Data on sexual abuse was not available for the older participants included in Paper I. However, results of that study indicating an association between sexual abuse and higher methylation of the exonic region of the *MAOA* ROI among the young women are consistent with, and further elaborated on in, Paper II. We lacked information about tobacco use in the women included in studies I-III. However, among men, tobacco use did not alter any result in Paper III. Interestingly, while a confounding effect of tobacco on associations between maltreatment and alterations to methylation of genes other than *MAOA* have been observed elsewhere[119,162], this effect was not observed in the study described in Paper III. Additionally, information on psychoactive medication was not available for men. However, the use of such medications did not alter significant findings among women in these studies.

In Paper III, the impact of including different types of participants in the study sample was negligible as the main analyses were adjusted for substance misuse and medication use. Follow-up analyses to replicate conditional effects among only the ex-clients yielded similar patterns in direction and magnitude as the conditional effects reported in the main models and models adjusted for covariates (data not shown). As such, including these participants increased statistical power and extended generalizability of the results through the benefit of having adequate data to adjust for important factors. In this study, aggressive behaviour was measured as the number of types of aggressive behaviours engaged in. While we were not able to directly assess the frequency or severity of aggressive behaviour, individuals who are severely antisocial engage in a greater litany of aggressive behaviours[21]. Further, there are distinct sex-differences in type and frequency of aggressive behaviours antisocial men and women engage in[11,21]. As such, the number of aggressive behaviours our study participants engaged in offered a suitably diverse measure of aggressive behaviours relevant to aggressive behaviour in both sexes.

The study reported in Papers I and III benefited greatly from well-defined and well-supported hypotheses, along with insights gained from the study in Paper II which helped us refine our statistical and theoretical approach to best use the data available such as the use of single-model testing of hypotheses

and use of empirical PCA-derived components of methylation to reduce the number of variables used in studies I and III. As such, despite small sample sizes, optimizing the use of the extensive data available helped to generate interpretable results.

Gold-standard molecular techniques.

All studies outlined in this thesis benefitted from the use of gold-standard genotyping methods using a primer design common to most studies of the MAOA-uVNTR[88], and has been validated in *in-vitro* analysis of the association of *MAOA* genotypes and reporter gene expression levels[89]. Similarly, Papers I-III benefited from the use of EpiTYPER, which offered optimal resolution for methylation analyses for these studies as it is particularly well-suited for examining patterns of methylation across genomic regions approximately 800 bp in length, while still offering CpG-specific resolution[220]. This offered an advantage over the most viable alternative, pyrosequencing, which produces similar data, has greater CpG specific resolution, but is less suitable for assessments of methylation patterns across >500 bp regions[220]. Rigorous quality control procedures conducted as part of methylation analyses also ensured that our analyses included high-quality methylation data. Finally, extant research of *MAOA* ROI methylation has primarily relied on EpiTYPER and pyrosequencing, thus allowing for consistency between our studies with others and offering data usable in future replication studies and meta-analyses.

Well-characterized MAOA ROI.

The *MAOA* ROI characterized and examined in Papers I-III was selected based on prior in-vitro analyses showing that methylation of this region associates with drastically reduced reporter gene expression[166], and that methylation levels of the ROI were associated with MAO enzymatic activity in the brain[165]. Further, genome-wide methylation studies have pointed to exonic/intronic junction regions, similar to the *MAOA* ROI, as remaining responsive to epigenetic alterations across the lifespan and are also associated with health outcomes[117,151]. As such, the *MAOA* ROI presents a compelling locus for future investigations.

The *MAOA* gene is among the most well-established candidate genes associated with behavioural phenotypes[11,51,78]. Converging lines of evidence from gene-environment studies and from DNA methylation studies have suggested that *MAOA* plays a role in antisocial behaviour and substance misuse[51,78,100,172], as well as other mental disorders[168,169,171]. As such, the comprehensive characterization of *MAOA* ROI methylation, taking account of *MAOA* genotypes, that is reported in Paper I and elaborated upon throughout Papers II and III contributes to furthering understanding of *MAOA* regulation in clinical populations and in our study population.

Methodological Considerations

During quality control procedures provided by Karolinska University Hospital's Mutation Analysis Core Facility (MAF) as part of the EpiTYPER procedure, to lower the risk of biased amplification of bisulfite-treated unmethylated over methylated DNA, PCR primers were designed to include a number of non-CpG C sites (the amplicon designed on the forward strand contained 4, whereas the amplicon designed on the reverse strand contained 6 non-CpG C sites). To further assess a possible amplification bias, fully methylated, unmethylated, and a 50% mix of DNA controls (EpiTect, Qiagen, Hilden, Germany) were included in the analysis. No amplification bias was observed in either of the two amplicons (results not shown).

Contribution to the Literature

General Relevance of Papers I-IV

The studies described in this thesis offer a comprehensive characterization of methylation in the *MAOA* ROI, initial evidence that methylation of a region of interest spanning the first exonic and partial first intronic region of *MAOA* contributes to increased risk for mental disorders and aggressive behaviours in a clinical population of men and women, that associations of maltreatment and aggressive behaviours may be moderated by both *MAOA* genotype and ROI methylation levels, and that consideration of positive environmental factors are important in understanding genetic susceptibility of the *MAOA* gene. Further, this work introduces the initial examinations of sex and age in association with *MAOA* ROI methylation. Together, this work offers the first attempt to unite gene-environment and epigenetic lines of research to expand our understanding of this important candidate gene in aggressive behaviour and other clinical conditions. In doing so, this work also highlights the importance of appreciating the complexity of how environmental factors interface with genes.

Understanding aggressive behaviours in males and females.

Aggression is a particularly sexually-dimorphic set of behaviours, also reflected among antisocial individuals who display a wide range of aggressive behaviours[21]. The studies in this thesis offer insight into how maltreatment, *MAOA* genotype, and *MAOA* ROI interface in aggression and related behaviours. Among the women in Paper II, although *MAOA* exonic methylation mediated the association of SA with current depression, no such mediation effect was observed in the association of SA with CD prior to age 15. Notably, most women with current depression also had a history of prior depression diagnoses and 37.5% also had CD prior to age 15. Evidence has shown that CD and

depression are often comorbid in adolescence[221] and CD in adolescence is associated with a high risk for depression among women in young adulthood[222]. As such, it may be expected that the women in Papers II and III would display more depressive than aggressive outcomes, which may help account for the weak associations with aggression described in Paper III. This interpretation of the Paper II and III findings also dovetails with evidence showing high heterogeneity in the etiology and presentation of both depression[223] and CD[224].

Further, the models including interactions with MAOA-L described in Paper IV in association with alcohol consumption which were no longer significant when some delinquent behaviours were adjusted for emphasizes the salience of both antisocial and substance misuse behaviours among this subset girls in adolescence. The transition from CD in adolescence to depression in adulthood among some women has been suggested to be driven, in part, by substance misuse[222]. Together, findings among females in Papers II, III, and IV may represent "snapshots" in time of a similar substance use driven progression from antisocial behaviours in adolescence to depression in early adulthood. This interpretation of the findings outlined in Papers II-IV is speculation, particularly since Papers II and III did not consider positive environment and methylation data was not available for Paper IV. However, this interpretation offers a compelling basis for follow-up studies in this healthy adolescent sample.

In men, the findings of Paper III echo those of GxE literature implicating the MAOA-S genotype[78] and of exonic hypermethylation observed in men with ASPD[166]. Notably, both the MAOA-S genotype and exonic hypermethylation are associated with diminished transcription[89,166]. As such, the findings of Paper III may suggest that both genotypic and epigenetic factors, in interaction with maltreatment, contribute to diminished gene expression. Notably, among maltreated men carrying MAOA-S with mean levels of methylation of CpGs 7-12, lifetime and current substance misuse explained, in part, the association of maltreatment and aggressive behaviour. Additionally, increased aggressive behaviours was also observed among maltreated men carrying MAOA-L with high methylation levels of the same sites, but this effect was not significant following adjustments for substance misuse covariates. Together these findings indicate that, in maltreated young adult men carrying MAOA-S, methylation of *MAOA* may play a stronger role in promoting aggressive behaviours than substance misuse. In contrast, substance misuse may have accounted for a larger proportion of the elevated aggression observed in the maltreated men carrying MAOA-L. These findings may suggest that, in a subgroup of susceptible men, a combination of genetic susceptibility and epigenetic alterations of *MAOA* may play a stronger role in promoting aggressive behaviours than substance misuse while others without similar susceptibility display aggressive behaviours primarily driven by substance misuse.

Uniting genotypic and epigenetic investigations of MAOA

Examinations of GxE interactions of *MAOA* on antisocial behaviour have implicated the gene in susceptibility to environmental factors that promote risk for antisocial behaviours and other behavioural phenotypes[11,78,80]. However, due to inconsistent findings from GxE studies of *MAOA* and the lack of putative mechanisms driving GxE interactions, epigenetic processes have been posited as an important avenue of further investigation[11,73,78]. A recently emerging set of epigenetic studies of the *MAOA* ROI have shown that methylation of this region is associated with several mental disorders[171], including ASPD[166], and with behaviours such as alcohol consumption[172]. Notably, altered methylation in this region has been associated with psychiatric treatment response in adult women[171,174]. Methylation of the ROI also shows promising functional viability as evidenced by altered transcription *in vitro*[166] and associations with MAO enzymatic activity in the adult brain[165]. Papers I-III offer an advancement in our understanding *MAOA* with a thorough characterization of *MAOA* ROI methylation, as well as an integration of GxE and epigenetic approaches to understanding the gene's role in aggressive behaviours. From this, we document an association of SA with exonic methylation among women consistent across studies I and II that was independent of genotype and robust to adjustment for covariates, and that MAOA-S and high exonic methylation in men moderated associations of maltreatment and aggressive behaviours among men in Paper III. Although methylation was not assessed for Paper IV, recent findings associating *MAOA* methylation with treatment response[171,174] offer a compelling foundation for future studies including positive environmental factors. Together, the studies outlined in this thesis offer a roadmap of how further integrated GxE and epigenetic studies can proceed and investigative approaches that can be adapted to studies of other candidate genes.

Relevance to Aggressive Behaviour.

The outcome-intersection phenomenon.

A recent review of *MAOA* GxE literature in antisocial behaviour points to an outcome-intersection phenomenon such that aggressive, antisocial, substance misuse and externalization behavioural phenotypes in conditions such as CD, as well as the comorbidities that often accompany it, may share a common underlying and unmeasured factor[11]. This can lead to confusion when interpreting cause and effect since the same factor could represent both an outcome, and a risk factor, for other related characteristics present in antisocial individuals, thus posing difficulty in interpreting results[11]. This phenomenon may have played a role in the results of studies II-IV presented in this thesis.

As reported in Paper II, among women, *MAOA* first exon hypermethylation mediated the association of sexual abuse with current depression but not CD. However, as the women with current depression in our sample displayed a particularly high prevalence of CD prior to age 15, and CD in adolescence is predictive of depression in adult women, particularly among those who engage in substance misuse[222], some of the observed effect may be attributable to antisocial behaviour occurring earlier in life among these women[89,225,226].

In Paper IV, we found that, among adolescent girls, the interaction of MAOA-L with maltreatment on alcohol consumption in models including FM was no longer significant once delinquent behaviours and use of other illicit substances were adjusted for, underscoring the salience of these factors in the adolescent age group. By contrast, models including NFM were robust to violent and non-violent delinquency at both waves. Such a finding may be expected since the context where risk factors are encountered often shifts from familial settings during childhood to peer settings throughout adolescence[3,42,227]. However, all models in Paper IV were ultimately rendered non-significant when other covariates such as nicotine use, were adjusted for. This limits conclusive interpretations. Similar to the study in Paper III, a composite maltreatment variable including FM and NFM may be helpful in elucidating the role delinquent behaviour in differential-susceptibility interactions.

The findings among the women in Papers II and IV, considered together, may be reflective of reports from others showing that age of onset for antisocial behaviours predicts[222] and precedes the age of onset for depression[228,229]. Further, it has been posited that the social and educational difficulties resulting from early onset antisocial behaviours promote subsequent onset of depression in early adulthood[230], an association exacerbated by substance misuse[222], and noted as a particularly salient phenomenon among females[231]. Such a phenomenon may also help account for the lack of findings regarding aggressive behaviours among the women in Paper III, as lower levels of aggression would be expected, thus reflecting in a weaker effect when examining aggressive behaviours. Nevertheless, the findings among females outlined in this thesis contribute to our understanding of aggression among females, which has typically remained understudied, as well as highlighting how aggressive behaviours may interrelate with other commonly co-occurring behaviours such as substance misuse and other psychiatric outcomes.

Relevance of substance misuse.

Substance misuse is strongly associated with aggressive behaviour and promotes the maintenance of persistent aggressive behaviour over time[38], and has also been associated with altered genome-wide DNA methylation[232,233] and of candidate genes[172]. Another study by our group in the same sample of men reported in Paper III further showed that MAOA-S carriers who experienced maltreatment and displayed lower *MAOA* first intronic methylation levels,

which would be expected to confer transcriptional downregulation[121,123], reported higher alcohol consumption than men carrying MAOA-L who experienced maltreatment[172]. As such, the interaction of maltreatment, MAOA-S, and lower *MAOA* intronic methylation that promoted alcohol consumption in these men may have also indirectly contributed to the aggressive behaviours otherwise accounted for by substance misuse as reported in Paper III. Findings from Paper III suggest that both *MAOA* genotype and methylation are involved in moderating associations of maltreatment and aggressive behaviours among men. However, there may be two subgroups among these men; one where associations of maltreatment, *MAOA* genotype, and *MAOA* methylation on aggression may also be reliant to some extent on the presence substance misuse though less so on *MAOA* genotype and methylation, and a second group where the association of this interaction on aggression occurs independently of substance misuse. It is notable that no significant interactions of *MAOA* genotypes with maltreatment and positive family environment on alcohol consumption among boys were reported in Paper IV, perhaps attributable to little variability in predictor variables between the low- and high-drinking boys.

The findings outlined in this thesis offer an important contribution to understanding aggression among men by providing deeper insight into how maltreatment, genetic and epigenetic factors influencing *MAOA*, as well as other important risk factors including alcohol and substance misuse converge to increase risk for aggressive behaviours. Although a robust body of evidence supports the involvement of the aforementioned factors in aggressive behaviours among men, the studies presented in this thesis represent an initial exploration into how they work together, thus producing novel findings and the identification of potential subgroups of men and women where the dynamics of these factors may differ.

Relevance of emotional reactivity.

Further, the outcome-intersection phenomenon may be the result of several underlying factors interacting to confer a single intermediate phenotype, which in turn feeds into numerous facets of mental health and behavioural outcomes such as antisocial aggression and substance misuse[11]. One proposed factor that may help account for the outcome-intersection phenomenon in antisocial populations is emotional reactivity[11,79]. Emotional reactivity is characterized by a propensity towards the experience of negative emotions, highly emotional reactions, and extended latency needed to return to baseline following emotional arousal[79,234], and has been identified as an endophenotype that increases risk for several facets of antisocial behaviour among those who experienced childhood maltreatment[79]. While reactive aggression is characterized by high emotional reactivity, instrumental aggression is typified by low emotional reactivity and is further associated with callous-unemotional

traits[21,235]. As such, regardless of the valence associated with aggressive behaviour type, emotional reactivity may represent a common factor in both reactive and instrumental types of aggressive behaviour. Both types of aggression are governed, in part, by similar frontal and subcortical limbic circuitry that regulates adaptive cognitive and emotional stress responses to environmental factors[21,23,24,50]. Further, 5-HT plays a crucial role in the development and activity of this neurocircuitry[71]. Dysregulation of 5-HT during critical periods of development disrupts these functions and contributes to the establishment of long-term behavioural consequences including antisocial aggression and the comorbid behaviours and mental disorders common to CD and ASPD, including depression and substance misuse[50,51,73]. Further, substance use, particularly chronic alcohol misuse, also contribute to further impairment of this neurocircuitry and thus promote the maintenance of persistent aggressive behaviour[31,33,34].

Diversity of aggressive behaviours and related outcomes.

Early studies of 5-HT dysregulation in humans and animals primarily reported associations with impulsive reactive types of aggressive behaviours[50,81,83,236]. However, these studies were conducted almost exclusively in samples of males[50], and may have thus overlooked similar associations of 5-HT and *MAOA* dysregulation with instrumental aggressive behaviours more prevalent in females[21]. More recent work has reported gene-environment interactions of *MAOA* in association with a wide variety of aggressive and antisocial behaviours among both males and females, as supported by meta-analyses[78,80], as well as other recent studies[96–99,105,210]. The results outlined in this thesis are also consistent with these more recent findings. Importantly, extant evidence collected across several decades points to a role of *MAOA* in a wide array of behavioural phenotypes aside from aggression[11,78], perhaps owing to its role in degrading dopamine[82], as well as 5-HT interactions with sex- and stress-hormones[21]. As such, *MAOA*'s role in behavioural phenotypes should be expected to be multi-faceted and include an array of behaviours that often accompany aggressive behaviours as has been reflected by recent reviews[11,51] and in the results reported in this thesis.

While maltreatment-linked *MAOA* dysregulation may be associated with a common intermediate phenotype such as emotional reactivity that then feeds into different facets of behaviours, which facet of behaviour that features more prominently may change over time. One example of this may be the proposed transition among some females from adolescent CD to depression in adulthood eluded to in this thesis and reported by others[222]. One recent study indicated that females carrying MAOA-L who experienced maltreatment displayed greater increases in emotional reactivity during adolescence, and that these higher levels of emotional reactivity served as an intermediate phenotype that predicted antisocial personality disorder severity in adulthood[79]. Thus, it may be that emotional reactivity was a missing factor of consideration

that prohibited us from observing more direct associations with CD diagnosis in Paper II, as well as a common factor in the aggressive behaviours examined in Paper III that allowed us to detect associations more consistent with extant literature. The influence of an underlying endophenotype may also be evident in the initial results of Paper IV indicating differential susceptibility of the MAOA-L genotype among girls. Carriers of *MAOA* susceptibility genotypes and those who experience maltreatment have been shown to present with elevated emotional reactivity in response to stressors[11,92,101], and one study reported heightened emotional reactivity among *MAOA* susceptibility allele carriers who experienced childhood stress[105].

Further, positive parent-child relationship is associated with better efficacy of emotional regulation among adolescents in high-risk environments[237]. As such, the initial results among girls carrying MAOA-L presented in Paper IV could be interpreted as the influence of good relationships with parents counteracting the negative influence of maltreatment resulting in lower levels of alcohol consumption as a function of these factors acting on emotional reactivity. By contrast, a poor parent-child relationship may confer difficulty in emotional reactivity, thus promoting higher alcohol consumption as a potential coping mechanism against maltreatment which may confer further vulnerability of developing alcohol use disorder in adulthood. Again, the results reported among girls in Paper IV findings were confounded by delinquent behaviors and substance use, which may also be associated with interactions of maltreatment and *MAOA* on emotional reactivity as an intermediate phenotype involved in several facets of antisocial behaviour[79].

As such, emotional reactivity may be an important factor to consider in future research to counter ambiguous findings conferred by the outcome-intersection phenomenon in cross-sectional studies of *MAOA* and aggressive behaviours, and how it influences a multitude of other behaviours that often accompany aggressive behaviours and how they manifest over time, may help increase the accuracy and interpretability of findings. In absence of a direct measure of emotional reactivity, it may also be prudent to form composite variables that include all data on relevant behaviours available.

Relevance of the predictor-intersection phenomenon.

The predictor-intersection phenomenon may have also played a role in the studies presented in this thesis. This phenomenon refers to frequently co-occurring predictors that may cumulatively contribute to the prediction of a given outcome[11]. This phenomenon poses a challenge to isolating and assessing independent effects of any single predictor, as doing so may also indirectly index other frequently co-occurring predictors[11]. The most relevant example for the studies outlined in this thesis is that individuals who experience one form of maltreatment are very likely to have experienced other forms of maltreatment which cumulatively associate with outcomes such as aggressive behaviour and depression[8,226,238]. Sex-differences in the experience of

maltreatment may also contribute to this phenomenon. For instance, girls are more likely to experience sexual abuse than boys and the experience of sexual abuse typically occurs with other forms of abuse, whereas boys are more likely to experience physical abuse only although boys who experienced sexual abuse are very likely to have experienced poly-victimization[8].

Paper II in this thesis offers an attempt to assess the unique influence of experiences of sexual abuse only, physical abuse only, and sexual and/or physical abuse on *MAOA* ROI methylation. To this end, the results offered some specificity to women who had experienced sexual abuse only as a particularly strong finding, though a large portion of women in this study had experienced both types of abuse. As such, a composite maltreatment variable including both forms of abuse was used in Paper III as a means to address the pattern of poly-victimization reported by others[8] and observed in our own sample in Paper II. In study I, which was conducted after those outlined in Papers II and III, any instance of sexual abuse was queried with the understanding that it was relevant to *MAOA* ROI methylation and would also indirectly index instances of physical abuse which frequently co-occurred with sexual abuse among participants in our sample.

In addition, in Paper IV we observed a large overlap between the familial and non-familial maltreatment measured at the two time-points among the girls, indicating that the context in which maltreatment occurred in reported at wave 2 may have confounded the effect of the three-way interaction on the alcohol consumption assessed using the wave 2 data. To address the predictor-intersection phenomenon, we adjusted the significant three-way interactions for co-occurring and re-occurring maltreatments, as recommended[11,239]. We found that non-familial maltreatment had a stronger impact than familial maltreatment on alcohol consumption among girls carrying MAOA-L. This finding is consistent with previous work indicating that individuals who experience persistent maltreatment are also more likely to face maltreatment outside of the home by peers and non-familial figures later in adolescence[3,42,227]. Furthermore, the study outlined in Paper IV showed that a good parent-child relationship may be protective against the deleterious impact of maltreatment imposed by a person outside of the family including non-familial maltreatment, bullying in school, or other negative life events. However, this protective effect may be diminished if familial maltreatment is ongoing.

Together, Papers I-III in this thesis illustrate the importance of poly-victimization in relation to aggressive behaviours and other co-occurring outcomes. Perhaps more importantly, these findings suggest that *MAOA* may be responsive to maltreatment in general, rather than specific types of abuse, in association with several co-occurring outcomes; a notion supported by previous meta-analyses[78,80] and reviews[11,51] of *MAOA*. Further, findings reported in Paper IV indicate that *MAOA* may also be responsive to both negative and positive environmental factors in association with alcohol consumption, a be-

haviour that both co-occurs with, and promotes, aggression[31,33,34,38]. Taken together the findings presented in this thesis present *MAOA* as a genetic locus responsive to a wide array of environmental influences and outcomes relevant to aggressive behaviour.

Relevance to the Biology of Aggression

The underlying biological mechanisms that confer environmentally-driven epigenetic alterations to genes and gene activity are not yet fully understood[107,108,119]. However, a recent review found little evidence of direct pathways between maltreatment and altered methylation of candidate genes (not including *MAOA)* that modify behavioural outcomes[119]. Rather, it is hypothesized that such associations are contingent on factors such as genotypic susceptibility that facilitate epigenetic lability of genes, along with other positive and negative environmental factors that in turn further modify methylation altered by maltreatment [119]. This is consistent with work showing that *MAOA* genotypes contribute to the neurodevelopment and serotonergic regulation of corticolimbic circuitry governing mood and behaviour[72,73], and sensitivity to maltreatment may be conditional on subtle neurodevelopmental alterations conferred by activity of *MAOA* susceptibility genotypes early in life[73,94,95]. Studies have shown that although *MAOA* genotype correlates poorly with MAO enzymatic activity in adult brains[106,240], methylation of the *MAOA* ROI does[165]. A recent review further notes that GxE interactions of *MAOA* associate more strongly with antisocial behaviour when exposure to adversity was recent as compared to earlier exposures[11], which may point to a more prominent role of epigenetic processes acting on *MAOA* later in life.

Genotypic and epigenetic susceptibility and MAOA

Extant literature suggests that *MAOA* susceptibility genotypes may incur subtle neurological perturbations in early development that render individuals particularly sensitive to risk and resilience factors experienced throughout life, which in turn modulates risk for behavioural outcomes such as aggression[51,74]. These risk and resilience factors may impact on gene expression through epigenetic processes[51,74,107]. As such, methylation may be anticipated to show stronger associations on behavioural outcomes than susceptibility genotypes. In Paper III, among the men who experienced maltreatment, only carriers of MAOA-S who also displayed high levels of exonic methylation, both of which would be anticipated to confer reduced transcriptional regulation, showed associations with aggressive behaviours. Men who experienced maltreatment, but who did not carry MAOA-S and did not present high methylation levels, did not show associations with aggressive behaviours. Replication of these findings in longitudinal studies is needed to adequately infer causal associations of maltreatment and methylation. Further, in Paper II, *MAOA* genotype

and sexual abuse, but not their interaction, was associated with methylation of the *MAOA* first exon. Given that methylation levels were more strongly associated with sexual abuse than with genotype, it may be that environmental factors experienced recently may have a greater impact on methylation of *MAOA* than genotype. This notion is also in line with recent evidence in adults showing that psychiatric treatment was associated with subsequent normalization of *MAOA* ROI methylation levels and symptom alleviation, which also occurred independently of *MAOA* genotype[137]. However, as Paper II was focused on identifying associations of abuse type on *MAOA* methylation, all participants who experienced physical abuse only, and those who experienced both types of abuse, were excluded from most analyses. As such, detection of an interaction of *MAOA* genotype and maltreatment in association with *MAOA* methylation levels may have been hindered by low statistical power resulting from the removal of these participants. Subsequent studies using larger sample sizes is needed to clarify these findings. Nevertheless, the findings reported in Papers II and III offer some support to the notion that the impact of maltreatment on *MAOA* methylation may further contribute to early perturbations conferred by genotypes by exacerbating *MAOA* transcriptional dysregulations, in turn, promoting aggressive behaviours among these susceptible individuals.

The results of the study outlined in Paper IV indicated that, among some adolescent girls, MAOA-L conferred susceptibility to both negative and positive environmental factors. Although methylation of the *MAOA* ROI was not assessed in this study, it may represent a putative mechanism contributing to the differential-susceptibility observed. Although follow-up studies assessing the role of *MAOA* methylation is needed, recent studies indicating responsiveness of *MAOA* methylation levels to psychiatric treatments[137,174] are promising that such an association may exist. However, it is vital to note that the differential-susceptibility effect initially observed in Paper IV was nullified when delinquent behaviours and substance/nicotine use were adjusted for. This finding may reflect the outcome-intersection phenomena and involve a common unmeasured intermediate phenotype, such as emotional reactivity[79]. Although no associations of epigenetic processes, emotional reactivity, and *MAOA* have yet been reported, genetic variations of genes that govern *de novo* methylation processes, such as DNMT3A, have shown an association with emotional reactivity to events occurring in daily life[241]. One review also noted a large range of convergent genetic and epigenetic factors that contribute to the regulation of positive emotions[242]. Findings of Paper IV, in conjunction with work illustrating associations of epigenetic processes with emotional regulation and positive emotions and the identification of emotional reactivity as an intermediate phenotype involved in GxE interactions of *MAOA*, support further examination of epigenetic processes as part of the differential-susceptibility framework in regards to *MAOA*.

Recent studies have shown that psychiatric treatment is associated with the return of *MAOA* ROI methylation to control levels accompanied by attenuation of anxiety symptoms among some adults[137,174], illustrating that positive environmental influences may also confer acute alterations to *MAOA* methylation in adulthood. As such, similar to genotypic factors, epigenetic factors impacting *MAOA* regulation may be also be consistent with the differential-susceptibility theoretical framework. Further, the contribution of epigenetics in differential susceptibility of *MAOA* could also help account for the absence of *in vivo* associations between transcriptional activity and MAOA-alleles[106] that has otherwise been reported *in vitro*[89]. Further, as noted in recent reviews, the contribution of genotype alone does not offer a mechanistic explanation of how *MAOA* dysregulation contributes to normative and abnormal developmental fluctuations in aggression[11,51]. As such, genotypic and epigenetic moderation of *MAOA* in associations of maltreatment and behavioural outcomes such as aggression should be considered together. The results of the studies included in this thesis offer additional support to this line of reasoning.

In Paper I, we found very few direct associations of age, or interactions of age and sex, on *MAOA* ROI methylation in an age-range spanning around three decades. These findings contrast with well-documented patterns of age-related reductions of genome-wide methylation[151]. However, some regions of the genome, particularly first exonic-intronic junctions similar to the *MAOA* ROI, may be exempt age-driven reductions in methylation[151–153]. These junction regions are suggested to be important loci that remain open to the influence of environmental influences on methylation levels across the lifespan and are implicated in a wide array of biomedical and behavioural outcomes[151–153]. Further, such exonic-intronic junctions are shown to be loci in which 5hmC is enriched[151], further indicating that methylation levels in such regions may be highly dynamic[122,151]. With these studies in mind, along with recent evidence showing that methylation levels of the *MAOA* ROI are malleable in response to psychiatric treatment in adults, the results presented in Paper I help establish the *MAOA* ROI as a particularly important locus to examine epigenetic processes.

Alcohol and MAOA

Chronic alcohol use plays a role in the maintenance of persistent antisocial and aggressive behaviours from adolescence into adulthood[17]. In some females, it also plays a role in driving the transition from adolescent CD to adult depression[222]. Such associations have been suggested to manifest, in men and women, due to the progressively deleterious impact of chronic alcohol use on corticolimbic circuitry that governs aggression and mood[17,26]. Chronic alcohol use also impacts reward centers in the brain that promote addiction and in the intrinsically rewarding aspects of aggressive behaviours[34]. The studies included in this thesis further highlight the importance of considering alcohol and substance misuse in relation to aggressive behaviour in investigations of

MAOA. Among women in Papers I and II, associations of sexual abuse and elevated exonic methylation, and the mediatory role of this hypermethylation on the association of maltreatment and current depression, were robust to adjustments for substance misuse. Although no significant interactions of maltreatment, genotype, and methylation were observed among women in Paper III, results in men indicated that such an interaction occurred independently of the influence of substance misuse. However, in other men in this study, the interaction on aggressive behaviours was better accounted for when substance misuse was adjusted for. In Paper IV, results showed an interaction of negative and positive environmental factors with MAOA-L in girls that was not robust to adjustments for other substance use and some delinquent behaviours. Considered together, these findings show a varied picture of how alcohol use associates with aggressive behaviour. This association appeared to be variably dependent on genotypic and epigenetic factors of *MAOA* and may differ by sex. However, such heterogeneity in the findings should be anticipated given that the presentation and etiology of antisocial behaviours, diagnoses of CD and ASPD, as well as other mental disorders, such as depression, are likewise highly heterogeneous[17,223].

Other biological considerations

It must also be emphasized that antisocial aggression involves many genes governing several neurotransmitter systems[50], that also interact with one another in an epistatic manner[243]. Heterogeneity observed in the brain circuitry involved in different types of aggression[17,21,24] would likewise be anticipated to also be reflected in diverse genetic and epigenetic profiles[11,51,114]. Multiple layers of epigenetic processes often work in conjunction[109,114–116] and with genetic polymorphisms[162] to modulate gene expression. While decades of research establishes an association between low serotonin and reactive aggression[50,51], the work on *MAOA* outlined in this thesis and reported by others[11,51,78–80] highlight the gene's role in numerous behavioural outcomes perhaps driven by a common unmeasured intermediate behavioural phenotype such as emotional reactivty[79]. As such, the studies outlined in this thesis should be interpreted as eluding to a more complex phenomenon. The work outlined in this thesis also helps provide a framework that may be used to study other candidate genes to expand our understanding of the biology of aggression.

Relevance to Sex-Differences in the Biology of Aggressive Behaviour

The presentation of aggressive behaviour is sexually dimorphic, with skews towards reactive aggression among males and instrumental aggression among

females, though both types of aggression are often exhibited by antisocial individuals[21]. Sex-differences in aggressive behaviours has been suggested to result from convergent evolutionary and environmental factors, the influence of stress- and sex-hormones on neurotransmitter systems and genotypic variations in candidate genes implicated in aggression[21]. The studies outline in this thesis offer further insight into sex-differences in the biology of aggression.

X-chromosome inactivation.

In Paper I, comparisons of women and men indicated differences in methylation of the *MAOA* ROI with women showing exonic methylation levels than men, indicative of X-inactivation[146]. These levels of *MAOA* first exon methylation among women are consistent with findings reported in Papers II and III, as well as studies by others[169,171]. Throughout Paper I, this sex difference in methylation remained consistent across analyses taking account of genotype, sexual abuse, and the interaction of genotype and sexual abuse. Notably, no differences in any measure of methylation were detected in comparisons of younger and older participants. Thus, results confirm sex differences in methylation of the *MAOA* ROI, which were generally not modified by age, save for two direct effects of age among women only at CpG13 and component 2 and among men at CpG15. These findings within the *MAOA* ROI are consistent with prior evidence of sex differences in methylation across the genome and extend knowledge by showing that within the *MAOA* ROI women present higher methylation levels than men for around three decades following puberty.

X-chromosome inactivation of *MAOA* is strongly suggested in the results outlined in Papers I-III, and has been posited as a factor contributing to sex-differences in aggressive behaviours[21] although how this association may occur is not currently understood. Notably, other factors could influence sex-differences in *MAOA* methylation on the active X-chromosome in women. Some evidence suggests that *MAOA* may be a genomic locus where sex-hormones influence serotonergic activity and behaviours that feature prominently in the sample used in Papers I-III, particularly antisocial behaviour and substance misuse[11]. For instance, one study found that the interaction of *MAOA* genotype and testosterone concentration in cerebrospinal fluid predicted antisocial behaviour and suggested that this interaction may have been mediated by direct effects on *MAOA* transcription[244]. Another study in healthy males showed that those carrying MAOA-S displayed increased risk-taking behaviours following administration of a topical gel containing testosterone[245]. Further, *MAOA* expression may be modified by interactions between testosterone levels and transcription factor Sp1 at its binding sites throughout the *MAOA* promoter region[178]. Given that DNA methylation can modify gene expression by disrupting transcription factor binding[111], the impact of sex-hormones on *MAOA* expression may be influenced by epigenetic processes. Although po-

tential influences of female sex-hormones on *MAOA* expression and serotonergic activity in such phenotypes remains unclear[11,21,51], investigations of such associations will be important to further elucidate sex-differences in *MAOA* regulation.

Sex-differences in MAOA-uVNTR susceptibility genotypes.

Extant evidence indicates that MAOA-S in males and MAOA-L in females, which confer lower and higher transcription, respectively, interact with childhood maltreatment to modify the risk of aggressive and antisocial behaviour in adolescence and adulthood[11,78,96–99,105,210], though some studies have reported contradicting results[78,90]. Neuroimaging studies have offered further evidence supporting the sex-difference in *MAOA* susceptibility genotypes in association with the corticolimbic circuitry of aggression[92,105]. The results reported in Papers III and IV offer some support to the findings these previous GxE studies.

In Paper III, our results indicated that component methylation within the *MAOA* ROI aligned closely with the first exonic and intronic regions of the *MAOA* gene. Among men, components 2 and 3 covered exonic CpGs 2-6 and 7-12 respectively. Among maltreated men carrying MAOA-S, those with higher exonic methylation levels, showed higher aggressive behaviours, that were not affected by covariates. The exonic hypermethylation observed among the men in this study would be anticipated to result in diminished gene transcription[121,123], concordant with the low-expressing MAOA-S allele. Although functional studies of methylation in the *MAOA* ROI regions implicated in this are necessary to verify our interpretation, these findings are consistent with previous GxE literature[78,79,92,96–99,105,210] and introduce DNA methylation as an additional factor that may further contribute to *MAOA* genotypic dysregulation to promote aggressive behaviours in men. Notably, models examining similar interactions among women revealed no significant results. The weaker models observed in women in Paper III may be attributable, to some extent, to less salience of aggressive behaviour in favour of elevated depression among these women, many of whom previously presented CD in adolescence. However, the study outlined in Paper IV does offer some support to the role of MAOA-L as a susceptibility genotype in females. Among the adolescent girls in the study, findings suggest that positive-parenting helped to counteract the impact of maltreatment leading to reduced alcohol consumption among MAOA-L carriers. This interaction was no longer significant following adjustments for some delinquent behaviours, and tobacco and substance misuse, a finding that highlights the co-occurrence of such behaviours among these adolescent girls.

Further, methylation was not examined as part of study IV. As such, inclusion of *MAOA* ROI methylation data in subsequent follow-up studies in this sample may help further elucidate sex-differences in *MAOA*'s role in antisocial and substance misuse behaviours. The findings outlined in Paper IV were

also sex-specific with no significant association of the three-way interactions observed among the boys, perhaps attributable to little variation across the main predictors, and sampling bias towards healthier participants than observed in prior studies[39,179]. The sex-difference observed in this study may also involve non-biological factors. For instance, sex-differences in the perception of stress and coping strategies, such that females often perceive daily and chronic stressors more stressful than males and prefer to seek emotional support as the coping mechanism[246–248] or sex-dependent influence of quality of parent-child relationship on adolescent's alcohol consumption such that females experience a stronger response than males[246].

While there is robust evidence associating 5-HT dysregulation with aggressive behaviours[21,51], a direct role of 5-HT in sex differences observed in aggressive behaviours has not been well supported[21,175]. However, it has been posited that concentrations of sex- and stress-hormones may regulate the associations between 5-HT activity and aggressive behaviours[21] by affecting sexually-dimorphic brain structures in the corticolimbic neural circuitry of aggression[249]. While this notion has yet to be fully supported in humans, emergent evidence suggests that *MAOA* may be a genomic locus that sex-hormones interface with to influence 5-HT associations with aggressive behaviours[178,244,245].

While heritability for antisocial behaviours is similar in males and females[46] the prevalence of CD is twice as high in males than in females[2]. The biological and environmental factors that account for this discrepancy are not currently well-understood[21]. While sex-differences in aggressive behaviours were not directly examined in Papers III and IV, the reported sex-differences in the interactions of maltreatment, *MAOA* genotypes, and *MAOA* ROI methylation levels that promoted aggressive behaviours offers an initial step towards this understanding, and a basis for future work along this line of inquiry. Consistent with the differential-susceptibility model, it will be particularly important to identify and examine the biological and environmental factors that promote antisocial behaviour in males but attenuate it in females.

Future Directions

While this thesis examines the impact of *MAOA* genotype and DNA methylation of the ROI on associations of maltreatment with aggressive behaviours and mental disorders, it is important to note that extant evidence shows that *MAOA* falls under the regulatory influence of numerous additional genetic and epigenetic factors. Gene polymorphism studies of aggressive behaviour have identified several candidate genes in the serotonergic, dopaminergic, and noradrenergic pathways[76] that share an epistatic relationship contributing to some of the additive genetic factors among antisocial individuals[243]. One recent

study further showed that polygenic interactions of positive and negative environmental factors with MAOA-uVNTR genotypes and other candidate gene polymorphisms modified the risk for antisocial behaviours in males and females[179]. As such, the MAOA-uVNTR is likely one component within a larger network of susceptibility genotypes in aggressive behaviours, which is reflected in the relatively modest individual contribution of *MAOA* GxE interactions to the overall variance in aggressive behaviours as noted in meta-analyses and reviews[11,78,80]. In addition to polygenic interactions, other *MAOA* polymorphisms have been implicated. One study found that physical and sexual abuse interacted with a set of *MAOA* single-nucleotide polymorphisms to predict increased violent and antisocial outcome severity, further noting that this interaction occurred relative to a high severity of maltreatment severity[202]. Another study showed that males carrying the "low-low" expressing combination of the MAOA-uVNTR and another functional MAOA-dVNTR polymorphism located in the gene-body, was associated with increased risk for nicotine dependence[250]. One other recent study showed that a better quality of parent-child relationship interacted with the MAOA-L genotype to predict improved effortful-control among adolescent boys, but not girls[182]. These findings point to several polymorphic factors regulating *MAOA* in association with, co-occurring, or potentially mitigating aggressive behaviours.

Multiple layers of epigenetic regulation may influence *MAOA* expression. The gene has been shown to fall under epigenetic influence by histone modifications at the transcriptional level[251] and MicroRNAs at the post-transcriptional level[252]. As such, regulation of *MAOA* expression may be subject to multiple layers of potentially interacting epigenetic processes.

Recent evidence supports multiple levels of epigenetic regulation of *MAOA*. A novel long noncoding RNA (lncRNA), known as MAOA-associated lncRNA (MAALIN), was identified in the *MAOA*/*MAOB* intergenic region in brain tissue[115]. Expression of *MAOA* is reduced by MAALIN in the brains of suicide completers and promotes impulsive-aggressive behaviours in mice[115]. Further, MAALIN was found to be regulated by both DNA methylation and chromatin modifications[115]. Another study found that elevated stress among male rats exposed to peripubertal stress, versus non-stressed rats, was accompanied by increased prefrontal *maoa* expression during the resident-intruder task and at basal[251]. This elevated prefrontal *maoa* expression was associated with chromatin modifications in *maoa* promoter region and pharmacological treatment with a monoamine oxidase inhibitor showed contrasting group effects such that non-stressed rats displayed increased aggression following treatment but stressed rats showed reduced aggression following the treatment[251]. No changes in aggression were observed when the stressing procedure was performed in another cohort of adult rats, suggesting the effects observed were limited to adolescence[251]. Taken together with the findings presented in this thesis, it is evident that the association of *MAOA* with

aggressive behaviour may be governed by several layers of genetic and epigenetic regulation that may act during different points in development and also vary by sex. As such, examination of these factors is vital in future studies of *MAOA* and aggression.

Sex-differences in the role of *MAOA* in aggressive behaviour present an important factor to examine in future studies. Together, Papers III and IV outlined in this thesis offer further support to previous studies indicating that MAOA-S in males and MAOA-L in females interact with environmental factors to predict behavioural outcomes[11,78]. However, while a concordant interaction of genotype and methylation, both suggestive of downregulated gene-expression, was supported among men in Paper III, the final models in females did not reach significance. The low strength of this final model in women may have resulted from the small sample size and potential prominence of depression rather than aggression of the young adult women as noted in Paper I. Although the model was not significant and thus excluded from the Paper, the interactions identified in subsequent conditional effects were significant such that maltreated women carrying MAOA-L who displayed low levels of exonic CpG methylation exhibited greater numbers of aggressive behaviours, a finding which was robust to adjustments for all covariates (data not shown). Low exonic methylation would be expected to confer increased gene expression[121,123], similar to the high-expressing MAOA-L allele implicated. While this finding must not be interpreted as supporting the hypothesis regarding women, further examination of such associations in sufficiently powered samples is warranted. Given the salience of current depression diagnoses among the women in this sample who had prior diagnoses of CD as noted in Paper I, a follow-up examination of interactions of maltreatment, *MAOA* ROI methylation, and *MAOA* genotype with depression symptoms may also be informative.

The Coronavirus-19 (COVID-19) pandemic represents a period of global crisis with far-reaching public health and socioeconomic consequences. While the biological mechanisms which determine the severity of infection outcomes among individuals, and long-term consequences of infection, is not currently well-understood[253,254], preliminary work has underscored the ubiquitous impact of the pandemic on daily life among many individuals[255]. Alterations in methylation levels among associated with large-scale crises have been documented. Two studies found that adolescent offspring of women who were pregnant during the 1998 Quebec ice storm, which trapped people in their homes for up to 3 weeks, presented with wide-spread hypermethylation across the genome[256,257]. The degree of hypermethylation observed was associated with the severity of objective hardship and cognitive appraisal of distress experienced, rather than subjective distress, and was enriched in genes related to immune response and HPA-axis function[256,257]. These findings highlight the importance of assessing the impact of large-scale crises, such as the COVID-

19 pandemic, on epigenetic signatures particularly in studies examining environmental adversity.

Recent studies have also indicated that, throughout aging, there is a global pattern of hypomethylation across the genome whereby the cumulative inter-individual differences in methylation gained from environmental experiences harmonize with gene regulation gradually adopted and maintained by other epigenetic processes[151,152]. Importantly, despite the pattern of genome-wide hypomethylation, age-related fluctuations in DNA methylation may remain more variable and open to environmental influences within regions that span first exonic/intronic boundaries[151], such as the *MAOA* ROI. Disturbances of normative epigenetic aging trajectories and processes have been implicated in risk for both mental and biomedical illnesses[151,155] and may be influenced by environmental factors[151], and differ by sex[157].

For instance, one study showed that low socioeconomic status (SES) children who were also exposed to protective environmental factors showed greater self-control which predicted less aggressive behaviour, depressive symptoms, rates of substance use, and externalizing problems along with increased academic success and better psychosocial adjustment in young adulthood relative to other low-SES children who were not exposed to protective factors[155]. Although the low-SES children exposed to protective factors showed greater resilience to these negative outcomes in early adulthood than their peers without exposure to protective factors, both low-SES groups presented with premature epigenetic aging trajectories, and no differences in poor cardiometabolic health indicators that predict biomedical illness later in life[155]. The authors of this study suggest a potential "skin deep" resilience, such that observed protective associations with outcomes that onset in late-adolescence and early adulthood mask an increased risk for other negative outcomes that may emerge over more protracted periods of time[155]. Another study in adult men and women found that high levels of aggression were associated with chronic arterial hypertension relative to those with chronic musculoskeletal disorders, with more pronounced effects observed among the men[258], highlighting the important associations between mental and biomedical health indicators.

While the results of Paper I report relative stability of *MAOA* ROI methylation levels in younger and older participants, this finding may support the importance of the *MAOA* ROI as an important locus where methylation remains malleable to environmental influences across time, given its location in a exonic/intronic junction[151,152]. Longitudinal studies and cross-sectional studies including individuals representing a wider range of ages may help elucidate the impact of aging on *MAOA* ROI methylation, as well as determine if it indeed remains labile to environmental alterations over time. Recent examinations of *MAOA* ROI methylation and treatment response in adult women[137,174] supports this notion. Although the role of epigenetic aging on

MAOA regulation in persistently elevated aggressive and antisocial behaviours, and other biomedical sequelae that may emerge over time, has not yet been examined, it may be a promising candidate gene for such future studies.

The outcome-intersection and predictor intersection phenomenon may have influenced the findings in the studies outlined in this thesis. A potential underlying factor contributing to this may be emotional reactivity, as suggested in a recent gene-environment study of *MAOA* and antisocial outcomes[79]. Subsequent work should explore the role emotional reactivity in analyses. However, if that measure is not available, a composite variable including co-occurring outcomes and predictors may also help yield clearer results. In addition, the use of composite variables may also help reduce the number of variables needed in analyses which would thus benefit studies that have smaller sample sizes.

CONCLUSIONS

- Women display higher *MAOA* ROI methylation levels than men, likely reflecting X-chromosome inactivation.
- Physical and sexual abuse associates with hypermethylation of the first exonic region of *MAOA* in women
- Hypermethylation of the *MAOA* first exon mediates associations between sexual abuse and current depression in women.
- Hypermethylation of the *MAOA* ROI in men exacerbates the impact of GxE interactions between MAOA-uVNTR genotypes and maltreatment to promote aggressive behaviours.
- The MAOA-uVNTR presents differential-susceptibility among female MAOA-L carriers, though examination of the outcome-intersection phenomenon in substance misuse and delinquent behaviours is needed.
- The findings presented here advance our understanding of how maltreatment interfaces with genotypic and epigenetic factors, in a sex-dependent manner, to promote aggressive behaviour and mental disorders among susceptible individuals.

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