The prevalence of the *MAOA* μVNTR alleles and their relationship to childhood behaviour and personality within a South African cohort

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Abstract

Historically, the X-linked *monoamine oxidase A* promoter variable number tandem repeat (*MAOA* μVNTR) low-activity (versus high-activity) alleles were thought to be associated with an increase in internalising and externalising behaviour problems in males. However, recent research has highlighted the importance of gene-environment (GxE) interactions as a modifier of this effect. Individuals with a low-activity allele, who were raised in a poor environment, show higher levels of behavioural problems, however, when these same individuals are in a supportive environment, they experience the lowest levels of behavioural problems compared to high-activity allele individuals. These crossover effects have been recently incorporated into a theory known as Environmental Sensitivity. This study aimed to genotype the *MAOA* μVNTR by PCR and gel electrophoresis within South African males (n = 543) and females (n = 593) of the Birth to Twenty Plus cohort. Genotypes were then correlated to historical cohort data on caregiver-rated childhood behaviour from the South African Child Assessment Schedule. When accounting for covariates (5-HTTLPR, gestational age, birth weight, maternal education, and household socio-economic status), linear regression analysis revealed that possessing a low-activity *MAOA* μVNTR allele was a predictor of internalising behaviour in males ($p < 0.05$) and females ($p < 0.05$) and externalising behaviour ($p < 0.05$) in males, but not sensory processing sensitivity (a psychological marker of environmental sensitivity) in either males ($p > 0.1$) or females ($p > 0.1$). Additionally, when maternal education was applied as an environmental modifier, a significant interaction ($p < 0.01$) revealed that males with a low-activity *MAOA* allele have externalising behavioural scores in direct proportion to maternal education levels. Moreover, males without a high-activity allele showed no change in externalising behaviour as a function of maternal education. When household socio-economic status was used as the environmental modifier in females, a significant interaction ($p < 0.01$) revealed a contrasting effect where low-activity allele carriers experienced an increase in internalising behaviour problems with an improved environment. However, high-activity allele carriers experience the opposite. These findings support the role of Environmental Sensitivity

theory through GxE interaction models in predicting behavioural development.

Declaration

I, Stephan Herman Wessels, declare that this Dissertation is my own, unaided work. It is being submitted for the Degree of Master of Science in Medicine at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other university.

Stephan Herman WESSELS

21 day of August 20 20 in Randburg

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bp	base pairs
BTT+	Birth To Twenty Plus
CNS	central nervous system
DsM	diathesis-stress model
DST	differential susceptibility theory
GxE	gene x environment
rGE	gene-environment correlations
HSPS	Highly Sensitive Person Scale
MAOA	*monoamine oxidase A*
MAOB	*monoamine oxidase B*
Maoa	*monoamine oxidase a*
MAOA μVNTR	*monoamine oxidase A* promoter variable number tandem repeat
MAR	missing at random
MNAR	missing not at random
NGS	next generation sequencing
PA	proportion affected
PCR	polymerase chain reaction
POI	proportion of interaction
QC	quality control
ROS	region of significance
SES	socio-economic status
SD	standard deviation

SPS	Sensory Processing Sensitivity
TBE	Tris-Borate-Ethylenediaminetetraacetic acid
VNTR	variable number tandem repeat
5-HT	serotonin
5-HTTLPR	serotonin transporter linked polymorphic region

1. Introduction

This chapter will introduce the reader to research aimed at understanding the complex factors leading to behavioural development. A particular focus will be to highlight the effects of childhood environment, the *MAOA* μVNTR, and how their interactions contribute to differences in individual behaviour, specifically internalising and externalising behaviour. Additionally, theories will be explored which aim to understand the relationship between genes and the environment. To conclude, the reader will be presented with the study rationale and the aims and objectives of the research to follow.

1.1 Human behaviour

Human behaviour encompasses a large set of observable behaviours and traits which can range from normal to pathological. Internalising and externalising behaviours stem from dysregulation of emotion, either over- (internalising) or under-regulation (externalising) problems (Eisenberg & Morris, 2002, Marsee & Frick, 2007). Internalising behaviours such as anxiety and depression represent problems which affect an individual's internal environment (Liu, Chen & Lewis, 2011). In contrast, externalising behaviours are those directed towards the external environment and include aggression and antisocial behaviour, *inter alia* (Liu, 2004, Merrell, 2013).

Human internalising and externalising behaviours, although a burden to society when poorly regulated, serve an evolutionary advantageous role by directing threat response to changing environments (Craig & Halton, 2009). Gasper & Clore, 2002 suggested that depression (an internalising behaviour) can be an advantageous trait leading to an increase in cognitive analysis and learning. This theory has been further supported through various experiments. Andrews & Thomson Jr (2009) evaluated the effect of depressive thoughts on complex problem-solving where they showed that students performed better on complex problems

when in a depressive state (Andrews & Thomson Jr, 2009). Additionally, regarding externalising behaviour, research suggests this behaviour is evolutionary advantageous through two systems, either interpersonal behaviour, such as reproductive success, or as a stress response mechanism (Hengartner, 2017). Externalising behaviours such as aggression has long served as a societal organiser, arranging individuals in a group with some having more reproductive success than others. Furthermore, these same externalising behaviours act as a threat response mechanism against social, external, and physical harm (Hengartner, 2017). Although these behaviours can be destructive, such as violence (externalising) or depression (internalising), they are ultimately advantageous when balanced (Gasper & Clore, 2002, Andrews & Thomson Jr, 2009).

However, these behaviours (internalising and externalising behaviours) still carry a large burden on both the South African health sector and for society as a whole if unregulated. A recent study by Bantjes, Kagee & Meissner (2017) showed that depression (internalising behaviour) accounted for $\pm$ 10% of all suicide-related deaths within South Africa. Additionally, a study by Khuzwayo, Taylor & Connolly (2018) across 16 schools in KwaZulu-Natal, South Africa showed that 12.6 % of grade ten learners had planned a suicide attempt, while 14.8 % had attempted suicide within the last 12 months. Similarly, violent behaviour (externalising behaviour) directed towards others carries an equally high burden in South Africa, where 15 000 murders and 273 000 cases of assault were reported to police within the 2015-2016 period. However, it has been suggested that these figures only account for 43% of the assaults within South Africa (Statistics South Africa, 2016). Elucidating the cause of these behavioural aberrations and the variation observed between individuals my thus help to mitigate the burden of such behaviours. Although behavioural development is driven by many factors, the field of behavioural genetics aims to understand the influence of genes on behavioural development (McGue, 2008). While historical research has divided the search for cause to either genetics or environmental factors, more recent research acknowledges the fact that behaviour is multifactorial and more so, a complex interplay between these factors (McGue, 2008).

1.2 Factors implicated in the development of behaviour

Human behaviour is complex, and so too are the factors accounting for variability in its expression (Rose, 1995). Although research has shown that many factors contribute to the development of behaviour, a large emphasis has been placed on the effects of environment and genetics (Caspi *et al.*, 2002, McGue, 2008).

1.2.1 Environment as a driver of behaviour

The environment is an important factor to consider when investigating individual differences in behaviour. Environmental effects on behaviour are viewed as either supportive (positive) or depriving (negative) (Turkheimer, 2000). While negative environments can lead to behavioural problems, positive environments can be protective, leading to improved behavioural outcomes (Walker *et al.*, 2011). Although, environmental influences affect behaviour through several mechanisms, childhood environment (environment during important developmental periods) is one of the largest predictors of behavioural development throughout life (Miles & Carey, 1997, Turkheimer, 2000). Within childhood environment, familial environment (shared components within families), and non-shared environment (non-familial or genetic effects such as the immediate environment) may also affect behavioural outcomes and behavioural development (Turkheimer, 2000). Firstly, a shared familial environment with a parental figure suffering from depression or anxiety is a known risk factor for developing behavioural problems, such as depression, *inter alia* (Biederman *et al.*, 2001, Tully, Iacono & McGue, 2008). The effect of shared environments has been extensively investigated as a risk factor for behavioural problems. However, the non-shared environment is a broad category which is difficult to quantify and includes non-shared familial environments, genes, and immediate stimuli (Anderson & Bushman, 2002, Marsee & Frick, 2007). While childhood environment is a significant risk factor for the immediate behavioural outcome, it also shapes developmental trajectories and therefore influences future behaviour (Teymoori *et al.*, 2018). Childhood risk factors, such as abuse, economic state, and parental education have been shown to lead to the development of both internalising (depression, and anxiety) and externalising (aggression) behavioural problems (Toth, Manly & Cicchetti, 1992, Lansford *et al.*, 2002).

Socio-economic status SES and parental education are among the most studied childhood environmental factors to influence behaviour (Tremblay *et al.*, 2004, Côté *et al.*, 2006). Research on the effects of SES has shown that individuals with lower SES experience a greater number of both internalising and externalising behavioural problems than individuals with higher SES levels (Tremblay, 1999). Additionally, there is evidence implicating social environment, particularly low parental education, as a potential risk factor for behavioural problems (Carneiro, Meghir & Parey, 2013, Harding, 2015). Consequently, the effects of low parental education are amplified in children living in poverty. Research by Singh & Ghandour (2012) demonstrated that children who grow up in poverty have a 3.7 fold increase in risk for behavioural problems. Moreover, children whose parents have an education level lower than high school experience a 1.9 fold increase in behavioural problems (Singh & Ghandour, 2012). Furthermore, a recent study by Hosokawa & Katsura (2018) investigated the effects of various socio-economic factors (household SES, paternal, and maternal education) on childhood behavioural problems (internalising and externalising behaviour). They were able to show that all measurements of SES were significantly associated with both internalising and externalising behaviours so that lower levels of SES lead to an increase in poor behaviour (Hosokawa & Katsura, 2018).

While the effect of the environment has been extensively investigated, twin studies have shown that behaviour has a strong heritable component (Haworth, Dale & Plomin, 2008). One possible mechanism by which genes influence behaviour is through neurobiological susceptibility (Hariri, 2009, Pluess, 2015). Neurobiological sensitivity is the sensitivity of the central nervous system (CNS) to environmental stimuli (Hariri, 2009).

1.2.2 The mechanism through which genes influence behaviour

Neurobiological sensitivity is the physiological trait which describes the variability in CNS responses to environmental stimuli, including socio-economic factors. Variability in CNS functioning may serve as a sensitivity factor for many neuropsychiatric conditions and aberrant behaviours (Hariri, 2009). A large body of evidence has linked areas of the brain, particularly the amygdala, in regulating both physiological and behavioural response to environmental

stimuli (LeDoux, 2000). Huey *et al.* (2015) investigated whether damage to specific brain regions would affect an individual's behaviour. They did this using self-report questionnaires and brain scans of individuals with penetrating brain injuries. From their research, they were able to show that damage to certain brain regions is associated with changes in either internalising or externalising behaviour. For instance, damage to the left amygdala and bilateral basal ganglia was associated with lower internalising problems, while damage to the bilateral hippocampal regions decreased externalising problems and the left medial orbitofrontal cortex increased externalising problems (Huey *et al.*, 2015). Therefore, these findings support the claim that variability in brain structure and functioning can contribute to changes in behaviour.

One biological mechanism which accounts for biological sensitivity within the CNS, particularly the serotonergic system, is neurotransmission (Hariri, 2009, aan het Rot, Mathew & Charney, 2009, Chen *et al.*, 2011, Acevedo *et al.*, 2014). Neurotransmitters, such as serotonin, function in the electrochemical signalling pathways within the CNS and are crucial for normal brain functioning and development (Sheffler & Pillarisetty, 2019). While several neurotransmitters and genes have been implicated in behavioural regulation, genes within the serotonergic pathway have been the focus of studies on behaviour (Levinson, 2006, Belsky *et al.*, 2009, Booij *et al.*, 2015). Although these neurotransmitters and genes are responsible for normal functioning, variation within these genes are believed to account for inter-individual differences in behaviour (Hariri & Weinberger, 2003, Belsky *et al.*, 2009).

Historically, behavioural research has only focused on the direct effect of genes on behaviour, particularly neurotransmitter gene variants, with varying results (Anguelova, Benkelfat & Turecki, 2003). As an example, Mann *et al.* (2000) investigated the relationship between the serotonin neurotransmitter and the serotonin transporter linked polymorphic region (5-HTTLPR; consisting of two primary alleles, a short and long allele, where the short allele is associated with reduced functioning) on major depressive disorder and suicide. They found lower binding of the serotonin neurotransmitter in brain biopsies of suicide victims with a short allele (Mann *et al.*, 2000). Additionally, when they tested for an association between the 5-HTTLPR alleles and major depressive disorder and suicide, they observed a significant

association between the 5-HTTLPR short allele and major depressive disorder, but not for suicide (Mann *et al.*, 2000). The serotonin 5-HTTLPR is explained in greater detail in Section 1.3.4.

1.2.3 Interactions between genes and environment

Many studies on behaviour have viewed its cause as either the distinct influence of genes or the environment (Hunter, 2005). However, the advancement of behavioural sciences has illustrated that neither genes nor environment act in isolation, but rather as a contextualised interaction of these factors (Turkheimer, 2000, Taylor & Kim-Cohen, 2007, Uher, 2008). This contemporary perspective has caused a paradigm shift from a single-factor to multifactorial view on behaviour and its causes (Taylor & Kim-Cohen, 2007, Uher, 2008). Caspi *et al.* (2002) performed one of the first gene x environment (GxE) interaction analyses in behaviour sciences and demonstrated how genetic influences on behaviour are contextualised by the environment (for a full breakdown of their results see Section 1.3.5) (Caspi *et al.*, 2002). The gene polymorphism they investigated was the *MAOA* μVNTR.

1.3 The *Monoamine Oxidase A* gene

The *monoamine oxidase A* (*MAOA*) gene encodes the mitochondrial catabolic enzyme, which is responsible for the degradation of biological amines serotonin (5-HT), dopamine, and norepinephrine, through oxidative deamination (Fowler, Mantle & Tipton, 1982, O'Carroll *et al.*, 1983). Located on the X chromosome between positions p11.23–11.4, *MAOA* has an internal structure of 15 exons with a total length of 4015 base pairs (bp) (Bach *et al.*, 1988, Hsu *et al.*, 1988, Manca *et al.*, 2018). Subsequent investigation revealed a sister gene, *monoamine oxidase B* (*MAOB*), which lies end-to-end with *MAOA* and shares approximately 93% amino acid identity with identical intron-exon structure (Grimsby *et al.*, 1991). Researchers theorise that both genes arose from a duplication event in a common ancestral gene (Grimsby *et al.*, 1991). Although both *MAOA* and *MAOB* are equally present in the CNS and peripheral organs, a region-specific expression profile has been observed, with *MAOA* expressed primarily in catecholaminergic neurons and *MAOB* in serotonergic and

histaminergic neurons (Westlund *et al.*, 1988, Shih & Thompson, 1999).

1.3.1 First association of the *Monoamine Oxidase A* gene to behaviour

The first association of the *MAOA* gene to behaviour was uncovered by Brunner *et al.* (1993a) within a Dutch family where males presented with slight mental delay along with high levels of aggressive behaviour (Brunner *et al.*, 1993a, Brunner *et al.*, 1993b). They uncovered a C to T point mutation at position 936 changing the codon sequence from glutamine (CAG) to a premature stop codon (TAG), inhibiting MAOA enzyme production (Brunner *et al.*, 1993a). Although urine tests on the affected individuals revealed changes in amine concentrations, the confirmation of the influence of this gene on behaviour came from animal studies. *Monoamine oxidase a* (*Maoa*) knockout mice exhibit heightened aggression and fear response mechanisms (Kim *et al.*, 1997). Moreover, these knockout mice models presented with altered brain structure and function along with higher levels of serotonin and dopamine neurotransmitters (Cases *et al.*, 1995).

1.3.2 Regulation of *MAOA* gene expression

The *MAOA* gene shows great genetic variability between individuals, and more so between sexes with females having two allelic copies compared to hemizygous males (Balciuniene *et al.*, 2001, Gilad *et al.*, 2002). Expression studies on X-linked genes have shown that in females, one copy of the *MAOA* gene undergoes X inactivation, however, disagreement exists whether the *MAOA* gene can escape this process (Hendriks *et al.*, 1992, Stabellini *et al.*, 2009). Consequently, understanding the *MAOA* gene's function in females is difficult with uncertainty as to which allele is actively expressed in females (Hendriks *et al.*, 1992, Stabellini *et al.*, 2009). Further inter-individual variation in MAOA enzyme expression has been attributed to variation within the *MAOA* gene, particularly a variable number tandem repeat (VNTR) within this gene termed the *monoamine oxidase A* promoter variable number tandem repeat (*MAOA* μVNTR) which controls the rate of MAOA enzyme expression as an upstream activator of transcription (Sabol, Hu & Hamer, 1998).

1.3.3 The *MAOA* Variable Number Tandem Repeat

The *MAOA* μVNTR was originally identified by Hinds *et al.* (1992) when looking at a highly polymorphic region surrounding exon one of the *MAOA* gene (Hinds *et al.*, 1992). Through sequencing, they were able to show that the μVNTR is located approximately 1.2 kilobase pairs upstream of the *MAOA* gene, 1142 bp upstream of the ATG translation initiation site, and is comprised of an internally repetitive core sequence ACC(A/G/C)G(C/T) of five repeats with a final length of 30 bp (Hinds *et al.*, 1992, Sabol, Hu & Hamer, 1998). Both Hinds *et al.* (1992) and Sabol, Hu & Hamer (1998) genotyped their participants in an attempt to group them into their respective allele groupings. Their investigations revealed the presence of four alleles characterised by a different number of repeats (3, 3.5, 4, and 5 repeats). However, current consensus is that at least five alleles commonly exist with the addition of a two repeat allele with final amplicon lengths: 2 = 290 bp, 3 = 320 bp, 3.5 = 335 bp, 4 = 350 bp, and 5 = 380 bp (Hinds *et al.*, 1992, Sabol, Hu & Hamer, 1998, Frazzetto *et al.*, 2007).

1.3.3.1 Function and expression of the μVNTR

Luciferase analysis was used to evaluate the effect of the various μVNTR alleles on the expression of the MAOA enzyme (Sabol, Hu & Hamer, 1998). Analysis revealed that the 3.5 and 4 repeat alleles showed higher activity levels and an increased expression ratio of 2.4-9.6 fold (Sabol, Hu & Hamer, 1998). Further expression analysis revealed that removing the promoter region revealed results similar to those seen for the 2, 3, and 5 repeat alleles, suggesting the 3.5 and 4 repeat alleles act as transcription activators (Sabol, Hu & Hamer, 1998). From this work, alleles have been grouped as either high-activity (3.5 and 4 repeats) or low-activity (2, 3, 5 repeat alleles) based on their effect on the transcription rate of the MAOA enzyme (Sabol, Hu & Hamer, 1998). Some studies have shown opposing results regarding the function of the five repeat allele given its rarity amongst most population groups ($< 5\%$) (Deckert *et al.*, 1999). The expression analysis performed by Sabol, Hu & Hamer (1998) suggested that the 5 repeat allele functions as a low-activity variant in three cell lines SY-5Y, SK-N-SH, and JAR. Opposing these findings, Deckert *et al.* (1999) performed a similar expression analysis, however, only in a single cell line (SH-SY5Y) where they found evidence that the 5 repeat allele acts as a high-activity allele rather than low-activity as proposed by

Sabol, Hu & Hamer (1998) (Deckert *et al.*, 1999). This uncertainty has resulted in researchers applying allele groupings inconsistently across the literature. Contemporary studies follow the allele classification proposed by Sabol, Hu & Hamer (1998) where the 5 repeat allele is considered a low-activity variant. Moreover, studies investigating the outcome of these different groupings found that using either functional classification did not change the outcome (Byrd & Manuck, 2014).

1.3.3.2 Population variation in the *MAOA* μVNTR alleles

Population variation at the *MAOA* gene locus shows high levels of linkage disequilibrium and significant inter- and intra-population variability (Balciuniene *et al.*, 2001, Gilad *et al.*, 2002, Vallender & Lahn, 2004). However, important to note is the lack of true African representation within the literature; to the reader's best knowledge, there have only been two studies reporting on μVNTR allele frequencies for Afrikaner Caucasians males (n = 196) and South African Black Xhosa males (n = 290) (Erasmus, Klingenberg & Greeff, 2015, Hemmings *et al.*, 2018).

1.3.4 The serotonin 5-HTTLPR

Serotonin (5-HTT) is an important neurotransmitter involved in electrochemical signalling throughout the central and peripheral nervous system, including the brain, gastrointestinal tract, and muscular systems (Adams, 1976, Bucher & Wightman, 2015). Apart from its role in normal functioning throughout the body, dysregulation of this neurotransmitter is associated with mood, behavioural, and psychological problems including depression and anxiety (Harris-Warrick & Cohen, 1985). Although the regulation of serotonin is complex, a variable number tandem repeat (VNTR) linked to serotonin is known to influence the rate of 5-HTT reuptake termed the serotonin-transporter-linked polymorphic region (5-HTTLPR). This VNTR is located upstream of the *solute carrier family 6 member 4* gene located on chromosome 17q12 and encodes the serotonin transporter protein (Gelernter, Pakstis & Kidd, 1995). The 5-HTTLPR contains multiple alleles with varying sizes, however, the two most common repeat sizes are 14 and 16 repeats (Heils, Mössner & Lesch, 1997). Expression studies have shown that the 14 repeat variant (short allele) is linked to decreased 5-HTT reabsorption compared to the 16 repeat (long allele) considered the wild type allele (Heils

et al., 1996). The relationship between the 5-HTTLPR and behaviour has been well established throughout the literature focusing on behaviour and psychological pathologies such as depression and anxiety (Caspi *et al.*, 2003).

1.3.4.1 The link between the 5-HTTLPR and *MAOA* μVNTR

The serotonin 5-HTTLPR and the μVNTR affect behaviour in parallel as both function within the serotonergic pathway, where they regulate the 5-HTT neurotransmitter concentration within the synaptic cleft and influence behaviour (Canli & Lesch, 2007). While the 5-HTTLPR regulates the rate of serotonin reuptake, the MAOA enzyme regulates the rate of serotonin digestion, as shown in Figure 1.1 (Canli & Lesch, 2007). Additionally, research has suggested possible epistatic effects between the μVNTR and the 5-HTTLPR (Priess-Groben & Hyde, 2013, Zhang *et al.*, 2017). Zhang *et al.* (2017) tested for possible interactions between the μVNTR and the 5-HTTLPR with aggression in victims of sexual abuse. They found a significant three-way interaction between these variables leading to an increase in aggression (Zhang *et al.*, 2017). Priess-Groben & Hyde (2013) investigated a possible four-way interaction between the μVNTR, 5-HTTLPR, life stress, and sex in predicting depression (Priess-Groben & Hyde, 2013). They found that although this four-way interaction predicted the depressive outcome, significant sex differences changed how the interaction influenced depression (Priess-Groben & Hyde, 2013). From their research, they showed that females with a short 5-HTTLPR allele and the low-activity μVNTR experienced higher levels of depressive symptoms than males. However, in males, the highest level of depressive symptoms was associated with a combination of the low-activity μVNTR and the long 5-HTTLPR allele (Priess-Groben & Hyde, 2013). Therefore, they concluded that the commonly reported 5-HTTLPR and stress interaction might only be limited to individuals with the low-activity μVNTR alleles (Priess-Groben & Hyde, 2013).

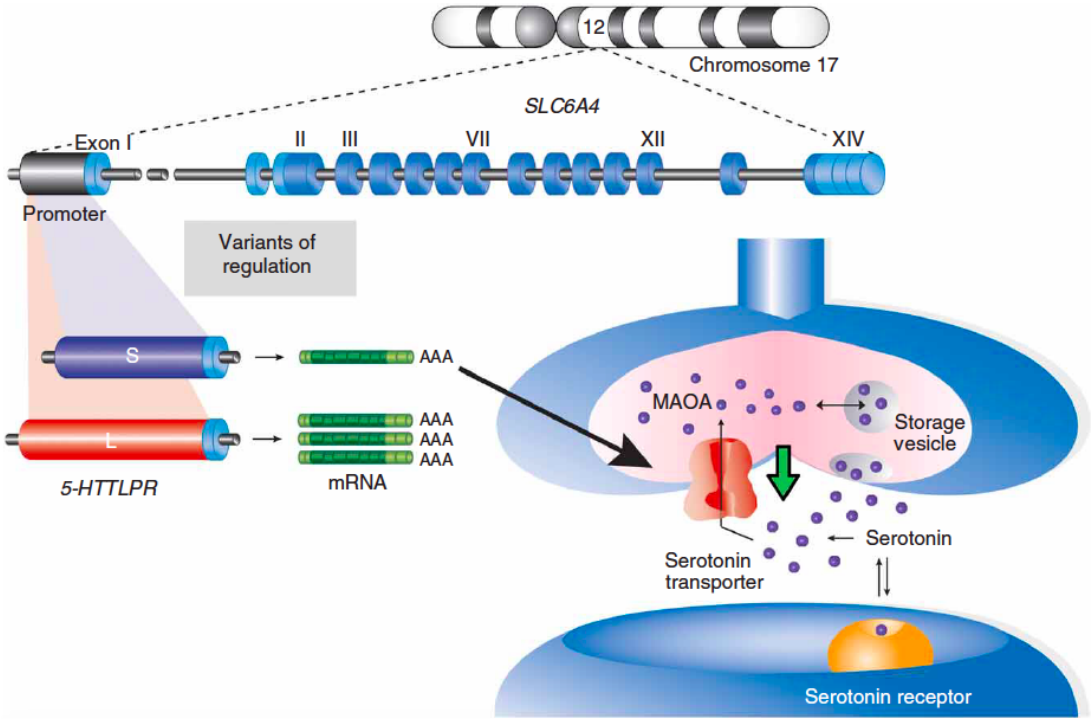

Figure 1.1: A synaptic view of the function of the *MAOA* enzyme and the serotonin transporter protein: The serotonin transporter protein (red) is responsible for the reabsorption of 5-HTT neurotransmitter from the synaptic cleft. The MAOA enzyme functions to degrade 5-HTT both pre and postsynaptically. Variation in the 5-HTTLPR loci controls the rate of 5-HTT reabsorption from the synaptic cleft and thus facilitates the role of the MAOA enzyme. Image taken from Canli & Lesch (2007) licence number: 4612431303642.

1.3.5 The *MAOA* μVNTR in gene x environment interactions

The seminal paper to demonstrate the interactive effects of the μVNTR and environment was published by Caspi *et al.* (2002). They investigated the interactive effects of the μVNTR and childhood abuse on adult conduct disorder, antisocial personality, and violent criminality in males. They showed that those with the low-activity μVNTR alleles had scored higher on an antisocial index compared to high-activity allele individuals. Additionally, of those physically abused, individuals with a low-activity μVNTR allele had the highest antisocial behaviour scores compared to non-abused low-activity allele carriers and high-activity allele carriers, regardless of abuse status. This study was the first to demonstrate that the μVNTR could confer risk to negative environments, thus the μVNTR low-activity allele renders an individual vulnerable to the effects of their environment (Caspi *et al.*, 2002). As the interest in these effects increased, so did the number of studies which aimed to replicate the original findings proposed by Caspi *et al.* (2002). Kim-Cohen *et al.* (2006) tested for the interaction between the μVNTR and physical abuse against children's mental health outcomes. Additionally, they

performed a meta-analysis of previous studies which aimed to replicate the findings of Caspi *et al.* (2002). Subsequently, they identified significant moderating effects of the μVNTR on physical abuse in children's mental health, attention deficit hyperactive disorder, and a near significant result predicting emotional problems (Kim-Cohen *et al.*, 2006). Through their study, they confirmed the effects identified by Caspi *et al.* (2002). They were able to demonstrate that of those who were abused, low-activity μVNTR allele individuals had scored significantly poorer on outcomes for mental health, attention deficit hyperactive disorder, and emotional problems compared to high-activity allele individuals (Kim-Cohen *et al.*, 2006).

Additionally, the meta-analysis they performed showed that although not all studies were able to replicate the results shown by Caspi *et al.* (2002) (Huizinga *et al.*, 2006, Haberstick *et al.*, 2014), the confluence of evidence from four supporting studies validates the existence of this gene x environment effect (Foley *et al.*, 2004, Kim-Cohen *et al.*, 2006, Nilsson *et al.*, 2006, Widom & Brzustowicz, 2006, Frazzetto *et al.*, 2007). With the strong evidence of the moderating effect of the μVNTR on the environment, the μVNTR has become a common variant included in studies focusing on negative behaviour, particularly violence, aggression, drug use, and criminality and has earned it the title of the "warrior gene" and the "criminal gene" (Lea & Chambers, 2007, Beaver *et al.*, 2010, Sohrabi, 2015). Subsequent work on gene x environment interactions has focused on understanding the mechanism by which the μVNTR and other variants, such as the 5-HTTLPR, increases an individual's vulnerability to environmental influences (Belsky *et al.*, 2009).

1.4 Theories related to the development of behaviour

The development of behaviour from childhood through to adulthood is ultimately contextualised on the state of the environment, either detrimental or supportive (a "poor environment" is any environment which is sub-optimal for healthy development) (Pluess, 2015). However, as described in Section 1.3.5, individuals differ in their responsiveness to environmental mediation, leaving some more, and others less responsive to changes in their environment (Monroe & Simons, 1991). These differences in sensitivity have in part been attributed to variation within neurotransmitter genes and have been investigated through

interaction analysis (Belsky *et al.*, 2009). While gene x environment interactions have been investigated within the aetiology of many diseases, within behaviour genetics, theories surrounding these effects are still currently being developed and adapted (Monroe & Simons, 1991, Hunter, 2005, Pluess, 2015).

1.4.1 Diathesis-stress

The first theory to account for differences in environmental sensitivity through gene x environment interactions was the diathesis-stress model (DsM) (Monroe & Simons, 1991). The diathesis-stress model was developed through the observation that some individuals appear vulnerable to the effect of stressful environments, while others do not. Under the DsM, it was acknowledged that not all individuals respond equally to the same environmental stressors and so even under the most adverse environments, some individuals remain unaffected while others are affected (Monroe & Simons, 1991). Consequently, this view that some are more vulnerable to adversity based on inherent mechanisms has made the DsM an ideal model for research within the gene x environment sphere (Belsky *et al.*, 2009).

The seminal study to investigate why certain individuals appear inherently more vulnerable to adverse environments was also the first to show a gene x environment interaction effect on behaviour (Section 1.3.5). Recall the study by Caspi *et al.* (2002), which showed that the μVNTR low-activity allele moderates the effect of abuse on externalising behaviours (antisocial behaviour and violence). While others have attempted to replicate this finding involving the μVNTR, Caspi *et al.* (2003) investigated this contextual effect on another variant, the serotonin 5-HTTLPR (Caspi *et al.*, 2003). They investigated the interaction of life stress measured through stressful life events and the 5-HTTLPR short allele on depression and suicidality (internalising behaviour). They showed that having at least one short allele moderates the effect of life stress, such that short allele carriers experience more depressive symptoms with an increase in the number of stressful life events. While both the μVNTR and the 5-HTTLPR function within the same pathway to reduce the concentration of 5-HTT within the synapse, Caspi and colleagues have further demonstrated that these two variants, the μVNTR (externalising behaviour) and the 5-HTTLPR (internalising behaviour), affect

behaviour independently (Caspi *et al.*, 2002, Caspi *et al.*, 2003). However, as noted in Section 1.3.4.1, there appears to be an epistatic effect between these two mechanisms as they both function within the serotonergic system.

Multiple attempts have been made to investigate the reproducibility of these gene x environment interaction effects first identified by Caspi *et al.* (2002) (Kim-Cohen *et al.*, 2006, Byrd & Manuck, 2014). Recall the study by Kim-Cohen *et al.* (2006), which showed how the μVNTR moderates life stress in mental health. They also showed support for this gene x environment interaction, through a meta-analysis, discussed in detail in Section 1.3.5. Their findings have since been corroborated by a second meta-analysis performed by Byrd & Manuck (2014). A summary of their meta-analysis is shown in Table 1.1. They showed that across 20 male cohorts, the μVNTR low-activity allele moderated the effect of adverse environments, with 16 studies showing significant interactions. They also investigated the potential publication bias that these effects are only seen within males. Furthermore, by stratifying the results from 12 female cohorts they showed that although an interaction was observed, these effects were largely contradictory between the sexes (Byrd & Manuck, 2014). Additionally, through the meta-analysis they conducted, they showed that studies combining males and females often fail to demonstrate a gene x environment interaction effect. Put differently, the μVNTR appears to have a sex specific effect. The authors ultimately concluded that these effects are seen more prominently in males and that this interaction appears to be specific to only severe environments such as maltreatment (sexual or physical abuse) (Byrd & Manuck, 2014).

Table 1.1: Summary of meta-analysis results from Byrd & Manuck (2014)

Sample sex	Number of studies (n)	GxE effect observed	Number of studies focused on childhood maltreatment	Number of Caucasian only studies
Male	20	16	13	17
Female	12	9	8	12
Mixed	4	3	4	2

1.4.2 Differential susceptibility

With more research being performed into the diathesis-stress effect of the μVNTR, researchers realised that the original studies by Caspi *et al.* (2002, 2003) had failed to identify a crucial interaction effect. These studies originally identified a diathesis-stress effect for both the μVNTR low-activity allele and the 5-HTTLPR short allele. However, in both studies, they failed to note that short allele (Caspi *et al.*, 2003) and low-activity allele (Caspi *et al.*, 2002) carriers had the fewest behavioural issues in the absence of stressful life events/abuse. As discussed in Section 1.3.5 and 1.4.1, Caspi *et al.* (2002) showed that μVNTR low-activity allele carriers are more vulnerable to abusive environments resulting in higher levels of antisocial behaviours. Pickles *et al.* (2013) showed that low-activity allele male carriers had higher levels of anger proneness under less maternal care, however, with more maternal care, these same low-activity males showed less anger proneness compared to high-activity males. This unrecognised finding suggests that these genes (μVNTR and 5-HTTLPR), rather than acting as a vulnerability factor to the environment, render an individual susceptible to the effect of both adverse and supportive ones (Belsky *et al.*, 2009). The realisation of the duality of these effects the variants have within gene x environment interactions led to the development of the differential susceptibility theory (DST) (Belsky, 1997, Belsky *et al.*, 2009). First conceptualised in 1991 by Belsky, Steinberg and Draper, differential susceptibility, similarly to DsM, theorises that a subset of people are susceptible to adverse environments, however, it further postulates that these same individuals also stand the most to gain from positive environments (Belsky, 1997, Belsky *et al.*, 2009). In the context of behavioural genetics, these genes confer susceptibility to both adverse and supportive environments under differential susceptibility (Belsky, 1997, Belsky *et al.*, 2009). Research focusing on gene x environment interactions has started acknowledging this shortcoming within the diathesis-stress model and shifted the research perspective to include the whole environmental spectrum (adverse and supportive) (Belsky *et al.*, 2009). This is further depicted within Figure 1.2 where a) depicts the vulnerability noted in diathesis-stress, while b) depicts the duality of the differential susceptibility theory with susceptible individuals showing an outcome proportional to their environment and resilient individuals outcome remaining unchanged (Belsky *et al.*, 2009).

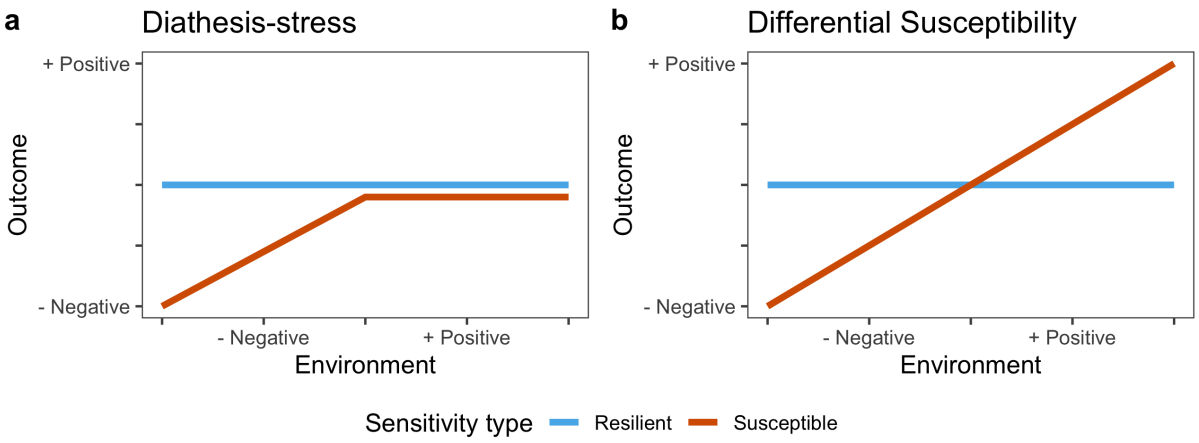

Figure 1.2: A comparison between the differential susceptibility cross-over effect and the diathesis-stress effect: Depicted in a) the diathesis-stress model and b) the differential susceptibility hypothesis. These graphs depicts the expected change in behavioural outcome as a product of GxE interactions as either a differential susceptibility or diathesis-stress effect.

Since then, several studies have investigated differential susceptibility effects regarding the μVNTR low-activity allele for several externalising behaviours (Nilsson *et al.*, 2018). Pickles *et al.* (2013) tested for possible gene x environment interactions between the μVNTR and maternal care in predicting infant anger proneness. They showed that the μVNTR moderates the effect of the environment bi-directionally. Males with the low-activity μVNTR allele expressed less anger proneness when receiving positive maternal care, while low-activity carrier males under negative maternal care showed more anger proneness, high-activity males showed no change regardless of the level of maternal care. The authors also tested this effect in females, however, females showed the opposite effect to males. The high-activity allele in females was associated with more susceptibility to the environment. Within this trend, the effect of maternal care also had the opposite effects between the sexes. Thus, boys with a low-activity allele and low maternal care showed an increase in anger proneness, while low maternal care resulted in lower anger proneness in females with a high-activity μVNTR allele (Pickles *et al.*, 2013).

Additionally, since many of these genes function within the same pathway, researchers have included multi gene x environment interaction models with a large degree of success (Nilsson *et al.*, 2018). Zhang *et al.* (2017) (Section 1.3.4.1) showed a significant moderating effect when considering the interaction between μVNTR the 5-HTTLPR and abuse. Similar interactions have been identified with other variants. Nilsson *et al.* (2015) identified significant

two, three, and four-way interactions between the brain-derived neurotrophic factor Val66Met, 5-HTTLPR, and μVNTR variants and sexual abuse leading to an increase in delinquent behaviour. Another approach has been to create polygenic risk scores to investigate the combined effect of these gene pathways. Simons *et al.* (2012) performed a cumulative plasticity allele analysis and showed that risk for aggression was moderated by the interaction of a hostile environment and plasticity allele count. Additionally, they showed that this moderating effect was enhanced by the addition of each plasticity allele so that those with more plasticity alleles showed greater susceptibility to the environment (Simons *et al.*, 2012). These studies present possible evidence to suggest that these genes could interact on an epistatic level, resulting in greater susceptibility (Nilsson *et al.*, 2018).

The existing diathesis-stress model saw individuals as vulnerable to their environment, but now, it appears that those vulnerable individuals who struggled the most in adverse environments also gain the most from positive ones (Belsky *et al.*, 2009). Once thought of as a vulnerability factor, these susceptibility genes/alleles appear rather as plasticity factors, increasing an individual's sensitivity to their environment (Belsky *et al.*, 2009, Pluess, 2015). This sensitivity to the environment appears to extend to intervention efforts as-well (Bakermans-Kranenburg & Van IJzendoorn, 2015). Different from traditional DST investigations, intervention efficacy experiments are based on the manipulation of a predictor, in this case, the environment (Bakermans-Kranenburg & Van IJzendoorn, 2015, Villiers, Lionetti & Pluess, 2017). Work by Morgan *et al.* (2017) has already demonstrated differential susceptibility in intervention efficacy within South Africa. The initial intervention aimed to improve mother-infant attachment, although, this only improved attachment with a modest effect size of 0.29. However, when the researchers retrospectively stratified the participants by their 5-HTTLPR genotype, those carrying either one or two copies of the short allele (plasticity allele) experienced an improved attachment at a large effect size of 0.75 compared to the homozygous long allele individuals at a small effect size of 0.03 (Morgan *et al.*, 2017).

1.4.2.1 Sensory processing sensitivity as a measurement of environmental sensitivity

One trait rooted in CNS sensitivity to external stimuli is the personality trait of Sensory Processing Sensitivity (SPS). Developed by Aron & Aron (1997), SPS is characterised by a deeper cognitive processing of external stimuli and a higher emotional reactivity (Aron & Aron, 1997, Chen *et al.*, 2011, Licht, Mortensen & Knudsen, 2011). While this trait is believed to be rooted in genetics (Aron, Aron & Jagiellowicz, 2012), only the 5-HTTLPR and dopamine-related genes have been linked to the trait, thus far (Licht, Mortensen & Knudsen, 2011). However, as with all behaviours and traits, the contributing factors are multifactorial and in the case of SPS, genetic and environmental factors have been suggested (Aron & Aron, 1997, Aron, Aron & Jagiellowicz, 2012, Acevedo *et al.*, 2014). While gene x environment interaction analysis is one method of investigating sensitivity to the environment, SPS measured through the Highly Sensitive Person Scale (HSPS) measurement is a well-validated measurement of general sensitivity to environmental stimuli (Aron & Aron, 1997, Smolewska, McCabe & Woody, 2006).

While SPS as a personality trait is not psychopathological, similar to environmental sensitivity theories, high SPS individuals show environmental specific effects (Greven *et al.*, 2019). Booth, Standage & Fox (2015) investigated the moderating effects of SPS and childhood experience on life satisfaction. They showed that SPS moderated the effect of the environment so that high SPS individuals showed less life satisfaction in severe childhood adversity compared to low SPS individuals (Booth, Standage & Fox, 2015). Conversely, Pluess and Boniwell (2015) tested whether SPS could moderate the effects of an intervention program directed at depression in school girls (Pluess & Boniwell, 2015). They showed that high SPS girls had significantly lower depression levels 12 months post-intervention compared to low SPS girls (Pluess & Boniwell, 2015). This showed that SPS as a measurement of environmental sensitivity moderates the effect of both supportive and adverse environment similar to DST (Pluess, 2015, Greven *et al.*, 2019).

1.5 Study rationale

Aberrant internalising and externalising behaviour imposes a large social and financial burden on South Africa (Section 1.1). While several factors have been implicated in the development of these behaviours, the effect of genes and environment have become the focus of research aimed at ameliorating these behaviours (Section 1.3.5). One genetic variant implicated in the dysregulation of both internalising and externalising behaviour is the μVNTR, particularly in interactions with childhood environment. However, the μVNTR has not been extensively studied within the environmental sensitivity framework, nor has SPS (a psychological measurement of sensitivity) been investigated in relation to the μVNTR alleles. Furthermore, the μVNTR and associated theories have not been comprehensively investigated within South Africa and its populations. Understanding the μVNTR and related theories in South Africa is important as similar variants within the environmental sensitivity framework have been implicated in therapeutic intervention efficacy through environmental moderation (Section 1.4.2). However, before variants such as the μVNTR and the environmental sensitivity theory can be applied to ameliorate aberrant behaviour, comprehensive investigation of its effects in South Africa have to be performed. Given South Africa's ethnic and genetic diversity, this study affords the opportunity to address the lack of studies of this nature in these populations.

1.6 Aims and objectives

Aim: This study aimed to investigate the possible main and interactive effects of the *MAOA* μVNTR on the development of internalising and externalising behaviour amongst members of the Birth To Twenty Plus (BTT+), which is the largest longitudinal cohort in South Africa. Ethical clearance was obtained from the University of the Witwatersrand Human Research Ethics Committee, clearance number:M180651 (Appendix A) and permission to use data from BTT+ Principle investigator (Appendix B).

Objectives:

1. Genotype the participants *MAOA* μVNTR alleles using PCR and gel electrophoresis.

2. Collate genetic data with historically collected data on BTT+ participants including

behavioural and SPS data.

3. Test for any main effect associations of the *MAOA* μVNTR to behaviour (internalising, and externalising) and sensory processing sensitivity (SPS).

4. To explore any interactions which moderates the effect of the *MAOA* μVNTR on behaviour, particularly gene x environment interactions with childhood environment.

2. Materials and Methods

The materials and methods chapter will introduce the reader to the sample and processing steps used within this research project. The chapter describes the Birth To Twenty Plus cohort participants, on whom this study was performed. Following on from this, the steps taken to measure and obtain the different variables used within this study will be identified, along with the procedure used to type the *MAOA* μVNTR alleles. This chapter will then describe the statistical procedures, including quality control, and regression models used to investigate the relationship between the μVNTR and behaviour. A general outline of the methods used in this study is captured in the flow diagram Figure 2.1. Additionally, ethical clearance was obtained from the University of the Witwatersrand Human Research Ethics Committee, clearance number: M180651 (Appendix A) and permission to use data from BTT+ principal investigator (Appendix B).

2.1 Participants

Partcipants from this study were drawn from the Birth To Twenty Plus (BTT+) study (formerly the Birth to Ten). The BTT+ study was conceptualised to investigate the health and development of children following the end of the Apartheid era. By the end of the Apartheid era, Black Africans were beginning to rapidly urbanise previously White city spaces. Researchers thus wanted to monitor the effect of this urbanisation on lifestyle, communicable and non-communicable diseases, socio-cultural changes and substance abuse within the "born free" generation (Richter *et al.*, 2009). The BTT+ is the largest longitudinal cohort in South Africa, which started in 1990 by recruiting pregnant woman from antenatal clinics in Johannesburg public hospitals. Approximately 2000 women were recruited, but a national hospital strike reduced the enrolment period such that only 1594 women were successfully enrolled (Richter *et al.*, 2007).

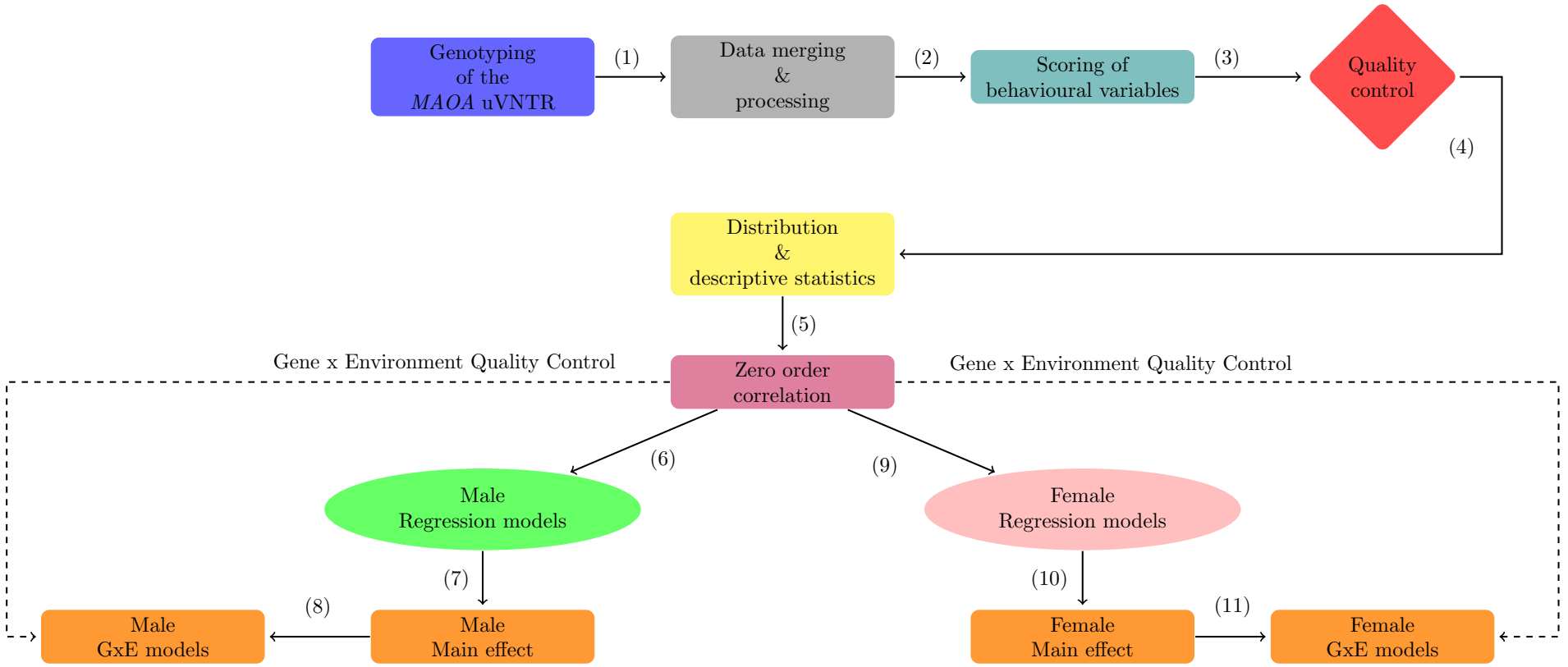

Figure 2.1: The flow of laboratory and statistical analysis steps performed in this study: This study started with the typing of the μVNTR alleles and merging of all relevant data sources, followed by the scoring of behavioural and personality measurements and quality control evaluation. Next, descriptive statistics and zero order correlations were calculated for each variable. This was followed by the linear regression analysis where males and females were separated and evaluated for both main effect and interaction associations.

Further participants were recruited from birth registries with 5449 live births recorded within the seven-week enrolment period. Of these, 1679 matched recruitment criteria for the BTT+ study (Richter *et al.*, 2007, Norris, Richter & Fleetwood, 2007). Since the start of the BTT+, over 21 data collection waves, each at a key developmental stage, have been conducted (Richter *et al.*, 2018). Within these data collection waves, several measures were recorded. Firstly, birth weight and gestational age were obtained from official hospital records. Data on household SES and maternal education were collected over the first two years of this study. Next, caregiver-rated behavioural scores were recorded when cohort members were seven years of age using the SACAS instrument. In the most recent data collection wave (2018-2019), when participants were aged 28, sensory processing sensitivity was assessed via the Highly Sensitive Person Scale (Section 2.2). Finally, in addition to the data mentioned above, the BTT+ cohort participants were previously genotyped for their 5-HTTLPR genotypes. While evidence has suggested that possible interactive effects exist between the *MAOA* μVNTR and 5-HTTLPR, this study lacked the power and sample size to detect gene-gene interactions. Furthermore, a preliminary analysis revealed that these interactions were non-significant. For a graphical representation of BTT+ data used in this study see Figure 2.2.

Although the BTT+ is the largest longitudinal birth cohort in South Africa, it still faces limitations experienced by all large studies of this nature. Firstly, the BTT+ has experienced attrition at a rate of 2.5% per data collection wave, by age 16. Furthermore, attrition has influenced the cohort in three ways, attrition attributed to death, intermittent attrition (due to cyclical migration), and study attrition (failure of the study to contact participant) (Richter *et al.*, 2007, Norris, Richter & Fleetwood, 2007). In addition to these issues, South Africa has a large inter- and intra-population genetic diversity, particularly the Soweto population, which has experienced significant admixture owing to its history as a genetic "melting pot" (May *et al.*, 2013). Although the BTT+ cohort includes members of several ethnicities, only Black South African members of the BTT+ cohort were selected for this study to avoid issues surrounding population stratification (Hellwege *et al.*, 2017). Moreover, compared to other countries, the South African environment is unique and characterised by high socio-economic,

and educational disparities stemming from its history of racial segregation during Apartheid (Barbarin *et al.*, 1998, Richter *et al.*, 2009). These unique factors make the BTT+ cohort an ideal sample to explore theories and genetic markers related to behavioural development in South Africa.

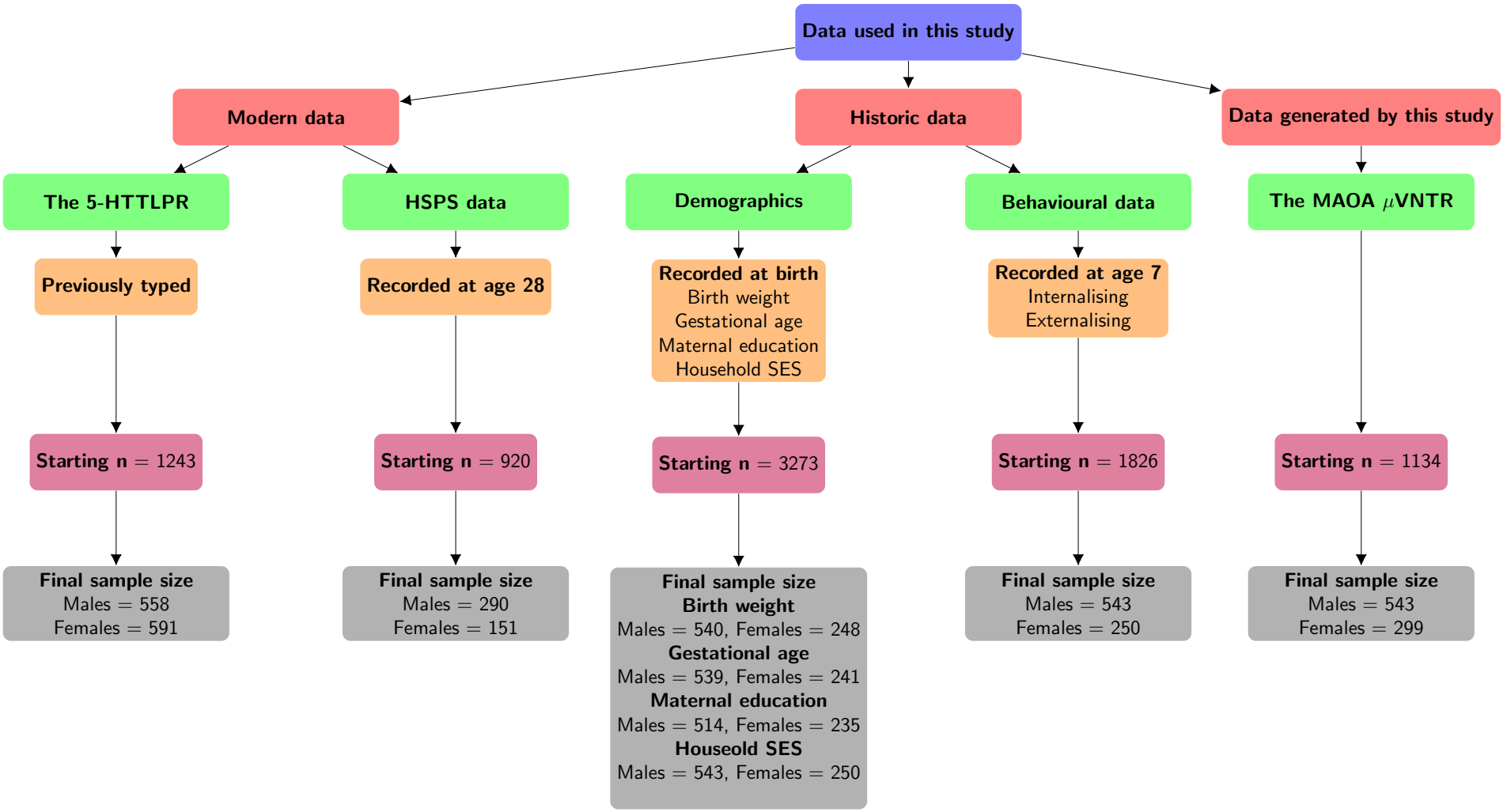

Figure 2.2: Flow diagram detailing the combination of data used in this study: This figure details the data sources used in this research and includes modern (recently collected) and historical data, and the μVNTR which was genotyped for this study. Modern data includes the 5-HTTLPR genotype data previously investigated in the BTT+ cohort and HSPS data which was previously collected during the 2018-2019 data collection wave. Next, historical BTT+ data is comprised of demographic data including: birth weight, gestational age, maternal education, and household SES and Behavioural data. Finally, the data generated by this study comprises the μVNTR alleles.

2.2 Instruments

This study used data from two psychological instruments; the South African Childhood Assessment Schedule and the Highly Sensitive person scale.

2.2.1 The South African Child Assessment Schedule

The South African Child Assessment Schedule is a caregiver-rated questionnaire that measures symptoms of child behavioural disorders including emotional disturbance, social competence, and academic adjustment Barbarin & Richter, 1999. The SACAS is composed of items from the International Classification of Diseases 10^{th} edition and the Diagnostic and Statistical Manual of Mental Disorders $4th$ edition as guidelines (Bell, 1994, World Health Organization, 2010). Additionally, the SACAS questionnaire items were developed from several validated instruments including the Achenbach-Connors-Quay questionnaire (Achenbach, Edelbrock, *et al.*, 1983), the Achenbach Child Behaviour Checklist (Achenbach & Edelbrock, 1991), the Problem Behaviour Index (Peterson & Zill, 1986), the Health Resources Inventory (Gesten, 1976, Hightower *et al.*, 1986), and the National Center for Education Statistics Kindergarten Teacher Survey on Student Readiness (National Center for Education Statistics, 1993). To supplement these measures above, an advisory group comprised of South African mothers was assembled to assess the language used and to suggest additional items concerned with aberrant behaviour (Barbarin & Richter, 1999). However, the SACAS measurement is not a clinical tool used to diagnose behavioural disorders, but rather a tool to highlight potentially problematic behaviour.

The final SACAS questionnaire uses a three-point scale to measure eight facets of childhood behaviour namely: 1) Anxiety-depression, 2) Self-regulation, 3) Aggression, 4) Opposition, 5) Affability, 6) Resilience, 7) Independence, and 8) Academic readiness. However, items from these scales have been combined to create a composite scale for the overarching patterns of internalising and externalising behavioural problems. The internalising scale is comprised of 32 items with a maximum possible score of 64, compared to the externalising scale which has 34 items with a maximum score of 68. Some example questions from the SACAS include 1) Does (CHILD) accept and listen to criticism calmly?, 2) Does (CHILD) avoid activities which

S/He is not good at? 3) Is (CHILD) unable to get his/her mind off certain thoughts? 4) Does (CHILD) complain of loneliness? 5) Does (CHILD) demand attention? The complete SACAS questionnaire can be found in Appendix C.

2.2.2 The Highly Sensitive Person Scale

The Highly Sensitive Person Scale (HSPS) is a 27 question scale measuring an individual's level of sensory processing sensitivity (SPS) (Aron & Aron, 1997). The HSPS uses a Likert scale where participants rate their agreement with the statement between 1 and 7 (where 1 implies not at all and 7 implies extremely), giving a maximum score of 189, with higher scores implying more sensitivity. As noted in Section 1.4.2.1, the HSPS is a measurement of sensitivity towards external stimuli and including loud noises, bright lights, overcrowded spaces, and a need to withdraw from overstimulating situations. Some questions in the HSPS include 1) Do other people's moods affect you? 2) Do you tend to be more sensitive to pain? 3) Do you startle easily? 4) Are you particularly sensitive to the effects of caffeine? 5) Do you find it unpleasant to have a lot going on at once? For the complete HSPS questionnaire see Appendix D.

2.2.3 Scoring of variables

The SACAS and HSPS items were scored to obtain a total measurement for each scale using the *Psych* package (Revelle, 2018) in R. Individual missing item responses were median imputed only for individuals missing 10% or less of item responses.

2.3 Genotyping

Typing of the μVNTR was performed by polymerase chain reaction (PCR) and gel electrophoresis on BTT+ participants DNA extracted at age 12 years. DNA was initially extracted using the salting-out method (Miller, Dykes & Polesky, 1988), after which the DNA was stored at 7°C at the Division of Human Genetics, National Health Laboratory Service, Braamfontein. The DNA was then quantified using the NanoDrop ND-1000 spectrophotometer supplied by Thermofisher Scientific, thereafter normalised, yielding a final DNA concentration of 50 μg/μl. A complete list of equipment and reagents used can be found in Table E.3 in

Appendix E.

2.3.1 Polymerase chain reaction

Polymerase chain reaction (PCR) for allele-specific amplification was successfully performed on 1134 DNA samples from the BTT+ cohort using a Bio-Rad T100 Thermal cycler, Accuprime GC-rich *Taq* polymerase (Invitrogen), and published primers (Integrated DNA Technologies), Forward Primer: 5'-ACA GCC TGA CCG TGG AGA AG-3', Reverse Primer: 5'-GAA CGG ACG CTC CAT TCG GA-3' (Sabol, Hu & Hamer, 1998). Reactions were set up using PCR string tubes and a PCR master mix as shown in Table 2.1. A Slowdown PCR cycle condition was used to optimise the amplification yield of the μVNTR given the GC rich nature of the region. PCR cycle conditions are shown in Table 2.2 (Frey *et al.*, 2008). Slowdown PCR utilizes a lower ramp rate $2.5°\text{C.s}^{-1}$ and lower cooling cycle $1.5°\text{C.s}^{-1}$ to reach the optimal denaturation and annealing temperatures allowing for optimal amplification of GC rich regions without the formation of secondary structures (Frey *et al.*, 2008). The expected amplicon band sizes are reported in Figure 2.3 with corresponding band sizes: 2 repeat (290 bp), 3 repeat (320 bp), 3.5 repeat (335 bp), 4 repeat (350 bp), and 5 repeat (380 bp) (Sabol, Hu & Hamer, 1998).

Table 2.1: MAOA μVNTR PCR amplification reagents

	Volume	Concentration
DNA Template	0.5 μl	50 ng
5" Forward Primer	0.25 μl	10 μmol
3" Reverse Primer	0.25 μl	10 μmol
5X Buffer A	2.5 μl	0.5X
Taq Polymerase	0.25 μl	1 U
Sterile water ddH2O	8.75 μl	
Total Reaction Volume	12.5 μl	

Table 2.2: Slow down PCR cycle conditions for amplification of the MAOA μVNTR alleles

Step Number	Step	Temperature	Ramp Rate	Time	Cycle
Initial Slow down PCR Protocol					
1	Initial Denaturation	95°C		5 minutes	1
2	Denaturation	95°C	Heating at 2.5°C.s^{-1}	30 seconds	
3	Annealing	68°C	Tm $\downarrow$ at 1°C every 3 cycles	30 seconds	48
4	Extension	72°C	Heating at 2.5°C.s^{-1}	40 seconds	
Additional 15 PCR cycles					
5	Denaturation	95°C	Heating at 2.5°C.s^{-1}	30 seconds	
6	Annealing	54°C	Cooling at 1.5°C.s^{-1}	30 seconds	15
7	Extension	72°C	Heating at 2.5°C.s^{-1}	40 seconds	
8	Final Extension	72°C	Heating at 2.5°C.s^{-1}	5 minutes	1

2.3.2 Gel electrophoresis

Allele band size determination was performed by gel electrophoresis. This was done by loading the PCR product onto a 2% agarose gel made from agarose powder and 1X Tris-Borate-Ethylenediaminetetraacetic acid (TBE) buffer. Samples were then run in an electrophoresis tank at 6.25 V/cm in 1xTBE running buffer for three hours to resolve bands adequately. Allele sizes were determined by comparison to the included 50 bp DNA molecular weight marker which ran alongside the samples. As discussed in Section 2.4.1.1 genotype results were called by two independent researchers and re-amplified until a consensus was reached. Figure 2.3 depicts the expected band size results following gel electrophoresis.

2.4 Data analysis

Data processing and statistical analysis was performed using R version 3.6.1 (2019-07-05) and RStudio version 1.2.1522.

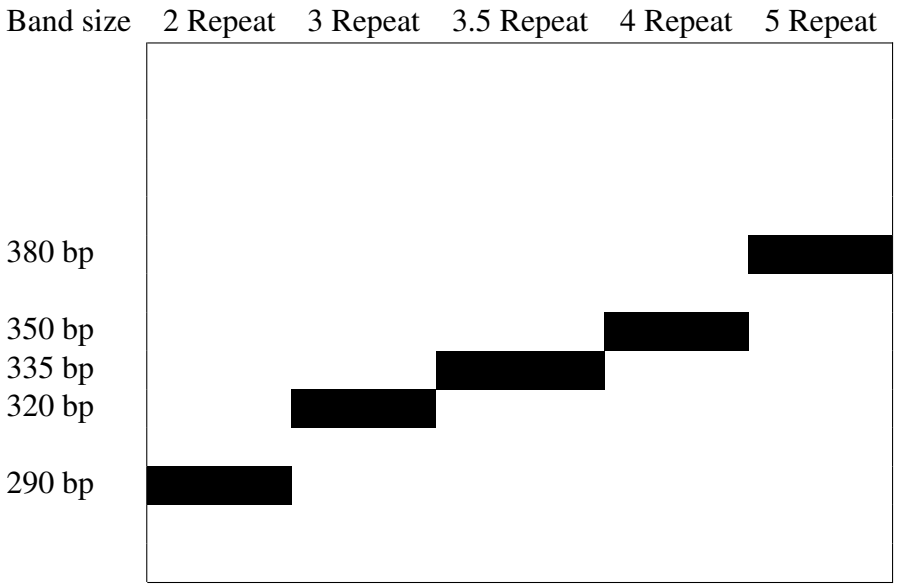

Figure 2.3: Expected results following PCR and gel electrophoresis procedures: Shown here are the expected view of allele size differences following gel electrophoresis of the μVNTR product. These alleles would be differentiated at band sizes 290 bp (2 repeat), 320 bp (3 repeat), 335 bp (3.5 repeat), 350 bp (4 repeat), and 380 bp (5 repeat).

2.4.1 Data missingness and quality control

Missing data are a complication of this study for various reasons, particularly, sample exclusions. Data missingness was assessed with a graphical representation for males and females using the *UpSetR* package (Gehlenborg, 2019). This was done to assess data missingness by sample (data missing at a single point) or data missingness per variable. The patterns of missing data are represented in Figure 2.4 for males, and Figure 2.5 for females. These figures demonstrate two important patterns, firstly, the absolute missing cases per variable (denoted by set size axis) and the result of overlapping missing data between variables. Within both male and female groups, the largest number of missing cases was seen in the HSPS measurement. Secondly, several variables showed overlapping missing cases with other variables including maternal education, gestational age, birth weight and HSPS. This result is important as it points to a data missingness structure of missing at random (MAR) compared to missing not at random (MNAR) which would have to be addressed. However, the only way to confirm either of these data structures would be to return to the sample and complete the measurements. Before missing values of the SACAS data set were imputed, total missingness for each participant was assessed to evaluate the reliability of imputation on these samples. As a general cut-off, imputation would not be performed if data missingness was

higher than 10% for an individual (Dong & Peng, 2013). The missingness evaluation revealed the maximum observed missing values for any participant was 5% amongst males and 7% amongst females. Imputation was thus performed for the SACAS variables, but not for demographic or HSPS data. The demographic data was recorded by trained hospital staff and very few missing values were observed. Since the HSPS scores obtained in the most recent data collection wave (2018-2019), the cohort was smaller than at the beginning of the study (1990) due to attrition.

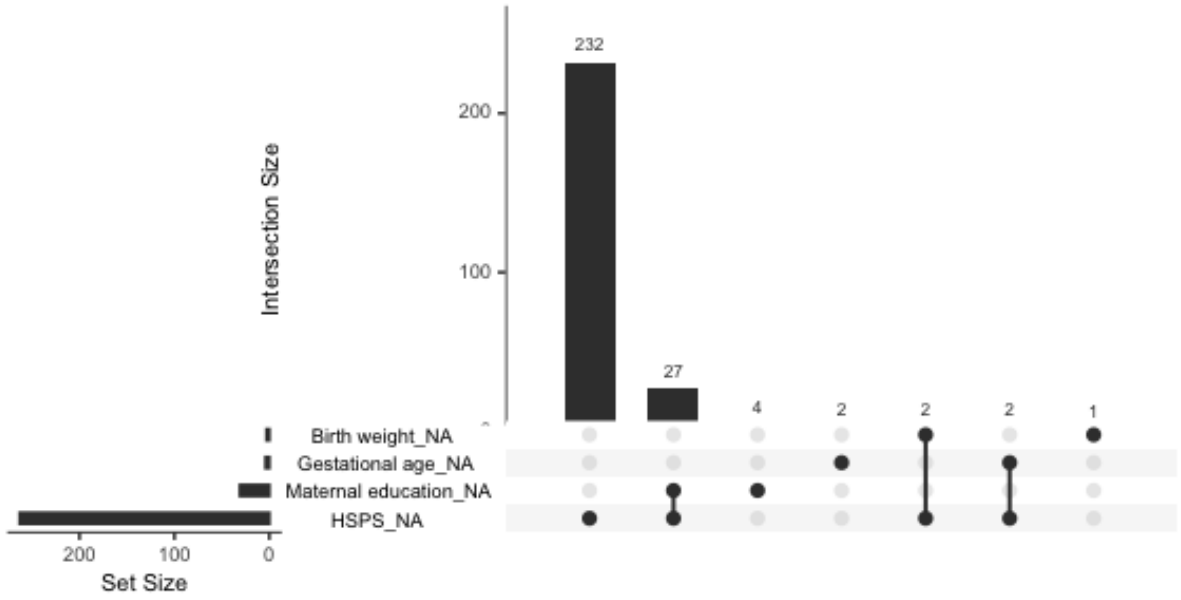

Figure 2.4: Data missingness structure for males: The data missingness plot for males shows that the HSPS measurement had the largest number of missing cases (over 200 missing observations). This is because the HSPS scores were collected in the most recent data collection wave (2018-2019) when the cohort was notably smaller than the study onset (1990). Other variables were only missing in a small proportion of individuals.

2.4.1.1 Genotype quality control

The genotyping quality control (QC) process started with the genotyping of the μVNTR alleles by having two independent researchers calling allele sizes and re-performing the amplification until a consensus was reached. To assess for genotyping error, a Hardy-Weinberg equilibrium test was performed using the *HardyWeinberg* package (Graffelman & Morales-Camarena, 2008, Graffelman, 2015). However, due to male homozygosity, only females could be assessed for Hardy-Weinberg equilibrium as a proxy for the BTT+ cohort (Hardy, 1908, Weinberg,

1908, Graffelman, 2015, Manca *et al.*, 2018).

An additional part of the QC process required the removal of all heterozygote females due to the uncertainty regarding which allele was active in females. This resulted in a further loss of approximately one-half of all female participants from the analysis. The removal of heterozygote females has resulted in a final sex distribution of 68.5% males and 31.5% females, which further complicated the data analysis and the effect of data missingness in female participants. Next, gene-environment correlations (rGE) were assessed as a prerequisite for any gene x environment interaction analysis. For a genetic and environmental variable to be assessed as an interaction, the rGE correlation should be near zero, (see Section 3.4.2 for male rGE correlations, and Section 3.5.2 for female rGE correlations).

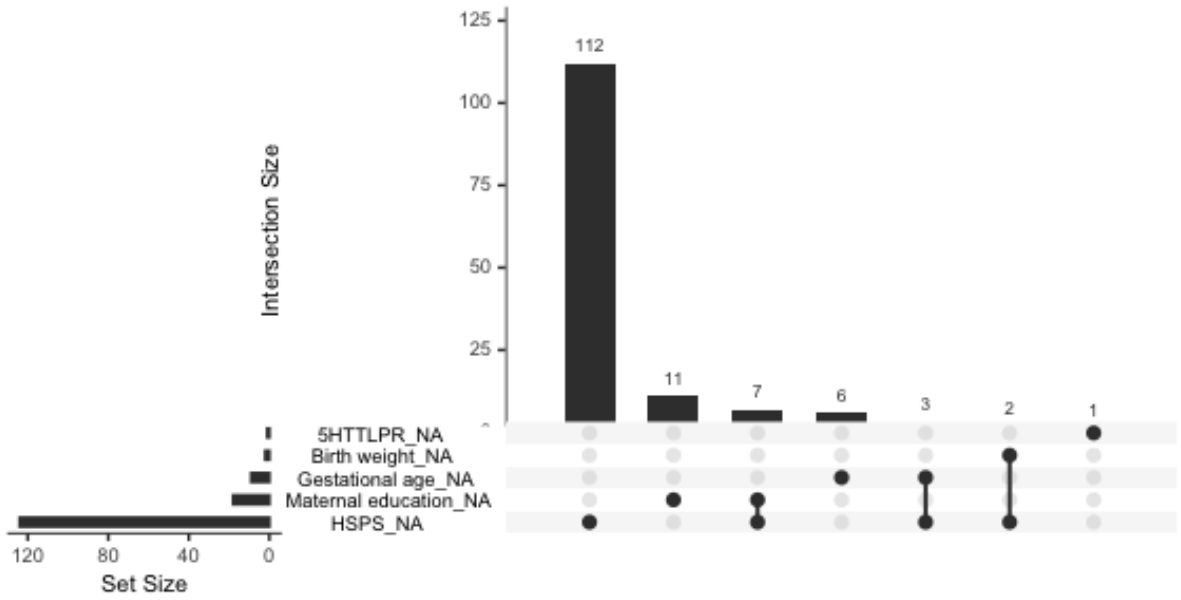

Figure 2.5: Data missingness structure for females: The data missingness plot for females, like males shows that the variable with the most missing cases is the HSPS (missing over 120 cases).

2.4.2 Allele and genotype encoding

Analysis of the μVNTR variation in relation to behaviour and other traits is challenging given that it is an X-linked variant with five possible alleles, and thus males are hemizygous but

females can either be hetero- or homozygous (Sabol, Hu & Hamer, 1998, Deckert *et al.*, 1999). One method employed to overcome these issues was to dichotomise participants according to a binary functional classification, either high- or low-activity variants. Female high-activity genotypes include the 4/4, 3.5/4, and 3.5/3.5 genotypes and low-activity genotypes include the 2/2, 2/3, 3/3, 3/5, and 5/5 genotypes, with males grouped into either high-activity (4, and 3.5 repeats) alleles or low-activity (2, 3, and 5 repeats) alleles (Sabol, Hu & Hamer, 1998, Frazzetto *et al.*, 2007). Furthermore, researchers conventionally divide samples by sex while also excluding females with high-activity/low-activity heterozygotes (2/4, 3/4, and 4/5) genotypes (Reti *et al.*, 2011). However, disagreement exists surrounding the functionality of the 5 repeat allele. Sabol, Hu & Hamer (1998) classify this allele as a low-activity allele, but research by Deckert *et al.* (1999) and Beach *et al.* (2010) suggests it acts as a high-activity allele. However, Byrd & Manuck (2014) discuss that given the rarity of the 5 repeat allele in most populations either classification (high- or low-activity) would not affect the analysis significantly. Additionally, a recent methodological analysis has shown that linear regression is unable to accurately model interactions with factor variables containing three or more levels, such as in genotype models, unless the regression equation is first re-parameterised (Aliev *et al.*, 2014). Nevertheless, the literature on the effects of the μVNTR suggests binary models of high versus low expression accurately represent the functional model by which the μVNTR affects behavioural variation (Kim-Cohen *et al.*, 2006, Byrd & Manuck, 2014).

2.4.3 Distribution of alleles and genotypes

Genotype and allele frequency distribution was assessed by constructing a frequency table along with a histogram representing these frequencies (Wickham & Chang, 2016). Males and females were grouped separately and allele frequencies were compared to those from other populations, both within South Africa and elsewhere.

2.4.4 Descriptive statistics for each variable

A demographic summary of the variables was obtained using the *Stargazer* package (Hlavac, 2018) and includes the number of observations, mean, and standard deviation. This was followed by assessing variable distribution, inspected both visually using the *ggplot2* package

(Wickham & Chang, 2016) and statistically through the Shapiro-Wilk normality test. Kurtosis and skewness tests was then performed to better understand the variable distributions (Revelle, 2018, R Core Team, 2019).

2.4.5 Zero-order correlation

Next, inter-variable zero-order correlations were performed to investigate the relationship between variables, without controlling for covariates. This step has a secondary function as a QC step for the interaction modelling performed later (Roisman *et al.*, 2012, Jolicoeur-Martineau *et al.*, 2017). However, different correlation statistics were used based on the nature of the variable tested i.e factor versus continuous variables. Genotype, allele, and sex correlations were performed using point-biserial correlation (R Core Team, 2019). For discrete variables with repeated measurements (ties within the data) including gestational age, birth weight, household SES, and maternal education, Kendal's τ statistic was applied (R Core Team, 2019). Finally, for other continuous variables correlations (internalising and externalising scores), Spearman's ρ correlation was used (R Core Team, 2019).

2.4.5.1 *Post hoc* power analysis

post hoc power analysis was conducted on significant results in line with the retrospective nature of the data. The main effect power was conducted using Quanto (version 1.2.4) (Gauderman & Morris, 2006), while the *pwr* package (Champely, 2018) was used to evaluate the power of the interaction models. In both instances, the power of each model was calculated on sample size and beta values obtained from the linear regression models.

2.4.6 Regression analysis

Linear regression analysis was performed in R studio using the *stats* package (R Core Team, 2019). This applied the general multiple linear regression equation 2.1, including adjusted R^2 as a measurement of effect size and the F statistic as model fit significance (Field, Miles & Field, 2012, Cohen, West & Aiken, 2014).

$$\hat{Y} = \beta_0 + \beta_1 \cdot x_1 + \beta_2 \cdot x_2 ..... + \beta_i \cdot x_i + \varepsilon \tag{2.1}$$

$\hat{Y}$ is the dependent variable

β_0 is the slope when x=0

β_i is the slope of the regression line

x_i is the predictor value

ε is the error or residuals

However, to apply a linear regression test, some assumptions have to be satisfied, including: 1) a linear relationship exists between independent (x) and dependant (y) variables; 2) the residuals or errors from the mean are assumed to be normally distributed, however, normality is not assumed for x and y variables; 3) no multicollinearity exists between independent variables (independent variables should not correlate with one another); 4) little or no autocorrelation exists between the data (where observations are related or dependent on one another); 5) the result of the regression is homoscedastic meaning the residuals (error) are equal across all values of x (Field, Miles & Field, 2012, Cohen, West & Aiken, 2014).

2.4.6.1 Regression modelling

Variable selection for inclusion in linear models was conducted using an exhaustive modelling approach (*leaps* package Lumley, 2017) to determine which combination of variables best explain variability in outcomes (HSPS, internalising, and externalising scores). The exhaustive modelling results were used in conjunction with fit statistics to determine the best model from available variables (Miller, 1984, Igarashi *et al.*, 2018). The fit statistics used to determine the final combination of variables in the models include the Akaike Information Criterion, adjusted R^2, Mallows Cp, and Residual Standard Error (R Core Team, 2019).

2.4.6.2 Main effect of the μVNTR on behaviour

The first step in determining the relationship between the μVNTR and behaviour was to assess individual scores under each genotype through graphical means (Wickham & Chang, 2016). Next, rGE correlations were examined to assess the correlation between predictors. This was done using Kendal's τ and point-biserial correlation tests (R Core Team, 2019). Finally, regression modelling was performed to determine which combination of predictors best explain the variance in behaviour through a direct effect.

2.4.6.3 Interaction effects of the *MAOA* μVNTR and environment on behaviour

To determine whether an interactive effect exists between the μVNTR and childhood environment, an interactive modelling approach was performed. Following the modelling approach discussed in Section 2.4.6.1, an interaction term between two variables was added to determine if a possible gene x environment interaction effect influenced behavioural outcomes. Both household SES and maternal education were investigated as possible environmental factors to interact with the μVNTR. However, these moderating effects can influence behaviour in various ways as depicted in Figure 2.6. These include differential susceptibility, diathesis-stress, contrasting effects, and the absence of an interaction. Firstly, differential susceptibility is characterised by a crossover effect where susceptible individuals score changes based on the nature of the environment (in a for-better and for-worse manner), while resilient individuals outcome remains stable across the environmental spectrum, (Figure 1.2b and Figure 2.6a). Furthermore, diathesis-stress is similar to differential susceptibility in that the resilient individual's score remains the same, however, the susceptible individual's outcome worsens as a product of the environment. Contrasting effects are identified when two linear models have opposite slopes. When an absence of interactive effect is present, the scores of resilient versus susceptible individuals scores do not change as a product of the environment, however, the absence of effect does not mean the scores are equal between resilient and susceptible individuals (Belsky, Bakermans-Kranenburg & Van Ijzendoorn, 2007).

2.4.6.4 Diagnostics and Outlier identification

Outliers of model fit diagnostics were identified by various methods including graphically through quantile-quantile plots, added variable plots, model fit plots, and influence index plots to identify samples that affect the model fit. Statistical methods used to identify these samples include Cook's distance, studentized residuals, and hat-values. Cook's distance was used as a measurement of an individual samples effect on the fitted line for both independent and dependent variables. Statisticians generally agree that samples whose Cook's distance are three times larger than the mean Cook's distance should be removed (Field, Miles & Field, 2012, Cohen, West & Aiken, 2014). While studentized residuals identify samples with extreme y values skewing the line, hat-values identify samples with extreme x values (Fox & Weisberg,

2011, Cohen, West & Aiken, 2014).

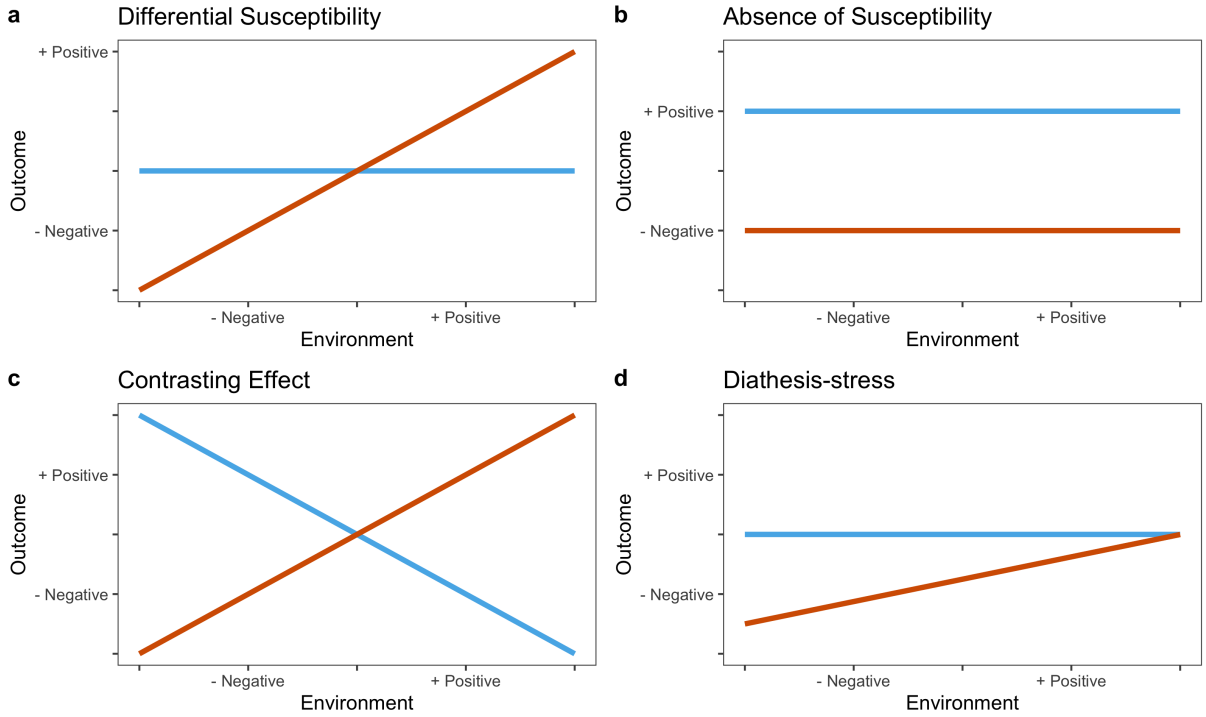

Figure 2.6: Different interaction models observable through gene x environment interaction analysis: The different effects observed through interactive models include a) differential susceptibility (where susceptible individuals scores change proportionally to their environment, while resilient individuals scores remain stable), b) an absence of effect (where individuals scores (susceptible and resilient) do not change throughout the environmental spectrum), c) a contrasting effect (where susceptible and resilient individuals scores change inversely with one's score improving while the other decreases with a change in environment), and d) dual risk/diathesis stress (where susceptible individuals experience a poorer outcome under negative environments and resilient individuals scores remain stable across the environment). Adapted from Belsky, Bakermans-Kranenburg & Van Ijzendoorn (2007).

3. Results

This chapter reports on the results of the various laboratory and statistical analyses performed. Firstly, scoring reliability, descriptive statistics, and the distribution evaluations are presented. This is followed by the genotyping results for the *MAOA* μVNTR within the BTT+ cohort participants. Next, the reader will be presented with the zero order correlation, and linear regression results. This includes the main effect regression models, as well as the interaction models between the μVNTR and environment (household SES and maternal education) in determining childhood behaviour. The results section concludes with the exploratory analysis of female gene x environment interactions that are often ignored in behaviour studies on the μVNTR.

3.1 Scoring of behavioural and personality variables

All three scales, internalising, externalising, and HSPS, showed good reliability values for both Cronbach's α (internalising = 0.72, externalising = 0.82, and HSPS= 0.8) and Guttman's λ^6 (internalising = 0.78, externalising = 0.86, and HSPS = 0.89) (Table E.1). These coefficients suggest a good internal consistency reliability, consistent with SACAS reports and the literature on HSPS (Aron & Aron, 1997, Barbarin & Richter, 1999).

3.1.1 Distribution

A deeper investigation of each variable distribution was performed using both visual (males, Figure E.1 and females, Figure E.2) and statistical methods (Table 3.1). From the Shapiro-Wilk tests performed, only the measurement of HSPS was normally distributed in both sexes, which meant non parametric tests had to be used to investigate the other variables. In terms of data skewness, both internalising and externalising behaviour were positively skewed (longer positive tail), but gestational age, birth weight, maternal education, and household SES were all negatively skewed. Notably, gestational age had a skewness value of

−2.2 which is considered highly skewed. Additionally, the kurtosis (weight of the tails compared to the rest of the distribution) of each variable was evaluated; this measurement is often used to evaluate the presence of outliers in a sample. Birth weight, household SES, maternal education, and internalising and externalising behaviour showed kurtosis values below three suggesting a platykurtic distribution with light tails and few outliers. Gestational age, however, had a kurtosis value of 8.33 which is indicative of a leptokurtic distribution with long heavy tails suggestive of outliers. While the distribution statistics suggested the presence of outliers in the data, these outliers were not removed for various reasons. Firstly, statistical practices suggest that outliers in the data exist as influential cases and the only reason to remove these samples would be as a result of measurement or recording error (Field, Miles & Field, 2012). There was no evidence of such error in this study and thus samples were not removed. Secondly, in the case of gestational age (leptokurtic distribution), values were obtained from official hospital records (not self-report) and given the outliers being due to premature or post-term pregnancies these samples were not removed.

Table 3.1: Statistics for Shapiro-Wilk test of normality, kurtosis, and skewness

Variables	Normality	Kurtosis	Skewness
Internalising behaviour	W = 0.96898 p < 0.001	0.10	0.62
Externalising behaviour	W = 0.98633 p < 0.001	−0.24	0,35
HSPS	W = 0.99554 p > 0.1	−0.14	−0.04
Gestational	W = 0.78036 p < 0.001	8.33	−2.20
Birth weight	W = 0.97595 p < 0.001	1.70	−0.35
Maternal education	W = 0.85884 p < 0.001	1.26	−0.8
Socio-economic status	W = 0.94802 p < 0.001	−0.19	−0.67

3.1.2 Descriptive statistics

A descriptive investigation of the data was performed and summarised in Table 3.2. Participants' mean scores for internalising behaviour were 15.45 ± 6.91 standard deviation (SD) in males

and 15.93 $\pm$ 6.6 SD in females out of a possible maximum of 64 (higher scores mean more behavioural problems). This compared to externalising behaviour where the mean scores in males was 23.78 $\pm$ 10.13 SD and 20.47 $\pm$ 9.04 SD in females out of 68. A further Wilcoxon-rank-sum test (Table E.2) revealed the observed difference in externalising behaviour score was significantly different between the sexes (W = 139240, $p < 0.001$), however, this sex difference was not observed in internalising behaviour. The level of behavioural problems in this cohort was relatively low amongst both males and females, which was expected given that the BTT+ cohort was a non-clinical sample. Furthermore, the level of sensitivity measured through the HSPS was 100.73 $\pm$ 21.03 SD in males and 110.67 $\pm$ 20.25 SD in females out of 189, which was significantly different between males and females (W = 67140, $p < 0.001$). Concerning the demographics of this cohort, males showed a higher household SES than females (male = 0.12 $\pm$ 0.95 SD and female = 0.04$\pm$ 0.98 SD), which was significantly different (W = 186110 , $p < 0.001$). However, no mean score difference was observed in maternal education (male = 4.32 $\pm$ 0.95 SD and female = 4.29 $\pm$ 1.00 SD) which was equally low in both sexes. The low household SES and maternal education level was, however, expected, given the historical levels of poverty in Soweto, where the cohort members were reared (Richter *et al.*, 2009).

3.2 Genotyping

Typing of the μVNTR alleles and genotypes was performed for approximately 1134 individuals, 561 males and 593 females. However, 18 samples labelled as males were genotyped as having heterozygous genotypes (only observable in females given male hemizigosity) and were removed after re-amplification confirmed this anomaly. While sequencing could have been used to validate this finding, sequencing all samples to investigate the true number of sample swaps would have been too costly. Following this removal, only 543 males remained with successful allele determinations. Although the number of successfully genotyped females remained as 593, the number of useable genotypes after removal of high-activity/low-activity allele heterozygotes was 299 genotypes. Figure 3.1 depicts the observed male alleles (2, 3, 4, 5) and female genotypes (2/2, 2/3, 2/4, 3/3, 3/4, 3/5, 4/5). Important to note is the lack of the 3.5 allele commonly reported in most populations, as shown in Table 3.3.

Table 3.2: Descriptive statistics of male and female participants

Variable	n	Mean	Median	Mad	SD	Min	Max
Males							
Internalising behaviour	543	15.45	15.00	5.93	6.60	2.00	47.00
Externalising behaviour	543	23.78	23.00	10.38	10.13	2.00	57.00
Sensory Processing Sensitivity	290	100.73	102.50	18.53	21.03	38.00	156.00
Birth weight (kg)	540	3.123	3.12	0.45	0.52	1.13	4.80
Gestational age (weeks)	539	37.96	38.00	1.48	1.75	28.00	44.00
Household SES	543	0.12	0.18	1.06	0.95	−2.65	1.94
Maternal education	514	4.32	4.00	1.48	0.95	1.00	6.00
Females							
Internalising behaviour	250	15.93	15.00	7.41	6.91	3.00	39.00
Externalising behaviour	250	20.47	21.00	8.90	9.04	0.00	45.00
Sensory Processing Sensitivity	151	110.67	111.00	19.27	20.25	58.00	174.00
Birth weight (kg)	248	2.99	3.00	0.45	0.52	1.10	4.92
Gestational age (weeks)	241	38.03	38.00	1.48	1.54	30.0	42.00
Household SES	250	0.04	0.18	1.13	0.98	− 2.65	1.69
Maternal education	235	4.29	4.00	1.48	1.00	1.00	6.00

Note: SD = Standard Deviation, Min = Minimum, Max = Maximum, Mad = Median absolute deviation

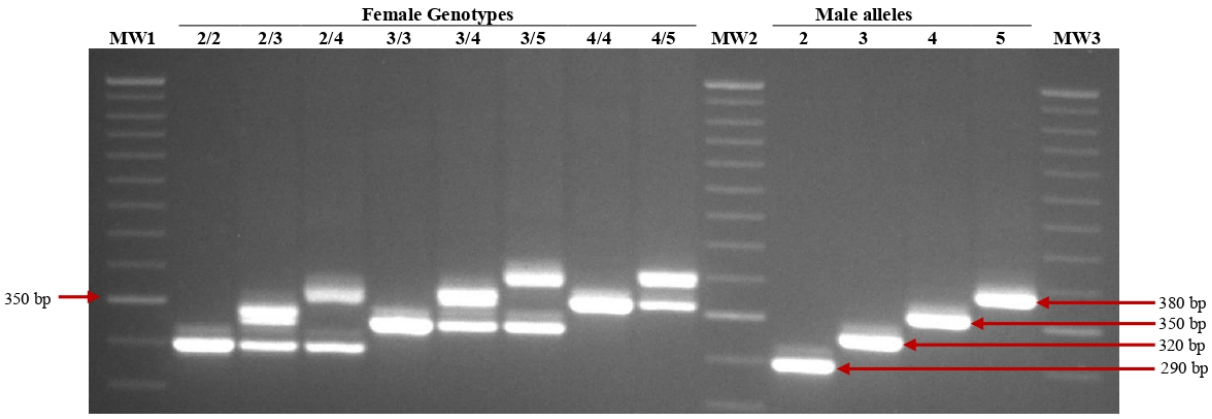

Figure 3.1: Gel electrophoresis image showing female genotypes and male alleles: Shown here are the female genotypes (left of MW2) and male alleles (right of MW2) as seen on the gel image. The amplicons were sized against a 50 bp DNA molecular weight marker. The 290 bp amplicon corresponds to the 2 repeat allele; the 320 bp amplicon corresponds to the 3 repeat allele; the 350 bp amplicon corresponds to the 4 repeat allele, and the 380 bp amplicon corresponds to the 5 repeat allele.

3.2.1 Allele and genotype frequency

From Table 3.4 (where allele and genotype frequencies are reported) and the results of a t-test, the observed allele frequencies were similar (χ^2 = 1.1721, p > 0.5) between males (2 = 7.2%,

3% = 40.7%, 4 = 50.1%, and 5 = 2%) and females (2 = 6.2%, 3 = 39.8%, 4 = 52.3%, and 5 = 1.7%). This similarity was important as Hardy-Weinberg calculations can only be performed in females and used as a proxy for the males (Kim-Cohen *et al.*, 2006, Byrd & Manuck, 2014).

Table 3.3: MAOA μVNTR allele frequences for selected populations across the globe

Population Group	Ethnic group	Number Tested	MAOA Allele frequency %						Reference
			1.5	2	3	3.5	4	5	
American	Caucasian	3356	0	0.03	34.4	1.6	62.6	1.3	Haberstick *et al.*, 2014
African American	Black	618	0	4.7	48.7	0.2	45.5	0.9	Reti *et al.*, 2011
African American	Black	960	0	4.8	51.0	0.01	43.3	0.7	Haberstick *et al.*, 2014
Chinese	Asian	214	0	0.5	57	0	42	0.5	Lu *et al.*, 2002
Italian	Caucasian	180	0	1.7	40	0	56.6	1.7	Deckert *et al.*, 1999
German	Caucasian	390	0	0.8	35.9	0.8	61	1.5	Kuepper *et al.*, 2013
New Zealand	Caucasian	442	0	0.2	33.7	1.1	62	2.9	Sabol, Hu & Hamer, 1998
Pacific islanders	Asian	82	0	0	61	1.2	37.8	0	Sabol, Hu & Hamer, 1998
Brazilian	Native	235	0	0.4	31.1	0.4	66.4	1.7	Contini *et al.*, 2006
Sweden	Caucasian	1522	0	0	36.6	1.4	61.4	0.5	Åslund *et al.*, 2011
South African	Caucasian	196	0	0	28.1	0	68.4	3.6	Erasmus, Klingenberg & Greeff, 2015
South African	Black	250	0	6.1	41.1	0	46	1.9	Hemmings *et al.*, 2018
Iraqi	Asian	440	1.1	0	35.8	0	52	11.1	AL-Tayie & Ali, 2018

Table 3.4: Allele and genotype frequencies of the MAOA μVNTR

Genotypes		2/2	2/3	2/4	3/3	3/4	3/5	4/4	4/5
Female n = 593	% (Count)	0.7 % (4)	6.2 % (37)	4.7 % (28)	14.2 % (84)	42.8 % (254)	1.4 % (8)	28 % (166)	2 % (12)
Alleles		2	3	4	5				
Females n = 1174	% (Count)	6,2 % (73)	39,8 % (467)	52.3 % (614)	1.7 % (20)				
Males n = 543	% (Count)	7.2 % (39)	40.7 % (221)	50.1 % (272)	2 % (11)				
Binary encoding		Low-activity = 0		High-activity = 1					
Females n = 299	Count	133		166					
Males n = 543	Count	271		272					
5-HTTLPR alleles		Short		Long					
Females n = 591	Count	221		370					
Males n = 558	Count	199		359					

Hardy-Weinberg in females: p 0.1

The Hardy-Weinberg test performed indicates that females were in Hardy-Weinberg equilibrium ($p > 0.1$). However, important to note from Table 3.4, is the number of usable observations reported as binary (high- vs low -activity) encoded alleles. This estimate showed that females had the smallest number of usable observations (n = 299) compared to males (n = 543). These allele frequencies were compared to selected populations as reported in Table 3.3. Due to the large inter-population genetic variability of the μVNTR alleles, frequencies could only be compared to other populations where the 3.5 repeat allele was not observed, such as Black South Africans, Italians, and some Chinese populations. Fisher's exact and Pearson's χ^2 tests demonstrated that the observed allele frequencies in the BTT+ cohort were similar to those observed in Xhosa South Africans (Fisher's exact, $p > 0.5$; $\chi^2 = 0.47882$, $p > 0.5$). However, these tests further demonstrated that the observed allele frequencies were significantly different from both Italian (Fisher's exact, $p < 0.05$; $\chi^2 = 8.2706$, df = 3, $p < 0.05$) and Chinese populations (Fisher's exact, $p < 0.001$; $\chi^2 = 26.536$, df = 3, $p < 0.001$).

3.3 Zero-order correlation

Zero-order correlations were performed between variables for both males and females and are represented in Figures 3.2 and 3.3. In females, significant female correlations were seen between internalising and externalising behaviour, internalising behaviour and birth weight, and internalising behaviour and the 5-HTTLPR. Within males, there were significant correlations between internalising and externalising behaviour, internalising behaviour and 5-HTTLPR, externalising behaviour and birth weight, externalising behaviour and gestational age, and highly sensitive person scale and birth weight. While both internalising and externalising behaviour had multiple correlations, neither were directly correlated with the μVNTR. Additional correlations between variables were examined and reported in Section 3.4.2 as they pertain to interaction QC. The complete correlation statistics for all inter-variable correlations performed can be found in Table E.4.

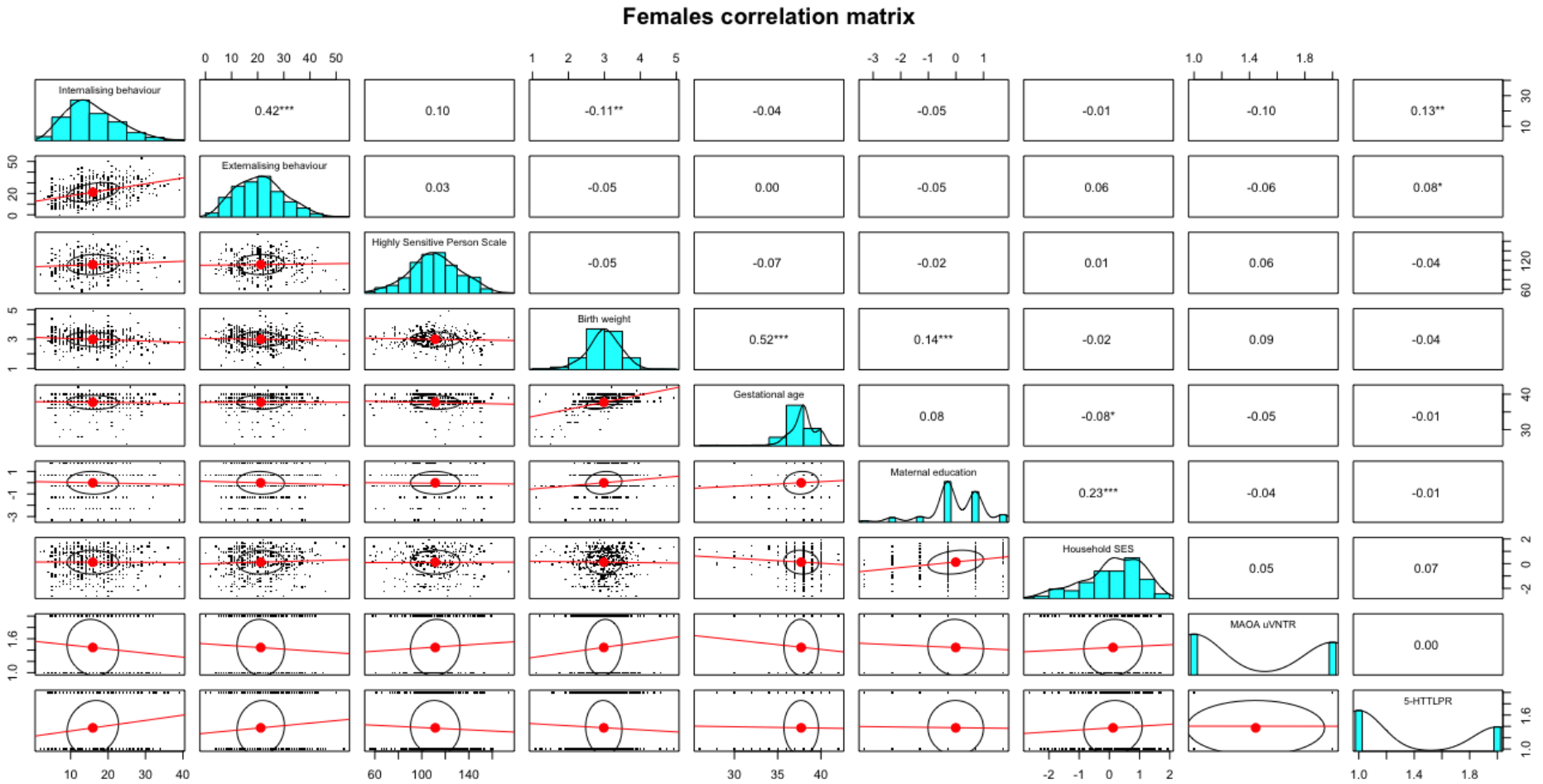

Figure 3.2: Correlation scatter matrix for females: This figure depicts the inter-variable correlation matrix (top right), a variable distribution graphs (diagonal), and an inter-variable scatter plot matrix (bottom left) in males. Important to note regarding the correlation matrix is the the significance notation added to the correlation statistics $^{*}p<0.1$; $^{**}p<0.05$; $^{***}p<0.01$.

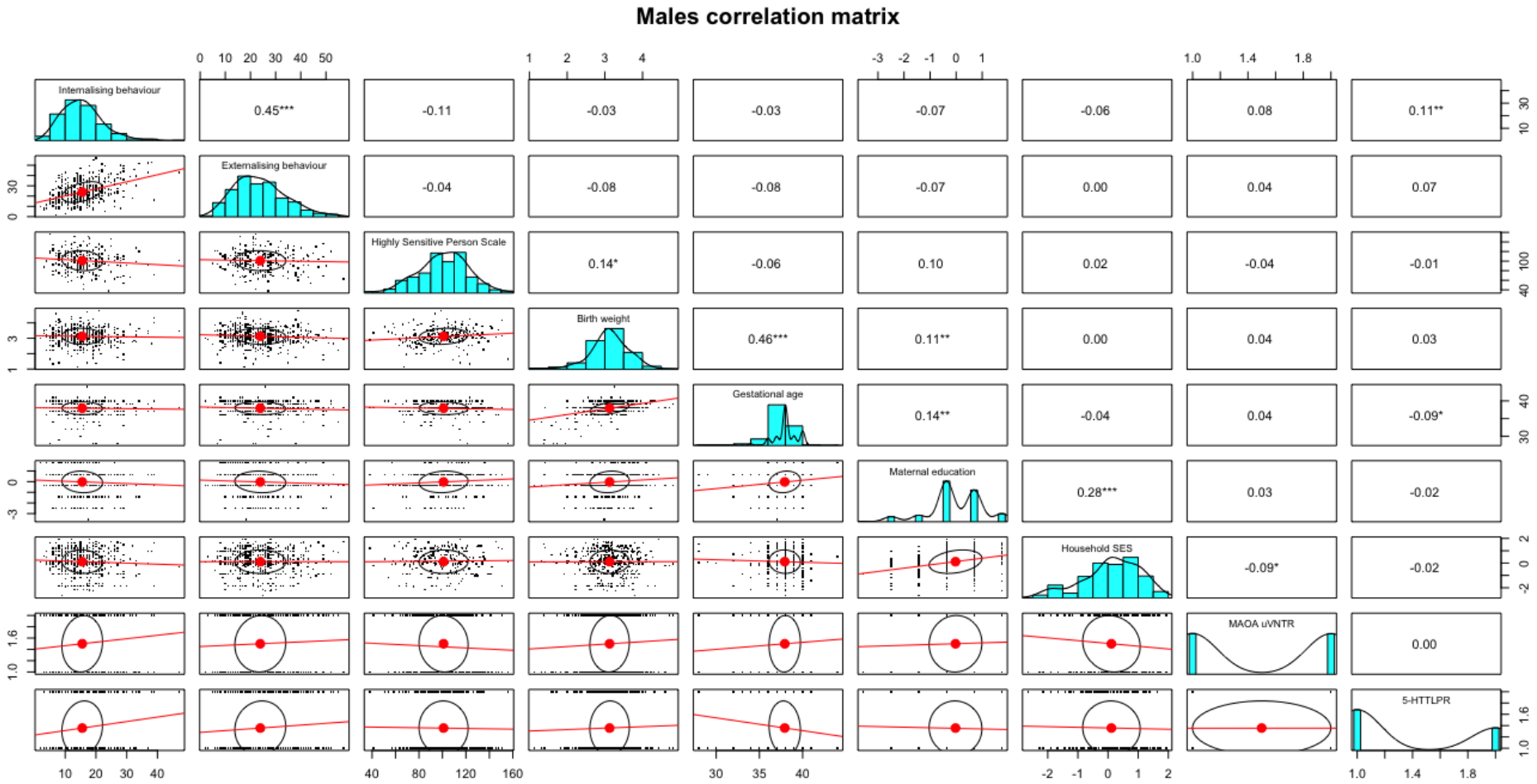

Figure 3.3: Correlation scatter matrix for males: This figure depicts the inter-variable correlation matrix (top right), a variable distribution graphs (diagonal), and an inter-variable scatter plot matrix (bottom left) in males. Important to note regarding the correlation matrix is the the significance notation added to the correlation statistics $^{*}p<0.1$; $^{**}p<0.05$; $^{***}p<0.01$.

3.4 The *MAOA* μVNTR allele effect on behaviour in males

To determine whether the μVNTR affected behaviour in males, tests were conducted to investigate outcome score differences between the high and low-activity allele groupings of the μVNTR variant. These mean score differences are represented in Figure 3.4, where a two-point mean score difference was observed in internalising and externalising behaviour between the high- and low-activity μVNTR alleles. Furthermore, regarding the HSPS measurement, a four-point mean score difference was observed between the different μVNTR alleles. However, a Wilcoxon-rank-sum test showed that the only significant score differences were seen in internalising behaviour, where the μVNTR low-activity allele carriers had higher internalising behaviour scores compared to high-activity allele carriers (W = 32334, $p < 0.05$).

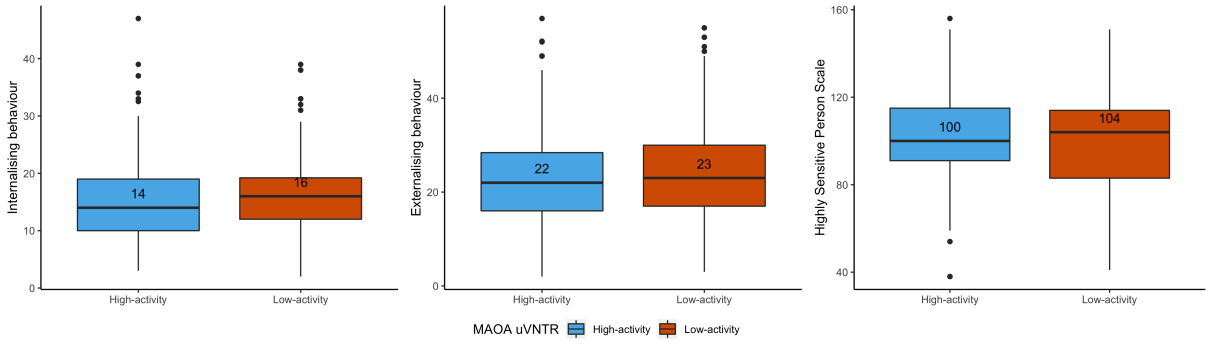

Figure 3.4: Outcome score change by μVNTR low- versus high-activity alleles in males: Score change for internalising, externalising, and HSPS measurements by a change in μVNTR functional encoding.

3.4.1 Male *MAOA* μVNTR main effect regression models

Following from Section 3.4, exhaustive modelling was performed to determine the most suitable combination of variables accounting for behavioural variation. The final variables included in each model and the regression results are reported in Table 3.5. Importantly, the μVNTR was significantly associated with changes in internalising (β = 0.09, $p < 0.05$) and externalising (β = 0.12, $p < 0.05$) behaviour scores Figure 3.5, but not for SPS. In addition, the 5-HTTLPR and maternal education showed a near significant association with internalising behaviour, while maternal education was also significantly associated with externalising behaviour. Variables significantly associated with HSPS included birth weight and gestational age but not maternal

education. Interestingly, in a main effect capacity, the μVNTR low-activity alleles appeared to led to an increase in behavioural problems for both internalising and externalising behaviour. The internalising behaviour model had an F(3.415) with p-value < 0.01. *Post hoc* power analysis revealed that this model was only 42% powered to identify a main effect of the *MAOA* μVNTR (B = 1.18 at n = 507). Similarly, the power analysis conducted on the externalising behaviour model F(3.224) with p-value < 0.05 suggests that this model was 38% powered to identify the main effect of the *MAOA* μVNTR (B = 2.30 at n = 280). Although not significant, within this model the low-activity μVNTR allele appeared to lead to a decrease in sensory processing sensitivity.

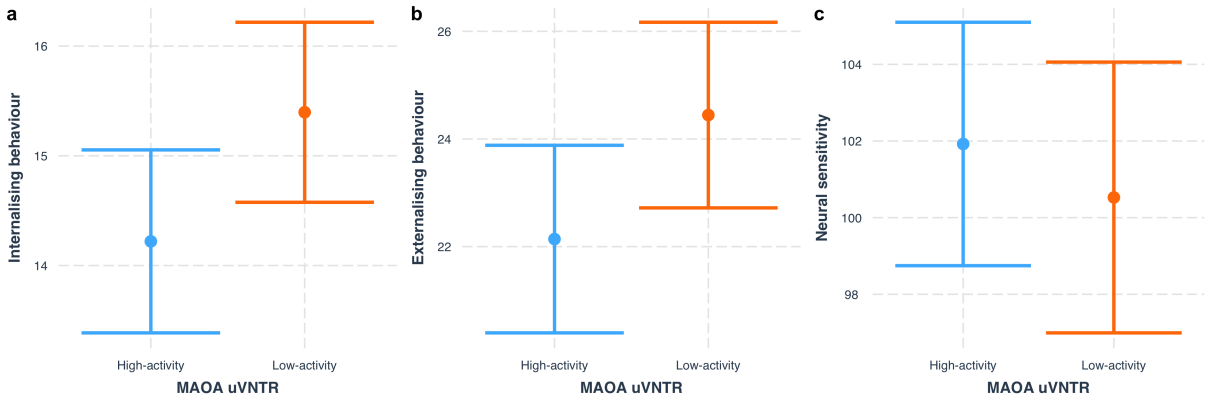

Figure 3.5: Main effect regression plots for males: Depicted here are the score differences of each μVNTR allele for behaviour in males. Males with a low activity allele appeared to score higher on a) internalising behaviour and b) externalising behaviour, but lower on c) SPS, when compared to males with a high activity allele.

3.4.2 Interaction effects of the *MAOA* μVNTR on behaviour in males

Similar to Section 3.4.1 regarding the main effect models in males, an exhaustive variable selection approach was used to determine the best combination of variables to include in the interaction analysis; a linear model was then constructed with these variables. Two measurements of childhood environment were investigated for possible interactive effects with the μVNTR and included household SES and maternal education. Here, as within the literature, maternal education was investigated as it is a strong predictor of child cognitive development, child well-being, and family financial status (Jackson, Kiernan & McLanahan, 2017). Additionally, as discussed in Section 3.3, before an interaction analysis can be

Table 3.5: Main effect regression models for males

	Dependent variable:		
	Internalising behaviour	Externalising behaviour	Highly Sensitive Person Scale
Constant	14.24 p = 0.00*** β = 0.00	24.90 p = 0.00*** β = 0.00	148.47 p = 0.00*** β = 0.00
MAOA μVNTR low-activity	1.18 p = 0.028** β = 0.09	2.30 p = 0.041** β = 0.12	−1.4 p = 0.568 β = −0.03
5-HTTLPR short allele	1.01 p = 0.07* β = 0.08	1.39 p = 0.23 β = 0.07	
Birth weight			7.74 p< 0.01*** β = 0.21
Gestational age			−1.86 p = 0.02** β = −0.16
Maternal education	−0.53 p = 0.056* β = −0.09	−1.52 p = 0.01** β = −0.15	2.24 p = 0.09* β = 0.10
household SES	−0.20 p = 0.50 β = −0.03		
HSPS		−0.03 p = 0.30 β = −0.06	
Observations	507	280	279
R^2	0.026	0.045	0.053
Adjusted R^2	0.019	0.031	0.039
Residual Std. Error	5.956 (df = 502)	9.179 (df = 275)	19.840 (df = 274)
F Statistic	3.415*** (df = 4; 502)	3.224** (df = 4; 275)	3.847*** (df = 4; 274)
Note:			*p<0.1; **p<0.05; ***p<0.01

performed, a rGE correlation investigation must be undertaken to ensure the interacting variables do not strongly correlate with one another. To test this assumption, point biserial correlations were performed between the μVNTR and both household SES and maternal education. This investigation showed that both maternal education (ρ = 0.055) and household SES (ρ = 0.053) were both poorly correlated to the μVNTR.

3.4.2.1 The interaction between the *MAOA* μVNTR and household SES in males

Table 3.6 contains the results of the interaction analysis performed to test for possible interactive effects between the μVNTR and household SES. No significant interaction was observed for any of the outcomes (internalising behaviour, externalising behaviour, nor the highly sensitive person scale). This result was supported by the graphical representation of

these models in Figure 3.6, where only a small score change was observed for either internalising behaviour (Figure 3.6a) or externalising behaviour (Figure 3.6c) as a product of the environment. However, a contrasting effect was observed in HSPS regarding the interaction between the μVNTR and household SES (Figure 3.6e), although, this was not supported by the regression analysis. According to the graph, sensitivity in individuals with a low-activity allele decreased as the environment became more supportive, while the inverse was seen in high-activity allele individuals.

Table 3.6: MAOA μVNTR household SES interaction models for males

	Dependent variable:		
	Internalising behaviour	Externalising behaviour	Highly Sensitive Person Scale
Constant	14.16 p = 0.00 *** β = 0.00	24.12 p = 0.00*** β = 0.00	120.58 p = 0.00*** β = 0.00
MAOA μVNTR low-activity	1.41 p = 0.01** β =0.11	2.01 p = 0.09* β = 0.10	−1.39 p = 0.57 β = −0.04
Household SES	−0.36 p = 0.38 β =−0.06	−0.61 p = 0.47 β =−0.06	0.82 p = 0.63 β = 0.04
5-HTTLPR short allele	1.35 p = 0.02** β = 0.10	2.02 p = 0.09* β =0.10	
Birth weight			8.25 p = 0.001*** β = 0.23
Gestational age			−1.18 p = 0.133 β = −0.10
Maternal education	−0.44 p = 0.129 β = −0.07	−1.60 p = 0.01*** β = −0.16	2.28 p = 0.08* β = 0.11
HSPS		−0.02 p = 0.52 β = −0.04	
MAOA μVNTR X Household SES	0.27 p = 0.64 β = 0.03	0.30 p = 0.81 β = 0.02	−2.12 p = 0.41 β = −0.07
Observations	508	283	277
R^2	0.032	0.049	0.060
Adjusted R^2	0.023	0.029	0.039
Residual Std. Error	6.182 (df = 502)	9.429 (df = 276)	19.207 (df = 270)
F Statistic	3.353*** (df = 5; 502)	2.390** (df = 6; 276)	2.854** (df = 6; 270)
Note:			*p<0.1; **p<0.05; ***p<0.01

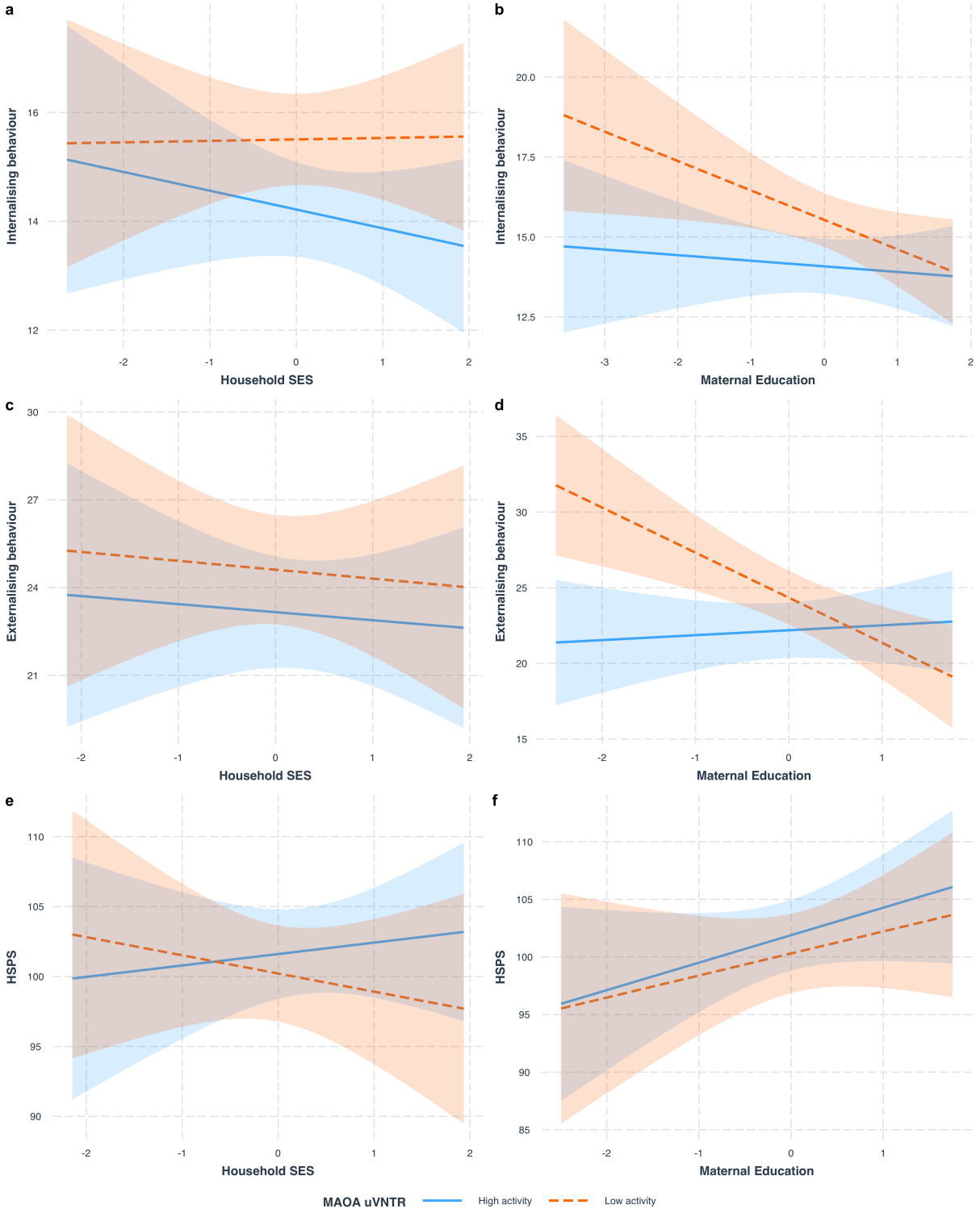

Figure 3.6: Interaction regression plots for males: The interaction plots in males for the μVNTR x household SES on the left and the μVNTR x maternal education on the right. Panel a) and b) deal with the interactions of internalising behaviour score while panel c) and d) deal with externalising behaviour. Lastly, panel e) and f) look at the HSPS outcome as a measurement of sensory processing sensitivity.

3.4.2.2 The interaction between *MAOA* μVNTR and maternal education in males

The results of the interaction analysis between the μVNTR and maternal education are reported in Table 3.7. In modelling this interaction, the only significant gene x environment interaction model was observed for externalising behaviour ($\beta = -0.23$, $p < 0.01$). The regression model had an F(3.353) with a p=value < 0.01. The *post hoc* power analysis suggests that this interaction model was more than 80% powered to detect an interaction with B = 3.35 at n= 280. Notably, these results were in line with environmental sensitivity theory. Supported by Figure 3.6b and Figure 3.6d, individuals with the low-activity allele showed higher levels of behavioural problems in a poor environment while in a positive environment they appeared lower than high-activity allele carriers whose behaviours remain relatively unaffected throughout the environmental spectrum. The HSPS measure showed no interactive effects in males as a product of maternal education with the graph only showing a small difference in HSPS score between the μVNTR high- and low-activity alleles.

3.5 The *MAOA* μVNTR allele effect on behaviour in females

Similar to tests performed in males (Section 3.4), female behaviour scores were assessed for a relationship to the μVNTR low-activity allele which is depicted in Figure 3.7. The μVNTR low-activity allele was associated with a one-point decrease in a) internalising and b) externalising behaviour, and a three and a half-point increase in c) sensitivity scores. A Wilcoxon-rank-sum test, however, revealed that the behavioural score change observed were not significant for internalising behaviour (W = 12120, p > 0.1), externalising behaviour (W = 11864, p > 0.1), nor the Highly Sensitive Person Scale (W = 3553, p > 0.1).

3.5.1 Female *MAOA* μVNTR main effect regression models

The female main effect regression model results are reported in Table 3.8 and graphically represented in Figure 3.8. Here, the μVNTR low-activity allele was associated with a decrease in internalising behaviour ($\beta = -0.17$, $p < 0.05$) but not externalising behaviour or HSPS measures. However, important to note is that the only model which had a significant fit was internalising behaviour (F = 4.85, $p < 0.01$). The *post hoc* power analysis suggests that this

Table 3.7: MAOA μVNTR maternal education interaction models for males

	Dependent variable:		
	Internalising behaviour	Externalising behaviour	Highly Sensitive Person Scale
Constant	14.24 p = 0.00*** β = 0.00	23.71 p = 0.00*** β = 0.00	148.89 p = 0.00*** β = 0.00
MAOA low-activity	1.21 p = 0.02** β = 0.10	2.09 p = 0.06* β = 0.11	−1.41 p = 0.56 β = −0.03
Maternal education	−0.19 p = 0.59 β = −0.03	0.28 p = 0.72 β = 0.03	2.58 p = 0.13 β =0.12
HSPS		−0.01 p = 0.66 β = −0.02	
5-HTTLPR short	1.11 p = 0.05** β = 0.09	1.13 p = 0.34 β = 0.06	
Household SES	−0.21 p = 0.46 β = −0.03		
Birth weight			7.94 p < 0.01*** β = 0.21
Gestational age			−1.89 p = 0.02** β = −0.17
MAOA low-activity X Maternal education	−0.83 p = 0.12 β = −0.09	−3.35 p < 0.01*** β = −0.23	−0.93 p = 0.72 β = −0.03
Observations	507	280	280
R^2	0.034	0.058	0.053
Adjusted R^2	0.024	0.040	0.036
Residual Std. Error	5.929 (df = 501)	9.213 (df = 274)	19.840 (df = 274)
F Statistic	3.483*** (df = 5; 501)	3.353*** (df = 5; 274)	3.088*** (df = 5; 274)
Note:			*p<0.1; **p<0.05; ***p<0.01

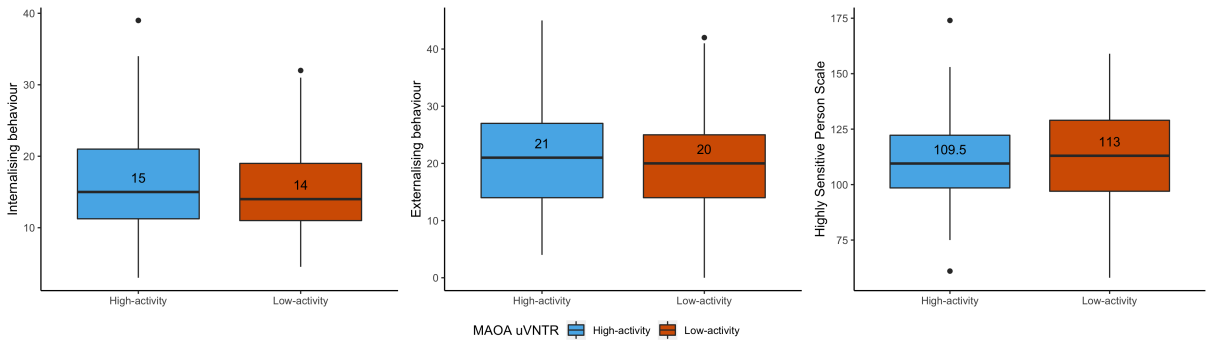

Figure 3.7: Outcome score change by μVNTR low- versus high-activity alleles in females: Score change for internalising, externalising, and HSPS measurments by a change in μVNTR functional encoding in females.

model was underpowered at 37% to detect the main effect of the *MAOA* μVNTR on internalising behaviour at B = 1.41 and n = 160. While low-activity allele females had significantly lower internalising behaviour scores compared to high-activity carriers, HSPS was the best predictor of internalising behaviour in females within this model (β = 0.24, p < 0.01).

Table 3.8: Main effect regression models for females

	Dependent variable:		
	Internalising behaviour	Externalising behaviour	Highly Sensitive Person Scale
Constant	32.57 p = 0.01** β = 0.00	38.38** p = 0.03** β = 0.00	112.14 p = 0.00*** β = 0.00
MAOA μVNTR low-activity	$-$2.04 p = 0.02** $\beta = -0.17$	$-$1.41 p = 0.28 $\beta = -0.09$	2.94 p = 0.31 β = 0.08
Birth weight	$-$1.21 p = 0.23 $\beta = -0.10$		
Gestational age	$-$0.35 p = 0.33 $\beta = -0.08$	$-$0.48 p = 0.31 $\beta = -0.08$	
HSPS	3.21 p < 0.01*** β = 0.24	3.27 p = 0.03** β = 0.18	
5-HTTLPR Short allele			$-$5.99 p = 0.04** $\beta = -0.16$
Observations	162	160	168
R^2	0.110	0.043	0.032
Adjusted R^2	0.087	0.025	0.020
Residual Std. Error	5.593 (df = 157)	8.134 (df = 156)	18.437 (df = 165)
F Statistic	4.845*** (df = 4; 157)	2.351* (df = 3; 156)	2.692* (df = 2; 165)
Note:			*p<0.1; **p<0.05; ***p<0.01

3.5.2 Interaction effects of the *MAOA* μVNTR on behaviour in females

Interaction modelling was performed in line with the analysis in males in Section 3.4.2. Once again, rGE correlations were checked before any interaction analysis was undertaken. Similarly to males, maternal education (ρ = 0.075) and household SES (ρ = 0.072) were both poorly correlated to the μVNTR.

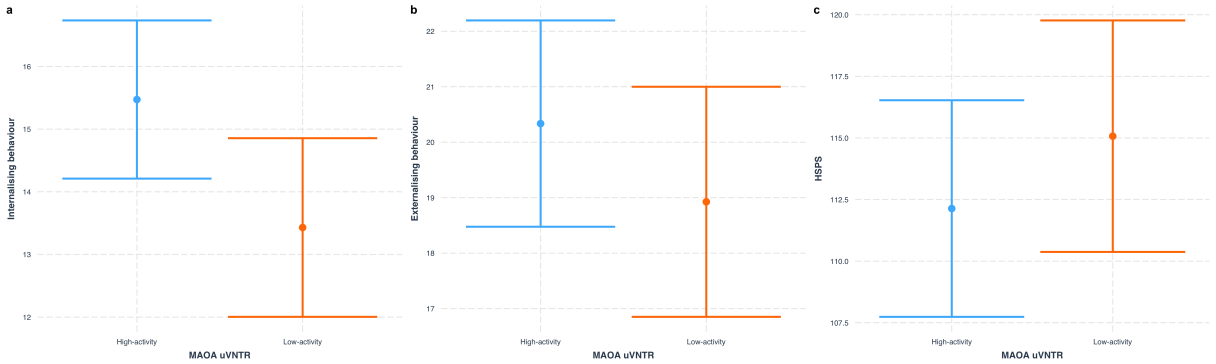

Figure 3.8: Main effect regression plots for females: Depicted here are the score changes in a) internalising and b) externalising behaviour and finally c) HSPS as a measurement of Sensory Processing Sensitivity in females.

3.5.2.1 The interaction between the *MAOA* μVNTR and household SES in females

Reported in Table 3.9 are the linear regression models for the interaction between the μVNTR and household SES in females. The only significant GxE interaction model observed here was for internalising behaviour (β = 0.33, p < 0.01) where the interaction between the low-activity allele was associated with an increase in internalising behaviour as household SES improved. This model had an F(4.837) with p-value < 0.01. *Post hoc* power analysis revealed that this model was over 80% powered to detect an interaction with B = 2.65 and n = 162. This is depicted in Figure 3.9a, which further shows that high-activity females' internalising behaviour scores decreased with an improved household SES, indicative of a contrasting effect.

3.5.2.2 The interaction between the *MAOA* μVNTR and maternal education in females

The results of the interaction regression models between the μVNTR and maternal education are shown in Table 3.10. The only significant GxE interaction model was observed for HSPS scores (β = $-$0.33, p < 0.01). Moreover, this model had an F(2.560) with p-value < 0.05. The subsequent *post hoc* power analysis suggests that this model was over 80% powered to detect an interaction with B = 8.984 and n = 163. Herein, possessing a low-activity allele was associated with a decrease in HSPS as maternal education improved. Represented in Figure 3.9f which resembles a contrasting effect, high-activity females show an increase in HSPS scores as maternal education improves while low-activity allele carriers HSPS scores

Table 3.9: MAOA μVNTR household SES interaction models for female

	Dependent variable:		
	Internalising behaviour	Externalising behaviour	Highly Sensitive Person Scale
Constant	37.94 p = 0.00*** β = 0.00	39.11 p = 0.03** β = 0.00	112.07 p = 0.00*** β = 0.00
MAOA μVNTR low-activity	−2.00 p = 0.02** β = −0.17	−2.18 p = 0.10* β = −0.13	2.92 p = 0.31 β = 0.08
Household SES	−1.25 p = 0.03** β = −0.23	0.76 p = 0.37 β = 0.10	2.82 p = 0.13 β = 0.16
HSPS	3.34 p < 0.01*** β = 0.25	3.36 p < 0.05** β = 0.18	
Birth weight	−1.27 p = 0.20 β −0.11		
Gestational age	−0.49 p = 0.16 β = −0.12	−0.49 p = 0.30 β = −0.08	
5-HTTLPR Short allele			−5.87 p = 0.05** β = −0.15
MAOA μVNTR X Household SES	2.65 p ,0.01*** β = 0.33	−0.67 p = 0.59 β = −0.06	−4.79 p = 0.08* β = −0.18
Observations	162	163	168
R^2	0.158	0.062	0.051
Adjusted R^2	0.125	0.032	0.028
Residual Std. Error	5.426 (df = 155)	8.188 (df = 157)	18.364 (df = 163)
F Statistic	4.837*** (df = 6; 155)	2.086* (df = 5; 157)	2.183* (df = 4; 163)
Note:			*p<0.1; **p<0.05; ***p<0.01

decreased within the same environment. Figure 3.9f depicts the modelled interaction between the μVNTR and maternal education, which showed a contrasting effect. This effect demonstrated that low-activity allele individuals experienced lower levels of sensitivity as maternal education increased, while those with a high-activity allele showed higher levels of sensitivity under the same environment.

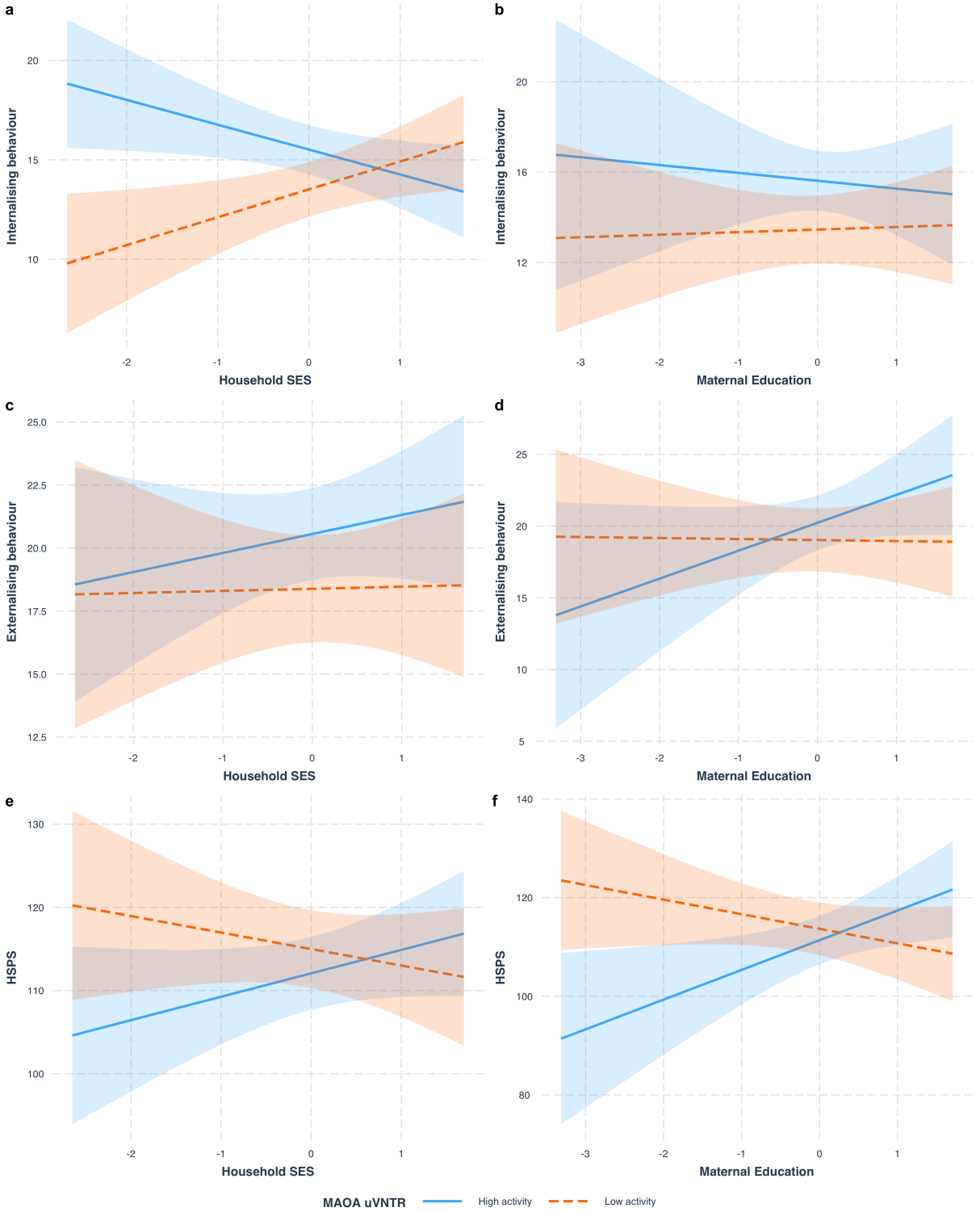

Figure 3.9: Interaction regression plots for females: The interaction plots in females for the μVNTR x household SES on the left and the μVNTR x maternal education on the right. Panel a) and b) deal with the interactions of internalising behaviour score while panel c) and d) deal with externalising behaviour. Lastly, panel e) and f) look at HSPS outcome as a measurement of Sensory Processing Sensitivity.

Table 3.10: MAOA μVNTR maternal education interaction models for female

	Dependent variable:		
	Internalising behaviour	Externalising behaviour	Highly Sensitive Person Scale
Constant	32.836 p = 0.01** β = 0	43.946 p = 0.02** β = 0	111.364 p = 0.00*** β = 0
MAOA μVNTR low-activity	$-$2.160 p = 0.02** β = $-$0.18	$-$1.203 p = 0.38 β = $-$0.07	2.317 p = 0.48 β = 0.06
Maternal education	$-$0.348 p = 0.69 β = $-$0.06	1.945 p = 0.09* β = 0.23	6.024 p = 0.02** β = 0.29
HSPS	3.142 p , 0.01*** β = 0.24	3.107 p = 0.05** β = 0.17	
Gestational age	$-$0.367 p = 0.32 β = $-$0.09	$-$0.625 p = 0.20 β = $-$0.10	
Birth weight	$-$1.090 p = 0.30 β = $-$0.09		
5-HTTLPR			$-$4.693 p = 0.16 β = $-$0.11
MAOA μVNTR X Maternal education	0.461 p = 0.67 β = 0.06	$-$2.016 p = 0.17 β = 0.19	$-$8.984 p , 0.01*** β = $-$0.33
Observations	152	152	163
R^2	0.105	0.061	0.061
Adjusted R^2	0.068	0.029	0.037
Residual Std. Error	5.687 (df = 145)	8.229 (df = 146)	20.350 (df = 158)
F Statistic	2.842** (df = 6; 145)	1.899* (df = 5; 146)	2.560** (df = 4; 158)
Note:			*p<0.1; **p<0.05; ***p<0.01

4. Discussion

This chapter will highlight the key findings from this study and discuss their relevance to the South African BTT+ cohort members, their environment, and human behaviour. Furthermore, these findings will be compared and contrasted against research on the *MAOA* μVNTR and its effects on behaviour with a particular focus on gene x environment interactions. Additionally, the limitations of this study will be discussed together with recommendations for future research.

4.1 Significant association findings

This study investigated the relationship between the *MAOA* μVNTR alleles and behaviour. The relationships investigated include the main effect of the *MAOA* μVNTR and the interaction of this variant with childhood environment. Participants were separated according to sex with significant associations observed amongst both males and females.

4.1.1 Main effect findings for males

Regarding the main effect of the μVNTR on behaviour in males, this study identified two significant associations, one for internalising behaviour (β = 0.09, p < 0.05) and another for externalising behaviour (β = 0.12, p < 0.05). From the analysis performed within this study, males possessing a low-activity allele scored higher on both internalising and externalising behaviour scales. This result implies that male BTT+ cohort members with a low-activity allele show higher levels of behavioural problems compared to high-activity allele males. The results reported in this study support what has been observed by most studies on the μVNTR and externalising behaviour in males but not internalising behaviour (as discussed below).

Armstrong *et al.* (2014) showed that within incarcerated men (n = 99 male inmates, age = 18-64 years), those with a low-activity allele had higher rates of serious violent crime than

high-activity allele inmates. This effect, where low-activity allele males show more criminal tendencies, has been further corroborated by Haberstick *et al.* (2014) (n = 3356 Caucasian and 960 African American males, age = 24-34 years). They reported that these low-activity allele males show higher rates of crime and more criminal convictions than high-activity allele men. While the results of this study correspond to the studies by Armstrong *et al.* (2014) and Haberstick *et al.* (2014), both these studies focused on self-reported criminality rather than just general externalising behaviour. Choe *et al.* (2014) (n = 83 African American and 106 Caucasian males, age = 17 years) showed that low-activity men had higher externalising behaviour scores than high-activity allele men. Nilsson *et al.* (2015) (n = 1337, 661 boys and 676 girls, age = 17-18 years) showed similar findings regarding externalising behaviour, however, they did not separate male and female participants during their analysis. These examples demonstrate the well-established relationship between the low-activity μVNTR allele and aberrant externalising behaviour. While the sample used within this study is non-clinical (not sampled from hospitals, clinics, or pathological patients), the same effect was observed where possessing the low-activity allele was associated with higher externalising behaviour in males.

Regarding internalising behaviour, very few studies have looked at the association between the μVNTR and internalising behaviour problems. Studies by Tzeng, Huang, Lee, *et al.* (2011) (n = 432 controls and 385 clinical cases) and Liu *et al.* (2017) (n = 148 boys and 193 girls, age = 4 years) suggested that possessing a low-activity μVNTR allele decreased a male's internalising behaviour problems while high-activity males' internalising behaviour was higher. These studies oppose what has been observed within this study where the low-activity allele was associated with an increase in internalising behaviour problems compared to high-activity groups within the literature (e.g. Kim-Cohen *et al.*, 2006). Some potential reasons for these differences are because Tzeng, Huang, Lee, *et al.* (2011) only detected a significant association in a clinical sample who already had depression; an association which was not shared in their control group. Furthermore, Liu *et al.* (2017) focused on a younger sample (age 4 years) which could influence this effect as the MAOA enzyme is important during neural development at a young age, especially during prenatal brain development (Wang *et al.*, 2011).

Regarding SPS, the main effect association between the μVNTR and HSPS (psychological measurement of sensitivity) showed no significant relationship (Figure 3.5c) ($\beta = -0.03$, p = 0.568). Furthermore, unlike the female models, HSPS in males was not a significant predictor of either internalising or externalising behaviour. Although Aron (1996) (who conceptualised SPS and the HSPS measure) postulated that the personality trait of SPS is rooted in one's genetic makeup, to the researcher's knowledge, only two studies have investigated genetic variants (the 5-HTTLPR, and dopamine-related variants) in predicting this trait (Chen *et al.*, 2011, Licht, Mortensen & Knudsen, 2011). While no study has investigated SPS as it relates to the μVNTR, a potential reason for the lack of association between HSPS and behaviour in males was the large temporal distance between the measurements of HSPS (age = 28 years) and behaviour (age = 7 years). Additionally, research suggests that males tend to under-report emotion and sensitivity. Both societal and cultural expectations have been implicated in this observation where males struggle to express emotion due to their perceived societal masculine roles (Boise & Hearn, 2017).

The effects observed within internalising and externalising behaviour correspond with what is known about the functioning of the MAOA enzyme and the μVNTR on this enzyme and related pathways (Sabol, Hu & Hamer, 1998). As discussed in Section 1.3.3.1, the MAOA enzyme is responsible for the degradation of serotonin, dopamine, and norepinephrine within the synapses (Fowler, Mantle & Tipton, 1982, O'Carroll *et al.*, 1983). Thus any reduction in the production of the MAOA enzyme (low-activity allele) corresponds to slower degradation of biological amines such as the 5-HTT neurotransmitter, and so doing, prolongs the effect of these neurotransmitters within the synapse. It is this prolonged effect of the neurotransmitters which is associated with higher neurosensitivity (CNS reactivity) and thus aberrant behaviour in a poor environment but positive outcomes in a nurturing environment (Pluess, 2015). Since the popularity of gene x environment studies has grown, the emphasis on the main effect of the μVNTR has declined. This is in part due to the realisation that variants such as the μVNTR do not act in isolation, but rather as one mechanism which increases CNS sensitivity. While many gene x environment interaction studies include main effect findings, the focus within the

literature has shifted to GxE interaction models (Pluess, 2015).

4.1.2 Observed gene x environment associations in males

Regarding the interaction analysis in males, this study identified a significant interaction between the *MAOA* μVNTR and maternal education ($\beta = -0.23$, $p < 0.01$). The interaction observed (Figure 3.6d) demonstrated a near differential susceptibility effect where low-activity allele males experienced higher levels of externalising behaviour compared to high-activity allele males. However, these low-activity individuals' externalising behaviour improved as maternal education improved, while high-activity males' scores remained stable throughout the environmental spectrum. Thus, possessing a low-activity variant was associated with susceptibility to environmental influences such that the outcome is proportional to the quality of the childhood environment. Research has shown that maternal education (not IQ) has been correlated with child cognitive development, child well-being, and family financial status, where the prevailing theory is that higher maternal education is associated with improved child development (Jackson, Kiernan & McLanahan, 2017). This might be a potential explanation for the interactive effect seen within the BTT+ cohort, which is consistent with Environmental Sensitivity theory.

The results reported here support the literature that the μVNTR does partake in interactions with childhood environment to cause aberrant behaviour. Although, this study further suggests that the effect follows the differential susceptibility hypothesis rather than a diathesis-stress model proposed by Caspi *et al.* (2002). As discussed in Section 1.4.2, under a differential susceptibility effect low-activity allele carriers experience a poor outcome under detrimental environments, while these same individuals also show the best outcomes in reinforcing ones (Belsky & Pluess, 2009). Pickles *et al.* (2013) (discussed in Section 1.4.2) investigated anger proneness in infants through the interaction of the μVNTR and maternal care (n = 1233 infant boys, age = 29 weeks - 14 months). Similar to the findings within this study, low-activity allele boys showed more anger proneness with less maternal care while these same boys also had the least behavioural problems with more maternal care, which is indicative of a differential susceptibility effect. The study by Pickles *et al.* (2013) is similar to this study in two ways, by

using a non-abusive childhood environmental variable, and in that their sample was non-clinical. Similarly, Cicchetti, Rogosch & Sturge-Apple (2007) (n = 184 males and 155 females, age = 16.7 years) investigated the interaction between the μVNTR and abuse on conduct disorders. From their analysis, they were able to show a similar differential susceptibility effect regarding low-activity allele carriers. While the effect observed within their study is similar to what was observed in this study, their research combined males and females into one group and used different forms of abuse as their environmental variable. As previously mentioned, the study by Armstrong *et al.* (2014) (n = 99 male inmates, age = 18-64 years) on delinquent behaviour in abused males - while their results were significant, they identified a purely diathesis-stress effect compared to a partial differential susceptibility one seen in this study. Although low-activity males did show more delinquent behaviour when abused, when not abused their behaviour did not improve over high-activity allele males (Armstrong *et al.*, 2014). One potential reason for their findings is that their environmental variable is solely and severely negatively focused on abuse, and thus, the effect of a supportive environment would not have been ascertainable through their analysis (Jolicoeur-Martineau *et al.*, 2017). Choe *et al.* (2014) (n = 83 African American and 106 Caucasian males, age = 17 years) investigated the moderating effect of the *MAOA* μVNTR on punitive discipline relating to antisocial behaviour and violent attitudes. Although the first interaction they identified (regarding antisocial behaviour) resembled a diathesis-stress model, the second interaction looking at violent attitudes resembled a differential susceptibility one. These violent attitudes, although higher in low-activity allele carrying boys, were also the lowest with less punitive discipline (Choe *et al.*, 2014). The discrepancy seen between antisocial behaviour and violent attitude could be due to the small sample size and severe environmental variable analysed. This work by Choe *et al.* (2014) further demonstrates the need for more comprehensive studies with more diverse behavioural outcomes. Nilsson *et al.* (2015) reported two significant interactions regarding the *MAOA* μVNTR on delinquency, one with family conflict and the other with sexual abuse. However, the researchers did not include any graphical models, and as such, it is difficult to determine the allele-specific effects. To highlight the different approaches used, a recent study by Zhang *et al.* (2017) (n = 546 males, age = $\pm$15 years) showed no significant two-way interaction, however, they did identify significant three- and four-way

interactions with the 5-HTTLPR alongside the μVNTR, sexual abuse, and physical abuse. The work done by Zhang *et al.* (2017), although negative regarding the μVNTR, further highlights the complexity of these traits and effects which need to be investigated in further detail.

Although most of the studies discussed here report significant interaction analyses, only a small subset of these has further investigated these relationships in the context of Environmental Sensitivity theory (Nilsson et al., 2018). As mentioned, the most common method used within the literature on the μVNTR is by graphical methods (as used within this study). To do so, the modelled pattern of each regression line (high- versus low-activity) are inspected to determine how the outcome varies across the environmental spectrum. Based on Figure 3.6d, the interaction between the μVNTR and maternal education resembles a partial differential susceptibility effect. To further investigate the possible differential susceptibility effect seen within this model, recently developed statistical protocols were applied as a *post hoc* analysis following GxE interaction modelling (Roisman *et al.*, 2012). The statistical tests include proportion of interaction (POI) (to investigate the proportion of the interaction situated on either side of the crossover), region of significance (ROS) (to determine for which values of x the modelled slopes are significant), and proportion affected (PA) (the proportion of high risk individuals influenced within the interaction) (Roisman *et al.*, 2012, Jolicoeur-Martineau *et al.*, 2017). Following this *post hoc* analysis (data not shown), the interaction model between the μVNTR and maternal education only met some of the requirements regarding POI, ROS, and PA needed to confirm a differential susceptibility effect, and was thus termed a partial differential susceptibility effect. This is in part due to the limited scope of the environmental component used which was negatively skewed.

The gene x environment effect observed (regarding externalising behaviour) in male BTT+ cohort members demonstrates a novel partial differential susceptibility finding of the μVNTR in a South African sample. This finding confirms that low-activity allele carrier males have heightened sensitivity to the effects of the South African environment which is characterised by high rates of poverty and other disparities (see Section 2.1). The effects seen in this study coincide with much of the literature on this effect (Byrd & Manuck, 2014), however, this study

is novel as it is the first to demonstrate this effect in a Black South African non-clinical sample applying a non-abusive environmental modifier. While the exact molecular mechanism is not entirely understood, it is believed that the combination of genes and the environment provides an individual with a more sensitive CNS. In the case of the *MAOA* μVNTR (as discussed in Section 4.1.1), reduced expression of the MAOA enzyme results in a slower turnover of neurotransmitters such as 5-HTT. This reduced efficacy, in turn, results in an individual processing stimulus more easily and in greater depth.

4.1.3 Main effect findings in females

Looking at the main effect of the μVNTR on behaviour in females, the only significant association observed was for internalising behaviour ($\beta = -0.17$, $p < 0.05$). Amongst females, possessing a low-activity allele was associated with less internalising behavioural problems compared to high activity allele females. Thus, female low-activity allele/genotype members of the BTT+ cohort showed less aberrant internalising behaviour than their high-activity allele counterparts. While this result supports what has been seen within the few studies in females, this result contradicts what is seen within males and the theory related to the effect of the μVNTR. In males, the low-activity μVNTR allele is generally associated with an increase in aberrant behaviour, however, in females, this effect appears to be inverted but in-line with the literature on females.

Deckert *et al.* (1999) (n = 209 German and Italian females) found that females with panic disorder had presented more frequently with a high-activity μVNTR allele or genotype. This was one of the first studies to perform an association test in females. Schulze *et al.* (2000) (n = 100 clinical females) showed that the high-activity μVNTR allele/genotypes were associated with heightened susceptibility to major depressive disorder in females with traumatic histories. Put differently, low-activity allele/genotype females showed fewer symptoms of major depressive disorder than high-activity grouped females. While their study was conducted in a clinical sample, the effect demonstrated here is supported by most studies on females regarding the μVNTR. Work done by Rivera *et al.* (2009) (n = 884 females) showed that high-activity μVNTR grouped females had higher levels of depressive symptoms than

low-activity grouped females among three different measures of depressive symptoms. Further work by McGrath *et al.* (2012) (n = 192 Caucasian females, age = 20+ years) investigated the relationship between the μVNTR alleles/genotypes on poor conduct. Their analysis showed that high-activity allele/genotype females showed significantly higher levels of problematic behaviour than low-activity allele females (McGrath *et al.*, 2012).

Although the effect observed within this study supports much of the literature on females where the high-activity μVNTR allele/genotype leads to an increase in aberrant behaviour, this inverted effect contradicts what was seen within males and with what is expected through the theories surrounding the *MAOA* μVNTR. The function of the μVNTR high- versus low-activity alleles and their mechanism is not well understood, however, two possible mechanisms accounting for the inverted effect have been proposed. Firstly, given the X-linked nature of this variant (Section 1.3.2) and the uncertainty regarding X-inactivation patterns in females (Hendriks *et al.*, 1992, Stabellini *et al.*, 2009), one hypothesis has been that incomplete X-inactivation could lead to an alternate *MAOA* expression profile (Byrd & Manuck, 2014). Alternatively, another explanation for the inverted allele effect is sex-specific methylation patterns. Philibert *et al.* (2008) (n = 96 females and 95 males) investigated the effect of methylation at the μVNTR locus had on its relationship with behaviour. They found that methylation status was significantly associated with alcohol and nicotine dependency in females but not males. They further showed that females were hypomethylated at this locus compared to males, with the three repeat allele (low-activity) showing the highest level of hypomethylation. This altered methylation status is suggested to cause an altered expression profile of the MAOA enzyme which results in observed sex-specific effects. Additionally, while the small sample size is one factor which could have lead to the negative findings regarding the relationship of the μVNTR and externalising behaviour and SPS, the negative finding for externalising behaviour is common throughout the literature (Nilsson *et al.*, 2018). Females tend to show less externalising behaviour, opting to rather internalise emotions compared to males (Craig & Halton, 2009).

4.1.4 Observed gene x environment associations in females

Regarding the females' GxE interaction models, only two interactions were significant, the interaction between the μVNTR and household SES in predicting internalising behaviour (β = 0.33, p < 0.01) and the interaction with maternal education predicting SPS (β = -0.33, p < 0.01). Looking at the interaction predicting internalising behaviour, similar to the female main effect models, an inverted allele effect was observed. Possessing a low-activity μVNTR allele/genotype was associated with an increase in internalising behaviour dysregulation as household SES improved. Inversely, high-activity females experienced a decreasing level of internalising behaviour within the same environment. This interaction depicted in Figure 3.9a resembles a contrasting effect with high-activity allele females scores decreasing and low-activity allele scores increasing as the environment improves. The second interaction looked at the level of sensory processing sensitivity as a product of the interaction between the μVNTR and maternal education. This model depicted in Figure 3.9f portrays a contrasting effect where low-activity allele/genotype females' SPS scores decrease as maternal education increases, while high-activity females' scores increases as maternal education improves. Put differently, high-activity allele/genotype females' sensitivity to the environment increases as the quality of the environment improves, while the inverse was seen in low-activity allele/genotype females.

The literature on female GxE interactions with the μVNTR is sparse, with conflicting results reported. However, the findings in this study regarding the interaction model on internalising behaviour in females is consistent with other reports within the literature. Firstly, the study by Eley *et al.* (2004) (n = 220 females and 157 males, age = 12-19 years) looking at depression and the study by Liu *et al.* (2017) (Section 4.1.1) looking at internalising behaviour were both unable to identify a significant interaction in females regarding the μVNTR. Furthermore, Lavigne *et al.* (2013) (n= 97 boys and 78 girls, age = 4 years) investigated depressive symptoms in males and females, however, none of the interactions within girls were significant. As discussed previously, the study by Cicchetti, Rogosch & Sturge-Apple (2007) investigated depressive symptoms through the interaction of various genes (including the μVNTR) and sexual abuse, while they did not initially identify any interaction, once they

grouped participants by the number of abusive incidents, an interaction was observed. The interaction identified showed that low-activity allele/genotype (grouped boys and girls) had more depressive symptoms compared to high-allele children. While some of the studies mentioned here do report significant findings, their results ultimately conflict with the results in this study as well as much of the literature on the μVNTR in females. The study by Cicchetti, Rogosch & Sturge-Apple (2007), although significant, showed that the low-activity allele was associated with behavioural aberrations rather the high-activity allele often noted in studies on females. Furthermore, many of the studies reported here, combine males and females into one group which could have altered the results observed.

Work done by Beach *et al.* (2010) (n = 280 females) on major depression looked at the interaction between the μVNTR and sexual abuse. They identified a significant interaction where high-activity allele/genotype females showed an increase in major depression when abused. Recall the study by McGrath *et al.* (2012) (which supports this finding) who investigated the interaction between the μVNTR and physical maltreatment leading to an increase in conduct problems. They showed that high-activity allele/genotype females who experienced physical abuse had significantly more conduct problems than low-activity grouped females in the same environment (McGrath et al., 2012). The interaction findings from this study regarding internalising behaviour in females corresponds with what has been observed within the literature. The high-activity allele/genotype is associated with more behavioural aberrations under poor environmental conditions. While these studies focused on abuse as its environmental modifier, their results still indicate that these same high-activity grouped females have the lowest behavioural problems in a supportive environment (Byrd & Manuck, 2014).

However, regarding the interaction between μVNTR and maternal education looking at sensory processing sensitivity, there was no obvious interpretation of the findings. To the researcher's knowledge, no other study has investigated SPS through a GxE interaction model with the μVNTR, and as such, no comparisons to previous literature could be made. However, this contrasting effect could aid in understanding the genetic contribution to this trait and

should be investigated further in future studies.

4.1.5 General implications for the BTT+ cohort

From the research presented here, it is clear that the *MAOA* μVNTR is involved in the development of aberrant behaviour in both male and female members of the Birth to Twenty Plus cohort. The low-activity variant had a direct association with an increase in internalising and externalising behaviour in males and a decrease in internalising behaviour in females. However, the μVNTRs' interaction with the environment revealed far more regarding the relationship between this variant and behaviour. In-line with Environmental Sensitivity effects, male externalising behaviour followed a differential susceptibility model, while female internalising behaviour resembled a contrasting effect. Given the historic economic and social disparities endured by cohort members in Soweto (Richter *et al.*, 2007), the adverse environment suggests that some (low-activity males and high-activity females) members are at risk for more aberrant behaviour. However, in line with theories on Environmental Sensitivity, so-called at-risk members could benefit from targeted intervention or precision medicine efforts. Recall the study by Morgan *et al.* (2017) (Section 1.4.2), who demonstrated the potential increase in treatment response experienced by susceptible individuals within a psychological intervention setting. Further evidence has suggested that the μVNTR variant is a possible target for a precision medicine approach, specifically in dosage response of Selective Serotonin Reuptake Inhibitors (SSRI). Younger *et al.* (2005) (n = 230 patients and 217 controls, age = 18-79) showed that low-activity allele/genotype females had responded significantly better than high-activity grouped females to a four-week treatment of fluoxetine. Although, the application of GxE interactions and Environmental Sensitivity models in precision therapy/medicine has been demonstrated in various studies (Belsky & IJzendoorn, 2015, Morgan *et al.*, 2017), applying these theories within South Africa would be challenging. Firstly, the *MAOA* μVNTR is only one variant implicated in sensitivity to the environment, the cost of typing this variant and others for the purpose of grouping individuals would strain the already overburdened health care sector within South Africa. Additionally, researchers within the field have highlighted the ethical conundrum surrounding such a policy; even if individuals could be grouped as susceptible or resilient, would serving only susceptible individuals be

acceptable (Belsky & IJzendoorn, 2015)? While the *MAOA* μVNTR has application in both intervention and pharmacogenetics, further investigation is needed regarding its utility, especially in a South African context.

4.2 Limitations

There are several limitations in this study worth noting. Firstly, except for the typing of the *MAOA* μVNTR, all other data were retrospectively collected. Consequently, this study had no control over the data collection, participants, or the instruments used. This resulted in skewed environmental measures resulting from an overly narrow selection criterion (Richter *et al.*, 2007). Since all participants were selected from the Soweto area, which was historically characterised by high poverty and other socio-economic disparities (Richter *et al.*, 2007), the environmental variables recorded at birth were highly negatively skewed. This was a large confounding factor within this study which is due to the criteria which differentiate between Environmental Sensitivity effects (differential susceptibility and diathesis-stress) and by causing a reduction in the statistical power to detect a differential susceptibility effect. The reason why skewed environmental variables affect such investigations is by limiting the spectrum in which these effects can be observed. Here, diathesis-stress is generally characterised by overly negative skewed environments, meaning the weight of the observations resides within the negative portion of the environment. Roisman *et al.* (2012) suggested that to observe a differential susceptibility effect, the observed range of the environment should be equal on either end of a variable's mean value. Often reported in standard deviations, differential susceptibility criteria recommend that two standard deviations on either side of the mean be visible for evaluation (Roisman *et al.*, 2012, Jolicoeur-Martineau *et al.*, 2017).

Another limitation resulting from this retrospective data is the large amount of data missingness which exacerbated the already small sample size. The work by Jolicoeur-Martineau *et al.* (2017) suggests that for small effect sizes ($R^2 = 0.05$) a study would need $n \geq 1000$ samples and medium effect sizes ($R^2 = 0.10$) $n \geq 500$ while large effect sizes ($R^2 = 0.15$) would need only $n \geq 250$ samples to have enough power to detect sensitivity effects through gene x environment interactions (Jolicoeur-Martineau *et al.*, 2017). The poor

power in this study is compounded by the small effect sizes observed and the small sample size due to the removal of various alleles/genotypes during the analysis. Although the BTT+ cohort offered a unique opportunity to investigate gene x environment interaction effects and differential susceptibility in a uniquely South African population, the retrospective nature of this study was ultimately a large limiting factor (Richter *et al.*, 2007). Firstly, while the BTT+ is one of the largest longitudinal cohorts in South Africa, a large temporal distance exists between the environmental data (collected at age 0 years) and the phenotypic data (collected at age 7 years). While this allowed for the investigation of the effects of childhood environment on later behaviour, a stronger effect could have been seen with data that were not so temporally distant. Another bias within the data existed in the characteristics of the data, the phenotypic behavioural data (SACAS) was caregiver-reported which is known to have an inherent social desirability bias (Sanzone *et al.*, 2013). At the time of this study, the statistical requirements for testing differential susceptibility effects (Roisman *et al.*, 2012) had not become available on statistical software such as R and R Studio. While the findings reported in this study supports the findings within the literature, the resulting models reported by this study would need to be validated in larger cohort.

4.3 Future directions

Comparing findings on the *MAOA* μVNTR to other studies is difficult based upon the various methods employed throughout the literature. Some studies exclude females, while those that include them remove all low-activity/high-activity (heterozygous) genotypes (Kim-Cohen *et al.*, 2006, Byrd & Manuck, 2014, Nilsson *et al.*, 2018). Additionally, a small subset of studies combine males and females into one group for analysis or only study individuals with a three or four repeat allele/genotype. Thus, future studies on the *MAOA* μVNTR should focus on all alleles in both males and females. However, the reasoning behind the various statistical strategies utilised within the literature lies in the fact that not enough basic studies have been performed on the *MAOA* μVNTR and the effect of each allele (Sabol, Hu & Hamer, 1998, Deckert *et al.*, 1999). Understanding the functional classification would allow future researchers to accurately group individuals into functional groups. Additionally, given the observed difference of effect between males and females (inverted effect of alleles), future

studies should include both males and females (separate analysis) of any study population so that these effects can be investigated and interrogated between sexes of the same population/community. Studies should further investigate mechanisms possibly responsible for the inverted allele effect at the *MAOA* gene (X-inactivation) and methylation and expression studies (Hendriks *et al.*, 1992, Philibert *et al.*, 2008, Stabellini *et al.*, 2009). Moreover, studies should separate participants by sex and not combine both sexes into one group given the difference in the effect of the μVNTR alleles. Although the data used in this study are longitudinal, this study did not make use of repeated measurements. Future studies should either make use of longitudinal data to investigate these effects throughout life or make use of data recorded concurrently to avoid issues related to temporal distance.

Modern behavioural genetics and work on *MAOA* started during the infancy of molecular genetic techniques, and as such, future research on the *MAOA* μVNTR and behavioural genetics should incorporate modern genetic techniques such as next generation sequencing (NGS). Firstly, the use of modern techniques, such as NGS, would allow for additional investigations into the contribution of genetics on behaviour. A recent paper by Palumbo *et al.* (2018) highlights the potential influence of epigenetic changes at the *MAOA* and 5HTTLPR loci, *inter alia*, on individual differences in behaviour. Herein they discuss the observations of research in animal models where altered epigenetic profiles at these loci further regulated the concentrations of these biological amines and so doing affected behavioural outcomes (Palumbo *et al.*, 2018). Although NGS in its current application is costly, the depth and breadth of information gathered through this technology far outweighs the reduction in cost offered by other techniques such as PCR based applications. An additional benefit from modern techniques lies in the ability to type many genes or variants. As behaviour and personality are multifactorial, some researchers have started creating polygenic risk scores to investigate polygenic plasticity through multi gene x environment interactions (Keers & Pluess, 2017). Moreover, various other genetic polymorphisms are located in the *MAOA* gene, such as the dVNTR (Manca *et al.*, 2018) or the SNP (rs72554632) identified by Brunner *et al.* (1993b). However, these variants comprise only a small percentage of cases such as Brunner syndrome identified in only two families and the alternative splice variant the dVNTR which

only comprises 5% of total MAOA transcripts. Future investigations should thus make use of NGS techniques to investigate the relationship between neurotransmitter genes and behaviour.

Another complication facing future studies of this nature is the required sample size. As previously mentioned, Roisman *et al.* (2012) and Jolicoeur-Martineau *et al.* (2017) discuss that the required sample size for gene x environment studies aimed at biological sensitivity is proportional to the effect size of the model (small = 1000, medium = 500, large = 250). An additional complication facing similar research in South Africa and abroad is the lack of reproducibility across populations/communities. Thus, when conducting gene x environment studies, researchers should investigate these effects within multiple populations/ethnicities to obtain an accurate measurement of these effects in each population group given the unique cultural and societal differences. In addition to investigating these effects across populations, there is a need for standardised instruments when evaluating behaviour, personality, and environment. This would allow for improved inter- and intra-study comparisons. An additional future recommendation would be to include more susceptibility variants such as the 5-HTTLPR as some studies suggest that the *MAOA* μVNTR moderates the interaction between the 5-HTTLPR and environmental stressors (Cicchetti, Rogosch & Sturge-Apple, 2007, Zhang *et al.*, 2017). Finally, while Roisman *et al.* (2012) have developed statistical measures for evaluating sensitivity (region of significance, the proportion of interaction, and the proportion affected) these measures have not been applied throughout the literature (Roisman *et al.*, 2012).

5. Conclusion

To conclude, this study identified significant relationships between the *MAOA* μVNTR and behaviour in the BTT+ cohort. The relationships observed took the form of both direct effects and interactions between genes (μVNTR) and childhood environments (household SES and maternal education). While studies aiming to elucidate these relationships have displayed various outcomes, theories regarding Environmental Sensitivity have produced meaningful observations. Similarly, within this study, a differential susceptibility and contrasting effect were observed which helped to explain inter-individual differences in behaviour. Furthermore, research has shown that differential susceptibility (an Environmental Sensitivity model) has clinical utility in treating behavioural dysregulation through psychological and pharmacological interventions. However, given the statistical requirements to uncover GxE and Environmental Sensitivity effects, this study only detected relationships of small effect size in a relatively small sample size. Additionally, a sex-specific effect of the μVNTR was observed in this study where the low-activity allele was associated to behavioural dysregulation in males while in females the high-activity allele was implicated. Future studies should thus investigate these effects in larger samples looking at a wider range of genetic variants in both sexes.

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A. Appendix A

UNIVERSITY OF THE
WITWATERSRAND,
JOHANNESBURG

R14/49 Mr Stephan Herman Wessels

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M180651

NAME: (Principal Investigator)	Mr Stephan Herman Wessels
DEPARTMENT:	Human Genetics National Health Laboratory Services (NHLS)
PROJECT TITLE:	The prevalence of the MAOA VNTR alleles and their relationship to aggression and sensory processing sensitivity amongst a South African cohort
DATE CONSIDERED:	29/06/2018
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Andrew May & Shelley Macaulay
APPROVED BY:	Doctor CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	03/09/2018

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary on the Third Floor, Faculty of Health Sciences, Phillip Tobias Building, 29 Princess of Wales Terrace, Parktown, 2193, University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in **June** and will therefore be due in the month of **June** each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature

10/09/2018
Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

B. Appendix B

DPHRU
MRC/Wits Developmental Pathways for Health Research Unit

4 June 2018

Professor C. Penny
Chairperson
Human Research Ethics Committee (Medical)
University of the Witwatersrand

Dear Professor Penny,

RE: Permission for Stephan Wessels to utilize Birth To Twenty Plus data for his MSc (Med) entitled "The prevalence of the *MAOA* VNTR alleles and their relationships to aggression and sensory processing sensitivity in black South Africans"

This letter serves to confirm that I give Stephan Wessels, under the supervision of Andrew May and Shelley Macaulay, permission to access and utilize the necessary Birth to Twenty Plus Cohort data including the South African Childhood Assessment Scale (SACAS) data and Highly Sensitive Persons Scale (HSPS) data for the purpose of his MSc (Med) project.

Yours sincerely,

Professor Shane Norris
Director
MRC/WITS Developmental Pathways for Health Research Unit
011-9331122
Shane.Norris@wits.ac.za

C. Appendix C

BIRTH TO TEN: 1997
SOUTH AFRICAN CHILD ASSESSMENT SCHEDULE (SACAS)

Today's Date | | | | | | | 1 | 9 | 9 | 7 |

BTT ID | | | | | | | |

CHILD'S NAME: _____
SURNAME: _____
INTERVIEWER: _____
WHO PROVIDED THE INFORMATION: _____
WHAT IS THE RELATIONSHIP OF THE INFORMANT TO THE CHILD? _____

I have some question I would like to ask you about problems and skills some children have. I would like you to tell me the extent to which it is true that your child has these. In responding tell me whether it is……

0 = NOT TRUE; 1 = SOMETIMES TRUE; 2 = OFTEN TRUE

0	1	2	1.	Does (CHILD) seem fragile or cry when an adult just looks at him/her?
0	1	2	2.	Does (CHILD) accept and listen to criticism calmly?
0	1	2	3.	Does (CHILD) accept restrictions from adults?
0	1	2	4.	Does (CHILD) act too young for His/Her age?
0	1	2	5.	Does (CHILD) adjust well to changes in the classroom routine?
0	1	2	6.	Is (CHILD) affectionate to others?
0	1	2	7.	Is (CHILD) an angry child?
0	1	2	8.	Does (CHILD) approach new experiences confidently, without fear?
0	1	2	9.	Does (CHILD) argue?
0	1	2	10.	Does (CHILD) avoid activities which S/He is not good at?
0	1	2	11.	Does (CHILD) brag or boast?
0	1	2	12.	Does (CHILD) bully or is S/He mean to others?
0	1	2	13.	Is (CHILD) unable to concentrate, pay attention for long?
0	1	2	14.	Is (CHILD) unable to get His/Her mind off certain thoughts?
0	1	2	15.	Is (CHILD) unable to sit still, does (CHILD) squirm?
0	1	2	16.	Can (CHILD) accept things not going His/Her way?
0	1	2	17.	Does (CHILD) carry out requests and directions responsibly?
0	1	2	18.	Does (CHILD) cling to adults, is (CHILD) too dependant?
0	1	2	19.	IS (CHILD) co-operative?

0	1	2	20.	Does (CHILD) complain of aches or pains in arms or legs?
0	1	2	21.	Does (CHILD) complain of dizziness?
0	1	2	22.	Does (CHIL) complain of headaches?
0	1	2	23.	Does (CHILD) complain of loneliness?
0	1	2	24.	Does (CHILD) complain of nausea of feeling sick?
0	1	2	25.	Does (CHILD) complain of stomach aches or cramps?
0	1	2	26.	Does (CHILD) complete homework?
0	1	2	27.	Is (CHILD) confused, in a fog (Does not keep up with others.)?
0	1	2	28.	Does (CHILD) cry without good reason?
0	1	2	29.	Is (CHILD) curious or enthusiastic about new activities?
0	1	2	30.	Does (CHILD) day dream or get lost in His/Her thoughts?
0	1	2	31.	Does (CHILD) defy authority or break rules?
0	1	2	32.	Does (CHILD) deliberately destroy things that belong to others?
0	1	2	33.	Does (CHILD) demand attention?
0	1	2	34.	Does (CHILD) destroy His/Her own things?
0	1	2	35.	Is (CHILD) disobedient at home?
0	1	2	36.	Is (CHILD) disobedient at school?
0	1	2	37.	Does (CHILD) do unusual original, creative work?
0	1	2	38.	Does (CHILD) eat poorly?
0	1	2	39.	Is (CHILD) easily jealous?
0	1	2	40.	Does (CHILD) express needs and feelings appropriately?
0	1	2	41.	Does (CHILD) face the pressures of competition well?
0	1	2	42.	Does (CHILD) have specific fears?
0	1	2	43.	Does (CHILD) fear H/She might do something bad?
0	1	2	44.	Does (CHILD) feel good about Him/Herself?
0	1	2	45.	Does (CHILD) feel S/He has to be perfect?
0	1	2	46.	Does (CHILD) feel too guilty?
0	1	2	47.	Does (CHILD) feel worthless or inferior?
0	1	2	48.	Does (CHILD) feel or complain that no one loves Him/Her?
0	1	2	49.	Does (CHILD) follow rules and directions?
0	1	2	50.	Does (CHILD) function well even with distractions?
0	1	2	51.	Is (CHILD) generally relaxed?
0	1	2	52.	Does (CHILD) get teased by other children?

0	1	2	53.	Is (CHILD) good at counting (MATHS)?
0	1	2	54.	Is (CHILD) happy?
0	1	2	55.	Is it too hard to understand what (CHILD) is saying?
0	1	2	56.	Does (CHILD) have a good sense of humor, smile a lot?
0	1	2	57.	Does (CHILD) have many friends?
0	1	2	58.	Does (CHILD) have strange ideas – if true Describe : _____
0	1	2	59.	Does (CHILD) hear things that aren't there? If true Describe : _____
0	1	2	60.	Does (CHILD) hesitate to try new things?
0	1	2	61.	Is (CHILD) impulsive or does (CHILD) act without thinking?
0	1	2	62.	Is (CHILD) independent, does (CHILD) like to do things without help?
0	1	2	63.	Is (CHILD) interested in school work?
0	1	2	64.	Is (CHILD) irritable?
0	1	2	65.	Is (CHILD) a good reader for His/Her Grade?
0	1	2	66.	Does (CHILD) know His/Her strengths and weaknesses?
0	1	2	67.	Does (CHILD) look unhappy without good reason?
0	1	2	68.	Is (CHILD) Loud, Noisy?
0	1	2	69.	Is (CHILD) loving, shows affection to others?
0	1	2	70.	Is (CHILDS) mood even and stable?
0	1	2	71.	Does (CHILD) make nervous movements or twitch?
0	1	2	72.	Is (CHILD) nervous, high strung or tense?
0	1	2	73.	Is (CHILD) able to take turns and share?
0	1	2	74.	Is (CHILD) not liked by other children?
0	1	2	75.	Is (CHILD) overactive, restless, unable to sit still?
0	1	2	76.	Is (CHILD) overtired, sleepy during the day?
0	1	2	77.	Is (CHILD) overweight?
0	1	2	78.	Does (CHILD) physically attack people?
0	1	2	79.	Does (CHILD) plat enthusiastically?
0	1	2	80.	Is (CHILD) polite and courteous?
0	1	2	81.	Does (CHILD) do poor work at school?
0	1	2	82.	Is (CHILD) poorly co-ordinated or clumsy?
0	1	2	83.	Does (CHILD) prefer playing with younger children?

0	1	2	84.	Does (CHILD) prefer to be alone?
0	1	2	85.	Does (CHILD) have problems with eyes, not corrected by glasses?
0	1	2	86.	Does (CHILD) require restrictions to control Him/Herself?
0	1	2	87.	Doe (CHILD) have rashes or other skin problems?
0	1	2	88.	Does (CHILD) refuse to talk in certain situations?
0	1	2	89.	Does (CHILD) repeat certain acts over and over?
0	1	2	90.	Doe (CHILD) resolve peer problems on His/Her own?
0	1	2	91.	Is (CHILD) sad or depressed?
0	1	2	92.	Doe (CHILD) scream?
0	1	2	93.	Is (CHILD) secretive, does (CHILD) keep things to Him/Herself?
0	1	2	94.	Does (CHILD) seem to think that others are out to get Him/Her?
0	1	2	95.	Does (CHILD) see things that are not really there?
0	1	2	96.	Is (CHILD) self-conscious or easily embarrassed?
0	1	2	97.	IS (CHILD) a self-starter, begins without waiting for adults?
0	1	2	98.	Does (CHILD) share things with others?
0	1	2	99.	Does (CHILD) show interest in people around Him/Her?
0	1	2	100.	Does (CHILD) Show off or Clown?
0	1	2	101.	Is (CHILD) shy or timid?
0	1	2	102.	Does (CHILD) stare blankly?
0	1	2	103.	Does (CHILD) stare into space or seem preoccupied?
0	1	2	104.	Does (CHILD) start fights?
0	1	2	105.	Does (CHILD) have strange behavior? Describe? _____
0	1	2	106.	Is (CHILD) stubborn, sullen or irritable?
0	1	2	107.	Does (CHILD) have sudden changes in mood or feelings?
0	1	2	108.	Does (CHILD) pull a face, sulk or pout?
0	1	2	109.	Is (CHILD) suspicious of others?
0	1	2	110.	Does (CHILD) talk too much?
0	1	2	111.	Does (CHILD) tease other kids?
0	1	2	112.	Does (CHILD) have temper tantrums or hot temper?
0	1	2	113.	Does (CHILD) threaten people?
0	1	2	114.	Is (CHILD) too fearful or anxious?
0	1	2	115.	Does (CHILD) try to help others?

0	1	2	116.	Is (CHILD) trustworthy?
0	1	2	117.	Is (CHILD) under active, slow-moving or lacks energy?
0	1	2	118.	Doe (CHILD) vomit, throw up?
0	1	2	119.	Is (CHILD) well liked by other children His/Her age?
0	1	2	120.	Is (CHILD) well behaved in school?
0	1	2	121.	Is (CHILD) withdrawn, doesn't get involved with others?
0	1	2	122.	Does (CHILD) work up to potential?
0	1	2	123.	Does (CHILD) work well with out adult support?
0	1	2	124.	Does (CHILD) suck His/Her thumb?
0	1	2	125.	Does (CHILD) do things to hurt Him/Herself (e.g. Bang head on wall)?
0	1	2	126.	Does (CHILD) worry?

B. Compared to other children His/Her age, how well does _____
(Childs Name)

127. . . Get along with children His/Her own age?

1	Worse than others	2	About the same	3	Better than other

128. . . Get along with His/Her brothers and sisters?

1	Worse than others	2	About the same	3	Better than other

D. Appendix D

QUESTIONNAIRE (HSP Scale)

INSTRUCTIONS: This questionnaire is completely anonymous and confidential. Answer each question according to the way you personally feel, using the following scale:

1	2	3	4	5	6	7
Not at All			**Moderately**			**Extremely**

____ 1. Are you easily overwhelmed by strong sensory input?

____ 2. Do you seem to be aware of subtleties in your environment?

____ 3. Do other people's moods affect you?

____ 4. Do you tend to be more sensitive to pain?

____ 5. Do you find yourself needing to withdraw during busy days, into bed or into a darkened room or any place where you can have some privacy and relief from stimulation?

____ 6. Are you particularly sensitive to the effects of caffeine?

____ 7. Are you easily overwhelmed by things like bright lights, strong smells, coarse fabrics, or sirens close by?

____ 8. Do you have a rich, complex inner life?

____ 9. Are you made uncomfortable by loud noises?

___ 10. Are you deeply moved by the arts or music?

___ 11. Does your nervous system sometimes feel so frazzled that you just have to go off by yourself?

___ 12. Are you conscientious?

___ 13. Do you startle easily?

___ 14. Do you get rattled when you have a lot to do in a short amount of time?

___ 15. When people are uncomfortable in a physical environment do you tend to know what needs to be done to make it more comfortable (like changing the lighting or the seating)?

___ 16. Are you annoyed when people try to get you to do too many things at once?

___ 17. Do you try hard to avoid making mistakes or forgetting things?

___ 18. Do you make a point to avoid violent movies and TV shows?

___ 19. Do you become unpleasantly aroused when a lot is going on around you?

___ 20. Does being very hungry create a strong reaction in you, disrupting your concentration or mood?

___ 21. Do changes in your life shake you up?

___ 22. Do you notice and enjoy delicate or fine scents, tastes, sounds, works of art?

___ 23. Do you find it unpleasant to have a lot going on at once?

___ 24. Do you make it a high priority to arrange your life to avoid upsetting or overwhelming situations?

___ 25. Are you bothered by intense stimuli, like loud noises or chaotic scenes?

___ 26. When you must compete or be observed while performing a task, do you become so nervous or shaky that you do much worse than you would otherwise?

___ 27. When you were a child, did parents or teachers seem to see you as sensitive or shy?

E. Appendix E

Table E.1 summarises the reliability estimates of the three variables measured through quantitative questionnaires. These estimates measure the reliability of the questionnaire to measure the variable/trait effectively. Higher scores denote higher reliability in the measured item, with acceptable reliability estimates reported above 0.7. Table E.2 contains the results of the Wilcox rank-sum tests performed to investigate possible sex differences between the measured variables. A significant p-value denotes significant sex differences and include the variables externalising behaviour, the highly sensitive person scale, birth weight, and maternal education.

Table E.1: Guttmans λ^6 and Cronbach's α reliability estimates

Outcome variable	Guttmans λ^6	Cronbach's α
Internalising behaviour	0.78	0.72
Externalising behaviour	0.86	0.82
Highly Sensitive Person Scale	0.89	0.80

Table E.2: Wilcox rank-sum test for sex differences

	W	p-value
Internalizing behaviour	166410	0.33
Externalizing behaviour	139240	8.09 x 10^{-5}
Highly Sensitive Person Scale	67140	2.19 x 10^{-9}
Birth weight	131640	3.58 x 10^{-7}
Gestational age	145670	0.02
Socio-economic status	162710	0.76
Maternal education	186110	2.2 x 10^{-16}

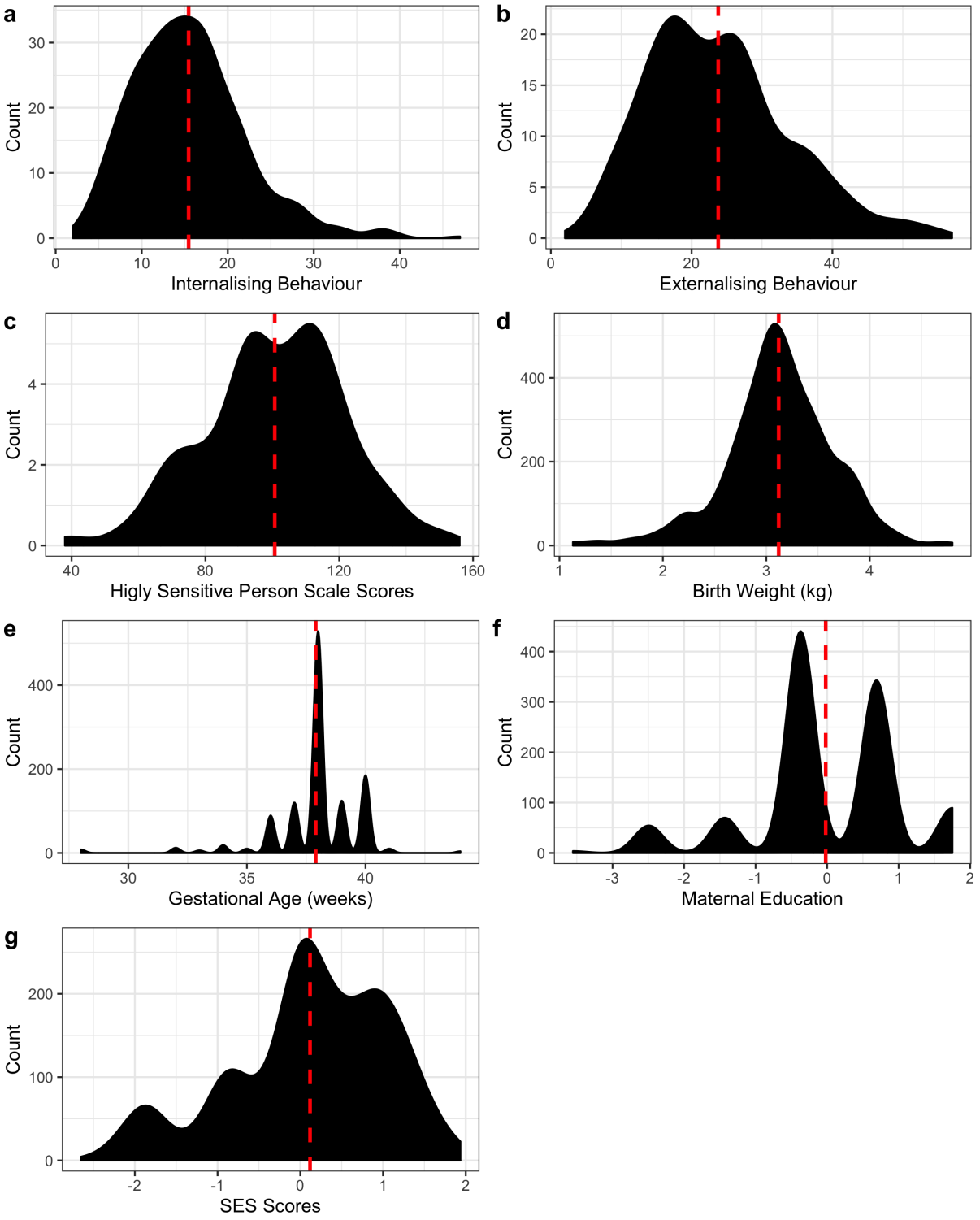

Figure E.1: Distribution of variables for males: Depicted here are the variable distribution graphs in males. The dashed red line denotes the mean score for each variable in males.

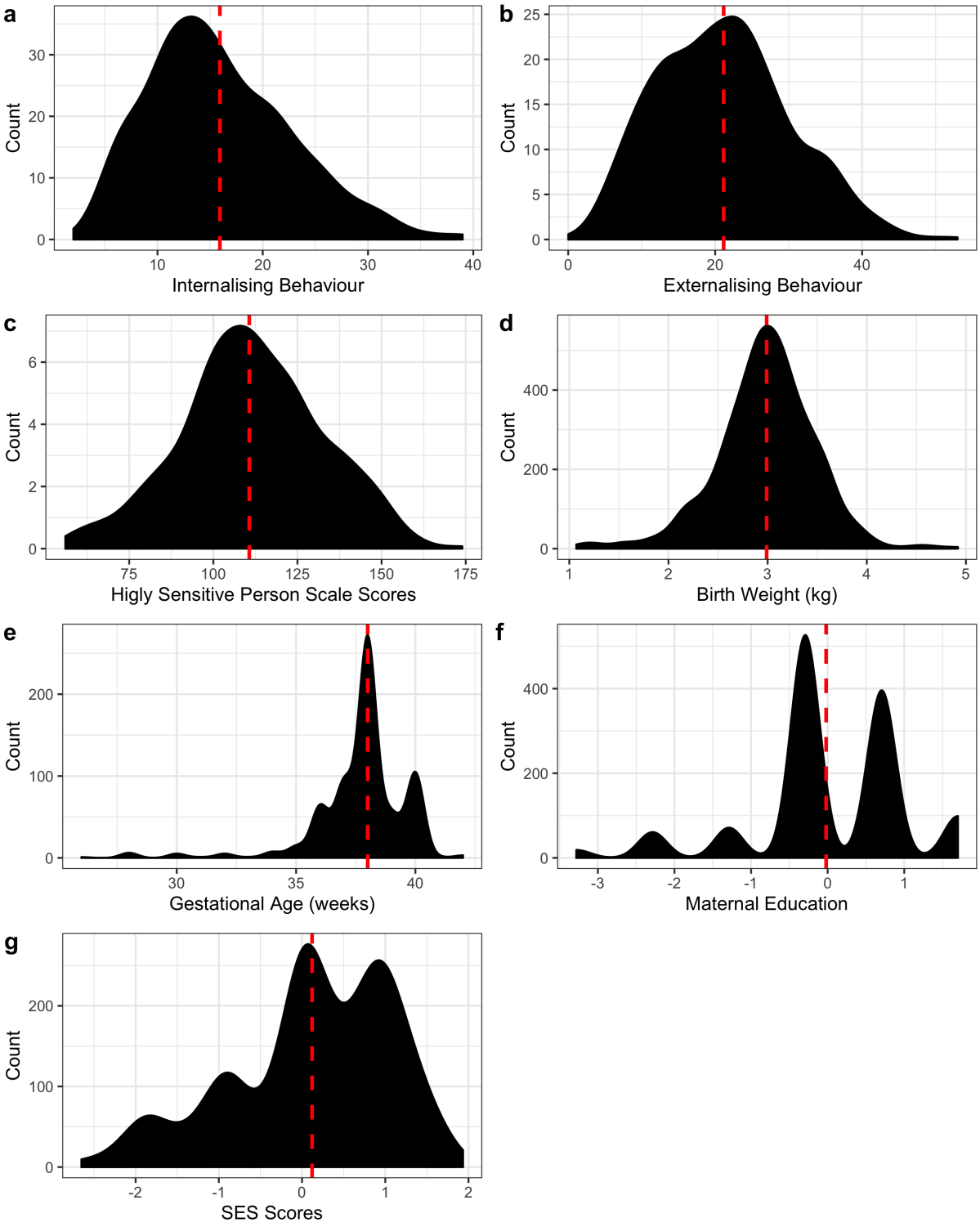

Figure E.2: Distribution of variables for female: Depicted here are the variable distribution graphs in females. The dashed red line denotes the mean score for each variable in females.

Table E.3: List of equipment and reagents used to type the *MAOA* μVNTR

Equipment Type	Equipmnt name	Manufacturer	Manufacturer Location
PCR Thermocycler	T100 Thermocycler	BIO-RAD	Califonia USA
Nano Drop 1000	Nano Drop 1000	Thermofisher	Massachusetts USA
Fluorometer	OMEGA FLUOR	Aplegen	California USA
Electrophoresis Tank	Gel Tank	Cleaver scientific	Warwickshire UK
Powerpack	powerpro-300	Cleaver scientific	Warwickshire UK
Balance	AA ADAM	Adam Equipment	Milton Keynes UK
Reagent Type	**Manufacturer**	**Storage condition**	**Manufacturer Location**
ACCUPRIME GC-RICH POLYMERASE 1000 RXNS	Invitrogen	-30°C	California USA
50 bp DNA Ladder	Invitrogen	-30°C	California USA
SeaKem Le Agarose	Lonza	Room Temp	ME USA
Ethidium Bromide	Sigma-Aldridge	Room Temp (Dark)	Steinheim DE

Table E.4: Correlation matrix for variables within females

	MAOA	5-HTTLPR	Internalising behaviour	Externalising behaviour	Sensory Processing Sensitivity	Birth weight	Gestational Age	Maternal education	Socio-economic Status
Internalising behaviour	rb = -0.12	rb = 0.17		***	**	**	**	**	
Externalising behaviour	rb = - 0.07	rb = 0.11	$\mathcal{T}$ = 0.3		*	*		*	
Sensory Processing Sensitivity	rb = 0.07	rb = -0.06	$\mathcal{T}$ = 0.09	$\mathcal{T}$ = 0.09					
Birth weight	rb = 0.11	rb = -0.05	$\mathcal{T}$ = -0.07	$\mathcal{T}$ = -0.05	$\mathcal{T}$ = -0.002		***	***	
Gestational Age	rb = - 0.07	rb = -0.01	$\mathcal{T}$ = - 0.065	$\mathcal{T}$ = - 0.013	$\mathcal{T}$= - 0.006	$\mathcal{T}$ = 0.28		**	
Maternal education	rb = - 0.05	rb = -0.02	$\mathcal{T}$ = - 0.02	$\mathcal{T}$ = -0.056	$\mathcal{T}$= - 0.02	$\mathcal{T}$ = 0.09	$\mathcal{T}$ = 0.09		***
Socio-economic Status	rb = 0.06	rb = 0.09	$\mathcal{T}$ = - 0.02	$\mathcal{T}$ = 0.02	$\mathcal{T}$ = -0.003	$\mathcal{T}$ = -0.002	$\mathcal{T}$ = - 0.04	$\mathcal{T}$ = 0.16	

Note: *p<0.1;**p<0.05;***p<0.01. rb denotes point biserial correlations (Pearsons correlation for factor variables), and $\mathcal{T}$ denotes kendals τ correlations.

Table E.5: Correlation matrix for variables within males

	MAOA	5HTTLPR	Internalising behaviour	Externalising behaviour	Sensory Processing Sensitivity	Birth weight	Gestational Age	Maternal education	Socio-economic Status
Internalising behaviour	rb = 0.1	rb = 0.13		***					
Externalising behaviour	rb = 0.05	rb = 0.09	$\mathcal{T}$ = 0.29			**	**		
Sensory Processing Sensitivity	rb = − 0.05	rb = − 0.02	$\mathcal{T}$ = − 0.06	$\mathcal{T}$ = − 0.02		***		*	
Birth weight	rb = 0.06	rb = 0.03	$\mathcal{T}$ = − 0.04	$\mathcal{T}$ = − 0.07	$\mathcal{T}$ = 0.13		***	***	
Gestational Age	rb = 0.05	rb = − 0.11	$\mathcal{T}$ = − 0.03	$\mathcal{T}$ = − 0.08	$\mathcal{T}$ = 0.02	$\mathcal{T}$ = 0.44		***	
Maternal education	rb = 0.04	rb = − 0.03	$\mathcal{T}$ = − 0.03	$\mathcal{T}$ = − 0.04	$\mathcal{T}$= 0.08	$\mathcal{T}$= 0.13	$\mathcal{T}$ = 0.15		***
Socio-economic Status	rb = 0.06	rb = 0.09	$\mathcal{T}$ = − 0.03	$\mathcal{T}$ = − 0.01	$\mathcal{T}$ = − 0.003	$\mathcal{T}$ = − 0.001	$\mathcal{T}$ = − 0.04	$\mathcal{T}$ = 0.28	

Note: *p<0.1; **p<0.05; ***p<0.01. rb denotes point biserial correlations (Pearsons correlation for factor variables), and $\mathcal{T}$ denotes kendals τ correlations.