Understanding and treating trauma and violence-related pathologies in South African townships: Emergence and modifiability of epigenetic and neural memories of traumatic stressors and appetitive offending

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DECLARATION – PLAGIARISM

I, Khethelo Richman Xulu, hereby declare that

1. The research work reported in this dissertation unless otherwise stated, is my original research

2. This dissertation has not been submitted for any qualification degree or examination at any other tertiary university in the world.

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Dedication of PhD work

I dedicate this work to all underprivileged people who keep on pushing beyond any imaginary mind. I know most of us know our own struggles at best, and we have to realise our passed test. I think about back at the times where meals at university were a luxury we could not afford. I remember the times where umbhubhudlo (sugar-water) was a luxury beverage to quench that thirst. Most of you, including me, had to endure the shame of cracked heels as we walked barefoot without a choice in our primary schools.
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I am in a verge of treasuring the exquisiteness of the valleys, mountains and rivers that could have limited any imagination of this presence. I vow to meditate on the lessons learnt from ukwelusa (livestock herding), playing of izindunku nokweqhathwa (stick fighting) and eating all those wild fruits such as amathunduluka, imiphafa, amanqayi, etc.., while surviving in the face of all wild animals like dangerous snakes. I would not be led by the circumstances of such triumphal pursuit to think and believe the worst about where I come from. I know the industriousness and conscientiousness of our people, whom had consistently been referred as lazy by the narrative. I shall not let my imagination be shattered and captured by any sense of narration. You and I, most of us, know how to rise and influence positively.

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ABBREVIATIONS

%	percentage
<	less than
>	greater than
µg/dl	micrograms per deciliter
µg/l	micrograms per liter
µM	micromolar
3’	3- prime end
5’	5- prime end
A	adenine
AAS	Appetitive Aggression Scale
APP	amyloid precursor protein gene
AUTS2	autism susceptibility candidate 2 gene
AVP	arginine vasopressin gene
BDNF	brain-derived neurotrophic factor
bp	base pair
C	cytosine
CBT	cognitive behavioural therapy
chr	chromosome
CI	confidence interval
COMT	catechol-O-methyltransferase gene
CTQ	childhood trauma questionnaire
DNA	deoxyribonucleic acid
dNTP	deoxyribonucleotide triphosphate
DSM-IV	Diagnostics and Statistical Manual of Mental Disorders IV
DSM-5	Diagnostics and Statistical Manual of Mental Disorders 5
DRD2	dopamine receptor 2 gene
FORNET	Narrative Exposure for Forensic Offender Rehabilitation
G	gigabases
g	grams
G	guanine
GABA	gamma-aminobutyric acid
GABRA2	gamma-aminobutyric acid receptor subunit alpha-2
GAD	generalised anxiety disorder
GAD1	glutamate decarboxylase gene

GLS2	glutaminase 2 gene
GRM2	glutamate receptor 2
GWAS	genome-wide association study
HOXA1	homeobox A1 gene
HPA	hypothalamus-pituitary-adrenal
hr	hour
HWE	Hardy-Weinberg equilibrium
IEG	immediate-early response gene
indel	insertion/ deletion
ISL2	islet 2 LIM homeobox gene
LDLR	low-density lipoprotein receptor gene
LHX5	LIM homeobox 5 gene
kb	kilobase pair
LSD	least significance difference
M	molar
MAOA	monoamine oxidase A
MAOI(s)	monoamine oxidase inhibitor(s)
MDD	major depressive disorder
*MECP*2	methyl CpG binding protein 2 gene
mg	milligrams
MgCl2	magnesium chloride
mGluRs	metabotropic glutamate receptors
min	minutes
ml	millilitres
mM	millimolar
mRNA	messenger ribonucleic acid
MS	maternal separation
NaCl	sodium chloride
ng	nanogram(s)
NGF	nerve growth factor
NGS	next generation sequencing
NR4A2	nuclear receptor subfamily 4, group A, member 2 gene
°C	degrees Celcius
P	p-value
PCR	polymerase chain reaction
PD	panic disorder

pH	percentage hydrogen
PTSD	posttraumatic stress disorder
PSS-I	PTSD symptom scale-interview
QC	quality score
QTL	quantitative trait loci
R	reverse primer
rpm	revolutions per minute
RELN	reelin gene
RPL39	ribosomal protein L39 gene
RPP21	protein subunit of nuclear ribonuclease P gene
SB	sodium tetraborate decahydrate
sec	seconds
SI	social interaction
SNP(s)	single nucleotide polymorphism(s)
SORBS3	sorbin and sh3 domains-containing protein 3 gene
SMS	sspermine synthase gene
SP	specific phobia
SSRI(s)	serotonin reuptake inhibitor(s)
T	thymine
Taq	*Thermus aquaticus*
TAU	Treatment As Usual
TCA(s)	tricyclics
TERT	telomerase reverse transcriptase
TFAM	transcription factor A, mitochondrial
Tm	melting point
U	units
UTR	untranslated region
v/v	volume per volume
w/v	weight per volume
WT	wild-type
µg	micrograms
µg/ml	microgram per millilitre
µl	microlitre

DISSERTATION ABSTRACT

Prolonged exposure to traumatic stress results in sustained activation of biological responses, which affect shared similar pathways, leading to posttraumatic stress disorder (PTSD), a serious mental illness; and behavioural problems such as appetitive aggression. Appetitive aggression is the perpetration of violence that induces feelings of arousal and excitement. Chronic exposure to stress can modify mechanisms such as DNA methylation and telomere length, which are environmentally sensitive. DNA methylation and telomere length, including genetic variants have been implicated in the aetiology of both aggression and PTSD. Therefore, studying DNA methylation, telomeres, and genetic variation is essential to better understand appetitive aggression and PTSD. The overarching aim of this study was to investigate DNA methylation, telomere length and genetic variation in association with appetitive aggression and PTSD. The secondary aim was to investigate changes in DNA methylation and telomere length that may be involved in the effectiveness of psychotherapeutic interventions by identifying key biological markers related to appetitive aggression and PTSD symptom severity in Xhosa Black South African men. The third aim was to investigate the association between appetitive aggression and genetic variations of both the serotonin transporter in the promoter region (5-HTTLPR) and monoamine oxidase A (*MAOA*).

Recruited participants with higher levels of appetitive aggression and higher PTSD symptom severity, as measured by the Appetitive Aggression Scale (AAS) and the PTSD Symptom Scale-Interview (PSS-1), respectively, were randomised to receive either Narrative Exposure Therapy for Forensic Offender Rehabilitation (FORNET), Cognitive Behavioural Therapy (CBT) and controls. Saliva collection occurred before the intervention (baseline) and at 8 and 16 months post-intervention, and genomic DNA was extracted at subsequent follow-up visits. The Human Mental Disorders EpiTect Methyl II Signature PCR Array was used to quantify DNA methylation. Telomere length was determined using quantitative polymerase chain reaction (qPCR). PCR was used for genotyping. The data were analysed using regression models, repeated measures of analysis of variance (ANOVA), mixed models and Fisher's least significant difference (LSD).

No significant changes in methylation were observed in the FORNET group. However, methylation in both autism susceptibility candidate 2 (*AUTS2*) (p=0.000) and reelin (*RELN*) (p=0.023) genes decreased significantly in the CBT group between first and second follow-up visits. In the catechol-o-methyltransferase (*COMT*) gene, methylation significantly

decreased between baseline and first follow-up (p=0.007), while between first and second follow-up visits there was a significant increase (p=0.038). Methylation in the *RELN* (r=0.38, p=0.02) gene exhibited strong positive correlation with appetitive aggression, while methylation in the *COMT* gene correlated with PTSD symptom severity (r=0.35, p=0.01). No significant correlation between appetitive aggression and telomere length (r=0.09, p= 0.121) was observed; however, increased telomere length correlated with higher PTSD symptom severity (r=0.13, p=0.039). The STin2 VNTR 12-repeat homozygous genotype and L'-STin2.12/STin2.12 were associated with appetitive aggression (p=0.003 and p=8.00 x 10^{-8}, respectively). This research provides insights into the biological mechanisms that may play a significant role in appetitive aggression and PTSD. DNA methylation and telomere length may be biological markers that are responsive to psychotherapeutic interventions. The STin2 VNTR 12-repeat may be a distinguishing marker of appetitive and reactive forms of aggression. These biological mechanisms provided preliminary insights into appetitive aggression and PTSD.

OPSOMMING

Langdurige blootstelling aan traumatiese stres het volgehoue aktivering van biologiese response tot gevolg, wat gedeelde soortgelyke paaie beïnvloed. Dit lei tot posttraumatiese stresversteuring (PTSV), 'n ernstige geestesiekte, en gedragsprobleme soos dranggedrewe aggressie. Dranggedrewe aggressie is geweldpleging wat gevoelens van stimulering en opwinding veroorsaak. Chroniese blootstelling aan stres kan meganismes soos DNS-metilering en telomeerlengte, wat omgewingsensitief is, verander. DNS-metilering en telomeerlengte, wat genetiese variante insluit, speel 'n rol in die etiologie van sowel aggressie as PTSV. Daarom is die bestudering van DNS-metilering, telomere en genetiese afwykings noodsaaklik vir 'n beter begrip van dranggedrewe aggressie en PTSV. Die oorkoepelende oogmerk van die huidige navorsing was om DNS-metilering, telomeerlengte en genetiese afwyking met betrekking tot dranggedrewe aggressie en PTSV te ondersoek. Die sekondêre oogmerk was om die veranderinge in DNS-metilering en telomeerlengte wat by psigoterapeutiese intervensie betrokke is, te ondersoek deur belangrike biologiese merkers wat met dranggedrewe aggressie en intensiteit van PTSV-simptome in Xhosa swart Suid-Afrikaanse mans verband hou, te identifiseer. Die derde oogmerk was om 'n verband tussen dranggedrewe aggressie en genetiese afwykings in sowel serotonientransport in die promotorgebied (5-HTTLPR) as monoamienoksidase-A (*MAOA*) te ondersoek.

Gewerfde deelnemers met sowel hoër dranggedrewe aggressie as hoër intensiteit van PTSV-simptome, onderskeidelik gemeet deur die Skaal vir Dranggedrewe Aggressie (AAS) en die Skaal vir PTSV-simptome – onderhoudsweergawe (PSS-i), is verewekansig om verhalende blootstellingterapie vir rehabilitering van forensiese oortreders (FORNET), kognitiewe gedragsterapie (KGT) en kontroles te ontvang. Speeksel is voor die intervensie versamel (basislyn) en 8 en 16 maande ná intervensie is genomiese DNS by opvolgsessies onttrek. Die *Human Mental Disorders EpiTect Methyl II Signature* PKR-toets is gebruik om DNS-metilering te kwantifiseer. Telomeerlengte is deur middel van kwantitatiewe polimerase-kettingreaksie (kPKR) bepaal. PKR is vir genotipering gebruik. Die data is deur middel van regressiemodelle, herhaalde-meting-variansieontleding (ANOVA), gemengde modelle en Fisher se kleinste betekenisvolle verskil (LSD) ontleed.

Daar was geen beduidende veranderinge in metilering in die FORNET-groep nie. Metilering in sowel die outisme-ontvanklikheidskandidaat-2- (*AUTS2*-) geen (p=0.000) en reelin-(*RELN*-)geen (p=0.023) het egter beduidend in die KGT-groep afgeneem tussen die eerste en tweede opvolgsessie. In die katesjol-O-metieltransferase- (*COMT*-) geen het metilering

tussen die basislyn en die eerste opvolgsessie beduidend afgeneem (p=0.007), terwyl dit tussen die eerste en tweede opvolgsessie beduidend toegeneem het (p=0.038). Metilering in die *RELN*-geen het sterk positiewe korrelasie met dranggedrewe aggressie getoon (r=0.38, p=0.02), terwyl metilering in die *COMT*-geen met intensiteit van PTSV-simptome gekorreleer het (r=0.35, p=0.01). Daar is geen beduidende korrelasie tussen dranggedrewe aggressie en telomeerlengte (r=0.09, p=0.121) waargeneem nie; groter telomeerlengte het egter met hoër intensiteit van PTSV-simptome gekorreleer (r=0.13, p=0.039). Die intron 2 (STin2) veranderlike getal tandem herhalings (VNTR) 12-herhaling homosigotiese genotipe en L'-STin2.12/STin2.12 het met dranggedrewe aggressie geassosieer (p=0.003 en p=8.00 x 10^{-8} onderskeidelik). Hierdie navorsing het insig gebied in die begrip van biologiese meganismes wat moontlik 'n belangrike rol in dranggedrewe aggressie en PTSV speel. DNS-metilering en telomeerlengte kan in wese merkers wees wat op psigoterapeutiese intervensies reageer. Die STin2 VNTR 12-herhaling kan tussen dranggedrewe en reaktiewe aggressie onderskei. Hierdie biologiese meganismes bied voorbereidende insig in die begrip van dranggedrewe aggressie en PTSV.

CHAPTER ONE: LITERATURE REVIEW

1.1 Association between exposure to violence and acts of aggression

Aggression is an inherent natural phenomenon that is associated with feelings of anger, which can lead to the perpetration of violence. This natural phenomenon of aggressive behaviour has both emotional and behavioural concomitants (Blanchard and Blanchard, 2003). Aggression in both animals and humans have several similarities; and there are also distinct types of aggression observed in animals such as play fighting, offensive aggression, defensive aggression, maternal aggression and predatory aggression (Gendreau and Archer, 2005; Glanc et al., 2006). This aggression is also observed to be a distinctly gender-specific behaviour in many species of animals, and it plays a pivotal role in survival, selection of sexual partners, and dominance (Georgiev et al., 2013; Kempes et al., 2005). However, unlike other animal aggression, human aggressive behaviour can be defined as the perpetration of aggression with an intention to cause harm (Bandura, 1983; Elbert et al., 2010; Kempes et al., 2005; Montagu, 1977). In animals, aggression refers to intra-species fighting (Gendreau and Archer, 2005; Glanc et al., 2006; Hinde, 1970), which can be perpetrated with an intention to protect and prevent harm in the same species, but not to intentionally cause deaths (Wright, 1970). Aggression within an animal species is often not the same as prey hunting, where one animal species may hunt another species for food (Glanc et al., 2006; Kempes et al., 2005; Wright, 1970). Rather, aggression against animals of the same species often consists of intimidating or repelling other animals by adopting specific postures or visual demonstrations of colour, or making sounds, causing the weaker animal to run away (Gendreau and Archer, 2005; Glanc et al., 2006).

Aggression in animals does not create violence that harms the community of the same animal species. On the contrary, aggression in humans is a multifactorial and complex behaviour (Georgiev et al., 2013; Tremblay, 2010), which can lead to a plethora of problems, such as gangsterism, criminal activities and the development of mental health disturbances (Abrahams and Jewkes, 2005; Kaminer et al., 2008). The literature suggests that experiencing violence as a victim or witnessing violence being committed, can be associated with the development of aggression (Crombach et al., 2013; Frazzetto et al., 2007; Hecker et al., 2015a; Nandi et al., 2015; Weierstall et al., 2013b). Individuals who are victims of childhood violence are susceptible to becoming violent offenders, thus perpetuating the cycle of violence (Catani et al., 2009; Elbert et al., 2006; Hinsberger et al., 2016b). Indeed, growing up under hostile and impoverished settings and experiencing trauma and/or sexual abuse, is associated with violent behaviour in adulthood (Abrahams and Jewkes, 2005; Norman et al., 2010).

Several studies have investigated exposure to violence and its association with the subsequent development of violent behaviour (Catani et al., 2009; Crombach et al., 2014; Hecker et al., 2013; Hinsberger et al., 2016b; Weierstall et al., 2013b). These studies have included individuals from different settings with diverse experiences of violence, such as child soldiers and former combatants in African countries (Hecker et al., 2015b; Köbach et al., 2015; Nandi et al., 2015; Weierstall et al., 2011). Although aggression has been widely studied in an ex-combatant context, it has also been found to occur in non-military settings. Individuals who are exposed to chronic traumatic stress and violence in South African townships, have also been found to develop aggression (Hinsberger et al., 2016b; Weierstall et al., 2013b). The consensus from these studies is that violence may in turn cause violent behaviour, which manifests as aggression.

Aggression can occur in two main forms: appetitive and reactive aggression, which are broadly characterised by the underlying motivation to perpetrate aggression (Hecker et al., 2015a; Köbach et al., 2015; Nandi et al., 2015; Schaal et al., 2014; Weierstall et al., 2013b, 2012a). Appetitive aggression is a subtype of instrumental aggressive behaviour characterised by the attraction to violence or seeking the key intrinsic joy of exhibiting aggressive activity in the absence of provocation or danger (Elbert et al., 2010; Vitiello and Stoff, 1997; Weierstall et al., 2011). This type of aggression is driven by the goal of attaining social status and the violent self-rewarding lust and enjoyment of inflicting pain through violence (Augsburger et al., 2017; Hermenau et al., 2013a; Weierstall et al., 2013b). Conversely, reactive aggression is an emotional responsive action usually occurring after provocation or in response to a life-threatening situation (Anderson and Bushman, 2002; Bushman and Anderson, 2001; Gilbert and Daffern, 2010; Hecker et al., 2012a; Weierstall et al., 2011). Reactive aggression is also associated with hyperarousal, negative emotions, and a drive for self-defence (Buss and Perry, 1992; Haer et al., 2013; Ramírez and Andreu, 2006; Weierstall et al., 2013a).

The characteristics of this kind of reactive aggression have been suggested to be associated with posttraumatic stress disorder (PTSD), a serious psychiatric disorder that can develop after experiencing stressful life events. However, appetitive aggression seems to provide a protective effect against the development of PTSD (discussed in Section 1.2) (Crombach and Elbert, 2014a; Nandi et al., 2015). It has been suggested that learned violent behaviour, i.e. appetitive aggression, may be beneficial for survival under adverse circumstances (Weierstall et al., 2012b). Conversely, those individuals who develop PTSD—a set of symptoms that are, by definition, maladaptive—tend to display reactive aggression (MacNair, 2002). For example, reactive aggression can be observed where an individual fantasises

about revenge, itself a symptom of PTSD (Kerig et al., 2016, 2012). Fantasising about harming someone who has inflicted pain can bring satisfaction. Initially, the defensive, reactive aggression serves to protect against immediate harm. However, this aggressive activity can progress into an intrinsically instrumental and proactive motivation to act violently in the absence of a situation that requires immediate self-defence (Kerig et al., 2016). Overall, the relationships between violence and aggression, both appetitive and reactive, suggests a cycle of violence caused by exposure to violent circumstances (Gäbler and Maercker, 2011).

1.2 Association between appetitive aggression and posttraumatic stress disorder

Continuous exposure to violence and different traumatic events can lead to the development of a variety of mental health disorders, including PTSD (Catani et al., 2009; Provençal et al., 2014; Weierstall et al., 2012a). PTSD is a psychiatric disorder characterised by extreme fear, coupled with significant behavioural changes (Kessler et al. 1995; American Psychiatric Association DSM-V 2013). The disorder is characterised by four behavioural symptoms, namely: re-experiencing the traumatic event while there is no danger, hyperarousal, decline in cognitive function coupled with avoidance of thoughts, feelings or conversations about the traumatic event, and fluctuation in mood (DSM-V American Psychiatric Association, 2013; Kessler et al., 1995; Kilpatrick et al., 2013). Overall, PTSD severely affects behaviour, normal functioning, and quality of life (Kessler et al., 1995; Neuner et al., 2004a; Weierstall et al., 2011).

The chances of an individual developing PTSD symptoms increase after exposure to cumulative traumatic events, such that a threshold trauma level is reached (Catani et al., 2008; Neuner et al., 2004a) referred to as the building block impact (Kolassa and Elbert, 2007; Neuner et al., 2004a; Schauer et al., 2003). Memories of traumatic events, which include both experiences of violence and reminders of experienced stress, involve molecular alterations in multiple brain regions. These regions of the brain are the frontal cortex, hippocampus, amygdala, and hypothalamus (Elbert and Schauer, 2002; McGowan et al., 2009; Neylan et al., 2014; Yehuda, 2006). Molecular changes that occur within these regions include epigenetic changes, e.g. in the promoter regions of genes, such as in the neuron-specific glucocorticoid receptor (*NR3C1*). McGowan et al. (2009) investigated these changes in the hippocampus of individuals who had completed suicide after a history of childhood abuse compared to controls who were suicide victims with no history of childhood abuse (McGowan et al., 2009). This study found decreased levels of glucocorticoid receptor mRNA from the hippocampal tissue of suicide completers with a history of childhood trauma and

abuse (McGowan et al., 2009). This decreased mRNA level in the hippocampal tissue samples included mRNA transcripts that are important for the glucocorticoid receptor 1F splice variant. This decrease in glucocorticoid receptor mRNA was associated with hypermethylation of the *NR3C1* promoter region (McGowan et al., 2009), suggesting that the changes in DNA methylation may have resulted in a change in the level of neurochemicals, which could have negatively affected the processing of trauma. This further suggests that there may be ongoing negative impacts for people who have experienced traumatic events.

Cumulative lifetime trauma, including early childhood experiences of violence, affects the anatomy of the brain, as well as its physiological response (Elbert et al., 2006; Vukojevic et al., 2014). Alteration in the cytoarchitecture of the brain can leads to cognitive dysfunction (Catani et al., 2009; Elbert et al., 2006), while gradual changes in brain activity can result in an altered state of mind (Kaminer et al., 2008; Provençal et al., 2014). Traumatic memories generate a fear network within the brain that is characterised by a complex web of emotional, sensory, cognitive, interoceptive, and circumstantial memories (Elbert and Schauer, 2002; Moran et al., 2014; Neylan et al., 2014; Yehuda, 2006). Therefore, chronic exposure to traumatic stress has negative impacts on biological responding, which may lead to altered physiological and psychological responses.

Studies have demonstrated that exposure to violence, serious injury and/or cruel environments can lead to trauma-related symptoms, including the development of PTSD (Crombach and Elbert, 2014b; Haer et al., 2013; Hecker et al., 2013; Hermenau and Hecker, 2013; Klasen et al., 2010; Weierstall et al., 2012a). Appetitive aggression is hypothesised to buffer and protect individuals against the development of PTSD by increasing their tolerance to violent and traumatic experiences (Crombach and Elbert, 2014b; Hecker et al., 2013; Weierstall et al., 2012a). However, if the traumatic load heightens to such an extent that a severity threshold is reached, the protective effects of appetitive aggression wane (Hecker et al., 2013; Weierstall et al., 2012b). This decline in resilience is in line with the building-block theory of PTSD, which states that constant exposure to traumatic events and violence is cumulative, leading to the development of PTSD over a period of time (Neuner et al., 2004a).

Individuals with PTSD have highly aroused sensory and emotional (“hot”) memories that are interconnected, while, their contextual and autobiographical (“cold”) memories remain disjointed (Elbert et al., 2010; Nell, 2006; Weierstall et al., 2013b). Sensory signals can activate traumatic memories without an individual having a clear sense of the “here and now,” resulting in flashbacks, dissociative episodes, and intrusive images or thoughts of traumatic events. The fear network is highly plastic and can constantly be remodelled and

reshaped (Elbert et al., 2006; Nell, 2006). With each subsequent experience, additional nodes are added to the network, and the associated connections are strengthened, increasing the risk for a traumatic memory to be activated on presentation of an otherwise harmless or superficially disconnected reminder (Kolassa et al., 2010). The addition of traumatic nodes in the fear network is in line with the building-block theory of PTSD (Kolassa and Elbert, 2007; Moran et al., 2014; Schauer et al., 2003).

Signals that trigger violent behaviour share similar sensations (cognitive and physiological responses) with the cues that elicit feelings of fear or horror (Elbert et al., 2010). Consequently, social stressors can stimulate synaptic activity in brain regions such as hippocampus and amygdala that have shared involvement in both PTSD and aggression (Elbert and Schauer, 2002; Moran et al., 2014). In individuals displaying appetitive aggression, violence signals, instead of being integrated into the fear network, become established into the so-called “hunting network,” an associative network that competes with the fear network (Elbert et al., 2010; Haer et al., 2013). This hunting network leads to signals, that would usually be associated with fear or horror, to become associated with positive emotions, such as excitement, which is linked to appetitive arousal (Elbert et al., 2010). This is seen in individuals with appetitive aggression, who develop symptoms similar to those found in addicts such as craving, seeking exposure to arousing stimuli, gradual development of tolerance, as well as psychological withdrawal symptoms (Elbert et al., 2010; Haer et al., 2013; Weierstall et al., 2013b).

Given the fact that the fear and hunting networks share many of the same physiological and neural substrates, elements within these networks may become intertwined (Elbert et al., 2010; Haer et al., 2013; Kolassa and Elbert, 2007). This interlinking of pathways is hypothesised to occur once the individual reaches a certain level of traumatic exposure (the “building block” effect previously referred). Signals that may have previously activated the hunting network become part of the fear network instead, serving to strengthen and extend the latter. This development of fear network can result in the development of trauma-related disorders like PTSD (Elbert et al., 2010; Haer et al., 2013). Thus, the dynamic influences contributing to the development of PTSD, as well as aggression, share underlying neurobiological responses (Figure 1.1) (McGowan, 2013; Provençal et al., 2014; Tremblay, 2010; Yehuda et al., 2008). A number of these neurobiological molecular responses modify brain chemistry and are affected by the individual’s genomic architecture and environmental interactions (McGowan, 2013; Provençal et al., 2014; Tremblay, 2010).

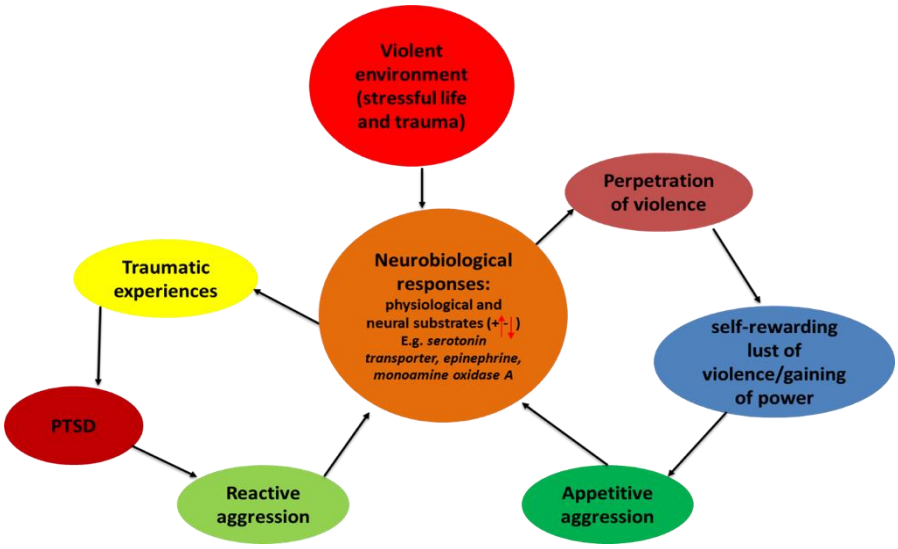

Figure 1.1: Schematic diagram showing the hypothesised neurobiological responses during the cycle of violence and their relationship to the development of appetitive and reactive aggression and posttraumatic stress disorder (PTSD). Red arrows with plus or minus indicate decreased or increased neurotransmitters.

The hypothesised overlapping neurobiological response in the development of both aggression and PTSD can be modified by violence and trauma (Crews, 2008; Lehrner et al., 2014; Neylan et al., 2014; Provençal et al., 2014; Radtke et al., 2011; Weder et al., 2014; Yehuda and Bierer, 2009). Memories of traumatic events can be stored at a molecular level in the brain. Thus understanding underlying and overlapping biological responses in both aggression and PTSD is vital if we wish to understand the onset of appetitive aggression and PTSD symptoms, and the interaction between these disorders. This includes studying complex biological responses contributing to behavioural change to better understand the molecular aetiology of PTSD and the association between appetitive aggression and PTSD phenotypes (Bjornsson et al., 2004; Crews, 2008; Lehrner et al., 2014; McGowan, 2013). Further understanding of the involvement of biological modifications, genetic variation, and gene-environment interactions will be helpful in elucidating the neurobiological underpinnings of PTSD, which will give us insights into the onset of appetitive aggression and PTSD symptom severity, particularly in individuals who are living in settings with constant violence and traumatic events. More specifically, the role of the hypothalamic-pituitary-adrenal (HPA) axis in mediating the development of PTSD, as well as appetitive aggression, is key.
The HPA axis plays a crucial role in regulating the stress response system (Lucassen et al., 2013; Wagner et al., 2011), through a cascade of reactions from the hypothalamus, the pituitary gland, and the adrenal cortex (Figure 1.2) (Brummett et al., 2008; Lucassen et al., 2013; McVicar et al., 2014a). The HPA axis regulates the release of cortisol in response to

stress via feedback and feed-forward mechanisms (McVicar et al., 2014b; Schatzberg et al., 2014; Sriram et al., 2012). The secretion of cortisol is preceded by the release of a corticotropin-releasing hormone (CRH) from the hypothalamus, which activates the anterior pituitary gland to secrete an adrenocorticotrophic hormone (ACTH). ACTH is transported to the adrenal cortex, where it triggers the secretion of cortisol (Brummett et al., 2008; Lucassen et al., 2013). Cortisol is a glucocorticoid with a high affinity for binding both glucocorticoid (GRs) and mineralocorticoid receptors (MRs) (Sapolsky et al., 1985; Yehuda et al., 1993). Cortisol binds to both GRs and MRs to inhibit the hypothalamus and anterior pituitary gland to decrease the secretion of CRH and ACTH, respectively (Figure 1.2).

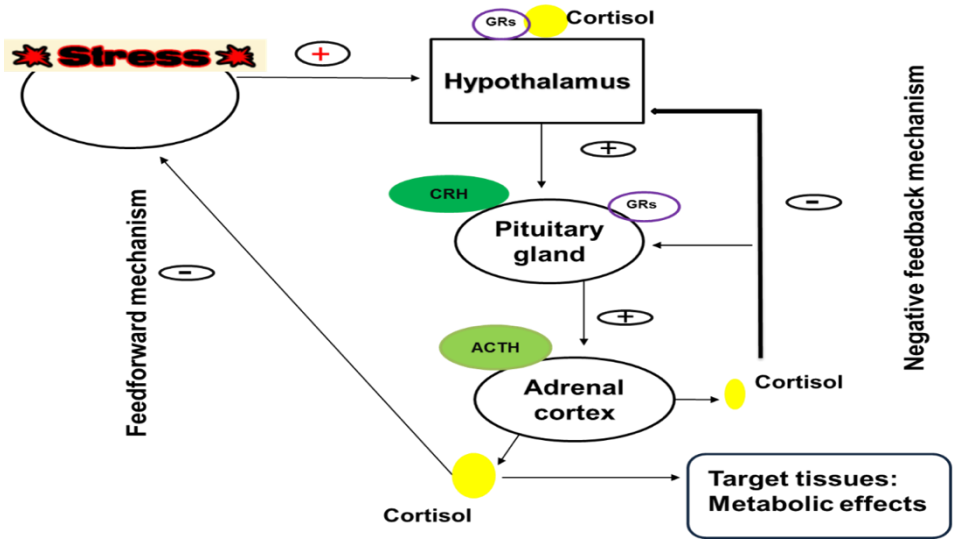

Figure 1. 2: Schematic diagram of the hypothalamic-pituitary-adrenal (HPA) axis. Stress triggers a feed-forward mechanism of the HPA axis by activating the hypothalamus to release corticotrophin-releasing hormone (CRH), which binds to the anterior pituitary gland and induces the secretion of adrenocorticotrophic hormone (ACTH). ACTH triggers adrenal cortex to release cortisol, which binds to glucocorticoid receptors (GRs) to activate metabolic effects for survival during a stressful event. Cortisol balances the feedforward and feedback mechanisms by deactivating both the hypothalamus and the pituitary gland.

Downregulation of the feed-forward mechanism of the HPA axis is crucial to ensuring the homeostasis of cortisol levels during stress (Sautter et al., 2003). Disruption of cortisol homeostasis can lead to an imbalance between feedforward and feedback mechanisms, with elevated negative feedback thought to result in blunted cortisol, as well as the development of both PTSD and aggression (Böhnke et al., n.d.; Raine, 1996; Sapolsky et al., 2000; Vachon-Presseau et al., 2013; Yehuda, 2002). There is a contradictory evidence with regards to the role of cortisol in aggression, with some studies demonstrating that decreased

circulating cortisol is associated with aggression (Loney et al., 2006; Platje et al., 2013), while other studies have demonstrated that individuals with aggressive behaviour have high cortisol levels (van Bokhoven et al., 2005). For example, individuals with conduct disorder were found to exhibit higher levels of cortisol than those without aggression (van Bokhoven et al., 2005). In contrast, individuals with chronic PTSD have lower cortisol levels than those without (de Kloet et al., 2007; Stoppelbein et al., 2012; Yehuda, 2002). Therefore, further investigations are required to clarify the role of stress hormones such as cortisol and their genes in the aetiology of aggressive behaviour and PTSD, especially since both aggression and PTSD share HPA axis dysregulation (Böhnke et al., n.d.; Raine, 1996; Sapolsky et al., 2000; Vachon-Presseau et al., 2013; Yehuda, 2002). Researchers have hypothesised that changes in DNA methylation in several genes that are essential in the HPA axis can lead to dysregulation of this system, with imbalances in neurohormones, such as cortisol.

Studies suggest that changes in the HPA axis and its associated neurobiological pathways, such as the serotonergic system, are associated with dysregulated cortisol as well as several genes involved in the stress response system (Yehuda et al., 2009). A number of genetic variations, as well as their interactions with environmental factors such as stress and trauma, can lead to dysregulation of cortisol (Böhnke et al., n.d.; T. Chen et al., 2014; Yehuda et al., 2009). Dysregulation of monoaminergic systems, including the serotonergic, noradrenergic, dopaminergic, and neurotrophic systems, have been suggested to contribute to HPA axis dysfunction (McGuffin et al., 2011; Popova, 2006; Porter et al., 2004). As stated above, the HPA axis is a complex pathway that is regulated by multiple converging molecular responses. For example, the serotonergic system, a key pathway during the activation of the HPA axis, is involved in the synthesis of a neurotransmitter, serotonin (5HT). Once 5HT is produced, a presynaptic membrane transporter, known as solute carrier family 6 member 4 (SLC6A4) [also referred as serotonin transporter (5HTT)] transports it back into presynaptic cells (Blier et al., 2002). Studies have demonstrated that dysregulated expression of the gene encoding 5HTT can lead to reduced levels of 5HTT and may result in negative consequences for an individual during a stressful situation (Caspi et al., 2010; Knuts et al., 2014). Reduced levels of 5HTT have been associated with blunted cortisol level, resulting in impaired HPA axis activity, which has been linked to psychiatric disorders including PTSD (Southwick et al., 1999; Yehuda et al., 1995). Therefore, different processes influence the HPA axis response to stress. Thus, this means the different neural substrates involved in the biological response to stress may be investigated is assessing genes that are essential for their synthesis.

1.3 Genetics of aggression and posttraumatic stress disorder

The perpetration of aggression is thought to be influenced by genetic predisposition and by a dynamic interaction between genes, psychological and social variability (Caspi, 2002; T. J. H. Chen et al., 2005; Ficks and Waldman, 2014; Frazzetto et al., 2007). There is evidence that aggressive behaviour is predominantly found in males compared to females (Craig and Halton, 2009; Vierikko et al., 2003). Research suggests that as individuals age the exhibition of aggressive behaviour traits increases, ranging from 55% between the age of 1–5 years to 65% between the age of 11–18 years (Craig, 2007; Craig and Halton, 2009; Miles and Carey, 1997; Vierikko et al., 2003). Genetic susceptibility is suggested to explain at least 65% of the variance of different forms of violent, aggressive, and impulsive behaviours (Burt, 2009; Mason and Frick, 1994; Miles and Carey, 1997; Rhee and Waldman, 2002).

Similarly, the development of PTSD has also been found to be influenced by genetic makeup (Koenen et al., 2003; Kremen et al., 2012; Lyons et al., 1993; Stein et al., 2002; True et al., 1993). Genetic factors that contribute to the development of PTSD have been estimated at between 28-46% (Ehlers et al., 2013; Sartor et al., 2012). Differences in genetic factors contributing to the development of PTSD have been shown by studying individuals who have experienced same stressful event (Chang et al., 2012; Nievergelt et al., 2015). These previous studies have shown that despite experiences of same stressful events, some individuals develop PTSD, whereas others do not develop PTSD but develop appetitive aggression (Hecker et al., 2013; Weierstall et al., 2012b); these differences have been attributed to genetic and environmental factors. Thus, interindividual differences in genetic predisposition contribute distinctly to PTSD as well as to the development of aggression.

This literature review will focus on discussing specific genes that have been studied in different types of aggression and PTSD. It is important to note at this juncture that previous studies have investigated these genes in populations originating largely from Europe and North America; therefore, African populations are under-represented. Furthermore, previous studies have investigated aggression in general, but less is understood about the association between appetitive aggression and genetic factors in the context of ongoing exposure to violence. In order to understand the genetic contribution to aggression and PTSD, in the context of childhood adversity exposure, several genes encoding for neuropeptides involved in neurotransmission, as well as hormones, enzymes and receptors have been investigated (Table 1.1). These studies considered a number of aggressive behaviours: conduct disorder, antisocial personality disorder, oppositional defiant disorder, reactive aggression, as well as proactive instrumental aggression (Table 1.1) (Caspi, 2002; Fernàndez-Castillo and

Cormand, 2016; Ficks and Waldman, 2014; Gorodetsky et al., 2014; Oades et al., 2008; Sysoeva et al., 2009). The most intensively investigated genes in these aggressive behaviours overlap with genes investigated in many psychiatric disorders such as PTSD, and studies have been conducted to investigate their contribution to the development of aggression and their role in the onset of PTSD (Schechter et al., 2016; Valente et al., 2011b).

Several neurobiological pathways such as serotonergic, noradrenergic, dopaminergic and neurotrophic systems are affected in psychiatric disorders (Brunello et al., 2001; T. Chen et al., 2005; Rakofsky et al., 2012). These neurobiological pathways are complex to understand and different genes play distinct roles in several physiological responses. Investigations seeking to understand genetic factors in psychiatric disorders and behavioural problems have included several genes. These genes have included brain-derived neurotrophic factor (*BDNF*) (Felmingham et al., 2013; Kretschmer et al., 2014; Musci et al., 2014), catechol-*O*-methyltransferase (*COMT*) (Calati et al., 2011; Hirata et al., 2013; Perroud et al., 2010), corticotrophin releasing hormone receptor 1 (*CRHR1*) (Amstadter et al., 2011; Boscarino et al., 2013), dopamine receptor 2 and 4 (*DRD2* and *DRD4*) (Buchmann et al., 2014; Dmitrieva et al., 2011; Guo et al., 2007; Vaughn et al., 2009), FK506 binding protein 5 (*FKBP5*) (Binder et al., 2008a; Boscarino et al., 2012, 2011; Sarapas et al., 2011; Wilker et al., 2014; Xie et al., 2010), monoamine oxidase A (*MAOA*) (Antypa et al., 2013; Armstrong et al., 2014; Beaver et al., 2014, 2010; Frazzetto et al., 2007; Gallardo-Pujol et al., 2013; Gorodetsky et al., 2014; Kuepper et al., 2013; Manuck et al., 2000; McDermott et al., 2009; Pingault et al., 2013; Reif et al., 2007; Verhoeven et al., 2012) and solute carrier family 6 member 4 (*SLC6A4*) (Heils et al., 1996; Kilpatrick et al., 2007; Lesch et al., 1996; Liu et al., 2015; Oades et al., 2008). These investigations are summarised in Table 1.1. Variants of some of these genes have been found to be associated with aggression related behaviours, i.e. conduct disorder and antisocial personality disorder; as well as PTSD. Although a number of genes have been investigated in both aggression and PTSD, from separate or combined samples, the discussion below will focus only on genes or variants that have been investigated in both aggression and PTSD including experiences of childhood adversity.

One of the genes that have been investigated is both aggression and PTSD is *BDNF*, which encodes brain derived neurotrophic factor (BDNF), a protein involved in neuroplasticity (Felmingham et al., 2013; Kowiański et al., 2017; Kretschmer et al., 2014; Musci et al., 2014). BDNF is essential in the brain and spinal cord as it plays a role for the survival of neurons by ensuring that their differentiation occurs adequately and that they are sufficiently matured. The activity of this protein occurs mostly between neurons, where cell-to-cell signalling occurs for information transmission to influence synaptic plasticity (Kowiański et

al., 2017; Liu and Nusslock, 2018). Genetic substitution of G to A nucleotides sequence at position 196 within codon 66, termed rs6265 (Lester et al., 2012) and normally referred to as Val66Metpolymorphism results in an amino acid variation from valine to methionine. Val66Met polymorphism has been found to be associated with the development of PTSD symptoms (Andero and Ressler, 2012; Hemmings et al., 2013; Pivac et al., 2012). This genetic variation modulates the synthesis of BDNF, which may alter its optimal intracellular function. Research indicates that carriers of Met66 variant are at risk of developing PTSD (Li et al., 2016; Sapolsky et al., 2000; Zhang et al., 2016).

In a Chinese population that had experienced the Wenchuan earthquake, Li et al. (2016) investigated the association between PTSD symptoms and Val66Met genotype longitudinally in the BDNF gene and found that carriers of Val66 polymorphism had lower symptoms of PTSD compared to carriers of Met66 polymorphism (Li et al., 2016). Furthermore, the Val66 allele has been indicated to be associated with improved symptoms of PTSD after exposure to therapy such as cognitive behavioural therapy (CBT). In a study by Felmingham et al. (2013), it was found that individuals who were carriers of Val66 polymorphism responded more positively to CBT compared to those who had the Met66 polymorphism (Felmingham et al., 2013). On the other hand, carriers of the homozygous genotype Met66Met polymorphism variant have also been linked to hostility, anger, and the perpetration of physical aggression in men compared to carriers of the homozygous genotype of Val66Val (Ilchibaeva et al., 2015; Kretschmer et al., 2014; Maynard et al., 2016; Musci et al., 2014). However, the Val66Met heterozygous genotype has not shown a different risk than Val66Val in the perpetration of aggression (Kretschmer et al., 2014). These findings indicate that the interaction between *BDNF* gene and environmental factors is essential in the stress response system and this interaction can facilitate behavioural changes. Therefore, variation in this gene may alter normal response to stress and worsen symptoms of PTSD as well as increase the risk of developing aggressive related behaviours.

It is interesting to note that *BDNF* has been found to interact with another gene, dopamine receptor D2 (*DRD2*). Dopamine receptor D2 (DRD2) is an essential protein in the brain, which facilitates memory formation and plasticity of neurons (Saab et al., 2009). *DRD2* gene has been shown to harbour a single nucleotide polymorphism (SNP) (rs1800497), which is located 10kb downstream from the ankyrin repeat and kinase domain containing 1 (*ANKK1*) gene (Comings et al., 1996; Young et al., 2002). This SNP is often referred as *Taq1A,* consisting of *T (A1)* and C *(A2)* variations (Comings et al., 1996). This SNP results in amino acid substitution of glutamate to lysine at position 713 in the protein sequence (Neville et al., 2004). It has been reported to be associated with PTSD development (Hemmings et al.,

2013) and behavioural changes such as addiction, alcoholism and aggression (Smith et al., 2008). Butovskaya et al. (2013) found that genetic variation of *DRD2* was associated with aggression and anger in Datoga men, a Tanzanian ethicnic group (Butovskaya et al., 2013). Furtheremore, Hemmings et al. (2013) reported an epistatic interaction between *BDNF* Val66Met and *DRD2 Taq1A* polymorphisms. This previous study hypothesised that the interaction between *BDNF* Val66Met and *DRD2 Taq1A* polymorphisms had a U-shaped function of dopamine (Hemmings et al., 2013). *DRD2 Taq1A* variant has been reported to affect the concentration of dopamine in different parts of the brain (Egan et al., 2003). It has been shown that the homozogous Val66Val genotype is associated with an increased activity of BDNF, in conjuction with the decreased DRD2 expression and linked to the *DRD2 Taq1A* genotype, contribute to increased concentration of dopamine (Jönsson et al., 1999; Ritchie and Noble, 2003). Research suggests that either too low or too high concentration of dopamine can disrupt congnitive function (Cools and D'Esposito, 2011; Goldman-Rakic et al., 2000; Koven and Carr, 2012). Moreover, it can be assumed that genetic variation in *DRD2* gene affects the normal reponse to stress, which has an impact on thought processing and can lead to the risk of PTSD development and behavioural disorders like aggression.

DRD4 encodes for dopamine receptor 4 (DRD4), another protein in the brain that functions to moderate neurons to form memory (Saab et al., 2009). Studies have shown that variation in *DRD4* impacts negatively on brain function and can lead to the risk of developing aggression (Buchmann et al., 2014; Dmitrieva et al., 2011; Schlomer et al., 2015) and PTSD (Dragan and Oniszczenko, 2009). Dmitrieva et al. (2011) found a variation in *DRD4* spanning exon 3, specifically 2 repeats (2R) that was associated with delinquency, anger and thrill seeking in a Russian sample (Dmitrieva et al., 2011). In another study, Buchmann et al. (2014) found that 7-repeats (7R) in exon 3 of *DRD4* was associated with aggression, externalizing behaviours, and delinquency (Buchmann et al., 2014). Another study found that this 7R in *DRD4* was associated with PTSD symptoms (Dragan and Oniszczenko, 2009). These findings of DRD4 association with both aggression and PTSD indicate that variations in *DRD4* can lead to the disruption of normal function for the DRD4 protein, which can affect the normal processing of memory.

Research has shed some light that genetic variation in the catechol-O-methyltransferase (*COMT)* gene is associated with development of PTSD and aggresion (Butovskaya et al., 2013; Valente et al., 2011a; Wagner et al., 2010). *COMT* is an essential gene in the neuroendocrine system and encodes an enzyme catechol-O-methyltransferase, which is responsible for degrading a number of neurotransmitters, such as norepinephrine, epinephrine, and dopamine (Lachman et al., 1996; Lonsdorf et al., 2010). This gene contains

a functional polymorphism, rs4680, harboured in codon 158, which leads to the amino acid substitution of valine to methionine (Val158Met). The Val158 variant is reported to have an optimal function than Met158 variant ; thus, the homozygous Val158 variant can catabolise neurotransmitters at high rate compared to Met158 variant (Schacht, 2016). Genetic variation in *COMT* is suggested to play a role in hyperarousal, a symptom of PTSD (Kolassa et al., 2010). In another study, it was reported that this variation in *COMT* was associated with PTSD symptoms in a Brazilian population that had experienced violence (Valente et al., 2011a). Furthermore, *COMT* variation has been found to be associated with physical aggression and impulsivity (Butovskaya et al., 2013; Wagner et al., 2010). Butovskaya et al. (2013) found that the interaction between polymorphisms of dopamine active transporter (DAT1, SLC6A3)-COMT pair genes, was associated with hostility and anger behaviour, which was linked to physical aggression in Tanzanian Datoga men. In addition, Wagner et al. (2010) found that carriers of *COMT* Val158Val variant had increased levels of impulsive aggresion (Wagner et al., 2010) compared to Val158Met and Met158Met carriers.

One of the first studies to investigate the association between specific genotypes in aggression was performed in a Dutch family in 1993 (Brunner et al., 1993a). The aggressive men in the family were found to harbour a mutation in the monoamine oxidase A (*MAOA*) gene. *MAOA* encodes for the enzyme, monoamine oxidase A enzyme, which catabolises neurotransmitters such as dopamine, serotonin, and epinephrine. This gene has a polymorphic upstream variable number of tandem repeats (uVNTR) located within the promoter region [1.2 kilobases (kb)] away from the coding region (Denney et al., 1994; Sabol et al., 1998). This polymorphic region has been associated with the development of aggressive behaviours (Chester et al., 2015; Sabol et al., 1998). The uVNTR polymorphism, *MAOA*-uVNTR, is comprised of variable numbers of 30 bp repeats referred as 2-, 3-, 3.5-, 4- and 5-repeat alleles (Antypa et al., 2013; Huang et al., 2004; Sabol et al., 1998). Two of these alleles, the 2- and 3-repeats (referred to as *MAOA-L*), have been associated with decreased transcription of *MAOA*, leading to reduced activity of MAOA, while 3.5- and 4-repeat alleles (*MAOA-H*) have been associated with increased expression, resulting in high activity of MAOA (Brummett et al., 2008; Deckert et al., 1999; Sabol et al., 1998). The functional impact of the 5-repeat allele has not yet been clearly characterised (Kim-Cohen et al., 2006).

MAOA-L has been associated with violent behaviour, aggression, and impulsivity (Beaver et al., 2013, 2010; Gallardo-Pujol et al., 2013; Kuepper et al., 2013; Manor et al., 2002; Reif et al., 2007; Tiihonen et al., 2014). Further studies have found an association between the *MAOA-L* and antisocial behaviour in maltreated children (Byrd and Manuck, 2014). Antisocial

personality disorder refers to a diverse collection of physical aggressive behaviours associated with bullying and fighting, which can also include irritability, rule-breaking, and oppositional behaviour (Moffitt et al., 2002; Piotrowska et al., 2015). In addition to the findings for both *MAOA-L* and *MAOA-H,* several single nucleotide polymorphisms (SNPs) in *MAOA* including rs5906893, rs5906957, rs2283725, rs2072744, and rs979605 have been associated with antisocial personality disorder (Ouellet-Morin et al., 2016). Although there are only limited studies that have investigated the association between PTSD and variants in *MAOA*, one study has investigated the association between *MAOA*-uVNTR and PTSD and found no association (Svob Strac et al., 2016a). However, an association between a variant of the monoamine oxidase B (*MAOB*) gene, rs1799836, was found to be linked with severity of PTSD symptoms in male war veterans (Svob Strac et al., 2016b). *MAOB* is structurally similar to the *MAOA* gene, and it encodes an isozyme, monoamine oxidase B (Sabol et al., 1998). MAOB has a similar function to MAOA as both metabolise dopamine, though MAOA catabolises other neurotransmitters such as serotonin, noradrenaline while MAOB does not (Youdim and Weinstock, 2004). The functional rs1799836 SNP has been found to be associated with negative emotional personality as well as depression (Dlugos et al., 2009). Therefore, since both *MAOA* and *MAOB* are functionally similar, their variation could alter the rate of metabolising neurotransmitters. This change could disturb the normal functioning of the stress response system, which has been implicated in the development of PTSD and aggression. Overall, genetic variation in both *MAOA* and *MAOB* can result in malfunction of the stress response.

SLC6A4, which spans 17q11.1–q12, encodes for the solute carrier family 6 member 4 (SLC6A4), also known as the serotonin transporter (Heils et al., 1996; Lesch et al., 1996). The 5HTT is responsible for the rapid reuptake of 5HT from the synaptic cleft, and it has a crucial role in regulating the concentration of serotonin in the brain (Risch and Nemeroff, 1992). *SLC6A4* has been extensively investigated for its role in various psychiatric disorders, including PTSD, depression, and aggression (Kilpatrick et al., 2007; Liu et al., 2015; Oades et al., 2008). The promoter region of this gene is characterised by a variable number of tandem repeats (VNTRs) of an insertion-deletion (indel) polymorphism namely the serotonin transporter-linked polymorphic region (5-HTTLPR) (Åslund et al., 2009; Heils et al., 1996; Lesch et al., 1994; Voyiaziakis et al., 2011). This polymorphism consists of two alleles that are often differentiated by at least 44 bp in length. These alleles are known as short (S) and long (L) and have been functionally characterised; the L-allele is associated with sufficient transporter transcription, while the S-allele has been linked to reduced expression (Greenberg et al., 1999; Heils et al., 1996; Lee et al., 2005; Lesch et al., 1996; Praschak-Rieder et al., 2007). A further SNP, rs25531, located in the 5-HTTLPR polymorphic region,

has been discovered (Hu et al., 2006). The rs25531 SNP has been suggested to alter the functionality of the L-allele by modulating transcription, resulting in reduced expression of *SLC6A4*. Researchers have found that the L-G haplotype is similar in function to the S-allele, thus resulting in decreased expression of *SLC6A4*, as opposed to the L-A haplotype, which is linked to increased *SLC6A4* transcription (Praschak-Rieder et al., 2007).

The SS genotype has been found to increase the risk of developing aggressive and violent behaviour, impulsive aggression, neuroticism, criminal behaviour, and anger (Beevers et al., 2009; Gerra et al., 2005; Gonda et al., 2009; Greenberg et al., 2000; Gyurak et al., 2013; Haase et al., 2015; Liao et al., 2004; Lopez-Castroman et al., 2014; Reif et al., 2007; Sysoeva et al., 2009). Other studies have found a significant association between the S-allele and conduct disorder (Brody et al., 2011; Malmberg et al., 2008; Sakai et al., 2010, 2006); antisocial behaviour, (Cicchetti et al., 2012; Ficks and Waldman, 2014; Garcia et al., 2010) and PTSD (Kimbrel et al., 2015; Liu et al., 2015; Mellman et al., 2009). These previous findings indicate that these genetic variants are linked in different mental illnesses and behavioural problems. Since the S-allele has been found to be associated with both aggression and PTSD, this association suggests that there are common mechanisms in the development of these disorders. The S-allele has been found to be associated with reduced expression of *SLC6A4,* leading to malfunctioning in the regulation of serotonin. This dysfunctioning in the modulation of serotonin results in an unbalanced neuroendocrine response, which is involved in both PTSD and aggression.

Another polymorphic VNTRs region located in intron 2 of *SLC6A4*, namely serotonin transporter intron 2 (STin2), has been investigated in both aggression and PTSD (MacKenzie and Quinn, 1999a; Oades et al., 2008; Sarosi et al., 2008a). This polymorphic region is characterised by different numbers of repeats that are 17 bp in length to produce 9-repeat (STin2.9), 10-repeat (STin2.10), and 12-repeat (STin2.12) variants (Lesch et al., 1994). The STin2.12 is referred to as the long allele (L), while both STin2.9 and STin2.10 are referred as short alleles (S). Studies have shown that STin2.10 is associated with reduced expression of the *SL6A4* gene, while STin2.12 has been linked to increased expression of this gene (Heils et al., 1996; MacKenzie and Quinn, 1999b; Sarosi et al., 2008b). Previous investigations have found that STin2.12 is linked to the perpetration of aggressive behaviours in individuals with impulsivity, persistent and pervasive aggression in both children and adults (Ali et al., 2010; Aluja et al., 2009; Beitchman et al., 2006; Davidge et al., 2004).

Studies have found an association between the short alleles of STin2 (STin2.9 and STin2.10) and PTSD symptoms. This association was enhanced by the combination of short STin2

variants and the effect of 5-HTTLPR genotypes together with SNP rs25531 (Grabe et al., 2009; Mercer et al., 2012). In addition to these *SLC6A4* variants, variation in the serotonin receptors 2A and 1B (*HTR2A* and *HTR1B*) genes have also been associated with aggression (Banlaki et al., 2015; Berggård et al., 2003; Dijkstra et al., 2013; Mik et al., 2007) and PTSD symptoms (Donaldson et al., 2016; Miller et al., 2016). The SNPs (rs977003 and rs7322347) of *HTR2A* were found to be associated with PTSD. In addition, rs7322347 was found to be associated with aggression (Banlaki et al., 2015). Moreover, variations in these genes can lead to the risk of developing PTSD and aggression.

FKBP5 encodes for the FK506 binding protein 5 (FKBP5), a functional protein that plays a pivotal role in stress regulation by modifying GRs activity and its sensitivity (Binder et al., 2008a; Klengel et al., 2013). The results of *FKBP5* investigations suggest a strong association between this gene and the development of PTSD (Binder et al., 2008b; Mehta et al., 2011). In a study comprised of civilian African-Americans, four SNPs (rs9296158, rs3800373, 1360780, rs9470080) were found to be associated with PTSD symptom severity (Binder et al., 2008a). These SNPs predicted PTSD severity in adults who had a history of childhood maltreatment, thus indicating the interaction between the gene and childhood abuse as risk for the development of PTSD symptoms (Binder et al., 2008a). These findings were partially replicated in another study of African-Americans who had a history of childhood adversity; however, only the rs9470080 SNP of *FKBP5* was associated with a risk of PTSD (Xie et al., 2010). Furthermore, another study found that a polymorphism within *FKBP5*, rs1360780, mediated the risk of developing PTSD and other psychiatric disorders.

The association between *FKBP5* rs1360780 variant and the development of PTSD was further shown to be exacerbated by the interaction between this polymorphism and DNA methylation, an epigenetic change within the gene in the development of PTSD, particularly in those who had a history of childhood abuse (Klengel et al., 2013). In addition to this relationship between the risk to develop PTSD and variants of *FKBP5*, interaction between epigenetic changes in the promoter region of this gene and genetic variants like *FKBP5* rs1360780 have been found to be associated with psychotherapy response (Yehuda et al., 2013). Other studies have found that *FKBP5* variants can be a risk factor for the development of aggression. Bevilacqua et al. 2012 found that the rs1360780 variant of *FKBP5* was associated with aggression in Italian Caucasians inmates who had childhood adversity, such as physical abuse (Bevilacqua et al., 2012). Another SNP of *FKBP5*, rs4713916, was found to be associated with externalising behaviour in a Dutch Caucasian sample (Bryushkova et al., 2016) and, in turn, related to aggressive behaviours (Bryushkova et al., 2016; Craig, 2007).

Other genes have also been investigated for their association with PTSD and aggression. These genes include nuclear receptor subfamily 3 group C member 1 (glucocorticoid receptor) (*NR3C1*) (Bachmann et al., 2005; Hauer et al., 2011; van Zuiden et al., 2012), oxytocin receptor (*OXTR*) (Feldman et al., 2014a; Sippel et al., 2017), gamma-aminobutyric acid (GABA) A receptor, alpha 2 (*GABRA2)* (Dick et al., 2009; Nelson et al., 2009), dopamine receptor 4 (*DRD4)* (Buchmann et al., 2014; Dmitrieva et al., 2011), corticotrophin releasing hormone receptor 1 (*CRHR1*) (Amstadter et al., 2011; B. Chen et al., 2014). The *NR3C1* encodes for glucocorticoid receptors (GRs), which are essential during the stress response as they bind to cortisol. A study involving Vietnam combat veterans found that the rs41423247, a SNP within *NR3C1*, was associated with a risk of developing PTSD (Bachmann et al., 2005). In a study by van Zuiden et al. (2012) in Dutch Caucasians, the rs41423247 SNP was also found to be associated with PTSD. Lie et al. (2017) investigated the association of variations in *NR3C1* and found that the rs1800445 was associated with aggressive behaviour in the Han Chinese population (Li et al., 2017).

OXTR encodes for the oxytocin receptor, another essential protein that binds to oxytocin, a neurotransmitter that activates dopaminergic neurons that has effects on sexual, social, and addictive behaviours (McGregor et al., 2008). Variations in *OXTR* have been found to be associated with persistent and pervasive aggressive behaviours (Malik et al., 2012) and PTSD (Sippel et al., 2017). In a study conducted by Malik et al. (2012), it was found that the rs6770632 and rs1042778 SNPs were associated with aggression (Malik et al., 2012). The follow-up study also identified the rs6770632 and rs237898 SNPs, which were associated with aggression (Malik et al., 2014). In other studies, it was found that the rs1042778, rs2254298, rs53576 (Feldman et al., 2014a) and rs53576 (Lucas-Thompson and Holman, 2013; Sippel et al., 2017) in *OXTR* were associated with PTSD. Interestingly, the rs53576 SNP has been implicated in both aggression and PTSD (Feldman et al., 2014b; Lucas-Thompson and Holman, 2013), indicating potential shared mechanisms in the development of these states. Therefore, variations in *OXTR* alters the product of this gene, which can lead to improper function of the produced protein that affects downstream mechanisms.

GABRA2 encodes for gamma-aminobutyric acid (GABA) A receptor, alpha 2 which also plays an essential role in mediating neural activity to process information in inter-neurons (Engin et al., 2012). Variation in *GABRA2* has been found to be associated with PTSD (Nelson et al., 2009) and aggression (Dick et al., 2009). The rs497068 SNP was found to be associated with externalising behaviour (Dick et al., 2009), while rs279836, rs279826, rs279858 and rs279871 have been found to be associated with PTSD (Nelson et al., 2009).

Indeed, it is interesting that these polymophysisms in *GABRA2* could be risk markers for PTSD and aggressive behaviour. It has previously been hypothesised that variation in *GABRA2* may play a role in disrupting excitability and inhibition in the CNS (Edenberg et al., 2004). Thus, these reported variations in *GABRA2* may be associated with disrupted homeostatic balance in the CNS (Begleiter and Porjesz, 1999; Edenberg et al., 2004). Therefore, variations in *GABRA2* could result in impaired homeostatic balance in the CNS, which may be hypothesised to be a risk for developing PTSD and behavioural problems.

Table 1. 1: Genetic alleles that have been investigated in aggression, anger, violent behaviour, childhood adversity and posttraumatic stress disorder in humans

Gene	Allele/Variant /	Associated phenotypes	Number of participants	Ethnicity (%)	Gender (%)	P-value	Reference
Brain-derived neurotrophic factor (*BDNF*)	rs6265 (valine66methione)	Antisocial behaviour and impulsivity	9244	Caucasians (95.9%) and Non-Whites (4.1)	Females and males	0.001	Kretschmer et al. 2014
	rs6265 (Val66Met)	Impulsive behaviour	678	African Americans (86.8%) and Caucasians (13.2%)	Females and males	0.05	Musci et al. 2014
	rs6265 (Val66Met))	Impulsive behaviour	315	Caucasians (90%), Chinese, Africans, Indians and Native-Americans (10%)	Females (20%) and males (80%)	0.008	Lee et al. 2007
	rs6265 (Val66Met)	PTSD	55	Caucasians	Females and males	—	Felmingham et al. 2013
	rs6265 (Val66Met)	PTSD	268 (107 with PTSD,161 controls)	Koreans	Female (58%) and male (42%)	0.81	Lee et al. 2006
	rs6265 (Val66Met)	PTSD	576 (370 with PTSD and206 controls)		Males	—	Pivic et al. 2012
Catechol-O-methyltransferase (*COMT*)	rs6269	Childhood-onset aggression, social behaviour	144	Caucasians (77.6%), African Canadians (5.6%), mixed ancestry (16.7%)	Female (27.8%) and male (72.2%)	0.019	Hirata et al. 2013
	rs4818	Childhood-onset aggression, social behaviour	144	Caucasians (77.6%), African Canadians (5.6%), mixed ancestry	Female (27.8%) and male (72.2%)	0.064	Hirata et al. 2013
	rs4680	Childhood-onset aggression,	144	Caucasians (77.6%), African Canadian (5.6%),	Female (27.8%) and male (72.2%)	0.173	Hirata et al. 2013

		social behaviour		mixed ancestry (16.7%)			
	rs4633	Childhood-onset aggression, social behaviour	144	Caucasians (77.6%), African Canadians (5.6%), mixed ancestry (16.7%)	Female (27.8%) and male (72.2%)	0.240	Hirata et al. 2013
	rs4680	Anger , trait anger	308	Koreans		—	Kang et al. 2008
	rs4680	Anger trait	875	Caucasians	Female (40.3%) and male (59.7%)	0.001	Perroud et al. 2010
	rs4680	Anger and aggression	477 (149 with aggression and anger, 328 controls)	Caucasians	Female (70.7%) and male (29.3%)	0.0002	Rujescu et al. 2003
	rs4680	Externalizing behaviour	502	Caucasians	Female (57.7%) and male (42.3%)	0.001	Shehzad et al. 2012
	rs4680	PTSD	412	Caucasians	Female (68.8%) and male (31.2%)	0.001	Boscarino et al.2013
	rs4680	PTSD	424	Caucasians	Female (68.8%) and male (31.2%)	0.0178	Boscarino et al. 2013
	rs4680	PTSD	270 (98 with PTSD and 172 controls)	Rwandan Africans	Female (68.9%) and male (30.2%)	0.000	Kolassa et al. 2010
	rs4680	PTSD	335 (65 with PTSD and 270 controls)	African Americans	Female (53.3%) and male (46.7%)	0.05	Norrholm et al. 2013
	rs4860	PTSD	99 65 with PTSD and 34 without PTSD)	Brazilians	Female (67.3%) and male (32.7%)	0.02	Valente et al. 2011
			55	American Caucasians	Female (38.2%) and male (61.8%)		
Corticotrophin releasing hormone receptor 1 (*CRHR1*)	rs4458044, rs242924, and rs1768996	Violent aggressive behaviour	459 177 with violent behaviour and 282 controls)	Chinese Han	Male	0.003	Chen et al. 2014
	rs4074461,	PTSD	103	Caucasians (40.8), African Americans (45.6%), unspecified (13.6%)	Female (73.8%) and male (26.2%)	0.02	Amstadter et al. 2011
	rs12944712	PTSD	103	Caucasians (40.8), African Americans (45.6%), unspecified	Female (73.8%) and male (26.2%)	0.005	Amstadter et al. 2011

				(13.6%)			
	rs12944712	PTSD	103	Caucasians (40.8), African American (45.6%), unspecified (13.6%)	Female (73.8%) and male (26.2%)	0.02	White et al. 2013
Dopamine receptor 2 (*DRD2*)	rs1800497	Pathological violent aggressive behaviour and violent delinquency	121 (30 with violence and 91 controls)	Unspecified	Unspecified	0.000	Chen et al. 2005
	rs1800497	Violent delinquency	3121	Africans and Hispanics	Male	0.000	Guo et al. 2007
	rs1800497	Pervasive childhood aggression	288 (144 aggressive children and 144 controls)	Unspecified	Unspecified	0.01	Zai et al. 2012
	rs1079598	Pervasive childhood aggression	288 (144 aggressive children and 144 healthy controls)	Unspecified	Unspecified	0.04	Zai et al. 2012
	rs1800497	PTSD	56 (32 with PTSD and 24 controls)	Unspecified	Unspecified	0.0001	Comings et al. 1996
	rs1800497	PTSD	212 (70 with PTSD and 130 did not meet PTSD threshold)	Armenian Caucasians	Females (57%) and males (43%)	0.56	Bailey et al. 2010
	rs2075652 and rs7131056	PTSD	834 (337 with PTSD and 497 controls)	Han Chinese	Females ((51%) and males (49%)	0.05	Duan et al. 2015
	rs1800497	PTSD	139 (52 with PTSD and 87 controls)	American Caucasians	Unspecified	0.52	Gelernter et al. 1999
	rs12364283	PTSD	1749 (1343 with PTSD and 406 controls)	Caucasians (68.6%) Unspecified Asian (5.1%), Aboriginal (1.4%) and mixed (24.9%)	Females (43%) and males (57%)	0.000	Nelson et al. 2014
	rs6277	PTSD	127	Caucasians	Unspecified	0.021	Voisey et al. 2009
	rs1800497	PTSD	151 (91 with PTSD and 51 controls)	Caucasians	Unspecified	0.009	Young et al. 2012

Dopamine Receptor 4 (*DRD4)*	7-repeat (7R)	Aggressive, externalizing behaviours, and delinquency	298	Unspecified	Females (53%) and males (47%)	0.032	Buchmann et al. 2014
	2-repeat (2R)	Delinquency, anger and thrill seeking	263	Russians (unspecified)	Females (50%) and males (50%)	0.05	Dmitrieva et al. 2011
	2-repeat (2R) allele	Anger trait	308	Koreans	Females (40%) and males (60%)	0.005	Kang et al. 2008
	7-repeat (7R)	Aggression and hostility	580	Caucasians	Females (54.8%) and males (45.2%)	—	Schlomer et al. 2015
	7-repeat (7R)	PTSD	118	Polish Caucasians	Females (53%) and males (47%)	.05	Dragan & Oniszczenko 2009
FK506 binding protein 5 (*FKBP5*)	rs1360780	Aggression, hostility and impulsivity	583	Italian Caucasians	Males	0.05	Bevilacqua et al. 2012
	rs4713916	Externalising behaviour	340 (170 aggressive participants and 170 controls)	Dutch Caucasians	unspecified	0.018	Bryushkova et al. 2016
	rs9296158, rs3800373, rs4713916 and rs1360780	PTSD	900	Caucasians (2.2%), African Americans (95.2%), Hispanics (0.6%), Asians (0.1%) mixed (0.9%), unspecified (1%)	Females (57.3%) and males (42.7%)	002	Binder et al. 2008
	rs9470080	PTSD	502	Caucasians	Unspecified	0.05	Boscarino et al. 2011
Gamma-aminobutyric acid (GABA) A receptor, alpha 2 (*GABRA2)*	rs497068	Externalising behaviour	585	Caucasians (81%), African American (17%), and unspecified other ethnicity (2%)	Females (52%) and males (48%)	0.007	Dick et al. 2009
	rs279836 rs279826 rs279858 rs279871	PTSD PTSD PTSD PTSD	238 247 241 209	Unspecified Unspecified Unspecified Unspecified	Unspecified Unspecified Unspecified Unspecified	<0.05 <0.05 <0.10 <0.05	Nelson et al. 2009 Nelson et al. 2009 Nelson et al. 2009 Nelson et al. 2009
Monoamine oxidase A (*MAOA)*	rs6323	Aggression, anger, and suicidality	578 (171 suicide attempters, 90 suicide completers, and 317 controls)	Unspecified (German)	Females (54.3%) and males (45.7%)	0.001	Antypa et al. 2013

	rs909525	Aggression, anger, and suicidality	578 (171 suicide attempters, 90 suicide completers, and 317 controls)	Unspecified (German)	Females (54.3%) and males (45.7%)	0.007	Antypa et al. 2013
	uVNTRs	Criminal activity, and delinquency	99	Unspecified	Unspecified	—	Armstrong et al. 2014
	uVNTRs	Gang-related fight and criminality	2196	Caucasian (67.6%), African American (17.4), other (15%)	Females (52.6%) and males (47.4%)	—	Beaver et al. 2010
	uVNTRs	Physical aggression	235	Caucasians	Females (65%) and males (35%)	0.75	Frazzetto et al. 2007
	uVNTRs	Aggressive behaviour	57	Caucasians	Males	0.04	Gallardo-Pujol et al. 2013
	uVNTRs	Aggressive behaviours, hostility, impulsivity	692	Italian Caucasians	Males	0.002	Gorodetsky et al. 2014
	uVNTRs	Reactive aggression	239	Caucasians	Females (63%) and males (37%)	<0.05	Kuepper et al. 2013
	uVNTRs	Hostility, impulsivity and lifetime aggression	110	Unspecified	Males	0.015	Manuck et al. 2000
	uVNTRs	Reactive aggression and anger	78	Unspecified	Males	0.065	McDermott et al. 2009
	rs5906957	Physical aggression	436	Canadian Caucasians	Unspecified	0.05	Pingault et al. 2013
	rs5953385	Physical aggression	436	Canadian Caucasians	Unspecified	0.09	Pingault et al. 2013
	rs2283725	Physical aggression	436	Canadian Caucasians	Unspecified	0.15	Pingault et al. 2013
	rs3027400	Physical aggression	436	Canadian Caucasians	Unspecified	0.52	Pingault et al. 2013
	rs2072744	Physical aggression	436	Canadian Caucasians	Unspecified	0.63	Pingault et al. 2013
	uVNTRs	Violence and aggressive behaviour	184	Caucasians	Males	0.027	Reif et al. 2007
	uVNTRs	Violent behaviour and impulsivity	174	Finnish Caucasians	Males	0.004	Tikkanen et al. 2009
	uVNTRs	Aggressive behaviour	114 (73 maltreated aggressive participants and 41 controls)	American Caucasians (24%), Hispanic (25%), African American (31%), and mixed race (19%)	Females (34%) and males (66%)	0.05	Weder et al. 2009
	uVNTRs	PTSD	249	Croatian Caucasians	Males	—	Svob Strac et al. 2016

Nuclear Receptor Subfamily 3 Group C Member 1 (glucocorticoid receptor) (*NR3C1*)	rs1800445	Aggressive behaviour and intentional interpersonal injury	—	Chinese Han	—	0.01	Li et al. 2017
	rs41423247	PTSD	160 (118 participants with PTSD and 42 control)	Vietnam veterans (unspecified)	Unspecified	0.048	Bachmann et al. 2005
	rs41423247	PTSD	126	Unspecified	Unspecified	0.03	Hauer et al. 2011
	rs41423247	PTSD	448 (35 participants with PTSD, 413 controls)	Dutch Caucasians	Females (10%) and males (90%)	0.001	van Zuiden et al. 2012
Oxytocin receptor (*OXTR*)	rs6770632 and rs1042778	Persistent and pervasive aggressive behaviour	236	Canadian Caucasians (82%), African Canadians (8%), mixed race (10%)	Females (31.4%) and males (68.6%)	0.032	Malik et al. 2012
	rs6770632	Persistent and pervasive aggressive behaviour	364	Caucasians	Females (18%) and males (82%)	0.028	Malik et al. 2014
	rs237898	Persistent and pervasive aggressive behaviour	364	Caucasians	Females (18%) and males (82%)	0.006	Malik et al. 2014
	rs1042778 rs2254298 rs53576	PTSD	232	Israelites (unspecified)	Females (52.4%) and males (47.6%)	0.01	Feldman et al. 2014
	rs53576	PTSD	704	Caucasians (74.9%), Africans (8.92%), Hispanic (8.92%), mixed race (7.19%)	Females (52.3%) and males (47.7%)	0.0001	Lucas-Thompson & Holman 2013
	rs53576	PTSD	2163	Caucasians	Unspecified	0.02	Sippel et al. 2017
Solute carrier family 6 member 4 (*SLC6A4*)	5HTTLPR	Violence , aggression, anger, impulsivity, hostility, neuroticism, externalising behaviour, delinquency, and criminal behaviour	1482	Unspecified	Unspecified	—	Åslund et al. 2009
	5HTTLPR	Externalising behaviour and impulsivity	87	—	—	—	Cadoret et al. 2003
	5HTTLPR	Aggression	381	Australian Caucasians (93%), mixed race (7%)	Females (61%) and males (39%)	—	Conway et al. 2012
	5HTTLPR	Persistent and pervasive	—	—	—	0.039	Davidge et al. 2004

		aggression					
	5HTTLPR	Temperamental traits and aggressiveness	216	Caucasians	Females (40.28%) and males (59.72%)	0.001	Gerra et al. 2005
	5HTTLPR	Hostility and aggression	169	—	Females	—	Gonda et al. 2009
	5HTTLPR	Emotional reactivity and expressive behaviour	163	Caucasian (70.63%) Asian (13.75%), Latino (6.75%), African American (3.13%), and mixed race (6.25%)	Females (66.88%) and males (33.12%)	0.01	Gyurak et al. 2013
	5HTTLPR	Aggressive behaviour	1187	Unspecified	Unspecified	—	Haberstick et al. 2006
	5HTTLPR	Violent behaviour	184 (72 violent participants and 112 non-violent)	Unspecified	Males	0.011	Reif et al. 2007
	5HTTLPR	PTSD	408	African Rwandan	Unspecified	—	Kolassa et al. 2010
	5HTTLPR	PTSD	589	Caucasian (90%) Asian (13.75%), Latino (3.9%), African American (3.9%), and mixed race (1.7%)	Females (63.5%) and males (36.5%)	—	Kilpatrick et al. 2007
	5HTTLPR	PTSD	116	Unspecified	Females (54%) and males (46%)	—	La Greca et al. 2013
	5HTTLPR	PTSD	297 (100 with PTSD and 197 controls)	Unspecified	Unspecified	—	Lee et al. 2005
	5HTTLPR	PTSD	1105 (517 with PTSD and 588 controls)	Caucasians (50.2%) and Non-Hispanic Africans (49.8%)	Females (31.6%) and males (68.4))	0.0025	Liu et al. 2015
	5HTTLPR	PTSD	118 (55 with PTSD and 63 controls)	African Americans	Females (66.1%) and males (33.9%)	—	Mellman et al. 2009
	5HTTLPR	PTSD	149	Caucasians (71.2%), Hispanic (10.3%), African Americans (9.6%), and mixed race (8.9%)	Females (58.9%) and males (41.1%)	0.001	Pietrzak et al. 2013
	5HTTLPR	PTSD	99 (65 with PTSD and 34 controls)	Brazilian (unspecified)	Females (66.7%) and males (33.3%)	0.3	Valente et al. 2011
	5HTTLPR	PTSD	388 (212	Caucasians	Females	0.03	Wang et al. 2011

	rs25531		with PTSD, 176 controls)	(69.8%), non-Caucasians (30.2%)	(13%) and males (87%)		
	5HTTLPR	PTSD	5178 (717 with PTSD, 4460 controls)	Caucasians (53.7%), African Americans (46.3%)	Females (44%) and males (56%)	0.019	Xie et al. 2012

5HTTLPR— serotonin-transporter-linked polymorphic region
N— number of participants
PTSD— posttraumatic stress disorder
uVNTRs— upstream variable number of tandem repeats

1.4 Gene-environment interaction in aggression and post-traumatic stress disorder

Gene-environment interaction refers to the dynamic interplay between genetic variations and environmental risk exposures in determining susceptibility to developing a disorder (Caspi, 2002; Kaufman et al., 2006). Several factors such as social and psychological factors and gene- environment interactions can influence biological responses that are associated with the development of mental health disorders as well as behavioural patterns (Legrand et al., 2008; Marceau et al., 2012). Studies have indicated that early childhood trauma and abuse, including prenatal stress exposure, may have a negative impact on mental health, and may lead to antisocial and aggressive behaviours, conduct disorders (Alia-Klein et al., 2009; Buckholtz and Meyer-Lindenberg, 2008; Fernàndez-Castillo and Cormand, 2016; Frazzetto et al., 2007) and an increased predisposition towards the development of several psychiatric disorders, including PTSD and depression (Derringer et al., 2010; Mehta et al., 2013; Nijmeijer et al., 2010; Reif et al., 2007; Uher, 2008).

In a sample of Caucasian men, it was found that the interaction between *MAOA*-uVNTR and environmental adversity increased the risk of developing aggressive behaviour (Gallardo-Pujol et al., 2013). Carriers of *MAOA-L* were found to exhibit more aggression than those who had *MAOA-H* (Gallardo-Pujol et al., 2013). Furthermore, other studies have investigated gene–environment interactions in several psychiatric disorders including PTSD. One of the most reported gene-environmental investigations involved *FKBP5* in PTSD (Binder et al., 2008b; Klengel et al., 2013; Mehta et al., 2011; Xie et al., 2010), Studies have focused on elucidating the effect of interaction between *FKBP5* genetic variations with childhood adversities. This line of evidence has shown that interaction between variation in *FKBP5* and environmental stressors increase the risk of developing PTSD (Klengel et al., 2013; Mehta et al., 2013). Therefore, these differences in gene-environment interactions can moderate behavioural changes and may partially explain why not all victims of maltreatment will develop aggression. Furthermore, gene-environmental interactions play a role in epigenetic

modification, such as DNA methylation, another essential biological change that can be affected by environmental stress (van Dongen et al., 2015; Guillemin et al., 2014; Provençal et al., 2014) and PTSD (Boks et al., 2014; Maddox et al., 2013; Norrholm et al., 2013; Rusiecki et al., 2012; Yehuda et al., 2014).

1.5 Epigenetics of aggression and posttraumatic stress disorder

Although multiple studies, including case-control and genome-wide association studies (GWAS), have sought to identify gene variants that are associated with behavioural and psychiatric disorders (including aggression), these studies have suggested a limited role for epigenetic and gene variations, explaining little of the heritability of complex disorders like PTSD and behavioural problems (including aggression). A number of environmental factors like childhood adversity and continuous exposure to stressful life events have been identified as risk factors for the development of aggression and PTSD. These risk factors are mediated by epigenetic changes in neural systems that regulate the stress response. Therefore, epigenetic modifications are an important element in better understanding the contribution of environmental factors to the development of anxiety-related disorders including PTSD and aggressive behaviour (Feil and Fraga, 2012; Feinberg, 2007; Yehuda et al., 2013).

Epigenetics is a branch of molecular investigations which research about biological modifications that happen without a change in the genomic sequence (Dupont et al., 2009; Kubota et al., 2012). Epigenetics play a significant role in different molecular pathways, particularly during early developmental stages of life such as the development and differentiation of embryo (Kubota et al., 2012). Therefore, aberrant epigenetic modifications can cause several defects and congenital disorders. Epigenetic modifications often are associated with environmental signals like dietary nutrients and traumatic experiences (Feil and Fraga, 2012; Hammamieh et al., 2017; Yehuda et al., 2014), which can underlie responses that can be observed, such as severity in disorders like PTSD (Hammamieh et al., 2017; Rusiecki et al., 2013). DNA methylation and histone modification are examples of epigenetics that play a key role in determining the configuration of chromatin, which has an essential role for the regulation of gene expression (Dupont et al., 2009; Kubota et al., 2012). Thus, epigenetic mechanisms through DNA methylation and histone proteins are responsible in regulating gene expression without changing the actual DNA sequence (Hou et al., 2012; Kubota et al., 2012). Therefore, the understanding of epigenetic modifications bridges a gap between genetic and environmental factors in shedding light on disease mechanisms, particularly in complex disorders, such as mental illness (Boks et al., 2014; Yehuda et al., 2013).

Epigenetic modification can also involve the regulation of protein production and packaging using histones, ubiquitination, acetylation, sumoylation, and phosphorylation, as well as microRNAs (miRNAs) and small interfering RNAs, which are non-coding RNA-mediated modifications (Bjornsson et al., 2004; Yehuda and Bierer, 2009). These epigenetic modifications are essential for the regulation of gene expression through several mechanisms such as the recruitment of cofactors and packaging of proteins, as well as chromatin conformation (Lister et al., 2013; Russo et al., 1996). Although there are several different possible epigenetic modifications, DNA methylation has thus far been the most extensively studied in relation to psychiatric disorders, such as PTSD and aggression. Therefore, DNA methylation could explain inter-individual variation in the response to trauma and may provide an insight into behavioural change.

DNA methylation is a reversible covalent binding of a methyl group ($–CH_3$) at the five prime cytosine-guanine dinucleotides (CpGs) in the DNA sequence. The attachment of a methyl group does not change the original DNA sequence (Ehrlich et al., 1982; Lister et al., 2013; Russo et al., 1996). The synthesis process of these methylations involves specialised enzymes called DNA methyltransferases (DNMTs) (Guo et al., 2014; Jia et al., 2007; Kadriu et al., 2012; Morris et al., 2014). DNA methyltransferases are a family of enzymes that recognise and methylate hemimethylated regions in the genome. There are two types of DNA methylation, methylcytosine (5mC) and hydroxymethylcytosine (5hmC), both occurring at the five prime end (Ehrlich et al., 1982; Guillemin et al., 2014; Weaver et al., 2005). DNA methylation plays a significant role in regulating gene expression for a particular purpose, such as in early development wherein specific cell types have to be developed (Borghol et al., 2012; Enoch et al., 2010). Several CpGs are methylated across the genes, but a cluster of these CpGs, called CpG islands—often found in the promoter regions of genes—are mostly unmethylated, allowing for the initiation of gene expression (McGowan et al., 2009).

Gene expression is dynamic and can be triggered and controlled by a variety of physiological mechanisms and environmental exposures (Keller et al., 2010; Wolf et al., 2016). It is normally initiated by transcription factors, i.e. homeobox domain proteins (vital proteins responsible for the switching on/off of gene to start of gene expression (Chen et al., 2011; Mitchell et al., 2015)). These transcription factors normally bind to the promotor regions of the gene and regulate the initiation of gene expression (Uddin et al., 2010; Weaver et al., 2007; Yehuda et al., 2011). However, if the promoter region is methylated, gene expression can be “switched off” (Nguyen et al., 2013). The position of methylation is essential in determining gene expression, as methylation of the promoter region prevents the initiation of

transcription, while methylation in the gene body does not affect gene expression, but rather it may stimulate transcription (Figure 1.3) (Duymich et al., 2016). DNA methylation, and by extension gene expression, may be mediated by environmental exposure. Thus, understanding DNA methylation may provide additional insight into environmentally-induced changes in gene expression (van Dongen et al., 2015; Guillemin et al., 2014; Provençal et al., 2014).

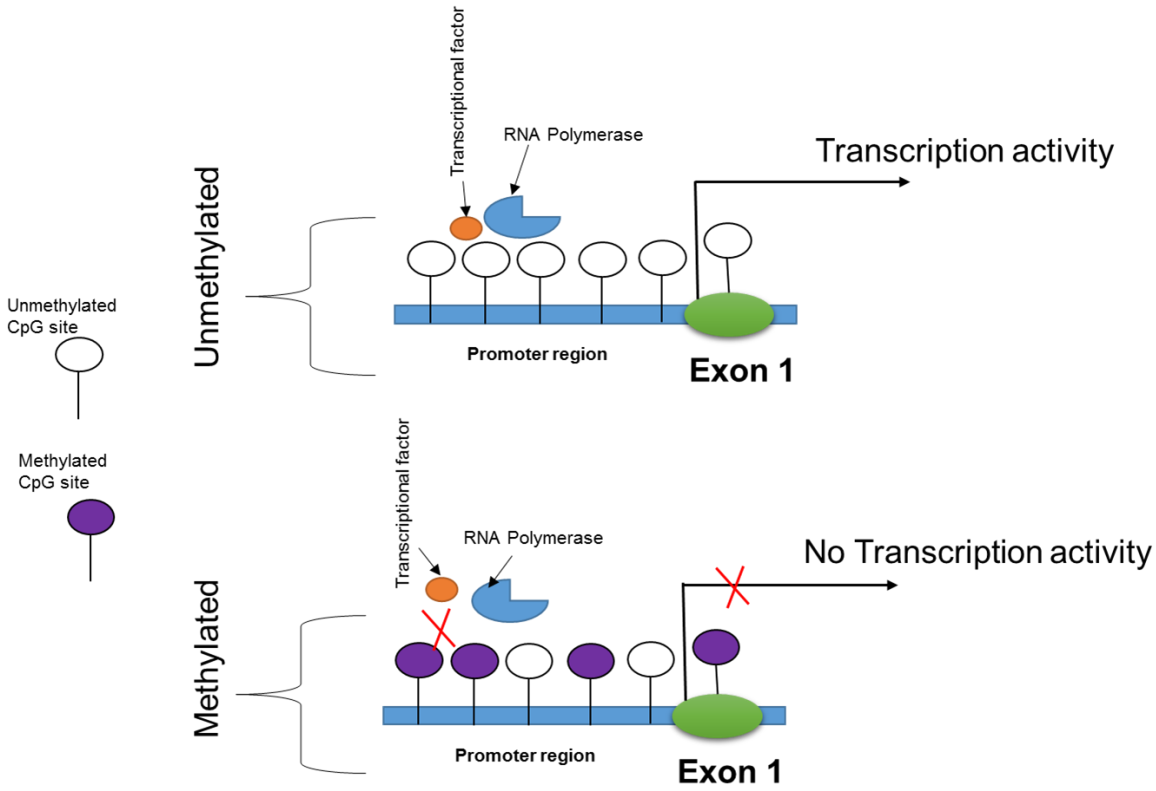

Figure 1. 3: **Schematic diagram of DNA methylation.**
Transcription activation occurs when transcription factors bind at promoter regions. However, in the presence of DNA methylation at promoter regions, there is a silencing of gene expression: the absence of DNA methylation allows activation of gene expression.

Several studies have investigated DNA methylation in order to understand its role in PTSD (Chang et al., 2012; Neylan et al., 2014; Wolf et al., 2016; Yehuda et al., 2013) and aggression (van Dongen et al., 2015; Guillemin et al., 2014; Provençal et al., 2014; Schechter et al., 2016; Tremblay and Szyf, 2010). These studies have elucidated an association between DNA methylation and different aggressive behaviours, such as physical aggression and antisocial behaviour (Guillemin et al., 2014; Provençal et al., 2014). Most of these genes, *SLC6A3* (Beach et al., 2011; Koenen et al., 2011; Provençal et al., 2013; Wang et al., 2012), *NR3C1* (Guillemin et al., 2014; Heinrich et al., 2015; Radtke et al., 2011;

Schechter et al., 2016; Vukojevic et al., 2014; Yehuda et al., 2015), *COMT* (Hu et al., 2018; Norrholm et al., 2013), are crucial in the stress response system (McGowan, 2013; Yehuda et al., 2013). As indicated in previous sections, all these genes are essential in the stress response system like HPA axis. They play a fundamental role and if dysregulated, the stress response system is also dysregulated. Results from studies that have investigated association between DNA methylation, aggression and PTSD are summarised in Table 1.2.

Although this literature review discusses methylation in *both* aggression and PTSD; methylation in other genes has been reported. These genes included genes such as arginine vasopressin receptor 1A (*AVPR1A*), 5-hydroxytryptamine receptor 1D *(HTR1D*) (serotonin receptor 1), dopamine receptor D1 (*DRD1*) and glutamate metabotropic receptor 5 (*GRM5*), interleukin 1 receptor antagonist (*IL1RN*) and interleukin 1 beta (*IL1B*) (Guillemin et al., 2014). Furthermore, other studies comparing individuals with PTSD and controls have also observed differences in methylation in genes. These genes included *ADCYAP1R1* (Ressler et al., 2011; Uddin et al., 2013), Alu element (*Alu*) insulin-like growth factor 2 (*IGF2),* interleukins, H19, long interspersed nuclear elements (*LINE-1*) (Rusiecki et al., 2013), annexin A2 (*ANXA2*), anaphase promoting complex subunit 5 (*APC5*), C-type lectin domain family 9, member A (*CLEC9A*); toll like receptor 8 (*TLR8*), and translocated promoter region (*TPR*) (Smith et al., 2011) and FKBP5 (Yehuda et al., 2013). Methylation of genes can be influenced by environmental exposures such that lasting imprints of these methylation modifications can be traced even in subsequent generations, which can predict risks related to the development of mental health disorders and aggressive behaviours (Meaney and Szyf, 2005; Mehta et al., 2013; Melas et al., 2013; Zhang et al., 2010).

In a study by Beach et al. (2011) that investigated methylation of 71 CpG sites in the 5HTTLPR of serotonin transporter promoter region in females, differential hypermethylation was associated with antisocial personality disorder (Beach et al., 2011). Another study also observed that hypermethylation in CpG sites in the 5HTTLPR region was associated with chronic physical aggression (Wang et al., 2012). Koenen et al. (2011) reported that hypomethylation in the 5HTTLPR region was observed to be associated with a risk of developing PTSD symptoms (Koenen et al., 2011). However, in the very same study, Koenen et al. (2011) showed that high exposure to traumatic events was associated with resilience against PTSD, which was associated with hypermethylation (Koenen et al., 2011). This variation in methylation in the 5HTTLPR region indicates that there could be inter-individual response towards stressful events (Zovkic et al., 2013). Thus, different individuals could respond differently with some developing PTSD while others not and may develop resilience against PTSD. This may be explained by inter-individual biological responses such

as methylation, as observed in the 5HTTLPR region of the *SLC6A3* (Koenen et al., 2011; Ouellet-Morin et al., 2013)

Gene methylation in *NR3C1* has been found in both PTSD (Vukojevic et al., 2014; Yehuda et al., 2015, 2014) and aggression (Heinrich et al., 2015; Parade et al., 2016). Methylation of 11 CpG sites (promoter region) in *NR3C1* has been found to be associated with externalising disorders in childhood and adolescence (Heinrich et al., 2015). Furthermore, Parade et al. (2016) showed that methylation of exons 1_D and 1_F regions, which forms part of the promoter region was associated with internalising and externalising disorder and other behavioural problems (Parade et al., 2016). Furthermore, another study observed that hypermethylation in the promoter region of *NR3C1* was associated with conduct disorder (Heinrich et al., 2015). Methylation in the 1_F region in *NR3C1* has been found to be associated with PTSD. Yehuda et al. (2013 and 2015) showed that altered methylation in *NR3C1* promoter region was associated with the development of PTSD. This methylation variation in *NR3C1,* like in other genes, indicates that there are underlying biological mechanisms involved in the response to experiences of traumatic events, which could affect the development of PTSD and aggression. Overall, variation in *NR3C1* and associated biological changes involved in the stress response may be associated with these disorders.

Studies investigating the methylation in *COMT* show an association between differential methylation and aggression (Hu et al., 2018) and PTSD (Norrholm et al., 2013). A study that investigating methylation in 41 CpG sites spanning the promoter region of *COMT* found that higher methylation was associated with the development of PTSD (Norrholm et al., 2013). In addition, participants with reduced methylation in this region demonstrated less fear to the safety signal compared than those who had high fear that was associated with higher methylation levels (Norrholm et al., 2013). In Chinese Han men, it was found that increased methylation was associated with homicide, a violent aggressive behaviour with the intent of murdering someone (Hu et al., 2018). Dysregulated methylation in *COMT* can lead to reduced production of COMT, resulting in increased production of metabolites like neurotransmitters due to decreased catabolism (Nohesara et al., 2011; Norrholm et al., 2013).

The development of PTSD and behavioural problems like aggression can be influenced by genetic and environmental factors (Kim-Cohen et al., 2006; Klengel et al., 2013; Zovkic et al., 2013). Although a combination of genetic and environmental factors can influence the risk of developing PTSD or resilience against it and aggressive behaviours, DNA methylation provides additional insights on the mediation between gene and environmental influences

(Kremen et al., 2012; McGowan et al., 2009; Russo et al., 2012; Weaver, 2009; Zovkic et al., 2013). Therefore, it is essential to investigate DNA methylation in a context of different environmental settings and interventions in order to understand the development of aggression and PTSD and facilitate the discovery of prognostic markers to monitor the response to interventions.

Table 1.2: DNA methylation modifications that have been investigated in relation to aggression, anger, violent behaviour, childhood adversity and posttraumatic stress disorder (PTSD)

Gene	Allele/variant/ site	Diagnostic characteristic s and associated phenotypes	Number of participants	Ethnicity	Gender (%)	P-value	Reference
Solute carrier family 6 (neurotransmitter transporter), member 4 (*SLC6A4*) (serotonin transporter)	CpG1— CpG71 in the 5HTTLPR	Antisocial Personality Disorder	155	Unspecified	Females	0.001	Beach et al. 2011
	CpG 5, 6, 11 and 12 in the 5HTTLPR	Chronic physical aggression	25 (8 aggressive participants and 17 controls)	French-Canadians	Males	0.001	Wang et al. 2012
	CpG 1 in the first intron of 5HTTLPR	PTSD	100	(N=79 African American, N=21 other)	Females (60%) and male (40%)	0.018	Koenen et al. 2011
Nuclear receptor subfamily 3 group C member 1 (*NR3C1*) (Glucocorticoid receptor)	CpG1—CpG11 (promoter region)	Externalising disorder in Childhood and Adolescence	192 (68 with behavioural problems and 124 controls)	Germans (unspecified	Females (55.65%) and male (44.35%)	0.009	Heinrich et al. 2015
	Exons 1_D and 1_F	Internalising and externalising disorder and behavioural problems	171	(N=39 American Caucasians, N=82 Hispanics, N=25 African Americans, N=25 other races)	Females (52%) and males (48%)	0.01	Parade et al. 2016
	CpG1—CpG10 (promoter region)	Intimate partner violence	49	Unspecified Germanic mothers (N=25) and their offspring 24)	Females and males	0.7	Radtke et al. 2011
	Exon 1_F	PTSD	122 (61 with PTSD and 61 controls)	(N=26 American Caucasians, N=48 Hispanics, N=26 African Americans)	Females and male (unspecified)	0.021	Yehuda et al. 2015

	CpG1—CpG 13 (promoter region)	PTSD	45 (25 with PTSD and 17 without PTSD)	European Caucasians	Females	0.006	Schechter et al. 2015
	Exon 1_F	PTSD	16	(N=26 American Caucasians, N=48 Hispanics, N=26 African Americans)	Females (12.5%) and males (87.5%)	0.045	Yehuda et al. 2013
	CpG1—CpG8	PTSD	108 (83 genocide survivors and 25 healthy controls)	African Rwandans	Males	0.008	Vukojevic et al. 2014
	CpG1—CpG8	PTSD	116 (60 genocide survivors and 47 healthy controls)		Females	0.553	Vukojevic et al. 2014
Catechol-O-methyltransferase (*COMT*)	Val158Met (rs4680) region	Impulsive behaviour and homicide	300 (100 participants with schizophrenic impulsive behaviour, 100 homicide behaviour and N=100 controls)	Chinese Han	Males	0.01	Hu et al. 2018
	CpG1—CpG41	PTSD	270 (98 participants with PTSD and 178 controls)	African American (90.8%) and other race (9.2%)	Females (64%) and males (36%)	0.0012	Norrholm et al. 2013

5HTTLPR— serotonin-transporter-linked polymorphic region
CpG— cytosine-guanine dinucleotide
N— number of participants
PTSD— posttraumatic stress disorder

1.6 Telomere length in PTSD and aggression

1.6.1 Telomeres as chromosomal protective barrier

Another biological factor that has been shown to be influenced by environmental exposures, such as trauma and stressful life events, is telomere length (Jergović et al., 2014; Küffer et al., 2016; Mitchell et al., 2014; O'Donovan et al., 2011; Shalev et al., 2013b). Similar to telomere length, DNA methylation, particularly methylation in the promoter regions of genes

have been indicated to be affected by environmental exposures (Jergović et al., 2014; Küffer et al., 2016; McGowan et al., 2009; O'Donovan et al., 2011; Yehuda et al., 2014). Telomere length and methylation of promoter region in certain genes have been reported to be associated with accelerated cellular ageing (Boks et al., 2014; Jergović et al., 2014). Association of accelerated shortening and differential methylation in promoter regions have been found in psychiatric disorders such as PTSD and behavioural problems like aggression (Jergović et al., 2014; Küffer et al., 2016; McGowan et al., 2009; O'Donovan et al., 2011; Yehuda et al., 2014). Therefore, studying both DNA methylation and telomere length can be crucial to understanding the underlying molecular mechanisms involved in psychiatric disorders like PTSD and behavioural problems such as appetitive aggression. Telomeres are repetitive nucleoprotein capping eukaryotic chromosomes (Aviv and Levy, 2012; Blackburn et al., 1989; Burdo et al., 2012). Chromosomes of eukaryotic organisms are prone to losing sequences of nucleotides during each cycle of cell division (Figure 1.4) (Chae et al., 2016; S. Chen et al., 2014; Cong et al., 2002; Epel et al., 2004). Thus, telomere length can be used as a proxy for cellular aging (Cawthon et al., 2003; Epel et al., 2004; Lindqvist et al., 2015).

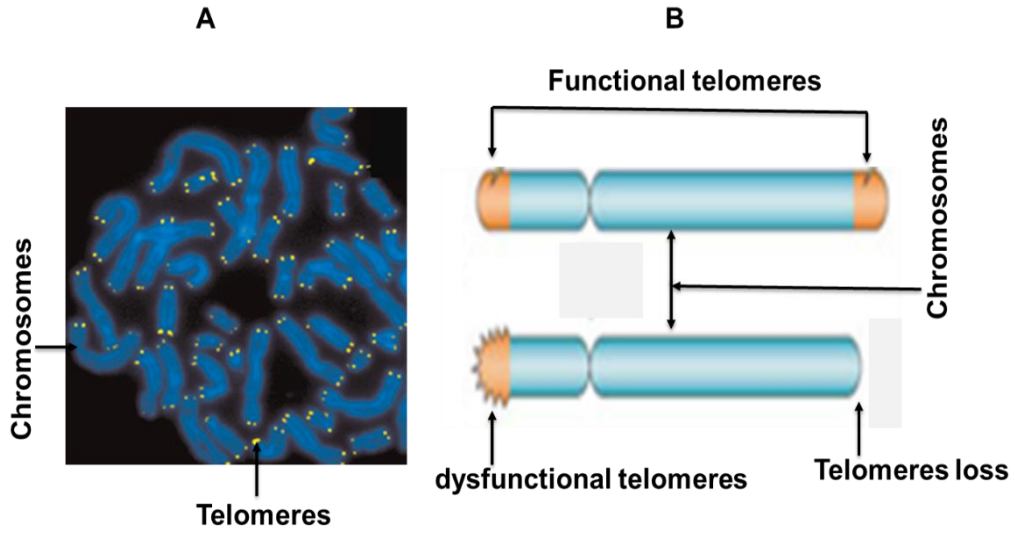

Figure 1. 4: **Telomeres at the end of chromosomes.**
Telomeres were microscopically visualised using fluorescence *in situ* hybridization (FISH). The four yellow dots indicate telomeres at either end of the chromosomes (blue) (A). Telomeres cap the end of chromosomes to protect against the loss of genomic information after each cycle of cell division (B). Figure adapted) and modified for illustrations (Calado and Young, 2008).

The synthesis of telomeres is a well-orchestrated process requiring a number of proteins belonging to the telomerase complex (Figure 1.5) (Blackburn et al., 1989; de Lange et al.,

2005; Martínez and Blasco, 2011). Telomeres are synthesised by the telomerase enzyme TERT, which is responsible for the addition of DNA sequence repeats to the 3' end of the telomere region (Cong et al., 2002; Young et al., 2006). TERT uses RNA components (*TERC*) as a template to synthesise telomeres (Calado and Young, 2008; Cong et al., 2002). If the telomerase complex is dysfunctional, the synthesis of telomeres will be incomplete, resulting in unfinished capping of the chromosome (Calado and Young, 2008; Yamaguchi et al., 2005). The incomplete synthesis of telomeres can trigger DNA repair processes or signal cellular apoptosis (Calado and Young, 2008; Young et al., 2006).

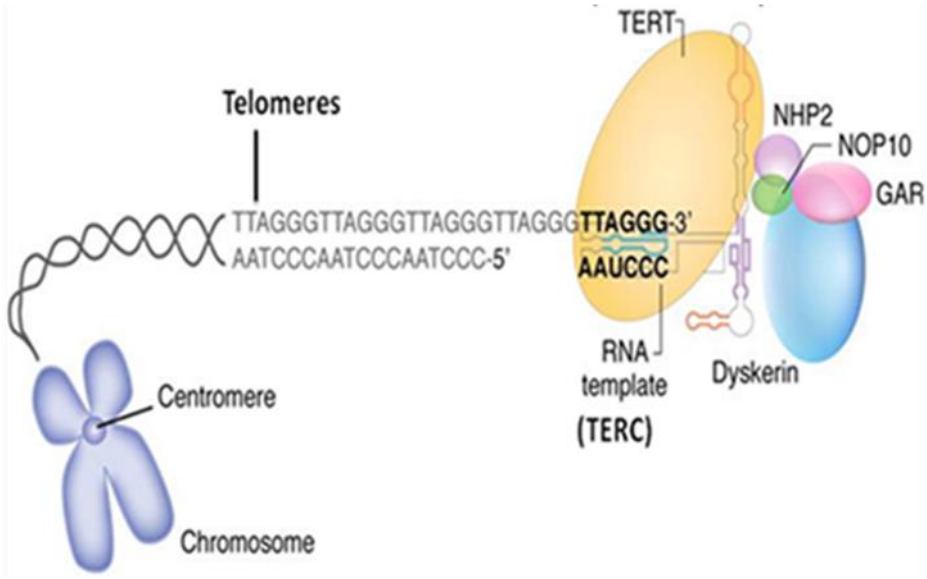

Figure 1. 5: **Schematic diagram of the telomerase complex.**
The telomerase complex is responsible for the synthesis of telomeres. The telomerase complex utilises telomerase (TERT) and RNA template (TERC) and is comprised of related proteins (dyskerin, NOP10, NHP2 and GAR1) that have a significant role in RNA stability. Telomeres cap the chromosomal ends of DNA in cells undergoing division. The above image was adapted from Calado et al and modified (Calado and Young, 2008).

1.6.2 Telomere length in aggression and posttraumatic stress disorder

Several psychiatric disorders, including PTSD, have been associated with accelerated biological aging (Boks et al., 2014; Jergović et al., 2014; Küffer et al., 2016; O'Donovan et al., 2011). Telomere length, a biomarker of cellular aging, has also been found to be associated with mental illness such as PTSD (Drury et al., 2014; Hoge et al., 2013; O'Donovan et al., 2011). Furthermore, studies have indicated that telomeres are sensitive to a number of environmental exposures, such as early childhood trauma and experiences of low socioeconomic status like poverty, as well as lifestyle factors such as chronic alcohol

consumption and regular exercise (Geronimus et al., 2015; Hoge et al., 2013; Njajou et al., 2012; O'Donovan et al., 2011). These environmental exposures and psychological stressors can lead to telomere shortening, which may be mediated by biological responses like inflammation as well as oxidative stress (Chae et al., 2016; Epel et al., 2004). It is important to differentiate between biological and chronological age (Diez Roux et al., 2009; Hunt et al., 2008; Ranjit et al., 2007). The former is defined by physiological activities, whereas the latter is measured using calendar units (Blackburn et al., 2015b). Physiological activities and natural ageing both affect telomere length.

Although genetics and physiological responses have a significant role in shaping telomere length and the activity of telomerase, a number of environmental factors can also influence telomere length (Boks et al., 2014; Drury et al., 2014; Needham et al., 2014; Shalev et al., 2013b; Watkins et al., 2016). Several lines of evidence indicate that decrease in telomere length is associated with exposure to violence, maltreatment in childhood, and PTSD symptoms (Epel et al., 2004; Humphreys et al., 2012; Jergović et al., 2014; Küffer et al., 2016). This modification in telomere length has been shown to be induced by different traumatic events, which trigger several biological pathways such as ataxia-telangiectasia mutated– and Rad3-related (ATR)–ATR-interacting protein (ATRIP) (Maréchal and Zou, 2013; Yang et al., 2017). ATRIP is a DNA damage sensing kinase complex that can elicit telomere length dysregulation (Entringer et al., 2011; Kroenke et al., 2011; O'Donovan et al., 2012; Song et al., 2013; Teyssier et al., 2012).

In a cross-sectional study that investigated the consequences of exposure to family violence and childhood traumatic events, results indicated a correlation between significantly reduced telomere length and exposure to violence (Drury et al., 2014). Family instability, interpersonal violence, and cumulative exposure to early childhood trauma were also associated with shortened telomere length (Chae et al., 2014; Drury et al., 2014; Geronimus et al., 2015). This shortened telomere length was evident even in children who had only witnessed family violence but who had not themselves been physically maltreated (Drury et al., 2014). A retrospective study that investigated telomere length in adults who had experienced early childhood stress found that self-reported lifetime traumatic experiences, in combination with early life stress, were associated with shortened telomere length (Savolainen et al., 2014). Similarly, it was found that exposure to violence was significantly associated with reduced telomere length (Shalev et al., 2013b). A further study of adverse early experiences found that physical, emotional, and sexual abuse in childhood was significantly associated with telomere length shortening, coupled with increased immunological inflammatory response biomarkers (Kiecolt-Glaser et al., 2011). Furthermore, telomere length was found to be

shortened in institutionalised children compared to those without institutional experience (Drury et al., 2012). Overall, studying environmental exposures such as violence and stressful life events, as well as telomere length as a biological marker, could be vital when seeking to understand the mechanisms underlying the response to stress and the development of PTSD and violent behaviour like appetitive aggression.

1.7 Therapygenetics

Therapygenetics is a developing research approach that aims to study the relationship between genetic variants harboured by different individuals and their response to therapy (Eley, 2014; Eley et al., 2012). Studies have shown that genetic variants in individuals are associated with a broad range of phenotypic expressions, and when exposed to certain environmental stimuli, these variants can be used to predict psychotherapeutic outcomes (Bakker et al., 2014; Eley et al., 2012; Lester et al., 2012; Yehuda et al., 2013). Identification of genes that are differentially expressed in individuals exhibiting aggression and PTSD could provide insight into the genes that can be targeted or are important in the response to therapy. Therapygenetics research can also provide a better understanding of the shared biological mechanisms and pathways underlying the development of violent behaviour and PTSD. This could assist in monitoring the response to therapy and how these responses may be mediated by genetic variants as well as alterations in DNA methylation.

Genetic variation and their influence on therapeutic response has been indicated in individuals with anxiety disorders who had received CBT. 5-HTTLPR in particular, has been found to predict symptom severity in people with anxiety disorders (Bakker et al., 2014; Eley et al., 2012; Lester et al., 2012; Yehuda et al., 2013). The SS genotype in 5-HTTLPR was associated with an improvement in anxiety disorder symptoms after receiving CBT, while individuals harbouring either SL or LL genotypes did not improve or respond positively to the psychotherapy (Eley et al., 2012). Individuals with panic disorder who had received CBT were shown to respond differently based on variation in 5-HTTLPR (Lueken et al., 2015) . The low-expression genotype of the serotonin transporter promoter region (i.e. the SS genotype) was associated with a positive response to CBT intervention, while those with high expression genotype (SL or LL genotypes) showed no symptom improvement (Knuts et al., 2014).

Felmingham et al. (2013) investigated the *BDNF* gene in order to understand the impact of its variation on the response to CBT in individuals with PTSD (Felmingham et al., 2013). Individuals harbouring the homozygous or heterozygous Met66 variants compared to those

homozygous Val66 variant genotype of *BDNF* showed improvement in symptoms of PTSD after receiving CBT when compared to those without this allele (Felmingham et al., 2013). Met66 variant had also been shown in both animal and human studies to be a genotype associated with a significant vulnerability to extinction learning (Soliman et al., 2010). Another study investigated the potential influence of the *COMT* gene variations on CBT response in individuals with panic disorder (Lonsdorf et al., 2010). This study found that those with Val158Met in the *COMT* gene responded poorly to CBT compared to those with Met158Met genotype (Lonsdorf et al., 2010). Recently, it was reported that a lower activity genotype of *MAOA* was associated with symptom improvement in response to CBT in individuals with panic disorder (Reif et al., 2014). Individuals who had alleles corresponding to a higher activity of *MAOA* responded poorly to CBT and showed no improvement (Reif et al., 2014).

Epigenetic mechanisms, particularly DNA methylation, have also been found to play a role in the response to psychotherapy such as Cognitive Behavioural Therapy (CBT) (Roberts et al., 2014; Yehuda et al., 2013; Ziegler et al., 2016). A study that investigated the relationship between the therapeutic response to a psychological therapy, CBT, and DNA methylation in participants with PTSD symptoms found reduced methylation (hypomethylation) of the *NR3C1* gene after prolonged exposure to CBT in participants with PTSD (Yehuda et al., 2013). In addition, there was significantly reduced methylation of the *FKBP5* gene in individuals who had shown improvement in PTSD symptoms after receiving CBT (Yehuda et al., 2013). Furthermore, researchers have shown that genetic variation in 5-HTTLPR, a promoter region in the serotonin transporter gene, could influence psychological treatment response (Eley et al., 2012; Lueken et al., 2015). These reports indicate the necessity of studying these molecular underpinnings, epigenetics and genetic variants in order to understand the response to therapeutic approaches, which could lay a foundation for the discovery of biomarkers for therapy interventions, including pharmacotherapies that could mediate psychotherapeutic interventions. This line of investigation could identify essential diagnostic and prognostic biological markers in mental health disorders as well as markers for treatment outcomes.

1.8 Treatment of PTSD and aggressive behaviour

There have been several approaches to treating symptoms of violent behaviour and PTSD using psychological interventions in conjunction with pharmacological therapies (van Dam et al., 2013; Jones et al., 2011; Lovibond, 2004). Psychotherapy often used in PTSD and aggression includes treatments such as CBT (Foa et al., 1991), eye movement

desensitization and reprocessing (Shapiro, 1995; Solomon and Shapiro, 2008), Narrative Exposure Therapy (NET), Supportive Psycho-education,(Neuner et al., 2002; Schauer et al., 2004a) and Narrative Exposure Therapy for Forensic Offender Rehabilitation (FORNET), an adaptation of NET (Elbert et al., 2012). The current regimen of pharmacotherapy for PTSD includes psychotropic medications such as antidepressants (e.g. selective serotonin reuptake inhibitors), as well as anxiolytics, mood stabilisers and benzodiazepines (Berlin, 2007; Brunello et al., 2001; Nakashita et al., 1997; Sansone and Sansone, 2014; Sofuoglu et al., 2014). A combination of both psychotherapy and pharmacotherapy may be more effective in improving health status in PTSD and aggression (Rappaport and Thomas, 2004). This combination is not yet well established and there are inconsistencies in its application across different settings (Hollander, 1999; Olabi and Hall, 2010). Since both appetitive agression and PTSD share similar biological reponses (Elbert and Schauer, 2002; Moran et al., 2014), studying therapy-induced biological changes could provide a better understading of the molecular responses underpinning both disorders.

1.8.1 Cognitive Behavioural Therapy

CBT for PTSD is based on the principle of exposure (Cohen et al., 2007; van Dam et al., 2013; Eley et al., 2012). It is offered as a first line psychotherapy for PTSD (Cohen et al., 2007; van Dam et al., 2013) and it is often coupled with other psycho-educational and other supportive measures (van Dam et al., 2013; Lovibond, 2004). CBT invokes memories of the trauma by consistently exposing an individual to a trauma stimulus in a controlled environment (van Dam et al., 2013; Feeny et al., 2002; Lovibond, 2004). This controlled environment allows CBT to focus on building skills to manage fear-generating activities (van Dam et al., 2013; Feeny et al., 2002) and allows an individual to learn how to control their behaviour and cognitive responses to anxiogenic stimuli (Lovibond, 2004).

CBT is also used to treat aggressive disorders such as externalising and internalising disorders, anger traits and conduct disorder (Lochman et al., 2011; Sukhodolsky et al., 2016a, 2016b). The purpose of CBT in aggression is to reduce aggression levels, provide learning strategies to control anger expression that can lead to aggressive behaviour (Sukhodolsky et al., 2016a). This copying strategy facilitate problem-solving, mediates socially acceptable behaviour and reduce aggression (Eyberg et al., 2008; Jacobs et al., 2010). Despite the efficacy of CBT, some people may not benefit from therapy or alternatively may relapse after treatment (Bernardy and Friedman, 2015; Feeny et al., 2002; Kubiak, 2004). Therefore, effective treatment for PTSD and other mental disorders, requires addressing addictive and criminal behaviours, including violence and aggression.

1.8.2 Narrative Exposure Therapy for Forensic Offender Rehabilitation

The treatment of trauma-related disorders requires disruption of fear networks and re-segregation of discrete traumatic events from the past, where these events have intermixed with each other during the process of traumatisation. Narrative Exposure Therapy for Forensic Offender Rehabilitation (FORNET) is an adaptation of Narrative Exposure Therapy (NET), an evidence-based intervention intended to reduce trauma (Elbert et al., 2012; Schauer et al., 2011). FORNET has been adapted to treat and rehabilitate traumatised violent offenders (Elbert et al., 2012; Hermenau et al., 2013b; Hinsberger et al., 2016a). In a situation where autobiographical memory is suppressed, such as is often the case in PTSD, the individual may not be able to narrate their traumatic experiences (Hermenau et al., 2013b; Neuner et al., 2004b; Zang et al., 2013). NET enables one to talk about traumatic events and to understand that these events have happened, thereby allowing individuals to understand the event as occurring in the "here and now," even though in reality, they could have taken place a number of years ago (Ehlers and Clark, 2000). Typically, in a NET session, the therapist asks questions aimed at invoking particular emotions, cognitive and/or sensory functions, and physiological reactions in order to connect the traumatic events to a wider autobiographical context (Neuner et al., 2004b; Schauer et al., 2004b).

The central objective of the approach is to link possible positive emotions and thoughts about perpetrating aggression and violence to the autobiographical context and past situation (Elbert et al., 2012; Neuner et al., 2004b; Schauer et al., 2011). The use of NET enables individuals to construct a sequential narrative of their entire lifespan (Neuner et al., 2004b; Onyut et al., 2005). NET focuses on exposure to traumatic stress by activating the "hot" memories, which are associated with an increased emotional response (Onyut et al., 2005; Schaal et al., 2009; Schauer et al., 2004a). With the assistance of the therapist, individuals are encouraged to activate the painful memories; therapists aim to prevent individuals from using familiarisation or avoidance techniques to cope with the memories (Bichescu et al., 2007; Hermenau et al., 2013b; Schauer et al., 2004a).

The adapted version of NET for criminal contexts, FORNET, can be effectively used for the treatment of mental distress caused by traumatic events and to reduce aggressive behaviour (Elbert et al., 2012; Hermenau et al., 2013b). This intervention is applicable across cultures and languages (Adenauer et al., 2011; Neuner et al., 2004b; Schauer et al., 2004b). FORNET has been shown to be successful in reducing trauma levels in individuals who have been exposed to trauma and stress, and it can be offered by laypersons with little or no

medical background (Neuner et al., 2008; Schauer et al., 2011). FORNET enhances the positive arousal network related to violence signals (Section 1.2.2) (Schauer et al., 2004a); thus, allowing for the narration of an event that requires an entire autobiographical memory (Elbert et al., 2012; Schauer et al., 2004b). As mentioned above, FORNET has been adapted to treat and rehabilitate traumatised violent offenders with appetitive aggression (Crombach and Elbert, 2015; Hermenau et al., 2013b; Hinsberger et al., 2016a). Studies evaluating the use of FORNET in individuals with appetitive aggression have included former child soldiers in Uganda who had appetitive aggression (Ertl et al., 2011), former Congolese combatants (Hermenau et al., 2013b; Köbach et al., 2015); and children living on the streets of Burundi (Crombach et al., 2014). Overall, these studies indicated a positive response in affected individuals with respect to appetitive aggression as well as PTSD symptoms. Therefore, this therapy allows individuals to develop positive emotions and decrease the perpetration of violence.

THE PRESENT STUDY

A considerable number of South Africans, especially children, adolescents, and young adults, are victims of poly-victimisation: that is, exposure to different types of traumatic events across multiple settings, consisting of complex violence activities (Seedat et al., 2009a; Stein et al., 2009). This repeated exposure to traumatic events is exacerbated by high levels of poverty and poor education that can be found in low socioeconomic settings. In these situations, exposure to hostile and traumatic experiences becomes a normal occurrence rather than a one-off event (Finkelhor et al., 2007). South African townships are human settlements with low socioeconomic status that were previously designated for non-White people during the political regime of apartheid that was in place before the dawn of the South African democratic government (Barbarin et al., 2001; Das-Munshi et al., 2016). Several groups of non-White people, especially Blacks, have undergone and experienced many types of violent activities (Das-Munshi et al., 2016; Kaminer et al., 2008). Ethnic segregation resulted in inequalities in economic opportunities, education, and even health care facilities (Das-Munshi et al., 2016). Living in these precarious conditions has exacerbated individuals' exposure to repeated violence, traumatic events, and criminal victimisation (Dinan et al., 2004). This exposure to violence and trauma often results in a cycle of violence (Samuels et al., 2001) wherein those who had been victims become perpetrators (Abrahams and Jewkes, 2005). This self-perpetrating cycle of violence may explain the high rates of violence and crime, especially amongst young men in present day low-socioeconomic South African communities (Barbarin et al., 2001). The level of PTSD in South African adolescents is considerably higher than the rates reported in developed

countries such as the United States of America (5%) and Germany (1%) (Kilpatrick et al., 2003; Perkonigg et al., 2005). This high level of PTSD in South African population can be caused by poly-victimisation that most adolescents experience (Seedat et al., 2009a; Stein et al., 2009).

The **primary aim** of the present study was to understand the epigenetic changes involved in the context of delivering psychotherapeutic interventions for PTSD and aggression and identify significant biological markers related to trauma and violence.

The secondary aims were:

1) To investigate DNA methylation changes in the context of FORNET and CBT in young Xhosa Black South African men with appetitive aggression and PTSD symptom severity
2) To investigate telomere length changes in the context of FORNET and CBT in young Xhosa Black South African men with appetitive aggression and PTSD symptom severity
3) To investigate an association between telomere length and appetitive aggression or PTSD in young Xhosa Black South African men with appetitive aggression
4) To investigate an association between genetic variation in the serotonin transporter promoter region and appetitive aggression in young Xhosa Black South African men

The content of this dissertation consists of four research articles, which overlap across the chapters as each chapter is presented as stand-alone *manuscript-readily* prepared for submission for publication or already published

1. DNA methylation profiles as a predictive tool for psychotherapeutic treatment response in South African males with appetitive aggression (unpublished work, *manuscript-ready*) (Chapter 2).

2. Telomere length alteration associates with psychotherapeutic response in individuals with appetitive aggression (unpublished work, *manuscript-ready*) (Chapter 3).

3. Investigation of telomere length in young Black South African men with appetitive aggression (unpublished work, *manuscript-ready*) (Chapter 4).

4. Appetitive and reactive aggression are differentially associated with the STin2 genetic variant in the serotonin transporter gene (published *manuscript*, Hemmings et al. 2018, *Scientific Reports* **8**, 6714) (Chapter 5).

CHAPTER 2

DNA METHYLATION PROFILES ASSOCIATED WITH POSTTRAUMATIC STRESS DISORDER IN XHOSA BLACK SOUTH AFRICAN MALES WITH APPETITIVE AGGRESSION WHO HAVE BEEN TREATED WITH PSYCHOTHERAPIES

ABSTRACT

Introduction

Appetitive aggression is a goal-driven behaviour that stimulates feelings of excitement and arousal by inflicting pain on others. Narrative Exposure Therapy for Forensic Offender Rehabilitation (FORNET) has been found to reduce appetitive aggression and the effects of violence-related trauma. DNA methylation, a modification that occurs without changing the sequence of the DNA, has been associated with posttraumatic stress disorder (PTSD) and aggression. The aim of this study was to investigate methylation changes in Xhosa Black South African men in response to FORNET.

Methods

Young Xhosa Black South African men (N=30) with high appetitive aggression and PTSD scores were assigned randomly in an observer-blinded intervention trial of FORNET (N=10), cognitive behavioural therapy (CBT) (N=10) (as a comparable intervention), and controls (N=10) to investigate DNA methylation changes over time. The severity of appetitive aggression and PTSD symptoms were measured in all participants at baseline and at two follow-up visits (mean: 8 months and 16 months post-therapy). The Appetitive Aggression Scale (AAS) and the PTSD Symptom Scale-Interview (PSS-1) were used to measure appetitive aggression and PTSD, respectively. The Human Mental Disorders EpiTect Methyl II Signature PCR Array (22 genes) was used to measure methylation using real-time polymerase chain reaction (qPCR). Repeated measures of analysis of variance (ANOVA), mixed models, and Fisher's least significant difference (LSD) were used to analyse the association between DNA methylation and appetitive aggression or PTSD symptom severity over time in each of the three groups.

Results

A significant reduction in PTSD symptom severity between baseline and first follow-up was observed in the FORNET group (p=0.048), although no change in appetitive aggression was noted for any of the treatment group in all the time points. Methylation in the reelin (*RELN*) (r=0.38, p=0.02) gene was positively correlated with appetitive aggression in overall analysis.

Methylation in the catechol-o-methyltransferase (*COMT*) gene was positively correlated with PTSD symptom severity (r=0.35, p=0.01). In the CBT group, methylation in autism susceptibility candidate 2 (*AUTS2*) (p= 0.000) and *RELN* (p= 0.023) genes decreased significantly between first and second follow-up, while COMT gene methylation decreased between baseline and first follow-up (p=0.007) but increased between first and second follow-ups (p=0.038).

Conclusion

Changes in methylation of different genes over time were associated with CBT but were not conclusively linked clinical outcomes. Replication of this study in a larger sample size of participants randomised to FORNET and CBT is needed to determine whether there is an association between DNA methylation and reduction in appetitive aggression, or PTSD symptoms, after treatment.

2.1 INTRODUCTION

Aggression in humans is a multifactorial and complex behaviour (Barker et al., 2007; Georgiev et al., 2013; Tremblay, 2010). Aggression can lead to the formation of dangerous gangs and criminal activities, which can results in mental health disorders such as posttraumatic stress disorder (PTSD) (van Heerden et al., 2009; Seedat et al., 2009b). PTSD is a psychiatric disorder that can develop after experiencing a traumatic event (Kessler et al., 1995). The perpetration of violence in communities can result in a cycle of repeated traumatic experiences, which can lead to the development of aggressive behaviour, such as appetitive aggression and PTSD (Hecker et al., 2015a; Hinsberger et al., 2016b; Tremblay, 2010; Weierstall et al., 2013b; Widom and Brzustowicz, 2006).

Appetitive aggression is a goal-directed aggression that is perpetrated to achieve a specific objective through inflicting pain on another; while the achievement of the goal stimulates feelings of excitement and arousal (Elbert et al., 2010; Hecker et al., 2012b; Weierstall et al., 2012b). Therefore, the individual continues to perpetrate this aggressive behaviour for self-pleasure (Hinsberger et al., 2016b; Sommer et al., 2016; Weierstall et al., 2013b). As a result, appetitive-aggressive behaviour leads to the perpetration of violence in the future (Weierstall et al., 2011; Weierstall and Elbert, 2011). According to Köbach et al. (2015), the total number of traumatic events/violent acts experienced by an individual can predict the development of both appetitive aggression and symptoms of PTSD (Köbach et al., 2015). In a sample of former Democratic Republic of Congo (DRC) combatants who belonged to armed groups, it was found that witnessing, perpetrating or experiencing extreme violence repeatedly was associated with appetitive aggression (Köbach et al., 2015). Thus, repeated exposure to extreme violence can enhance the likelihood of developing appetitive aggression.

Several studies have investigated strategies that may serve as interventions to reduce stress, violence-related trauma and aggressive behaviour. These interventions include Narrative Exposure Therapy for Forensic Offender Rehabilitation (FORNET) (Crombach and Elbert, 2015; Hermenau et al., 2013b) and Cognitive Behavioural Therapy (CBT) (Cohen et al., 2004; Yehuda et al., 2013). The FORNET intervention is a short-term psychotherapy intended to alleviate consequences of violent and criminal behaviour that influence psychological responses in an environment with prolonged trauma exposure (Adenauer et al., 2011; Neuner et al., 2004b). A study by Ertl et al. (2011) investigated the efficacy of

FORNET in Ugandan former child soldiers and found that there was a significant reduction in PTSD symptoms in this group compared to a control group that was given a supportive counselling treatment that did not include a trauma-focused therapeutic component (Ertl et al., 2011). Similarly, another study investigated a sample of former armed groups (child soldiers and adult veterans) from the DRC who had high levels of appetitive aggression and PTSD symptom severity (Hermenau et al., 2013b). This study found that both PTSD symptom severity and appetitive aggression were significantly reduced in individuals who had received FORNET compared to those who had received a supportive counselling treatment as the control treatment (Hermenau et al., 2013b). These previous studies have demonstrated that FORNET can be an essential psychotherapeutic intervention to reduce PTSD and appetitive aggression levels, particularly in individuals who had experienced complex stress and trauma such as combat or similar violence related events. This reduction in PTSD symptom severity or appetitive aggression could be linked to molecular mechanisms, such as the involvement of cortisol and serotonergic pathways that have been implicated in the processing of stress and traumatic experiences (Bremner et al., 2003; Stoppelbein et al., 2012; Yehuda et al., 2011).

The literature suggests that several molecular alteration , such as genetic variation and epigenetics in genes encoding for essential molecules such as neurohormones, may be involved in the processing of traumatic events associated with the susceptibility to develop PTSD or aggressive behaviour (Boscarino et al., 2011; Hygen et al., 2015; Pivac et al., 2012; Provençal et al., 2014; Yehuda et al., 2013). Epigenetics is a biological mechanism that controls gene expression without affecting the DNA sequence (Bjornsson et al., 2004; Jones, 2001). These epigenetic changes are formed through methylation and acetylation of DNA, histones, and non-coding RNA (van Dongen et al., 2015; Guillemin et al., 2014; Neylan et al., 2014; Provençal et al., 2014; Sasaki and Matsui, 2008; Tremblay, 2010).

DNA methylation is the most well studied epigenetic mechanism and involves the methylation of cytosine in the DNA's (5mC) cytosine-guanine dinucleotides (CpGs). DNA methylation plays a crucial role in the regulation of gene expression as a response to certain environmental exposures, such as stress and trauma. Several studies have provided evidence for the association between DNA methylation and aggression (Guillemin et al., 2014; Provençal et al., 2014) and PTSD symptom severity (Labonté et al., 2014; Rusiecki et al., 2013; Wolf et al., 2016). Thus, DNA methylation can be activated during the development of PTSD or aggression. DNA methylation can also be induced by several other environmental factors such as drugs, psychotherapeutic interventions and lifestyle factors (McGowan et al., 2009; Yehuda et al., 2013). Therefore, DNA methylation is a dynamic and

reversible process that could provide insights to understand complex disorders such as PTSD and behavioural problems like aggression.

Differential DNA methylation has been found to be able to predict changes in symptoms of trauma in individuals with PTSD (Yehuda et al., 2013), as well as in other mental illness (Roberts et al., 2015; Ziegler et al., 2016). Yehuda et al. (2013) investigated differential DNA methylation in response to Prolonged Exposure Therapy (PE), a behavioural intervention coupled to CBT in combat veterans who had PTSD. Lower DNA methylation in exon 1F promoter region in *NR3C1* at pre-treatment was associated with positive outcomes of the psychotherapeutic intervention (Yehuda et al., 2013). Interestingly, decreased methylation in the exon 1 promoter region in the *FKBP5* gene was associated with decreased PTSD symptom severity in combat veterans (Yehuda et al., 2013) . Therefore, differential DNA methylation could be an essential biological mechanism involved in the response to psychotherapeutic interventions, and may potentially be utilised as biomarker to predict symptom severity, inform treatment and treatment outcomes (Yehuda et al., 2013).

The aim of this present study was to determine differential DNA methylation in twenty-two (22) mental-health-related genes in participants who had received either FORNET or CBT, as well as a control group that had received TAU, at pre-treatment and post-treatment and investigate the association between DNA methylation and (i) PTSD symptom severity and (ii) appetitive aggression. Participants were from Khayelitsha and Gugulethu townships in Cape Town. Participants in the study had repeatedly been exposed to a number of traumatic events since their living conditions and socioeconomic background predispose them to violence and crime. Previously, Hinsberger et al. (2016) investigated the effectiveness of FORNET and CBT in reducing PTSD symptom severity and appetitive aggression in the same sample of participants investigated in the present study (Hinsberger et al., 2016a).

2.2 MATERIALS AND METHODS

2.2.1 Clinical and demographic information

All participants were recruited with the assistance of a locally operating organisation, the Rebuilding and Life-skills Training Centre (REALISTIC), a community-based organisation, which assists young former offenders, some of who are ex-prisoners and at risk of committing crime and violence. This program trains young people in life skills aimed at preventing them from committing crimes and supporting them to avoid drug addiction. A priori sample size estimation using g-power (Faul et al., 2009) was performed for the parent study (Hinsberger et al., 2016a), in which the current study was nested. All participants were recruited from Khayelitsha and Gugulethu townships in Cape Town, South Africa. “Township”

in a South African context refers to an under-developed urban residential area, which were historically designated for non-White people. Townships are characterised by socio-economic inequalities with high rates of poverty and unemployment (Dinan et al., 2004). All participants were kept safe over the 3-week camps to eliminate drug abuse, and ensure adequate nutrition and shelter during the therapy program (Hinsberger et al., 2016a). The 3-week camp was comprised of individuals receiving the intervention, as well as various staff members (facilitators, social workers, and security personnel) who provided assistance during the camp.

Our study consisted of a subsample (N=30) of young Xhosa Black South African men who were selected from a clinical intervention trial of FORNET that comprised 39 participants who were drawn from a larger sample and were identified based on high PTSD and high appetitive aggression, as measured by the Appetitive Aggression Scale (AAS) (Weierstall and Elbert, 2011) and the PTSD Symptom Scale-Interview (PSS-I) (Foa and Tolin, 2000). In order to protect the confidentiality of participants, therapy sessions were conducted in separate rooms within the camp setting. Participants from three treatment groups (FORNET, CBT, and TAU) were given recreational activities of their choice, which amongst others included playing soccer and walking on the beach. All these activities were separate to the psychotherapeutic interventions (Hinsberger et al., 2016a). The clinical baseline and first follow-up data on intervention outcomes have been reported by Hinsberger et al. (2016) in a previous publication (Hinsberger et al., 2016a). The subsample that we have included in the current study comprised participants who had been assessed at three time points: baseline (prior to the intervention), first follow-up (8 months post-intervention) and second follow-up (16 months post-intervention). Participant samples included in the current DNA methylation analysis were randomly selected from all participants who completed all intervention assessments.

Relevant sociodemographic information for each participant, including ethnicity, age and education level are provided in Table 2.1. No participants had any acute psychosis. For the interventions, all participants had to meet both criteria for PTSD symptom severity and appetitive aggression (Hinsberger et al., 2016a). Thus, only participants who met the criteria of a minimum of 9 points on the AAS (Weierstall and Elbert, 2011) (Table 2.2) and a minimum of 8 points on the PSS-I (Foa and Tolin, 2000)(Table 2.3) were included in the current study.

Table 2.1: Demographic information of young Black South African men who were investigated for changes in DNA methylation in association with changes in appetitive aggression and PTSD symptom severity pre- and post- psychotherapeutic intervention.

Variables	M	SD
Age (range: 18–40 years)	23 years	5.4 years
Education (range: 1–16 years)	10.33years	2.12 years

M— Mean
SD— standard deviation

The perpetration of appetitive aggression was assessed using the AAS (Weierstall & Elbert 2011). The AAS consists of 15 questions examining instrumental aggression, addiction behaviour and craving to cause or inflict pain, as rated on a 5-point Likert scale. The overall AAS score is a summary of the scores of the 15 items (Table 2.2).

Table 2. 2: Descriptive statistics for appetitive aggression measured by AAS in treatment groups across three time points

Treatment Group	N	Mean (AAS)	SD	SE	95% Confidence Interval for Mean	
					Lower	Upper
Baseline						
FORNET	10	23.40	7.56	2.39	17.99	28.80
CBT	10	31.40	13.35	4.22	21.84	40.95
TAU	10	24.40	12.27	3.88	15.61	33.18
First follow-up						
FORNET	9*	24.00	13.82	4.60	13.37	34.62
CBT	10	24.30	16.13	5.10	12.76	35.84
TAU	6*	25.66	17.00	6.94	7.82	43.50
Second follow-up						
FORNET	10	15.700	11.78	3.72	7.26	24.13
CBT	3*	23.50	19.19	9.59	-7.03	54.03
TAU	10	24.200	10.87	3.43	16.42	31.97

FORNET— Narrative Exposure Therapy for Forensic Offender Rehabilitation
CBT— Cognitive Behavioural Therapy
TAU: Treatment As Usual
N— number of participants
SD— standard deviation
SE— standard error
AAS— Appetitive Aggression Scale
*: Participants indicated with an asterisk that were groups of less than 10 due to dropouts
Baseline— time point 1, 0 months prior to the intervention
First follow-up— time point 2, 8 months on average post-intervention

Second follow-up— time point 3, 16 months on average post-intervention

PTSD symptom severity was examined using PSS-I (Foa and Tolin, 2000). The PSS-I assesses participants on their experiences of 17 PTSD symptoms encountered in the previous two weeks, in accordance with Diagnostic and Statistical Manual of Mental Disorders criteria (Foa and Tolin, 2000). Descriptive data for each treatment group over the three-time points are shown in Table 2.3.

Table 2.3: Descriptive statistics for posttraumatic stress disorder symptom severity measured by PSS-I in the three treatment groups across the three time points.

Treatment Group	N	Mean (PSS-I)	SD	SE	95% Confidence Interval for Mean	
					Lower	Upper
Baseline						
FORNET	10	23.40	9.69	3.06	16.46	30.33
CBT	10	20.40	6.67	2.10	15.62	25.17
TAU	10	15.60	6.46	2.04	10.97	20.22
First follow-up						
FORNET	9*	14.00	10.86	3.62	5.65	22.34
CBT	10	18.22	13.71	4.57	7.67	28.76
TAU	6*	15.83	10.94	4.46	4.34	27.31
Second follow-up						
FORNET	10	18.60	12.13	3.83	9.91	27.28
CBT	3*	19.25	21.66	10.83	-15.23	53.73
TAU	10	23.00	14.49	4.58	12.63	33.36

FORNET— Narrative Exposure Therapy for Forensic Offender Rehabilitation
CBT— Cognitive Behavioural Therapy
TAU— Treatment As Usual
N— number of participants
SD— standard deviation
SE— standard error
PSS-I— PTSD Symptom Scale-Interview
*: Participants indicated with an asterisk that was groups of less than 10 due to dropouts
Baseline— time point 1, 0 months prior the intervention
First follow-up— time point 2, 8 months on average post-intervention
Second follow-up— time point 3, 16 months on average post-intervention

Trauma exposure was assessed using an adaptation of the Childhood Exposure to Community Violence Checklist (CECV) (Amaya-Jackson, 1998). The CECV comprises of a 33-item self-report checklist, which measures children's levels of witnessing, experiencing or hearing about traumatic events. To reflect the types of violence in low socioeconomic income areas of South African townships, the questionnaire was adapted to indicate physical assault, armed robbery and sexual abuse. Several studies have utilised the CECV in South African populations (Fincham et al., 2009a; Weierstall et al., 2013b). Traumatic events can

be categorised as self-experienced or witnessed trauma, with a total score demonstrating the severity of community violence. The measure had a high reliability (coefficient omega = 0.87; 95 CI=0.84-0.89) as reported by (Sommer et al. 2017). All participants in the FORNET intervention had to meet both criteria for appetitive aggression and PTSD symptom severity (Hinsberger et al., 2016a). The age of participants ranged between 18–40 years, which fis the age group that is vulnerable to gang activity. The study received ethical clearance from the ethics review boards of Stellenbosch University (Ethics Reference number: N13/01/006), the University of Konstanz and the University of Cape Town.

2.2.2 DNA methylation

Genomic DNA was isolated from saliva collected in Oragene™ DNA self-collection kits (OG-500, DNA Genotek, Ontario, Canada) using the Prep-It L2P reagent (DNA Genotek, Ontario, Canada) as per manufacturer's instructions. Of the 39 participants who were included in the randomised controlled intervention trial, only 30 (FORNET=10, CBT=10 and TAU=10) (Figure 2.1) were randomly selected for the investigation of DNA methylation of 22 mental health-related genes (Table 2.4), using the EpiTect DNA Methyl for mental health disorders (Qiagen, Germany).

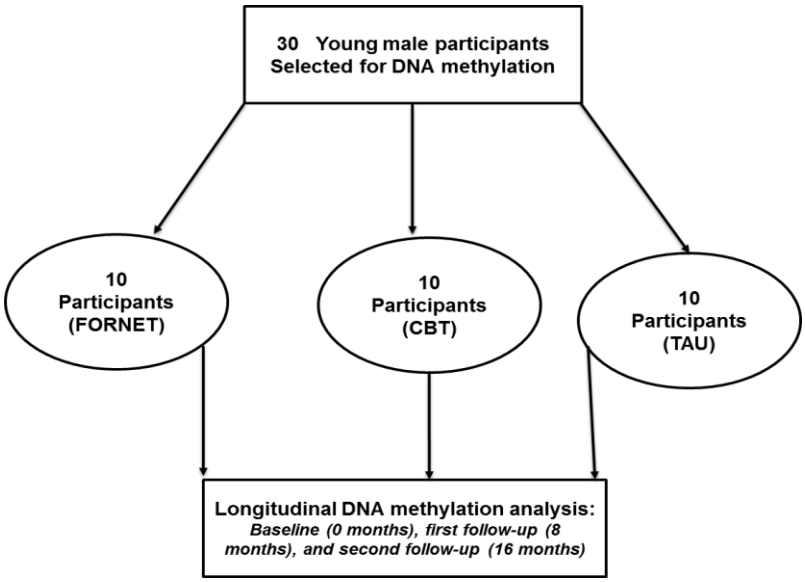

Figure 2.1: Schematic diagram demonstrating a selection of participants for longitudinal DNA methylation analysis amongst three treatment groups, Narrative Exposure Therapy for Forensic Offender Rehabilitation (FORNET), Cognitive Behavioural Therapy (CBT), and a control group receiving Treatment As Usual (TAU).

Table 2.1: List of genes in the EpiTect® Methyl II Signature PCR Array (22) Human Mental Disorders (Cat. no. 335212 EAHS-601ZE) pathway-bases assay that were investigated to identify methylation changes after psychotherapy

Number	Gene symbol	Reference sequence	PCR amplicon size (bp)	Chromosome	Assay chromosome location
1	*TFAM*	NM_003201	205	10	60145034
2	*BDNF*	NM_001709	246	11	27721819
3	*DRD2*	NM_016574	193	11	113345881
4	*GLS2*	NM_013267	255	12	56882484
5	*LHX5*	NM_022363	254	12	113909800
6	*ISL2*	NM_145805	182	15	76629117
7	*LDLR*	NM_001195803	150	19	11201186
8	*NR4A2*	NM_006186	297	2	157190532
9	*GAD1*	NM_013445	233	2	171673862
10	*AVP*	NM_000490	181	20	3063385
11	*APP*	NM_201414	194	21	27543083
12	*COMT*	NM_000754	161	22	19929342
13	*GABRA2*	NM_000807	156	4	46392308
14	*TERT*	NM_198253	200	5	1295423
15	*RPP21*	NM_024839	288	6	30312999
16	*HOXA1*	NM_153620	248	7	27136177
17	*AUTS2*	NM_001127231	290	7	69062848
18	*RELN*	NM_173054	176	7	103630017
19	*SORBS3*	NM_005775	297	8	22408886
20	*SMS*	NM_004595	234	X	21959275
21	*RPL39*	NM_001000	270	X	118925617
22	*MECP2*	NM_004992	168	X	153362836

TFAM— transcription factor A, mitochondrial
BDNF— brain-derived neurotrophic factor
DRD2— dopamine receptor 2
GLS2— glutaminase 2
LHX5— LIM homeobox 5
LDLR— low-density lipoprotein receptor
NR4A2— nuclear receptor subfamily 4, group A, member 2
GAD1— glutamate decarboxylase
AVP — arginine vasopressin
COMT— catechol-O-methyltransferase
GABRA2— gamma-aminobutyric acid receptor subunit alpha-2
TERT— telomerase reverse transcriptase
RPP21— protein subunit of nuclear ribonuclease P
AUTS2— autism susceptibility candidate 2
RELN— reelin
SORBS3— sorbin and sh3 domains-containing protein 3
SMS— spermine synthase
RPL39— ribosomal protein L39
MECP2— methyl CpG binding protein 2
APP— amyloid precursor protein
HOXA1— homeobox A1
ISL2— islet 2 LIM homeobox

2.2.3 The EpiTect® Methyl II Signature PCR Array (22) Human Mental Disorders (Cat. no. 335212 EAHS-601ZE)

This study investigated DNA methylation using a pathway-based approach the EpiTect® Methyl II Signature PCR Array (22) Human Mental Disorders (Qiagen, Germany). This pathway-based approach consists of 22 mental health-related genes (Table 2.4). The principle of the EpiTect® Methyl II Signature PCR Array (22) Human Mental Disorders is based on the restriction enzyme digestion of genomic DNA input. The system quantifies residual DNA input remaining after the enzymatic digestion using specific oligonucleotides that are flanking a promoter region of interest for the investigated genes. This process was performed as follows: four reactions were conducted in parallel for each sample: 1) single restriction enzyme digestion with methylation-sensitive enzyme (Ms), 2) single restriction enzyme digestion with methylation-dependent enzyme (Md), 3) double enzyme digestion with methylation-sensitive enzyme and methylation-dependent enzyme (Msd), and 4) the mock reaction with no digestion enzymes (Mo), using water as an input (Oakes et al., 2006; Ordway et al., 2006; Esteller 2007; Hashimoto et al., 2007). The results obtained from the qPCR are presented as a percentage of the methylated and a percentage of the unmethylated portion of genomic DNA that was used as the original input before restriction enzyme digestion.

2.2.3.1 Restriction enzyme digestion reactions

A restriction digestion reaction mixture without enzymes was prepared using 0.5 µg of genomic DNA, 26 µL of 5X restriction digestion buffer, and nuclease-free water, making the final reaction volume of 125 µL. Four reaction mixtures (Mo, Ms, Md, and Msd) were prepared for digestion using the specific enzyme for each reaction. Each reaction mixture contained 28 µL of the earlier prepared restriction digestion reaction mixture and 2 µL of nuclease-free water for Mo digestion; 28 µL of the previously prepared reaction mixture, 1 µL of methylation sensitive enzyme A and 1 µL of nuclease-free water for Ms digestion; 28 µL of the previous prepared reaction mixture, 1 µL methylation-dependent enzyme B and 1 µL of nuclease-free water for Md digestion; 28 µL of the same previously prepared reaction mix, 1 µL of each enzyme (both methylation sensitive enzyme A and methylation-dependent enzyme B), making the final volume of 30 µL for all restriction digestion reaction mixes. All digestion reaction mixes were incubated at 37 ºC overnight (approximately 16 hours) (Figure 2.2). The digestion reaction was terminated by heat-inactivating the enzymes at 65°C for 20 minutes. Tubes containing the reaction mixture were vortexed and briefly centrifuged to ensure that the contents were at the bottom of the tube.

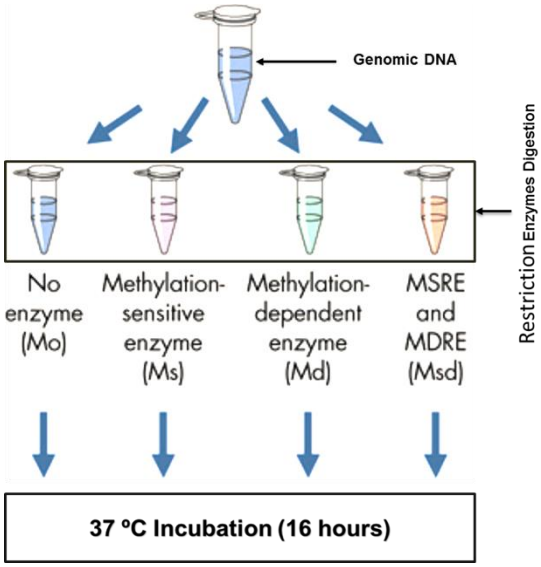

Figure 2.2: Schematic diagram adapted from Qiagen manual (Qiagen, Germany), illustrating restriction digestion of genomic DNA using EpiTect restriction enzymes [(methylation-sensitive enzyme (Ms) and methylation-dependent enzyme (Md)] for the investigation of changes in DNA methylation with psychotherapy.

2.2.3.2 Methylation qPCR

Each participant had three DNA samples in total from each time point (baseline, and first, and second follow-up visits). PCR reaction mix was prepared using a PCR master mix provided in the EpiTect DNA Methyl for mental health disorders kit (Qiagen, Germany). The digested DNA reaction mixture were used in the EpiTect Methyl II Signature PCR Array 384-well plate (Figure 2.3) (Qiagen, Germany), according to the manufacturer's recommendations. Each digested DNA mixture of 30 µl from each digestion reaction was mixed with 170 µl of prepared PCR master mix and 140 µl to make a final volume of 340 µl for each qPCR reaction.

The 384-well plate consisted of 16 rows of wells (A-P rows) (Figure 2.3) enabling investigation of four genomic DNA samples. Ten microlitres of PCR reaction mix with each digested DNA sample was added into the 384-well plate. The first digested DNA reaction sample PCR mix was used in the first four set of rows (A-D), the second digested DNA reaction sample PCR mix was used in the second set of four rows (E-H), the third digested DNA reaction sample PCR mix was used in the third set of four rows (I-L), the fourth digested DNA reaction sample PCR mix was used in the fourth set of rows (M-P), making a total of 16 rows. DNA methylation was investigated from time points 1 (baseline, 0 months), 2 (first

follow-up, 8 months on average) and 3 (second follow-up, 16 months on average). To reduce plate/batch effect during PCR reactions, samples of all participants from three treatment groups (FORNET, CBT, TAU), which were collected at three time-points (baseline, first and second follow-ups) were randomly placed in experimental plates to investigate DNA methylation at baseline and at each of the follow-ups.

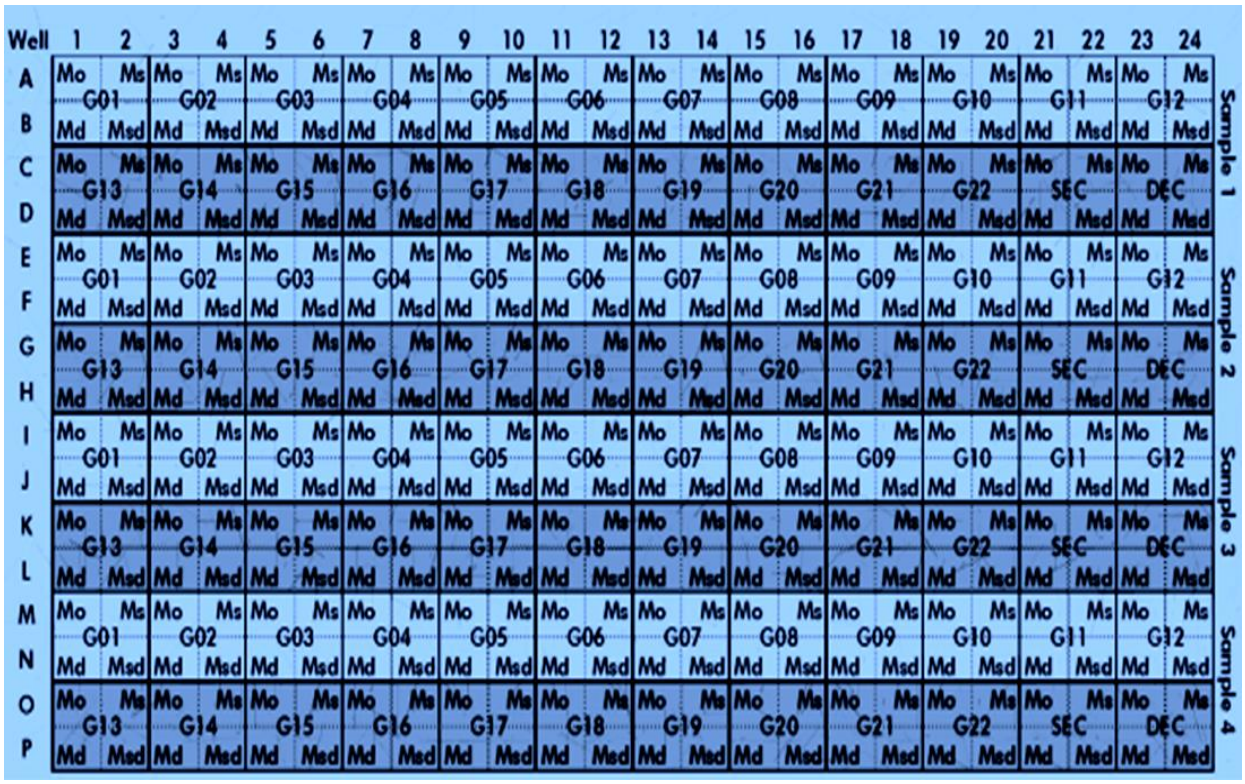

Figure 2.3: Schematic diagram indicating a 384-wells plate the EpiTect® Methyl II Signature PCR Array (22) Human Mental Disorders (Qiagen, Germany). Wells from A-P consist of reactions: Ms: methylation sensitive enzyme, Md: methylation-dependent, Msd: methylation sensitive-dependent, SEC methylation sensitive enzyme control, DEC: methylation-dependent enzyme control. Symbol (G) with numbers represents each gene spanning across a 384-wells plate. This 384-wells plate investigates four independent human genomic DNA samples.

2.2.4 Statistical data analysis

The clinical outcomes of interest were defined as a change in aggressive behaviour and a change in PTSD symptom severity, as assessed on the AAS and PSS-I, respectively. A probability plot was constructed to verify the normality of data. The data were transformed using winsorization to reduce the effect of extreme outlier values. To test if DNA methylation levels were associated with these main clinical outcomes, we performed a one-way analysis

of variance (ANOVA) and analysis of covariates (ANCOVA) over three-time points. To account for repeated measurements over time, mixed model analyses were performed using treatment group and time as fixed effects, and participants nested in a treatment group as a random effect. Post-hoc analyses were performed using Fisher's least significant difference (LSD), which corrects for multiple pairwise comparisons. We did not adjust for ethnicity, age, and environment as covariates as all participants belonged to the same ethnicity; participants were of similar age; none of the participants was currently on drugs, and no participants developed depression over the course of the study. Statistical significance of variances was restricted at 95% confidence interval with an alpha (α) of less than 0.05 ($p<0.05$). To better reflect the findings, a trend towards a statistical significance was set at less than 0.1 ($p<0.1$).

2.3 RESULTS

2.3.1 Clinical, demographic and descriptive measures

Descriptive clinical information of the treatment groups is provided in Tables 2.2 and 2.3. This information includes the change in appetitive aggression (as measured by the AAS) and PTSD symptom severity (as measured by the PSS-I) scores over treatment. Appetitive aggression did not improve in either of the treatment groups after the administration of psychotherapeutic intervention.

Appetitive aggression

No significant reduction in appetitive aggression was observed in the FORNET group across any of the time points (between baseline and first and second follow-up visits) (Table 2.5) (Figure 2.3). Similarly, we did not observe a significant reduction in appetitive aggression across any of the time points in the group that received CBT (Table 2.5) (Figure 2.4). In addition, no significant changes in appetitive aggression were found in the waitlisted TAU controls when time points 1 and 2, time points 1 and 3 and time point 2 and 3 were compared (Table 2.5) (Figure 2.5).

Table 2. 2: Fisher's least significant differences multiple comparisons of appetitive aggression measured by AAS in the three treatment groups between time points.

Within groups	
Treatment groups	P-value
F1 vs F2	0.977
F1 vs F3	0.106
F2 vs F3	0.124
C1 vs C2	0.135
C1 vs C3	0.592
C2 vs C3	0.586
T1 vs T2	0.962
T1 vs T3	0.966
T2 vs T3	0.991

F1, F2, F3— Narrative Exposure Therapy for Forensic Offender Rehabilitation (FORNET) group at baseline, first follow-up and second follow-up respectively
C1, C2, C3—Cognitive Behavioural Therapy (CBT) group at baseline, first follow-up and second follow-up respectively
T1, T2, T3— Treatment As Usual (TAU) group at time points 1, 2 and 3, respectively
AAS— is the Appetitive Aggression Scale
Time points 1, 2, 3— represent baseline (prior to the intervention), first follow-up (eight months on average) and second follow-up (16 months on average)

PTSD Symptom Severity

No significant reduction in PTSD symptom severity was found in the FORNET group between time points 2 and 3 (p=0.315) (Table 2.6). However, PTSD symptom severity was significantly reduced (albeit nominally) in the FORNET group between time points 1 and 2 (p=0.048) (Table 2.6) (Figure 2.3). No reduction in PTSD symptom severity, as measured using the PSS-I, was found in the CBT group between any of the time points (Table 2.6) (Figure 2.4). No significant difference in the reduction in PTSD symptom severity was found when each time point was compared to each other in the TAU controls (Table 2.6) (Figure 2.5).

Table 2.3: Fisher's least significant differences multiple comparisons of posttraumatic stress disorder measured by PSS-I in the three treatment groups between three-time points.

Within groups	
Treatment groups	P-value
F1 vs F2	**0.048***
F1 vs F3	0.298
F2 vs F3	0.315
C1 vs C2	0.731
C1 vs C3	0.928
C2 vs C3	0.735
T1 vs T2	0.991
T1 vs T3	0.112
T2 vs T3	0.173

F1, F2, F3— Narrative Exposure Therapy for Forensic Offender Rehabilitation (FORNET) group at baseline, first follow-up and second follow-up respectively
C1, C2, C3— Cognitive Behavioural Therapy (CBT) group at baseline, first follow-up and second follow-up respectively
T1, T2, T3— Treatment As Usual (TAU) group at time points 1, 2 and 3, respectively
PSS-I— Posttraumatic stress disorder (PTSD) Symptom Scale-Interview administered through the interview method
*: $p < 0.05$— indicates statistical significance
Time points 1, 2, 3— represent baseline (prior the intervention), first follow-up (eight months on average post-intervention) and second follow-up (16 months on average post-intervention)

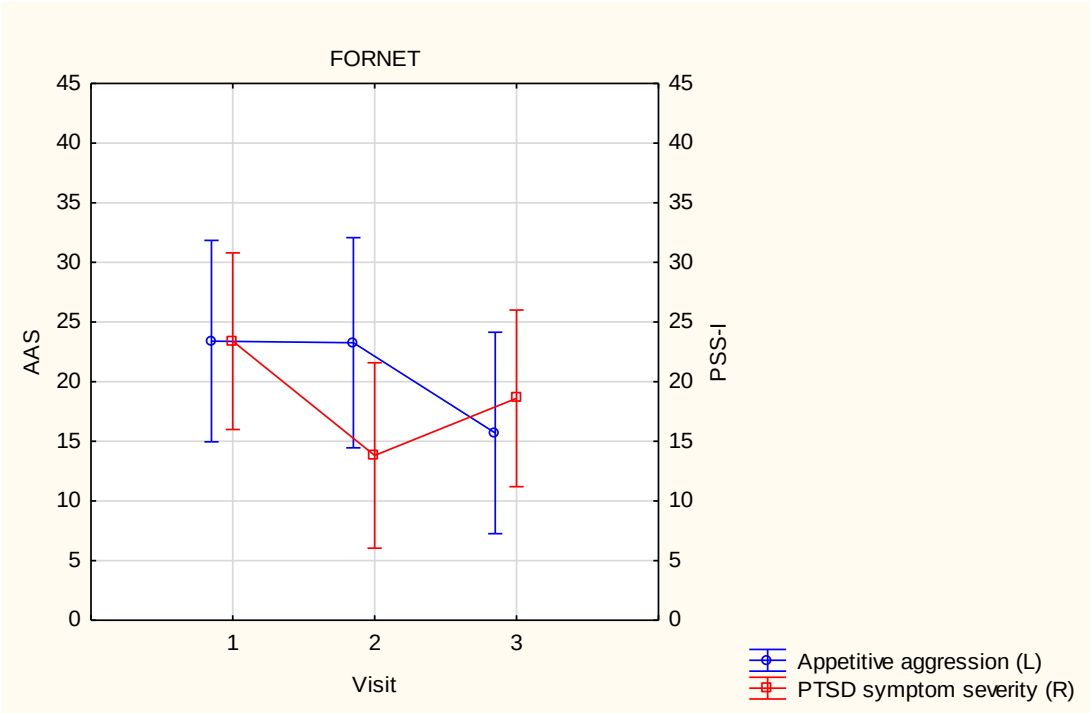

Figure 2.4: Longitudinal analysis of appetitive aggression, as measured by the Appetitive Aggression Scale (AAS) and posttraumatic stress disorder (PTSD), as measured by the PTSD Symptom Scale-Interview (PSS-I), in participants who received narrative exposure therapy for forensic offender rehabilitation (FORNET). L and R represent left and right Y-axis on the graph. Visit 1, 2 and 3 indicate time points 1 (0 months, prior to the intervention), 2 (approximately 8 months post-intervention), and 3 (approximately 16 months post-intervention), respectively. PTSD symptom severity was significantly different between time points 1 and 2 (p=0.048). However, there was no significant reduction or increase in appetitive aggression between time points 1 and 2 or between time points 2 and 3.

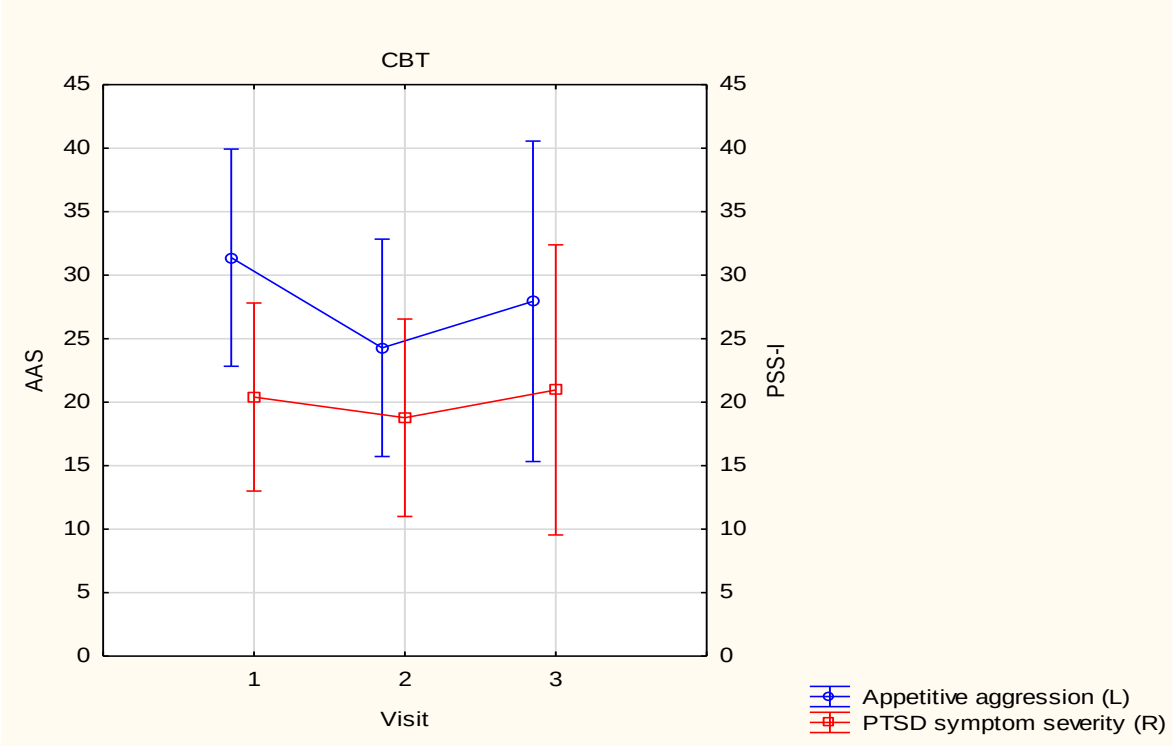

Figure 2.5: Longitudinal analysis of appetitive aggression measured by the Appetitive Aggression Scale (AAS) and posttraumatic stress disorder (PTSD) measured by the PTSD Symptom Scale-Interview (PSS-I) in participants who had received cognitive behavioural therapy (CBT). L and R represent left and right Y-axis on the graph. Visits 1, 2, and 3 refer to time points 1 (0 months, prior the intervention), 2 (approximately 8 months post-intervention), and 3 (approximately 16 months post-intervention), respectively. No significant differences in either appetitive aggression or PTSD symptom severity were found between time points 1 and 2 and between time points 2 and 3, respectively.

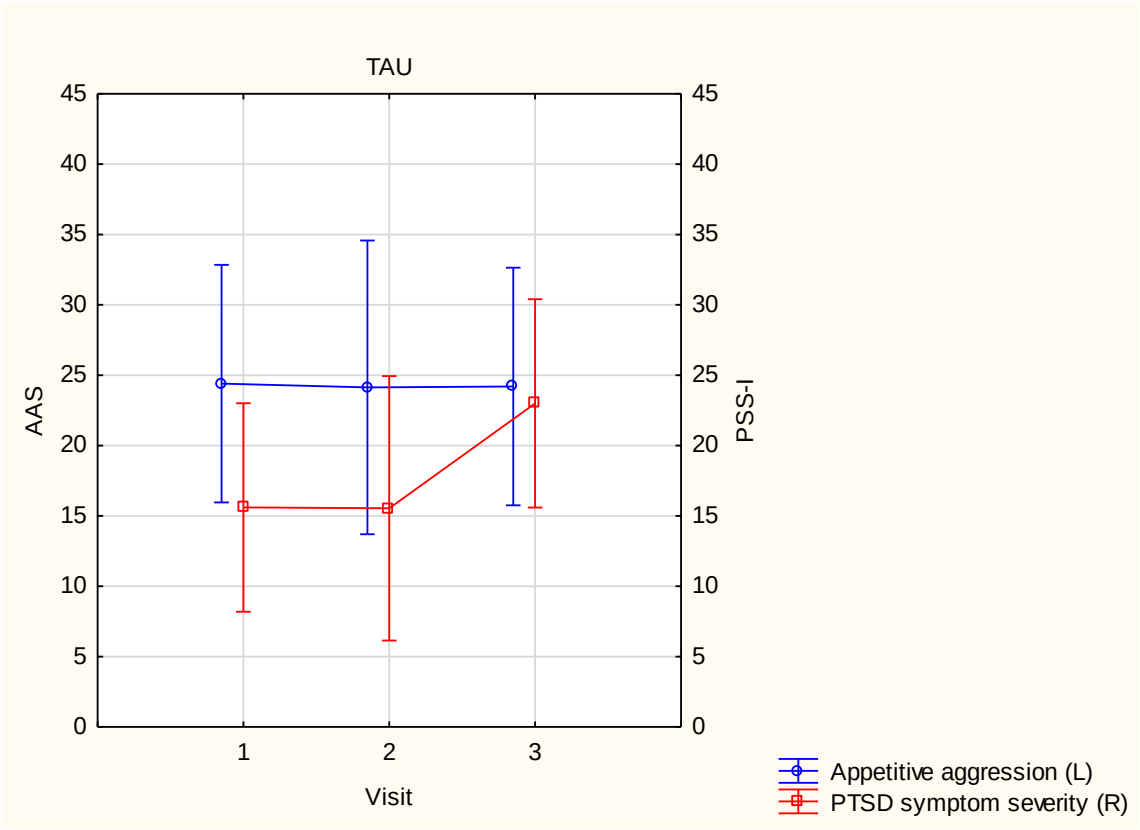

Figure 2.6: Longitudinal analysis of appetitive aggression, as measured by the Appetitive Aggression Scale (AAS), and posttraumatic stress disorder (PTSD), as measured by the PTSD Symptom Scale-Interview (PSS-I), in participants who received treatment as usual (TAU). L and R represent left and right Y-axis on the graph. Visits 1, 2, and 3 indicate time points 1 (0 months, prior the intervention), 2 (approximately 8 months post-intervention), and 3 (approximately 16 months post-intervention), respectively. No significant change in either appetitive aggression or PTSD symptom severity was observed between time points 1 and 2 or between time points 2 and 3.

2.3.2 Association between DNA methylation and changes in appetitive aggression or PTSD symptom severity

DNA methylation is reported as the change in percentage methylation in each gene at each time point, in individuals who received FORNET, CBT or were waitlisted in the TAU group. DNA methylation was assessed at time point 1 (baseline, 0 months) prior to the administration of any intervention to assess if there was any association with clinical outcomes of appetitive aggression or PTSD symptom severity following treatment. DNA methylation changes between time points was assessed to determine if there was an association with changes in appetitive aggression or PTSD symptom severity in the three groups (after therapy for the FORNET and CBT). There was a significant difference in

methylation levels of three genes when the groups were compared over three time points (baseline, two follow-ups). Here, the autism susceptibility candidate 2 (*AUST2*) (p=0.036), reelin (*RELN*) (p=0.029), and catechol-O-methyltransferase (*COMT*) (p=0.035) were found to have a significant difference in methylation over the three time points.

2.3.2.1 RELN gene methylation

DNA methylation change was compared with the change in appetitive aggression and the change in PTSD symptom severity in the FORNET, CBT and TAU groups over the three time points. Descriptive information on DNA methylation changes for the *RELN* gene is reported in Table 2.7.

Table 2.4: DNA methylation changes (%) in *RELN* for the FORNET, CBT and TAU treatment groups between the three time points

Treatment Group	N	Mean (% DNA methylation)	SD	SE	95% Confidence Interval for Mean	
					Lower	Upper
Baseline						
FORNET	10	0.90	0.22	0.07	0.74	1.06
CBT	10	0.93	0.14	0.04	0.82	1.03
TAU	10	0.53	0.40	0.12	0.25	0.82
First follow-up						
FORNET	9*	0.96	0.05	0.01	0.92	1.00
CBT	10	0.92	0.13	0.04	0.82	1.02
TAU	6*	0.84	0.35	0.14	0.46	1.21
Second follow-up						
FORNET	10	0.94	0.11	0.03	0.86	1.03
CBT	3*	0.58	0.42	0.24	-0.47	1.64
TAU	10	0.74	0.24	0.07	0.57	0.91

FORNET— Narrative Exposure Therapy for Forensic Offender Rehabilitation
CBT— Cognitive Behavioural Therapy
TAU— Treatment As Usual
N— number of participants
SD— standard deviation
SE— standard error
RELN— is the reelin gene
* Participants indicated with an asterisk that were groups of less than 10 due to dropouts
Baseline— time point 1, 0 months prior the intervention
First follow-up— time point 2, eight months on average
Second follow-up— time point 3, 16 months on average

2.3.2.1.1 FORNET

No significant change in DNA methylation was observed in the *RELN* gene between time points 1 (baseline, 0 months) and 2 (first follow-up, 8 months on average post-intervention) or between time points 2 (first follow-up, 8 months on average post-intervention) and 3 (second follow-up, 16 months on average post-intervention) in the FORNET group (Figure 2.6) (Table 2.8). *RELN* DNA methylation was not found to change significantly across time, even though a significant decrease in PTSD symptom severity was found in the FORNET group (p=0.048) (Table 2.6). However, this significant decrease in PTSD symptom severity was not correlated with a change in DNA methylation in *RELN* (r=-0.143, p=0.50) (Figure 2.7).

Table 2.5: Fisher's least significant differences multiple comparisons for DNA methylation in the *RELN* gene in FORNET, CBT and TAU treatment groups between three time points.

Within groups	
Treatment groups	P-value
F1 vs F2	0.549
F1 vs F3	0.665
F2 vs F3	0.857
C1 vs C2	0.957
C1 vs C3	**0.021***
C2 vs C3	**0.023***
T1 vs T2	**0.025***
T1 vs T3	**0.022***
T2 vs T3	0.774

F1, F2, F3— Narrative Exposure Therapy for Forensic Offender Rehabilitation (FORNET) group at baseline, first follow-up, and second follow-up, respectively
C1, C2, C3 — Cognitive Behavioural Therapy (CBT) group at baseline, first follow-up and second follow-up, respectively
T1, T2, T3— Treatment As Usual (TAU) group at baseline, first follow-up, and second follow-up, respectively
*p< 0.05 and *p< 0.01 indicate statistical significance
RELN: is the reelin gene
Time points 1, 2, 3— represent baseline (prior the intervention), first follow-up (eight months on average) and second follow-up (16 months on average)

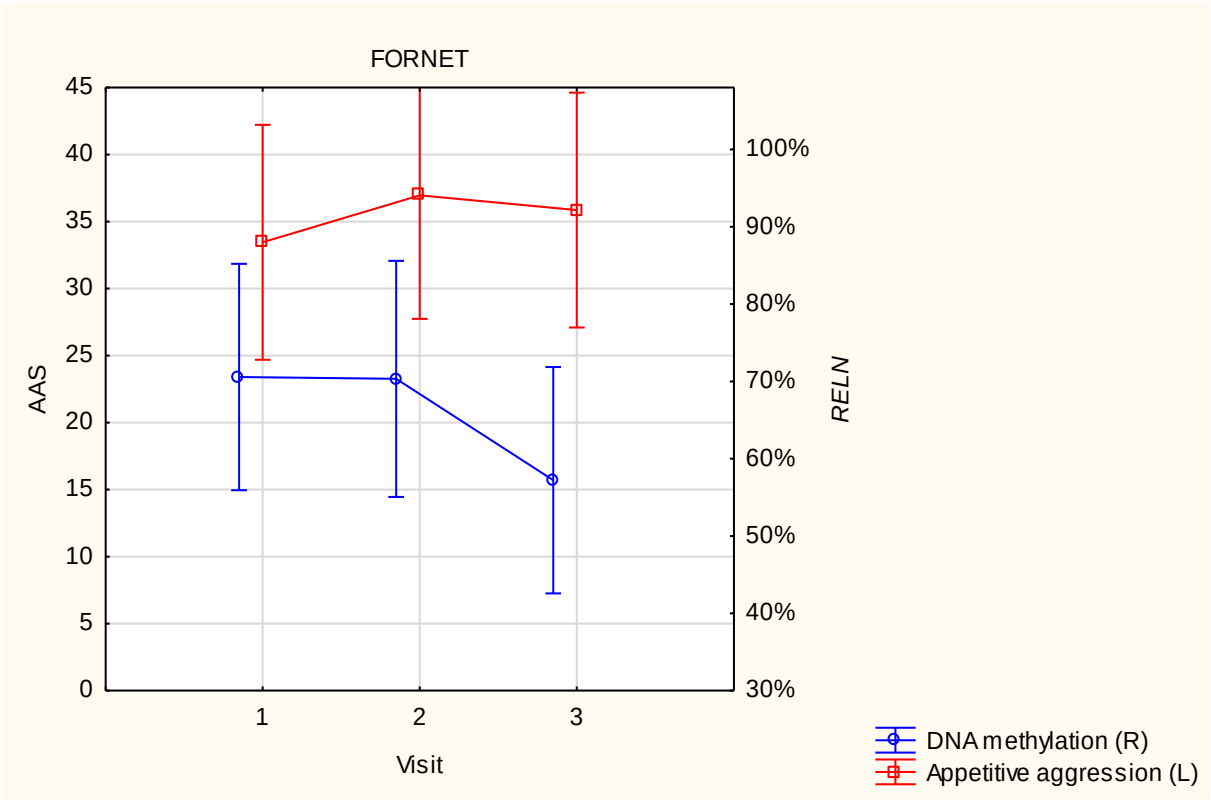

Figure 2.7: Longitudinal analysis of the changes in appetitive aggression as measured by the Appetitive Aggression Scale (AAS) and change in DNA methylation in the reelin (*RELN*) gene after participants had received narrative exposure therapy for forensic offender rehabilitation (FORNET). L and R represent left and right Y-axis on the graph. Visits 1, 2, and 3 indicate baseline (0 months, prior the intervention), first follow-up (approximately 8 months post-intervention), and second follow-up (approximately 16 months post-intervention), respectively. No significant change in appetitive aggression (L) or in DNA methylation were observed in the *RELN* gene (R) between time points 1 and 2 or between time points 2 and 3.

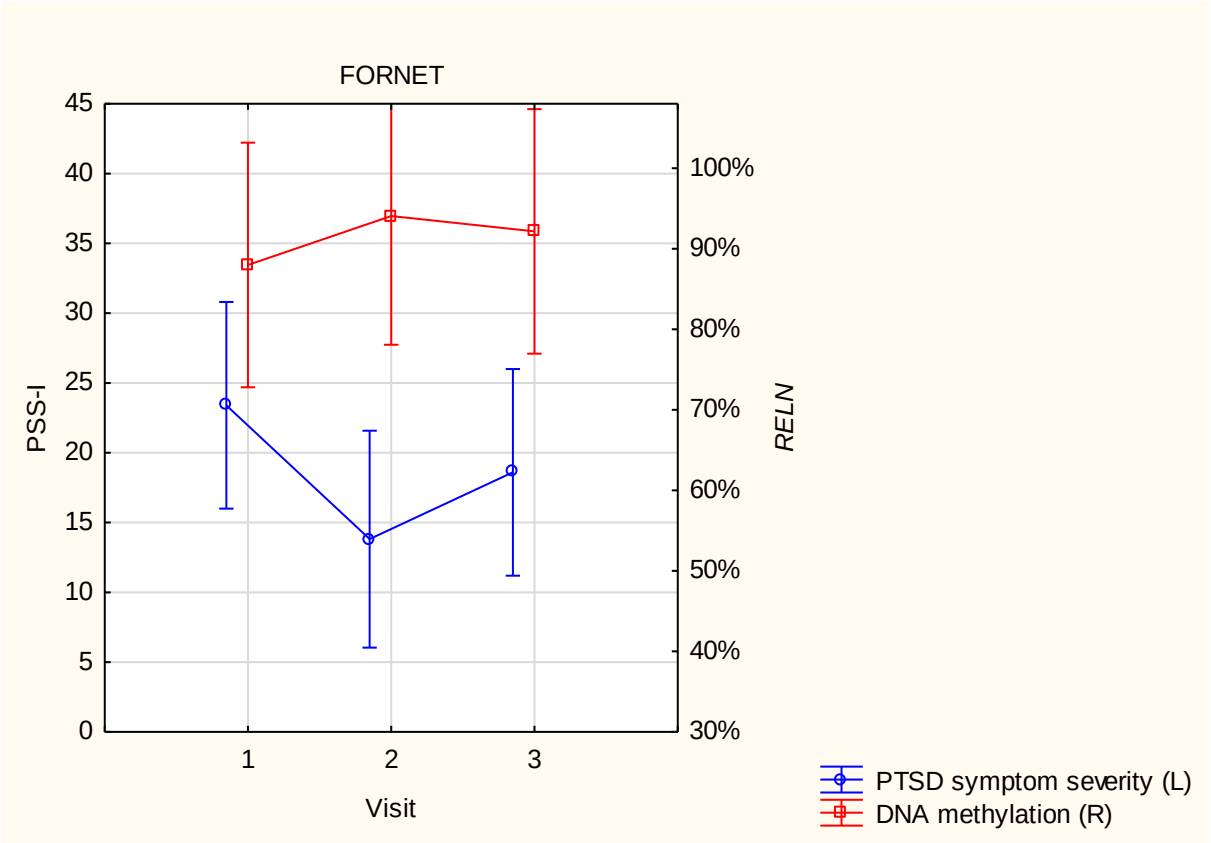

Figure 2.8: Longitudinal analysis of posttraumatic stress disorder (PTSD) measured by the PTSD Symptom Scale-Interview (PSS-I) in association with DNA methylation in the reelin (*RELN*) gene after participants had received narrative exposure therapy for forensic offender rehabilitation (FORNET). L and R represent left and right Y-axis on the graph. Visits 1, 2, and 3 indicate baseline (0 months, prior the intervention), first follow-up (approximately 8 months post-intervention) and second follow-up (approximately 16 months post-intervention), respectively. PTSD symptom severity was significantly reduced between time points 1 and 2. No significant change in DNA methylation in the *RELN* gene between time points 1 and 2 or between time points 2 and 3.

2.3.2.1.2 CBT

Significantly decreased methylation in the *RELN* gene was found between time points 2 (first follow-up, 8 months on average post-intervention) and 3 (second follow-up, 16 months on average post-intervention) in the CBT group (p=0.023) as well as between time points 1 (baseline, 0 months) and 3 (second follow-up, 16 months on average post-intervention) (p=0.021) (Figure 2.8 and 2.9) (Table 2.8). However, there was a no significant change in DNA methylation in the *RELN* gene between time points 1 and 2 (p=0.957). The significant decrease in DNA methylation that was found between time points 2 and 3 was not

associated with a change in appetitive aggression (r=-0.30, p=0.24). Likewise, methylation changes observed in this gene were not associated with changes in PTSD symptom severity observed between any time points (r=-0.143, p=0.50) (Figure 2.8). There was a significant dropout of participants at first follow-up as some of these individuals could not be traced in their communitities due to a veriety of reasons such as low-socieconomic level, high unemployment and relocation to other places of residence. Therefore, the number of participants was significantly reduced from10 to 3 participants.

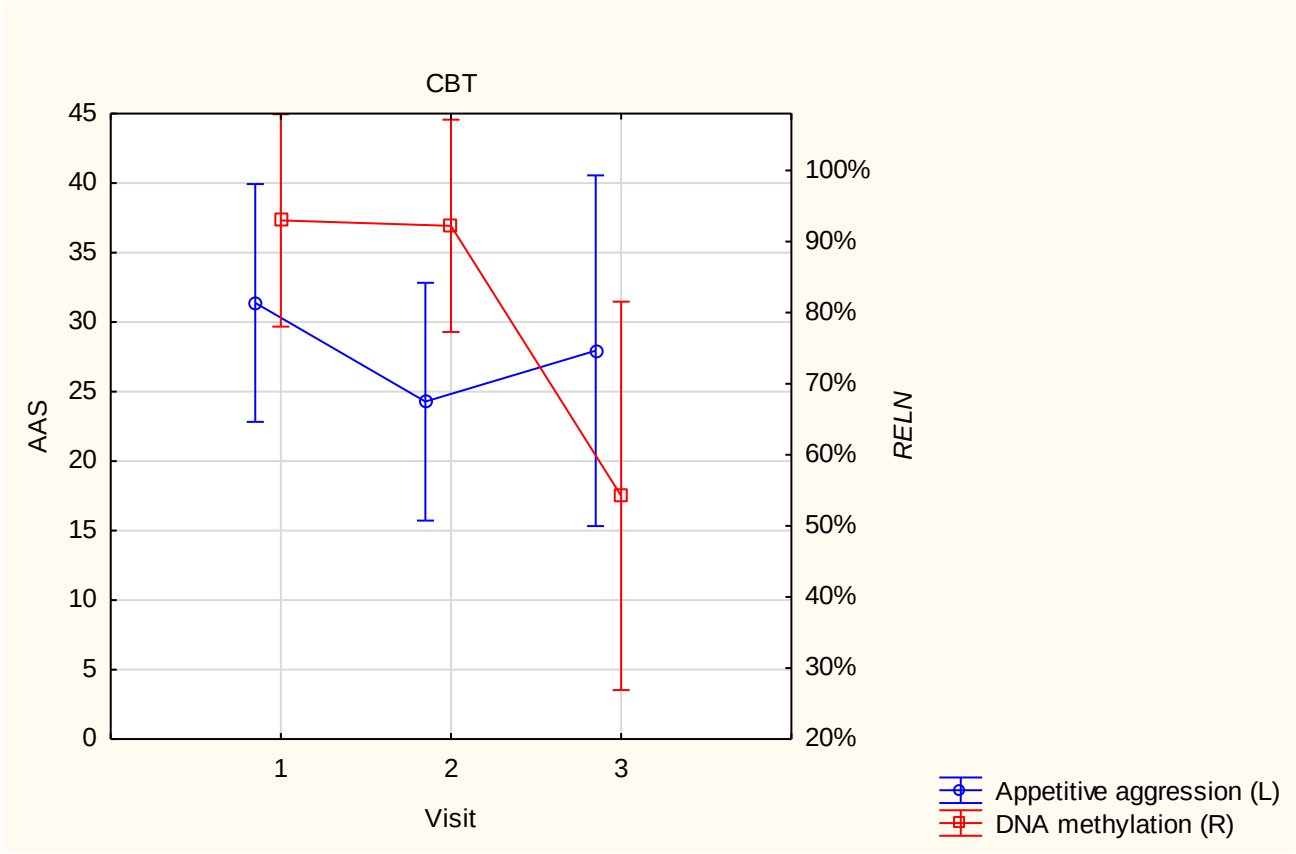

Figure 2.9: Longitudinal analysis of appetitive aggression measured by the Appetitive Aggression Scale (AAS) in association with DNA methylation changes in the reelin (*RELN*) gene after participants had received cognitive behavioural therapy (CBT). L and R represent left and right Y-axis on the graph. Visit 1, 2, and 3 refer to baseline (0 months, prior the intervention), first follow-up (approximately 8 months post-intervention), and second follow-up (approximately 16 months post-intervention), respectively. No significant change in appetitive aggression (L) between time points 1 and 2 or between time points 2 and 3. Significantly reduced DNA methylation in the *RELN* gene (R) between time points 2 and 3 (p=0.023) was found.

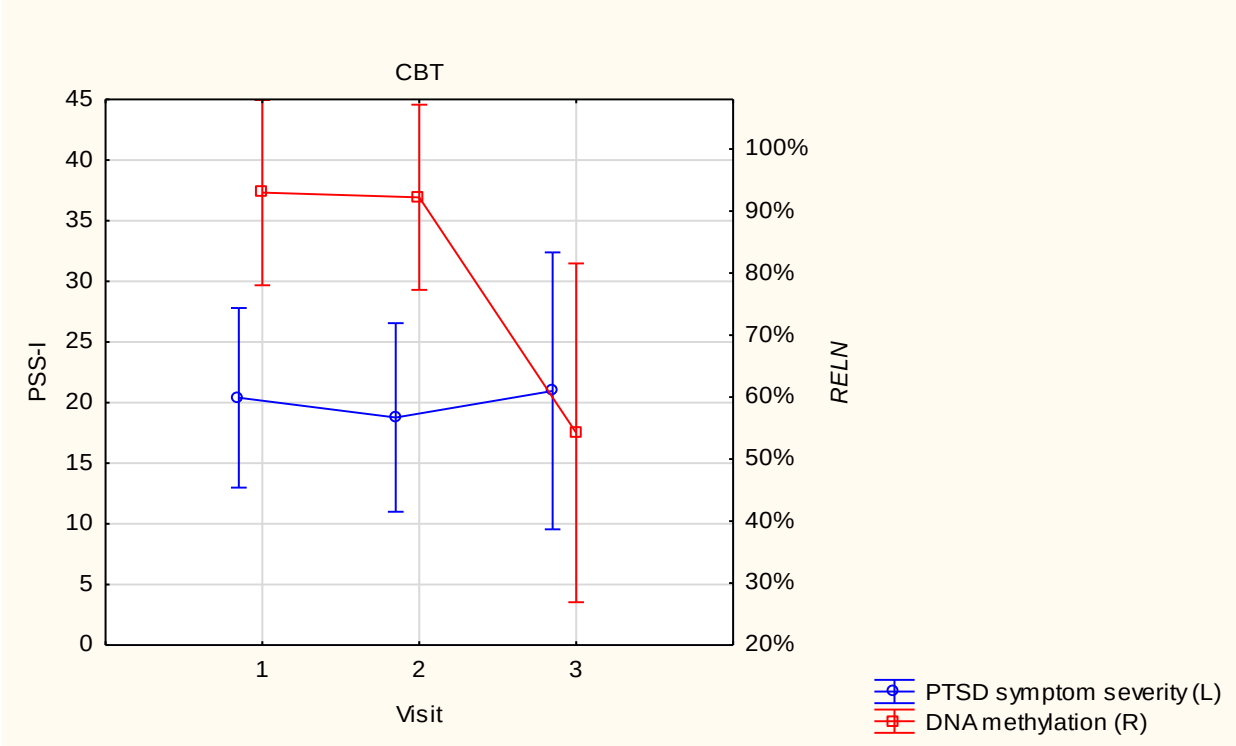

Figure 2.10: Longitudinal analysis of posttraumatic stress disorder (PTSD) measured by the PTSD Symptom Scale-Interview (PSS-I) in association with DNA methylation in the reelin (*RELN*) gene after participants had received cognitive behavioural therapy (CBT). L and R represent left and right Y-axis on the graph. Visits 1, 2, and 3 refer to baseline (0 months, prior the intervention), first follow-up (approximately 8 months post-intervention), and second follow-up (approximately 16 months post-intervention), respectively. No change in PTSD symptom severity between the three-time points (L). DNA methylation was significantly reduced in the *RELN* gene between time points 2 and 3 (p=0.023) (R).

2.3.2.1.3 TAU

Significantly increased methylation in the *RELN* gene was found in the TAU group between time points 1 (baseline, 0 months prior to the intervention) and 2 (first follow-up, 8 months on average post-intervention) (p=0.025) (Figure 2.10) (Table 2.8). No significant change in appetitive aggression or PTSD symptom severity was found in the TAU group over the three time points.

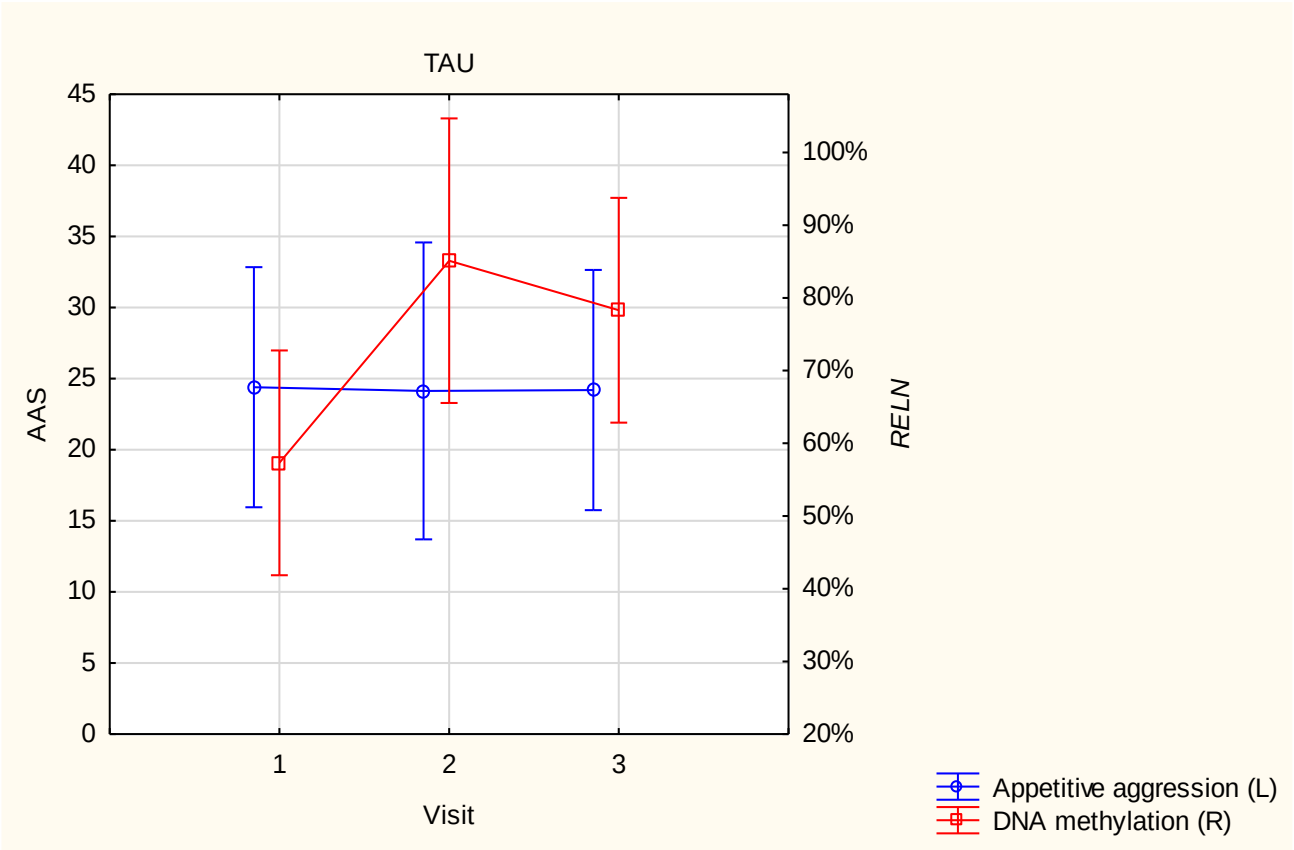

Figure 2.11: Longitudinal analysis of appetitive aggression measured by the Appetitive Aggression Scale (AAS) in association with DNA methylation of reelin (*RELN*) gene after participants had received treatment as usual (TAU). L and R represent left and right Y-axis on the graph. Visits 1, 2, and 3 indicate time points 1 (baseline, 0 months, prior the intervention), 2 (first follow-up, approximately 8 months post-intervention), and 3 (second follow-up, approximately 16 months post-intervention), respectively. No change in appetitive aggression (L). No change in appetitive aggression (L). Significantly increased methylation in the *RELN* gene was found between time points 1 and 2 (p=0.025) (R).

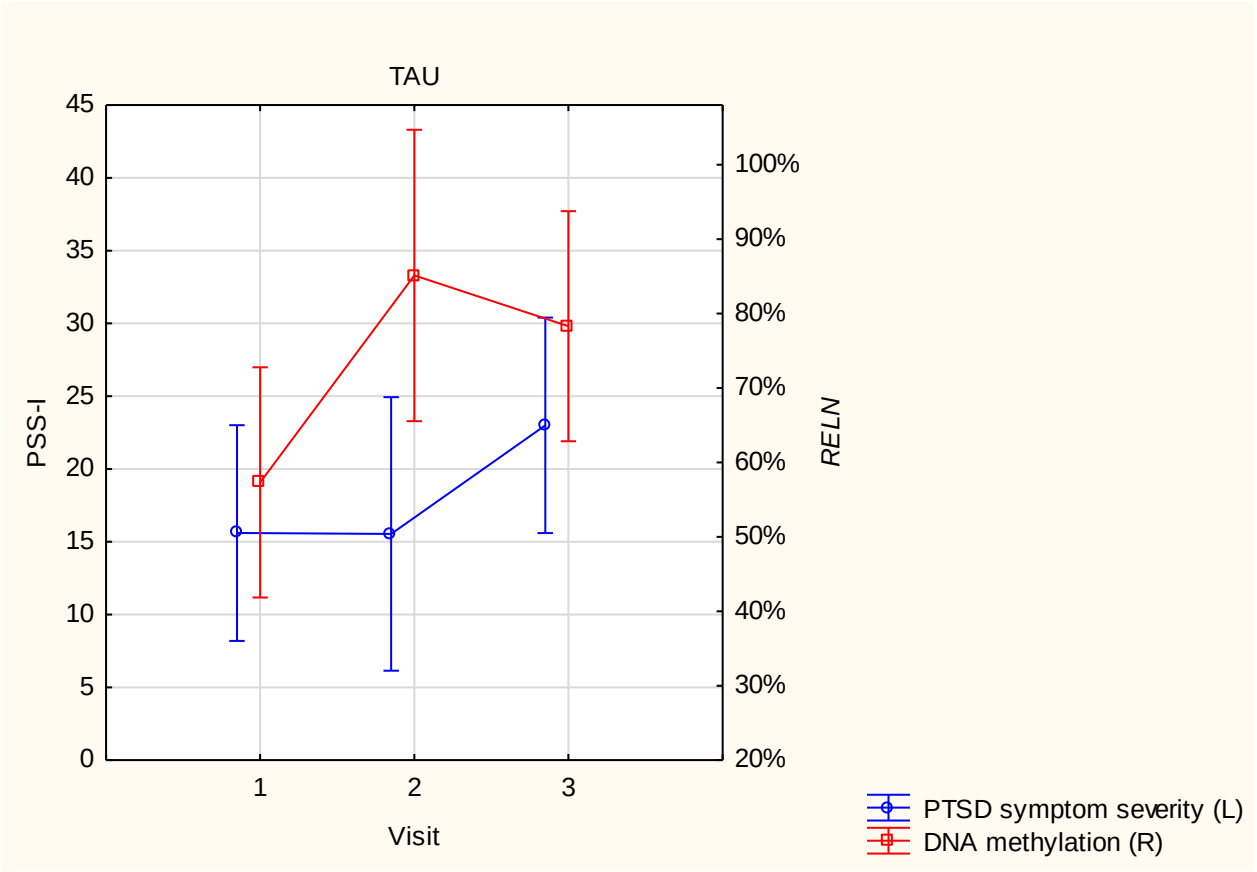

Figure 2.12: Longitudinal analysis of posttraumatic stress disorder (PTSD), as measured by the PTSD Symptom Scale-Interview (PSS-I), in association with DNA methylation of reelin (*RELN*) gene after participants had received treatment as usual (TAU). L and R represent left and right Y-axis on the graph. Visits 1, 2, and 3 refer to time points 1 (baseline, 0 months, prior the intervention), 2 (first follow-up, approximately 8 months post-intervention), and 3 (second follow-up, approximately 16 months post-intervention), respectively. No significant changes in PTSD symptom severity (L). No change in appetitive aggression (L) over the three-time points. Methylation in the *RELN* gene significantly increased between time points 1 and 2 (p=0.025) (R).

2.3.2.2 *AUTS2* gene methylation

DNA methylation changes in the *AUTS2* gene were compared to the changes in appetitive aggression and PTSD symptom severity in the treatment groups over the three-time points. Descriptive information on the change in DNA methylation in the *AUTS2* gene is shown in Table 2.9. None of the treatment groups exhibited significant differences in *AUTS2* DNA methylation at baseline (Table 2.10).

Table 2. 6: Descriptive statistics in DNA methylation changes in the *AUTS2* gene for the FORNET, CBT and TAU treatment groups between three time points

Treatment Group	N	Mean (% DNA methylation)	SD	SE	95% Confidence Interval for Mean	
					Lower	Upper
Baseline						
FORNET	10	0.97	0.04	0.01	0.94	1.00
CBT	10	0.98	0.02	0.00	0.96	1.00
TAU	10	0.94	0.10	0.03	0.87	1.02
First follow-up						
FORNET	9*	0.94	0.14	0.04	0.82	1.05
CBT	10	0.91	0.16	0.05	0.79	1.03
TAU	6*	0.99	0.01	0.00	0.97	1.00
Second follow-up						
FORNET	10	0.87	0.19	0.06	0.73	1.01
CBT	3*	0.55	0.00	0.00	0.55	0.55
TAU	10	0.85	0.20	0.06	0.70	1.00

FORNET— Narrative Exposure Therapy for Forensic Offender Rehabilitation
CBT— Cognitive Behavioural Therapy
TAU— Treatment As Usual
N— number of participants
SD— standard deviation
SE— standard error
* Participants indicated with an asterisk that were groups of less than 10 due to dropouts
Time points 1, 2, 3— represent baseline (prior the intervention), first follow-up (eight months on average) and second follow-up (16 months on average)
AUTS2— is the autism susceptibility candidate 2 gene

2.3.2.2.1 FORNET

There was no significant change in DNA methylation in the *AUTS2* gene in the FORNET group between time points 1 (baseline, 0 months) and 2 (first follow-up, 8 months on average) or between time points 2 and 3 (second follow-up, 16 months on average) (Figures 2.12 and 2.13) (Table 2.10). Methylation in the *AUTS2* gene was not associated with changes in appetitive aggression severity (r=-0.11, p=0.) or PTSD symptom severity (r=-0.067, p=0.75).

Table 2.7: Fisher's least significant differences multiple comparisons for DNA methylation in the *AUTS2* gene in the FORNET, CBT and TAU treatment groups between three time points.

Within groups	
Treatment groups	P-value
F1 vs F2	0.567
F1 vs F3	0.069
F2 vs F3	0.225
C1 vs C2	0.173
C1 vs C3	**0.000****
C2 vs C3	**0.000****
T1 vs T2	0.560
T1 vs T3	0.107
T2 vs T3	0.053

F1, F2, F3—Narrative Exposure Therapy for Forensic Offender Rehabilitation (FORNET) group at baseline, first follow-up and second follow-up respectively
C1, C2, C3— Cognitive Behavioural Therapy (CBT) group at baseline, first follow-up, and second follow-up, respectively
T1, T2, T3— Treatment as usual (TAU) group at baseline, first follow-up, and second follow-up post-intervention, respectively
*p < 0.05 and *p < 0.01 indicate statistical significance
AUTS2— is the autism susceptibility candidate 2 gene
Time points 1, 2, 3— represent baseline (prior the intervention), first follow-up (eight months on average post-intervention) and second follow-up (16 months on average post-intervention)

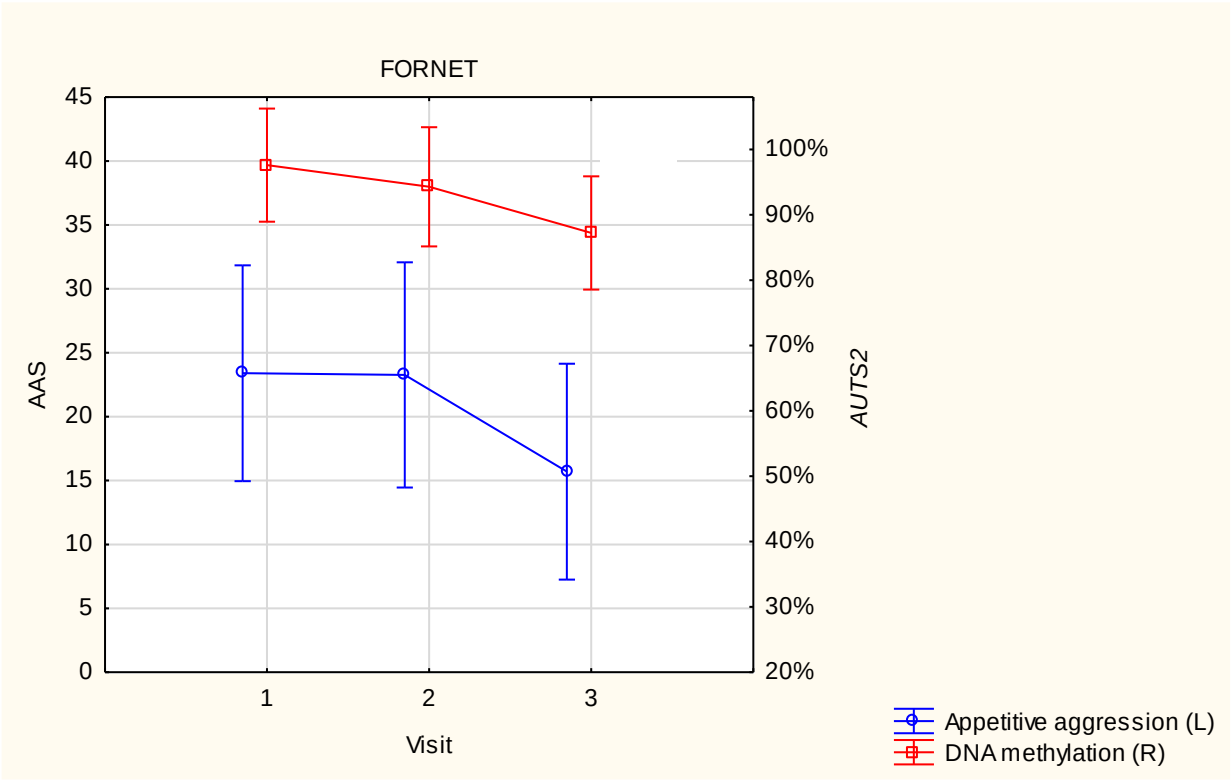

Figure 2.13: Longitudinal analysis of appetitive aggression, as measured by the Appetitive Aggression Scale (AAS), in association with DNA methylation in the autism susceptibility candidate 2 (*AUTS2*) gene, after participants had received Narrative Exposure Therapy for Forensic Offender Rehabilitation (FORNET). L and R represent left and right Y-axis on the graph. Visits 1, 2, and 3 indicate time points 1 (baseline, 0 months, prior the intervention), 2 (first follow-up, approximately 8 months post-intervention), and 3 (second follow-up, approximately 16 months post-intervention), respectively. No significant changes in appetitive aggression (L) and no significant change in DNA methylation (R) over the three-time points.

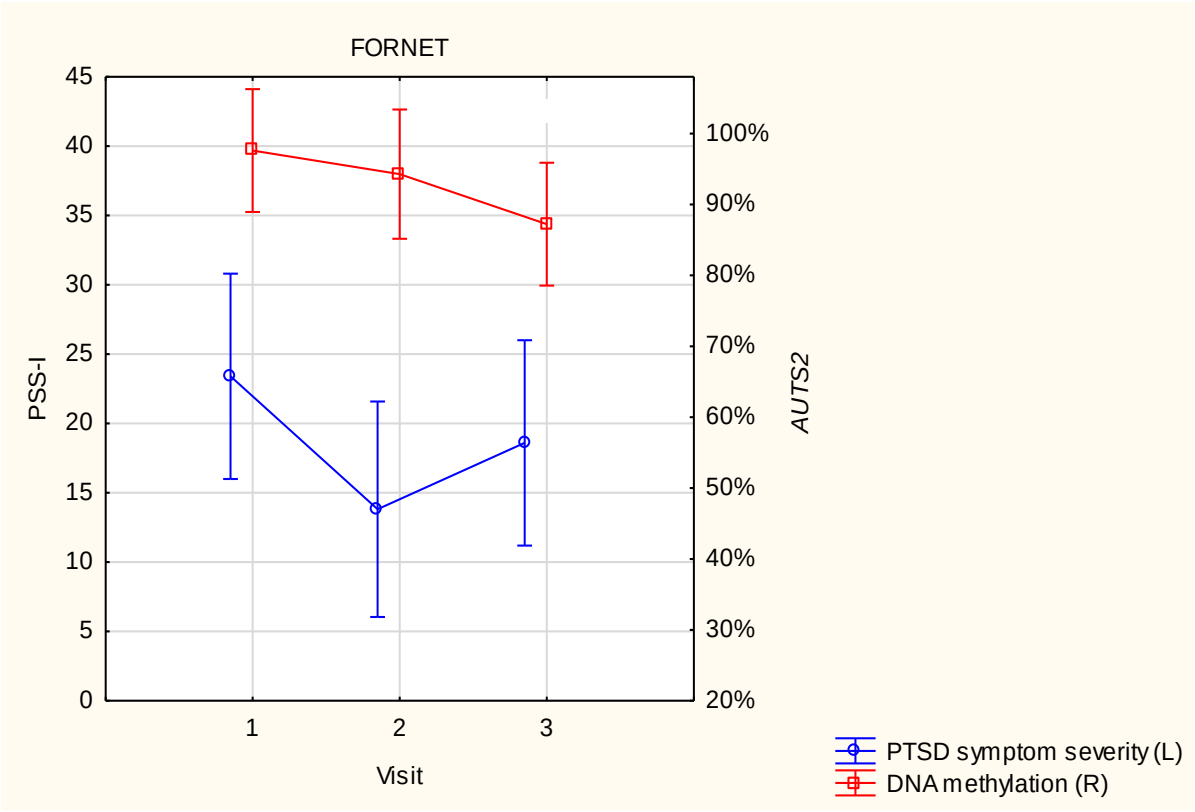

Figure 2.14: Longitudinal analysis of posttraumatic stress disorder (PTSD), as measured by the PTSD Symptom Scale-Interview (PSS-I), in association with DNA methylation in the autism susceptibility candidate 2 (*AUTS2*) gene after participants had received Narrative Exposure Therapy for Forensic Offender Rehabilitation (FORNET). L and R represent left and right Y-axis on the graph. Visits 1, 2, and 3 indicate time points 1 (baseline, 0 months, prior the intervention), 2 (first follow-up, approximately 8 months post-intervention), and 3 (second follow-up, approximately 16 months post-intervention), respectively. PTSD symptom severity (L) was significantly reduced between time points 1 and 2 (p=0.048). No significant change in DNA methylation (R) between the time points.

2.3.2.2.2 CBT

DNA methylation in the *AUTS2* gene was significantly decreased in the CBT group between time points 2 (first follow-up, 8 months on average) and 3 (second follow-up, 16 months on average) (p=0.000) (Figure 2.14 and 2.15) (Table 2.10). Methylation in this gene was negatively correlated with appetitive aggression between time point 2 and 3 (r=-0.52, p=0.03), while PTSD symptom severity was not associated with this methylation change (r=0.19, p=0.50).

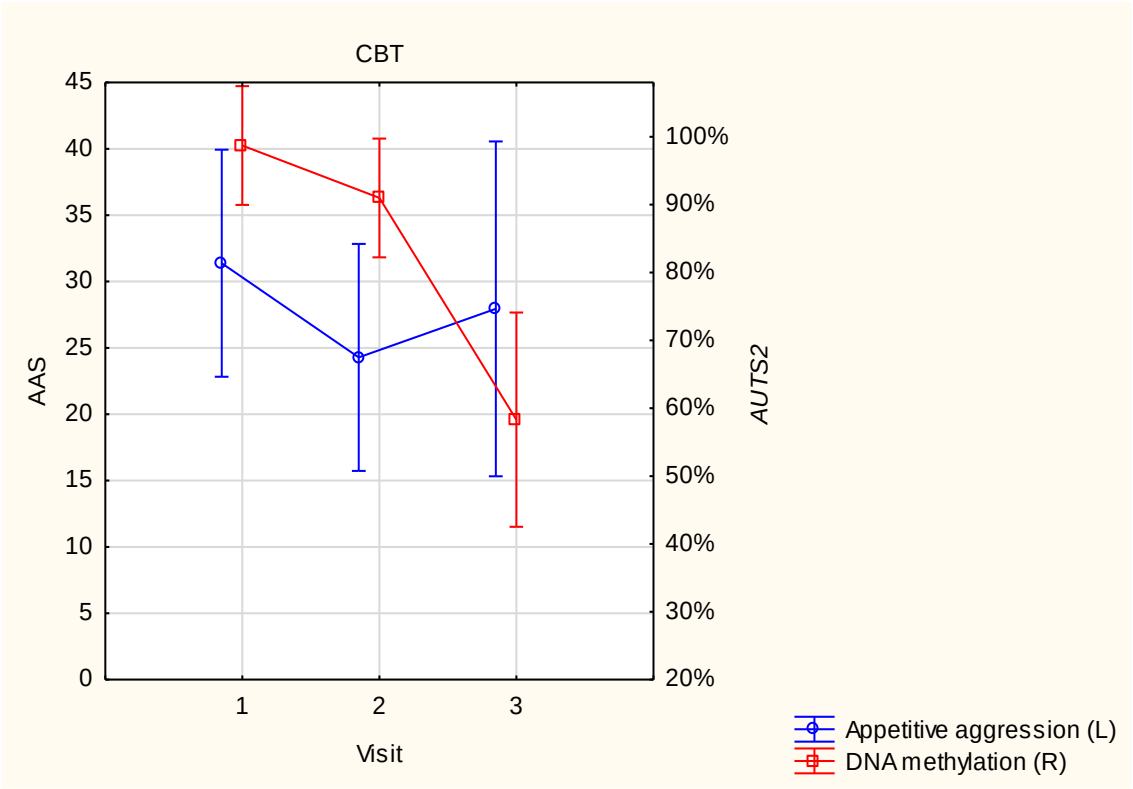

Figure 2.15: Longitudinal analysis of appetitive aggression, as measured by the Appetitive Aggression Scale (AAS), in association with DNA methylation in the autism susceptibility candidate 2 (*AUTS2*) gene, after participants had received Cognitive Behavioural Therapy (CBT). L and R represent left and right Y-axis on the graph. Visits 1, 2, and 3 indicate time points 1 (baseline, 0 months, prior the intervention), 2 (first follow-up, approximately 8 months post-intervention), and 3 (second follow-up, approximately 16 months post-intervention), respectively. No significant change in appetitive aggression (L) over the time points. DNA methylation in the *AUTS2* gene (R) was significantly reduced between time points 2 and 3 (p=0.000).

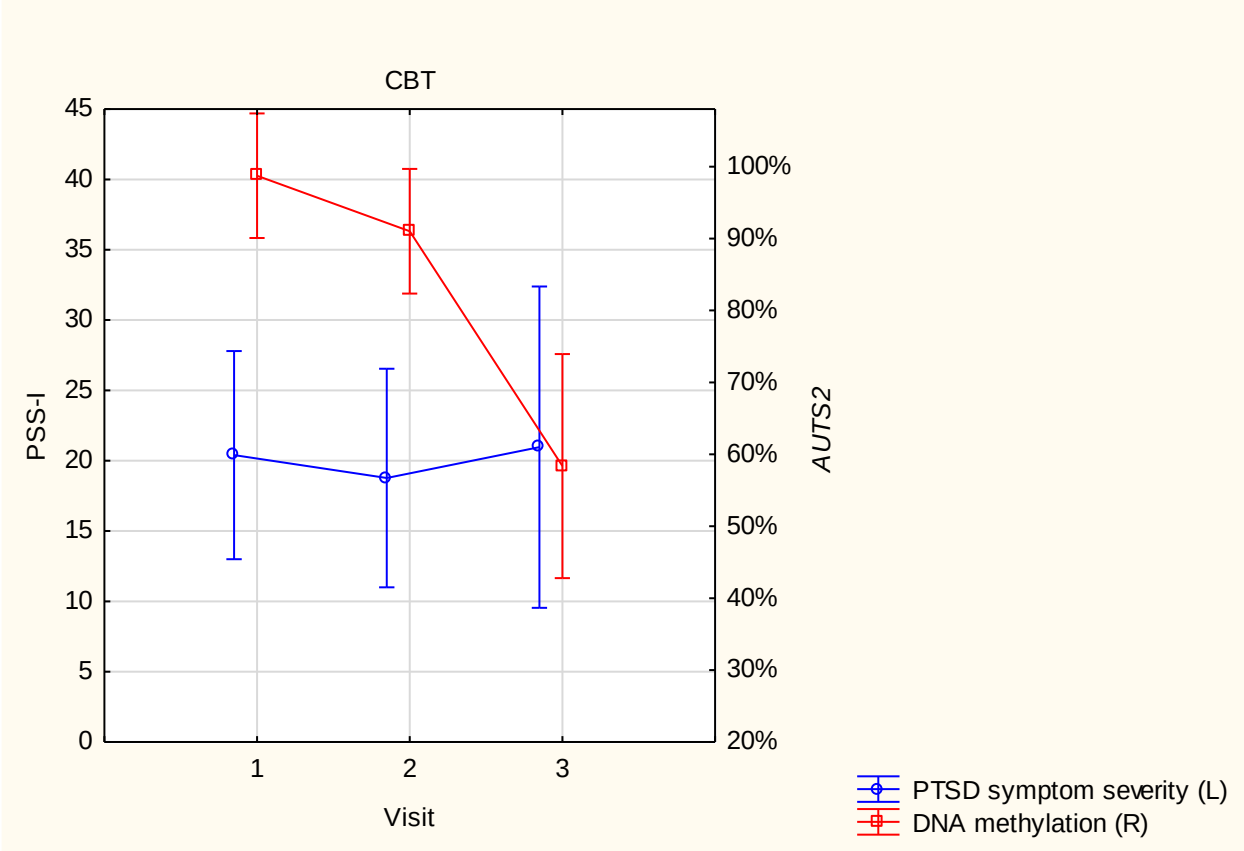

Figure 2.16: Longitudinal analysis of posttraumatic stress disorder (PTSD), as measured by the PTSD Symptom Scale-Interview (PSS-I), in association with DNA methylation in the autism susceptibility candidate 2 (*AUTS2*) gene participants had received Cognitive Behavioural Therapy (CBT). L and R represent left and right Y-axis on the graph. Visits 1, 2, and 3 correspond to time points 1 (baseline, 0 months, prior the intervention), 2 (first follow-up, approximately 8 months post-intervention), and 3 (second follow-up, approximately 16 months post-intervention), respectively. No significant change in PTSD symptom severity (L). No change in PTSD symptom severity (L) over the time points. DNA methylation in the *AUST2* gene (R) was significantly reduced between time points 2 and 3 (p=0.000).

2.3.2.2.3 TAU

There was no significant change in DNA methylation in the *AUTS2* gene in the TAU group across the three-time points (Figure 2.16 and 2.17) (Table 2.10). Although no significant change in DNA methylation was found in this gene in the TAU group, a trend towards decreased methylation in this gene was observed between time points 2 (first follow-up, 8 months) and 3 (second follow-up, 16 months) in this group (p=0.053) (Table 2.10).

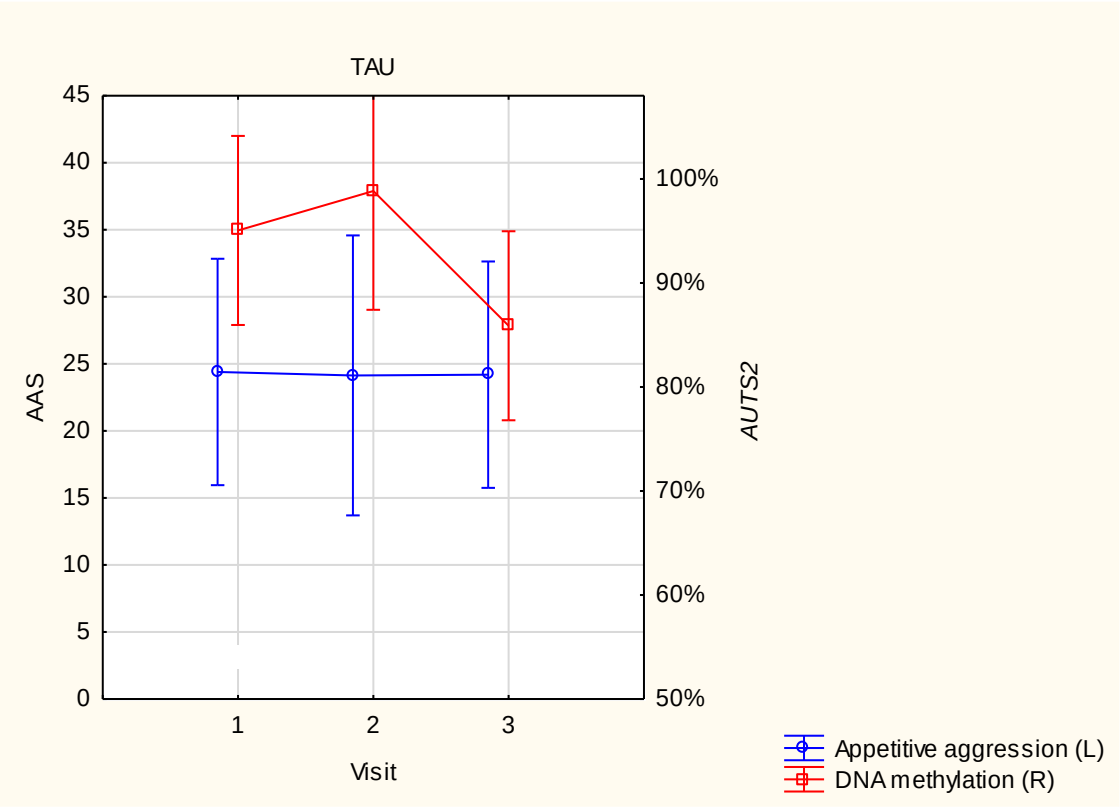

Figure 2.17: Longitudinal analysis of appetitive aggression, as measured by the Appetitive Aggression Scale (AAS), in association with DNA methylation in the autism susceptibility candidate 2 (*AUTS2*) gene, after participants had received Treatment As Usual (TAU). L and R represent left and right Y-axis on the graph. Visit 1, 2, and 3 correspond to time points 1 (baseline, 0 months, prior the intervention), 2 (first follow-up, approximately 8 months post-intervention), and 3 (second follow-up, approximately 16 months post-intervention), respectively. There was no significant change in appetitive aggression (L) over the time points. DNA methylation in the *AUTS2* gene (R) was not significantly changed across the three-time points.

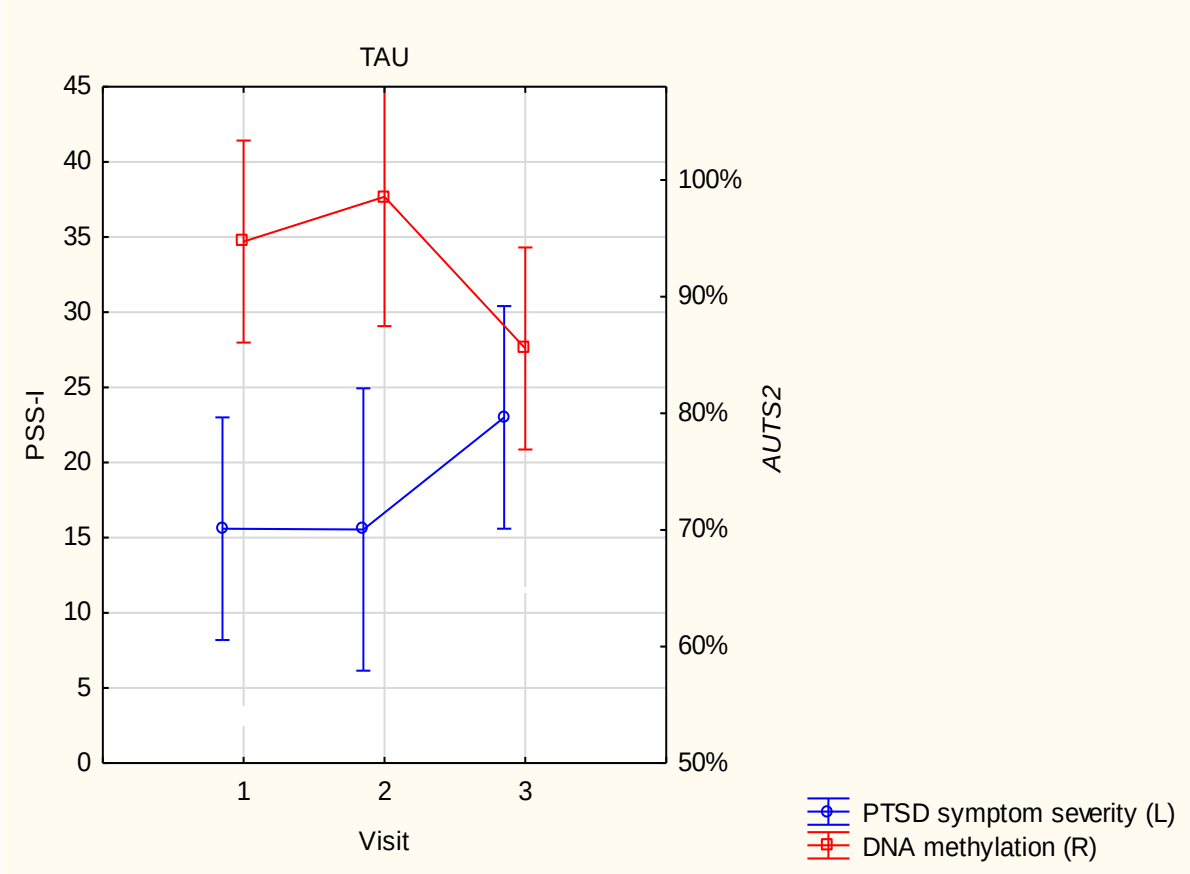

Figure 2.18: Longitudinal analysis of posttraumatic stress disorder (PTSD), as measured by the PTSD Symptom Scale-Interview (PSS-I), in association with DNA methylation in the autism susceptibility candidate 2 (*AUTS2*) gene, after participants had received Treatment As Usual (TAU). L and R represent left and right Y-axis on the graph. Visits 1, 2, and 3 refer to time points 1 (baseline, 0 months, prior the intervention), 2 (first follow-up, approximately 8 months), and 3 (second follow-up, approximately 16 months), respectively. There were no significant changes in PTSD symptom severity (L) were found over the time points. No significant change in methylation in *AUTS2* gene (R) over the time points.

2.3.2.3 COMT gene methylation

Description of the change in DNA methylation in the *COMT* gene is provided in Table 2.11.

Table 2.8: Descriptive statistics in DNA methylation changes in the *COMT* gene for the FORNET, CBT and TAU treatment groups between three-time points

Treatment Group	N	Mean (% DNA methylation)	SD	SE	95% Confidence Interval for Mean	
					Lower	Upper
Baseline						
FORNET	10	0.98	0.01	0.00	0.97	0.99
CBT	10	0.99	0.01	0.00	0.98	1.00
TAU	10	0.98	0.01	0.00	0.97	0.99
First follow-up						
FORNET	9*	0.96	0.04	0.01	0.93	0.99
CBT	10	0.95	0.03	0.01	0.93	0.98
TAU	6*	0.99	0.00	0.00	0.98	1.00
Second follow-up						
FORNET	10	0.98	0.01	0.00	0.97	0.99
CBT	3*	0.99	0.00	0.00	0.99	0.99
TAU	10	0.96	0.03	0.01	0.94	0.99

FORNET— Narrative Exposure Therapy for Forensic Offender Rehabilitation
CBT— Cognitive Behavioural Therapy
TAU— Treatment as usual
N— the number of participants
SD— standard deviation
S—standard error
* Participants indicated with an asterisk that were groups of less than 10 due to dropouts
Baseline— time point 1, 0 months prior the intervention
First follow-up— time point 2, eight months on average
Second follow-up—time point 3, 16 months on average
COMT— is the catechol-O-methyltransferase gene

2.3.2.3.1 FORNET

There was no significant change in DNA methylation in the *COMT* gene between time points 1 (baseline, 0 months) and 2 (first follow-up, 8 months post-intervention) or between time points 2 (first follow-up, 8 months post-intervention) and 3 (second follow-up, 16 months post-intervention) in the FORNET group (Figure 2.18 and 2.19) (Table 2.12). There was no statistically significant association between the change in methylation in this gene and the change in appetitive aggression or PTSD symptom severity.

Table 2.9: Fisher's least significant differences multiple comparisons for DNA methylation in the *COMT* gene for the FORNET, CBT and TAU treatment groups between threetime points

Within groups	
Treatment groups	P-value
F1 vs F2	0.114
F1 vs F3	0.770
F2 vs F3	0.065
C1 vs C2	**0.007****
C1 vs C3	0.806
C2 vs C3	**0.038***
T1 vs T2	0.754
T1 vs T3	0.060
T2 vs T3	0.055

F1, F2, F3— Narrative Exposure Therapy for Forensic Offender Rehabilitation (FORNET) group at baseline, first follow-up and second follow-up respectively
C1, C2, C3— Cognitive Behavioural Therapy (CBT) group at baseline, first follow-up and second follow-up respectively
T1, T2, T3— Treatment As Usual (TAU) group at baseline, first follow-up and second follow-up respectively
*p<0.05 and *p <0.01 indicate statistical significance
COMT— catechol-O-methyltransferase gene
Time points 1, 2, 3— represent baseline (prior the intervention), first follow-up (eight months on average post-intervention) and second follow-up (16 months on average post-intervention)

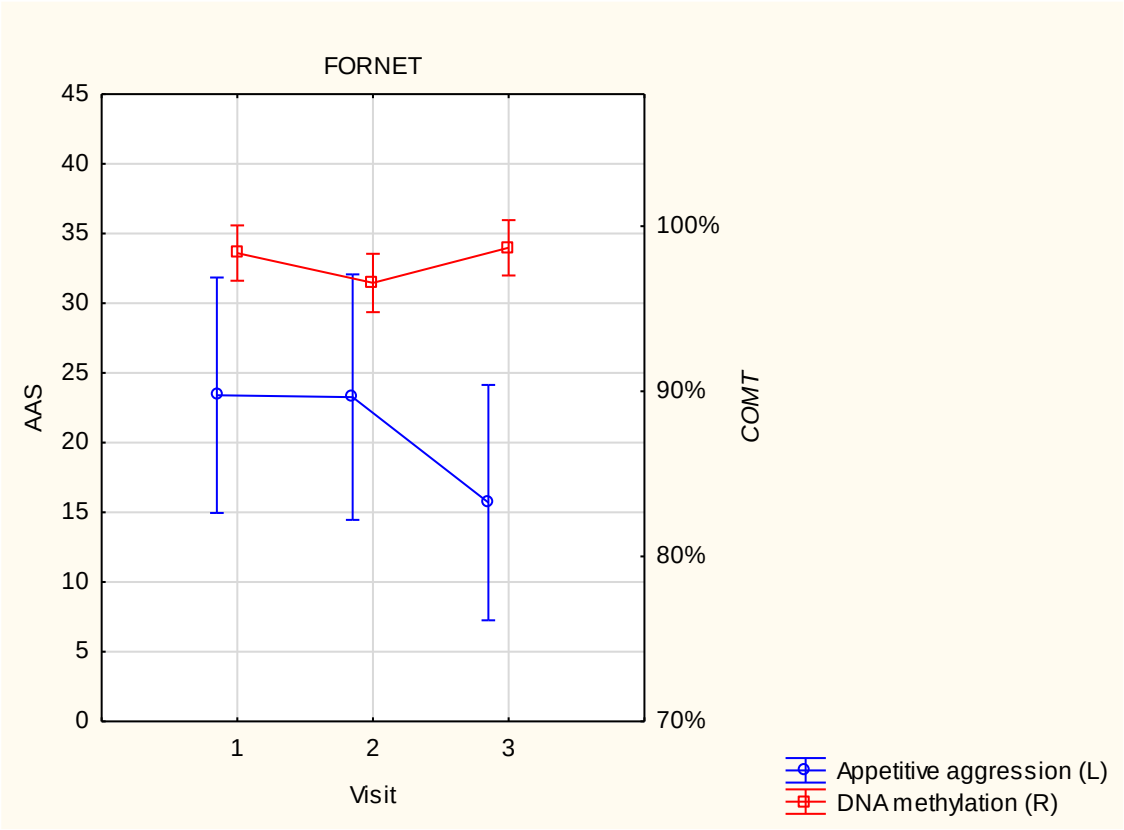

Figure 2.19: Longitudinal analysis of appetitive aggression, as measured by the Appetitive Aggression Scale (AAS), in association with DNA methylation changes in the catechol-O-methyltransferase (*COMT*) gene, after participants who had received Narrative Exposure Therapy for Forensic Offender Rehabilitation (FORNET). L and R represent left and right Y-axis on the graph. Visits 1, 2, and 3 correspond to time points 1 (baseline, 0 months, prior the intervention), 2 (first follow-up, approximately 8 months post-intervention), and 3 (second follow-up, approximately 16 months post-intervention), respectively. There was no significant association between the change in appetitive aggression (L) and the change in DNA methylation (R) over the three-time points.

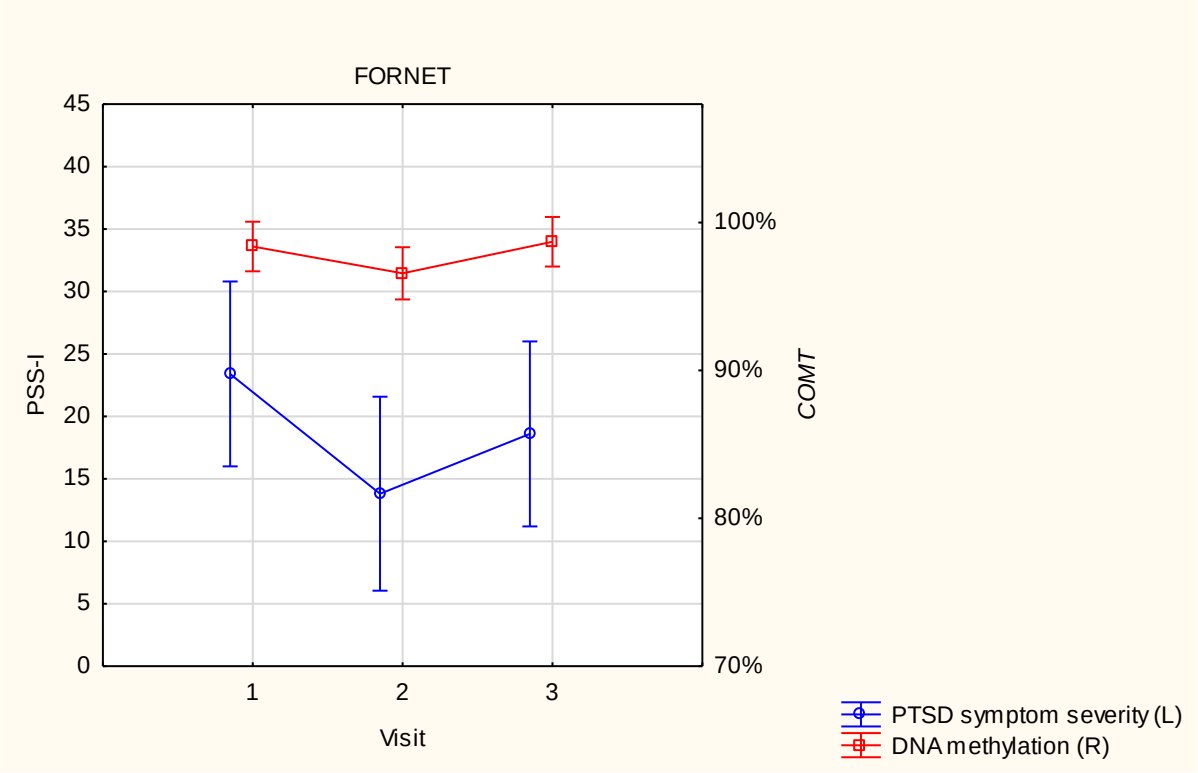

Figure 2.20: Longitudinal analysis of posttraumatic stress disorder (PTSD), as measured by the PTSD Symptom Scale-Interview (PSS-I), in association with DNA methylation in the catechol-O-methyltransferase (*COMT*) gene after participants had received Narrative Exposure Therapy for Forensic Offender Rehabilitation (FORNET). L and R represent left and right Y-axis on the graph. Visits 1, 2, and 3 matches with time points 1 (baseline, 0 months, prior the intervention), 2 (first follow-up, approximately 8 months post-intervention), and 3 (second follow-up, approximately 16 months post-intervention), respectively. No significant change in PTSD symptom severity (L). PTSD symptom severity was significantly reduced between time points 1 and 2 (L) (p=0.048). There was no significant change in DNA methylation (R) over the time points.

2.3.2.3.2 CBT

DNA methylation was significantly reduced in the *COMT* gene between time points 1 (baseline, 0 months) and 2 (first follow-up, 8 months post-intervention) (p=0.007) (Table 2.12) (Figure 2.20 and 2.21). However, between time points 2 and 3 (second follow-up, 16 months on average post-intervention), DNA methylation in this gene increased significantly (p=0.038) (Figure 2.20 and 2.21) (Table 2.12). None of these methylation changes were found to be significantly associated with either appetitive aggression between the three-time

points (r=-0.23, p=0.28).However, we have noted that increased methylation in the *COMT* gene was negatively correlated with PTSD symptom severity over time (r=-0.35, p=0.10).

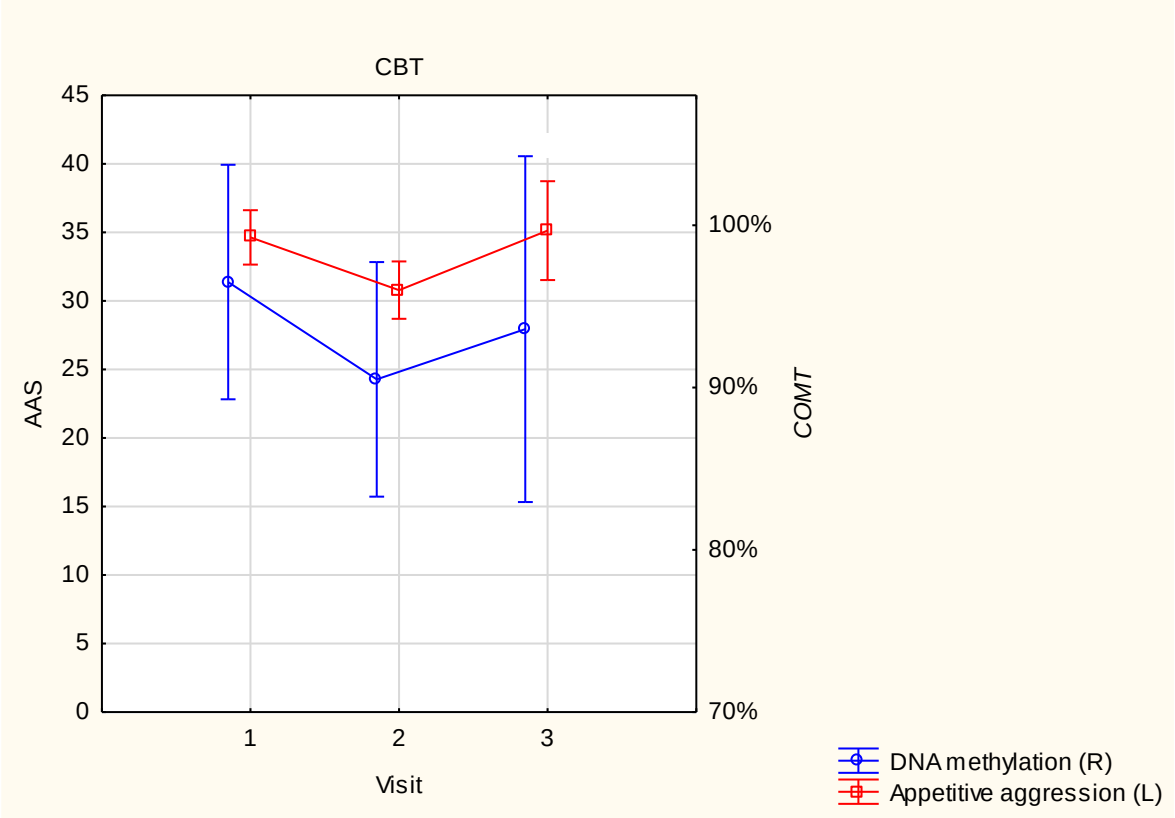

Figure 2.21: Longitudinal analysis of appetitive aggression, as measured by the Appetitive Aggression Scale (AAS), in association with DNA methylation changes in the catechol-O-methyltransferase (*COMT*) gene, after participants had received Cognitive Behavioural Therapy (CBT). L and R represent left and right Y-axis on the graph. Visit 1, 2, and 3 correspond to time points 1 (baseline, 0 months, prior the intervention), 2 (first follow-up, approximately 8 months), and 3 (second follow-up, approximately 16 months), respectively. No significant change in appetitive aggression (L) across the three-time points (R). DNA methylation was significantly reduced between time points 1 and 2 (p=0.007), and became significantly increased between time points 2 and 3 (p=0.038).

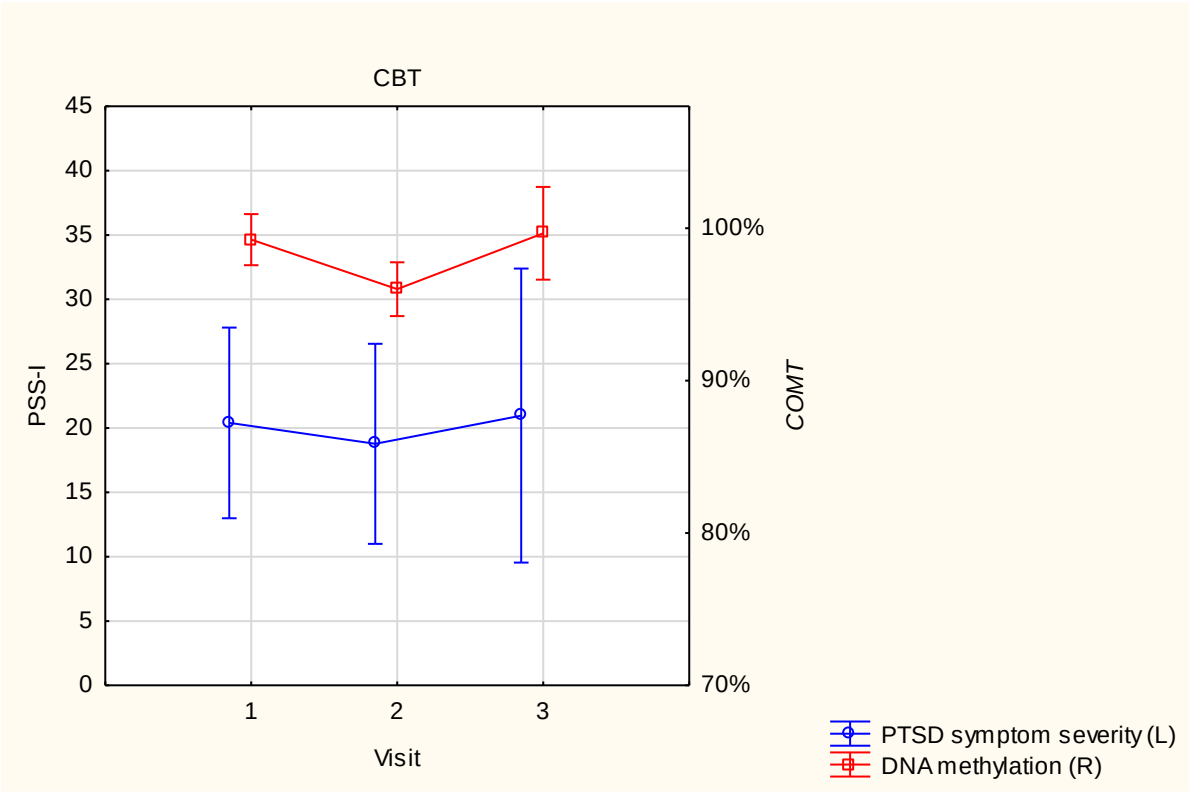

Figure 2.22: Longitudinal analysis of posttraumatic stress disorder (PTSD), as measured by the PTSD Symptom Scale-Interview (PSS-I), in association with DNA methylation in the catechol-O-methyltransferase (*COMT*) gene after participants had received Cognitive Behavioural Therapy (CBT). L and R represent left and right Y-axis on the graph. Visits 1, 2, and 3 correspond to time points 1 (baseline, 0 months, prior the intervention), 2 (first follow-up, approximately 8 months), and 3 (second follow-up, approximately 16 months), respectively. PTSD symptom severity (L) did not change significantly across the time points. Significant reduction in DNA methylation (R) between time points 1 and 2 (p=0.007) and significantly increased methylation in the COMT gene between time points 2 and 3 (p=0.038) was found.

2.3.2.3.3 TAU

No significant change in DNA methylation was found in the *COMT* gene across the three-time points (Figure 2.22 and 2.23).

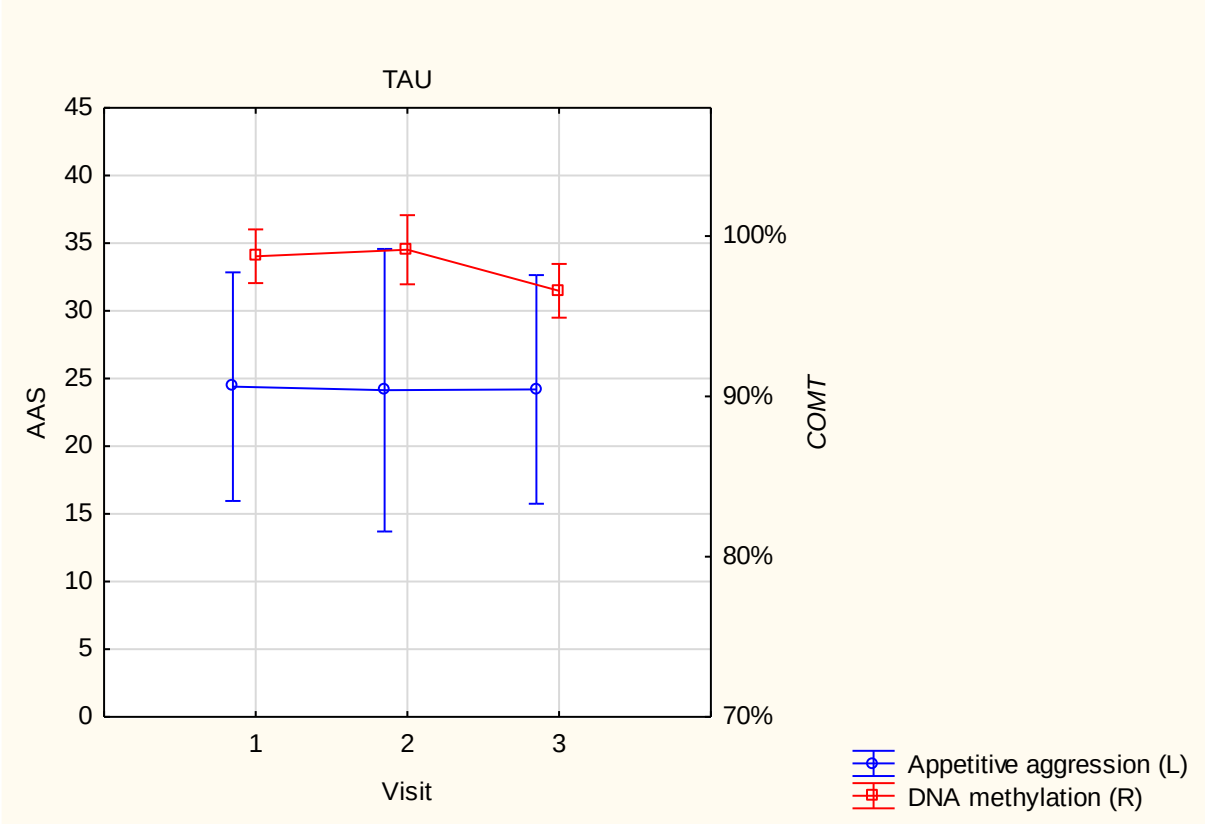

Figure 2.23: Longitudinal analysis of appetitive aggression, as measured by the Appetitive Aggression Scale (AAS), in association with DNA methylation changes in the catechol-O-methyltransferase (*COMT*) gene after participants had received Treatment As Usual (TAU). L and R represent left and right Y-axis on the graph. Visits 1, 2, and 3 correspond to time points 1 (baseline, 0 months, prior the intervention), 2 (first follow-up, approximately 8 months), and 3 (second follow-up, approximately 16 months), respectively. No significant association between change in appetitive aggression (L) and DNA methylation change (R) across the three-time points.

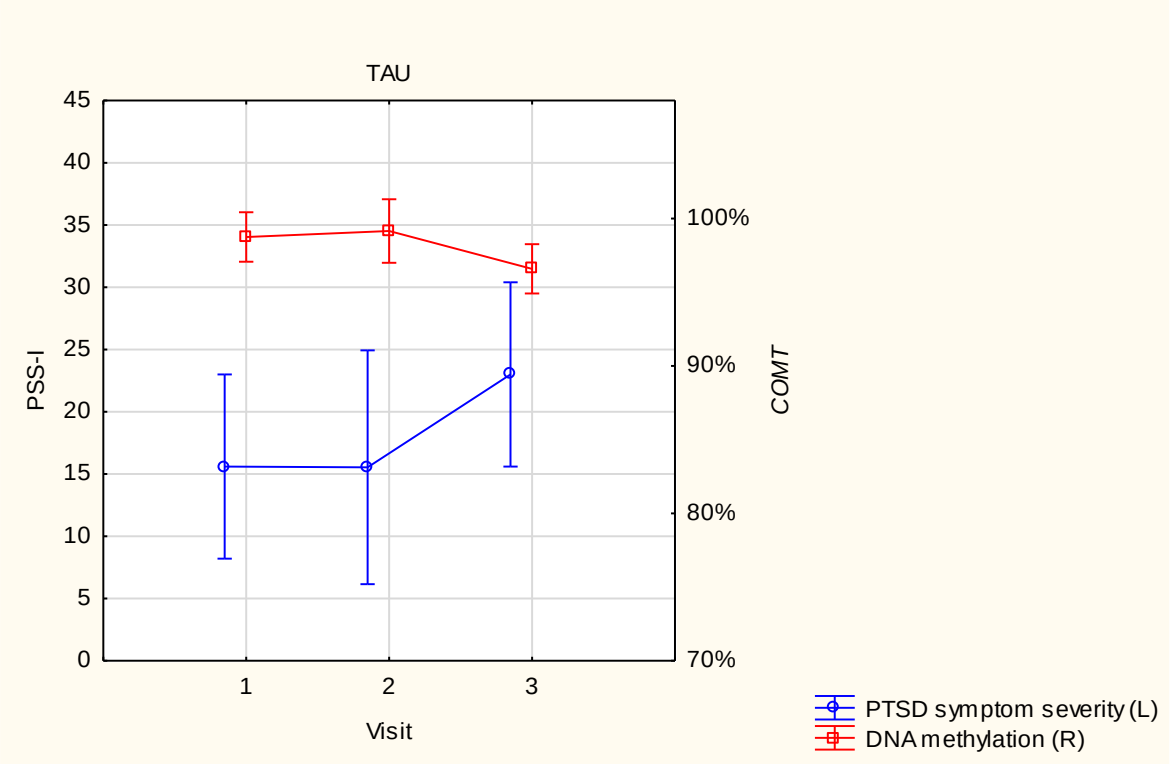

Figure 2.24: Longitudinal analysis of posttraumatic stress disorder (PTSD), as measured by the PTSD Symptom Scale-Interview (PSS-I), in association with DNA methylation in the catechol-O-methyltransferase (*COMT*) gene after participants had received Treatment As Usual (TAU). L and R represent left and right Y-axis on the graph. Visit 1, 2, and 3 indicate time points 1 (baseline, 0 months, prior the intervention), 2 (first follow-up, 8 months on average) and 3 (second follow-up, 16 months on average), respectively. No significant association of between PTSD symptom severity change (L) and DNA methylation change (R) was found over the time points.

2.4 DISCUSSION

Several studies have investigated the relationship between environmental exposure and traumatic events such as childhood abuse, violent and aggressive behaviour to the methylation of genes (Checknita et al., 2015; van Dongen et al., 2015; Guillemin et al., 2014; Provençal et al., 2013; Schechter et al., 2016). Methylation of certain genes has been shown to be associated with the perpetration of aggression (Guillemin et al., 2014; Provençal et al., 2013) and the development of PTSD (Rusiecki et al., 2013; Wolf et al., 2016; Yehuda et al., 2013; Zannas et al., 2015). A study by Provençal et al. (2014) identified genes that were differentially methylated in the promoter regions in individuals who had experienced childhood trauma. Hypermethylation of these genes was associated with perpetration of chronic physical aggression in adulthood (Provençal et al., 2014). Genes that have been

found to be associated with aggressive behaviour include *DRD1* (Våge et al., 2010), *AVPR1A* (Ferris et al., 2006) and *SLC6A3* (Vaughn et al., 2009). All of these genes are essential in the neuroendocrine system and play a crucial role in the proper functioning of the brain. Other studies have identified genes that are methylated in individuals with severe PTSD symptoms associated with various traumatic exposures. These genes include *COMT* (Norrholm et al., 2013) and the long interspersed nuclear element-1 (*LINE-1*) gene (Rusiecki et al., 2012). *LINE-1* methylation has been associated with an instability in chromosomes and has been reported in certain cancers (Dunn, 2003; Eden et al., 2003).

In this study, we investigated the effects of FORNET on the changes in DNA methylation of candidate genes that have been found to be involved in mental health and behavioural disorders (Beunders et al., 2013; Dammann et al., 2011; Guidotti et al., 2016; Labouesse et al., 2015; Maddox et al., 2013; Roth and Sweatt, 2011). CBT was included as the comparative psychotherapeutic intervention as it is an evidence-based treatment for PTSD, while TAU was used as a control and comprised non-clinically focused counselling (van Dam et al., 2013; Watts et al., 2015). We found that *RELN*, *AUTS2*, and *COMT* genes were differentially methylated at different time points of assessment in the different groups when methylation was analysed longitudinally in participants receiving therapeutic interventions.

RELN

There were no significant changes in methylation of the *RELN* gene across the different time points of assessments in individuals who had received the FORNET intervention. We found that individuals who had received the CBT intervention had significantly decreased methylation of *RELN* gene between time points 1 and 3, and between time points 2 and 3. *RELN* encodes for reelin, a protein that plays an essential role during neuronal migration, the branching of axons, synapse formation, memory formation, and cell signalling (Abdolmaleky et al., 2005; Levenson et al., 2006; Niu et al., 2008). These processes are essential for the proper function of the brain (Guidotti et al., 2016; Hashimoto-Torii et al., 2008; Maloku et al., 2010).

Individuals who had received the FORNET intervention exhibited reduced PTSD symptom severity. The FORNET intervention may have stabilised molecular responses associated with trauma processing, primarily as this intervention targets violence signals and corrects a fragmented autobiographical memory that is often seen in PTSD (Crombach and Elbert, 2015; Elbert et al., 2012; Hermenau et al., 2013b). Although we did not find a link between *RELN* methylation and clinical outcomes for the CBT group, our findings demonstrate that there may be an effect at molecular level regarding the processing of trauma, as indicated by

a change in gene methylation profile in *RELN*. Participants in this study were offered a course of CBT at the camp, and then released back to their communities after three weeks where there was a high risk of re-exposure to traumatic events and exacerbation of PTSD symptoms. Re-experiencing of traumatic stress may have contributed to PTSD symptom severity remaining relatively unchanged, despite the change in methylation. *RELN* methylation may also be differentially affected when there is a high co-occurrence of appetitive aggression and elevated PTSD symptoms.

Previous studies have documented an association between hypermethylation of the *RELN* gene and the development of psychiatric disorders, such as schizophrenia and bipolar disorder (Abdolmaleky et al., 2005; Grayson and Guidotti, 2013; Guidotti et al., 2016; Nabil Fikri et al., 2017; Tamura et al., 2007). Abdolmaleky et al. (2005) investigated methylation of the *RELN* gene in patients with schizophrenia with cognitive behavioural problems and found that this gene was hypermethylated in these patients compared to controls. Our findings have shown that *RELN* methylation was reduced over time after CBT intervention, which could indicate that CBT affects molecular pathways containing reelin protein. Reduced methylation could indicate differential gene expression; thus, the amount of reelin protein may have increased or decreased, depending on the effect of methylation on gene regulation. Hypermethylation of the promotor region of *RELN* gene has been associated with low gene expression (Abdolmaleky et al., 2006, 2005; Grayson et al., 2006). *RELN* is generally essential during early developmental stages (Curran and D'Arcangelo, 1998; D'Arcangelo et al., 1995), and its differential methylation after psychotherapeutic intervention may suggest that aggressive behaviour and/or PTSD may be neurodevelopmental disorders. Neurodevelopmental processes are continuous mechanisms, which could be impacted by environmental influences such as traumatic stress, genetic makeup, and gene-environmental interactions (Guidotti et al., 2016; Sugden et al., 2006). Therefore, differential methylation in *RELN* gene may be a response towards traumatic stress, which could modulate behaviour in aggressive behaviour and PTSD symptom severity. Overall, differential methylation in the *RELN* gene may have a role in the response to traumatic stress that is observed in appetitive aggression as well as in PTSD symptom severity, mainly since these disorders share similar pathways (Elbert and Schauer, 2002; Moran et al., 2014).

AUTS2

AUTS2 gene is part of a developmental cluster of genes, such as polycomb repressive complex 1 (*PRC1*), which encodes for a polycomb group of proteins that remodel chromatin through epigenetic modification, which in turn can lead to the silencing of gene expression (Gao et al., 2014; Son et al., 2013). This gene is not only essential during the developmental

stages, but plays an essential role even in adulthood, where it aids in the regeneration of neurons and maintaining their optimal function (Hori et al., 2015). Expression of *AUTS2* gene has been implicated in regulating activation of certain genes, including phosphatidylinositol 3, 4, 5-trisphosphate-dependent Rac exchanger 1 (*PREX1*) and engulfment and cell motility gene 2 (*ELMO2*), which are activated to induce expression of ras-related C3 botulinum toxin substrate 1 (*RAC1*) gene (Hori et al., 2014; Oksenberg et al., 2014). In turn, this activation aids in mechanisms that maintain neuronal development and migration (Hori et al., 2014; Katoh et al., 2006; Li et al., 2015; Sadok and Marshall, 2014; Yoshizawa et al., 2005). On the other hand, the expression of *AUTS2* gene suppresses the activation of the cell division control protein 42 homolog (*CDC42*) gene through interaction with intersectin-1 (*ITSN*) gene, which is involved in the synthesis of clathrin-coated vesicles and plays a role in synaptic vesicle recycling (Hori et al., 2014). Therefore, methylation in the *AUTS2* gene could play a significant role in regulating mechanisms involved in both appetitive aggression and PTSD.

Dysregulation of *AUTS2* gene expression can affect a number of processes, although most of them are not well characterised. Importantly, in the context of mental disorders, disruption of the normal functioning of *AUTS2* gene has been associated with the development of symptoms of schizophrenia, behavioural changes in anxiety-related disorders, drug addiction and the development of autism spectrum disorder (Dang et al., 2014; Hori et al., 2015; Zhang et al., 2014). Methylation in *AUTS2* could play a significant role in regulating underlying mechanisms involving this gene, which may also be essential in these mental disorders. Therefore, investigating methylation in the *AUTS2* gene after a psychotherapeutic intervention may shed light on neuronal processes that are involved in the therapeutic response to psychotherapies.

We found that methylation in *AUTS2* was reduced between time points 2 and 3 in individuals who received CBT intervention. We also observed a negative association between methylation in *AUTS2* and appetitive aggression between time points 2 and 3 ($r=-0.52$, $p=0.03$). Methylation reduction in the promoter region in the *AUTS2* may be associated with increased expression of this gene, which may have been triggered by the CBT intervention. An alternative explanation is that expression of *AUTS2* may have been below a level physiologically required to impact neurobiological processes. CBT may have induced normal gene expression of *AUTS2* between baseline (prior intervention) and first follow-up. Thus expression of *AUTS2* may have been increased over time. Expression of *AUTS2* could affect important processes such as neuronal migration and cell division, which are essential for the recycling of synaptic vesicle (Hori et al., 2014). Therefore, change in methylation after

intervention may alter regulation of this gene and affect molecular processes responding to stressful events.

COMT

COMT gene encodes for an essential enzyme, catechol-*O*-methyltransferase (COMT), which is responsible for the degradation of a number of neurotransmitters such as dopamine, epinephrine, norepinephrine and catecholamines in the CNS (Eisenberg et al., 1999; Lotta et al., 1995). Dysregulation of this gene may lead to increased levels of neurotransmitters that can result in imbalances in the CNS (Barnett et al., 2011; Hygen et al., 2015; Lonsdorf et al., 2010; Perroud et al., 2010). Some studies have found an association between the reduction of COMT enzyme activity and increased neurotransmitters such as dopamine in the synaptic cleft due to hypermethylation of the *COMT* gene (Bilder et al., 2004; Norrholm et al., 2013; Ursini et al., 2011). In contrast, other studies have shown that genetic variation or aberrant methylation in the promoter regions of the *COMT* gene could lead to low activity of this gene (Norrholm et al., 2013; Shifman et al., 2004; Strous et al., 2003). Indeed, studies have identified genetic variation (Met158Met allele) in the *COMT* gene to be associated with lower expression of COMT enzyme and decreased resilience against PTSD (Kolassa et al., 2010; Lonsdorf et al., 2010). In addition, reduced expression of the *COMT* gene has been associated with aggressive behaviour in patients with schizophrenia (Hu et al., 2018; Nohesara et al., 2011). Therefore, hypermethylation of *COMT* gene may result in reduced expression of the COMT enzyme.

We found that methylation in the promoter region of the *COMT* gene was significantly reduced between time points 1 (baseline, 0 months) and 2 (first follow-up, 8 months on average) but increased between time points 2 and 3 (second follow-up, 16 months on average) in the CBT group. Individuals in this group did not show any reduction in appetitive aggression or PTSD symptom severity despite a reduction in methylation in the *COMT* gene. Decreased *COMT* methylation between time points 1 and 2 after CBT intervention could indicate a positive effect of CBT in the direction of reducing appetitive aggression. It is important to note that even though appetitive aggression was not significantly reduced after CBT, there was a trend towards reduction ($p=0.135$). Furthermore, there was no significant association between appetitive aggression and *COMT* methylation over the three time points ($r=-0.23$, $p=0.28$). Although, we did not find an association between either appetitive aggression or PTSD and *COMT* methylation over time, previously, it has been shown that increased *COMT* methylation in the promoter region is associated with impaired fear inhibition in individuals with PTSD (Norrholm et al., 2013). Increased *COMT* methylation between time points 2 and 3 could be because of re-experiencing traumatic stress since

participants were released back to their communities after the three-week camp. This re-experiencing of traumatic stress may have triggered molecular pathways that could have increased *COMT* methylation that was observed between time points 2 and 3. Therefore, our finding may suggest that CBT intervention affected molecular pathways that are essential for necessary responses to traumatic stress between time points 1 and 2, which resulted in decreased *COMT* methylation. Therefore, analysing changes in methylation in the *COMT* gene may serve as an indicator of an individual's response to therapeutic intervention. However, more studies are required to confirm this finding.

This study yields key findings about DNA methylation changes; however, these findings have to be interpreted with caution considering the study's limitations. Firstly, the study investigated therapeutic interventions in a specific population and gender, so the results cannot be generalised across different ethnicities and genders without further research. Secondly, the interpretation of the study should also consider the effect size and the statistical approaches that were implemented; thus, future replication studies will be needed to confirm whether there are robust associations between DNA methylation and appetitive aggression and PTSD symptom severity in an adequately powered cohort. Although the Fisher's LSD corrects for multiple pairwise comparisons and avoids type 2 errors, stringent statistical approaches such as Bonferroni may be necessary in future studies with larger samples. Thirdly, participants should perhaps also be re-assessed after a shorter period (i.e. on a monthly basis after therapeutic intervention) to determine an association between clinical and biological variables. In our study, we assessed participants on an average of eight months post-intervention, whereas there may have been changes in appetitive aggression or PTSD symptom severity in the first few months post-intervention that are not captured by these data. Lastly, future studies should interrogate gene expression and assess how gene methylation may correlate with gene regulation. Investigating both DNA methylation and gene expression in future studies will allow us to directly compare the two mechanisms and may provide insights into how DNA methylation and gene expression interplay in the etiology of mental health disorders.

We selected a pathway-based DNA methylation kit comprising of 22 genes, which has been previously found to be associated with a range of mental health disorders (Labouesse et al., 2015; Maddox et al., 2013; Murgatroyd and Spengler, 2014). This limits the detection of other genes that may be differentially methylated and have important role in stress response system, thus, approaches such as whole epigenome investigation (e.g. Illumina's Infinium Methylation EPIC Kit) should be considered, if one has sufficient sample sizes to overcome multiple testing correction. It is important for us to stress that in our study we had a small

sample size and limited funds so a pathway-based DNA methylation approach was the only viable approach.

In conclusion, we showed that CBT intervention resulted in decreased DNA methylation in *RELN*, *AUTS2* and *COMT* genes over the three time points in a cohort of young Black South African men who had appetitive aggression and elevated PTSD symptoms. However, a change in DNA methylation in these genes was not significantly associated with changes in appetitive aggression or PTSD symptom severity. These findings lay the foundation to pursue investigations to better understand the role of DNA methylation in different genes over time in trauma-exposed indidividuals, as well as their potential future use as biomarkers for conditions such as PTSD and appetitive aggression.

CHAPTER 3

INVESTIGATION OF TELOMERE LENGTH IN RESPONSE TO NARRATIVE EXPOSURE THERAPY FOR FORENSIC OFFENDER REHABILITATION IN XHOSA BLACK SOUTH AFRICAN MEN WITH APPETITIVE AGGRESSION

ABSTRACT

Background: The impact of psychological interventions on telomere length has not been well investigated in individuals with posttraumatic stress disorder (PTSD) and aggressive behaviours, such as appetitive aggression. PTSD is a serious mental disorder that can occur after experiencing life-threatening traumatic events. Appetitive aggression is a type of instrumental aggression characterised by perpetrating violence for intrinsic pleasure. Exposure to violent traumatic events, such as ongoing community and gang-related violence, can result in appetitive aggression. Violent traumatic events have also been found to be associated with the development PTSD. Telomeres are non-coding nucleoproteins found at the end of chromosomes, and act to prevent the loss of crucial information during cell replication. Studies show that psychological interventions, such as mindfulness and meditation, have been found to reduce levels of traumatic stress, and decrease symptoms of PTSD and aggressive behaviour. Studies also indicate that telomere length may be a marker of response to psychotherapeutic interventions. Accelerated shortened telomere length is reported to be associated with accelerated cellular ageing, which is linked to old age diseases such as heart attack. The present study aimed to investigate relative telomere length and (i) appetitive aggression and (ii) PTSD symptom severity in response to Narrative Exposure Therapy for Forensic Offender Rehabilitation (FORNET) and Cognitive Behavioural Therapy (CBT) in young Xhosa Black South Africa men.

Methods: A subsample of young Xhosa Black South African men (N=30) was drawn from a concurrently conducted study of an intervention trial and who either received FORNET (N=10), CBT (N=10), or were waitlisted controls (Treatment As Usual, TAU) (N=10). Clinical outcomes were appetitive aggression and PTSD, which were assessed by the Appetitive Aggression Scale (AAS) and PTSD Symptom Scale-Interview (PSS-1), respectively, at baseline (prior to the intervention) and two post-intervention follow-ups (8 and 16 months). Quantification of relative telomere length was achieved by using real-time quantitative polymerase chain reaction (qPCR). The Fisher's least significant difference was applied to the analysis of data.

Results: PTSD symptom severity was significantly decreased in the FORNET group, but not in the CBT or TAU groups, while appetitive aggression remained unchanged in all groups.

Relative telomere length was not significantly altered between baseline and follow-up in the post-intervention FORNET group while relative telomere length increased between baseline and the second follow-up post-intervention in both CBT (p=0.005) and TAU (p=0.012) groups. There was no significant association between relative telomere length and appetitive aggression or PTSD symptom severity between baseline and subsequent follow-ups post-intervention.

Conclusion: There was no significant association between relative telomere length and appetitive aggression or PTSD symptom severity in the FORNET group had. However, there was an increase in relative telomere length in both CBT and TAU groups although not associated with any of the clinical outcomes. While this requires further exploration, telomere length could shed a light as a molecular response to violent threats.

3.1 INTRODUCTION

Appetitive aggression refers to the perpetration of aggressive behaviour with the purpose of achieving internal feelings of joy, arousal, and excitement (Elbert et al., 2010). It is a type of instrumental aggression, where violent behaviour is internally motivated, such as in hunting and killing, for the sake of excitement. Individuals who are constantly exposed to violence, stressful life events, and social adversity, such as in informal settlements and low-income areas in South Africa, are at high risk of developing appetitive aggression (Hinsberger et al., 2016b; Weierstall et al., 2013b). Exposure to childhood trauma, abuse, and hostile activities may result in a variety of these aggressive and cruel behaviours, which perpetuate the cycle of violence (Elbert et al., 2010; Hinsberger et al., 2016b). Furthermore, exposure to violent traumatic events and stressors triggers a number of biological as well as psychological responses (Epel et al., 2010), which may play a significant role in the stress response caused by this environment (Epel et al., 2004). These responses include alteration in telomere length, which has been found to be associated with the development of several mental disorders like posttraumatic stress disorder (PTSD) (Jergović et al., 2014; Kim et al., 2017; Ladwig et al., 2013) and behavioural problems, such as aggression (Barik et al., 2013; Mcewen, 2000; Worthman and Panter-Brick, 2008).

Telomeres are non-coding nucleoprotein structures found on chromosomal endings, and prevent the loss of crucial information during cell replication (Blackburn, 2005; de Lange et al., 2005). They protect against premature termination of cell replication by maintaining chromosomal stability, although they shorten naturally during each somatic cell division and are associated with chronological ageing (Blackburn et al., 2015b). However, when telomere length is critically shortened, it results in chromosomal and genomic instability, which then leads to the initiation of DNA damage responses that would result in premature cell death, a mechanism associated with accelerated ageing (Blackburn, 2005; de Lange et al., 2005). The synthesis of telomeres involves genes such as telomerase reverse transcriptase (*TERT*) and telomerase RNA component (*TERC*) found in the telomerase complex (de Lange et al., 2005). Together with other associated genes in the complex, *TERT* and *TERC* play a key role in the maintenance of telomere length during cell replication (Chiang et al., 2006; Mirabello et al., 2010; Pellatt et al., 2013).

There are a few studies that have investigated the role of telomere length in aggression and posttraumatic stress disorder (PTSD) (Jergović et al., 2014; Kim et al., 2017; Küffer et al., 2016; Watkins et al., 2016; Zalli et al., 2014). Researchers who have investigated telomere length in association with PTSD or aggression have not examined both clinical variables in

tandem. PTSD and aggression are multifactorial disorders that are influenced by genetic and environmental factors (Chae et al., 2014; Ficks and Waldman, 2014; Nievergelt et al., 2015; Oades et al., 2008; Shalev et al., 2013a). Environmental factors include the level of trauma experienced, chronic exposure to stressful life events and differences in socioeconomic status in populations across different settings (Beach et al., 2014; Chae et al., 2014; Epel et al., 2004; Needham et al., 2014).

Although telomere length shortens as people age, there is an inter-individual difference in the rate of change in telomere length (Chae et al., 2016; Eitan et al., 2014; Geronimus et al., 2015). Psychological distress and a number of environmental factors, including psychological interventions, can influence the rate at of telomere length shortening (Blackburn et al., 2015a; Chae et al., 2014; Epel et al., 2010; Kim et al., 2017; O'Donovan et al., 2012). Therefore, telomere length could be a potential biomarker of risk associated with psychological stress as well as a biomarker of response to psychological interventions (Boks et al., 2014; Epel et al., 2016; Hoge et al., 2013; Jacobs et al., 2011; Sindi et al., 2017). Childhood trauma has been found to be associated with accelerated telomere length shortening in adults (Küffer et al., 2016; O'Donovan et al., 2011). In addition, individuals with stressful experiences in childhood and have experienced chronic exposure to stressful life events have been found to possess shorter telomeres compared to controls (Epel et al., 2004).

Stressful life events and adverse childhood traumas can affect telomere length with studies finding an association between accelerated telomere length reduction and PTSD in individuals with a stressful childhood compared to individuals who had less stressful chiildhood (Drury et al., 2012; Humphreys et al., 2012; Jergović et al., 2014; Ladwig et al., 2013; O'Donovan et al., 2011; Tyrka et al., 2010). In a study by Küffer et al. (2016), childhood trauma was found to be a risk factor for accelerated telomere length shortening in adults with PTSD (Küffer et al., 2016). An exploratory study conducted by Kim et al. (2017) found that severe trauma and PTSD were associated with shorter telomere length in combat veterans who were exposed in the Vietnam War (Kim et al., 2017). Although accelerated shortening of telomere length has been shown to be associated with PTSD (Jergović et al., 2014; Ladwig et al., 2013), there is contradictory evidence showing that longer telomere length is associated with PTSD (Boks et al., 2014; Küffer et al., 2016). This conflicting evidence underscores the complexity of telomere length as a marker for accelerated ageing in PTSD (Boks et al., 2014).

Reduced telomere length has been found to be associated with impulsivity, hostility, internalising and extrenalising disorders (Fleckman et al., 2016; Mason et al., 2015; de Souza Costa et al., 2015; Watkins et al., 2016). A study by Brydon et al. (2012) found that shortened telomere length was associated with hostility. Hostility is a form of aggression where an individual fails to control his anger and temper (Brydon et al., 2012; Elbogen et al., 2010). Another study found that manifestation of hostility in U.S. military veterans was associated with reduced telomere length (Watkins et al., 2016). Reduction in telomere length has also been found to be associated with other diseases linked to ageing, such as cardiovascular diseases (Fitzpatrick et al., 2007; Weischer et al., 2012), inflammation, obesity (Njajou et al., 2012), diabetes, and metabolic syndrome (Révész et al., 2016; Weischer et al., 2012). Several factors such as smoking (Huzen et al., 2014) gender differences (Révész et al., 2016; Strandberg et al., 2011), and lifestyle factors (Denham et al., 2016; Østhus et al., 2012) can also contribute to telomere length alterations.

Although telomere length has been investigated in stress-related diseases, there is still insufficient knowledge about its association with PTSD and certain types of aggression like appetitive aggression. Therefore, further investigations are needed in different settings to broadly examine the potential of telomere length as a predictor of environmental stress. Investigating telomere length in South African men who are regularly exposed to threats of violence could provide insights into the association of telomere length and violent traumatic related behaviours and PTSD. Understanding telomere length and their changes could inform intervention strategies and the development of tailored preventative measures to alleviate the risk of developing PTSD. Psychotherapeutic interventions such as mindfulness and supportive-expressive therapy have been found to be associated with stable telomere length over time (Alda et al., 2016; Carlson et al., 2015; Conklin et al., 2018; Hoge et al., 2013; Jacobs et al., 2011). Hoge et al. (2013) found that women who had been meditating for at least four years had longer telomere length compared to controls, and had lower stress levels. Furthermore, Alda et al. (2016) found that meditators with at least ten years of experience had longer telomeres and lower psychological negative emotions and thoughts compared to matched controls. Carlson et al. (2015) found that reduced stress was associated with stable telomere length in cancer survivors after receiving a mindfulness intervention. Taking into consideration these aforementioned studies, telomere length could be an essential biomarker for response to psychological interventions and exposure to stressful events.

The present study is part of a clinical trial of Narrative Exposure Therapy for Forensic Offender Rehabilitation (FORNET) and Cognitive Behavioural Therapy (CBT) in individuals

with appetitive aggression (Hinsberger et al., 2016a). We used the same sample as Hinsberger et al. (2016), who found that FORNET did not reduce symptoms of appetitive aggression, but reduced PTSD symptom severity. Although Hinsberger et al. (2016) did not find reduced appetitive aggression after FORNET, previous studies had found that FORNET contributed to the reduction of attraction to violence and appetitive aggression symptoms in youth samples of Burundians and former Congolese combatants, respectively (Crombach and Elbert, 2015; Hermenau et al., 2013b). Therefore, the aim of this study was to investigate change in telomere length as a treatment outcome or a predictor of clinical symptom changes in association with appetitive aggression or PTSD symptom severity in Xhosa Black South African men in a randomised control trial of FORNET compared to CBT and waitlisted controls (TAU).

3.2 MATERIALS AND METHODS

3.2.1 Sociodemographic and clinical data

Xhosa Black South African men (N=405) were initially recruited via a local reintegration centre for offenders and young people at risk of gang violence and substance abuse related activities in Khayelitsha and Gugulethu townships of Cape Town (Hinsberger et al., 2016a; Sommer et al., 2017). For the FORNET intervention trial, firstly, 89 participants who met inclusion criteria for appetitive aggression (≥ 9 points on the Appetitive Aggression Scale, AAS) and PTSD (≥ 8 points on the PTSD Symptom Scale-Interview, PSS-I) were selected (Hinsberger et al., 2016a; Sommer et al., 2017). To determine a priori sample size estimations, g-power was performed (Faul et al., 2009) for the parent study. Participants (N=54) who attended at least one of the two post-intervention examinations (8 and 16 months on average) were included in the study (Hinsberger et al., 2016a; Sommer et al., 2017). The present study only investigated those participants who were present for all assessments [baseline (0 months, prior intervention), first (8 months on average, post-intervention) and second (16 months on average, post-intervention) follow-ups]. The study received ethical clearance from the ethics review boards of Stellenbosch University (Ethics Reference number: N13/01/006), the University of Konstanz, and the University of Cape Town.

3.2.2 Quantitative polymerase chain reaction (qPCR) for telomere length

DNA samples were isolated as described in Section 2.2.2 (Chapter 2). DNA was diluted to 20 ng/µl and amplified using Sybr-Fast Mastermix (2X) ABI PRISM PCR kit (KAPA Biosystems, Massachusetts, USA), using a protocol developed by Cawthon (2002) (Cawthon, 2002).

Telomeric repeats were amplified using primers adopted from this previous investigator (Cawthon, 2002). A single copy reference gene (S), human beta globin, with stable gene expression, was correspondingly quantified during the telomeric repeats experiment, and the sequence of primers was adapted from Epel et al. (2004) (Epel et al., 2004). This quantification of telomeric repeats and the human beta-globin gene was performed to provide a relative measurement of telomere length in each genomic DNA sample; thus, relative telomere length was represented as a ratio of telomere copy number to single copy reference gene (T/S). Each qPCR reaction for relative telomere length determination consisted of SYBR FAST MASTERMIX (2X) ABI PRISM (5 µL), forward primer (0.27 µM), reverse primer (0.9 µM), genomic DNA (20 ng), and nuclease-free water to make for a final volume of 10 µL.

The qPCR reactions for the human beta-globin gene were composed of the same reagents and quantities as for the telomere experiments, except that both forward and reverse primers were included at the same concentration (0.4 µM). The amplification of both qPCR reactions of the telomeric repeats and the human beta-globin gene was performed on the same 384-well plates but in different wells. The qPCR amplification was conducted on the ABI 7900HT platform (Applied Biosystems, Foster City, CA). For both telomeric repeats and human beta globin, thermal cycling was as follows: 95ºC for initiation (5 min); followed by 40 amplification cycles of 95ºC for 3 sec and 65ºC for 30 sec to allow primer annealing (30 sec); and finally the melting cycling of 95ºC (15 sec), 64ºC (15 sec), and 95ºC (15 sec). The amplification of each DNA sample was performed in triplicate for technical replicates. The mean values for each triplicate reaction were calculated.

Equal amounts of DNA samples from each participant were pooled to prepare a standard sample in order to generate a standard curve. Serial dilutions of 1.68 fold were performed to yield calibration standard DNA amounts ranging from 50 ng/µl to 0.79 ng/µl (Cawthon, 2002) These calibration standards were separately amplified using telomere repeat and human beta-globin gene primers, yielding nine points with which to construct separate standard curves for both telomeric repeats and human beta globin. A standard linear curve was constructed using the threshold cycle (Ct) values versus the log value of the input amount of serially diluted DNA. Subsequently, the efficiency of each qPCR reaction was determined using the standard curve for that specific reaction.

All Ct values for triplicates were corrected, and the difference was restricted to 0.5 Ct value. Interpolate calibrations were performed. The calculated efficiency values were subsequently used to correct for interplate variation using GenEx software (http://www.gene-quantification.de/datan.html). The mean relative telomere repeat copy number (T) was

normalised to a single copy reference gene (S) to control for different input amounts of DNA and to provide a measure of relative telomere length. A T/S ratio of greater than one (T/S >1) indicates that the telomere length is greater than the reference sample, while a T/S ratio of less than one (T/S <1) shows that the average telomere length is less than that of the reference sample.

3.2.3 Statistical data analysis

All participants were of the same ethnicity (African black) and were of a similar age with no significant difference in age across the groups (Table 2.1). None of the participants was currently on drugs and none had a diagnosis of depression. Clinical improvement was defined as a significant reduction in aggressive behaviour and PTSD symptom severity utilising measures of the AAS (statements) and PSS-I, respectively. A probability plot verified the normality of data. Transformation of data through winsorization reduced the effect of extreme outlier values. In order to test if relative telomere length was associated with treatment outcomes, we performed a one-way analysis of variance (ANOVA) and an analysis of covariates (ANCOVA) over three-time points. We performed mixed model analyses using treatment group and time as fixed effects, and nesting participants within a treatment group as the random effect aided to account for repeated measurements over time. Post-hoc analyses by Fisher's least significant difference (LSD) determined significance and corrected for multiple pairwise comparisons. Childhood trauma, witnessed trauma, self-experienced trauma were treated as fixed variables during analysis, while we did not adjust for ethnicity, age, or the environment as covariates.

Statistical significance of variances was set at a 95% confidence interval with an alpha (α) of less than 0.05 ($p< 0.05$). A trend towards a statistical significance was set at less than 0.1 ($p< 0.1$). Correlation analyses between relative telomere length and appetitive aggression and between telomere length and PTSD symptom severity were conducted in mean values of subtracted mean values from time points 2 and 1, 3 and 1, and 3 and 2 of relative telomere length, appetitive aggression and PTSD symptom severity.

3.3 RESULTS

3.3.1 Demographic, clinical, and descriptive measures

No significant reduction in appetitive aggression was observed in the FORNET, CBT and TAU groups between time points 1 (baseline, 0 months, prior intervention) and 2 (8 months

on average, post-intervention), or between time points 1 and 3 (16 months on average), and between time points 2 and 3. Clinical data was analysed as described in Section 2.3.1 (Chapter 2). A statistically significant reduction in PTSD symptom severity was found between time points 1 and 2 for the FORNET group (p= 0.048) (Table 2.6, Section 2.3.1, Chapter 2). Loss to follow-up of some participants (between time points 1 and 2 and between time points 2 and 3) (Table 2.2 and 2.3, Section 2.2.1, Chapter 2) resulted in a smaller number of participants for the analysis. This mirrored the parent study by Hinsberger et al. (2016), where a subset of participants was drawn from for the analysis and biological investigations.

3.3.2 Relative telomere length changes in comparison to appetitive aggression and PTSD symptom severity after intervention

Telomere length was quantified at baseline for participants in each treatment group: FORNET (N=10), CBT (N=10), and TAU (N=10). The correlation between relative telomere length and (i) appetitive aggression (as measured using AAS) (Weierstall and Elbert, 2011) and (ii) PTSD symptom severity (as measured by PSS-I) (Foa and Tolin, 2000) was assessed for the three groups across time. Relative telomere length was investigated at three time points in each treatment group. Relative telomere length changes are described in Table 3.1. Changes in appetitive aggression, PTSD symptom severity and relative telomere length were also determined across the time points.

Scores of appetitive aggression and PTSD symptom severity and values of relative telomere length obtained at first follow-up (8 months on average at post-intervention) were subtracted from baseline (0 months, prior to the intervention), those at second follow-up (16 months on average at post-intervention) were subtracted from those in first follow-up, and likewise for time points 3 and 1. Change scores in appetitive aggression and PTSD symptom severity were used to determine the correlation between these clinical variables and relative telomere length between the time points. There was no statistically significant correlation found between relative telomere length and either appetitive aggression or PTSD symptom severity across all groups (Table 3.2). However, there was a trend towards a positive correlation between relative telomere length and appetitive aggression in the control group (TAU) between time points 1 and 2 (r=0.31, p=0.09) (Table 3.2). A comparison of relative telomere length change between time points for the FORNET, CBT and TAU groups is shown in Table 3.3. These changes in relative telomere length were then compared to changes observed in appetitive aggression or PTSD symptom severity between time points across all treatment and control groups.

Table 3.1: Descriptive statistics for relative telomere length changes investigated before and after psychotherapeutic interventions within treatment groups at the three time points

Treatment Group	N	Relative telomere length mean	SD	SE	95% Confidence Interval for Mean	
					Lower	Upper
Baseline						
FORNET	10	0.424093	0.446656	0.141245	0.1046	0.74361
CBT	10	0.216475	0.169914	0.060074	0.0744	0.35853
TAU	10	0.251501	0.101342	0.032047	0.1790	0.32400
First follow-up						
FORNET	9*	0.436578	0.546854	0.172930	0.0454	0.82777
CBT	10	1.509504	2.576183	0.814660	-0.3334	3.35239
TAU	6*	3.826949	5.310363	2.655182	-4.6230	12.27692
Second follow-up						
FORNET	10	1.517835	1.498660	0.473918	0.4458	2.58991
CBT	3*	3.405615	2.564659	1.047018	0.7142	6.09706
TAU	10	3.100636	2.254781	0.713024	1.4877	4.71361

FORNET— Narrative Exposure Therapy for Forensic Offender Rehabilitation
CBT— Cognitive Behavioural Therapy
TAU— Treatment As Usual
N— Number of participants
SD— Standard deviation
SE— Standard error
* Participants indicated with an asterisk that were groups of less than 10 due to dropouts
Baseline— time point 1, 0 months prior the intervention
First follow-up— represents time point 2, eight months on average post-intervention
Second follow-up— represents time point 3, 16 months on average post-intervention

Table 3.2: Longitudinal correlations analysis in relative telomere length changes investigated before and after psychotherapeutic interventions within each treatment group and at the three time points.

Average relative telomere length (T/S)	Clinical outcomes	Spearman's correlation (r)	P= value
F2-F1	AAS (F2-F1)	-0.28	0.18
F2-F1	PSS-I (F2-F1)	-0.05	0.81
F3-F1	AAS (F3-F1)	-0.06	0.77
F3-F1	PSS-I (F3-F1)	0.08	0.70
F3-F2	AAS (F3-F2)	0.08	0.75
F3-F2	PSS-I (F3-F2)	-0.09	0.75
C2-C1	AAS (C2-C1)	0.08	0.67
C2-C1	PSS-I (C2-C1)	-0.15	0.48
C3-C1	AAS (C3-C1)	0.18	0.38
C3-C1	PSS-I (C3-C1)	0.07	0.74
C3-C2	AAS (C3-C2)	0.01	0.97
C3-C2	PSS-I (C3-C2)	-0.17	0.52
T2-T1	AAS (T2-T1)	0.31	0.09
T2-T1	PSS-I (T2-T1)	-0.14	0.48
T3-T1	AAS (T3-T1)	0.06	0.75
T3-T1	PSS-I (T3-T1)	-0.28	0.12
T3-T2	AAS (T3-T2)	-0.10	0.61
T3-T2	PSS-I (T3-T2)	-0.22	0.29

AAS— Appetitive Aggression Scale
PSS-I— Posttraumatic stress disorder (PTSD) Symptom Scale-Interview
F1, F2, F3—Narrative Exposure Therapy for Forensic Offender Rehabilitation (FORNET) group at baseline, first follow-up and second follow-up respectively
C1, C2, C3— Cognitive Behavioural Therapy (CBT) group at baseline, first follow-up, and second follow-up, respectively
T1, T2, T3— Treatment As Usual (TAU) group at baseline, first follow-up, and second follow-up post-intervention, respectively

3.3.2.1 FORNET

No statistically significant change in relative telomere length for the FORNET group was observed between time points 1 (baseline, 0 months) and 2 (first follow-up, 8 months on average post-intervention) (p=0.750) (Table 3.3). This group had no statistically significant change in relative telomere length between time points 1 and 3 (second follow-up, 16 months on average post-intervention) (p=0.777) (Table 3.3), or between time points 2 and 3 (p=0.963) (Figures 3.1 and 3.2).

Table 3.3: Fisher's least significant differences multiple comparisons for changes in relative telomere length on different treatment groups that had received psychotherapy, assessed between three-time points

Within groups	
Treatment groups	P-value
F1 vs F2	0.750
F1 vs F3	0.777
F2 vs F3	0.963
C1 vs C2	0.084
C1 vs C3	**0.005****
C2 vs C3	0.121
T1 vs T2	0.057
T1 vs T3	**0.025***
T2 vs T3	0.832

F1, F2, F3: Narrative Exposure Therapy for Forensic Offender Rehabilitation (FORNET) group at baseline, first follow-up and second follow-up, respectively
C1, C2, C3: Cognitive Behavioural Therapy (CBT) group at baseline, first follow-up and second follow-up, respectively
T1, T2, T3: Treatment As Usual (TAU) group at baseline, first follow-up and second follow-up, respectively
*p <0.05 and *p <0.01 indicate statistical significance
Time points 1, 2, 3-represent baseline (prior the intervention), first follow-up (eight months on average post-intervention) and second follow-up (16 months on average post-intervention)

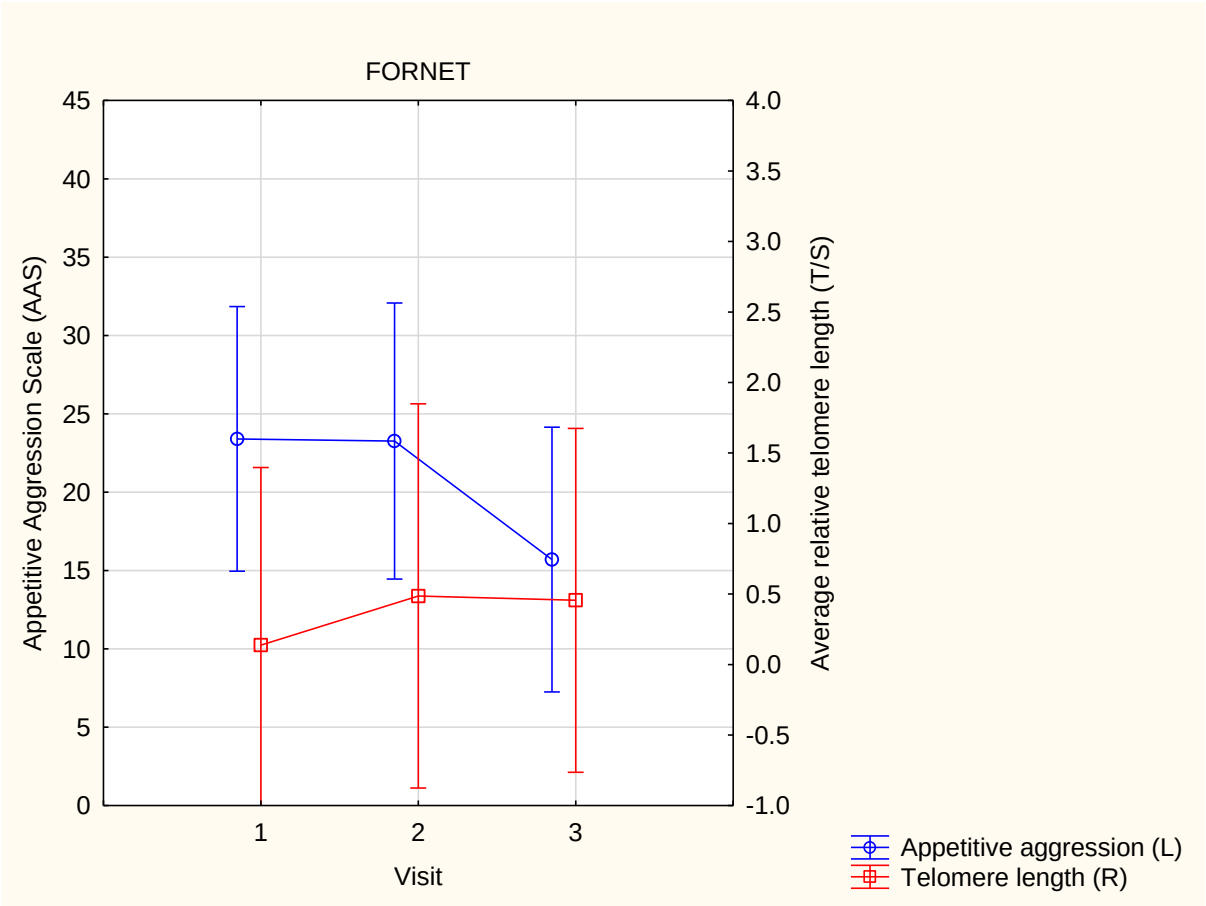

Figure 3.1: Longitudinal analysis of changes in appetitive aggression, as measured by the Appetitive Aggression Scale (AAS), and change in relative telomere length in Xhosa Black South African men who received narrative exposure therapy for forensic offender rehabilitation (FORNET). “L” and “R” represent left and right Y-axis on the graph, respectively. X-axis represents visits 1, 2 and 3, which indicate baseline (0 months, prior to the intervention), first follow-up (8 months on average post-intervention), and second follow-up (16 months on average post-intervention), respectively. No statistically significant changes in appetitive aggression (L) or relative telomere length (R) were noticed between any of the time points.

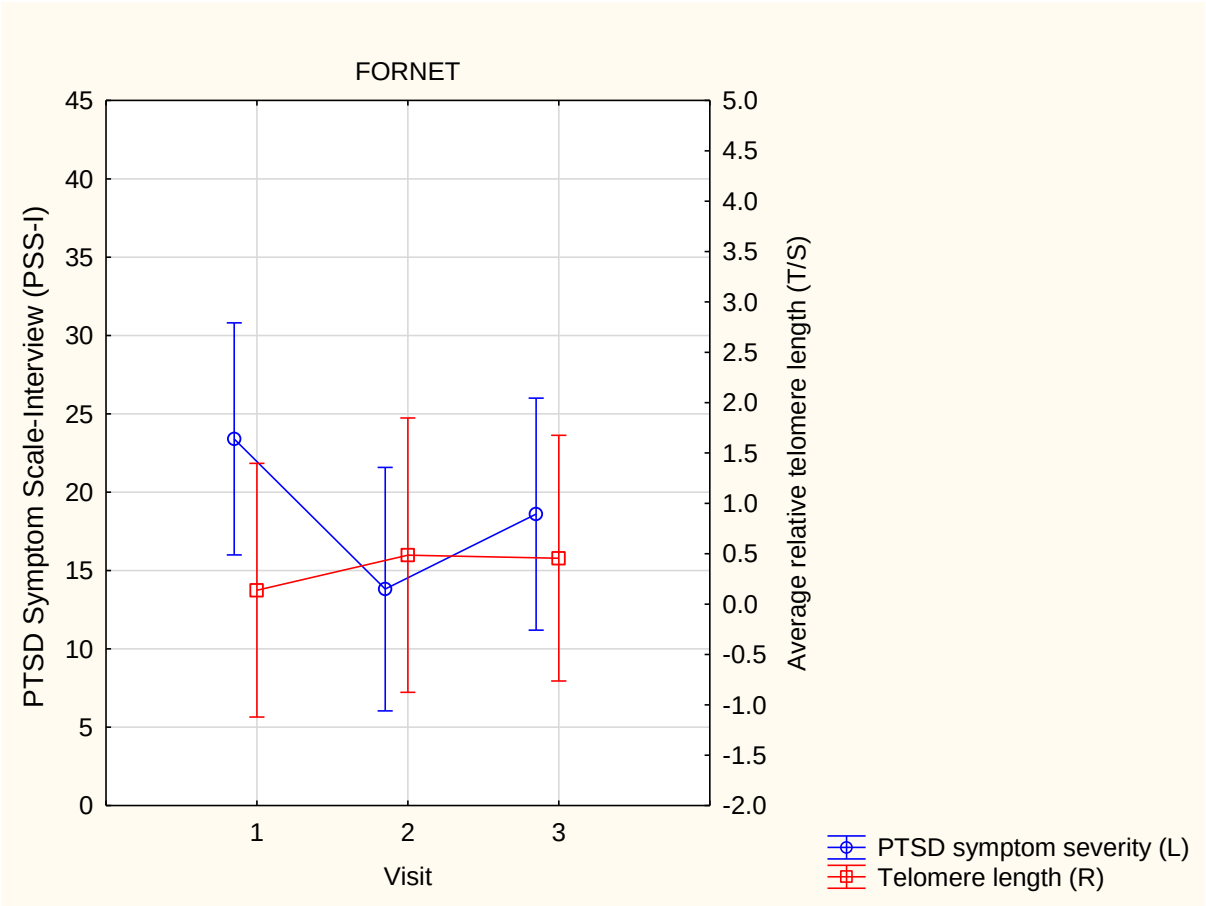

Figure 3.2: Longitudinal analysis of posttraumatic stress disorder (PTSD), as measured by the PTSD Symptom Scale-Interview (PSS-I), and relative telomere length in Xhosa Black South African men who had received narrative exposure therapy for forensic offender rehabilitation (FORNET). "L" and "R" represent left and right Y-axis on the graph, respectively. X-axis represents visit 1, 2 and 3, which indicate baseline (0 months, prior to the intervention), first follow-up (8 months on average post-intervention), and second follow-up (16 months on average post-intervention), respectively. No statistically significant changes in appetitive aggression (L) or relative telomere length (R) were noticed between any of the time points. PTSD symptom severity was statistically significantly reduced between time points 1 and 2 (p=0.048). No statistically significant changes in relative telomere length were observed between time points 1 and 2, between time points 1 and 3, or between time points 2 and 3.

3.3.2.2 CBT

In the CBT group, relative telomere length increased significantly between time points 1 (baseline, 0 months) and 3 (second follow-up, 16 months on average) (p=0.005) (Table 3.3)

(Figure 3.3 and 3.4). However, between time points 1 and 2 (first follow-up, 8 months on average), only a trend towards significance was observed (p=0.084) in this group.

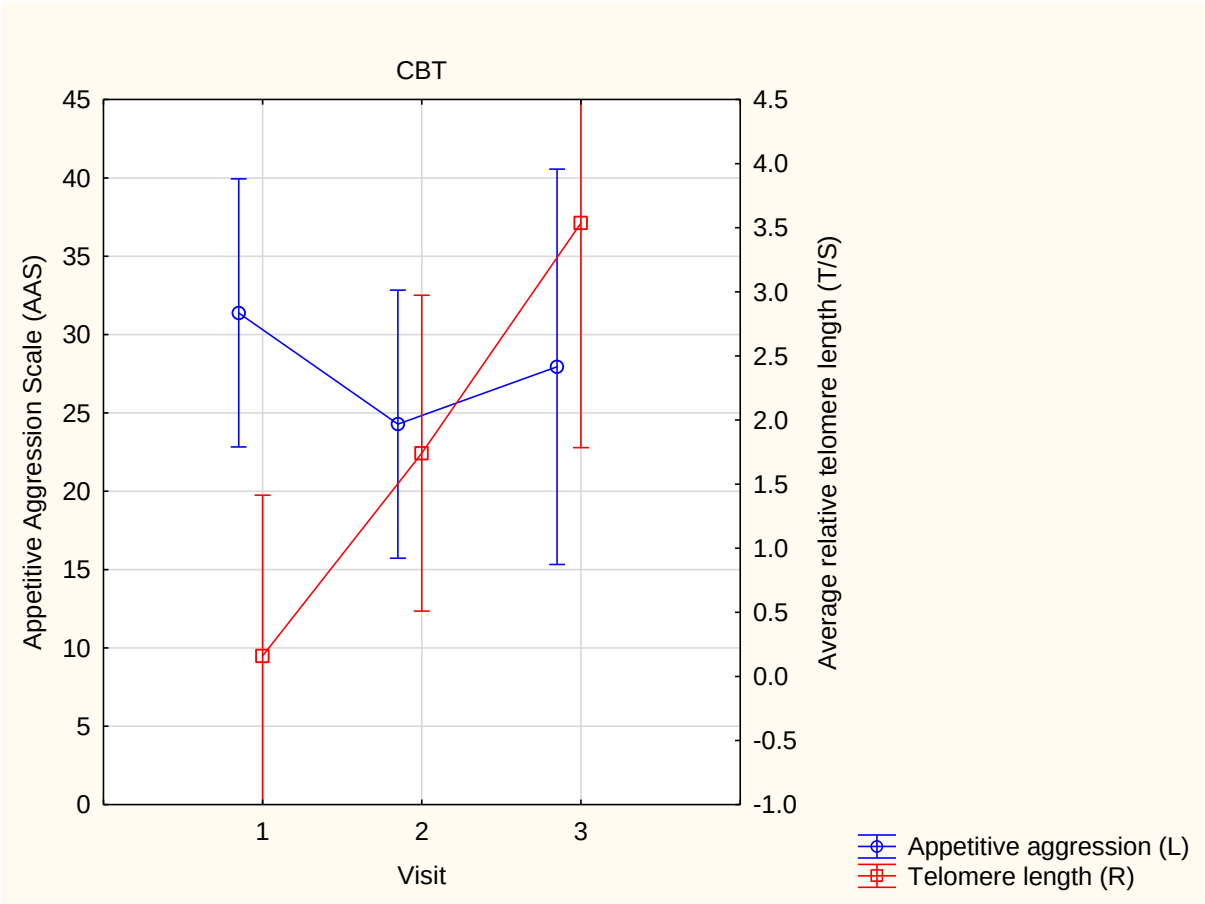

Figure 3.3: Longitudinal analysis of appetitive aggression measured, as measured by the Appetitive Aggression Scale (AAS), and relative telomere length changes in young Xhosa Black South African men who had received Cognitive Behavioural Therapy (CBT). “L” and “R” represent left and right Y-axis on the graph, respectively. X-axis represents visit 1, 2 and 3, which indicate baseline (0 months, prior to the intervention), first follow-up (8 months on average post-intervention), and second follow-up (16 months on average post-intervention), respectively. No statistically significant changes in appetitive aggression were observed between time points 1 and 2, between time points 1 and 3, and between time points 2 and 3. Relative telomere length was statistically significantly increased between time points 2 and 3 (p=0.005).

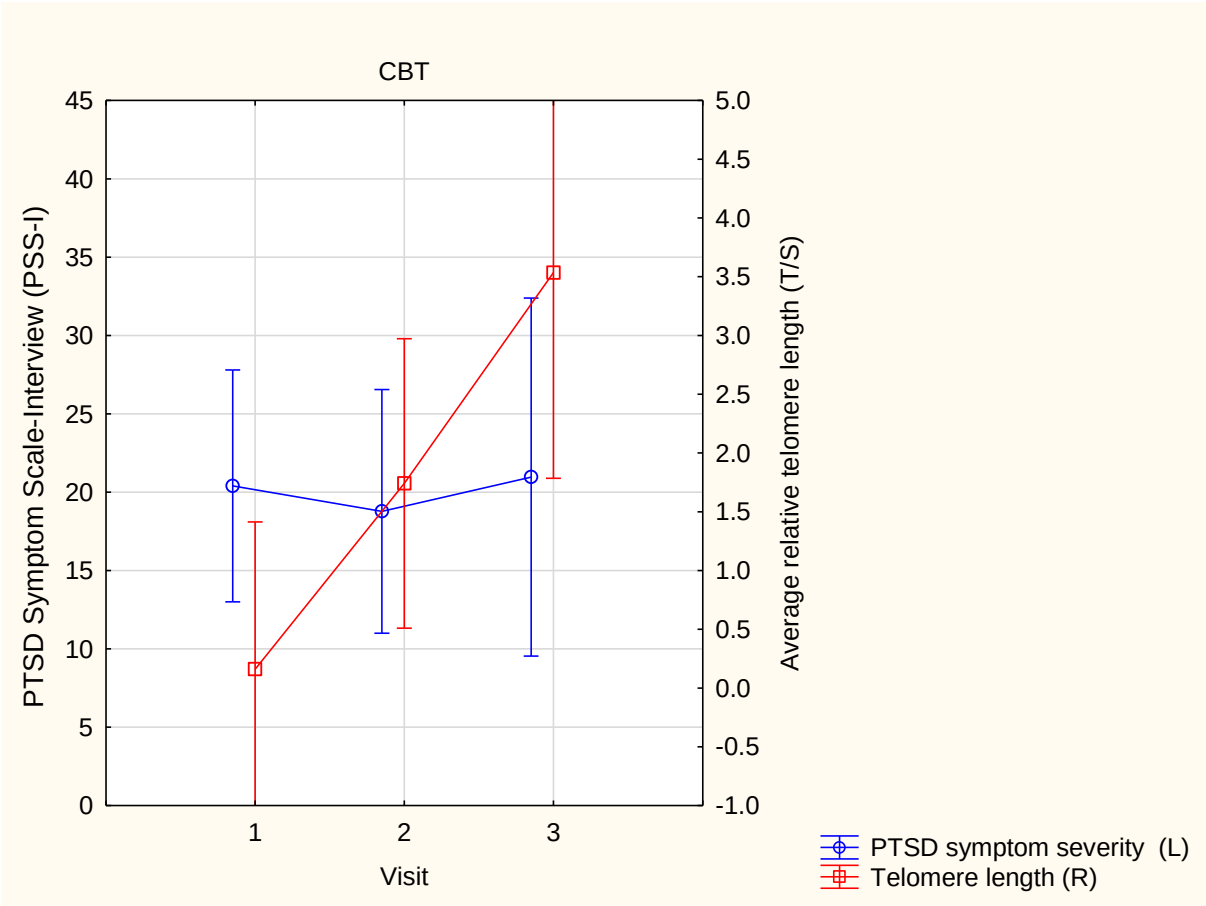

Figure 3.4: Longitudinal analysis of posttraumatic stress disorder (PTSD), as measured by the PTSD Symptom Scale-Interview (PSS-I), in comparison with relative telomere length in young Xhosa Black South African men who had received cognitive behavioural therapy (CBT). "L" and "R" represent left and right Y-axis on the graph, respectively. X-axis represents visit 1, 2 and 3, which indicate baseline (0 months, prior to the intervention), first follow-up (8 months on average post-intervention), and second follow-up (16 months on average post-intervention), respectively. No statistically significant change in PTSD symptom severity was observed between the three time points (L). Relative telomere length increased significantly between time points 2 and 3 (p=0.005).

3.3.2.3 TAU

Relative telomere length increased significantly between time points 1 (baseline, 0 months) and 3 (16 months on average post-intervention) in the TAU control group (p=0.012) (Table 3.3) (Figures 3.5 and 3.6). However, there was a trend towards statistical significance that was observed between time points 1 and 2 (8 months on average post-intervention) (p=0.057) (Table 3.3) in this group with no statistically significant differences between time points 2 and 3 (p=0.832).

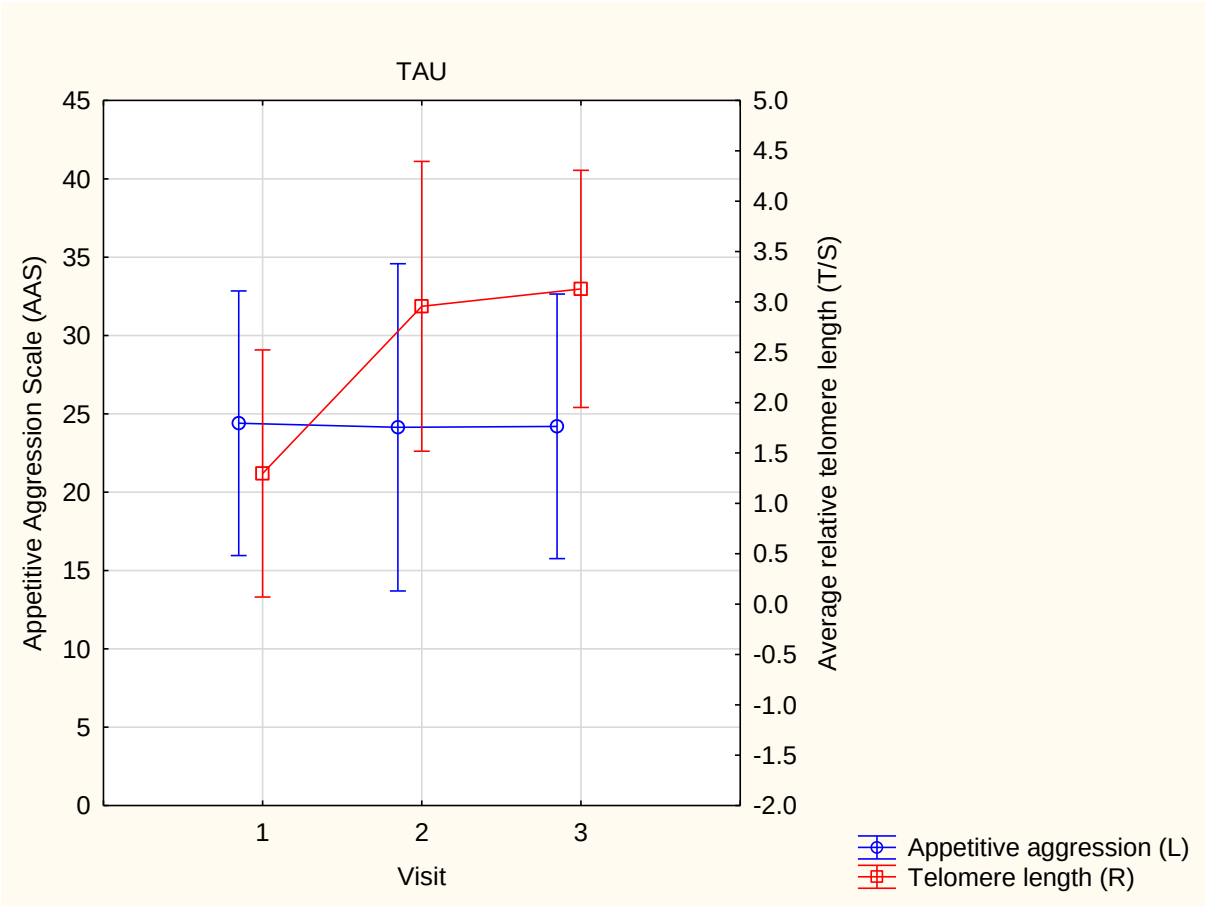

Figure 3.5: Longitudinal analysis of appetitive aggression, as measured by the Appetitive Aggression Scale (AAS), and telomere length changes in young Xhosa Black South African men who had received treatment as usual (TAU). “L” and “R” represent left and right Y-axis on the graph, respectively. X-axis represents visit 1, 2 and 3, which indicate baseline (0 months, prior the intervention), first follow-up (8 months on average), and second follow-up (16 months on average), respectively. Appetitive aggression did not change significantly across the three-time points. Relative telomere length increased significantly between time points 1 and 3 (p=0.025), while between time points 1 and 2 and 2 and 3, there was no statistically significant change (R).

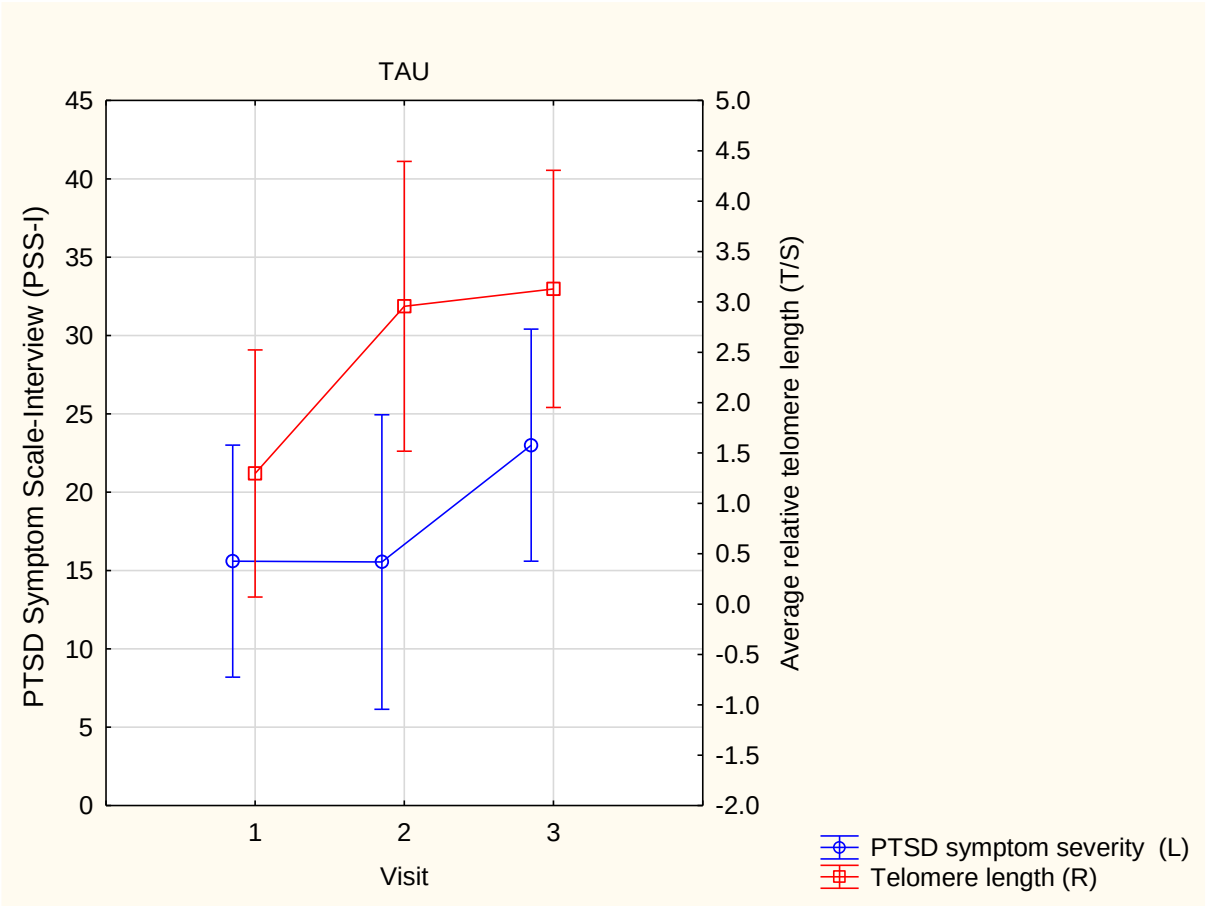

Figure 3.6: Longitudinal analysis of posttraumatic stress disorder (PTSD), as measured by the PTSD Symptom Scale-Interview (PSS-I) and telomere length in young Xhosa Black South African men who had received treatment as usual (TAU). “L” and “R” represent left and right Y-axis on the graph, respectively. X-axis represents visit 1, 2 and 3, which indicate baseline (0 months, prior the intervention), first follow-up (8 months on average), and second follow-up (16 months on average), respectively. PTSD symptom severity did not change significantly between the three-time points (L). Relative telomere length increased significantly between time points 1 and 3 ($p=0.025$), while between time points 1 and 2 and 2 and 3 there was no statistically significant change (R).

3.4 DISCUSSION

The present study investigated relative telomere length changes in response to FORNET, in the context of a clinical intervention trial conducted in young Xhosa South African men with high appetitive aggression (as measured by the AAS) (Weierstall and Elbert, 2011) and high PTSD symptom severity (as measured by the PSS-I) (Foa and Tolin, 2000). Participants had been ongoing exposed to violence in their communities (Hinsberger et al., 2016a; Sommer et al., 2017). Previous studies have shown that exposure to traumatic stress such as childhood

adversity and continuous exposure to violence can affect telomere length (Humphreys et al., 2016; Küffer et al., 2016; Shalev et al., 2014; Verhoeven et al., 2015). Psychotherapeutic interventions have also been found to affect telomere length, particularly to stabilise and reduce accelerate reduced telomere length (Carlson et al., 2013; Jacobs et al., 2011; Tolahunase et al., 2017). Accelerated reduction of telomere length is associated with premature ageing, which could lead to an early onset of diseases such as cardiac disease and diabetes (Blackburn et al., 2015b; De Meyer et al., 2018, 2011).

This study had two treatment groups, FORNET and CBT as well as a control group, TAU. Participants were assessed for clinical outcomes (appetitive aggression and PTSD symptom severity) and relative telomere length as a biological variable at baseline prior to the administration of an intervention, and at two follow-up visits post-intervention (eight and sixteen months on average). Relative telomere length was found to increase significantly across the three time points in the CBT ($p=0.005$) and TAU groups ($p=0.025$). However, this change in telomere length in the CBT or TAU groups was not associated with either appetitive aggression or PTSD symptom severity over the time points. Although the FORNET group demonstrated a significant decrease in PTSD symptom severity between baseline and first follow-up ($p=0.048$), no significant change was found in relative telomere length over the time points. Moreover, psychotherapeutic interventions may play a role in biological responses, which could be essential to understanding their effect and role in appetitive aggression and PTSD symptom severity.

Previous investigations have found that psychological interventions such as mindfulness, meditation and yoga can affect relative telomere length over a couple of months post-intervention (Carlson et al., 2015; Daubenmier et al., 2012; Hoge et al., 2013; Jacobs et al., 2011). Hoge et al. (2013) found that Loving-Kindness Meditation (LKM), a practice derived from the Buddhist practices, which focuses on unselfish kindness and warmth towards other people, was associated with longer relative telomere length in participants compared to controls who did not practice LKM. Furthermore, longitudinal studies have found an upregulation of telomerase activity in participants who received some form of mindfulness meditation retreat and yoga (Daubenmier et al., 2012; Jacobs et al., 2011; Lavretsky et al., 2013). Jacobs et al. (2011) studied the effect of intensive daily meditation over a period of three months and found that individuals who were meditating had increased telomerase activity compared to controls. Furthermore, Lavretsky et al. (2013) investigated the effect of yoga and meditation longitudinally and found that individuals who were meditating had an elevated telomerase activity compared to controls. Telomerase activity is an important indicator of telomere length maintenance as this enzyme is responsible for the synthesis of

telomeres (Blackburn, 2005; Smogorzewska and de Lange, 2004). Thus, it stands for reason that psychological interventions may trigger molecular mechanisms that could increase telomerase activity and in turn result in increased synthesis of telomeres, and/or stabilised telomere length.

We found no significant association between reduced PTSD symptom severity and relative telomere length in the FORNET group, suggesting that telomeres may have been unaffected after FORNET. The FORNET intervention may have affected mechanisms mediating the stress response, which could have affected traumatic stress levels resulting in reduced PTSD symptom severity. Reduction in PTSD symptom severity and unchanged relative telomere length may be indicative of a stable stress molecular response system that prevents accelerated relative telomere length reduction (even though appetitive aggression remained high in these participants). The living conditions of participants may have played a role in masking the beneficial effects of FORNET since we know that appetitive aggression can be adaptive and act as a coping mechanism against trauma (Weierstall et al., 2013b). It can also be maladaptive, leading individuals to perpetrate a cycle of violence, which in turn results in re-exposure to trauma (Shields et al., 2008; Weierstall et al., 2013b).

The stress response system, such as the hypothalamic pituitary adrenal axis (HPA axis), involves cortisol which is often abnormal in both PTSD (Basu et al., 2013; Gill et al., 2008; Yehuda et al., 2005) and aggression (van Bokhoven et al., 2005; Hennig et al., 2005; Montoya et al., 2012). Reduced cortisol levels have been associated with lower activated telomerase, an enzyme responsible for the synthesis of telomeres (Choi et al., 2008). An *in-vitro* study by Choi et al. (2008) treated human T lymphocytes with cortisol, measured the activity of telomerase in both CD4 and CD8 T lymphocytes and found that cortisol levels were associated with increased expression of *TERT*, a gene encoding telomerase catalytic components that is responsible for telomere maintenance (Blackburn, 2005; Denham et al., 2016). Therefore, findings of statistically significant reduction in PTSD symptom severity with no change in relative telomere length observed after FORNET may suggest that one of these mechanisms was at play, which may have buffered a change in relative telomere length through preventing accelerated shortening. This finding may suggest that immunological responses may have been altered during FORNET intervention (Lin et al., 2012). It has been found that constant exposure to stressful life events triggers mechanisms such as inflammation, an immunological response, which in turn could affect telomere length (Bellavance and Rivest, 2014; S. Chen et al., 2014; Choi et al., 2008; Lin et al., 2012). Chronic exposure to stressful life and experiences of constant trauma may result in a

dysregulated stress response, which may also affect immune pathways (Bellavance and Rivest, 2014).

The CBT intervention did not sufficiently affect PTSD symptom severity or appetitive aggression although it significantly increased relative telomere length over time. It is unclear how CBT and supportive counselling (TAU) may have played a role in triggering an increase in telomere length. Telomere biology is complex and involves several mechanisms (Beery et al., 2012; Choi et al., 2008); thus, alternative pathways may have been involved in CBT and supportive counselling (TAU). Our findings are not consistent with the findings of other studies (Drury et al., 2012; Epel et al., 2004; Küffer et al., 2016) and may indicate an alternative pathway involved in the stress response system (Beery et al., 2012). Previous studies have found an association between increased telomere length and PTSD symptom severity as well as exposure to ongoing stressful life events (Boks et al., 2014; Küffer et al., 2016). In a cohort of Dutch soldiers, Boks et al. (2014) found that relative telomere length increase was associated with PTSD symptom severity at post-deployment in Afghanistan (Boks et al., 2014). This underlies the complexity of understanding telomere length in disorders like PTSD and behavioural problems such as appetitive aggression.

The challenge in understanding the underlying molecular mechanisms in telomere length biology in response to stressful and traumatic life events in the onset of PTSD and aggressive behaviour may be compounded by comorbidity with other disorders such as heart failure and other mental health disorders (Ahmadi et al., 2011; Fudim et al., 2018; Vaccarino et al., 2013). Previous reports have shown that reduced telomere length is associated with age-related disorders such as cardiovascular diseases and coronary artery diseases (van der Harst et al., 2007; Kark et al., 2013; Mainous et al., 2010; De Meyer et al., 2011). Since multiple disorders can alter telomere length, understanding their contribution to the onset and maintenance of PTSD and aggression requires further invetsigation (Kang et al., 2017a; Kim et al., 2017; Watkins et al., 2016). However, there is consensus in the literature that of reduced telomere length is a risk factor for several psychiatric disorders such as major depressive disorder (MDD) (Gotlib et al., 2014; Hartmann et al., 2010) and PTSD (Jergović et al., 2014; Kim et al., 2017; Küffer et al., 2016; Ladwig et al., 2013; O'Donovan et al., 2011). Furthermore, other studies have shown that reduced telomere length is associated with aggressive behaviours (Kang et al., 2017a; Watkins et al., 2016; Zalli et al., 2014).
In most South African townships, like in many low-income settings in the world, violence persists and affects the fabric of communities. Understanding the effects of stressful life events at a molecular level and the impact of interventions in responding to these situations is essential in designing community-tailored psychotherapeutic interventions, where

molecular changes in relative telomere length, may be a biomarker with utility in predicting clinical outcomes. Such interventions are highly crucial for communities with constant exposure to threats, such as in low-income South African townships. The findings of stable, unchanged telomere length in the FORNET group adds to the existing literature that psychological interventions can play a role in the maintenance of telomere length (Carlson et al., 2015; Epel et al., 2009; Jacobs et al., 2011; Schutte and Malouff, 2014). Therefore, further investigations are required in a larger sample size where additional robust analyses can be applied.

3.4.1 Limitations

One of the limitations of this study was that it was carried out in participants of a specific gender (males), an exclusive ethnic group (Xhosa Black South Africans), and in a particular community setting (township). Therefore, the specificity of this population limits generalisation to the broader population comprised of different ethnic groups and genders. In addition, the high rate of dropout observed during follow-up visits reduced the number of participants in groups, and affected statistical power. Therefore, further studies are required to replicate these findings in a broader and more diverse population to overcome these limitations. Future studies would need to be sufficiently powered for statistical analysis so that even small differences can be detected with stringent statistical methods. In addition to these limitations, the present study did not have data on diet, lifestyle and normal daily physical activities of participants. Relative telomere length is sensitive to the environment and a variety of stressful life events. These environmental stressors, lifestyle activities and nutritional status could activate several different pathways ranging from neurobiological to immunological mechanisms that could affect telomere length (Chae et al., 2016; Diez Roux et al., 2009; Fairlie et al., 2016; García-Calzón et al., 2014; De Meyer et al., 2018; Needham et al., 2014). Lastly, it would have been preferable to include the medical history of participants and their medication treatment in the analysis as these factors may affect relative telomere length.

3.4.2 Conclusion

In this study, we found no statistically significant change in relative telomere length and appetitive aggression over time in participants who had received FORNET, though PTSD symptom severity was significantly reduced using Fisher's LSD. However, relative telomere length was significantly increased in those who had received CBT and a non-clinically focused supportive TAU counselling over the three-time points (0, 8 and 16 months on

average). Telomere length could be reflecting mechanisms responding to stress. Stable telomere length over time after the intervention, while clinical outcomes changes such as reduced PTSD symptom could be indicative that the intervention has worked to slow or moderate pathways that would affect telomere length when responding to the stress. Overall, relative telomere length is an essential environmentally sensitive biological marker that may assist us to understand effects of psychotherapeutic interventions and the development of mental disorders after exposure to traumatic stress.

CHAPTER 4

INVESTIGATING RELATIVE TELOMERE LENGTH IN YOUNG XHOSA BLACK SOUTH AFRICAN MEN WITH APPETITIVE AGGRESSION

ABSTRACT

Background: Appetitive aggression is a type of goal-driven instrumental aggression that is perpetrated with the intention of experiencing joy by committing violent behaviour. Childhood abuse and victimisation have been found to be associated with a variety of mental health and behavioural problems. Chronic exposure to several violent traumatic stresses due to the ongoing community and gang-related violence, as well as family disruptions and interpersonal violence, can result in alterations in telomere length. Telomeres are essential, non-coding ribonucleoproteins that cap chromosomal ends to prevent loss of critical functional DNA sequences during cellular replication, and studies suggest that accelerated shortened telomere length is correlated with cumulative exposure to violence and stress. The aim of this study was to investigate the association between appetitive aggression and telomere length in young South African isiXhosa-speaking men.

Methods: Young Xhosa Black South African men (N=290) were assessed for, amongst others, exposure to different types of violent traumatic events and community violence using the Appetitive Aggression Scale (AAS) and the posttraumatic stress disorder (PTSD) Symptom Scale-Interview (PSS-I). Relative telomere length quantification was performed by real-time quantitative polymerase chain reaction (qPCR).

Results: Regression and correlation analyses found no statistically significant association ($p=0.500$) or correlation ($r=0.09$, $p=0.121$) between appetitive aggression and relative telomere length. However, longer relative telomere length was found to be correlated ($r=0.13$, $p=0.039$) and associated ($p=0.046$) with higher PTSD symptom severity. No statistically significant interaction was found between appetitive aggression and PTSD symptom severity, which was contributing to relative telomere length.

Conclusion: This study is the first to examine the role of telomere length in appetitive aggression in young Xhosa Black South African men. These preliminary findings suggest no statistically significant association between appetitive aggression and telomere length. However, PTSD symptom severity was found to have a considerable effect on telomere length.

4.1 INTRODUCTION

Young South African males living in townships, which are low-income and poverty-stricken urban settlements, are continuously exposed to high violence and various criminal activities (Norman et al., 2007; Seedat et al., 2009b). Serious crime and gang activity coupled with drug abuse have escalated in South African townships (Maxson et al., 1998; Dos Santos et al., 2014; Shields et al., 2008). Distinguishing between the perpetration of violence and victimisation under these traumatic circumstances can be challenging, as they co-occur in the same environment (Kaminer et al., 2008; Lockhat and Van Niekerk, 2000; Maxson et al., 1998; Shields et al., 2008). Continuous exposure to violent traumatic activities has been found to lead to the development of aggression. There are broad categories of aggressive behaviour, and the two main categories are appetitive aggression and reactive aggression (Elbert et al., 2010; Fontaine et al., 2007).

Appetitive aggression is characterised by a desire to perpetrate more violence, which triggers feelings of excitement and arousal (Elbert et al., 2010; Weierstall et al., 2013c). On the other hand, reactive aggression is a negative emotional response, which occurs if an individual has been provoked or there is a danger against him/her; thus happens as a self-defensive response (Elbert et al., 2010). Appetitive aggression can emerge after an individual has been exposed to cruelty, chronic traumatic events, stressful life, and may develop as a survival strategy within a violent environment (Hecker et al., 2012a; Weierstall et al., 2013b). Thus, appetitive aggression may reduce the feeling of victimisation and vulnerability to traumatic stress, which fosters a cycle of violence (Elbert et al., 2010; Hecker et al., 2013; Weierstall et al., 2013b).

Appetitive aggression can decrease the development of mental illnesses such as posttraumatic stress disorder (PTSD) (Hecker et al., 2013; Weierstall et al., 2013b). It has been found to provide a protective buffer against PTSD (Hecker et al., 2012a; Weierstall et al., 2012b). However, this buffering effect of appetitive aggression could decline should the trauma load increase and become too high (Hecker et al., 2012a; Weierstall et al., 2012b), in line with the building-block theory of PTSD (Neuner et al., 2004a). The building block theory states that cumulative exposure to a variety of traumatic events can increase the risk of developing PTSD symptoms (Neuner et al., 2004a). The interaction between different types of aggression and the development of PTSD is complex and requires a comprehensive model to better understand the different pathways contributing to its development.

Studies have shown that continuous exposure to psychological stressors could lead to accelerated ageing (Epel et al., 2004; O'Donovan et al., 2012). Constant exposure to traumatic events, combined with harmful lifestyle factors, low socioeconomic conditions, and the risk of infectious diseases, can act as factors that render people more susceptible to accelerated ageing, compromising the immune system and increasing the risk of cardiovascular disease (Carroll et al., 2013; Epel et al., 2004; Needham et al., 2014; Shalev et al., 2013a). Telomere length is a biological marker, which has been widely investigated to understand the effect of psychological stressors in different environments (Epel et al., 2004; Hajat et al., 2010; Needham et al., 2014).

Telomeres are nucleoprotein structures, which protect the loss of crucial coding DNA information during each cell replication (Blackburn, 2005; de Lange et al., 2005). They maintain the stability of chromosomes by preventing senescence and premature cell death (Blackburn et al., 2015b). If telomere length is critically shortened, genomic and chromosomal stability gets affected, resulting in the onset of DNA damage response mechanisms that could result in senescence (Blackburn, 2005; de Lange et al., 2005). Telomerase, an enzyme responsible for the synthesis of telomere length works together with other accessory proteins and genes in the telomere biology to ensure that telomeres are properly synthesised to maintain telomere length during each cell replication, though few nucleotides are trimmed (Blackburn, 2005; Chiang et al., 2006; de Lange et al., 2005; Mirabello et al., 2010; Pellatt et al., 2013).

The effect of psychological stress on telomere length (Jergović et al., 2014; Küffer et al., 2016; O'Donovan et al., 2011), as well as the association between telomere length and aggressive behaviour, such as hostility, has been subject to intense investigation (Kang et al., 2017a; Watkins et al., 2016; Zalli et al., 2014). Studies have demonstrated that psychological stress and the development of mental illness such as PTSD is associated with accelerated telomere length shortening (S. Chen et al., 2014; Epel et al., 2010; Jergović et al., 2014; Küffer et al., 2016; O'Donovan et al., 2011; Shalev et al., 2014). In addition, some studies have shown that accelerated shortened telomere length is associated with aggressive related behaviours such as hostility (Kang et al., 2017a; Watkins et al., 2016; Zalli et al., 2014).

Telomere length can be affected by a variety of factors such as the trauma load and the environmental setting under which such traumatic psychological stress is experienced (Carroll et al., 2013; Kim et al., 2017; Shalev et al., 2013b). For example, a study by Boks et al. (2014) found that Dutch soldiers who were deployed to combat activity had longer

telomere length, which was associated with worsening of PTSD symptoms (Boks et al., 2014). Another study found that veterans who had hostility, a form of aggression, had shorter telomere length (Watkins et al., 2016). These opposing findings indicate the complex role played by telomere length in the development of PTSD and aggression, particularly as mentioned above that appetitive aggression could play a role in buffering against PTSD. This interaction between PTSD and appetitive aggression and their role in telomere length had been fully explored. Therefore, the aim of this study was to investigate the association between relative telomere length, appetitive aggression and PTSD in young Xhosa Black South African men.

4.2 MATERIALS AND METHODS

4.2.1 Sociodemographic and clinical data

This chapter (4) cross-sectionally investigated relative telomere length in association with appetitive aggression or PTSD symptom severity, whereas Chapter 3 longitudinally investigated these outcomes as part ofa clinicla trial. Similarly to Chapter 2 and 3, firstly, young Xhosa Black South African men (N=405) were recruited in collaborations with staff members belonging to the Rebuilding and Life Skill Training Centre (REALISTIC) from Khayelitsha and Gugulethu townships of Cape Town (Hinsberger et al., 2016a; Sommer et al., 2017). REALISTIC is a reintegration program that assists former offenders who have been incarcerated and those individuals who are at risk of committing crime and violence. Participants consisted of former offenders (20%) and those at risk of committing crime (80%). Only 19% of the participants had completed secondary education while the remaining 81% did not (Sommer et al., 2016).

The Childhood Exposure to Community Violence Checklist (CECV) adapted from Amaya-Jackson (1998) (Amaya-Jackson, 1998a) was used to assess exposure to trauma. The CECV is comprised of a 33-item self-reporting checklist that measures levels of witnessed, experienced, and second-hand trauma in childhood. This study adapted the questions in the CECV to reflect the types of violence that are experienced in a typical low socioeconomic status South African township, including questions concerning physical and sexual abuse. The CECV has been previously utilised in South African population (Fincham et al., 2009b; Weierstall et al., 2013b). Exposure to a traumatic event is categorised as either witnessed or experienced, with the sum of both indicating the cumulative severity of exposure to traumatic events and community violence. The reliability of the CECV was high in this cohort with a Cronbach's alpha coefficient of 0.92 and an inter-rater reliability of 0.85 (Hinsberger et al., 2016b). The Appetitive Aggression Scale (AAS, ≥ 9 points) was used to assess appetitive

aggression (Weierstall and Elbert, 2011). The PTSD Symptom Scale-Interview (PSS-I, ≥ 8 points) was used to assess PTSD symptom severity (Hinsberger et al., 2016a; Sommer et al., 2017). South African townships are historically characterised by poverty, high unemployment rates, and were previously designated for non-White people like Black South Africans and other populations. Ethical clearance for this study was obtained from the ethics review boards of Stellenbosch University (Ethics Reference number: N13/01/006), the University of Konstanz, and the University of Cape Town.

4.2.2 Quantitative PCR for telomere length

PCR experiments were performed as described in Section 3.2.2 (Chapter 3) to quantify relative telomere length.

4.2.3 Statistical data analysis

Previous studies by Hinsberger et al. (2016) and Sommer et al. (2016) that were conducted concurrently with the present study included clinical data in their publications. Appetitive aggression and PTSD symptom severity were main clinical outcome variables as described in other publications (Hinsberger et al., 2016b; Sommer et al., 2016). The biological outcome variable was relative telomere length, which was investigated to determine its association with clinical variables. The normality of relative telomere length data was assessed using a constructed probability plot. To minimise the effect of outlier values, the relative telomere length data was log-transformed. Correlation analysis between relative telomere length and appetitive aggression or PTSD symptom severity was determined using a scatter plot. Generalised linear regression models were used to determine if there was an association between relative telomere length, appetitive aggression, and PTSD symptom severity. Furthermore, the interaction between appetitive aggression and PTSD symptom severity was determined to assess its effect on telomere length. Statistical significance of variances was set at 95% confident intervals (CI) with an alpha (α) less than 0.05 ($p<0.05$).

4.3 RESULTS

4.3.1 Demographic, clinical, and descriptive measures

The age of participants was normally distributed between 14 and 40 years [mean (M)=21.96 and standard deviation (SD)=4.53] (Hinsberger et al., 2016b; Sommer et al., 2016). The majority of participants did not finish secondary school while few participants had some tertiary education (Hinsberger et al., 2016b; Sommer et al., 2016). Demographic information

of all participants is reflected in Table 4.1. Concurrent studies have analysed some clinical outcomes (Hinsberger et al., 2016b; Sommer et al., 2016). The current study investigated clinical outcomes in relation to relative telomere length. The perpetration of violence was found to be indirectly influenced by both witnessed and self-experienced traumatic events, which was linked to appetitive aggression, which in turn had an effect on the development of PTSD (Hinsberger et al., 2016b; Sommer et al., 2016). Therefore, appetitive aggression was significantly positively correlated with both witnessed and self-experienced trauma (respectively: r=0.35, p<0.001; r= 0.40, p<0.001) (Hinsberger et al., 2016b). This relationship between both witnessed and self-experienced trauma, which correlated with appetitive aggression was indirectly correlated with PTSD (r= 0.28, p< 0.001) (Hinsberger et al., 2016b).

Table 4. 1: Descriptive sociodemographic information, appetitive aggression and PTSD symptom severity of young Xhosa Black South African men (N=290), adapted from Hinsberger et al. (2016)

Demographic Information	N	%	Mean	Median	SD
Age (14–40 years)	–	–	–	10	4.5
Formal education (range: 1–16 years)	–	–	10	–	1.8
Completed primary school	227	78.3	–	–	–
Completed secondary school	52	17.9	–	–	–
Completed tertiary school	4	1.4	–	–	–
Participated in reintegration program	148	51	–	–	–
Never participated in reintegration program	142	49	–	–	–
PSS-I (range: 8–37)	–	–	19.15	–	8.33
AAS (range: 9–52)	–	–	27.72	–	11.44

AAS— Appetitive Aggression Scale,
PSS-I— Posttraumatic stress disorder (PTSD) Symptom Scale-Interview,
SD— standard deviation,
N— number of participants,
%— percentage

4.3.2 The analysis between relative telomere length and appetitive aggression or posttraumatic stress disorder

We analysed clinical variables [appetitive aggression (measured using the AAS) and PTSD symptom severity (measured using the PSS-I)] in relation to relative telomere length to determine if there was a correlation or association between relative telomere length and appetitive aggression or PTSD symptom severity. No statistically significant difference was found between relative telomere length and childhood trauma or other traumatic events using regression analysis (Table 4.2). There was no correlation between relative telomere length

and appetitive aggression (r=0.09, p=0.121) (Figure 4.1). However, longer relative telomere length was correlated with PTSD symptom severity (r=0.13, p=0.04) (Figure 4.2).

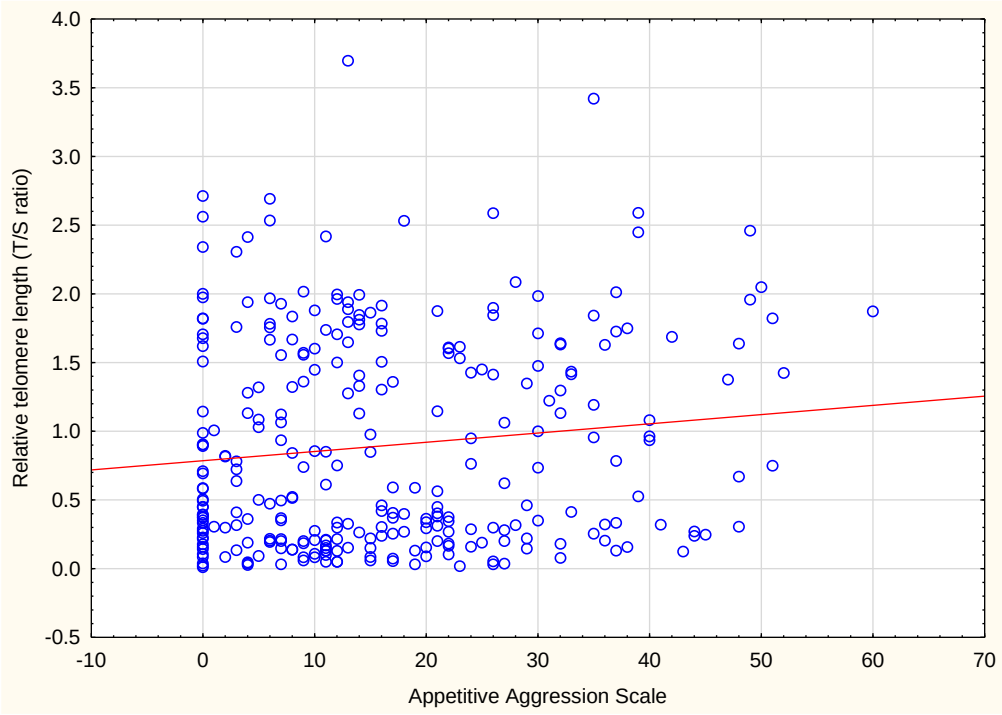

Figure 4.1: Scatter plot demonstrating the correlation between relative telomere length and appetitive aggression measured by the Appetitive Aggression Scale (AAS). There was no significant correlation found between relative telomere length and appetitive aggression (r=0.09, p=0.14). The T/S ratio represents telomere copy number to single copy reference gene (T/S).

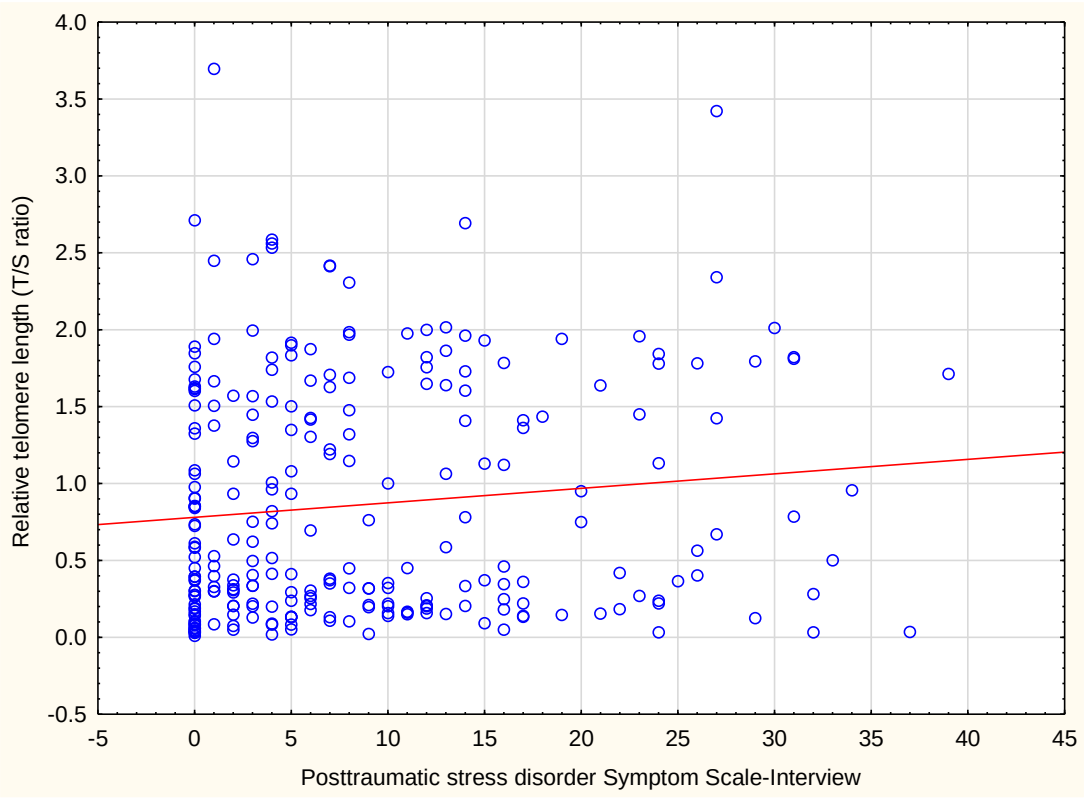

Figure 4.2: Scatter plot demonstrating the correlation between relative telomere length and posttraumatic stress disorder (PTSD) symptom severity measured by PTSD Symptom Scale-Interview (PSS-I). There was a positive correlation between relative telomere length (r=0.13, p=0.046). The T/S ratio represents telomere copy number to single copy reference gene (T/S).

Using generalised multiple regression analysis, we did not find any statistically significant difference between relative telomere length and appetitive aggression (p=0.500) (Table 4.2). However, relative telomere length was found to be associated with PTSD symptom severity (p=0.046) (Table 4.2). Furthermore, we analysed the interaction between both appetitive aggression and PTSD symptom severity to assess their contribution towards relative telomere length. No statistically significant interaction was found between appetitive aggression and PTSD symptom severity (p=0.735) (Figure 4.3).

Table 4.2: Multiple linear regression analysis of relative telomere length in association with appetitive aggression and PTSD symptom severity of young Xhosa Black South African men (N=290)

Outcome variables	b	SE	t	*P*-value
Age	-0.007	0.013	-0.568	0.570
Childhood trauma	-0.002	0.013	-0.182	0.855
Traumatic events	0.002	0.026	0.111	0.911
PSS-I	0.012	0.006	2.005	0.046*
AAS	0.011	0.014	0.779	0.436

N— number of participants,
SE— standard error,
b— beta coefficient,
t— difference representative units of standard error,
*p-value ≤ 0.05— indicates significance,
AAS— Appetitive Aggression Scale,
PSS-I— posttraumatic stress disorder (PTSD) Symptom Scale-Interview.

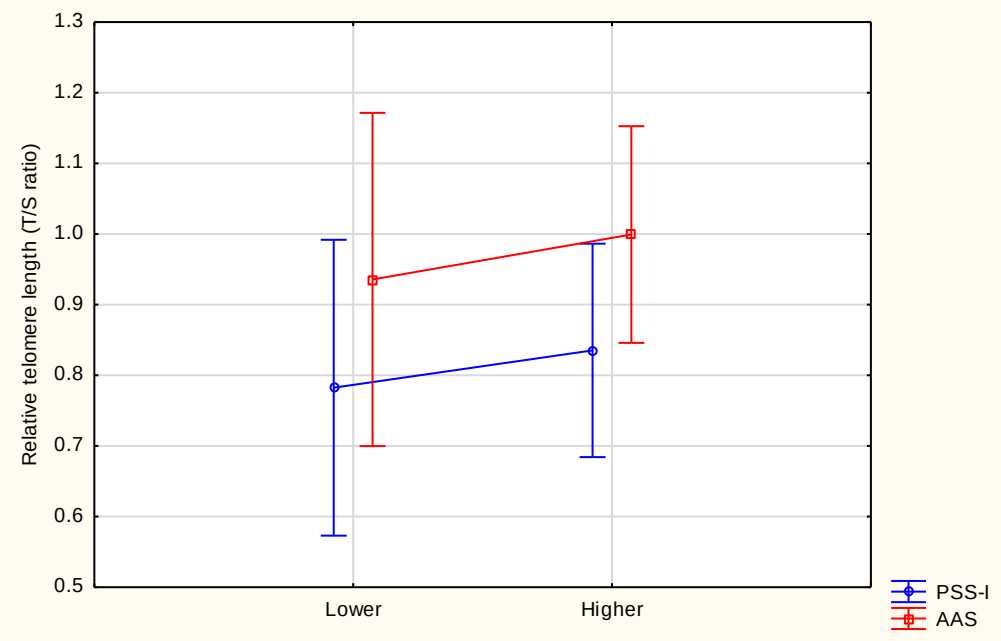

Figure 4.2: Graph depicting relative telomere length in relation to both appetitive aggression and posttraumatic stress disorder (PTSD). No significant interaction between appetitive aggression and PTSD and the difference in relative telomere length difference. Appetitive aggression and PTSD were measured using instruments: Appetitive Aggression Scale (AAS) and PTSD Symptom Scale-Interview (PSS-I), respectively. The T/S ratio represents telomere copy number to single copy reference gene (T/S).

4.4 DISCUSSION

The objective of the present study was to investigate the relationship between relative telomere length, the development of appetitive aggression and PTSD symptom severity in a cohort of Xhosa Black South African men at high risk of continuous exposure to violent traumatic events. In addition, this study intended to elucidate the contribution of the interaction between appetitive aggression and PTSD symptom severity in relative telomere length since most of the participants displayed both appetitive aggression and PTSD symptom severity. Appetitive aggression was assessed using the AAS (Weierstall and Elbert, 2011) and PTSD symptom severity was measured by the PSS-I (Foa and Tolin, 2000). Quantitative PCR was used to determine relative telomere repeats to quantify their length using a published method that had been used in several other studies (Cawthon, 2002; Malan-Muller et al., 2013; Pellatt et al., 2013).

To our knowledge, this is the first study to investigate an association between relative telomere length and appetitive aggression and between relative telomere length and PTSD. Relative telomere length can be sensitive to environmental exposures such as chronic stress and traumatic events (Carroll et al., 2013; Chae et al., 2016; Entringer et al., 2011; Shalev et al., 2013b). Furthermore, exposure to traumatic situations such as violence can lead to stress-related disorders such as PTSD and behavioural problems like appetitive aggression (Hecker et al., 2015a; Hermenau et al., 2013b; Köbach et al., 2015). Participants were categorised as having high or low appetitive aggression based on the AAS scores (Weierstall and Elbert, 2011), as well having high or low PTSD symptom severity based on PSS-I (Foa and Tolin, 2000). This categorisation of participants was intended to determine the association between these clinical variables and relative telomere length. These clinical outcomes were also used in combination to determine their contribution to relative telomere length. The present study did not find an association between appetitive aggression and relative telomere length when multiple regression analyses were performed. Furthermore, no correlation was found between relative telomere length and appetitive aggression in this investigated group. However, a statistically significant correlation was found between longer relative telomere length and PTSD symptom severity. Interestingly, this finding of an association between longer telomere length and PTSD symptom severity is in line with some previous reports (Boks et al., 2014; Küffer et al., 2016), although it differs with other studies (Jergović et al., 2014; Kim et al., 2017; Ladwig et al., 2013; O'Donovan et al., 2011). Some studies have widely reported that shortened telomere length is associated with PTSD symptom severity (Kim et al., 2017; Ladwig et al., 2013; O'Donovan et al., 2011), while other researchers have reported longer telomere length to be associated with PTSD symptom

severity (Boks et al., 2014; Küffer et al., 2016). The differences in findings on telomere length may reflect distinct differences trauma-related experiences (including age of onset, type of trauma, trauma severity, number of traumas).

A study by Ladwig et al. (2013) found that individuals with PTSD had shorter telomeres. Another study by Kim et al. (2017) found that shorter telomere length was associated with PTSD in combat veterans, with combat veterans who were using antidepressants having longer telomere length (Kim et al., 2017). These findings concur with our finding of an association between shorter telomere length and PTSD symptom severity. However, Boks et al. (2014) reported that longer telomere length was associated with higher PTSD symptom severity in Dutch combatants who had been deployed in war zones of Afghanistan and who were assessed longitudinally for telomere length and PTSD symptom severity. Furthermore, a study by Küffer et al. (2016) had results that were in agreement with Boks et al. (2014), whereby longer telomere length was associated with PTSD symptom severity in adults who were indentured Swiss child labourers (Küffer et al., 2016). These previous studies indicate the complex relationship of telomere length and PTSD symptom severity. Further investigations and analyses that include variables such as level of education, lifestyle, nutritional information, and others in different settings may shed light to the understanding of underlying pathophysiological pathways and their involvement in telomere changes.

It is still not well understood how PTSD symptom severity affects telomere length or vice versa. The complexity in understanding the association between PTSD symptom severity and telomere length is exacerbated by the multifactorial nature of the disorder and comorbidity with depression and other age-related disease (e.g. heart disease) (van der Harst et al., 2007; Kark et al., 2013; De Meyer et al., 2011). Furthermore, literature shows that individuals with PTSD symptom severity often have an abnormal autonomic nervous system response, which is also associated with hyperarousal even in situations with no threat (Kim et al., 2017; Williamson et al., 2015). This hyperarousal is due to a highly activated hypothalamic-pituitary-adrenal (HPA) axis, a pathway responsible for response to stressful events, which may lead to increased cortisol, an essential glucocorticoid during traumatic stress (Bellavance and Rivest, 2014; Cain and Cidlowski, 2017; Miller and Sadeh, 2014; Nguyen et al., 1998). The HPA axis is involved in the stress response whereby cortisol plays a significant role in coping with stressful life events (Lucassen et al., 2013; Wagner et al., 2011). Increased cortisol levels are associated with oxidative damage to neuronal cells, which may be associated with accelerated cellular ageing (Miller and Sadeh, 2014). Overall, the complexity of telomere length and PTSD leaves room for further investigation to shed

light on the pathophysiology of PTSD and appetitive which share similar neurobiological pathways.

Our findings that longer relative telomere length is associated with PTSD symptom severity, but not appetitive aggression, is interesting and further demonstrates the complexity of understanding telomere length in different populations and settings. Our sample had chronic exposure to stressful life with constant exposure to violent threats and have been exposed to childhood trauma (Hinsberger et al., 2016b; Sommer et al., 2017). These factors may affect telomere length differently. Telomere length is dynamic and can be affected by several factors including environmental stressors like childhood adversity and many biological pathways such as the HPA axis that have been found to be linked to both PTSD symptom severity and aggression (Bellavance and Rivest, 2014; Böhnke et al., n.d.; Cain and Cidlowski, 2017; Lopez-Duran et al., 2009; Nguyen et al., 1998). It is still not clear how the co-occurrence of both PTSD symptom severity and appetitive aggression may have influenced relative telomere length. Literature suggests that certain biological mechanisms responding to stressful events, which are associated with fear sensations that are shared in both appetitive aggression and PTSD (Elbert et al., 2010). Thus, the combination of signals that are triggered by appetitive aggression which are associated with excitement, may become part of fear and hyperarousal, which may activate trauma-related phenotypes, such as seen in PTSD (Elbert et al., 2010; Haer et al., 2013). Therefore, the co-occurrence of appetitive aggression and PTSD may be affecting telomere length differently.

Previous reports have suggested that the linkage between the physiological and neural responses seen in both appetitive aggression and PTSD symptom severity are reached when an individual reaches a certain traumatic state (Elbert et al., 2010; Hecker et al., 2013). The dynamic impact involved in both appetitive aggression and PTSD symptom severity on telomere length can be complex since underlying neurobiological responses in these clinical variables can vary at times. This variation in neurobiological responses can be influenced by environmental interactions and an individual's genomic architecture, which may be linked to changes in brain chemistry (McGowan, 2013; Provençal et al., 2014; Tremblay, 2010) and may have a different impact in telomere length at different points in time.

The co-occurrence of appetitive aggression and PTSD may have a particular impact in relative telomere length. The effect could be mediated through pathways like the HPA axis since both aggressive behaviour and PTSD share similar phenotypic responses which may, in turn, trigger similar neuroendocrine responses (Elbert and Schauer, 2002; Haer et al., 2013; Moran et al., 2014). Chronic exposure to stress and development of PTSD symptom

severity in our sample may have triggered upregulation of telomerase, an enzyme responsible for telomere synthesis. This upregulation of telomerase may have occurred as a compensatory mechanism in response to stress (Beery et al., 2012; Epel et al., 2010). Previous studies have shown that exposure to stress can increase the activity of telomerase, which in turn can increase the synthesis of telomeres, leading to increased telomere length (Beery et al., 2012; Epel et al., 2010). Beery et al. (2012) found that exposing rats to chronic stress increased the activity of telomerase (Beery et al., 2012). Furthermore, Epel et al. (2010) found that exposing individuals to stress increased telomerase activity (Epel et al., 2010). Therefore, our findings of longer relative telomere length in individuals with gretaer PTSD symptom severity are in line with the aforementioedn findings.

4.4.1 Limitations

This study was novel as it investigated the association between relative telomere length, appetitive aggression, and PTSD in a unique cohort of Xhosa Black South African men who had experienced constant exposure to violent threats. Our results must be interpreted with caution since the study participants were of the same ethnicity, which makes it difficult to generalise our observations to other ethnic groups. We only had male participants in this study; hence, our findings cannot be extrapolated to other genders. A study by Shalev et al. (2014) found an association between shortened telomere length and externalising behavioural disorder in men, while there was no association in women (Shalev et al., 2014). This study underscores the importance of investigating both genders so that we can better understand the aetiology of aggressive behaviour and the associated molecular mechanisms. Furthermore, we did not collect lifestyle information such as diet, physical activity and daily routines of the participants; hence, in future studies these variables would need to be considered. Lastly, we did not collect information on the medical histories of participants, including their use of the medications, which may be confounding influences. Therefore, a more comprehensive analysis that incorporates these factors into account is required.

4.4.2 Conclusion

In conclusion, this study demonstrated that there was an association between longer relative telomere length and PTSD symptom severity. However, no association was found between relative telomere length and appetitive aggression in participants at high risk of repeated exposure to violent threats and traumatic events. Given the small sample, our findings require replication in a broader and more robust scientific study, which would include diverse

ethnicities, genders, and experiences. However, our findings of an association between relative longer telomere length and PTSD symptom severity provide insights on the biological mechanisms associated with PTSD symptom severity and appetitive aggression.

CHAPTER 5

THE *SCL6A4* STIN2 VARIANT IS ASSOCIATED WITH APPETITIVE AGGRESSION IN A COHORT OF YOUNG BLACK MALES EXPOSED TO CONTINUOUS THREAT

ABSTRACT

Introduction: Appetitive aggression is a sub-category of instrumental aggression characterised by the primary intrinsic enjoyment of aggressive activity. Studies have indicated that aggression is heritable, with serotonergic and monoaminergic neurotransmitter systems contributing to the underlying molecular mechanisms. The aim of the present study was to investigate the role that genetic variants in the serotonin transporter gene (*SLC6A4*) and monoamine oxidase A (*MAOA*) play in the levels of appetitive aggression in South African Xhosa males (n = 290).

Methods: Participants were assessed for, amongst others, exposure to traumatic events and community violence, and appetitive aggression. *SLC6A4* 5-HTTLPR, rs25531, and STin2 variants, as well as *MAOA*-uVNTR were investigated for their association with levels of appetitive aggression using Poisson regression analysis.

Results: STin2 VNTR 12-repeat homozygous genotype and L'-STin2.12/STin2.12 genotype combination were found to be associated with increased levels of appetitive aggression ($p = 0.003$ and $p = 8.00 \times 10^{-8}$, respectively).

Conclusion: This study is the first to investigate genetic underpinnings of appetitive aggression in a South African population, with preliminary evidence suggesting the involvement of *SCL6A4* variants in the aetiology of appetitive aggression. Although the results require replication, they shed some preliminary light on the molecular underpinnings of appetitive aggression.

5.1 INTRODUCTION

Aggression comes in two flavours: the reactive form motivated to reduce threat, and the appetitive form which refers to the internally motivated urge, i.e. to fight just for the sake of it. The latter drives behaviour such diverse as ego-shooter gaming, hunting and killing (Elbert et al., 2017) (Elbert et al., 2018). Appetitive aggression is a sub-category of instrumental aggression in which the aggressive behaviour is exerted to achieve a particular goal, and on achievement of the goal, induces feelings of arousal and excitement (Elbert et al., 2010). Although the environment affects the development of aggression, studies indicate that genetic factors explain up to 65% of the variability in violent, aggressive and impulsive behaviour (Burt, 2009; Ferguson, 2010; Mason and Frick, 1994; Miles and Carey, 1997; Rhee and Waldman, 2002). Interestingly, the heritability of aggressive traits has been found to increase with age. In a recent meta-analysis, Burt et al. (Burt, 2009) noted an increase in heritability from 55% at ages 1-5 years to 65% at 11-18 years of age. Moreover, heritability of aggressive traits has been shown to be higher in males compared to females (Craig and Halton, 2009; Miles and Carey, 1997; Vierikko et al., 2003).

The majority of genetic association studies in aggression have employed a candidate gene approach, whereby genes are selected for investigation based on current knowledge of the biology of the disorder or trait. Numerous lines of evidence indicate that low or impaired serotonin function underlies the traits of aggression and impulsivity (Alenina et al., 2009; Angoa-Pérez et al., 2012; Heiming et al., 2013; Holmes et al., 2002; Mosienko et al., 2012; Stanley et al., 2000). Serotonin may be involved in the withdrawal from dangerous or aversive situations, thus a hypofunctioning of the serotonergic system (the so-called “serotonin deficiency hypothesis”) could result in impaired avoidance of aversive stimuli or undesirable situations, and could lead to impulsive, aggressive and violent behaviour and responses (Linnoila and Virkkunen, 1992; Tops et al., 2009).

The serotonin transporter (also known as solute carrier family 6 member 4 (SLC6A4)) plays an important role in the regulation of serotonin concentration in the brain (Risch and Nemeroff, 1992). The gene encoding SLC6A4 is located on chromosome 17q11.1–q12 (Heils et al., 1996), and has been extensively investigated in the context of anxiety and depressive disorders. The gene contains an insertion-deletion (indel) polymorphism known as the serotonin transporter-linked polymorphic region (5-HTTLPR) (Heils et al., 1996; Lesch et al., 1994). This polymorphism comprises a short (S) allele and a long (L) allele, which differ in length by 44 base pairs (bp). 5-HTTLPR is reportedly functional, with the L-allele

facilitating more efficient transcription than the S-allele (Greenberg et al., 1999; Heils et al., 1996; Lee et al., 2005; Lesch et al., 1996). Recently, Hu et al. (Hu et al., 2006) identified a single nucleotide polymorphism (SNP), rs25531, situated within the 5-HTTLPR variant. This SNP has been found to modulate the functionality of the L-allele, such that the L-G haplotype results in reduced *SLC6A4* expression (comparable to that of the *S*-allele), whilst the L-A haplotype is associated with increased *SLC6A4* expression, and increased SLC6A4 binding potential in the putamen (Praschak-Rieder et al., 2007). Many researchers, therefore, have indicated that it is functionally more sound to group subjects who carry the L-G haplotype with those carrying S-alleles (Hu et al., 2006; Wendland et al., 2006). This is particularly important in populations with African ancestry, as the frequency of the L-G haplotype has been found to be much higher than in other populations (Enoch et al., 2013).

Another *SLC6A4* variant that has been investigated for its role in aggression is a variable number of tandem repeats (VNTR) polymorphism located in intron 2, referred to as STin2. This polymorphism comprises various repeats of a 17 bp motif, with the most common alleles represented by the 9-repeat (STin2.9), 10-repeat (STin2.10) and 12-repeat (STin2.12) variants (Lesch et al., 1994). Studies have found that STin2.12 enhances transcription of *SLC6A4* (Bah et al., 2008; Fiskerstrand et al., 1999; Heils et al., 1996; MacKenzie and Quinn, 1999b), and STin2.10 has been associated with less efficient serotonin turnover (MacKenzie and Quinn, 1999b; Sarosi et al., 2008a). The STin2.12 allele has been found to be associated with increased aggression in children (Beitchman et al., 2003; Davidge et al., 2004) and adults (Aluja et al., 2009; Payer et al., 2012), although association between STin2.12 allele and aggression in the latter two studies was observed only in combination with at least one 5-HTTLPR S-allele. Indeed, recent evidence suggests that the 5-HTTLPR and STin2 VNTR polymorphisms may be controlled by the same regulatory pathway (Ali et al., 2010), with the S-STin2.12 allele combination resulting in increased SLC6A4 expression compared to the L-STin2.10 allele combination.

Monoamine oxidase A (MAOA) is the main enzyme responsible for serotonin degradation and thus plays an important role in the regulation of serotonin levels. The gene encoding MAOA is located on the X chromosome, and has long been associated with aggression. Brunner et al. (Brunner et al., 1993b) observed that a *MAOA* point mutation in exon 8 was associated with Brunner Syndrome, characterised by increased antisocial behaviour, aggression, and very high levels of disruptive and violent outbursts in affected males, in a large Dutch kindred. Subsequent studies found that adult male mice lacking *MAOA* exons 2 and 3 (resulting in MAOA deficiency) exhibited significantly increased levels of aggressive behaviour (Cases et al., 1995). These results are in line with those observed in a mouse

model with a novel, spontaneous nonsense mutation in exon 8 (effectively resulting in *MAOA* deletion) (Scott et al., 2008). More recently, Palmer et al. (2016) observed, amongst others, episodic explosive aggression in individuals with *MAOA* loss-of-function mutations.

MAOA contains a polymorphism located in the promoter region, approximately 1.2 kilobases (kb) upstream of the coding region (Denney et al., 1994). This VNTR polymorphism (*MAOA*-uVNTR) is characterised by variable numbers of 30 bp repeats, and commonly comprises 2-, 3-, 3.5-, 4- and 5-repeat alleles (Huang et al., 2004; Kim-Cohen et al., 2006; Sabol et al., 1998). The MAOA-uVNTR 2- and 3-repeat alleles are associated with reduced transcriptional efficiency compared to the 3.5- and 4-repeat alleles (Deckert et al., 1999; Denney et al., 1999; Jonsson et al., 1999; Kim-Cohen et al., 2006; Sabol et al., 1998). The functionality of the 5-repeat allele is not clear (Kim-Cohen et al., 2006). Evidence abounds for a sexually dimorphic role of the *MAOA*-uVNTR in aggression. Males possessing the low-activity alleles have been found to be at greater risk for increased aggressive and impulsive reactions to stressful stimuli, whilst females carrying the high-activity alleles have been found to possess an increased risk of aggression, but only if they have also been exposed to increased levels of early adversity (reviewed in Godar et al. (Godar et al., 2016)).

South Africa is characterised by social and economic inequalities, with poorer communities experiencing daily traumatic stressors. Currently the rates of homicide, gender-based violence and gang-related violence in South Africa are amongst the highest in the world (Seedat et al., 2009b). Exposure to stressors results in the activation of biological and psychological responses that are necessary for adaptation to the environment. Repeated and prolonged exposure to violence and stressful events can, however, result in sustained activation of biologically-mediated responses, with the result that the individual becomes susceptible to an array of both physical and psychological complications (Mcewen, 2000; Worthman and Panter-Brick, 2008). Early childhood abuse and adversity, which coincides with the cycle of violence, facilitates the development of violent behaviour and cruelty (Elbert et al., 2010).

The current study follows on from a recent study by Hinsberger et al. (Hinsberger et al., 2016c), who investigated attraction to violence in the context of continuous traumatic stress exposure in the same study sample originating from townships in Cape Town, South Africa. Here, it was found that appetitive aggression scores were predicted by witnessed as well as self-experienced traumatic events. In the current study, we aimed to investigate whether genetic variants in *SLC6A4* and/or *MAOA* accounted for, at least partially, some of the

remaining variance in appetitive aggression score, after correcting for severity of witnessed and self-experienced traumatic events.

5.2 MATERIALS AND METHODS

5.2.1 Demographic and clinical measures

The cohort of 290 male Xhosa participants were all recruited from the townships of Khayelitsha and Gugulethu in the Western Cape, South Africa. “Township” in South Africa refers to an underdeveloped urban residential area that was historically reserved for non-white inhabitants. The socio-economic conditions of most townships in South Africa are poor, and unemployment rates are very high. Sociodemographic information was obtained from each participant and included age, ethnicity and educational background (Hinsberger et al., 2016c; Sommer et al., 2016). All participants were recruited with the assistance of staff at the Rebuilding and Life Skill Training Centre (REALISTIC) in Gugulethu. The final sample comprised those individuals who were attending the reintegration program at the time (20%) and those who were identified as having a high risk for perpetrating violence or becoming victims of crime (80%).

The study was approved by the ethics review boards of the University of Konstanz, Stellenbosch University and University of Cape Town. Participants received compensation for taking part in the study. All participants over the age of 18 years gave informed consent to participate in the study, and informed consent was obtained from parents or caretakers for participants under the age of 18 years. Trauma exposure was measured using an adapted Childhood Exposure to Community Violence Checklist (CECV) (Amaya-Jackson, 1998b). The CECV is a 33-item self-report checklist that assesses children’s levels of witnessing, experiencing or hearing about trauma. The questionnaire was adapted to reflect types of violence typical of low-income areas in South Africa, such as sexual and physical assault. The CECV has been used previously in South African populations (Fincham et al., 2009a) and offender populations (Weierstall et al., 2013b) . The events can be categorised as either “witnessed” or “experienced”. The total score on the CECV indicates an individual’s severity of exposure to traumatic events and community violence. The reliability of the CECV score in the current sample, measuring internal consistency using the McDonald’s omega, has been found to be 0.79 (95% CI: 0.75-0.82) (Sommer et al., 2016).

Appetitive aggression was measured using the Appetitive Aggression Scale (AAS) (Weierstall and Elbert, 2011). Here, responses to 15 questions on instrumental aggression, addiction behaviour and desire to do harm were rated on a 5-point Likert scale, and the total AAS score calculated by summing the scores of the 15 items. The reliability of the score has

been found to be high in the current sample (coefficient omega = 0.87; 95 CI: 0.84-0.89) (Sommer et al., 2016). Reactive aggression was measured using the Buss-Perry Aggression Questionnaire (BPAQ) score (Buss and Perry, 1992). The BPAQ is a 29-item inventory scored on a 5-point Likert-type scale from 1 to 5. Higher scores on the BPAQ indicate higher levels of trait aggression.

5.2.2 Genotyping

Genomic DNA was extracted from saliva collected in Oragene™ DNA self-collection kits (OG-500, DNA Genotek, Ontario, Canada) using the Prep-It L2P reagent (DNA Genotek, Ontario, Canada) as per manufacturer's instructions. The 5-HTTLPR and rs25531 polymorphisms were genotyped by employing a two-stage genotyping procedure, as previously described (Voyiaziakis et al., 2011). The STin2 VNTR polymorphism was amplified using previously published primer sequences adapted from (Battersby et al., 1996). The forward primer was fluorescently labelled with the fluor HEX, to facilitate genotyping by capillary electrophoresis. The 20 µl PCR reaction mixture comprised 12.5 µl KAPA ReadyMix (Kapa Biosystems, Wilmington, MA, USA), 0.15 µM each of the forward and reverse primers, and 20 ng template DNA, made up to a final volume of 20 µl using bi-distilled water. PCR conditions were as follows: an initial 5-min denaturation step at 95°C, 35 cycles of 95 °C for 60 s, 60°C for 30 s, and 72 °C for 45 s and a final 7-min extension step at 72°C. PCR amplicons were separated by agarose gel electrophoresis and visualised by ethidium bromide staining to assess the PCR success. The loci containing the 5-HTTLPR/rs25531, and STin2 VNTR polymorphisms were amplified separately. Following agarose gel electrophoresis to determine the success of each PCR, the amplicons (STin2 and *5*-HTTLPR) were combined in a 1:1 ratio for capillary electrophoresis. *MAOA*-uVNTR amplification was performed using published primer sequences adapted from Sabol et al. (Sabol et al., 1998), which generates PCR amplicons of sizes 291 bp (2-repeat allele), 321 bp (3-repeat allele), 336 bp (3.5-repeat allele), 351 bp (4-repeat allele), and 381 bp (5-repeat allele). The forward primer was fluorescently labelled using VIC™ in order to facilitate capillary electrophoresis. For statistical analyses, *MAOA*-uVNTR alleles were grouped according to low activity (2-repeat [291 bp] and 3-repeat [321 bp] alleles) and high-activity (4-repeat [351 bp] and 5-repeat [381 bp]) alleles.

Capillary electrophoresis was performed on an ABI Prism 3730 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) at the Central Analytical Facility (CAF-SUN Unit, Stellenbosch, South Africa). The 5-HTTLPR and rs25531 variants have been found to represent putatively functional haplotypes (Hu et al., 2006), with the L-G haplotype exhibiting

similar transcriptional activity to the S-allele. Therefore, for statistical analysis, haplotypes containing these two variants were collapsed into two groups: those with high transcriptional activity (L-A alleles [designated as H']) and those with low transcriptional activity (L-G + S-alleles [designated as L']). Given that the *SLC6A4* 5-HTTLPR and STin2 variants have been found to possess a combined effect on gene expression, and because our results indicated that the STin2.12/STin2.12 genotype and 5-HTTLPR L'-allele were risk factors for appetitive aggression, we combined the 5-HTTLPR-rs25531 and STin2 VNTR variants according to the number of risk factors each variant combination possessed (Aluja et al., 2009; Payer et al., 2012): no risk factors (H'/H' + STin2.10), one risk factor (H'/H' + STin2.12/STin2.12, H'/L' + STin2.10), two risk factors (H'/L' + STin2.12/STin2.12, L'/L' + STin2.10) or three risk factors (L'/L' + STin2.12/STin2.12). To verify the genotypes from each variant investigated, 10% of the sample was randomly selected, PCR amplified and sequenced using primers flanking each of the variants. The sequences were analysed using the BIOEdit software (http://www.mbio.ncsu.edu/BioEdit/bioedit.html).

5.2.3 Statistical data analysis

Hinsberger et al. (Hinsberger et al., 2016c) previously conducted a path analysis in order to investigate the role of attraction to violence in the context of ongoing stress, and found that attraction to violence was predicted by the witnessed traumatic events as well as victimisation (Hinsberger et al., 2016c). In addition, the aim of the study was to identify genetic variants associated with appetitive aggression, and because instrumental / proactive aggression is usually significantly correlated with reactive aggression (Weierstall and Elbert, 2011), we included the BPAQ score in the regression model as a covariate. The outcome data (level of appetitive aggression, assessed by means of the AAS score) were distributed as a bounded rare event, i.e., a Poisson-like distribution. Therefore, we regressed AAS score against genotype, adjusting known AAS covariates in this sample, namely witnessed and self-experienced trauma (Hinsberger et al., 2016c), as well as age and BPAQ score. All analyses were performed using R v3.2.2 and base functions of R (R Core Team, 2017). Multiple testing correction was implemented using Benjamini-Hochberg false discovery rate (FDR) (Benjamini and Hochberg, 1995).

5.3 RESULTS

5.3.1 Demographic and clinical variables

Participants ranged in age between 14 and 40 years, with a median of 21 years (IQR: 19-24 years). All participants were of isiXhosa ethnicity, and the majority (80.7%; n = 238) had not

completed high school. Of the 19.3% who had completed high school, four (1.4%) attended college (Hinsberger et al., 2016c). On average, participants witnessed 10.2 (SD = 2.6) traumatic events. The average number of self-experienced trauma types was 8.4 (SD = 3.0). The AAS scores ranged from 0 to 60, with a median of 12 (IQR: 6-23.5) (Hinsberger et al., 2016c; Sommer et al., 2016). The AAS total score was found to be negatively correlated with age ($r = –0.11$; $p = 0.07$), although this was not statistically significant. As in Hinsberger et al. (Hinsberger et al., 2016c), both self-experienced trauma and witnessed trauma were found to be positively correlated with AAS total score ($r = 0.38$ [$p < 0.001$] and $r = 0.32$ [$p < 0.001$], respectively).

5.3.2 Genetic variables

All genotype calls were 100% concordant with sequencing results. Genotypes for STin2 and *MAOA*-uVNTR genotypes were in HWE ($p=0.734$ and $p=1.0$, respectively). The genotypes for 5-HTTLPR and rs25531 were not in HWE ($p<0.001$ for both variants). Genotype distribution summaries for the variants are provided in Table 5.1.

Table 5. 1: Genotype counts and frequencies (%) of SLC6A4 and MAOA variants. P-values are from exact tests of Hardy-Weinberg equilibrium.

Variant	Genotype	Count (%)	Mean residual value (SD)	HWE p-value
rs25531	AA	209 (72.1)	-0.45 (3.0)	<0.001
	AG	50 (17.2)	-0.63 (3.1)	
	GG	31 (10.7)	-0.261 (3.1)	
5-HTTLPR	LL	122 (42.1)	-0.47 (2.9)	<0.001
	LS	160 (55.2)	-0.58 (3.0)	
	SS	8 (2.8)	1.86 (2.7)	
5-HTTLPR-rs25531	L'L'	86 (29.7)	-0.17 (3.1)	<0.001
	L'H'	115 (39.7)	-0.68 (3.1)	
	H'H'	89 (30.6)	-0.46 (3.0)	
STin2*	12/12	174 (60.0)	-0.41 (3.0)	0.734
	12/10	103 (35.5)	-0.68 (3.1)	
	10/10	13 (4.5)	0.64 (2.6)	
MAOA-uVNTR	*MAOA*-L	136 (49.4)	-0.33 (3.1)	0.660**
	MAOA-H	139 (50.5)	-0.59 (2.9)	

Abbreviations: *5*-HTTLPR*:* serotonin transporter-linked polymorphic region; *MAOA:* monoamine oxidase A; HWE: Hardy-Weinberg equilibrium. *Alleles are represented as repeats: 12 repeat = 301 bp (high expressing); 10 repeat = 267 bp (low-expressing allele). **HWE was calculated based on ungrouped *MAOA* allele frequencies; individual frequencies are as follows: 291 bp (2-repeat) = 19 (6.9%), 321 bp (3-repeat) = 117 (42.5%), 351 bp (4-repeat) = 134 (48.7%), 381 bp (5-repeat) = 5 (1.8%). *MAOA*-L: *MAOA*-uVNTR low-expressing allele (2-repeat + 3-repeat alleles); *MAOA*-H*: MAOA*-uVNTR high-expressing allele (4-repeat + 5-repeat alleles)

5.3.3 Statistical analysis

The known covariates of appetitive aggression (experienced and witnessed trauma) in the current sample (Hinsberger et al., 2016b), as well as age, BPAQ score and each of the genetic variants (additive inheritance model) were regressed on AAS total score, using Poisson regression.

Under the additive genetic model, the AAS score increased, on average, by 1.04 points (95% confidence interval (CI): 1.00–1.08) with the addition of each L' 5-HTTLPR-rs25531 allele

combination (p=0.057) (Table 2). The STin2 VNTR was found to be significantly associated with AAS score, with the addition of each STin2.12 allele increasing the AAS score by a factor of 1.09 points (95% CI: 1.03–1.14) (p=0.003). When the three *SLC6A4* variants were investigated for their combined effects on appetitive aggression, AAS score was found to increase by a factor of 1.12 (95% CI: 1.077–1.080) with the addition of each genetic risk factor (L' and STin2.12/STin2.12) to the 5-HTTLPR-rs25531-STin2 combination (p=8.00 x 10^{-8}). *MAOA* uVNTR was not found to be associated with appetitive aggression in the present study (p=0.728) (Table 5.2).

Table 5. 2: Association between SLC6A4 and MAOA variants under investigation in the current study, using additive genetic models.

Variant	Risk allele	β (SE)	95% CI	Nominal p-value	Corrected p-value
5-HTTLPR-rs25531 (additive)	L'	0.039 (0.019)	0.0013–0.077	0.043	0.057
STin2	STin2.10	-0.008 (0.026)	-0.133– -0.0.31	0.002	0.003
5-HTTLPR-rs25531-STin2 VNTR	L' STin2.12/STin2.12	0.114	0.074 – 0.154	2.88 x 10^{-8}	8.00 x 10^{-8}
MAOA	*MAOA*-L	0.0108 (0.031)	-0.050– -0.072	0.728	0.728

Abbreviations: *5-HTTLPR*: serotonin transporter-linked polymorphic region; L' refers to the 5-HTTLPR-rs25531 low-expressing allele combination*; MAOA*: monoamine oxidase A; STin2.10 refers to the 10-repeat allele (267 bp); *MAOA-L* refers to the low-expressing *MAOA*-uVNTR allele combination (2- and 3-repeat alleles)

5.4 DISCUSSION

This is the first study to investigate the relationship between the *SLC6A4* and *MAOA* genes and appetitive aggression in a South African male population of Xhosa ethnicity. Serotonin has long been implicated in the aetiology of aggression (Duke et al., 2013), and numerous publications have investigated the association between serotonergic genes and various forms of aggression. However, appetitive aggression has been largely excluded from these investigations.

We observed a significant association between the STin2 VNTR variant and levels of appetitive aggression, measured using the AAS (Weierstall and Elbert, 2011). Here, the STin2.12 repeat allele was found to increase the AAS score by 9% ($p = 0.003$). It is interesting to note that, while STin2 VNTR was associated with reactive aggression in our population as well, the association was found to be in the opposite direction, with the STin2.12 allele reducing BPAQ score by 5% ($p = 7 \times 10^{-5}$). Aggression is a heterogeneous multi-dimensional construct, which can be very broadly divided into reactive (hostile-affective) and proactive (instrumental-predatory) aggression (Reif et al., 2007; Vitiello and Stoff, 1997). Appetitive aggression is a form of proactive aggression, which is defined as goal-oriented, proactive and controlled (Elbert et al., 2018; Miller and Lynam, 2006). Reactive aggression, on the other hand, occurs in response to a perceived threat. Although the value of distinguishing between the two types of aggression has been debated (Bushman and Anderson, 2001; Elbert et al., 2018), the differentiation may have implications regarding intervention, diagnosis and prevention (Kempes et al., 2005). Indeed, unique risk factors have been found to be associated with both types of aggression, and differences in serotonergic functioning may partially underlie the dichotomy. Our results provide the first evidence that the two forms of aggression can be differentiated by *SLC6A4* STin2 genetic variants.

Genes containing the STin2.12 allele have been found to have higher rates of SLC6A4 transcription compared to those with the STin2.10 allele (Fiskerstrand et al., 1999; Hranilovic et al., 2004; MacKenzie and Quinn, 1999b). Increased rates of transcription would, theoretically, result in more efficient clearing and thus reduced availability of serotonin in the synaptic cleft. Serotonin is a key neurotransmitter in the central nervous system, and has been found to be important in numerous brain functions, including neurogenesis, apoptosis and synaptic plasticity. Alterations in serotonin concentrations could thus have consequences for brain function and behaviour (Lesch and Waider, 2012). In line with the currently debated serotonin deficiency hypothesis of human aggression, reduced serotoninergic tone has been found to be associated with increased risk for pathological aggression (Alenina et al., 2009; Audero et al., 2013; Hendricks et al., 2003; Mosienko et al., 2012), although a recent hypothesis by Montoya et al. (2012) suggests that it is the ratio of testosterone to cortisol that predisposes one to aggressive behaviour, and the levels of serotonin that tip the scale in favour of either reactive (low serotonin levels) or instrumental (high serotonin levels) aggression.

Given the latter hypothesis, our present results are notable, in that we observed an association between STin2.12 (associated with lower levels of serotonin) and appetitive

aggression, with the opposite effect observed for reactive aggression. It is, however, important to keep in mind that serotonergic regulation in the central nervous system is highly complex – factors modifying the regulation of serotonin (including genes and environment) will vary between study populations, and depending on interaction between the modifiers, may result in association between variables that differ between studies. What these results do indicate, however, is that there seems to be a U-shaped “Goldilocks” effect, where either too much or too little serotonin is associated with either reactive or appetitive aggression, and may therefore may be useful in differentiating between proactive (appetitive) and reactive aggression.

Literature abounds regarding the association of the *MAOA*-uVNTR low-expressing alleles and aggression (Caspi et al., 2002; Foley et al., 2004; Haberstick et al., 2005). However, no significant association was observed between *MAOA*-uVNTR and either appetitive or reactive aggression. The lack of evidence of association between *MAOA*-uVNTR and appetitive aggression in our study may be due to a number of factors, including a lack of power to detect possibly small effects, lack of LD of the *MAOA*-uVNTR with the actual causal variant, and a difference in study designs.

The lack of HWE for the 5-HTTLPR and rs25531 variants is interesting, although perhaps unsurprising in the present context, given the sample used in the investigation. Deviation from HWE may indicate, amongst others, population stratification, inbreeding or genotyping error. For HWE to be fulfilled, a number of assumptions, including random mating, lack of selection according to genotype and absence of mutation or migration should be met. It is unlikely that deviation from HWE in the present study is due to genotyping error, as we validated genotype results by sequencing a randomly selected 10% of the sample for all the variants investigated.

We did not formally test for population stratification in our sample; however, all participants were of Xhosa ethnicity, a group of predominantly Bantu-speaking individuals in South Africa. The Xhosa population is currently the second-largest ethnic group in the country, constituting approximately 18% of the South African population (Drögemöller et al., 2010). Although no in-depth analysis has been performed to study the underlying genetic substructure in the Xhosa population, the Niger-Kordafarian linguistic subgroup to which they belong has been found to exhibit relative genetic homogeneity (Bryc et al., 2010; Tishkoff et al., 2009; Veeramah et al., 2012). In addition, the Xhosa population is also characterised by cultural and ethnic isolation and thus less likely to present with genetic and phenotypic heterogeneity(Niehaus et al., 2005).

Deviations from HWE in the present study are thus likely to be the result of selection bias. Participants were a highly select group of males recruited from two low-socioeconomic areas, in the Western Cape, known for high levels of violence and PTSD (Seedat et al., 2009b). Recruitment focussed on males who were former young offenders (identified via a reintegration programme) or were at risk of perpetrating crimes (recruited via police stations or concerned family members) (Hinsberger et al., 2016c; Sommer et al., 2016). The 5-HTTLPR and rs25531 variants may thus be associated with an as yet uninvestigated trait in this specific population, causing HWE to deviate significantly.

The current study is a novel one, which yields interesting findings. However, the results should be interpreted in the context of some important limitations. First, the specificity of the population in the current study invalidates generalisation to other populations, necessitating replication of the results in samples recruited from the general population. Second, as mentioned above, we did not correct for population stratification in our Xhosa sample. Although there is historical and cultural evidence to suggest that the Xhosa population may be, for all intents and purposes, genetically homogenous, this needs to be tested empirically, in a sample with sufficient power to provide the required resolution. In addition, the effect sizes for each of the significant genetic associations reported are small, necessitating replication studies using increased numbers of samples to attain sufficient power to identify robust associations between STin2 and appetitive and reactive aggression.

The study represents the first to investigate the association between genetic variants in *SLC6A4* and *MAOA* and appetitive aggression in male South Africans of Xhosa ethnicity who were identified as being at high risk for perpetrating violence. Although the selected variants have been widely studied in reactive or impulsive types of aggressive behaviour, the current study is the first to investigate 5-HTTLPR, STin2 and *MAOA*-uVNTR polymorphisms in the context of appetitive aggression. We provide, for the first time, preliminary evidence suggesting that STin2 VNTR may be important in distinguishing appetitive from reactive aggression. However, given the aforementioned limitations, the present results should be interpreted with caution until such time as they are replicated in a scientifically rigorous manner.

CHAPTER 6

6.1 GENERAL DISCUSSION AND CONCLUSION

This research aimed to investigate and understand the epigenetic (DNA methylation), telomere length and genetic variations involved in appetitive aggression and PTSD symptom severity in a cohort of young Black South African men of Xhosa ethnicity. All participants were recruited from Khayelitsha and Gugulethu townships of Cape Town, South Africa. In a South African context, “townships” is a low-socioeconomic setting, often characterised by continuous exposure to violent threats and traumatic stress (Hinsberger et al., 2016b, 2016a; Sommer et al., 2016). Exposure to constant violent threats has been found to result in several complex behavioural problems, such as aggression, and mental health disorders, such as PTSD (Abrahams and Jewkes, 2005; Crombach et al., 2013; Hecker et al., 2013; Kaminer et al., 2008). Previous studies have observed an association between the development of aggression and exposure to violent threats, either as a victim or a witness of violent activities (Crombach et al., 2013; Hecker et al., 2015a; Hinsberger et al., 2016b; Nandi et al., 2015; Weierstall et al., 2013b). Furthermore, exposure to violent threats during childhood has been shown to lead to a cycle of violence, whereby victims of violence become violent offenders themselves (Catani et al., 2009; Crombach and Elbert, 2014b; Elbert et al., 2006; Hinsberger et al., 2016b). Overall, growing up under impoverished settings with hostility, and experiences of trauma is associated with violent behaviour and PTSD development (Abrahams and Jewkes, 2005; Atwoli et al., 2013; Norman et al., 2010).

PTSD is a devastating psychiatric disorder, which can be caused by experiencing stressful traumatic events such as violence. On the other side, appetitive aggression is a goal-driven aggressive behaviour with an intention of gaining a social status through a self-rewarding lust and joy of inflicting pain by acting violently without any danger or provocation (Augsburger et al., 2017; Elbert et al., 2010; Vitiello and Stoff, 1997; Weierstall et al., 2011). Appetitive aggression has been found to have a buffering effect against the development of PTSD (Crombach and Elbert, 2014a; Nandi et al., 2015). The protective effect provided by appetitive aggression may benefit the individual to cope under constant exposure to violent threats (Weierstall et al., 2012b). However, individuals who develop PTSD tend to exhibit reactive aggression, another form of aggression characterised by maladaptive symptoms such as a desire to seek revenge (Kerig et al., 2016, 2012; MacNair, 2002). Reactive aggression initially develops as a defensive mechanism against immediate harm. However,

reactive aggressive behaviour can advance into an internal motivation to perpetrate violence in the absence of a harmful situation (Kerig et al., 2016). Overall, aggression and PTSD can severely affect the normal functioning and quality of life of an individual (Kessler et al., 1995; Neuner et al., 2004a; Weierstall et al., 2011).

Continuous exposure to traumatic events can affect the anatomy of the brain, together with its molecular responses and memory (Elbert et al., 2006; Vukojevic et al., 2014). The change in the structure of the brain can result in cognitive dysfunction, leading to change in the brain's activities and the state of mind (Catani et al., 2009; Elbert et al., 2006). Several parts of the brain such as the frontal cortex, hippocampus, amygdala, and hypothalamus are affected by exposure to traumatic stress; and this trauma is embedded in memories (Barik et al., 2013; McGowan et al., 2009; Neylan et al., 2014; Yehuda, 2006; Yehuda and LeDoux, 2007). Memories of trauma stimulate a sense of fear within the brain's network, which is characterised by a complex web of emotions, sensory and cognitive circumstantial memories (Elbert and Schauer, 2002; Moran et al., 2014; Neylan et al., 2014; Yehuda, 2006). Thus, cumulative exposure to traumatic stressful life events can have a negative impact on molelular responses, and may manifest through changes in psychological and physiological responses.

Literature shows that sensations that trigger violence are shared with those that are activating feelings of fear seen in PTSD (Elbert and Schauer, 2002; Moran et al., 2014; Weierstall et al., 2012c). Individuals with PTSD often display sensations and emotions that are highly activated while their contextual meaning is elusive. This simply means that sensory signals can stimulate memories of trauma in the absence of harm, leading to an individual having no clear sense of the "here and now"; consequently, images of traumatic experiences reappear, resulting to flashbacks and dissociation (Elbert et al., 2010; Nell, 2006; Weierstall et al., 2013b). On the other hand, individuals with appetitive aggression tend to channel signals of violence into the hunting network, a competitive network to the fear network. Thus, signals that are often associated with fear become linked to a positive thrill of excitement that are associated with appetitive aggression (Elbert et al., 2010; Haer et al., 2013).

The fear and hunting network could be linked because they both share several physiological, psychological and neural substrates (Elbert et al., 2010; Haer et al., 2013; Kolassa and Elbert, 2007). It can be hypothesised that molecular responses found in both PTSD and appetitive aggression could be changed by traumatic and violent events (Crews, 2008; Neylan et al., 2014; Provençal et al., 2014; Weder et al., 2014; Yehuda and Bierer,

2009).These traumatic memories can be retained in the brain and kept at a molecular level. Therefore, investigating the underlying molecular mechanisms that drive both appetitive aggresion and PTSD is essential to elucidate overlapping molecular responses.

Investigating of biological mechanisms in complex disorders like PTSD in conjunction with behavioural problems such as aggression in different settings is vital for understanding common pathways that may be driving the onset of the disorder. Furthermore, investigation of DNA methylation, telomere length and genetic variations could shed light on mechanisms that may distinguish these disorders in different environmental settings. Previous studies have found an association between genetic variations and both PTSD and aggression-related disorders such as impulsivity, conduct disorder and hostility (Brydon et al., 2012; van Dongen et al., 2015; Kang et al., 2017b; Kim et al., 2017; Maddox et al., 2013; O'Donovan et al., 2011; Provençal et al., 2014; Rusiecki et al., 2012; Watkins et al., 2016; Yehuda et al., 2013). Futheremore, an association between DNA methylation or telomere length and PTSD or aggression has been reported (Boks et al., 2014; Provençal et al., 2014; Rusiecki et al., 2013). This previous evidence shows that both DNA methylation and telomere length may be key to understanding mechanims during PTSD and agression.

Studies show that both DNA methylation and telomere length are environmentally sensitive and can be affected by a variety of factors (Boks et al., 2014; Epel et al., 2004; Lansdorp, 2006; McLennan et al., 2016; Yehuda et al., 2013). Furthermore, it has been shown that both DNA methylation and telomere length can be affected by psychological interventions such as CBT (Carlson et al., 2015; Hoge et al., 2013; Jacobs et al., 2011; Tolahunase et al., 2017; Yehuda et al., 2013; Ziegler et al., 2016). Psychotherapeutic interventions such as CBT could be advantageous since they may exact biological changes without the introduction of exogenous factors like medications (Watts et al., 2015; Yehuda et al., 2013). There are other psychotherapeutic interventions, like FORNET that may exact molecular changes in a similar way as CBT. The effect of psychotherapeutic interventions at a molecular level is not well established, particularly since there are inconsistent reports (Hollander, 1999; Olabi and Hall, 2010). Therefore, it is necessary to investigate intervention-induced molecular changes in both PTSD and appetitive aggression since they both share similar molecular responses and pathways.

The current research consisted of longitudinal and cross-sectional studies in young Xhosa Black South African men who were constantly exposed to violent threats. These participants were assessed for appetitive aggression and PTSD symptom severity, using AAS (Weierstall and Elbert, 2011) and PSS-I (Foa and Tolin, 2000), respectively. The longitudinal studies

investigated changes in DNA methylation and relative telomere length in randomised individuals who had received psychotherapeutic interventions and in controls that received non-clinical support. The cross-sectional studies investigated the association between appetitive aggression or PTSD symptom severity and relative telomere length or genetic variations in the serotonin transporter promoter region (5-HTTLPR), intron 2 region (STin2) and *MAOA* gene in a cohort that was initilaly recruited to select participants who met the criteria of high appetitive aggression and high PTSD sympton severity for the longitudinal studies.

Participants were chosen based on high appetitive aggresion and high PTSD symptom severity and were randomised to receive either CBT or FORNET, and compared with a control group. DNA methylation and relative telomere lenght were longitudinally investigated in the three groups. CBT and FORNET are both psycho-behavioural interventions that are based on the principle of exposure (Cohen et al., 2007; van Dam et al., 2013; Elbert et al., 2012; Schauer et al., 2004b). Although both interventions are exposure-based, CBT requires an extensive period and is conducted by a clinically trained personnel such as a clinical psychologist, while FORNET is a briefer intervention that may be offered by a non-clinically trained person (Neuner et al., 2008; Schauer et al., 2011); especially in under resourced settings such as the South African townships. Furthermore, FORNET is designed to treat and rehabilitate traumatised aggressive individuals (Elbert et al., 2012; Hermenau et al., 2013b), and can be applicable across cultures and languages (Adenauer et al., 2011; Neuner et al., 2004b; Schauer et al., 2004b).

We observed differential changes in DNA methylation in three genes in participants who had received CBT and in controls in our longitudinal analysis, while no changes were found in those who received FORNET. DNA methylation was significantly decreased in both *RELN* and *AUTS2* genes over a period of 16 months (on average) post-intervention in the CBT group, while did not significantly change between baseline (prior to the intervention) and first follow-up post-intervention in the same group. In both FORNET and control groups, methylation in the *AUTS2* gene did not change significantly over time. However, methylation in the *RELN* gene significantly increased in the control group (TAU) over time. Furthermore, we found reduced DNA methylation in the *COMT* gene between baseline and first follow-up post-intervention in the CBT group, while methylation was significantly increased between first and second follow-up visits post-intervention in the same group. We anticipated this change in DNA methylation since previous studies have reported changes in DNA methylation over a period of time due to enviromental stressors such as combat (Boks et al.,

2014) or after receiving a psychotherapeutic intervention such as CBT (Roberts et al., 2014; Yehuda et al., 2013).

Another enviromentally sensitive biological marker, telomere length, similar to DNA methylation was also observed to differentially change. Telomere length increased significantly in both CBT and control groups over time, while no significant changes in telomere length was observed in participants who received FORNET intervention. Changes in telomere length after psychotherapeutic intervention were similarly expected as with DNA methylation. Previous studies have found signficant modification in telomere length when individuals were longitudinally analysed after exposure to stressful life events (Humphreys et al., 2016; Küffer et al., 2016; Shalev et al., 2014; Verhoeven et al., 2015) or after receiving psychotherapeutic interventions (Carlson et al., 2013; Jacobs et al., 2011; Tolahunase et al., 2017).

Results of both DNA methylation and telomere length were not significantly associated with either appetitive aggression or PTSD symptom severity in all groups. Individuals who had received FORNET were found to have reduced PTSD symptom severity at first follow-up post-intervention, though at second follow-up post-intervention, symptom scores did not differ significantly with those found at first follow-up, although remained lower compared to baseline. We did not observe a reduction in appetitive aggresion after FORNET, despite previous studies having shown that this intervention reduces aggression and attraction to violence in youth Burundians and former Congolese combatants, respectively (Crombach and Elbert, 2015; Hermenau et al., 2013b). Looking at both DNA methylation and telomere length, and their relationship with PTSD symptom severity, we can hypothesise that the effect of intervention influenced the stability of both DNA methylation and telomere length in those individuals who received FORNET, though we did not find a statistically significant association after longitudinal analysis. This stability in both DNA methylation and telomere length may be linked to the improvement in PTSD symptom severity. Indeed, a number of complex pathways, such as the stress response pathway (HPA axis) are affected during stressful encounters, which could lead to changes in telomere length and DNA methylation of specific genes (Boks et al., 2014; Jergović et al., 2014; Roberts et al., 2015; Yehuda et al., 2013).

We found that CBT intervention did not reduce PTSD symptom severity or appetitive aggression as we had anticipated. A study by Yehuda at al. (2013) found that differential DNA methylation was associated with symptom improvement in PTSD. Individuals who had improved PTSD symptom severity after receiving the PE, a psychological intervention used in conjunction with CBT, were found to have decreased methylation in the promoter region in

the *FKBP5* gene. Thus, the decrease in methylation of this gene was associated with reduction in PTSD symptom severity after intervention (Yehuda et al., 2013). Furthermore, we did not find an association between relative telomere length and both appetitive aggression and PTSD symptom severity in any of the investigated groups. Previous studies have observed that psychotherapeutic interventions can contribute to telomere length change (Carlson et al., 2015; Daubenmier et al., 2012; Hoge et al., 2013; Jacobs et al., 2011).

We expected to observe changes in telomere length, which could be associated with changes in appetitive aggression or PTSD symptom severity. However, we observed significantly increased relative telomere length in participants who had received CBT intervention, which was not associated with changes in either appetitive aggression or PTSD symptom severity. Psychotherapeutic interventions such as Mindfulness, Meditation and Yoga have been reported to affect telomere length (Carlson et al., 2015; Daubenmier et al., 2012; Jacobs et al., 2011). Individuals who have been subjected to these interventions have been shown to have improved symptoms of chronic stress and anxiety, which was also associated with increased or stable telomere length compared to non-treated participants (Daubenmier et al., 2012; Lavretsky et al., 2013). Epel et al. (2009) had predicted that individuals receiving psychotherapeutic interventions such as Meditation training had increased cognitive behaviour, which would increase positive attitude, thus in turn, might influence telomere length to remain stable.

Changes in relative telomere length observed in the CBT group suggest that the intervention may be influencing molecular responses that are essential in the processing of trauma load and trauma memories. The relationship between relative telomere length and PTSD symptom severity is not clear in individuals who received FORNET intervention; despite this PTSD symptom severity was significantly reduced. It is still possible that FORNET may have affected molecular mechanisms causing relative telomere length to remain stable, while reducing PTSD. Previously, Carlson et al. (2015) found that cancer survivors who had been given a mindfulness intervention had stable telomere length compared to controls. This study corroborates our findings in this cohort of young Xhosa men.

We cannot provide an explanation for the significant increased relative telomere length in the control group. These findings further demonstrate the complexity of studying environmentally sensitive biomarkers like telomere length and DNA methylation in complex disorders such as PTSD and comorbid problems like appetitive aggression. It is known that appetitive aggression can be adaptive and serve as a coping strategy against stressful life events

(Weierstall et al., 2013b). Therefore, since our cohort consisted of participants who are constantly exposed to violent threats as seen in the townships of South Africa, we could extrapolate that this may have served as protection against PTSD (Hinsberger et al., 2016c; Sommer et al., 2016; Weierstall et al., 2013b). Appetitive aggression may have triggered similar mechanisms affecting telomere length as observed after CBT (Hecker et al., 2013; Weierstall et al., 2012a). This increase in telomere length in controls may also suggest that an alternative molecular response system may be induced by the stressful situations such as living in the townships. Beery et al. (2012) found that stressed animals had elevated telomerase compared to controls, which may have been indicating a resilient mechanism against stress.

Variation in DNA methylation in *AUTS2, RELN* and *COMT* genes across time points further indicates the complexity of mechanims involved during stress processing as seen with relative telomere length. Interestingly, both *AUTS2* and *RELN* genes are essential during early development of life, and also play a significant role even in adulthood (Abdolmaleky et al., 2005; Niu et al., 2008). *RELN* gene encodes for a protein, reelin, that has a crucial role in neuronal migration, the branching of axons, as well as the formation of synapses (Abdolmaleky et al., 2005; Levenson et al., 2006; Niu et al., 2008). *AUTS2* gene encodes for a protein, AUTS2, which is responsible for the activation and maintenance of proper neuronal development and migration (Hori et al., 2014; Katoh et al., 2006; Li et al., 2015; Sadok and Marshall, 2014; Yoshizawa et al., 2005). On the other hand, *COMT* gene encodes for the COMT, an enzyme responsible for the degradation of several neurotransmitters such as dopamine, epinephrine, norepinephrine, and catecholamines in the CNS (Eisenberg et al., 1999; Lotta et al., 1995).

RELN gene methylation has been observed in the development of psychiatric disorders such as schizophrenia and bipolar disorder (Abdolmaleky et al., 2005; Grayson and Guidotti, 2013; Guidotti et al., 2016; Nabil Fikri et al., 2017; Tamura et al., 2007). It has been shown that *RELN* hypermethylation is associated with cognitive impairment in patients with schizophrenia compared to controls (Abdolmaleky et al., 2005). We found that *RELN* methylation was significantly reduced after CBT, which suggests that molecular responses involving reelin protein expression may be affected. It is possible that the reduced methylation in *RELN* could potentially increase the expression of this gene as it hypermethylation in the promoter regions of this gene is associated with low gene expression (Abdolmaleky et al., 2006, 2005; Grayson et al., 2006). Therefore, decreased methylation in the *RELN* gene after CBT may suggest that the expression of this protein was reduced. This

is why in future studies, we need to analyse protein expression to determine the effects of differential methylation on protein expression

AUTS2 gene methylation can affect normal expression of this gene, which could affect several molecular pathways involving AUTS2 protein. Dysregulation of the *AUTS2* is associated with changes in behaviour, particularly in anxiety-related disorders such as autism spectrum disorder and drug addiction (Dang et al., 2014; Hori et al., 2015; Zhang et al., 2014). AUTS2 protein has been shown to affect neuronal development and migration (Hori et al., 2014; Oksenberg et al., 2014). Therefore, methylation in the *AUTS2* gene may impact these aforementioned pathways and lead to the development of psychiatric disorders such as PTSD or behavioural problems like aggression. In addition, differential methylation of this gene post intervention could suggest that the response to psychotherapy involves the function of this gene. This is unsurprising since this gene has been shown to be essential for development, particularly early in life (Oksenberg et al., 2014).

Previous investigations have shown that methylation in the promoter region of *COMT* gene was associated with impaired fear inhibition in individuals with PTSD (Norrholm et al., 2013). Furthermore, animal studies have shown that methylation of *COMT* gene was linked with decreased gene expression, and was associated with stress response in rats (Ursini et al., 2011). We observed reduced methylation in the *COMT* gene after CBT intervention between baseline and first follow-up post-intervention. This may suggest that CBT has an effect on molecular responses. However, this did not translate to changes in clinical outcomes such as appetitive aggression and PTSD symptom severity over time. We think that further studies are required to clarify the association between DNA methylation and appetitive aggression or PTSD symptom severity in robust and appropriately powered scientific studies.

The comorbidity of appetitive aggression and PTSD may complicate the understanding of biological responses such as DNA methylation and telomere length mechanisms in response to psychotherapeutic interventions. Although appetitive aggression can buffer against PTSD development (Hecker et al., 2013; Weierstall et al., 2012c), it is still not clear how far this interaction may affect biological reponses. We did not find any significant interaction between appetitive aggression and PTSD symptom severity, and their effects on DNA methylation or relative telomere length. Given the limitations of our study, further studies are required to robustly analyse the comorbidity of appetitive aggression and PTSD symptom severity and their effect on molecular mechanisms such as telomere length and DNA methylation.
This study represents the first to investigate the association between genetic variants in *SLC6A4* and *MAOA* and appetitive aggression in male South Africans of Xhosa ethnicity who

were identified to be at high risk of perpetrating violence (Hemmings et al., 2018). Although the selected variants have been widely studied in reactive or impulsive types of aggressive behaviour, the current study is the first to investigate 5-HTTLPR, STin2 and *MAOA*-uVNTR polymorphisms in the context of appetitive aggression. We provide, for the first time, preliminary evidence suggesting that STin2 VNTR may be important in distinguishing appetitive from reactive aggression (Hemmings et al., 2018). However, given the aforementioned limitations, the present results should be interpreted with caution until they are replicated in a scientifically rigorous manner.

Cross-sectional investigation of relative telomere length further enhanced our understanding of biological interaction between appetitive aggression and PTSD, while genetic variation added an important understanding to differentiate appetitive aggression and reactive aggression. The integration of enviromentally sensitive markers is vital since both appetitive aggression and PTSD share similar biological mechanisms (Elbert et al., 2010; Haer et al., 2013). It can provide a better understading of the onset of appetitive aggression or PTSD. It is important to understand the contribution of genes such as *SLC6A4* and *MAOA*, which have been implicated in the development of both aggressive behaviour (Duke et al., 2013; Tatarelli et al., 2014) and PTSD (Antypa et al., 2013; Svob Strac et al., 2016a; Wang et al., 2011; Xie et al., 2012). Although previous studies have shown an association between these genes and different types of aggression as well as in PTSD, no studies have looked at both appetitive aggression and PTSD, and their association with genetic variations and telomere length. Moreover, most studies have been conducted overseas (Antypa et al., 2013; Baker et al., 2008; Craig and Halton, 2009; Godar et al., 2016), and few studies have been done on African populations.

Our findings of longer relative telomere length association with higher PTSD symptom severity is in agreement with previous studies (Boks et al., 2014; Küffer et al., 2016). However, other studies have reported the opposite of shortened telomere length in PTSD symptom severity (Jergović et al., 2014; Kim et al., 2017; Ladwig et al., 2013; Malan-Muller et al., 2013; O'Donovan et al., 2011). The inconsistencies of the relationship between telomere length and PTSD symptom severity should be interpreted with caution as investigated participants are coming from different environmental settings. For example, experiences of violent trauma in combat by soldiers differs from experiences of trauma in communities.This effect of different traumas could alter the relationship between telomere length and the comorbid of appetitive aggression and PTSD, which could have an effect on biological mechanisms such as DNA methylation and telomere length.

Biological responses triggered by traumatic stress can be difficult to understand since they involve complex pathways such as the HPA axis. Moreover, hormones like cortisol have been shown to highly expressed during stress response (Lucassen et al., 2013; Wagner et al., 2011), and have been implicated in affecting telomere length and immunological pathways (Bellavance and Rivest, 2014; Cain and Cidlowski, 2017; Deslauriers et al., 2017; Neigh and Ali, 2016; Rohleder and Karl, 2006). Researchers have shown that elevated cortisol can result in shortened telomere length after acute stress via deactivating immunological responses in cells (Choi et al., 2008; Norrback and Roos, 1997). In turn, these immunological cells are unable to produce sufficient telomerase enzyme that is responsible for the production of telomere repeats and thus resulting in shortened telomeres (Choi et al., 2008; Norrback and Roos, 1997). However, the extent of these immunological responses on the stress response system is not known. Therefore, more studies are required to investigate the link between immune cells, telomere length and psychiatric disorders like PTSD and aggressive behaviour such as appetitive aggression. Future studies will need to take environmental factors, geographical settings, genetic variations, gene-environmental interactions into consideration, and the specificity of genes that may play a significant role during telomere length maintenance.

Previous studies have demonstrated a link between genetic variation in crucial genes and the development of aggressive behaviour (Aluja et al., 2009; Oades et al., 2008). We also found that genetic variation in the *SLC6A4* gene in the STin2 VNTR, specifically the STin2.12 allele, was associated with reactive aggression (Hemmings et al., 2018). Moreover, other studies have shown that the STin2.12 repeat is associated with increased impulsivity (Aluja et al., 2009; Oades et al., 2008), and increased aggression in children (Beitchman et al., 2003; Davidge et al., 2004). Our finding showing an association between STin2.12 and reactive aggression is important in distinguishing different types of aggression and how these aggression types relate to PTSD symptom severity (Hemmings et al., 2018).

Previous studies have shown the association of the *MAOA*-uVNTR low-expressing alleles and aggression (Caspi et al., 2002; Foley et al., 2004; Haberstick et al., 2005). However, we did not find a significant association between *MAOA*-uVNTR and either appetitive or reactive aggression. The lack of an association between *MAOA*-uVNTR and appetitive aggression in our study could be due to the ethnicity and culture of our studies population and possible gene-environment interactions. Previously, Butovskaya et al (2013) found that the differences in cultures of Hadza and Datoga tribes in Tanzanian population had differential effects on aggression. It was shown that there was an association between aggression and genetic variation in Dagota men, while there was no association found in Hadza men

(Butovskaya et al., 2018, 2013). Generally, Dagota men are known to be physically aggressive while Hadza men are peaceful and hunter-gatherers (Butovskaya et al., 2013).

Taken together, our findings from longitudinal studies of DNA methylation and relative telomere length in relation to psychotherapeutic interventions, and cross-sectional studies of genetic variations and relative telomere length demonstrate the complex interplay between molecular mechanisms and the development of psychiatric disorders such as PTSD and behavioural problems like appetitive aggression.

6.2 General limitations to the study

The findings from this research are novel and provide insights into the association between DNA methylation, telomere length and genetic variations in relation to appetitive aggression or PTSD symptom severity. Although we have obtained interesting results, these findings need to be interpreted cautiously in the context of certain limitations of the study. Our longitudinal study that aimed to investigate an association between DNA methylation, telomere length and appetitive aggression or PTSD symptom severity in randomised groups of participants consisted of a number of limitations; the number of participants per groups was small, which was exacerbated by dropouts of some participants during the course of the intervention. This dropout was worsened by the fact that the majority of the participants were unemployed and were from unstable families, and locating them proved challenging. Some of the participants in the intervention trial had relocated to other provinces while some were incarcerated for other offences that they had committed in their communities. Furthermore, participants were released from eth camp into their communities after the intervention, where they experienced traumatic and stressful events as before.

Analyses of appetitive aggression and PTSD symptom severity were conducted a couple of months after the interventions. This delay in analysis may have buffered the effect of the intervention, as participants continued to re-experience violent trauma and stressful life events in their community. Future studies should consider follow-up over a shorter time period (e.g., at two or three months) to assess whether there are shorter term maintenance effects of the interventions.

The statistical power was compromised by the use of a liberal approach of analysis, the Fisher's LSD (Meier, 2006). However, the Fisher's LSD has strengths to account for repeated measures in multiple comparisons and ensure that even a small significance can be detected. If we had enough participants, it would have been better to use a more stringent

approach such as Bonferroni's correction (Armstrong, 2014; Armstrong et al., 2011) [ref]. Therefore, our results from the longitudinal studies of both DNA methylation (Chapter 2) and telomere length (Chapter 3) need to be interpreted with caution and require replication with larger cohorts.

This research did not investigate gene expression in association with DNA methylation. Gene expression data could have provided additional insights. Therefore, for a better understanding of the impact of DNA methylation and potential mechanisms involved, it would be best to investigate both these biological mechanisms concurrently in future studies.

Another limitation of this study was the selected sample in terms of its gender and ethnicity, which limits the generalisability of the results to other groups. Finally, although all clinical questionnaires were translated into the IsiXhosa language, it is possible that we could have lost information as the researchers were not IsiXhosa speaking. In addition, it has to be noted that most of the participants did not have Grade 12 education. These factors may affect the quality of information obtained during clinical assessment.

6.3 Conclusion

In conclusion, we have shown that DNA methylation and relative telomere length may change differentially in response to different psychotherapeutic interventions. Taken together these longitudinal studies and cross-sectional studies of genetic variation and relative telomere length demonstrate the complex interplay between molecular mechanisms and PTSD and behavioural problems like appetitive aggression. Future studies should consider a larger sample for increased statistical power. In addition, it will be essential to consider different genders and ethnicities to ensure greater representativity and generalisability. Overall, our findings lay a foundation to the understanding of appetitive aggression and PTSD symptom severity in relation to molecular changes such as DNA methylation, telomere length and genetic variation.

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