Effect of adverse childhood experiences and occurrence of uVNTR polymorphism in monoamine oxidase A gene in recidivist violent offenders: Forensic implications

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by

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DECLARATION

I, Siva Prasad, M. S. hereby declare that this thesis entitled "***Effect of adverse childhood experiences and occurrence of uVNTR polymorphism in monoamine oxidase A gene in recidivist violent offenders: Forensic implications***" is the report of the original research work carried out by me under the supervision of Dr. Y. Shibu Vardhanan, Associate Professor and Head, Department of Zoology, University of Calicut for the award of the degree of Doctor of Philosophy in Zoology of the University of Calicut and further that this thesis contains no materials previously submitted for any degree, diploma, associateship, fellowship or other similar title of any other Universities or Institutes.

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This thesis is dedicated to the society

"We owe our children – the most vulnerable citizens in any society – a life free from violence and fear. In order to ensure this, we must be tireless in our efforts not only to attain peace, justice and prosperity for countries, but also for communities and members of the same family. We must address the roots of violence"

***Nelson Mandela* (2002)**

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ABBREVIATIONS

$	Dollar
%	Percentage
µg	Microgram
µl	Microlitre
µM	Micromolar
2R	Two repeats
3R	Three repeats
4R	Four repeats
5-HIAA	5-hydroxyindoleacetic acid
5-HIET	5- hydroxyindolethanol
5-HTOL	5-hydroxytryptophol
A	Adenine
A_{230}	Absorbance at 230nm
A_{260}	Absorbance at 260nm
A_{280}	Absorbance at 280nm
ACE-IQ	Adverse Childhood Experiences-International Questionnaire
ADH	Alcohol dehydrogenase
AL	Adolescence-limited
ALDH	Aldehyde dehydrogenase
ALR	Aldehyde reductase
ANOVA	Analysis of variance
APA	American Psychological Association
ASPD	Antisocial personality disorder
ATP	Adenosine Triphosphate
AUD	Alcohol Use Disorders
B	Regression coefficient
bp	Base pair
Buffer AE	DNA elusion buffer, Qiagen
C	Cytosine
CAG	Glutamine codon
CDC	Centre for Disease Control, U.S.
CI	Circle Inspector
CSF	Cerebrospinal fluid
DA	Dopamine
DARE	Drug Abuse Resistance Education

df	Degrees of freedom
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide triphosphate
DSM	Diagnostic and Statistical Manual of Mental Disorders
DySP	Deputy Superintendent of Police
E	Epinephrine
EDTA	Ethylenediaminetetraacetic acid
Enz	Enzyme
FAD	Flavin Adenine Dinucleotide
$FADH_2$	Flavin Adenine Dinucleotide Reduced
FASTA	Fast Alignment Search Tool Algorithm
FFT	Functional Family Therapy
fMRI	Functional magnetic resonance imaging
G	Guanine
GxE	Gene-Environment interaction
H_2O_2	Hydrogen Peroxide
HRBs	Health risk behaviours
HVA	Homovanillic acid
IPC	Indian Penal Code
K.D	Known Depredator
KAAPA	Kerala Anti-Social Activities (Prevention) Act, 2007
Kb	Kilobase
kDa	Kilo Dalton
Km	Michaelis constant
KO	Knockout
LCP	Life-course-persistent
M	Mean
M	Molar
MAOA	Monoamine oxidase A (Human and other primate gene)
Maoa	Monoamine oxidase A (Mouse gene)
Maob	Monoamine oxidase B (Mouse gene)
MAO-A	Monoamine oxidase A (Protein)
MAOA-H	High expressing MAOA allele
MAOA-L	Low expressing MAOA allele
MAOA-u	Monoamine oxidase A-upstream
MAO-B	Monoamine oxidase B (Protein)
MAPK	Mitogen-activated protein kinases

ml	Millilitre
mM	Millimolar
MPQ	Multidimensional Personality Questionnaire
MST	Multisystemic therapy
N	Number of participants
$NAD(P)^+$	Nicotinamide adenine dinucleotide phosphate
NCBI	National Center for Biotechnology Information
ND	Norrie disease
NDP	Norrie disease pseudoglima
NE	Norepinephrine
NH_4^+	Ammonium
NIMH	National Institute of Mental Health
nm	Nanometre
O_2	Molecular Oxygen
ºC	Degree Celsius
OCD	Obsessive-compulsive disorder
OD	Optical density
OG-500	Oragene DNA kit
P value	Probability value
PACT	Positive Achievement Change Tool
PCR	Polymerase Chain Reaction
PD	Parkinson's disease
PEA	2-phenylethylamine
PET	Positron Emission Tomography
PFC	Prefrontal Cortex
pH	Power of Hydrogen ion
PMT	Parent management training
Project TND	Project Towards No Drug Abuse
r	Correlation coefficient
R^2	Coefficient of determination
RDoC	Research Domain Criteria
rpm	Rotations per minutes
SAP	Special Assistance Programme
SD	Standard Deviation
SE	Standard Error
SPSS	Statistical Package for Social Science
SSRIs	Serotonin selective reuptake inhibitors
SULT	Sulfotransferase
SVC	Serious, violent and chronic delinquency
T	Thymine

T1AM	3-iodothyronamine
TAG	Stop codon
TBE	Tris Boric acid EDTA Buffer
Tris	Trishydroxymethylaminomethane
U.S.	United States
UGC	University Grants Commission
UGT	UDP-Glucuronyltransferase
UV	Ultra violet
V	Volt
VMA	Vanilliylmandelic acid
VNTR	Variable number of tandem repeat
WHO	World Health Organization
X	X Chromosome
Xp	The short arm of the X Chromosome
β	Standardized coefficient of regression
χ^2	Chi-square analysis

ABSTRACT

Aggression and violent behaviour are widespread in the world and causes serious threats to public safety. Violence among youth is a global health problem which includes various acts from physical fight, bullying, to more severe physical and sexual assault to homicide. Some offenders even after severe sentences of imprisonment show violent criminal recidivism. Several researches have been conducted worldwide to identify biological subtypes of pathological aggression and to develop new kind of taxonomy for mental disorders based on modern research approaches in neuroscience, genetics and behavioural science. Candidate gene-environment (cGxE) is associated with the development of pathological aggression and related violent behaviour.

In the present study, the 30 bp repeat polymorphism in the upstream region in the promoter of *MAOA* gene (*MAOA*-uVNTR), adverse childhood experiences (ACEs) and health risk behaviours (HRBs) of male recidivist violent offenders (n = 35) and control group (n = 32) were analyzed. It was assumed that there will be high occurrence of short repeat allele of *MAOA*-uVNTR polymorphism, more ACEs and HRBs in recidivist violent offenders. The aims of this thesis were to identify the interaction of allelic variants of *MAOA*-uVNTR polymorphism with adverse childhood experiences (ACEs) and also the interaction of ACEs with HRBs in the recidivist violent offenders in order to gain a better understanding of these hypotheses in the samples in Kerala population.

Genotyping of the DNA from buccal swabs taken from the participants (n = 67) revealed high occurrence of 3.5 repeat (3.5R) of *MAOA*-uVNTR polymorphism in cases (100 %) and controls (84.4 %). Remaining controls were identified with 4.5 repeat (4.5R) of *MAOA*-

uVNTR polymorphism. Though 4.5R allele was not identified in cases, the noted difference was statistically significant ($p = 0.015$). The data suggested that the 3.5R variant of *MAOA*- uVNTR polymorphism, the gene coding for the key regulatory enzyme, MAOA of the serotonergic neurotransmission system, is associated with violent criminal behaviour in the male recidivist violent offenders. Assessment of 13 categories of ACEs with modified ACE-IQ questionnaire revealed that mean scores of each categories of ACEs and total ACE were significantly high ($p < 0.001$) in cases. The data showed that cases experienced extreme ACEs. A significant association ($p < 0.05$) between ages of onset of crime depending upon the ACE exposure in cases was also found. Most of the ACE categories were interrelated and largest correlation was found between the presence of collective violence and household violence ($r = 0.813$). All ACE categories except emotional neglect and community violence, showed a significantly high positive correlation with violent criminality ($r > 0.30$). The highly significant correlation ($r = 0.927$, $p < 0.001$) of total ACE score and violent criminality in cases suggested that adverse childhood experiences influence violent criminal behaviour. Using total ACE score as the predictor variable, 86.0 % of the variability in the violent criminality as the dependent variable was accounted ($p < 0.001$). Whereas, in multiple regression analysis, collective ACEs significantly predicted violent criminality (F (13, 21) = 34.693, $p < 0.001$) and was found to be the best-fitting model ($R^2 = 0.956$). The result suggested that ACEs can be used as a predictor for violent criminality in cases.

Prevalence of health risk behaviours (HRBs) showed a significant association ($p < 0.001$) between cases and controls. Also there was early onset of HRBs in cases and exposure to extreme ACEs favored early onset of criminality. Mean score of HRBs were

significantly high ($p < 0.001$) in cases which suggested that cases were highly involved in these behaviours. Interrelationship with each HRB categories and total ACE score were found and the total scores were significantly correlated (r (67) = 0.684, $p < 0.001$) which showed an increase of HRBs with exposure to ACEs. No significant relations were found between total violent criminality and HRBs in cases (r = 0.128, p = 0.232). Total ACE score was found to be a good predictor of onset of HRBs in participants ($p < 0.001$, $R^2 = 0.468$).

The participants were grouped on the basis of allelic variants of *MAOA*-uVNTR polymorphism and the mean total ACE score were analyzed among them. The results showed that cases with 3.5R allele of *MAOA*-uVNTR experienced significantly more ACEs ($p < 0.001$) than controls with 3.5R and 4.5R of *MAOA*-uVNTR. Also, mean total ACE score of the controls with 3.5R and 4.5R *MAOA*-uVNTR were found to be slightly significant ($p = 0.051$) between each other.

In conclusion, born with the short allele (3.5R) *MAOA*-uVNTR genotype and growing up by facing frequent high ACEs before the age of 18, have developed pathological aggression and related problem behaviour leading to antisocial behaviour, violence and criminality in recidivist violent offenders in this study. These findings could be viewed in line with the Moffit's developmental taxonomy theory and life-course persistent (LCP) offending group. The moderating effect of high activity allele (4.5R) of *MAOA*-uVNTR on the impact of childhood maltreatment in the development of antisocial behaviour in males was also observed in this study. Strengths, limitations, policy implications and further scope of the study are also discussed.

Keywords: Aggression, violence, offenders, recidivism, *MAOA*-uVNTR, adverse childhood experiences.

SIVA PRASAD M. S. “EFFECT OF ADVERSE CHILDHOOD EXPERIENCES AND OCCURRENCE OF UVNTR POLYMORPHISM IN MONOAMINE OXIDASE A GENE IN RECIDIVIST VIOLENT OFFENDERS: FORENSIC IMPLICATIONS”. THESIS. DEPARTMENT OF ZOOLOGY, UNIVERSITY OF CALICUT, 2018.

Chapter 1
INTRODUCTION

According to the report of the World Health Organization, it is estimated that 0.2 million homicide occur worldwide each year among youths aged between 10-29 years (World Health Organization, 2016). This constitutes 43% of the total number of homicides happening globally each year. Most of these homicides happen in low and middle income countries. Majority of the perpetrators of the homicide are males in all countries and globally 83% of victims of homicide are youth males (World Health Organization, 2016). World Health Organization defined violence as the "intentional use of physical force or power, threatened or actual, against oneself, or against a group or community that either results in or has a high likelihood of resulting in injury, death, psychological harm, maldevelopment or deprivation" (Krug *et al*., 2002). Violence among youth is a global health problem which includes various acts from physical fight, bullying, to more severe physical and sexual assault to homicide.

Aggression and violent behaviour are widespread in the world and causes serious threats to public safety (Ramsay *et al*., 2014; Shrivastava *et al*., 2016; Vazsonyi *et al*., 2018). The problems associated with aggressive behaviour or pathological aggression has devastating consequences on individuals, families and socioeconomic sector (Brown *et al*., 2015). Due to the non-fatal violent crimes, the total cost estimated to exceed $180 billion/ year in U.S. alone (McCollister *et al*., 2010). This include direct costs for legal and medical procedures, incarceration of perpetrator, etc., and indirect costs like lost earnings, time, productivity, etc. The current strategies

employed to treat pathological aggression in U.S. are based on empirical approaches which include the combination of anticonvulsant and antidepressant medications with behavioural/ cognitive therapy, which are effective moderately only (Volavka *et al*., 2006). This is due the inadequate understanding of the dimensional nature of externalizing disorders and aggressive behaviours (Krueger *et al*., 2005).

Anderson and Bushman (2002) defined aggression as "any behaviour directed toward another individual that is carried out with the *proximate* (immediate) intent to cause harm. In addition, the perpetrator must believe that the behaviour will harm the target and that the target is motivated to avoid the behaviour". On the other hand, violence is considered to be a subset of aggression. The extreme form of aggression that has severe physical harm as its goal is termed as violence (Bushman & Huesmann, 2010). Aggressive and violent behaviours can be better conceptualized as being on a range of severity with comparatively minor acts of aggression (e.g., pushing) at the low end of the spectrum and violence (e.g., homicide) at the high end of the spectrum (Allen & Anderson, 2017). Although one could argue that aggression and violence are not exactly the same, the concepts are clearly related. When using the term 'aggression' in this thesis, this refers to the aggressive behaviour leading to violence. Also main focus is given on the legal outcomes of this behaviour like convictions for violent offences which includes wrongful restrain, assault, hurt, rape, attempt to murder and murder.

Diagnostic criterias defined by the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) is not distinguishing the dimensional commonalities in the conditions which are predominantly characterized by aggressive psychopathology. These conditions include antisocial personality disorder and intermittent explosive disorder in adulthood; conduct disorder and oppositional defiant disorder in childhood and adolescence (Godar *et al*., 2016). To overcome these conceptual restrictions, National Institute of Mental Health (NIMH), U.S has started a project named Research Domain Criteria (RDoC) in 2010. The aims of RDoC is to identify biological subtypes of pathological aggression and to develop new kind of taxonomy for mental disorders based on modern research approaches in neuroscience, genetics and behavioural science (Morris & Cuthbert, 2012; Carcone & Ruocco, 2017).

Within the broader group of negative valence systems, aggression is regarded as a core frustrative, non-reward construct, by the RDoC framework (Godar *et al*., 2016). Based on the autonomic responses, information processing mechanisms, emotional and motivational state of the perpetrators, RDoC has recognized a preliminary difference between proactive and reactive aggression (Connor *et al*., 2004; Vitaro & Brendgen, 2011; Fite *et al*., 2012).

In the case of proactive aggression, an offender typically initiates it in an instrumental fashion which is directed towards a positive outcome for rewarding or attaining power or dominance (Vitaro *et al*., 1998; Vitaro & Brendgen, 2011). Proactive aggression is linked to callous-unemotional traits which is characterized by lack of

remorse, empathy and flattened emotional responses for other's suffering (Frick *et al*., 2003). Robust environmental and genetic underpinning has been shown for this temperament (Viding *et al*., 2007). Substance abuse and social learning from models of violent behaviour are also linked to proactive aggression (Dodge *et al*., 1997; Connor *et al*., 2004).

Reactive aggression is characterized by exaggerated and/or uncontrollable defensive responses to perceived threat or provocation (Eisenberg & Fabes, 1992). Individuals with reactive aggression have higher propensity to recognize threat or provocation in response to neutral stimuli (Vitaro & Brendgen, 2011). Responses of the reactive aggression are hostile in nature and are typically associated with impairments in emotion regulation, social information processing, impulse control, verbal intelligence and executive functioning (Dodge *et al*., 1997; Lemerise & Arsenio, 2000; Connor *et al*., 2003; Dodge & Pettit, 2003; Marsee & Frick, 2007; Arsenio *et al*., 2009). Reactive aggression has been associated with child abuse and adverse childhood experiences (Shields & Cicchetti, 1998; Connor *et al*., 2004). In the developmental trajectory, the prevalence and severity of this subtype shows a peak at young ages and followed by a gradual remission which signifies a larger ability to repress emotional outbursts.

According to the report of National Crime Records Bureau, in the state of Kerala, the rate of violent crimes has increased from 28.3 % in 2005 to 37.9 % in 2016 (Ministry of Home Affairs, 2018). Also, the rate of recidivism amongst persons arrested under IPC crimes has increased from 1.8 % in 2005 to 3.7 % in 2016 in Kerala. In the

country, the quantum of total violent crimes is continuously increasing from 2009 to 2016 (Ministry of Home Affairs, 2018).

In criminal justice context recidivism can be defined as the reversion of an individual to criminal behaviour after the person has been convicted of a prior offense, sentenced and presumably corrected. Violent criminal recidivism rates remain very high among certain groups of offenders. Many offenders, even after severe sentences of imprisonment, repeatedly fail to desist from crime and reintegrate into the community as law-abiding citizens. Imprisonment, in itself, is incapable of addressing the offender's social integration issues. Even when solid prison programs have helped offenders to achieve some progress during detention, that progress is often lost in some offenders.

Traditionally, in criminological theory and research, environmental explanations for aggression and violent behaviour are examined. The sources of antisocial behaviour in neighbourhoods, parenting practices, peer association etc., are generally analyzed by mainstream criminological theories. Recently, in this field there has been a shift for exploring the possibility of genetic factors that influence aggression and violent behaviour (Boccio *et al*., 2018).

1.1 Behavioural genetics

In behavioural genetic research, quantitative genetic methodologies are used to estimate the influence of genetic and environmental influences on variations in human behaviour. In this field several researches are being conducted in twins to know their behaviour in connection with the shared and non-shared environments

(Plomin *et al*., 2013). To obtain the heritability estimates behavioural geneticists compared the behaviour of twins living in the same or different household through twin and adoption studies respectively. Thus, by incorporating twins with different levels of genetic relatedness, the genetic and environmental influence on variance in human behaviour is estimated (Beaver, 2007; Beaver, 2011; Barnes *et al*., 2014). In general, the findings from twin and adoption studies revealed that the variance in behavioural phenotype is approximately 50 % attributable to genetic factor (Polderman *et al*., 2015). Similarly, several studies have also demonstrated that the variance in antisocial phenotypes, including aggression and violent behaviour is 50 % attributable to genetic factors and remaining 50 % is due to the non-shared environmental factors (Mason & Frick, 1994; Miles & Carey, 1997; Rhee & Waldman, 2002; Ferguson, 2010; Boccio *et al*., 2018). Information related to the specific genes involved in the creating phenotypic variance are not provided by the methodologies and analytical approaches used in behavioural genetic research.

1.2 Molecular genetics

Studies in molecular genetics pinpointed the genes involved in the phenotypic outcomes and revealed the associations between possessing particular genetic marker and behavioural outcomes (Boccio *et al*., 2018). Also, using the methodologies in molecular genetics, the linkage of several genes with antisocial phenotypes including aggressive and violent behaviour has been demonstrated (Caspi *et al*., 2002; Guo *et al*., 2007).

Small subsets of genes in the human genome are 'polymorphic' and alleles of such polymorphic genes within a population accounts for the variations in physical and behavioural phenotypes observed between people (Mielke, 2006). In a population most of the polymorphic genes do not appear to cause functional differences in phenotypes as the difference between the available alleles do not lead to change in protein structure (Boccio *et al*., 2018). However, two kind of polymorphisms, single nucleotide polymorphisms (SNPs) and variable number of tandem repeats polymorphisms (VNTRs) correspond to functional differences that lead to the production of different phenotypes (Mielke, 2006). Also, these polymorphisms in genes lead to the synthesis of different amino acids that affect the protein's structure and function. The polymorphisms affect phenotypes in several ways. In the polygenic effect of polymorphic genes, multiple genes work together probabilistically to influence an individual's phenotype. Aggression and violent behaviour are likely the result of polygenic effect of genes and several genes associated with neurotransmission have been shown to influence these behaviours including antisocial behaviour (Gill *et al*., 1997; Beaver *et al*., 2008; Beaver *et al*., 2009; Boccio *et al*., 2018).

Most of the genes thought to be related to extreme violence are involved in the detection, transportation and breaking down of neurotransmitters, especially serotonin. The relationship between cognition, emotion and aggression is documented and neural circuitries such as the serotonergic system have been shown to play a key role in regulating aggressive behaviour (Reif *et al*., 2007). There have been

many major studies published so far in the area of antisocial behaviour and brain chemistry which indicates the role of serotonin in the aetiology of aggressive and antisocial behaviour (Raine, 1993; Vassos *et al*., 2014; Nilsson *et al*., 2018).

1.3 Serotonin deficiency and aggression

Serotonin or 5-hydroxytryptamine (5-HT) is a neurotransmitter that has inhibitory properties and act as the body's natural brake system (Virkkunen & Linnoila, 1993). The release of serotonin modulates behaviours by dampening innate drives and instincts, mainly the impulsive behaviours (Siegel, 2004). Serotonin is produced by the raphe nuclei in the middle of the brain stem which send projections through the cerebral hemispheres. Serotonin does not cross blood-brain barrier and hence in the brain it is synthesized in the selective cell bodies in the brain stem. The substrate for serotonin synthesis is dietary tryptophan. Tryptophan in plasma passes through the blood brain barrier and it is hydroxylated to tryptophan hydroxylase at the 5^{th} position, then decarboxylated by L-amino acid decarboxylase, forming serotonin and stored in vesicles for future release (Siegel, 2004).

Serotonin has been shown to play a key role in the initiation, execution and treatment of aggressive acts (Berman *et al*., 1997; Gowin *et al*., 2010). Several studies have confirmed a negative correlation between serotonin levels in the brain and aggression and commonly known as the serotonin-deficiency hypothesis of aggression (Berman *et al*., 1997). According to this hypothesis, aggression is characterized by low brain serotonin levels. Given that extreme

violence is often unplanned and spontaneous there has been a lot of interest in examining the precise role that the serotonergic system plays in the development of antisocial behaviours (Gottfredson & Hirschi, 1990). The original serotonin deficiency hypothesis was based on a negative correlation between trait like impulsive aggression/ violence and the CSF concentration of the serotonin metabolite in humans and other primates (Brown & Goodwin, 1986; Berman *et al*., 1997).

In humans, low levels of the serotonin metabolite, 5-hydroxyindole acetic acid in the cerebrospinal fluid have been associated with aggression and other forms of antisocial behaviour including assault, arson, murder, child abuse and as well as in violent forms of suicide (Berman *et al*., 1997). Moreover, a substantial number of non-primate animal studies have revealed that the propensity to exhibit excessive and abnormal forms of aggression is similarly linked to long-term reduced brain serotonin activity (Miczek *et al*., 2002). In addition, studies have shown that chronically lowering or heightening brain serotonin provokes increased or reduced levels of aggressive behaviour respectively, further supporting the serotonergic deficiency hypothesis of aggressive behaviour (Weinshenker & Siegel, 2002). Similar studies in humans and non-human primates confirm a negative correlation between serotonin turnover and aggressive tendencies (Brown *et al*., 1982). Although the findings have been mixed, several meta-analyses found statistically significant negative association between serotonin levels and extreme violence (Balaban *et al*., 1996; Moore *et al*., 2002; Duke *et al*., 2013). In other words, lower levels of

serotonin were found to correspond with greater involvement in acts of extreme violence.

Diets which are low in tryptophan have been shown to increase aggression in animals and humans (Cleare & Bond, 1995). The relevance of the serotonergic pathway in violent behaviour is further supported by the ability of parachlorophenylalanine, an irreversible inhibitor of serotonin biosynthesis to heighten aggression in animals (de Boer & Koolhaas, 2005). The extracellular levels of serotonin also depend on its reuptake and metabolic degradation by monoamine oxidase (MAO) enzymes (Fornai *et al*., 1999). Serotonin selective reuptake inhibitors (SSRIs), which increase the availability of serotonin in the brain and are used commonly for the treatment of depression, were also effective for the treatment of aggression (Olivier *et al*., 1989). The depletion of *Maoa* gene in rodents produced an increase in aggressiveness (Cases *et al*., 1995). These findings indicate that availability of serotonin at the synapses influences aggressive behaviour.

1.4 Role of Monoamine oxidase (MAO) enzyme in aggression

Monoamine oxidase (amine: oxygen oxidoreductase (deaminating) (flavine-containing); MAO; EC 1.4.3.4) is a mitochondrial-bound flavoprotein, catalyzing the oxidative deamination of monoamine neurotransmitters and trace amines (Peter, 1986). MAO also serves a cytoprotective role by degrading exogenous amines, which exert their toxicity by affecting cardiovascular and endocrine homeostasis. Monoamine Oxidase was discovered by Mary

Hare (later Mary Bernheim) in 1928. This unknown enzyme was named Tyramine Oxidase (Hare, 1928) and later renamed MAO by E.A. Zeller (Zeller, 1938; Slotkin, 1999).

1.4.1 Functions of MAO

MAO catalyses the oxidative deamination of monoamines to the corresponding aldehydes:

$$RCH_2NH_2 + O_2 + H_3O^+ \rightarrow RCHO + NH_4^+ + H_2O_2$$

Flavin adenine dinucleotide (FAD) is required as a covalently bound redox cofactor for this reaction and there are three major steps involved in this reaction (Edmondson *et al*., 2009). After the formation of a FAD-substrate adduct, the cofactor is reduced to its hydroquinone form ($FADH_2$) and the amine is transformed into the corresponding imine:

$$Enz - FAD + RCH_2NH_2 \rightarrow Enz - FADH_2 + RCH = NH$$

The imine is spontaneously hydrolyzed when it is dissociated from the enzyme with the production of aldehyde and ammonium:

$$RCH = NH + H_3O^+ \rightarrow RCHO + NH_4^+$$

The rate- limiting step of the whole enzymatic process is the formation of hydrogen peroxide and molecular oxygen as result of the reoxidation of $FADH_2$ to FAD:

$$Enz - FADH_2 + O_2 \rightarrow Enz - FAD + H_2O_2$$

The key brain neurotransmitters such as serotonin (5-hydroxytryptamine, 5-HT), dopamine (DA), norepinephrine (NE) and epinephrine (E) are the endogenous substrates of MAO. Also, a number of trace amines such as tryptamine, tyramine, octopamine, 2-phenylethylamine (PEA) and 3-iodothyronamine (T1AM) are also metabolized by MAO (Bortolato & Shih, 2011). Thus the role of MAO is essential to modulate the neuroendocrine regulation of the central nervous system, many peripheral organs and in the homeostasis of these compounds.

Aldehydes produced as a result of the enzymatic action of MAO are toxic species which need to be converted into less harmful metabolites (O'Brien *et al*., 2005). These aldehydes are oxidized to the corresponding carboxylic acid by NAD $(P)^+$ dependent aldehyde dehydrogenase (ALDH) which is functionally coupled with MAO enzyme. Alternatively, aldehydes are reduced to alcohols or glycols by aldehyde reductase (ALR) or alcohol dehydrogenase (ADH) depending on the location and the intracellular conditions.

1.4.2 Metabolism of serotonin by MAO

In the metabolism of serotonin, by the joint action of MAO and ALDH, serotonin is converted to 5-hydroxyindolacetic acid (5-HIAA) which is rapidly eliminated by diffusion into the bloodstream and excreted through the kidneys by glomerular filtration and active tubular excretion (Udenfriend *et al*., 1956; Despopoulos & Weissbach, 1957) (Figure 1.1).

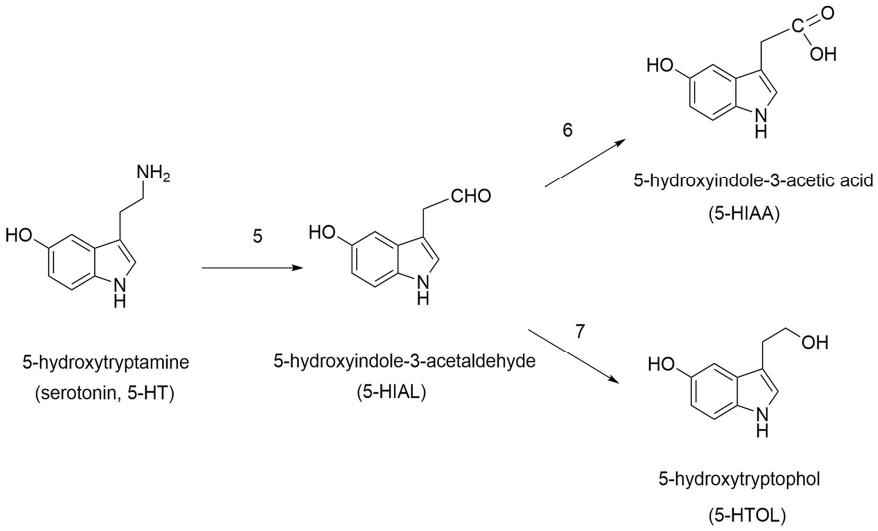

Figure 1.1: Biotransformation of serotonin by phase I enzymes. Oxidative deamination of serotonin by monoamine oxidase (5) produces 5-hydroxyindole- 3-acetaldehyde, which can be further oxidized to 5-hydroxyindole-3-acetic acid by aldehyde dehydrogenase (6) or can be reduced to 5-hydroxytryptophol by alcohol dehydrogenase (7) (Szeitz & Bandiera, 2018).

For the measurement of plasma serotonin content urinary levels of 5-HIAA are used as an index. Only 1-5% of serotonin is converted to 5- hydroxyindolethanol/ 5-hydroxytryptophol (5-HIET/ 5-HTOL) by either ALR or ADH (Svensson *et al*., 1999). Compounds like ethanol that compete with endogenous serotonin metabolite for ALDH can enhance the amount of 5- HIET (Helander *et al*., 1993). Before being excreted in urine, serotonin and its metabolites undergo conjugation with glucuronic acid or sulphate (Figure 1.2).

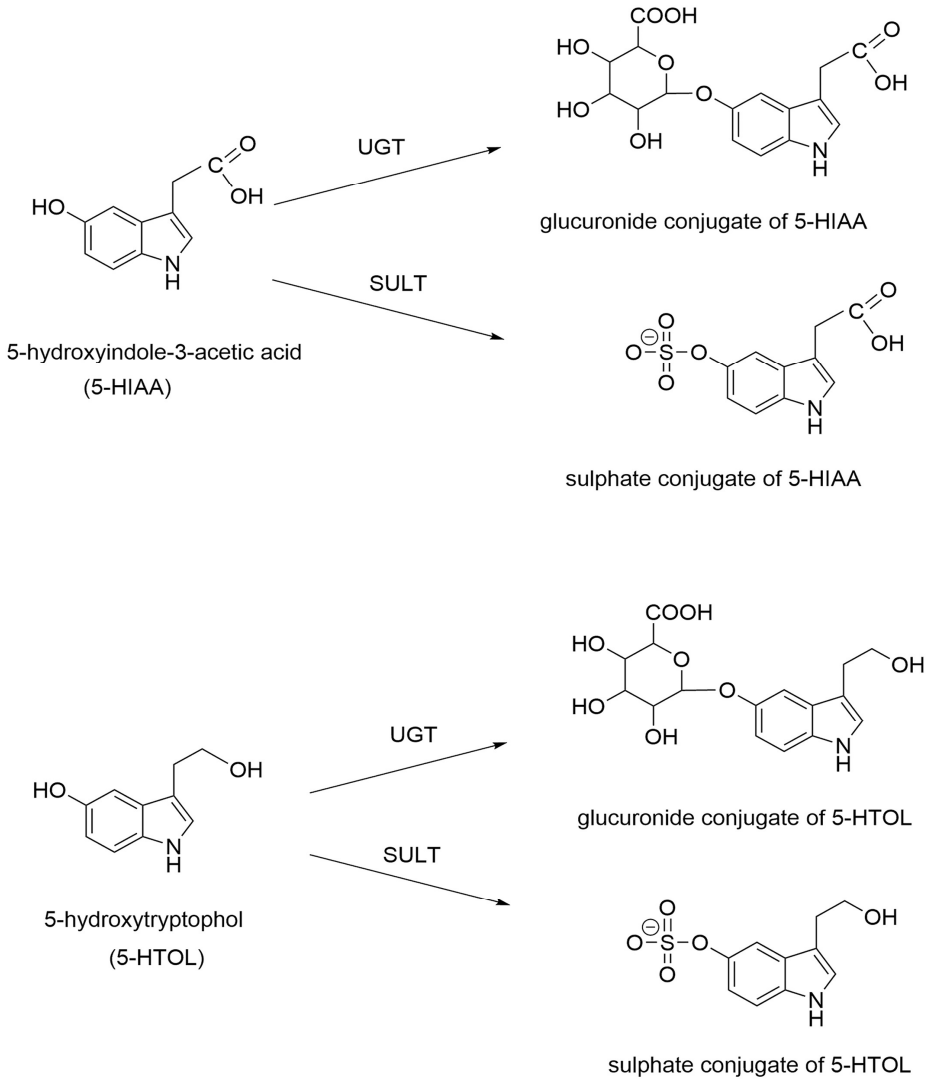

Figure 1.2: Biotransformation of serotonin and its major phase I metabolite by phase II enzymes. Conjugation catalyzed by glucuronyl transferase (UGT) or sulfotransferase (SULT) produces the glucuronide or sulfate conjugate of serotonin and 5-hydroxyindole-3-acetic acid (Szeitz & Bandiera, 2018).

The phenolic hydroxyl group of serotonin, 5-HTOL and 5-HIAA is the major site of conjugation by glucuronyl transferase (UGT) or sulfotransferase (SULT) enzymes (Heiander *et al*., 1995).

Both these enzymes also play a vital role in the detoxification and excretion of a large number of endogenous and xenobiotic compounds. 5-HIAA and 5-HIAA-sulfate are the most abundant metabolites of serotonin in human brain and cerebrospinal fluid (Suominen *et al*., 2013; Szeitz & Bandiera, 2018).

1.4.3 Role of MAO in maintaining the redox state of neurons

For the regulation of intracellular redox state in neurons and other cells, the function of MAO enzyme is highly essential. Hydrogen peroxide, one of the by-product of MAO mediated reaction, is a potent oxidizer. Even though hydrogen peroxide is converted into water and molecular oxygen by catalase (Jones & Suggett, 1968), superoxide radicals and other reactive oxygen species that are formed as a result of the triggering of hydrogen peroxide, induces mitochondrial and cytoplasmic damage. Antioxidant enzymes like superoxide dismutase, glutathione peroxidase and catalase keeps the overall redox potential in equilibrium under physiological conditions. However, high concentration of the other by-product of the reaction such as ammonia decreases the activity of these antioxidant enzymes and lead to the formation of superoxide radicals (Kosenko *et al*., 1997). In brain, the detoxification of ammonia largely relies on glutamine synthesis in the astrocytes by glutamine synthetase (Rose *et al*., 2013).

In the central nervous system, excess of oxidizing species leads to the death of neurons, glia and thus causes permanent damages which results in neurodegenerative disorders like Parkinson's disease (PD), dementias and Alzheimer's (Danielczyk *et al*., 1988; Grunblatt *et al*., 2005). Thus MAO enzyme serves as the detoxification machinery in the central nervous system by degrading primary, secondary and some

tertiary xenobiotic amines and thus prevents their cardio and neurotoxicity.

1.4.4 Isoenzymes of MAO

MAO family contains two isoenzymes which are termed as MAO-A and MAO-B. Even though there is significant structural overlap between these isoenzymes, they are differentiated by their remarkable difference in preference for substrate, pharmacological responsiveness to inhibitors, anatomical allocation and functional role in behavioural regulation. Initially, a number of experimental data based on the different neurochemical effects of various inhibitors described the existence of multiple forms of MAO isoenzymes. Low doses of clorgyline selectively blocks MAO-A (Johnston, 1968) and nanomolar concentration of (R)-deprenyl inhibits MAO-B (Knoll & Magyar, 1972). Electrophoretically, these two isoenzymes can be separated (Shih & Eiduson, 1969). Later studies showed that MAO-A had high affinity for serotonin and to a lower degree to norepinephrine. The substrates of MAO-B are 2-phenylethylamine and benzylamine. Both MAOs mediates the degradation of dopamine, tyramine and tryptamine, which depends on the species and tissue under consideration (Fornai *et al*., 1999).

Also, in terms of the substrate preference, the dichotomy between MAO-A and MOA-B is not absolute because in the absence of one isoenzyme, some amounts of its non-preferred substrates are deaminated by other (Chen *et al*., 2004). Unequivocal demonstration in the partial compensation mechanism and the physiological significance of these two isoenzymes came in 1988 with the cloning of human *MAOA* and *MAOB* genes (Bach *et al*., 1988). Further studies identified

the structural configuration of both genes. These studies revealed that the genes responsible for these isoenzymes are specifically mapped to Xp11.23-11.4, in a tail-to-tail arrangement with the 3'-coding sequences separated by about 50 kb (Ozelius *et al*., 1988; Chen *et al*., 1992). The study by Grimsby and colleagues (1991) revealed the structure and evolution of these isoenzymes with a suggestion that both genes were derived from duplication of a common ancestor gene (Figure 1.3).

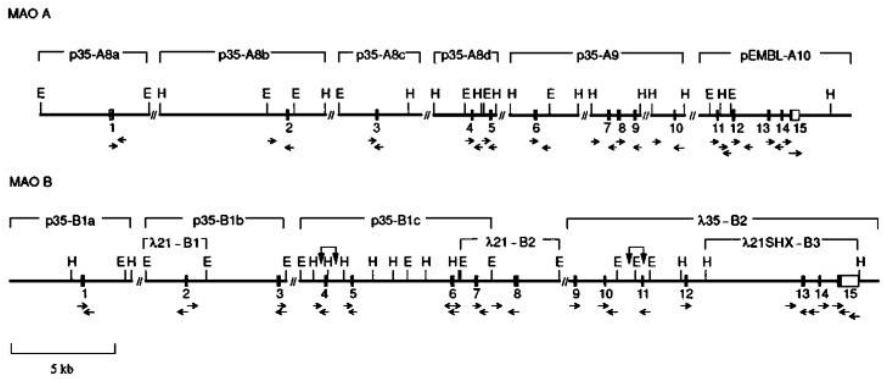

Figure 1.3: Partial structural map of the monoamine oxidase (MAO) A and B genes showing the location of exons. (Filled bars) Coding regions; (unfilled bars) untranslated regions of the exons. Exon numbers are below the bars. (Horizontal arrows) the regions sequenced; (vertical arrows) equivocal assignment of an exon to either restriction fragment. The prefix "λ," λ bacteriophage clones; the prefix "p," pUC19 sub clones of λ phage DNA; E, EcoRI restriction site; H, HindIII restriction site; //, intron gap (Grimsby *et al*., 1991).

According to this study, two proteins of 527 and 520 amino acids with molecular weights of 59.7 and 58.8 kDa were encoded by *MAOA* and *MAOB* respectively. These two genes share approximately 70% sequence identity and an identical intron-exon organization with 15 exons and 14 introns (Grimsby *et al*., 1991). Phylogenetic studies showed the existence of only one MAO in early eukaryotes (Schilling

& Lerch, 1995), teleost fish (Setini *et al*., 2005) and invertebrates (Boutet *et al*., 2004). MAO-A is predominant in frogs in the tadpole stage and MAO-B expression increases though metamorphosis (Nicotra & Senatori, 1988). MAO-B has a much higher Km (lower affinity) for oxygen (~ 250 μM) than MAO-A (~ 6 μM) (Edmondson *et al*., 2004). Both enzymes are anchored to the mitochondrial outer membrane through a transmembrane helix which is located within the carboxyl terminal domain (De Colibus *et al*., 2005). Both MAOA and MAOB are dimeric in their membrane-bound conformations (Binda *et al*., 2007; Upadhyay *et al*., 2008).

1.4.5 Transcription and translation of MAO

Promoters of *Maoa* and *Maob* genes, their mechanisms of activation and repression are extremely different (Shih *et al*., 1994). Human MAO-B expression is activated by protein kinase C and MAPK signaling transduction pathways (Wong *et al*., 2002), while it is decreased by methylation (Wong *et al*., 2003). A key role in the regulation of the expression of both genes is played by the family of the transcription factor Sp1 (Zhu *et al*., 1994; Wong *et al*., 2001). A novel repressor, R1 of *Maoa* gene expression is also identified (Chen *et al*., 2005). Both Sp1 and R1 activities are modulated by glucocorticoids and androgens (Ou *et al*., 2006).

Like other mitochondrial proteins, MAOs are synthesized by free ribosomes in the cytosol (Sagara & Ito, 1982). Both isoenzymes have the signal sequence in the N-terminal domain typical of mitochondrial protein precursors (Roise & Schatz, 1988). The C-

terminus of MAO-B contains the sequence for targeting the mitochondrial outer membrane (Mitoma & Ito, 1992) and attaching to it (Rebrin *et al*., 2001). Even though the specific mechanism of insertion of both MAO-A and MAO-B into the outer mitochondrial membrane is still poorly understood, the same process requires ubiquitin and ATP (Zhuang *et al*., 1988; Zhuang & McCauley, 1989; Zhuang *et al*., 1992).

1.4.6 Distribution of MAO-A and MAO-B

Both the isoenzymes, MAO-A and MAO-B are expressed in most of the mammalian tissues. MAO-A is normally abundant in fibroblasts and placenta, whereas MAO-B is only expressed in platelets and lymphocytes (Bond & Cundall, 1977; Donnelly & Murphy, 1977). In brain most of the regions contain MAO-A and MAO-B, but certain areas display only one isoenzyme. MAO-A is found mainly in catecholaminergic neurons and in particular in the nucleus accumbens, locus coeruleus, hypothalamus and mammillary complex. MAO-B is only expressed in the cell bodies of serotonergic and histaminergic neurons and astrocytes (Konradi *et al*., 1989). In the *in vivo* studies serotonin is mainly metabolized by MAO-A. Hence it is hypothesized that MAO-A protein is segregated to the axon terminals after the translation in cell body (Bortolato *et al*., 2010). Subsequent studies discovered that MAO-B is absent in the mitochondria of the axon terminals (Arai *et al*., 2002) and also documented MAO-A mRNA in the serotonergic cells (Luque *et al*., 1995; Filipenko *et al*., 2002; Wylie *et al*., 2010). The hypothesis for these discrepancies is that in

serotonergic neurons MAO-A proteins are synthesized in the cell body (axon hillock) (Figure 1.4).

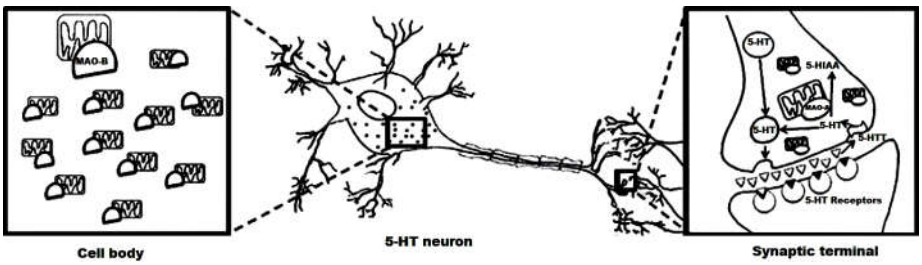

Figure 1.4: Hypothetical compartmentalization of MAO-A and MAO-B in serotonergic neurons. The localization of MAO-B (represented as a semi-oval shape in the left box) is limited to the mitochondria of the cell body and dendrites of the serotonergic neuron. Conversely, MAO-A (schematized as an elliptic shape on the mitochondria in the right box) may be mainly distributed in the synaptic terminals, where it would degrade the serotonin (5-HT) after its reuptake operated by 5-HT transporter (5-HTT) (Bortolato *et al.,* 2010).

These proteins which are anchored to mitochondria are transported by microtubules or other mechanisms to the axon terminals located in different brain areas such as the amygdala, cortex, hippocampus, etc., (Jacobs & Azmitia, 1992). This possibility is indirectly supported by the finding that MAO-B expression is rich in the mitochondria located in the somata of serotonergic neurons, but completely absent from the mitochondria in their axon terminals (Arai *et al.,* 2002). The differential sub cellular location of MAO-B containing and MAO-B free mitochondria reflects their different interactions with kinesins, the motor proteins in charge of anterograde mitochondrial transport through microtubules (Hollenbeck & Saxton, 2005). The identification of MAO-A containing mitochondria in axon terminals suggests that they are efficiently transported to this compartment by microtubules (Westlund *et al.*, 1993). Further investigations regarding the possible interactions between MAO

isoenzymes and kinesin receptors are reasonable to understand the molecular mechanisms behind these differences. The proposed compartmentalization of MAO isoenzymes in serotonergic neurons responds to specific physiological requirements for the proper processing of serotonin synthesis, vesicular uptake and metabolism. Various studies point out the independent regulation of formation and degradation of serotonin in the cell body and axon terminals of serotonergic neurons (Neckers, 1982; Daszuta *et al.*, 1984a; Daszuta *et al.*, 1984b).

Even though serotonin synthesis occurs in the axon terminal, it is physiologically more abundant in the cell body (Daszuta *et al.,* 1984a; Pivac *et al.,* 2003). Thus, in consideration of the high affinity of MAO-A for serotonin, the localization of this enzyme in the soma participates with the gradient-based mechanism of serotonin vesicular uptake. The presence of MAO-A in the synaptic terminal facilitates the degradation of serotonin after its reuptake mediated by serotonin transporter (5-HTT). On the other hand, the presence of MAO-B in the body of serotonergic neurons may prevent the uptake of wrong amine neurotransmitters in the vesicles (Bortolato *et al*., 2010). This compartmentalization of MAO-A facilitates the specific degradation of serotonin in the synaptic terminals and expression of MAO-B in the somata of serotonergic neurons serves protective functions of serotonin (Bortolato *et al*., 2010). Thus, MAO-A is positioned to govern both the availability of monoamine neurotransmitters for vesicular sequestration and their subsequent extra synaptic inactivation following release (Figure 1.5) (Buckholtz & Meyer-Lindenberg, 2008).

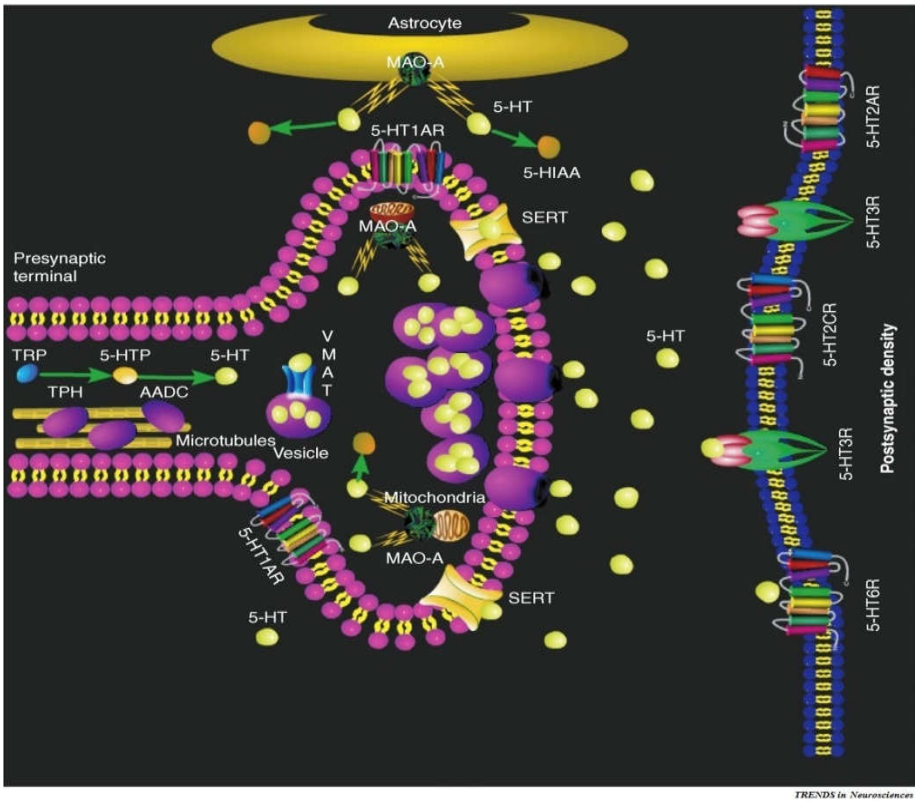

Figure 1.5: Idealized serotonergic synapse depicted here demonstrates the role of MAO-A in the catabolism of serotonin. Serotonin (5-HT) is packaged into synaptic vesicles by the vesicular monoamine transporter (VMAT) and degraded presynaptically by mitochondrial monoamine oxidase A (MAO-A) or extrasynaptically by glially expressed MAOA into 5-hydroxyindoleacetic acid (5-HIAA). Once released, synaptic serotonin can be cleared by the serotonin transporter (SERT) or bind to one of seven classes of serotonin receptors residing on both the pre and postsynaptic membranes (Buckholtz & Meyer-Lindenberg, 2008).

1.5 Role of MAO in behavioural regulation

The development and design of MAO inhibitors was originally driven by the accidental discovery of iproniazid which is a derivative of isoniazid. Antitubercular properties of iproniazid exerted remarkable mood-enhancing properties (Fox & Gibas, 1953) since it was also a

potent irreversible MAO blocker (Zeller & Barsky, 1952). Several researchers postulated that there is a link between MAO, its substrates and mood control and this theory was substantiated by the antidepressant activity of novel irreversible MAO inhibitors such as nialamide (Rowe *et al.,* 1959) and phenelzine (Saunders *et al.*, 1959). The successful use of MAO inhibitors in clinical practice also resulted in many side effects like 'cheese reaction', characterised by lethal hypertensive crisis caused by the absorption of tyramine or other sympathomimetic amines present in fermented food such as cheese, wine, etc., (Anderson *et al.*, 1993). In this condition, high concentration of dietary amines will be circulated in the bloodstream due to the inactivation of intestinal MAO and results in extreme sympathetic activation and systemic vasoconstriction. This paved the way for the development of new categories of antidepressant drugs like tricyclics and selective serotonin reuptake inhibitors (SSRIs) with better tolerability.

Production of reversible MAO-A inhibitors has revived the interest for this category of drugs since these inhibitors can be replaced by dietary amines and do not induce the cheese effect. MAO-A inhibition has been shown to increase serotonin concentrations in the synaptic cleft (Sharp *et al.,* 1997) and to affect firing of serotonergic neurons in the dorsal raphe nucleus (Aghajanian *et al.*, 1970; Blier & de Montigny, 1985). There is a general agreement that the antidepressant properties of MAO inhibitors are actually due to the obstruction of MAO-A, particularly in its action on serotonin

metabolism and the subsequent ability to counter the decline in serotonin observed in depression.

Antidepressant effects of MAO-A inhibitors make them useful for the treatment of number of anxiety spectrum disorders like agoraphobia, social phobia, panic disorder, post-traumatic stress disorder (Cyr & Farrar, 2000), obsessive-compulsive disorder (OCD) (Jenike *et al.,* 1983) and aggression (Welch & Welch, 1968; Sofia, 1969; Raj, 2004) which have been shown to depend on alterations of serotonergic signaling. The involvement of serotonin in the antidepressant effects of MAO-A inhibitors is also indirectly confirmed by the selective inhibition of MAO-B by deprenyl which induces only modest mood-enhancing effects. This may be due to the action of this isoenzyme on oxidative stress also (Bortolato *et al.,* 2008). Thus, MAO-A inhibition reduces the oxidative metabolism of monoamines and presumably increases the availability of serotonin and other monoamines in the brain (Quadros *et al.,* 2010).

1.6 Polymorphisms in *MAOA* gene

Sequencing of *MAOA* gene allowed the classification of its variants (Shih & Thompson, 1999) and their different controls in behavioural regulation. Several polymorphisms of the *MAOA* gene have been identified and among the numerous allelic variants, four polymorphisms have been particularly studied as possible risk factors/ biomarkers for psychiatric disorders (Bortolato & Shih, 2011):

1. *MAOA* (*CA*)n, a dinucleotide repeat polymorphism in intron 2 (Black *et al.,* 1991).

2. 23 bp variable-number tandem repeat (VNTR) near exon 1 (Hinds *et al.,* 1992).

3. *Fnu*4HI and *Eco*RV, two restriction fragment length polymorphisms (Lim *et al.,* 1994).

4. *MAOA*-uVNTR, a 30 bp VNTR polymorphism located 1.2 kb upstream of *MAOA* transcription initiation site (Sabol *et al.*, 1998).

Variants of the first three polymorphisms have been associated with higher susceptibility to alcoholism/ substance abuse in males (Vanyukov *et al.,* 1995) and mental conditions like bipolar disorder (Lim *et al.,* 1994, Lim *et al.,* 1995; Kawada *et al.,* 1995; Rubinsztein *et al.,* 1996). Although these associations were not confirmed by few studies (Craddock *et al.,* 1995; Muramatsu *et al.,* 1997), but was also supported by a meta-analysis (Furlong *et al.,* 1999). The most extensively studied polymorphism related to *MAOA* is the *MAOA*-uVNTR promoter polymorphism.

1.6.1 *MAOA*-uVNTR polymorphism

Genetic studies on the several polymorphic variants of *MAOA* gene have given bulk of clinical evidence towards the association between *MAOA* and aggression (Godar *et al.,* 2016). The functional role of *MAOA* in aggression is evidently proven by the discovery of a variable number tandem repeats (VNTR) polymorphism which is featured by 30-bp repeats of alleles located approximately 1.2 kb upstream of the transcription initiation site (Sabol *et al.,* 1998). This

polymorphism was termed as *MAOA*-upstream (*MAOA*-u) VNTR (Sabol *et al.,* 1998).

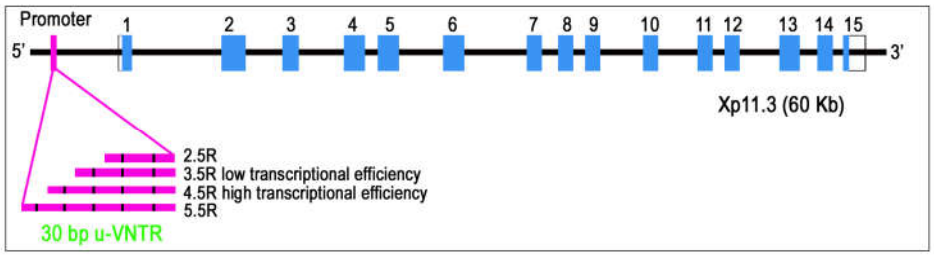

Figure 1.6: Diagrammatic representation of the *MAOA* gene. Showing 15 exons and position of the investigated polymorphism, *MAOA*-u VNTR (Das, 2008).

Sabol and colleagues (1998) reported four variants, "3R", "3.5R", "4R" and "5R" of the VNTR, which corresponds to "3.5R", "4R", "4.5R" and "5.5R" respectively as observed by Jorm and colleagues (2000) in Australian population. Similar findings were reported in the Indian population also (Figure 1.6) (Das *et al*., 2006; Das, 2008; Bhowmik, 2010; Das *et al*., 2011). Out of the seven different allelic variants (2, 3, 3.5, 4, 4.5, 5 and 6) (Sabol *et al.,* 1998; Huang *et al.,* 2004; Das *et al.,* 2006) that have been characterized in the human population, the most common are the three repeats (3R) and four repeats (4R) (Sabol *et al.,* 1998; Deckert *et al.,* 1999; Jonsson *et al.,* 2000). It is estimated that 35-39% and 59-63% of Caucasians is harbouring 3R and 4R of *MAOA*-uVNTR alleles respectively. On the other hand majority of Asian (53-61%), African (52-59%), Hispanic (70%) and Americans are having 3R variants (Sabol *et al.,* 1998; Widom & Brzustowicz, 2006; Rosenberg *et al.,* 2006; Beaver *et al.,* 2013).

The importance of *MAOA*-uVNTR polymorphism arises from its functional nature. There are a number of transcription factor binding sites in the both the 5' and 3' flanking regions of the *MAOA* VNTR (Zhu *et al.,* 1992) and hence this polymorphism affects transcriptional activity of the *MAOA* promoter (Sabol *et al.,* 1998; Deckert *et al.,* 1999). The 2R and 3R allele is associated with the low transcriptional efficiency of the *MAOA* promoter and hence the enzymatic activity of MAO-A is lower than that of 4R variant (Sabol *et al.,* 1998; Deckert *et al.,* 1999; Jonsson *et al.,* 2000), suggesting an optimal length for the regulatory region in *MAOA* gene.

The first studies on VNTR variants in *MAOA* promoter consistently documented higher *MAOA* gene transcription and enzyme activity in association with the 4R allelic variant in transfected cells (Sabol *et al.*, 1998) and in cultures of human male skin fibroblasts (Denney *et al.*, 1999). Conversely, 4R carriers display higher levels of 5-hydroxyindoleacetic acid (5-HIAA) in the cerebrospinal fluid (Jonsson *et al.,* 2000).

Considering the differences in transcriptional efficiency of various alleles of *MAOA*-uVNTR polymorphism, the researchers have commonly pooled the alleles into two groups. The alleles (2R and 3R) that correspond to low MAO-A activity is included in the short allele or low-expressing group (MAOA-L) and the alleles (4R and 5R) which correspond to high MAO-A activity in long allele or high-expressing group (MAOA-H) (Caspi *et al.,* 2002; Buckholtz & Meyer-Lindenberg, 2008). Also, it has to be noted that the transcriptional

efficiency of long repeat allele is still inconsistent (Deckert*et al.,* 1999; Jonsson *et al.,* 2000; Yirmiya *et al*., 2002).

Due to the complications from natural random X-chromosome inactivation in human females, most of the *MAOA* gene studies have focused on males as the males are hemizygous (Hendriks *et al*., 1992; Hendriks *et al*., 1993; Denney *et al.*, 1999; Yirmiya *et al*., 2002; Beaver *et al*., 2013; Buades & Gallardo, 2014; Xu *et al*., 2017).

Human studies have shown that the 2R and 3R variants are associated with behavioural features linked to low MAO-A activity like multi facets of aggression including antisocial personality and hostility (Oreland *et al.,* 2007; Buckholtz & Meyer-Lindenberg, 2008; Williams *et al.,* 2009; Weder *et al.,* 2009). Several impairment in the neurocognitive functions like processing of facial expressions affecting the activation of limbic regions (Lee & Ham, 2008) and blunted response to stress (Brummett *et al.,* 2008) were also related to *MAOA*-uVNTR polymorphism. Results of the studies that are done towards the investigation of association of *MAOA*-uVNTR variants and personality traits like neuroticism, conscientiousness and straightforwardness, are largely conflicting (Garpenstrand *et al.,* 2002; Samochowiec *et al.,* 2004; Contini *et al.,* 2006; Jacob *et al.,* 2005; Rosenberg *et al.,* 2006; Tochigi *et al.,* 2006; Kim *et al.,* 2006), and suggests a specific involvement of *MAOA*-uVNTR polymorphism in aggression (Godar *et al.,* 2016).

1.7 Candidate Gene-Environment interaction (cGxE)

From the time of ancient Greece, the nature-versus-nurture debate has been ongoing (Aristotle, 1984). The contribution of both genetic and environmental factors to health and behaviour is widely accepted today (Pinker, 2003; Nilsson *et al*., 2018). In gene-environment interaction (GxE), depending on the genetic makeup, the ability of the individual may vary to cope with stressful experiences and environments (Craig, 2007). Specific gene associated with certain phenotype is identified in candidate gene-environment interaction (cGxE) and several such studies have identified association of short allele of *MAOA*-uVNTR polymorphism with aggressive and antisocial phenotype (Byrd & Manuck, 2014; Dick *et al*., 2015; Boccio *et al*., 2018; Nilsson *et al*., 2018).

The base of cGxE research is the diathesis-stress hypothesis, which assumes that the risk of adverse outcomes in stressful environments is increased in certain genotypes (Dick, 2011; Manuck & McCaffery, 2014). In relation to aggressive and antisocial behaviour, there are strong evidence for increased chances of developing this behaviour in males who have been to childhood traumas/ adverse experiences and carriers of short allele of *MAOA*-uVNTR (Kim *et al*., 2006; Choe *et al*., 2014; Byrd & Manuck, 2014; Gorodetsky *et al*., 2014; Zhang *et al.*, 2016).

1.8 Childhood Trauma and Maltreatment

Childhood trauma is experienced by most of the youths worldwide. It is estimated that during childhood, more than half of the

children suffer from at least one traumatic or adverse experience (Felitti *et al*., 1998; Copeland *et al*., 2007). Traumatic or adverse experience ranges from different types of abuses, neglect, dysfunctional household environments like parental separation, family violence, household mental illness, household incarceration, household substance abuse, peer group violence and community violence. Growing child is affected in different ways by these traumatic events. Effects of childhood trauma on the possibility of various harmful adolescent and adult outcomes are being studied in many contemporary empirical researches. These researches have continuously shown that early traumatic experiences can lead to a large number of developmental noises for the individual throughout their life course (Lamphear, 1985; Trickett & McBride-Chang, 1995; Cicchetti & Toth, 1995).

The word 'trauma' means "any event that harms the body, self or spirit. It covers a broad range of hurtful experiences including traumas that involve the physical, sexual, mental, or emotional realms of our being" (Whitfield, 1998). The most commonly discussed forms of childhood trauma include physical abuse, sexual abuse, psychological abuse, physical neglect and emotional neglect. The concepts of child maltreatment and childhood trauma are constantly intertwined in the empirical literature (De Bellis, 2001).

The prevalence of child maltreatment as a major problem was not recognized by the society prior to 1960s. During that time views of parents on conventional disciplinary practices were favored and rights of children were largely ignored (Cicchetti & Carlson, 1989). Parents

often consider children as their property and as a result, negligent care and harsh corporal punishments were imposed on children in many families (Crosson, 2013) which potentially impacting the development of a child's life. Despite the continued occurrence of child abuse in the history, prior to the middle of the 20th century, it was not mentioned in the medical literature (Dubowitz & Newberger, 1989).

In the examination of developmental psychology and childhood development, child abuse, neglect and other traumas has been critically considered over the past few decades. The role of related and casual factors in the incidence of child trauma and maltreatment, have begun to be scientifically examined (Garbarino, 1977; Pelton, 1978). Poverty, class and other social factors as correlates for childhood traumatic experiences had empirically investigated (Gil, 1970). Beyond the causes of maltreatment, the effects and consequences of trauma in childhood and throughout life (Aber & Cicchetti, 1984), including the prevention and treatment for those effected children were studied (Kempe & Kempe, 1978).

As a result of these investigations, the study of trauma or maltreatment or adverse childhood experiences has become an integral part of the psychological, sociological, biological and various other scientific literatures. Researches on childhood trauma have examined a multitude of various aspects of role of trauma on the development of brain (Glaser, 2000), behavioural problems (Garnefski & Diekstra, 1997) and mental illnesses (Mueser *et al*., 1998). Vital to the discussion of behavioural problems is the concept of the "cycle of violence" (Widom, 1989a). According to this concept, individuals

those who are maltreated and abused early in life are more likely to engage in violence later in life (Widom, 1989b; Maxfield & Widom, 1996). This hypothesis was empirically examined and has found a persistent effect of various types of childhood trauma on violent behaviour, including serious, chronic and violent delinquency (Widom, 1989a; Widom, 1989b; Zingraff *et al*., 1994; Duke *et al*., 2010; Fox *et al*., 2015).

1.8.1 Developmental psychopathology

Developmental psychopathology is defined as "the study of the origins and course of patterns of behavioural maladaptation, whatever the age of onset, whatever the causes, whatever the transformations in behavioural manifestation, and however complex the course of the developmental pattern may be" (Sroufe & Rutter, 1984). Many earlier researches did not utilize a theoretical approach in empirical examinations and estimation of childhood trauma. Although many theories indicates the importance of normal development and discuss childhood trauma's effects on delinquent and criminal behaviour, the step-by-step developmental effects of early life trauma on a number of adverse outcomes throughout childhood and adolescence is examined on theoretical perspective is through developmental psychopathology which was originated from the field of developmental psychology (Aber *et al*., 1989).

Different challenges faced at each age by a child throughout the child's growth which determine the causes for normal and abnormal developmental processes is specifically examined in developmental

psychopathology (Causadias, 2013). The work of developmental and clinical psychologist Dante Cicchetti in 1980s largely shaped the developmental psychopathological perspective on child maltreatment (Cicchetti, 1984; Cicchetti & Toth, 1995). Interdisciplinary in nature, Cicchetti's principle is that early-life trauma can lead to adverse outcomes in a variety of different facets of life (Toth & Cicchetti, 2013). Since child abuse is such a negative experience, it can have enormous consequences for such a vulnerable population. Childhood maltreatment may lead to antisocial behaviour, depression, personality disorder and future victimization. Child's adjustment throughout development is adversely affected by the frequency and severity of childhood trauma. Accordingly, children who experience neglect, abuse or some other type of traumatic experience are predicted to be more likely to experience developmental difficulties in, personal and peer relationships, emotion regulation, self-concept, school adaptation and display psychopathological traits throughout their lives (Cicchetti & Toth, 1995).

1.8.2 Organizational perspective on development

Another theory under the developmental psychopathology regarding the sensitive risk of antisocial behaviour as a result of childhood trauma is the 'organizational perspective of development' (Werner, 1957; Sroufe, 1990; Cicchetti, 1990). According to this theory, "development occurs as a progression of qualitative reorganizations within and among the biological, social, emotional, cognitive, representational, and linguistic systems proceeding through differentiation and subsequent hierarchical integration" (Cicchetti &

Toth, 1995). In this perspective the development of each stages of childhood is predicted by the preceding stages and children who are experiencing normal development in early childhood are more likely to be successful in adjusting at the later life stages and vice versa (Sroufe & Rutter, 1984).

1.8.3 Ecological-transactional model

According to the ecological- transactional model which was developed by Cicchetti and Lynch (1993), "an increased presence of risk factors associated with the occurrence of maltreatment at any or at all ecological levels represents a deviation from the conditions that promote normal development" (Cicchetti & Toth, 2005). According to this model, throughout each stages of life, children who are maltreated are predicted to experience developmental difficulties which hinder their normal maturation. Such maltreated children may develop complications with learning to regulate their emotions, adapting to school, developing normal social relationships with peers, etc. Constant developmental noises initiated by the traumatic experiences continuously affect the child's development. This theory also predicts that future positive development is suggested by the early positive development and some individuals may change paths depending on other conditions. Due to certain resiliency factors in life, some children are able to resist maladaptive and antisocial behaviour (Cicchetti & Rogosh, 1997). Serious maladaptive behaviours were less likely to be demonstrated by children those who possessed higher self-esteem and more emotional control. A child's development is a multi-faceted process according to the developmental psychopathology perspective

(Cicchetti & Toth, 2009) and this has led to an understanding different maladaptive behaviours and the variety of pathways that can lead to such behaviours as in the case of ADHD (Sroufe, 1989), mental illnesses (Kendall *et al*., 1993) and conduct disorders (Richters & Cicchetti, 1993). Thus the onset of problem behaviours can be predicted using ecological- transitional model.

1.8.4 Problem behaviour theory

Problem behaviour theory which was developed by Richard Jessor is widely used in social-psychological frame work to explain maladaptation during adolescence (Jessor & Jessor, 1977). According to this theory problem behaviour is a "behaviour that is socially disapproved by the institutions of authority and that tends to elicit some form of social control response whether mild reproof, social rejection, or even incarceration" (Jessor, 1987). Many problem behaviours like deviant peer imitation, alcohol and drug abuse, high-risk sexual behaviours, school difficulties and different forms of delinquencies were examined using this theory (Bensley *et al*., 1999; Ary *et al*., 1999). The problem behaviours that are related and that any single problem behaviour such as gang involvement, criminal activities, illicit drug abuse etc., must be viewed within the complex system of both adaptive and problem behaviour, personality and perceived environment (Milkman & Wanberg, 2012). Studies have shown that maltreatment, family dysfunction and parental conflict during childhood can be important predictors in the development of problem behaviours later in life such as antisocial or aggressive behaviour (Mobley & Chun, 2013). Early life experiences of trauma

can instigate proneness towards maladaptations that contribute to antisocial personality development and as a result of each additional problem behaviour the youth is even more susceptible to further and more serious deviance (Cicchetti & Toth, 2005).

1.8.5 Moffitt's developmental taxonomy

Considering the developmental noises that encompass the lives of certain children, Moffitt (1993) proposed the theory of developmental taxonomy which was drawn from neuropsychology and developmental psychology. This theory gave an alternate explanation for antisocial and delinquent behaviour. According to this theory there are two types of antisocial behaviour: life-course-persistent (LCP) and adolescence-limited (AL) (Moffitt, 1993). In LCP delinquents the deviance is pathological and the deviant behaviour is initiated at very young age and persists throughout their life. These individuals are responsible for most of the serious violent crimes, even though they represent a small percentage of population (Moffitt, 1993). Onsets of delinquent behaviour in AL delinquents are in adolescences and eventually age-out. These individuals represent the majority of delinquent behaviour and generally commit less serious delinquency. Due to the maturity gap AL delinquents engage in deviant behaviour by mimicking the behaviour of others. Whereas, as a result of neurodevelopmental deficiencies, cognitive deficits and learning difficulties are developed in LCP delinquents and they engage in deviant criminal behaviour. During childhood they are temperamental and begin to engage in deviant development very early. Temperamental and impulsive parents lead to ineffective childrearing

which suggested a genetic link in LCP behaviour. LCP individuals have little chance to reform due to the culmination of adverse factors which leads to constant deviance. The neurodevelopmental and biological issues that Moffitt (1993) hypothesized contributed to temperamental children and seriously delinquent adolescents and the same was demonstrated in the study of Barnes *et al*., (2011) in which genetic factors explain between 56-70 % of the variation in LCP classifications. Children those who are aggressive, impulsive and deviant in a number of aspects of behaviour and often violent, have genetic differences which contribute to such behaviour (Moffitt & Caspi, 2001). Additionally, during childhood these children may be difficult for parents to manage which contributes to increase the amount of adverse experiences suffered during their early life (Moffitt, 1993).

1.8.6 Bronfenbrenner's ecological model

It has been identified that the health of an individual is not just the product of biology and genetics alone. The physical environment, interactions with the society, relationships and economic status have a great influence in the wellbeing and health of a person. Environments and experiences during infancy, childhood and adolescence have tremendous impact on the future behaviour and life quality of a person (Lerner & Castellino, 2002). According to this model human development occurs via a reciprocal interaction between individuals and proximal processes such as persons, objects etc., (Lerner & Castellino, 2002). So the theoretical models of developmental changes occurring through the multiple level systems require an inter-level

approach. This inter-level approach includes the interaction between individual and their immediate social and cultural environment and the same is explained by Bronfenbrenner's ecological model environment. These proximal processes vary as a function of both the characteristics of the developing individual and the characteristics of the environment (Bronfenbrenner, 1994).

1.9 Essential Criminological Perspectives

Various criminological theories have discussed the role of youth's development, onset of problem behaviours and subsequent involvement in delinquent and criminal behaviour.

1.9.1 Impulsivity and the General Theory of Crime

Self-control theory was developed by Gottfredson and Hirschi (1990) and according to this theory delinquent and criminal behaviour is the manifestation of an individual's low self-control. The ability to resist impulses and delay gratifications is defined as the self-control (Gottfredson & Hirschi, 1990). Several researches have established an empirical support for the relationship between self-control and delinquency (Arneklev *et al*., 1999; Gottfredson, 2009; Bouffard *et al*., 2015). Self-control is built through efficient parenting practices, parents' ability to monitor the child's behaviour, recognize any deviance and discipline the child appropriately (Gottfredson & Hirschi, 1990). Even though child's lower level of self-control is predicted by ineffective childrearing (Hay, 2001), relationship between parenting and delinquency is only partially mediated (Burt *et al*., 2006). Parenting practices of a family is the crucial factor which determines

the child's level of self-control and child reared by traumatic parenting practices or in a dysfunctional environment is less likely to develop enough self-control and produces a youth who is more likely to engage in violent delinquent behaviours (Azar, 2002). In the household, beyond the effects of maltreatment, the abuse of different substances can reduce the effectiveness of parenting practices and lead to more parental dysfunction (Mayes & Truman, 2002). Effectiveness of parenting practices which have a vital role in the creation of self-control, is also depends on the impact of serious parental conflicts, parental separation due to divorce or incarceration (Wilson & Gothman, 2002).

1.9.2 Deviant Peers and Social Learning Theory

The main concepts of criminological learning theories are rooted in the theory of differential association developed by Sutherland (1939). According to this theory criminal behaviour is learned through interactions with intimate personal groups. This theory only put forward the likelihood of delinquent behaviour which will be increased when an individual associates with more delinquent peers.

In the year 1966 Robert Burgess and Ronald Akers developed a revision to differential association theory by incorporating concepts of operant conditioning to this theory which expended its predictive power and rebranded the theory as social learning theory (Burgess & Akers, 1966; Akers *et al*., 1979). Apart from the learning of delinquent and criminal behaviour through peer associations, social learning theory included reinforcement and imitation (Krohn, 1999). In

reinforcement, reward and punishment for our behaviour is considered from those we have personal relationships with. Imitation is the replication of the behaviour of others. Many empirical literatures have substantiated the learning theories. Considering a variety of delinquent behaviours, consistent association of antisocial individuals has been found with other antisocial individuals (Akers *et al*., 1979; Pratt *et al*., 2010; Cochran *et al*., 2011).

1.10 Significance of the study

Even though many genetic and psychological researches related to aggressive and antisocial behaviour have been done worldwide, no combined researches among offenders are conducted in India. There is no survey or research till date done in India regarding the interaction of allelic variants of *MAOA*-uVNTR polymorphism and adverse childhood experiences (ACEs) in the development of aggressive and antisocial behaviour in recidivist/ habitual offenders. As per the know data only two researches were conducted in the country in the related area regarding the influence of *MAOA*-uVNTR polymorphism in the etiology of Attention Deficit Hyperactivity Disorder (ADHD) in psychiatric patients. First study showed that the short repeat allele of the *MAOA* gene is probably associated with ADHD in the studied population and could be the reason for making boys prone to ADHD as compared to girls (Das *et al*., 2006). In the second study *MAOA* gene variants could be considered as risk factors for ADHD in the Indo-Caucasoid population from eastern India (Karmakar *et al*., 2017).

If the basic causes of the violent crimes can be identified in a grass root level, the corrective measures can be implemented so as to prevent the increasing rate of violent criminality and recidivism in the country. Hence this study was focused on identifying the allelic variants of *MAOA*-uVNTR polymorphism and its interaction with adverse childhood experiences (ACEs) in the recidivist violent offenders in the state of Kerala.

1.11 Statement of problem

This study aimed at finding out the interaction between allelic variations of *MAOA*-uVNTR polymorphism and adverse childhood experiences (ACEs) in recidivist violent offenders. The interaction between adverse childhood experience (ACEs), health risk behaviours (HRBs) and violent criminality in the offenders and in the comparing group were also analyzed.

1.12 Objectives of the study

1. To know the life and crime history of the recidivist offenders.

2. To extract and isolate genomic DNA from the buccal samples drawn from the recidivist offenders and documentation.

3. To genotype the isolated DNA for the *MAOA* gene.

4. To study the degree of criminality, adverse childhood environmental condition in relation to uVNTR polymorphism of *MAOA* gene in recidivist offenders.

1.13 Process of Research

This thesis is presented as per the American Psychological Association (APA) style. The present study was done in two parts (Part-I and Part-II) and studies were conducted simultaneously in both the parts. Initially the study was aimed at identifying the allelic variants of *MAOA*-uVNTR polymorphism in the participants. For this purpose, part-I was done in two phases. In the first phase, a pilot study was conducted to identify the best biological sample collection procedure for obtaining high quality genomic DNA from the participants and also for standardization of laboratory protocols. On the basis of the results of the phase- I, in the second phase, the main study was conducted to identify the participants and genotype the participants based on the 30 bp repeats of *MAOA*-uVNTR polymorphism. Material and methods of genotyping are given in chapter 3. The results related to the association of allelic variants of *MAOA*-uVNTR with violent criminality are discussed in chapter 4.

In the part-II, the study was conducted to understand the demographic information; to assess the adverse childhood experiences (ACEs), health risk behaviours (HRBs) of the participants and the criminal history of the recidivist violent offenders. For this purpose, part-II was done in two phases. In the first phase, a pilot study was conducted to standardize the data collection tools. On the basis of the results of phase-I, in the second phase, the main study was conducted to understand the demographic information, to assess the adverse childhood experiences (ACEs) in the participants; to understand the violent crimes and age of onset of crime in offenders; health risk

behaviours (HRBs) of the participants, followed by the onset of age of HRBs in the participants. These analyses were retrospective. Data were collected through face-to-face interview with the help of Adverse Childhood Experiences International Questionnaire (ACE-IQ) and health risk behaviour questions. Official crime records of the offenders were collected from the police stations to understand violent crimes done. Methods of data collection are given in chapter 3 and the results are discussed in chapter 4.

Review provided in the chapter 3 suggested that there is occurrence of short allele of *MAOA*-uVNTR polymorphism in violent offenders and also there is high frequency of ACEs and HRBs in them. Further designing of the research was done to identify the independent variables for violent criminality.

The association and mean scores of each category of ACEs, total ACE and HRBs were measured in participants. Relationships of ACEs with HBRs in participants and with violent criminality in offenders were measured. ACEs were the independent variables for predicting the violent criminality in the offenders. Finally, the mean score of ACE categories and total ACE were evaluated between the participants grouped on the basis of allelic variants of *MAOA*-uVNTR polymorphism to know the candidate gene-environment interaction (cGxE).

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Chapter 2
REVIEW OF LITERATURE

2.1 Phenotypical outcomes of MAO deficit

Human MAO deficiency was first reported in some patients affected by Norrie disease (ND), which is X- linked. Mutation of the Norrie disease pseudoglima (NDP) gene located in Xp 11.4 resulted in this genetic disorder characterized by congenital blindness, cataracts, progressive hearing loss and development delays in motor and cognitive skills (Warburg, 1975). As a result of the close proximity of NDP to the *MAO* genes, some individuals affected by ND were found to harbour deletions of these genes (Sims *et al.,* 1989; Chen *et al.,* 1995) followed by the loss of MAO function in ND which results high urinary concentrations of β-phenylethylamine and tyramine metabolites (Murphy *et al.,* 1990; Collins *et al.,* 1992). Deletion of *MAOA* and *MAOB* in ND patients is accompanied by a range of atypical symptoms such as growth failure, alterations of sleep pattern, severe mental retardation and autistic-like symptoms (Sims *et al.,* 1989; Murphy *et al.,* 1990; Collins *et al.,* 1992).

2.2 Behavioural phenotypes and MAO-A deficiency in human

First insight into the phenotypical outcomes of selective MAO-A deficiency was described in 1993 by Brunner and colleagues in eight males of large Dutch kindred with a behavioural syndrome (Brunner *et al.,* 1993; Brunner *et al.,* 1996). The genetic defect was a point mutation (C936T) in exon 8 of the *MAOA* gene, resulting in the substitution of a glutamine codon (CAG) with a stop codon (TAG) at position 296 of the amino acid sequence (Bortolato & Shih, 2011).

This X-linked recessive disorder was named Brunner syndrome. Borderline mental retardation, maladaptive regulation of impulsive aggressiveness, antisocial behaviour (including attempted rape, murder, arson, exhibitionism and voyeurism) and violent responses to minor environmental stressors were the nosographic characteristics of this complex behavioural syndrome (Brunner *et al.,* 1993). Also, stereotyped hand movements, sleep disturbances, borderline intellectual disabilities were shown by the affected males. This was paralleled by a set of other abnormalities in the urinary concentrations of monoamine metabolites like fivefold increase in urinary levels of serotonin, decreased content of 5-HIAA, HVA and VMA.

Discovery of this mutation was a breakthrough and instrumental for the resurrection of biological criminology (Godar *et al.,* 2016). But for several years, there was no progress in the further clinical characterization of the phenotypic consequences of *MAOA* deficiency due to the lack of nosographic details on the cognitive and psychological characteristics of these patients (Hebebrand & Klug, 1995). Also, several attempts to discover other cases of this disorder were unsuccessful (Mejia *et al.,* 2001) until 2014, when a second case of this syndrome was identified (Piton *et al.,* 2014).

2.3 Behavioural outcomes in *Maoa*-knockout mice

Development of first mutant or transgenic line of *Maoa* knockout (KO) mice in the year 1995 helped in the progress on the characterization of the neurobehavioural correlates of MAO-A deficits (Cases *et al.,* 1995). Following the integration of an interferon β

cassette, serendipitous deletion of exon 2 and 3 of the *Maoa* gene in C3H/ HeJ took place which resulted in mutant mice with high levels of aggressive behaviour towards both familiar and foreign conspecifics. Age- dependent increase in the levels of serotonin, dopamine and norepinephrine were found during the analysis of the neurochemical correlates of these alterations. Similar findings were obtained in a second line of 129S6 mice having a spontaneous mutation of exon 8 (Scott *et al.,* 2008). A marked reduction in social and environmental explorations was displayed by these KO mice (Bortolato *et al.,* 2011; Godar *et al.,* 2011). In particular, these KO mice showed exaggerated defensive, neophobic responses to minor stimuli (Godar *et al.,* 2011); lower response to stressful, threatening contingencies, physical restraint and cold temperature (Godar *et al.,* 2015); marked reduction in other measures of risk evaluation and threat assessment (Bortolato *et al.,* 2011; Godar *et al.,* 2011); greater retention of aversive memories (Dubrovina *et al.,* 2006) and core autistic symptoms (Bortolato *et al.,* 2013).

These preclinical findings were confirmed with the discovery of new case of Brunner syndrome in 2014 in a boy with low-functioning autism spectrum disorder exhibiting intermittent self-aggression in response to stress and severe cognitive impairments (Piton *et al*., 2014). Recently, loss-of-function mutations of *MAOA* gene in males, identified with episodic explosive aggression, attention deficits, mild intellectual disability, introverted and obsessive traits (Palmer *et al.,* 2016) confirms that there is a strong dysregulations in early neurodevelopmental processes in Brunner syndrome. *MAOA*

plays a key role in the frequency of aggressive manifestations (Farmer & Aman, 2011), ontogenesis of sensory, communication deficits and arousal regulation problems in autistic boys (Cohen *et al*., 2011). These studies suggest that there is a potential relationship between aggression and sensory-communicative problems. Hence, there is a strong likelihood that aggression arises as a defensive response driven by poor social information processing (Crick & Dodge, 1996).

2.4 Allelic variants of *MAOA*-uVNTR in the ontogeny of aggression

Majority of the clinical studies have focused on the genetic relationship of *MAOA* gene in aggression. The studies that have investigated about the specific role of the enzymatic activity of MAO-A in antisocial and violent behaviours had the key limitations since they have measured the peripheral MAO-A activity in platelets, which only express MAO-B (Bortolato *et al.,* 2008). However, *Maob* deficiency in mice is associated with behavioural disinhibition, but not aggression (Grimsby *et al.,* 1997; Bortolato *et al.,* 2009).

Various researches on the psychological phenotypes have found out the association of different *MAOA*-uVNTR alleles with specific subtypes of aggression. Low activity variants of *MAOA*-uVNTR are associated with reactive aggression with a greater inclination to engage in hostile retaliatory acts against the provocations of alleged opponents and competitors (McDermott *et al.,* 2009; Kuepper *et al.,* 2013).

2.5 Association of short allele of *MAOA*-uVNTR with psychopathy and aggression

Many studies have found a strong association between short allele, especially 2R allele of *MAOA*-uVNTR polymorphism with criminal behaviour and psychopathy (Guo *et al.,* 2008; Beaver *et al.,* 2009; Beaver *et al.,* 2010; Beaver *et al.,* 2013; Beaver *et al.,* 2014; Stetler *et al.,* 2014; Armstrong *et al.,* 2014; Tiihonen *et al.,* 2015). The association of the short allele with psychopathy followed by callous-unemotional traits and proactive aggression has garnered this allele the questionable name of 'psycho gene' (Raine *et al.,* 2006; Nouvion *et al.,* 2007). Several aspects of impulsivity are shown by psychopathic trait and hence psychopathy is not just entirely associated with proactive aggression (Morgan *et al.,* 2011). Researchers have found out that psychopathy are of two types, primary psychopathy characterized by low anxiety and lack of ethics and secondary psychopathy related with high anxiety, negative affect and impulsivity (Blackburn, 1975; Newman *et al.,* 2005; Skeem *et al.,* 2007). These two types of psychopathies are underpinned by different genetic and environmental factors (Blonigen *et al.,* 2005; Hicks *et al.,* 2012); particularly secondary psychopathy is highly connected with environmental risk factors (Skeem *et al.,* 2003).

Even though the difference between reactive and proactive aggression is well supported by clinical evidence (Card & Little, 2006), several studies point to moderate consensus of both subtypes (Polman *et al.,* 2007) indicating a large overlap of their genetic predisposition factors (Brendgen *et al.,* 2006). Significantly higher

tendency to engage in impulsive aggressive reactions to negative affect was shown by the individuals with short alleles of *MAOA*-uVNTR (Chester *et al.,* 2015). Carriers of short *MAOA*-uVNTR alleles may attain psychopathic traits which results from social learning and this can lead to a greater predisposition to reactive aggression. This may result to the repeated engagement in violent acts and development of instrumental antisocial behaviours (Godar *et al.,* 2016).

2.6 Neurobiology of *MAOA*- uVNTR variants

Major line of evidence for the association between MAO-A activity and aggression comes from positron emission tomography (PET) studies using radiolabeled MAO-A inhibitors (Fowler *et al.,* 2007; Alia-Klein *et al.,* 2008). In these studies it was shown that in healthy adult males, MAO-A activity in multiple sub cortical and cortical brain regions was inversely related with indices of trait aggression in the Multidimensional Personality Questionnaire (MPQ) (Fowler *et al.,* 2007; Alia-Klein *et al.,* 2008). Independent studies have confirmed that anger and hostility are negatively related with MAO-A binding in multiple sub regions of the prefrontal cortex (PFC) (Soliman *et al.,* 2011). These results emphasize the importance of MAO-A in antisocial behaviour and point to this enzyme as a biomarker for abnormal aggression. Also, it was found out that MAO-A distribution volume is lower in patients affected by antisocial personality disorder (Kolla *et al.,* 2015).

Even though the comparison was not statistically significant, post-mortem analyses showed that the average MAO-A catalytic activity in brain samples of 3R carriers is numerically lower than that of 4R controls (Balciuniene *et al.,* 2002). No direct association was found between the *MAOA*-uVNTR alleles and MAO-A brain levels in adults (Fowler *et al.,* 2007). MAO-A activity in the cortical and subcortical brain regions was inversely correlated with the degree of self- reported aggression in men (Alia- Klein *et al.,* 2008). Functional magnetic resonance imaging (fMRI) studies showed a link between *MAOA*-uVNTR polymorphic variants and structural and functional differences between the brains of 3R and 4R carriers (Meyer-Lindenberg *et al.,* 2006). Functional abnormalities in cortical limbic regions, including amygdale, prefrontal cortex and hippocampus were found in individuals with carriers of low alleles of *MAOA*-uVNTR (Passamonti *et al.,* 2006). Especially, male individuals with the 3R haplotype exhibit morphological alterations of the orbitofrontal cortex (Cerasa *et al.,* 2008; Cerasa *et al.,* 2010). When taken together, this suggests that the variants of *MAOA*-uVNTR were expected to predispose to the different levels of MAO-A enzymatic activity in brain and the same may be particularly important in the early life stages when the effect of this enzyme reaches its peak concentration (Tong *et al.,* 2013).

2.7 Environmental factors influencing *MAOA*-uVNTR activity

MAOA activity may vary over time due to the exposure to multiple environmental factors such as diet (Jahng *et al.,* 1998), tobacco smoking (Fowler *et al.,* 1996), social environment (Filipenko

et al., 2002), physical exercise (Morishima *et al.,* 2006) and stress (Marquez *et al*., 2013). It was well documented that MAO-A activity is reduced in exposure to smoke (Fowler *et al.,* 1996; Berlin & Anthenelli, 2001) during maternal smoking which results in conduct disorder and antisocial behaviour (Baler *et al.,* 2008; Wakschlag *et al.,* 2010). The level of methylation of *MAOA* promoter regulates the activity of MAO-A which suggests that environmental factors, especially during early stages, influence MAO-A activity through epigenetic mechanisms. This was supported by the discovery of elevated levels of *MAOA* promoter methylation in violent offenders (Checknita *et al.,* 2015). Hence the nature of the association between *MAOA* and aggression is neurodevelopmental and early life exposure to environmental factors that may reduce MAO-A activity leading to a greater predisposition to aggression (Godar *et al.,* 2016).

Considering the multifactorial nature of aggression, various studies have recognized that the antisocial behaviour is shaped by the interaction of *MAOA* with other vulnerability factors. The first study that documented gene-environment (GxE) interaction in antisocial behaviour was conducted by Caspi and colleagues (2002). In this seminal study on New Zealanders it was found that individuals with a history of child abuse and 3R allele of *MAOA*-uVNTR had a significantly higher incidence of antisocial behaviours than individuals with only one risk factor. Multiple subsequent studies have confirmed this interaction (Huang *et al.,* 2004; Foley *et al.,* 2004; Nilsson *et al.,* 2006; Frazzetto *et al.,* 2007; Weder *et al.,* 2009; Derringer *et al.,* 2010; Beach *et al.,* 2010; Edwards *et al.,* 2010; Aslund *et al.,* 2011).

Thirty years longitudinal study have remarkably replicated the finding of Capsi and colleagues, by showing that abused children with low activity *MAOA* variants, at the age of around 16 developed conduct problems and hostility (Fergusson *et al.,* 2011).

Interactions of *MAOA-u*VNTR and childhood maltreatment in the aetiology of antisocial behaviour have contrasting evidence too (Prichard *et al.,* 2008; Haberstick *et al.,* 2014). Low activity *MAOA* alleles interacts with maternal stress to influence negative emotionality in infants which points towards the synergetic effects of stress and *MAOA* which occur throughout prenatal development (Hill *et al.,* 2013). Three meta-analyses have proved a clear interaction between *MAOA*-uVNTR polymorphism and childhood adversities on antisocial outcomes (Kim-Cohen *et al.,* 2006; Byrd & Manuck, 2014; Ficks & Waldman, 2014). The study that explained the psychological processes underlying GxE have reported that low-activity *MAOA* variants interacts with early maltreatment by determining the susceptibility to negative events (Nillson *et al.,* 2015).

The nature of GxE is sex-dimorphic and most of the studies done in females have shown varying results in the interaction between *MAOA*-uVNTR polymorphism and environmental adversities in the development of antisocial behaviour and aggression (Ducci *et al.,* 2008; Kinnally *et al.,* 2009; Beach *et al.,* 2010; Aslund *et al.,* 2011; Verhoeven *et al.,* 2012; McGrath *et al.,* 2012; Holz *et al.,* 2014).

The evidence on the variants of *MAOA*-uVNTR has clearly revealed that male carrier of low activity alleles of this gene are

predisposed to negative bias in the interpretation of social stimuli which results in a greater susceptibility for aggressive and impulsive reactions to provocation and stress. Depending on this hypothesis, several independent studies have shown that the synergism of this psychological substrate with early life traumas or stressors or experiences, clearly increases the susceptibility to develop antisocial behaviours from adolescence onwards (Godar *et al.,* 2016).

2.8 Adverse childhood experiences (ACEs)

To the criminological discourse the concept of ACE entered recently only (Baglivio *et al.,* 2014; Baglivio *et al.,* 2015; Fox *et al.,* 2015). The concept of ACE stems from public health and intersects with psychology and biology and through its application to juvenile justice, this concept have emanated into criminological literature.

Negative events occurring within the first 18 years of an individual's life that results in disrupted neurodevelopment leading to social, emotional and cognitive impairments are called as adverse childhood experiences (ACEs). Assessment of ACEs was developed by Felitti and colleagues in 1998, by conducting a survey over 17,000 adults who used Kaiser-Permanente health insurance in San Diego, California. This was to examine the relationship between childhood trauma and the most common cause of death in San Diego. The assessment included measures of physical abuse, emotional abuse, sexual abuse, household substance abuse, witnessing household violence, household mental illness and having an incarcerated member of the family. Subsequent studies included emotional neglect, physical

neglect and parental separation or divorce. All together, these experiences of childhood trauma consist of an assessment of ten distinct items and individual ACE score was calculated by summing the total number of ten ACEs during childhood (Felitti *et al*., 1998). In this study they found that during childhood more than half of the respondents were exposed to at least one ACE. Throughout the life, increased health risks for drug abuse, alcoholism, etc., were experienced by individuals with higher levels of ACEs. These findings indicated that an exponentially more damaging health effects were seen when exposed to multiple ACEs (Felitti *et al.,* 1998).

Using the ACE assessment several further researches have examined the effects of childhood traumas. From these studies it was understood that higher ACE scores have been related to a variety of destructive behaviours such as alcoholism (Dong *et al.,* 2005), smoking (Anda *et al.,* 1999), depression (Dube *et al.,* 2003), mental illness (Chapman *et al.,* 2007; Felitti & Anda, 2010), adolescent pregnancy (Hillis *et al.,* 2004) and risky sexual behaviour (Hillis *et al.,* 2001). Another research has found that several ACEs are co-occurring and exposure to ACE differs by gender and race/ ethnicity (Baglivio & Epps, 2016). It was found that ACEs have both direct and indirect effect on criminal recidivism (Wolff & Baglivio, 2017).

In infant rats, it was found that an early unexpected trauma and maternal deprivation increased the death of both neurons and glia cells in the cerebral and cerebellar cortexes (Zhang *et al*., 2002). In the paediatric imaging studies it was found that the size of the cerebral and cerebellar volumes was smaller in youths who were exposed to ACEs

(De Bellis *et al*., 2002). The cellular, cognitive and brain development is adversely influenced by the dysregulation in the biological stress systems (De Bellis *et al*., 1999) initiated by the childhood adverse events (De Bellis & Zisk, 2014). Also, several studies have found differences in DNA methylation in somatic tissue as a result of exposure to childhood abuse (Lutz & Turecki, 2014; Roberts *et al*., 2018).

2.9 Background Demographics and ACEs

Based on the various demographic factors affecting the family's dynamics, the adverse early- life experiences differs. Various investigations have showed that the level and type of trauma suffered by a child depends on the gender, their family characteristics like socioeconomic status and race/ ethnicity (Gil, 1970). An inverse relationship was found between many types of child abuse and neglect with social class (Brown, 1984). Even though this relationship was weak, it was demonstrated that child sexual abuse occur 17 times more often in families with low-income in the study conducted by U.S. Department of Health and Human Services in 1996 (Sedlak & Broadhurst, 1996). Similar results were found in the subsequent studies and this shows that there is more likelihood of facing higher levels of both child abuse and neglect by children of lower educated and parents with lower occupational prestige (Brown, 1998). In a study conducted by Scher and colleagues (2004), it was found that female children in White families faced more emotional abuse, physical neglect and higher prevalence of sexual abuse along with several types of contemporaneous maltreatment. Parental incarceration was also an

increasing traumatic experience for many African American children (Wildeman, 2009) that affected child's development (Miller, 2006).

2.10 Deviant Peer Imitation

During the formative peer encounters of a child, the child's circumstances surrounding in the home life have a direct impact on the type of individual they interact and imitate. In a study conducted by Fergusson and Horwood (1999), it was evident that parental violence and conflict, parental substance abuse and childhood abuse could be used as predictors of later deviant peer association. In children, lower levels of peer antisocial behaviour was shown during the positive parental disciplinary practices (Dishion *et al.,* 1991). Greater number of deviant peer affiliations was associated with more traumatic parenting practices (Brody, 2001).

During childhood, both physical and emotional neglect were found to be predictive of peer adjustment problems and associating with deviant peers (Chapple *et al.,* 2005). Reduced social competence and peer interactions were noticed in children growing up in a household with someone suffering from a mental illness (Billings & Moos, 1983; Thomas *et al.,* 1995). Margolin and Gordis (2004) found that exposure to family violence correspond with peer conflict resolution difficulties.

It was more probable that children who have experienced trauma could either withdrawn toward other children in their age group or be aggressive and commonly disliked by majority of their peers (Mueller & Silverman, 1989; Anthonysamy & Zimmer-Gembeck,

2007). It was found that abused children show higher levels of impulsivity and during adolescence they associate with deviant peers (Wright *et al*., 1999; Baron, 2003). Child's intellectual abilities were affected by the traumatic experiences, resulting in significantly weakened school comprehension and performance (Erickson *et al.,* 1989; Eckenrode, 1993). It was more likely that children, who experienced trauma during childhood, get in trouble in school and higher rates of dropping out prior to graduation (Kendall-Tackett & Eckenrode, 1996; Kokko *et al.,* 2006).

2.11 ACEs and Maladaptive Personality Development

Researchers have shown that during the formative years, abuse and neglect could even affect child's brain which hinders the normal development and could lead to certain maladaptive and problematic behaviour patterns (Cicchetti & Toth, 2005). More problematic conduct disorders were developed as a result of the related problem behaviours (Jessor & Jessor, 1977; Jessor, 1987). Traumas other than abuse and neglect also affect the developmental process of a child in a variety of facets of their life (Reed & Reed, 1997; Murray *et al.*, 2012). As a result of the early childhood traumatic experiences, creation of two traits in childhood were hypothesized and these traits were aggression and impulsivity, which were relatively stable throughout life (Olweus, 1979; Gottfredson & Hirschi, 1990; Farrington, 1994).

2.12 ACEs and Serious, Violent, Chronic (SVC) Delinquency

Several researches have examined the occurrence of ACEs in juvenile delinquents. Group of juvenile delinquents that commit

highest rate of violent offenses in any given population were known as serious, violent and chronic (SVC) delinquents (Loeber & Farrington, 1998). Even though this group represent a very small proportion of total offenders, more than half of all serious violent offences were committed by less than one-tenth of these juvenile delinquents (Piquero, 2011). It was more likely that the violent behaviour persist in adulthood also, in the group of juvenile delinquents who involve in serious violent behaviour at an early age (Elliott, 1994). More acts of antisocial behaviour were shown by the violent criminals and they had a higher likelihood of reoffending (Farrington, 1982). It has been found that the factors that contribute to chronic persistent delinquency closely mirror to the contributing factors for violent delinquency (Brame *et al.,* 2001; Piquero *et al.,* 2012). The children who develop aggression earlier in life would be expected to be more likely to advance into more serious and violent delinquent behaviour during their adolescent years (Loeber *et al*., 1998).

Grevstad (2010) showed that the ACE scores were three times higher in juvenile delinquents than the score reported in the study of Felitti and colleagues. There were more chances for the youth with higher ACEs to both offend and reoffend than the youth with lower ACEs. Hence there was a need of policy implications, screening and addressing the ACEs in juveniles for preventing them in reoffending (Baglivio *et al.,* 2013). It was often found that numerous developmental, psychological and social risk factors heighten the propensity of SVC delinquents in developing chronic violence throughout life (Fox *et al.,* 2014). In a study among 64,000 adjudicated

juvenile offenders, Baglivio and colleagues (2015) identified different offending trajectories and distinguished the patterns of early onset and chronic offending from mid-to-early onset, based on the exposure to ACEs. It has been shown that the possibility of serious, violent and chronic (SVC) delinquency in juveniles could be predicted by analysing higher ACE scores (Fox *et al.,* 2015). Among early onset juvenile offenders who have committed homicide and attempted homicide, there was a link between these offences and ACE such as living with household metal illness (Baglivio *et al.,* 2017).

It was substantiated by several longitudinal studies that chronic adolescent violence had relationship with childhood traumas (Farrington, 1989; Widom, 1989; Smith & Thornberry, 1995; Lansford *et al.,* 2007). In the self-reported violence, emotional and psychological abuse has been shown to be predictive of violent behaviour (Song *et al.,* 1998). For the perpetration of chronic violence, the most consistent predictor was physical abuse (Mass *et al.,* 2008). This view was opposed by Yun and colleagues (2011) and they concluded that for chronic violence, physical abuse alone was unrelated, but instead sexual abuse and childhood neglect were each independently predictive. Herrenkohl and colleagues (2008) found that the effects of child abuse and domestic violence simultaneously have a "double whammy" or trigger compounding effect on violent behaviour. In the study that tested the effects of occurrence of ACEs all together on youth's commission of violence, independent associations were found with general adolescent violent crimes, fighting, bullying, dating violence, weapon carrying and sexual abuse,

physical abuse, household substance abuse (Duke *et al.,* 2010), where the risk of each types of violence was increased 35% to 144% depending on each additional ACE.

Higher levels of SVC delinquency were shown by children with higher levels of childhood trauma as a result of greater imitation of antisocial siblings and delinquent peers (Maguin *et al*., 1995). During early adolescence, weak attachment to school and poor grades had been found to predict higher levels of violence all through the subsequent years of adolescence (Saner & Ellickson, 1996; Dahlberg, 1998; Ellickson & McGuigan, 2000). Among the male high school dropouts, one-quarter became SVC delinquents and nearly half became a serious delinquent (Huizinga & Jakob-Chien, 1998). Ikomi (2010) reported that chronic rates of violent felony referrals were more among school dropouts.

2.13 Aggression

Aggressions in children have long been linked to the childhood trauma experiences (Aber *et al.,* 1989). It was found that harsh discipline and the model of the cycle of violence, where the children who were aggressive and violent towards others, were victimized early in life (Dodge *et al.,* 1990). Klika and colleagues (2013) reported that physical abuse in childhood results in early aggressive and antisocial behaviour which persist throughout life. Another important predictor of higher levels of childhood aggression was childhood neglect (Kotch *et al.,* 2008). Increase in the levels of aggression at age 5 was noticed in the children who were subjected to minor corporal punishment like spanking at age 3 (Taylor *et al.,* 2010). Emotional abuse during

childhood also predicted later forms of aggression towards others (Allen, 2011). Child's risk for aggression significantly increases when there is dual exposure to both child abuse and witnessing domestic violence (Sousa *et al*., 2010). The effects of parental incarceration on childhood aggression were studied in a meta-analysis which showed a consistent relationship (Murray, 2012).

One of the significant risk factors for persistent adolescent violence was childhood aggression (Dahlberg, 1998; Dahlberg & Potter, 2001). It was well documented that aggression and violence have stability throughout childhood and adolescence (Farrington, 1994; Loeber & Hay, 1997). SVC delinquents throughout their childhood demonstrated significantly higher aggression (Huizinga & Jakob-Chien, 1998). Another study revealed that more than half of the children before the age 9, who have showed aggressive and violent tendencies, became serious violent adolescent delinquents (Thornberry *et al.,* 1995). This high level of stability among childhood aggression and adolescent violence did not necessarily mean that all children those who were aggressive would become aggressive adults, but it definitely indicates an association between them. Researchers have theorized that some aggressive children may have certain resiliency factors that dampen subsequent violence. Herrenkohl and colleagues (2003) demonstrated that early aggression and subsequent violence was diminished in persons who had a positive family environment and positive school experiences. Also, the relationship between early aggression and subsequent violence heightened in children who experienced other risk factors such as antisocial peers. It was more likely that individuals with higher levels of early-childhood aggression continue their violent behaviour into adolescence (Brame *et al.,* 2001).

When compared to proactively aggressive individuals, reactively aggressive individuals were more susceptible to impulsive and angry outbursts to external events (Conner *et al.,* 2003).

2.14 Impulsivity

The level of impulsivity of a child is an important determinant for the consequent behaviour and conduct. Effective parenting practices were the only cause of developing self-control and resistance to immediate impulses in a child (Gottfredson & Hirschi, 1990; Gibbs *et al.,* 1998; Unnever *et al.,* 2003). Childhood trauma severely damages the developing brain of a child and the key inhibitors required for the effective resistance and regulations of emotions are diminished (Braquehais *et al.,* 2010).

Study conducted by Farrington (1989) indicated that perpetration of consequent violence in later adolescence was able to be predicted by impulsivity during childhood. Another study showed that when controlling for other key demographic risk factors, for violent delinquency low impulse control was a strong predictor (Baron, 2003). When provoked, it was more likely that individuals with low self-control behave violently (DeWall *et al.,* 2007).

2.15 ACEs and Violent Behaviours

Experiences of childhood maltreatment and trauma can ultimately direct to serious juvenile violent behaviour. This behaviour can be expressed in two ways: the adolescent can express the behaviour outwardly and act violently towards others, or on the other hand, the problems can be internalized by the adolescent and become inwardly violent. Adverse childhood experiences were associated with

self-injurious, suicidal and violent delinquent behaviour (Malinosky & Hansen 1993; Brown *et al.,* 1999; Dube *et al.,* 2001). It was found by Moylan and colleagues (2010) that the child's risk for both externalizing and internalizing their negative emotions were increased when subjected to the traumatic experiences in the childhood, such as witnessing domestic violence and abuse.

2.16 Health Risk Behaviours (HRBs)

According to World Health Organization (2002) modifiable health risk behaviours such as smoking, alcohol intake and substance abuse, both individually and collectively accounts for substantial morbidity and mortality throughout life. Critically, the frequency of these risk behaviours rises with increasing age, especially during teenage and often continues into early adulthood (Smith & Bradshaw, 2005). Several studies have demonstrated the clustering of health risk behaviours such as smoking, alcohol intake and substance abuse during adolescence (Smith & Bradshaw, 2005).

According to the Cumulative Risk Theory, the outcome of the exposure to adverse events by a child will in a dose-dependent manner (Sameroff *et al*., 1999). Consistent with this theory, several researchers have found that exposure to multiple adverse childhood experiences (ACEs) are predictive of many of the leading causes of health risk behaviours (Dube *et al*., 2003; Garrido *et al*., 2011; Strine *et al*., 2012)

2.16.1 Alcohol

In order to cope with their emotions, there is an increased risk of utilizing alcohol by the children who have experienced early childhood trauma. In the children who have experienced childhood

adversities an independent association between earlier drinking onset and sexual abuse, physical abuse, household substance abuse, household mental illness and parental separation were seen (Rothman *et al.,* 2008). Alcohol use disorders (AUD) and problem drinking behaviours were found in children who were abused (Dube *et al.,* 2002; Simpson and Miller, 2002). Problem drinking behaviour exists in the form of either dependence or abuse. Children having these two AUDs were likely to have a history of sexual abuse 18 to 21 times more and physical abuse, 6 to 12 times more (Clark *et al.,* 1997). Beyond the effect of growing up with an alcoholic parent, various adverse childhood experiences could be used as a predictive of later-life alcoholism (Anda *et al.,* 2002). These findings suggest that it is more likely to provide a traumatic home environment by the alcoholic parents which further fuel the child's consequent alcohol dependence and abuse. The role of alcohol in violent crime was proved in a review conducted by Parker (2004). In a survey conducted by United States Department of Justice in 2010, it was found that approximately one-third of crimes were linked to alcohol use, especially in the areas of juvenile delinquency, sexual assault, domestic violence and homicide (Horvath & LeBoutillier, 2014).

2.16.2 Drugs

Varieties of other mood-altering substances are used by children in connection to childhood adversity. Use of marijuana, alcohol and polysubstance abuse was a predictive of anxiety, depression and conduct disorders (Greenbaum *et al.,* 1991; Neighbors *et al.,* 1992). Substance abuse could exacerbate the symptoms of

depression, anxiety or other mental illnesses. The co-occurrence of these two problem behaviours intensifies the effect of both and propels youths towards severe violent and antisocial behaviours (Deykin *et al.,* 1987; Simons *et al.,* 1988). Higher rates of disruptive behaviour disorders and depressive disorders were shown by the juveniles with greater levels of alcohol abuse (Rohde *et al.,* 1996). Hence it was more likely that the onset of these behaviours is rooted in variety of adverse experiences during childhood. Two to four times increase in the early initiation of illicit drug use, drug addiction and drug use problems were found with each adverse childhood experience (Dube *et al.,* 2003). Intravenous drug and substance use were found in children those who were physically and sexually abused (Ompad *et al.,* 2005; Kerr *et al.,* 2009). Exposures to several ACEs were a predictive of multiple substance use like the use of illicit drug use and alcohol simultaneously (Harrison *et al.,* 1997). Rebellion towards traditional social values may be manifested by children who express their anger through early life aggression (Brook *et al.,* 1992). This rebellion could take place in the form of drug experimentation and abuse. It was found that conduct problem and aggression is related to chronic substance abuse and dependence which persist in adulthood also (Fergusson *et al.,* 2007). The initiation, abuse and dependence of mood-altering substances like alcohol, nicotine and illicit drugs are associated with higher levels of impulsivity (Dawe & Loxton, 2004; Gullo & Dawe, 2008; Verdejo-Garcia *et al.,* 2008).

SIVA PRASAD M. S. “EFFECT OF ADVERSE CHILDHOOD EXPERIENCES AND OCCURRENCE OF UVNTR POLYMORPHISM IN MONOAMINE OXIDASE A GENE IN RECIDIVIST VIOLENT OFFENDERS: FORENSIC IMPLICATIONS”. THESIS. DEPARTMENT OF ZOOLOGY, UNIVERSITY OF CALICUT, 2018.

Chapter 3
MATERIALS AND METHODS

PART-I : GENOTYPING OF uVNTR POLYMORPHISM OF *MAOA* GENE

Phase- I : Standardization of laboratory protocols (Pilot study)
- Participants
- Collection, isolation, quantification and PCR analysis of genomic DNA
- Statistical Analysis

Phase- II : Main study
- Hypothesis
- Research design
- Participants
- Procedure of Sample collection, Genotyping and *in silico* sequence analysis
- Statistical Analyses

PART- II: : ASSESSMENT OF ADVERSE CHILDHOOD EXPERIENCES (ACEs) AND HEALTH RISK BEHAVIOURS (HRBs)

Phase- I : Standardization of data collection tools (Pilot study)
- Participants
- Data collection tools

Phase- II : Main study
- Hypotheses
- Research design
- Participants
- Changes made in ACE-IQ for the main study
- Procedure
- Statistical Analyses
- Ethical Consideration

This chapter provides the details of materials and methods used in this study. This comprises of participants, DNA collection techniques, methodology used for genotyping, data collection tools and the statistical techniques employed. This chapter has been presented in two parts.

Each part has two phases. Part-I, deals with the methodology used for genotyping all the participants for identifying the allelic variants of *MAOA*-uVNTR polymorphism. First phase in the part-I explains the methodology adopted for conducting the pilot study for the standardization of laboratory protocols. Pilot study has been executed to identify the best biological sample collection procedure for obtaining high quality genomic DNA from the participants and for amplifying the desired sequence of *MAOA*gene using PCR technique. In the second phase of the part- I, the main study, methodology used for identifying the participants, buccal swab collection and genotyping, identification of the 30 bp repeats of *MAOA*-uVNTR polymorphism and *in silico* sequence analysis have been explained.

Methods used for standardization of data collection tools, assessment of demographic information, adverse childhood experiences (ACEs), health risk behaviour (HRBs) of the participants and the criminal history of recidivist violent offenders has been explained in the part-II. First phase of part- II explains the methodology adopted for conducting the pilot study for the standardization of data collection tools. Second phase of the part- II, the main study, consists of the methodology for experimental research adopted for assessing the ACEs and HRBs of the participants. Also, this phase explains the procedure for estimating the violent criminality

of the recidivist offenders. Even though, the study is described in two parts, all the processes were conducted simultaneously.

PART- I: GENOTYPING OF uVNTR POLYMORPHISM OF *MAOA* GENE

Aim of this part of the study was to identify the allelic variants of *MAOA*-uVNTR polymorphism in the participants.

3.1 Phase- I: Standardization of laboratory protocols (Pilot study)

This phase explains the necessity of the pilot study and the methodology employed for identifying the best biological sample collection technique for obtaining high quality genomic DNA from the participants. As the participants of this study were humans, especially violent offenders, it was necessary to adopt a convenient and cost effective method for collecting biological samples for obtaining sufficient quantity and quality of DNA in field conditions.

3.1.1 Participants

Ten male volunteers belonging to the various departments of University of Calicut were identified and included in the pilot study.

3.1.2 Collection, isolation, quantification and PCR analysis of genomic DNA

3.1.2.1 Collection, preservation and transport of biological samples

After getting the informed written consent from the participants, the biological samples included buccal cells, dried blood and saliva were collected by commercially available collection kits. Buccal epithelial cells were collected using two sterile swabs per

participant. One swab was ‘Sterile Foam Tipped Applicator’ (Figure 3.1) from Puritan, USA, with plastic handle and dry transport system. Other was ‘Sterile Foam Tipped Swab’ (Figure 3.2) from HiMedia Laboratories Pvt. Ltd., India. All the participants were asked to desist from eating or drinking 1 hour prior to buccal cell collection. Inside cheeks of each participant were brushed for 45 s with each sterile swab. Blood was collected from same participants on blood sample storage card, ‘NucleoSave’ (Figure 3.3) from Macherey-Nagel GmbH & Co. KG, Germany. Finger puncture method was followed to collect blood using a sterile lancet. Aseptic conditions were maintained while blood collection.

Whole saliva was collected from same ten participants using whole-saliva collection kit ‘Oragene DNA (OG-500)’ (Figure 3.4a) from DNA Genotek, Inc., Ottawa, Ontario, Canada, following the manufacturer's instructions (Birnboim, 2004). Approximately 2 ml saliva was deposited by the participants into the collection cup (Figure 3.4b). Four hour gap was maintained between buccal swab and saliva collection procedure. The cap attached along with the collection cup was securely fastened after the collection of sufficient quantity of saliva, so that solution stored in the cap was released and mixed with the saliva. Buccal cells, blood and whole-saliva were collected from the participants on the same day. Buccal swabs and blood storage cards were air dried immediately after the sample collection and stored at room temperature for seven days including the saliva samples in order to mimic the exact field conditions.

Figure 3.1: Sterile Foam Tipped Applicator, Puritan, USA.

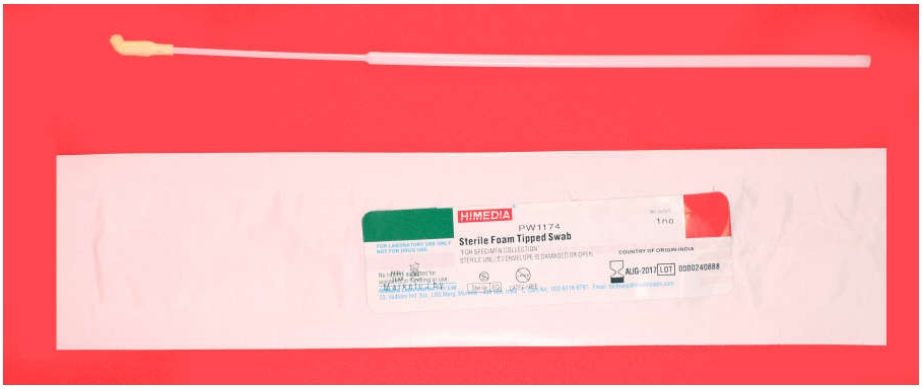

Figure 3.2: Sterile Foam Tipped Swab, HiMedia Laboratories Pvt. Ltd., India.

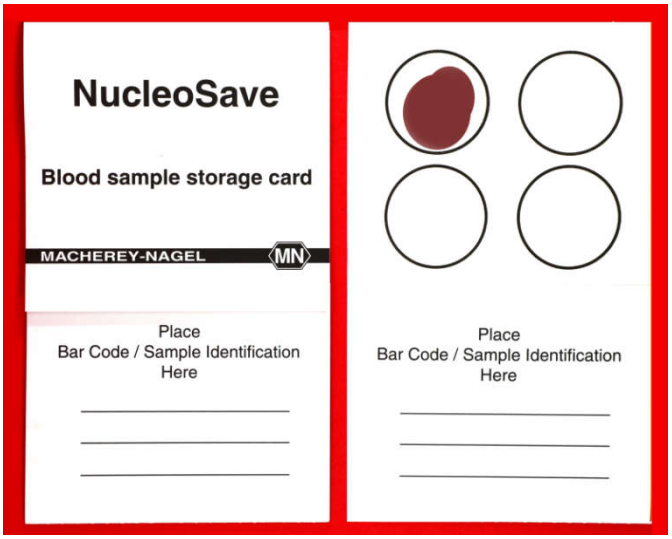

Figure 3.3: NucleoSave, Macherey-Nagel GmbH & Co. KG, Germany. Illustrative blood spot shown.

Figure 3.4a: Oragene DNA (OG-500), DNA Genotek, Inc., Ottawa, Ontario, Canada.

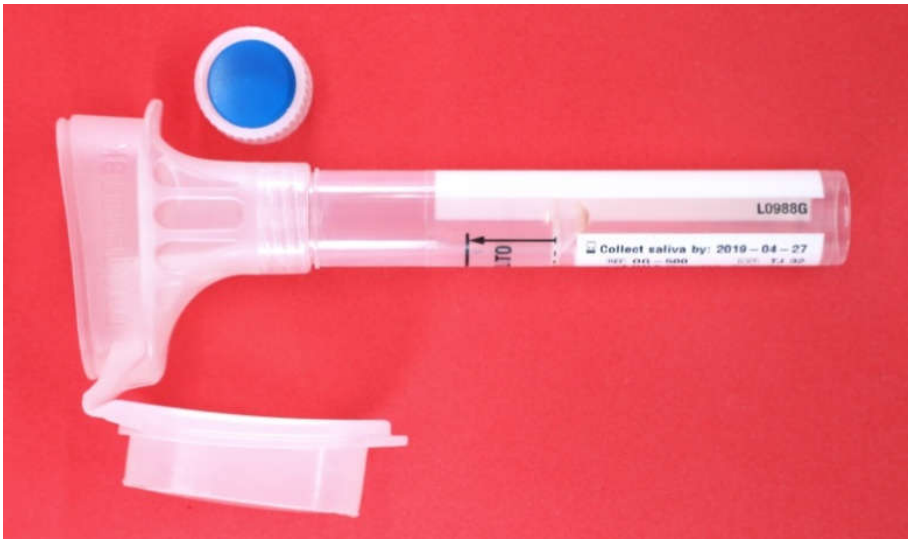

Figure 3.4b: Oragene DNA (OG-500) saliva collection cup with blue coloured cap.

3.1.2.2 Isolation of genomic DNA from samples

a) Buccal swab

Swab containing tip along with the plastic handle was cut using a sterile scissor and was placed in 1.5 ml microcentrifuge tube. To each samples 400µl phosphate buffered saline (pH 7.4) was added. DNA was extracted from the buccal cells using the QIAamp DNA Mini Kit (Qiagen) following the buccal swabs protocol (Appendix 1) with minor modifications in the final elution step. The final elution was done in two steps by adding 75µl Buffer AE and centrifuged at 8,000 rpm for 1 minute instead of elution in single step by adding 150µl Buffer AE. Also, Buffer AE was heated for 60 ºC before adding to the QIAamp Mini spin column. After adding the heated buffer AE, the column was incubated at room temperature for 10 minute in the modified steps before centrifugation. Eluted DNA was stored at -20ºC until further use.

a) Saliva

Isolation of genomic DNA from whole-saliva collected in Oragene DNA (OG-500) kit was done as per the manufacturer's protocol (Appendix 2). Extracted DNA was dissolved in 100µl AE buffer provided in QIAamp DNA Mini Kit (Qiagen) and stored at -20ºC.

b) Dried blood

From the centre of blood soaked region of NucleoSave, 0.5 cm^2 area was cut using a sterile surgical blade and added to 1.5 ml micro

centrifuge tube. Genomic DNA was then eluted using the QIAamp DNA Mini Kit (Qiagen) following the dried blood spots protocol (Appendix 3) with the same modifications in the final elution step as mentioned in the case of buccal swab. Eluted DNA was stored at -20ºC until further use.

3.1.2.3 Quantitative and qualitative assessment of DNA

a) UV spectrophotometry

The yield and quality of genomic DNA extracted from the buccal cells, dried blood and saliva were determined using UV spectrophotometry (Eppendorf BioSpectrometer® basic 6135, Germany) following the manufacturer's instructions. The concentration and purity of genomic extracted DNA were measured by spectrophotometric absorbance of ultraviolet light at wavelengths of 260, 230 and 280 nm. The ratio of absorbance of about 1.8 for the optical density (OD) at 260 nm/ 280 nm was considered to be the presence of high quality of nucleic acid with less protein and organic contamination. A higher value (~2.0- 2.2) for the ratio of absorbance for the OD at 260 nm/ 230 nm was preferred, which indicates limited salt and alcohol contamination (Mackey & Chomczynski, 1997; Rogers *et al*., 2007).

b) Agarose gel electrophoresis

The integrity of extracted DNA was evaluated by running 8 µl of each sample on 0.9 % agarose gel supplemented with ethidium bromide (0.5 µg/ml final concentration) (Sigma Aldrich Chemical Pvt.

Ltd., India) in TBE buffer (890 mM Tris base, 890 mM Boric acid, 0.5 M EDTA at final pH 8.3) at a constant current at 105 V for 45 minute. Bands of DNA were visualized in gel documentation system (G: BOX, F3, SYNGENE, UK) for examining the level of DNA degradation and digital photographs were taken. The bands were compared against 1.5 kb known molecular weight marker (50 DNA ladder, HiMedia).

c) Polymerase chain reaction for *MAOA*-uVNTR amplification

PCR amplification of the 30-bp repeat polymorphism in the promoter region of the *MAOA*-uVNTR gene was performed using PCR Master Mix (Qiagen HotStarTaq Master Mix Kit) containing a final concentration of 1.5 mM MgCl2, 1x PCR Buffer, 200 µM of each dNTP, 2.5 units HotStarTaq DNA Polymerase. Previously published primer (Sabol *et al*., 1998) sequences for *MAOA*- uVNTR were used, forward primer (MAOaPT1): 5'ACAGCCTGACCGTGGAGAAG-3', reverse primer (MAOaPB1): 5'-GAACGGACGCTCCATTCGGA-3' (Sigma Aldrich Chemical Pvt. Ltd., India). Through several standardization processes, total reaction volume of PCR was able to be reduced to 12.5 µl from 50.0 µl by varying the master mix volume, primer concentration and sample dilution. PCR reactions were conducted in the gradient thermal cycler (Sure Cycler 8800, Aligent Technologies, USA) with the following amplification protocol and reaction volume as shown in tables 3.1 and 3.2.

Table 3.1: PCR cycling conditions

Step	Time	Temperature
Initial heat activation	15 minute	95˚C
3-step cycling with 35 cycles		
Denaturation	1 minute	95˚C
Annealing	1 minute	62˚C
Extension	1 minute	72˚C
Final extension	10 minute	72˚C
Holding	Infinite	4˚C

Table 3.2: Standardized PCR reaction volume

Reaction mix	Volume	Concentration
Master mix	6.25µl	-
Forward primer	1.25µl	0.5 µM final concentration
Reverse primer	1.25µl	0.5 µM final concentration
Sterile double distilled water	3.25µl	-
Template DNA (sample)	1.25µl	0-20 times diluted (depends on the concentration of DNA)
Total reaction volume	**12.5µl**	-

Amplification of the desired sequence was confirmed by running 6 µl PCR products in 2 % agarose gel supplemented with ethidium bromide (0.5 µg/ml final concentration) (Sigma Aldrich Chemical Pvt. Ltd., India) in TBE buffer. Bands of amplified products were visualized in Gel documentation system and sizes were compared against 50 bp DNA Ladder (HiMedia, India) with visible bands of length 1000, 900, 800, 700, 600, 500, 400, 300, 250, 200, 150, 100, 50 bp.

3.1.3 Statistical technique

Statistical analyses of the data were performed using SPSS 20.0 for Windows. The yield and purity of DNA extracted from samples collected by four different collection methods were compared by one-way ANOVA with post-hoc Scheffe test. Criterion for statistical significance was set at $p < 0.05$ and 0.001.

3.2 Phase- II: Main study

This phase describes the procedure developed for the selection of participants as per the objectives of the study, procedures undergone for identifying and approaching the participants for sample collection, details of sample collected and methods used for genotyping. Methods used for analysing the tandem repeats and *in silico* sequence analysis of *MAOA*-uVNTR polymorphism of the participants are also explained in this phase.

3.2.1 Hypothesis

Based on the second and third objectives of this study and reviews of various research studies described in the previous chapter, the hypothesis was formulated. Following was the hypotheses formulated for the part- I of the study.

i. There will be significant association between the violent criminality and allelic variants of *MAOA*-uVNTR polymorphism in the participants.

3.2.2 Research design

Simple random sampling method was used in this study since investigator had a list of all subjects in the target population and drawn a random sample from the list of subjects in the sampling frame. In this method of sampling every subject has an equal chance of being selected for the study (Elfil & Negida, 2017). Also, the characteristics of a population can be well understood through interview schedule in this method (Suresh *et al*., 2011).

3.2.3 Participants

a) Universe of the study

The universe of the study was offenders from Thrissur, Palakkad, Malappuram, Calicut and Kottayam districts of Kerala. The population of the study were male habitual/ recidivist violent offenders and age matched controls, termed as case and control respectively.

b) Case

This group included male habitual/ recidivist violent offenders. A male recidivist violent offender in this study referred to an individual, who met the inclusion and exclusion criteria. Out of the fifty two recidivist violent offenders identified, only thirty five gave informed written consent for participating in this study during this period. Hence the first group, case consisted on 35 participants.

In the State of Kerala, the followings Acts and Rule deal with the habitual/ recidivist offenders.

- Habitual Offenders Act, 1960.
- Kerala Anti-Social Activities (Prevention) Act, 2007 (KAAPA Act).
- Chapter XIV, Section 200-205 & 207 of the Kerala Prisons Rules, 1958.

Inclusion and exclusion criteria regarding the selection of participants of this study were developed with the help of Criminologist, Kerala Police.

i) Inclusion criteria

Since the outcome of the aggressive behaviour is in the form of offences against person and property mostly, recidivist violent offenders of this proposed research met three criteria:-

- Above the age of 18 and willing to provide informed written consent.
- Convicted for offences punishable under Chapter XVI (Offences affecting the Human Body) or Chapter XVII (Offences against property) or both of Indian Penal Code, 1860.
- Included in the history sheet of 'Known Depredator (K.D)'/ Habitual offender'/ 'Ex-convicts and Jail released list' in the police stations of Kerala State, as per the Acts/ Rules as mentioned above.

ii) Exclusion criteria

- Individuals with reported psychiatric disorders were excluded from the study.
- Females were excluded from the study.

c) Control

This group included 32 individuals who were the neighbours of the cases.

i) Inclusion criteria

- Individuals of same age, sex, socio-demographic background.
- No specified antisocial behaviour and registered criminal cases.

ii) Exclusion criteria

- Individuals with reported psychiatric disorders were excluded from the study.
- Females were excluded from the study.

3.2.4 Procedure of sample collection, genotyping and *in silico* sequence analysis

a) Sample collection from Cases

Since the details of offenders were available in records in the various prisons, police stations, state and district Crime Records Bureaus, a request to grant permission to the investigator to visit all prisons and police stations for data collection and conduct this study among the recidivist offenders was sent through the Registrar, University to the Principal Secretary to Government, Department of Home, Government of Kerala.

Initially permission was not granted and on briefing about this study by the investigator before the then Director General Police (Prisons), Kerala and submitting a clarification letter, permission was granted to visit all prisons to identify and interview the prisoners. Also, the permission was limited to collect biological samples from released prisoners only.

With an intention to conduct pilot study among the offenders, the investigator visited Central and District Prisons located at Viyyur, Thrissur district of Kerala; sub- jail, Kakkanad, Ernakulam district of Kerala. Details of the released recidivist violent offenders meeting the inclusion criteria and exclusion criteria were obtained from the prisons and the investigator approached these individuals at their residence or convenient places for interview and sample collection. The responses and behaviour from some of these individuals were bitter and threatening. Also, the investigator found it very difficult to reach these individuals who were hard-core violent offenders. Hence an appeal to collect buccal samples from the prison inmates itself was again submitted before the Government, with detailed description about the sample collection procedures and confidentiality maintained to protect the human rights of the prison inmates. This appeal was also rejected by the Government.

As the investigator already got the permission to conduct this study among the released prisoners, letters were sent by the investigator to the District Police Chiefs of Thrissur, Palakkad, Malappuram, Calicut and Kottayam districts of Kerala, requesting for the assistance of Police to conduct the study. Permission was granted

to verify the records of the offenders in these respective District Crime Records Bureaus. The investigator with the help of the Deputy Superintend of Police (DySP) and Circle Inspectors (CI) of these districts made a preliminary list containing details of seventy recidivist violent offenders. Some of the offenders belonging to this list died due to gang wars during the period of this study, some were imprisoned again for committing crime and few were not approachable due to the security reasons. Thus the investigator was able to approach fifty two recidivist violent offenders only.

On prior intimation by the police officials, these offenders were asked to be present in the police stations or called upon to their convenient place or visited at their residence. During the first meeting the investigator self introduced, described about the study and informed about the right of the participant to withdraw from the study voluntarily at any time. After creating a good rapport with the offender, investigator requested for their voluntary willingness to be participant of this study. On getting the voluntary willingness, written informed consent were obtained from them. After noting down the demographic information, face-to-face interview was conducted and the details of the same are explained in the following section 3.4.6a. After the interview, buccal swabs were collected from each participant by the standardized collection procedure using 'Sterile Foam Tipped Applicator', Puritan, USA. Tubes containing the buccal swabs were coded and preserved at room temperature. They were taken to the UGC- SAP laboratory facility available in the Department of Zoology, University of Calicut, within seven days for processing and the DNA extraction.

b) Sample collection from controls

After identifying the controls, it was confirmed by the investigator with the concerned police station that there was no history of any specified antisocial behaviour or criminal cases registered against them. Thirty two individuals belonging to controls were met at their residence and all the procedures that were done for the sample collection from cases were followed in this group also.

c) Genotyping the samples from cases and controls

DNA was extracted from the buccal swabs in batch wise depending on the availability and field work. Standardized extraction protocol was followed (Prasad & Vardhanan, 2018). Presence of sufficient quantity of DNA was confirmed by running agarose gel electrophoresis and visualized in Gel documentation system. Eluted DNA was stored at -20ºC until further use.

PCR amplification of desired sequence in the uVNTR regions of the *MAOA* gene was performed in sixty seven samples by the standardized protocol. In the case of non successful amplification, the template DNA was diluted up to 20 times depending on the concentration of DNA. Proper amplification of the desired sequence and quality of the PCR products were analysed through agarose gel electrophoresis and visualized. Remaining quantity of PCR products were stored at 4˚C in a refrigerator until sequenced.

For sequencing, 6 µl of PCR products were sent to Regional Facility for DNA Fingerprinting at Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram, Kerala, India. PCR products were treated with EXoSAP-IT (GE Healthcare) for removal of

unwanted primers and dNTPs. For this cleaning procedure 5 µl of PCR product was mixed with 2 µl of ExoSAP-IT and incubated at 37^{o}C for 30 minutes followed by enzyme inactivation at 80^{o}C for 15 minutes. Sequence analysis of the PCR products were done in Applied Biosystems® 3500 Genetic Analyzer. The sequence quality was checked using Sequence Scanner Software v1 (Applied Biosystems). Sequence alignment and required editing of the obtained sequences were carried out using Geneious Pro v5.1 (Drummond *et al*., 2010).

d) *In silico* sequence analysis

Chromatograms obtained were converted to FASTA format and the 30 bp tandem repeats in the DNA sequence were analysed using online program, Tandem Repeats Finder (Benson, 1999). Multiple alignments of the sequences were done using MultAlin software (Corpet, 1988). All the individual nucleotide sequences were deposited to GenBank ®, the genetic sequence database of National Institutes of Health (NIH), Department of Health and Human Services, United States. *MAOA* promoter sequence was retrieved from NCBI (GenBank M89636) (Zhu *et al*., 1992) and compared with all the sequences obtained in this study.

3.2.5 Statistical analysis

A χ^2- test was performed to compare the association between allelic variants of *MAOA*-uVNTR polymorphism and violent criminality across categorical variables (case and control). All analyses were conducted using SPSS 20.0 for Windows. Criterion for statistical significance was set at $p < 0.05$ and 0.001.

PART-II: ASSESSMENT OF ADVERSE CHILDHOOD EXPERIENCES (ACEs) AND HEALTH RISK BEHAVIOURS (HRBs)

Aim of this part of the study was to know the demographics and to assess the Adverse Childhood Experiences (ACEs) and Health Risk Behaviours (HRBs) of the participants. The crime history of the recidivist violent offenders was also examined.

3.3 Phase- I: Standardization of data collection tools (Pilot study)

This phase explains the methodology employed for conducting the pilot study, which was carried out to:-

1. To detect the possible flaws in the data collection tools like, interview time, limits etc.

2. To identify ambiguous or unclear questions in the data collection tools.

3. To test the objective that leads to testing more precise objective in the main study.

4. To establish whether the sampling frame and techniques are effective.

5. To check the reliability and validity of results.

The adverse childhood experiences; use of tobacco, alcohol and street drugs/ substance abuse during the first 18 years of life of the

participants were assessed retrospectively using various data collection tools.

3.3.1 Participants

Twenty male participants were included in the pilot study. This consisted of 10 numbers of incarcerated recidivist violent offenders meeting the inclusion and exclusion criteria and 10 numbers of age matched volunteers belonging to the various departments of University of Calicut. The 10 volunteers were the same participants as mentioned in the section 3.1.1.

3.3.2 Data collection tools

Adverse Childhood Experiences International questionnaire (ACE-IQ) was used in the pilot study to assess common aspects of childhood traumas. Questions used to define Health Risk Behaviours like tobacco use, alcohol use and street drugs/ substance abuse were adapted from the male version of Family Health History Questionnaire of the CDC-Kaiser Permanente Adverse Childhood Experiences (ACE) Study (Centre for Disease Control and Prevention, 2014). All the questions in both the data collection tools were translated into regional language, Malayalam and standardized. Translation was done with the help of psychology and language experts.

1) Adverse Childhood Experiences International questionnaire (ACE- IQ)

ACE-IQ was developed by World Health Organization (WHO) (World Health Organisation, 2014) with an intention to measure

adverse childhood experiences (ACEs) in all countries. This questionnaire was designed for administrating to people aged 18 years older and measures the adverse childhood experiences retrospectively. ACE- IQ contains 9 sections, Demographic information, Marriage, Relationship with Parents/Guardians, Family environment, Peer violence, Witnessing community violence, Exposure to War/ Collective Violence.

Using the original ACE-IQ, 13 categories of childhood experiences can be assessed, which include physical abuse (2 questions); emotional abuse (2 questions); contact sexual abuse (4 questions); alcohol and/or drug abuser in the household (1 question); incarcerated household member (1 question); someone chronically depressed, mentally ill, institutionalized or suicidal (1 question); household member treated violently (3 questions); one or no parents, parental separation or divorce (2 questions); emotional neglect (2 questions); physical neglect (3 questions); bullying (3 questions); community violence (3 questions); collective violence (4 questions) (World Health Organisation, 2014). Demographic information includes sex, date of birth, age, ethnic group, education level, employment, civic status and questions about marriage.

Experts of WHO developed latest version of ACE- IQ based on the field studies done in seven countries. Study done using ACE- IQ among adolescents in Vietnam showed good concurrent validity (Tran *et al*., 2015). As a part of broader health surveys, this questionnaire is being validated in several countries (Almuneef *et al*., 2014; Bellis *et al*., 2014).

2) Health Risk Behaviour questions

Questions regarding the use of tobacco, alcohol and street drugs/ substance abuse used in the CDC-Kaiser Permanente Adverse Childhood Experiences (ACE) Study **(**The Centers for Disease Control and Prevention, 2014), were used in this study. There were six questions, three of were 'yes' or 'no' questions and others were regarding the age of onset of using tobacco, alcohol and street drugs/ substance abuse. The participants reported whether they started the habit of using tobacco, alcohol and street drugs/ substance abuse before the age of 18.

ACE questionnaires used in the CDC-Kaiser Permanente Adverse Childhood Experiences (ACE) Study had demonstrated good reliability, strong internal consistency and concurrent validity (Murphy *et al*., 2014).

3.3.3 Definitions of Adverse childhood experiences

There were 13 categories of adverse childhood experiences (ACEs) and 3 categories of health risk behaviour (HRBs) measured using ACE- IQ data collection tool and HRB questions respectively. All questions about ACEs and HRBs were related to the participant's first 18 years of life. Definitions of each category of ACEs and HRBs are described below.

ACE 1: Physical abuse

There were two questions under this category. Each participant was asked "During the first 18 years of your life**,** did a parent, guardian

or other household member spank, slap, kick, punch or beat you up?" and "Did a parent, guardian or other household member hit or cut you with an object, such as a stick (or cane), bottle, club, knife, whip etc?" The participants could respond 'many times' or 'a few times' or 'once' or 'never' depending on the intensity of these adverse experiences.

ACE 2: Emotional abuse

There were two questions under this category. Each participant was asked "During the first 18 years of your life**,** did a parent, guardian or other household member yell, scream or swear at you, insult or humiliate you?" and "did a parent, guardian or other household member threaten to, or actually, abandon you or throw you out of the house?" The participants could respond 'many times' or 'a few times' or 'once' or 'never' depending on the intensity of these adverse experiences.

ACE 3: Contact sexual abuse

There were four questions under this category. Each participant was asked "During the first 18 years of your life**,** did someone touch or fondle you in a sexual way when you did not want them to? Did someone make you touch their body in a sexual way when you did not want them to? Did someone attempt oral, anal, or vaginal intercourse with you when you did not want them to? Did someone actually have oral, anal, or vaginal intercourse with you when you did not want them to?" The participants could respond 'many times' or 'a few times' or 'once' or 'never' depending on the intensity of these adverse experiences.

ACE 4: Alcohol and/or drug abuser in the household

There was one question under this category. Each participant was asked "During the first 18 years of your life**,** did you live with a household member who was a problem drinker or alcoholic or misused street or prescription drugs?" The participants could respond either "yes" or "no" to these questions.

ACE 5: Incarcerated household member

There was one question under this category. Each participant was asked "During the first 18 years of your life**,** did you live with a household member who was ever sent to jail or prison?" The participants could respond either "yes" or "no" to these questions.

ACE 6: Someone chronically depressed, mentally ill, institutionalized or suicidal

There was one question under this category. Each participant was asked "During the first 18 years of your life**,** did you live with a household member who was depressed, mentally ill or suicidal?" The participants could respond either "yes" or "no" to these questions.

ACE 7: Household member treated violently

There were three questions under this category. Each participant was asked "During the first 18 years of your life, did you see or hear a parent or household member in your home being yelled at, screamed at, sworn at, insulted or humiliated? Did you see or hear a parent or household member in your home being slapped, kicked, punched or beaten up? Did you see or hear a parent or household

member in your home being hit or cut with an object, such as a stick (or cane), bottle, club, knife, whip etc.?" The participants could respond 'many times' or 'a few times' or 'once' or 'never' depending on the intensity of these adverse experiences.

ACE 8: One or no parents, parental separation or divorce

There were two questions under this category. Each participant was asked "During the first 18 years of your life, were your parents ever separated or divorced?" and "did your mother, father or guardian die?" The participants could respond either "yes" or "no" to these questions.

ACE 9: Emotional neglect

There were two questions under this category. These were negative questions. Each participant was asked "During the first 18 years of your life, did your parents/guardians understand your problems and worries?" and "did your parents/guardians really know what you were doing with your free time when you were not at school or work?" The participants could respond 'always' or 'most of the time' or 'sometimes' or 'rarely' or 'never' depending on the intensity of these adverse experiences.

ACE 10: Physical neglect

There were three questions under this category. Each participant was asked "During the first 18 years of your life, how often did your parents/ guardians not give you enough food even when they could easily have done so? Were your parents/guardians too drunk or

intoxicated by drugs to take care of you? How often did your parents/ guardians not send you to school even when it was available?" The participants could respond 'many times' or 'a few times' or 'once' or 'never' depending on the intensity of these adverse experiences.

ACE 11: Bullying and physical fight

There were two questions under this category. The meaning of bullying and physical fight as mentioned in the ACE- IQ was explained and made the participants understand about it. Then each participant was asked "During the first 18 years of your life, how often were you bullied?" and "how often were you in a physical fight?" The participants could respond 'many times' or 'a few times' or 'once' or 'never' depending on the intensity of these adverse experiences.

ACE 12: Community violence

There were three questions under this category. The meaning of community violence as mentioned the ACE- IQ was explained and made the participant understand about it. The each participant was asked "During the first 18 years of your life, did you see or hear someone being beaten up in real life? Did you see or hear someone being stabbed or shot in real life? Did you see or hear someone being threatened with a knife or gun in real life?" The participants could respond 'many times' or 'a few times' or 'once' or 'never' depending on the intensity of these adverse experiences.

ACE 13: Collective violence

There were four questions under this category. The meaning of collective violence as mentioned the ACE- IQ was explained and made

the participant understand about it. The each participant was asked "During the first 18 years of your life, were you forced to go and live in another place due to any of these events? Did you experience the deliberate destruction of your home due to any of these events? Were you beaten up by soldiers, police, militia, or gangs? Was a family member or friend killed or beaten up by soldiers, police, militia, or gangs?" The participants could respond 'many times' or 'a few times' or 'once' or 'never' depending on the intensity of these adverse experiences.

3.3.4 Definition of Health Risk Behaviours

Tobacco use

There were two questions under this category. Each participant was asked "During the first 18 years of your life, do you have a habit of smoking cigarette? and "how old were you when you began to smoke cigarettes fairly regularly?" The participants could respond either "yes" or "no" to the first question. Participants responded about the age to the second question.

Alcohol use

There were two questions under this category. Each participant was asked "During the first 18 years of your life, have you ever drunk alcohol?" and "how old were you when you had your first drink of alcohol other than a few sip?" The participants could respond either "yes" or "no" to the first question. Participants responded about the age to the second question.

Street drug/ substance abuse

There were two questions under this category. Each participant was asked "During the first 18 years of your life, have you ever used street drugs?" and "how old were you the first time you used them?" The participants could respond either "yes" or "no" to the first question. Participants responded about the age to the second question.

3.3.5 Scoring of data collection tools

Two method of analysis are proposed by WHO for calculating the ACE scores and this includes a Binary version and a Frequency version (World Health Organisation, 2014). In ACE- IQ, majority of the questions in the categories have response format in frequency. The participants report their adverse events of experiences during childhood as once, a few times, or many times. In the Binary version, answer "yes" (no matter the frequency) to the questions scored one and answer "no" to the questions scored zero.

In the frequency version of analysis, if the participants answered "rarely" or "never" for the questions assessing emotional neglect, "many times" for the questions assessing physical abuse, emotional abuse, physical neglect, household member treated violently, bullying, community violence and collective violence, which scored one. An answer "yes", "a few times" or "many times" scored one for the remaining ACEs. In both version of analysis, total ACE score was calculated by summing up the resulting scores of 13 ACE categories and the total score ranges from 0 to 13. Frequency version of analysis was employed in the pilot study.

Values 2 and 1 were assigned respectively to ‘yes’ and ‘no’ in dichotomous scale questions in the HRB questions. The values were summed up to get overall HRBs and these ranges from 3 to 6.

3.3.6 Procedure

ACE- IQ assessment tool and HRB questions were employed to collect data from both the groups. As it was difficult to get data from the offenders who were released from the prison, the investigator visited the Central and District prison, Thrissur, Kerala for conducting the pilot study among the recidivist violent offenders inside the prison. Similarly the investigator visited 10 numbers of age matched volunteers in the various departments of University of Calicut, Kerala.

The investigator self introduced and briefed the participants about the research work. The participant were also assured that, the data collected from them will be used for research work only and will be kept confidential at all times. Written informed consents were obtained from the participants who were willing to participate voluntarily and then they were interviewed face-to-face individually with the help of ACE- IQ assessment tool and HRB questions. The investigator asked the participants to provide their understanding about the questions, whether there were any confusion, discrepancies and inappropriate questions in the assessment tool. The survey took a span of 30 to 45 minutes to complete one participant. They were asked to give subjective opinion about the assessment tool employed for the study. Response received from the offenders contained some valuable information, which was taken into consideration.

3.4 Phase- II: Main study

This phase describes the procedure developed for accessing the demographic information, ACEs and HRBs of the participants. The procedure adopted for estimating the violent criminality is also explained in this phase.

3.4.1 Hypotheses

Based on the first and fourth objectives of this study and reviews of various research studies described in the previous chapter, the hypotheses were formulated. Following were the hypotheses formulated for the part- II of the study.

ii. There will be significant difference in the frequency and prevalence of adverse childhood experiences (ACEs) between cases and controls.

iii. There will be significant correlation between variables of adverse childhood experiences (ACEs) in the participants.

iv. There will be significant correlation between adverse childhood experiences (ACEs) and violent criminality in cases.

v. Adverse childhood experiences (ACEs) can significantly predict violent criminality in cases.

vi. There will be significant difference in the prevalence of health risk behaviours (HRBs) between cases and controls.

vii. There will be significant correlation between adverse childhood experiences (ACEs) and health risk behaviours (HRBs) in the participants.

viii. There will be significant correlation between health risk behaviours (HRBs) and violent criminality in cases.

ix. Adverse childhood experiences (ACEs) can significantly predict health risk behaviours (HRBs) in the participants.

x. There will be significant difference in the interaction of allelic variants of *MAOA*-uVNTR polymorphism and adverse childhood experiences (ACEs) and in cases and controls.

3.4.2 Research design

Simple random sampling was employed in this phase also as mentioned in the section 3.2.2.

3.4.3 Participants

The universe and population of this study was exactly same as mentioned in the section 3.2.3. This included the same 35 numbers of cases and 32 numbers of controls.

3.4.4 Changes made in ACE-IQ for the main study

Since the development of ACE-IQ is at the pilot stage (World Health Organisation, 2014), after analysing the responses and data obtained from the pilot study, some modifications in the response

categories of questions and scoring of ACE-IQ were done on expert opinion and applied for the main study as follows:-

1. Questions under the section, marriage in the demographic information were eliminated.

2. The response category, 'most of the time' was eliminated from the two response categories, 'always' and 'most of the time', to the questions belonging to the category of emotional neglect.

3. The response category 'refused' was also eliminated from all the questions. Thus, in the modified ACE- IQ, questions had four response categories only.

4. Question regarding the involvement in physical fight was also included along with the bullying when the total ACE was calculated.

3.4.5 Scoring used for the main study

Upon the expert opinion, except to the questions belonging to emotional neglect category, all other questions were positively scored on 4-point Likert-type scale according to the frequency with which experiences occurred (Bernstein *et al*., 1994; Zhang *et al.*, 2016), by assigning the values 4, 3, 2 and 1 respectively to 'many times', 'a few times', 'once' and 'never'. Since the questions belonging to emotional neglect are negative questions, reverse scoring was done on 4-point Likert-type scale by assigning the values 1, 2, 3 and 4 respectively 'always', 'sometimes', 'rarely' and 'never'. Values 2 and 1 were assigned respectively to 'yes' and 'no' in dichotomous scale questions.

The total ACE score was calculated by summing up the values of each question in Likert scale and in dichotomous scale in 13 sub categories of ACEs (Zhang *et al*, 2016). Thus the total ACE score ranges from 30 to 110 and categorized as given in the table 3.3.

Table 3.3: Categorization of total ACE score

Total ACE score	**ACE exposure**
Less than or equal to 40	Low
Between 41- 50	Moderate
Between 51- 60	Medium
Between 61- 70	High
Greater than or equal to 71	Extreme

3.4.6 Procedure

a) Data collection from Cases and Controls

Demographic information of the participants was noted, which included age, religion, education, work status over last 12 months and civic status. With the help of modified and translated version of ACE-IQ (Appendix 4 & 4.1) and HRB questions (Appendix 5 & 5.1), structured face-to-face interview were conducted. The rationale for using this method was that, this method is one of the most efficient methods to gather accurate information and it is free from biasness. Mainly it could be used for educated as well as uneducated participants. Some of the participants were visited 2-3 times to complete the data collection process, since they were intoxicated with alcohol or drugs of abuse during the meetings. The interviews were conducted by the investigator only during day time in the presence of police personnels and with the assistance of criminologist. It took a

span of 45 to 60 minutes to complete the interview of each participant with the help of modified ACE- IQ assessment tool and HRB questions. Name of each participants were coded and data collected through interview were compiled and stored in a password protected personal computer.

Data were collected from the controls through the same procedure as mentioned in the section 3.2.4b. Two buccal swabs were collected from each participant just after the interview.

b) Crime history of Cases

The history sheet of 'Known Depredator (K.D)', 'Habitual Offenders' and 'Ex-convicts and Jail release list' maintained in the police stations were verified in detail by the investigator to know the details of the crimes committed by the offenders included in this study. Also, the age at which first crime committed was taken into record. As per the expert opinion, number of crimes committed under the sections in the Chapter XVI (Offences affecting the Human Body) of Indian Penal Code (IPC) was summed up to determine the violent criminality of the cases (Table 3.4).

Table 3.4: Crimes considered for calculating violent criminality

Crime	IPC Sections
Murder	300
Attempt to murder	307
Hurt	319
Assault	351
Wrongful restrain	339
Rape	375

3.4.7 Statistical Analyses

Data collected from participants belonging to cases and controls were consolidated and entered into SPSS for Windows version 20.0. All the hypotheses were tested at both 0.05 and 0.001 level of statistical significance.

Descriptive statistics, bivariate analysis and multivariable analysis were carried out for data processing. Initially, descriptive statistics included frequencies, percentages, mean and standard deviation which were employed to understand the demographic information, response of ACE questions; smoking, alcohol and drug abuse habits of cases and controls. Subsequently bivariate analysis was performed using independent sample t-test for estimating mean differences of scores of ACE categories, total ACE and HRBs in cases and controls. Pearson correlation was carried out to analyse the linear relationship within ACE categories in the participants and between ACE categories and violent criminality in cases. In addition Pearson correlation estimated the relationship between total ACE score and HRBs. Multivariable analyses were conducted with linear and multiple regression for predicting violent criminality (Dependent variable) using ACE categories or total ACE score as predictors. As well as, total ACE score (Predictor) was also used to predict HRBs (Dependent variable). Candidate gene (allelic variants of *MAOA*-uVNTR) - Environment (ACEs) interaction was evaluated by performing one-way ANOVA, followed multiple comparisons performed using Scheffe procedure at 0.05 and 0.001 significance level.

3.5 Ethical consideration

This study was approved by the institutional ethics committee, the Calicut University Human Ethical Committee (Ref. No.: 003/CUEC/CR/2013-12-CU dated 25.04.2014) (Appendix 6). Ethical guidelines of Indian Council of Medical Research to involve human participants in research were followed throughout this study. Written informed consents were obtained from all the participants (Appendix 6.1 & 6.2). Permission to conduct this research in released prisoners was obtained from the Department of Home, Government of Kerala (Letter no. 71726/B1/2014/Home of the Additional Chief Secretary, Government of Kerala to the Registrar, University of Calicut dated 27.11.2014) (Appendix 6.3).

SIVA PRASAD M. S. “EFFECT OF ADVERSE CHILDHOOD EXPERIENCES AND OCCURRENCE OF UVNTR POLYMORPHISM IN MONOAMINE OXIDASE A GENE IN RECIDIVIST VIOLENT OFFENDERS: FORENSIC IMPLICATIONS”. THESIS. DEPARTMENT OF ZOOLOGY, UNIVERSITY OF CALICUT, 2018.

Chapter 4
RESULTS AND DISCUSSION

PART-I	**:**	**GENOTYPING FOR *MAOA*-uVNTR**
Section I	:	Identification of suitable method for DNA collection, extraction and quantification
Section II	:	Allelic variants of *MAOA*-uVNTR polymorphism and *in silico* sequence analysis in participants
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Section I	:	Pilot study for standardising data collection tools, Demographic information of participants; crime history and age of onset of crime in cases and ACEs
Section II	:	Relationship between Adverse Childhood Experiences (ACEs) and violent criminality
Section III	:	Prevalence and relationship between Health Risk Behaviours (HRBs) and violent criminality
PART- III	**:**	**CANDIDATE GENE-ENVIRONMENT (cGxE) INTERACTION**
Section I	:	Interaction between allelic variants of *MAOA*-uVNTR polymorphism and ACEs in cases and controls

This chapter is presented in three parts. Part-I illustrate the results obtained and discuss the findings related to *MAOA*-uVNTR polymorphism. This part is divided into two sections. The results of the pilot study that was done to identify the best biological sample collection procedure for obtaining DNA for analysing allelic variants of *MAOA*-uVNTR polymorphism are given in section- I. In section- II, the results and discussions of the main study related to the *in silico* sequence analysis of allelic variants of *MAOA*-uVNTR polymorphism in cases and controls are given.

Part- II consists of the results and discussions related to adverse childhood experiences (ACEs), health risk behaviours (HRBs) and crime. This part is divided into three sections. In section-I, the inferences of pilot study, followed by the demographic information, crime history and age of onset of crime of cases; frequency and prevalence of ACEs of all the participants in the main study are given. Section- II deals with the results and discussions of the main study which assessed the interrelationships of ACE categories, relationship between ACEs and violent criminality. Prevalence, relationship between HRBs, ACEs and violent criminality in the cases are given in section- III.

Part- III illustrates the interaction between allelic variants of *MAOA*-uVNTR polymorphism and adverse childhood experiences (ACEs) in cases and controls. In all the parts, the results obtained during the analysis are presented through tables, graphs and figures along with their discussions.

PART- I

GENOTYPING FOR *MAOA*-uVNTR POLYMORPHISM

4.1 Section I: Identification of suitable method for DNA collection, extraction and quantification

This part consists of the results obtained during the pilot study conducted for finding out the best biological sample collection procedure for obtaining high quality genomic DNA. Comparison between the quality and quantity of DNA extracted from various sources and its PCR amplification results are illustrated and discussed.

4.1.1 Yield and purity of DNA from buccal cells, saliva and dried blood

As the yield of DNA is quite high (typically 10–15 μg/ml) in whole blood and hence is the tissue of choice traditionally (Quinque *et al*., 2006). Obtaining blood sample is an invasive procedure that requires training in phlebotomy, it is painful for the donor and there are chances of infection too. These constraints limit the suitability of blood collection as a reference sample for some populations, which in turn has led to a search for alternative sources of DNA. Thus, many laboratories often use cheek swabs/ buccal swabs collection rather than drawing blood (Richards *et al*., 1993).

In this study the yield and integrity of DNA extracted from ten participants were checked by agraose gel electrophoresis. Degradation was observed more for the DNA from saliva samples with long smear when compared to the sharp bands in buccal cell DNA. No sharp bands were observed in the case of DNA from dried blood (Figure 4.1).

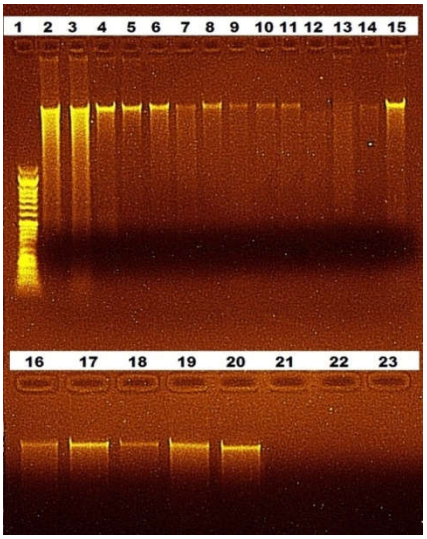

Figure 4.1: Representative agarose gel (0.9%) of genomic DNA extracted from saliva, buccal cells and dried blood. Lane 1 contains 50bp DNA ladder (HiMedia) of 17 bands ranging in size from 50 to 1500bp, with bold reference bands of 200, 500 and 1200 bp. Lanes 2-6, 7-11, 16- 20 were DNA extracted from saliva (Oragene OG-500), buccal cells from (HiMedia foam swabs) and Puritan foam swabs respectively. Lanes 12- 14, 21 and 22 were DNA extracted from dried blood (NucleoSave). Lane 15 and 23 were positive control and blank respectively. 8 µl of DNA was mixed with 2 µl 6X agarose DNA loading dye prior to loading the gel.

Also, the yield and purity of extracted DNA was estimated spectrophotometrically. One-way ANOVA was performed to identify the mean difference in the yield of DNA from four collection methods. The average yield of DNA was found to be different across sections, ($F(3,36) = 1354.55$, $p < 0.00$). The Scheffe multiple comparisons performed at the 0.05 significance level found that the mean yield of genomic DNA extracted from saliva (Oragene, OG-500) (M = 362.70, SD = 28.23, N = 10) was significantly greater than all other sample types in this study.

There was statistically significant difference in the mean yield of DNA from buccal cells collected by Puritan foam swab (M = 43.86, SD = 8.15, N = 10) than HiMedia (M = 17.36, SD = 3.06, N = 10) foam swab and dried blood collected on NucleoSave (M = 0.7, SD = 0.24, N = 10). Specifically, the Puritan swab seems to have better average DNA yield than the HiMedia swab and NulceoSave (Table 4.1). It was observed that high quantity of DNA was able to be extracted from all the buccal cells and dried blood with a modification

in the final elution step in the DNA extraction protocol of Qiagen. The quantity of DNA was much higher than the claims of Qiagen kit manufacturers. The mean yield of DNA from buccal cells collected by Puritan foam swab in this study was much higher than the results from other studies using spit wads (Ehli *et al*., 2008), buccal swabs (Meulenbelt *et al*., 1995; Freeman *et al*., 1997; King *et al*., 2002) and cytobrushes (Garcia-Closas *et al*., 2001; Saftlas *et al*., 2004). The mean number of epithelial cells per ml of saliva is about 4.3 x 10^5 (Dawes, 2003), whereas the number of nucleated cells in 1 ml of whole blood is about 4.5–11 x 10^5 (Dacie, 2006). On an average, every 2.7 hour, the surface layer of epithelial cells in mouth is replaced. Hence, the turnover of epithelial cells is quite extensive in the mouth, suggesting that there is likely to be intact genomic DNA in saliva and buccal samples (Dawes, 2003). Moreover, human genomic DNA can be reliably obtained from buccal cells (King *et al*., 2002; Brenton *et al*., 2018; Buscaino *et al*., 2018).

Table 4.1: Comparison of total DNA yield between collection methods

Method of collection	Puritan Foam Swab	HiMedia Foam Swab	NucleoSave	Oragene (OG-500)
Sample type	Buccal cells	Buccal cells	Dried blood	Saliva
N*	10	10	10	10
Mean ± SD[a]	43.86 ± 8.15[b,f]	17.36 ± 3.06[b]	0.70 ± 0.24[b]	362.70 ± 28.23[c,d,e]
Median	44.3	15.9	0.7	372
Minimum	32.2	14	0.4	314
Maximum	56.4	22.3	1	390

*N= Number of participants; SD = Standard Deviation; [a]Mean DNA yields (µg/ml) were compared using One-way ANOVA, Scheffe was done for multiple comparisons; [b]Mean DNA yield was significantly different from Oragene (OG-500); [c]Significantly different from Puritan Foam Swab; [d]Significantly different from HiMedia Foam Swab; [e]Significantly different from NucleoSave; [f]Significantly different from HiMedia foam swab and NucleoSave.

Purity of DNA can be affected by methods of collection (integrity and protein contamination) and extraction techniques (alcohol, salt and organic and protein contamination). Mean ratios of A_{260}/A_{280} and A_{260}/A_{230} for DNA extracted from dried blood were 1.81 ± 0.04 and 2.03 ± 0.03 respectively. This was in the criterion range (~1.8 and ~2.0-2.2 respectively) which showed that the quality of DNA was high. Contamination was high in the DNA extracted from saliva (A_{260}/A_{280} ratio = 1.65 ± 0.07; A_{260}/A_{230} ratio = 1.06 ± 0.07). Even if high molecular weight DNA was able to be extracted from saliva collected in Oragene DNA (OG-500) kit, the integrity and purity were less and it might be due to DNA degradation, carry over food particles and ethanol residues in the Oragene DNA extraction protocol (Feigelson *et al*., 2001). It was found that when compared with saliva, the contamination of DNA was less in buccal cells in Puritan (A_{260}/A_{280} ratio = 1.74 ± 0.10; A_{260}/A_{230} ratio = 1.76 ± 0.05) and HiMedia foam swabs (A_{260}/A_{280} ratio = 1.71 ± 0.08; A_{260}/A_{230} ratio= 1.71 ± 0.09). Also, there was not much difference in the purity between these collection methods (Figure 4.2).

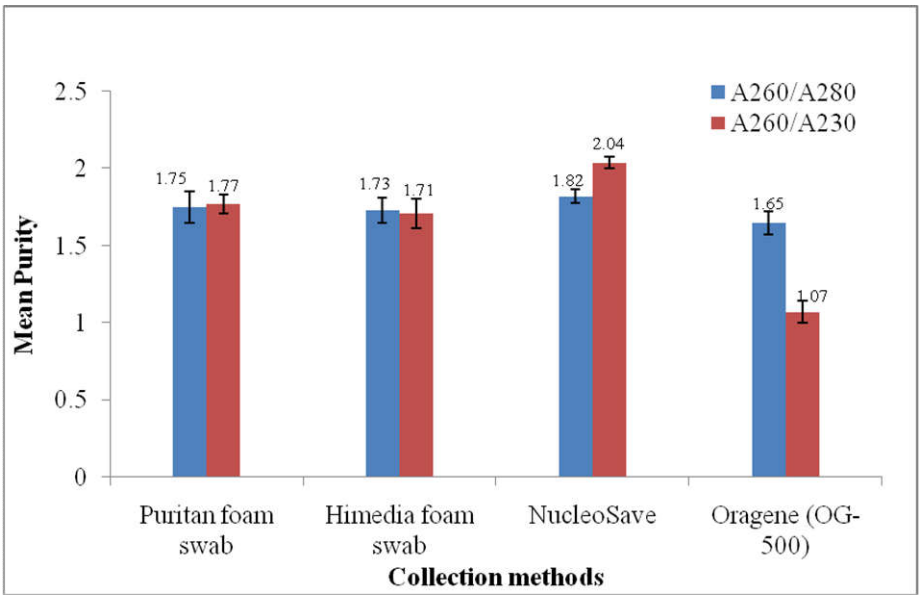

Figure 4.2: Comparison of mean purity of DNA from collection methods. A_{260}/A_{280}, ratio of absorbance at 260 nm to absorbance at 280 nm (measure of protein and organic contamination); A_{260}/A_{230}, ratio of absorbance at 260 nm to absorbance at 230 nm (measure of salt and alcohol contamination).

It is estimated that for PCR amplification reaction of majority of polymorphisms, DNA fragments of nearly 1 kb is only required and the quality of genomic DNA can also be measured using PCR success (King *et al*., 2002). As the main purpose of collecting DNA in this study was for future genotyping, the quality of extracted genomic DNA was also assessed by PCR success using primers specific for 30 bp uVNTR in the promoter of *MAOA* gene. The expected size of the amplified PCR products of *MAOA*-uVNTR polymorphism approximately ranges from 300bp to 400 bp (Das *et al*., 2006). DNA samples were subjected to PCR amplification and fragments were identified concordant in agarose gel electrophoresis (Figure 4.3).

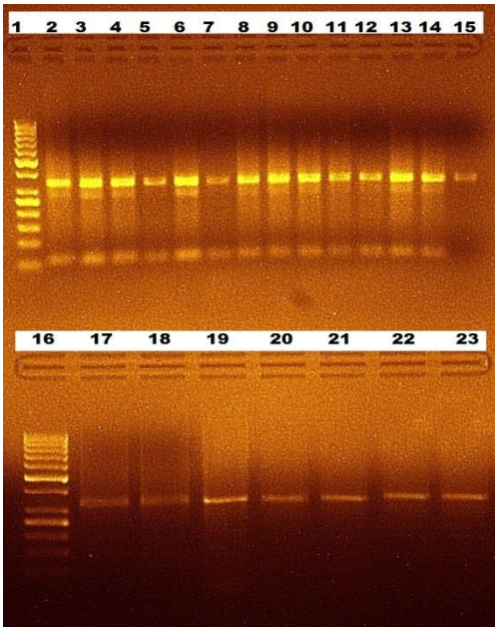

Figure 4.3: Representative agarose gel (2 %) of *MAOA*-uVNTR polymorphism PCR products from genomic DNA. Lane 1 and 16 contains 50 bp DNA ladder (Thermo Scientific) of 13 bands, with bold reference band of 250 bp and 500 bp. Lanes 2-6, 7- 11, 12- 15 and 17; 18- 22 were PCR products generated from DNA extracted from buccal cells collected by 'Puritan' and 'HiMedia' foam swabs, dried blood on 'NucleoSave' and saliva in 'Oragene OG-500' respectively. Lane 23 was a positive control of *MAOA*-uVNTR polymorphism. 6 µl of PCR reaction mix was mixed with 2 µl 6X agarose DNA loading dye (Thermo Scientific) prior to loading the gel.

The success rate of PCR was 100 % with the extracted genomic DNA from all samples as a template. Though the PCR amplifications were successful for all the samples collected by four collection methods, on visualizing the quality of DNA bands of PCR products from buccal cells collected by Puritan foam swabs were better than other PCR products. Also, when the yield and purity of DNA collected by Puritan foam swabs was compared with the others, the efficacy of this collection method remained better. Moreover, this noninvasive technique was more acceptable for the participants and hence selected for the main study.

4.2 Section II: Allelic variants of *MAOA*-uVNTR polymorphism and *in silico* sequence analysis

This session illustrates the genotyping and *in silico* sequence analysis results and related discussions based on the following hypothesis.

Hypothesis: There will be significant association between violent criminality and occurrence of allelic variants of *MAOA*-uVNTR polymorphism in the participants.

Yield and integrity of DNA extracted from sixty seven buccal swabs of the participants belonging to cases (N= 35) and controls (N= 32) were checked by agaraose gel electrophoresis. DNA bands were observed in all the samples (Appendix 7). PCR amplification of the desired sequence of *MAOA*-uVNTR in the DNA of the sixty seven participants was checked by agarose gel electrophoresis (Appendix 8). Depending upon the position of the PCR products in the gel, two types of alleles were visually observed (Figure 4.4). PCR products of all the samples were found to be concordant to the range of the reported size of allelic variants of *MAOA*-uVNTR (Deckert *et al*., 1999; Jorm *et al*., 2000; Huang *et al*., 2004; Das *et al*., 2006). Sequence analysis with Tandem Repeats Finder (Benson, 1999) for the presence of 30 bp tandem repeats revealed two variants of *MAOA*-uVNTR polymorphism only: 3.5 (252 bp) and 4.5 (297 bp) repeats (Appendix 9). In the samples of this study, 2, 2.5, 3, 4, 5, 5.5 and 6 repeat alleles were not identified. In the participants belonging to controls, 3.5 (3.5R) and 4.5 (4.5R) repeat alleles were present. All the participants belonging to cases had 3.5 (3.5R) repeats only. Accession numbers were assigned from GenBank ® for all the 67 nucleotide sequences of this study (Appendix 10).

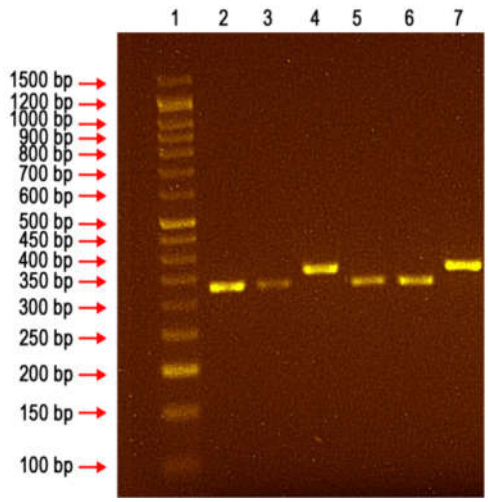

Figure 4.4: Representative agarose gel (2 %) of allelic variants of *MAOA*-uVNTR observed in this study. Lane 1 contains 50 bp DNA ladder (HiMedia, India) of 17 bands ranging in size from 50 to 1500 bp, with bold reference bands of 200, 500 and 1200 bp. Lanes 2, 3, 5 and 6 contains 3.5R allele and lanes 4 and 7 contains 4.5Rallele. 6 µl of PCR product was mixed with 2 µl 6X agarose DNA loading dye (Thermo Scientific) prior to loading the gel.

4.2.1 Allelic variation of *MAOA*-uVNTR polymorphism in different populations

Four different repeat alleles of *MAOA*-uVNTR with repeat numbers of 3, 3.5, 4 and 5 were reported by Sabol and colleagues (1998). All studies related to *MAOA*-uVNTR followed this pattern. The reference sequence available with GenBank (Accession number: M89636) (Zhu *et al*., 1992) actually contains 4.5 repeats of 30 bp repeat sequence of *MAOA*-uVNTR, while it was reported that the region has 4 repeats only. Allowing this adjustment, the 3.5 and 4.5 repeats identified in this study corresponds to 3 and 4 repeats as reported by Sabol *et al*.(1988) and followed by Jorm *et al*., 2000; Das *et al*., 2006; Das, 2008; Bhowmik, 2010; Melas *et al*., 2013; Lim *et al*., 2018. Allele frequencies of *MAOA*-uVNTR polymorphism in different ethnic groups are presented in table 4.2 and table 4.3. The frequencies of 3.5 and 4.5 repeat alleles in our study are 92.5 % and 7.5 % respectively.

Table 4.2: Allelic frequency of *MAOA*-uVNTR in different population groups (Repeat numbers as per the norms of Jorm *et al*., 2000; Das *et al*., 2006)

Population groups	Alleles					N
	2.5	3.5	4	4.5	5	
Australian Caucasian[l]	3	436	13	832	19	1303
Indian, West Bengal[m]	1	153	0	90	1	245
Indian, Kerala (Present study)	0	62	0	5	0	67

Sources: [l]Jorm *et al*., 2000; [m]Das *et al*., 2006; Alleles 2.5 to 5 refer to the number of 30 bp repeat is repeated; N is the total sample size.

Table 4.3: **Allelic frequency of *MAOA*-uVNTR in different population groups (Repeat numbers as per the norms of Sabol *et al*., 1998).**

Population groups	Alleles					N
	2	3	3.5	4	5	
Hispanic/ Latino[a]	0	27	0	65	0	92
Afrikaner[b]	0	55	0	134	7	196
White/non- Hispanic[a]	0	539	8	1056	26	1629
New Zeland, European origin[c]	3	658	9	1238	32	1940
German, European origin[d]	0	47	1	80	3	131
German, European origin[e]	3	140	3	238	6	390
Italian, European origin[d]	3	72	0	102	3	180
Chinese[f]	1	122	0	90	1	214
Asian/Pacific Islander[a]	0	50	1	31	0	82
African American[a]	0	52	2	32	2	88
Sweedish[g]	1	39	3	82	0	125
Israeli[h]	0	297	0	434	1	732
British Caucasian[i]	1	75	4	136	7	223
Japanese[j]	6	465	0	313	2	787
Han Chinese[k]	0	213	0	110	0	323
American White[l]	10	1152	52	2100	43	3356
American Black[l]	46	490	1	416	7	960

Sources:[a]Sabol *et al*.,1998; [b]Erasmus *et al*.,2015; [c]Caspi *et al*.,2002; [d]Deckert *et al*.,1999; [e]Kuepper *et al*.,2013; [f]Lu *et al*.,2002; [g]Jönsson *et al*.,2000; [h]Manor *et al*.,2002; [i]Lawson *et al*.,2003; [j]Ito *et al*.,2003; [k]Younger *et al*.,2005; [l]Haberstick *et al*., 2014; Alleles 2 to 5 refer to the number of 30 bp repeat is repeated; N is the total sample size.

Higher frequency of 3.5R allele was noted in cases (100 %) than that of controls (84.4 %). Only 15.6 % of the controls were with 4.5R allele and none of the cases were noticed with this 4.5R. Eventhough 4.5R allele was not identified in cases, the noted difference was statistically significant (χ^2 = 5.9, df = 1, N = 67, p = 0.015). Hence the hypothesis is accepted.

Allelic distribution pattern of *MAOA*-uVNTR polymorphism obtained in this study were compared with various populations and found to be similar to that of Asian/ Pacific Islander (Sabol *et al*., 1998), African American (Sabol *et al*., 1998), Japanese (Ito *et al*., 2003), Han Chinese (Younger *et al*., 2005), Australian Caucasian (Jorm *et al*., 2000), Indian, West Bengal (Das *et al*., 2006) and American Black (Haberstick *et al*., 2014). An opposite trend was observed in Hispanic/ Latino (Sabol *et al*., 1998), Afrikaner (Erasmus *et al*.,2015), White/ non- Hispanic (Sabol *et al*., 1998); New Zeland, European origin (Caspi *et al*., 2002); German, European origin (Decker *et al*., 1999; Kuepper *et al*., 2013); Italian, European origin (Decker *et al*., 1999); Sweedish (Jönsson *et al*.,1999), Israeli (Manor *et al*., 2002), British Caucasian (Lawson *et al*., 2003) and American White (Haberstick *et al*., 2014).

Even though there were no allelic variants other than 3.5 and 4.5 repeats of *MAOA*-uVNTR polymorphism in this study, the allelic variants and frequency pattern were similar to that of the variants reported by Das and colleagues (2006) in the population of West Bengal State in the eastern part of India. Since India is having a wide range of ethnic groups and diversities within population and the

samples of the present study were from the southern part of India, allelic frequency of *MAOA*-uVNTR polymorphism needs further investigation.

4.2.2 *In silico* sequence analysis

When multiple aligned and compared with the reference sequence (Zhu *et al*., 1992), sequences of this study revealed different single nucleotide variations in the upstream and downstream to the repeat region (Figures 4.5a, 4.5b, 4.6a, and 4.6b) (Appendix 11 and 12).

All the nucleotide sequences obtained in this study were compared with the mostly cited sequence of *MAOA* (GenBank, M89636) (Zhu *et al*., 1992) in association to aggression and antisocial behaviour (Godar *et al*., 2016). All the base variations in the 4.5R allele (N = 5) as same as reported by Das *et al*., (2006) and also four extra base substitutions (at -1307C > G, -1308G > A, -1330A > G and -1331G > A) at the upstream flanking region before the repeat region were observed in this study. These extra base substitutions were probably also noticed by Das *et al*. (2006) since they had described the sequence variations in the flanking regions of 4.5R of *MAOA*-uVNTR that differed by three bases with the published sequence (GenBank, M89636) (Zhu *et al*., 1992); however the extra base substitutions were not reported by Das *et al*. (2006).

Majority of the nucleotide sequences of this study belonged to 3.5R allele (N = 62). When compared with the reference sequence (Zhu *et al*., 1992), variation noticed in the sequences of this study was

only the difference in the repeat numbers. In the downstream flanking region after the repeat region there were two extra bases (at -1107G and -1118C) and absence of five bases (at -1124G, -1125G, -1126G, -1127A and -1134C). There were three base substitutions (at -1133A > G, -1138A > G and -1139G > A) also. Upstream flanking region after the repeat region also showed base substitutions (at -1309C > G, -1310G > C, -1332A > G and -1333G > A) and absence of one base (at -1284T). After the 3.5 repeat region, two base substitutions were also noticed (at -1153G > A and -1154A > G). Even though Das *et al*. (2006) reported 3.5 repeat allele of *MAOA*-uVNTR, the sequence variations were not mentioned. Hence, this study is reporting the sequence variations in the 3.5 repeat allele of *MAOA*-uVNTR in Indian population. The 4.5 and 3.5 repeats and flanking region observed in our study matched totally with the published sequences, GenBank, LN813020.1 (Erasmus *et al*., 2015) and GenBank, LN813022.1 (Erasmus *et al*., 2015) respectively.

In this study, the presence of 3.5R allele was seen both in cases and controls. But, 4.5R allele was present only in the controls which indicated that 3.5 allelic variants of *MAOA*-uVNTR polymorphism had a statistically significant association (p = 0.015) in contributing to violent criminality in cases. However, candidate gene - environment interaction (cGxE) might be a reason for the development of psychopathology in violent offenders (Dick *et al.*, 2015) which was supported by meta-analysis (Byrd & Manuck, 2014).

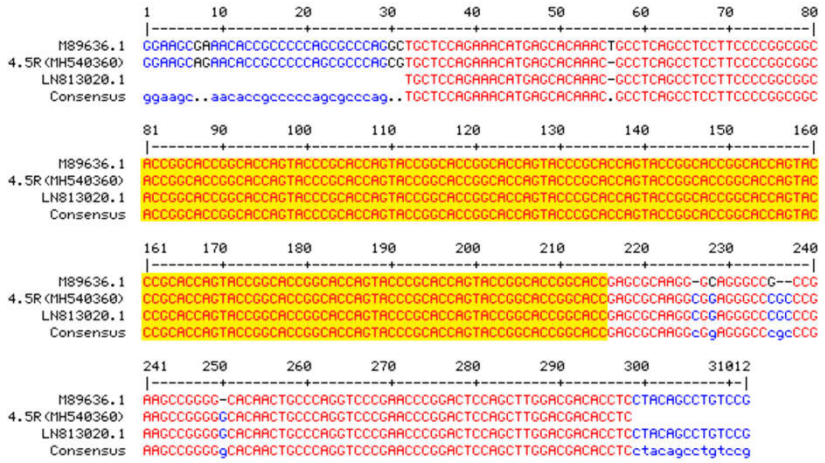

Figure 4.5a: Multiple alignment of representative 4.5R allele of *MAOA*-uVNTR (MH540360) of present study and reference sequences available with GenBank; M89636.1, Author: Zhu *et al*., 1992; LN813020.1, Author: Erasmus *et al*., 2015. Alignment was done by using MultAlin software (Corpet, 1988). Repeat regions are highlighted in yellow.

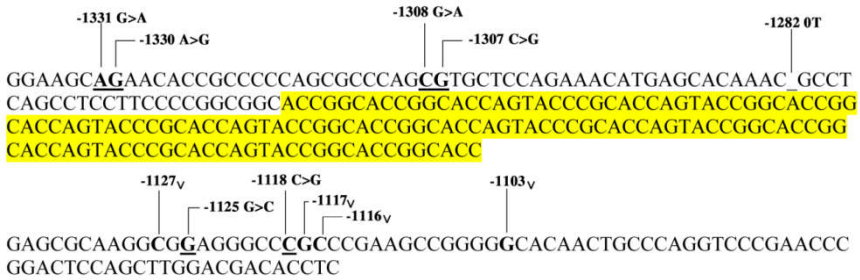

Figure 4.5b: Sequence variations in upstream and downstream flanking regions of 4.5R allele of *MAOA*-uVNTR. Repeat regions are highlighted in yellow. Symbols '>', '0' and 'v' indicates base substitution, absence of base and addition of base respectively.

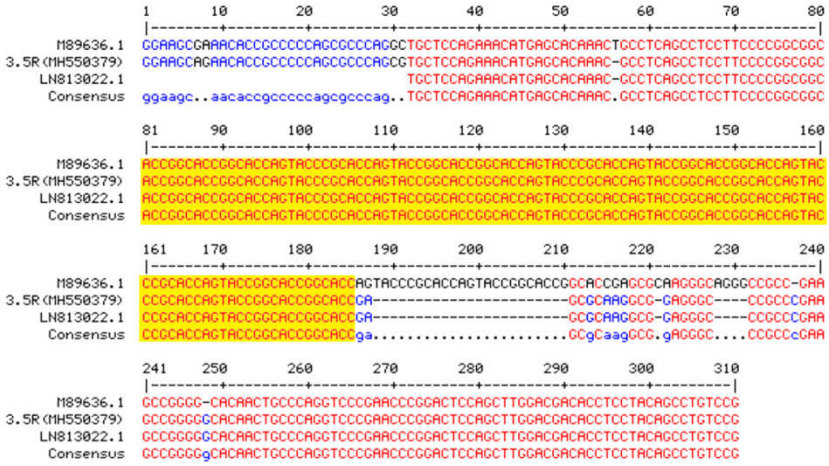

Figure 4.6a: Multiple alignment of representative 3.5R allele of *MAOA*-uVNTR (MH550379) of present study and reference sequences available with GenBank; M89636.1, Author: Zhu *et al*., 1992; LN813022.1, Author: Erasmus *et al*., 2015. Alignment was done by using MultAlin software (Corpet, 1988). Repeat regions highlighted in yellow.

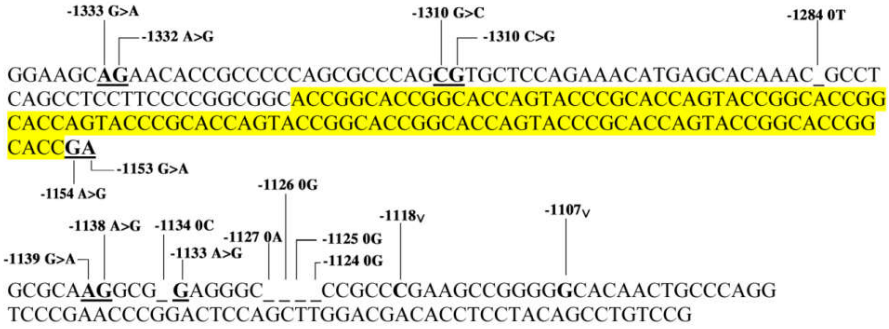

Figure 4.6b: Sequence variations in upstream and downstream flanking regions of 3.5R allele of *MAOA*-uVNTR. Repeat regions are highlighted in yellow. Symbols '>', '0' and 'v' indicates base substitution, absence of base and addition of base respectively.

PART- II

ADVERSE CHILDHOOD EXPERIENCES (ACEs), HEALTH RISK BEHAVIOURS (HRBs) AND CRIME

4.3 Section I: Pilot study for standardising data collection tools, demographic information of participants; crime history and age of onset of crime of Cases and ACEs

This section consists of the outcome and inferences of the pilot study conducted for the standardization of data collection tools, followed by the results of the main study obtained through the research on the demographic information of participants, including data collected on crime history of the cases, violent criminality and age of onset of crime. Frequency and prevalence of ACEs among cases and controls are also illustrated and discussed. For this purpose this section is sub divided into five.

4.3.1 Pilot study and questionnaire validation

This part consists of the outcome and inferences of the pilot study conducted for the standardization data collection tools. The major information obtained from the participants is given below.

1. Participants were very concerned about their identity and requested to keep their identity confidential.

2. Questions about the marriage were confusing, not clear and did not receive proper responses from the participants.

3. Participants responses showed that there was no difference in reporting between ‘always’ and ‘most of the time’, to the questions belonging to the category of emotional neglect.

4. None of the participants refused in providing the responses to the any of the questions in the ACE- IQ.

5. As per the ACE- IQ analysis guidelines, physical fight under the section Peer violence was not included for calculating total ACE score.

6. Participants reported the frequency of ACEs they faced during their first 18 years of life. Frequency version of analysis that were employed for calculating total ACE score did not give importance to the actual frequency of the adverse events experienced by the participants and hence this method of analysis was found to be inappropriate for the present study.

Based on the inferences, modifications were made in the ACE-IQ and the scoring pattern used in this study.

4.3.2 Demographic information of the participants

Demographic information of the participants belonging to controls and cases are provided in the table 4.4. Out of 67 participants, 32 were controls and 35 were cases. The average age of controls was 29.94 (±3.82) and ranged from 22 to 38. In cases the average age was 32.17(±5.15) and ranged from 24 to 43. Regarding education, 65.60 % of the controls have completed college/ University education and 54.30 % of cases have primary education only. Most of the participants belonging to cases were self-employed (80.0 %), 8.60 % of cases were unemployed even though they were able to work. In cases, 48.60 % were married and 45.70 % reported that they were single.

Table 4.4: Demographic information of the participants

	Controls	Cases	Overall
Total No. of participants	32	35	67
Mean Age ±SD	29.94 ±3.82	32.17 ±5.15	31.10 ±4.67
Median Age	29	31	30
Range	22-38	24-43	22-43
Education			
Less than primary school	0.0	8.60	4.50
Primary school completed	0.0	54.30	28.40
Secondary/High school completed	34.40	28.60	31.30
College/University completed	65.60	8.60	35.80
Work status over the last 12 months			
Government employee	3.10	0.0	1.50
Non-government employee	3.10	8.60	6.00
Self-employed	59.40	80.0	70.10
Student	34.40	0.0	16.40
Unemployed (able to work)	0.0	8.60	4.50
Unemployed (unable to work)	0.0	2.90	1.50
Civic Status			
Married	18.80	48.60	34.30
Living as couple	0.0	2.90	1.50
Divorced or separated	0.0	2.90	1.50
Single	81.20	45.70	62.70

Note: Data of education, work status over the last 12 months, civic status are given in percentage.

4.3.3 Crime history of cases

Table 4.5 and figure 4.7 show that among the total number of crimes committed by the cases, majority of the offences were against human body (67 %). Details of crime committed by cases are given in Appendix 13. Wrongful restrain (33 %) and attempt to murder (25 %) were the major violent crimes committed by the cases.

Table 4.5: Total crimes committed by Cases

Type of crimes	Total crimes committed	Percentage (%)
Murder	45	4
Attempt to murder	119	10
Hurt	105	9
Assault	49	4
Wrongful restrain	153	13
Total offenses against human body	**471**	**40**
Other IPC crimes	237	20
Total number of crimes committed	**708**	**100**

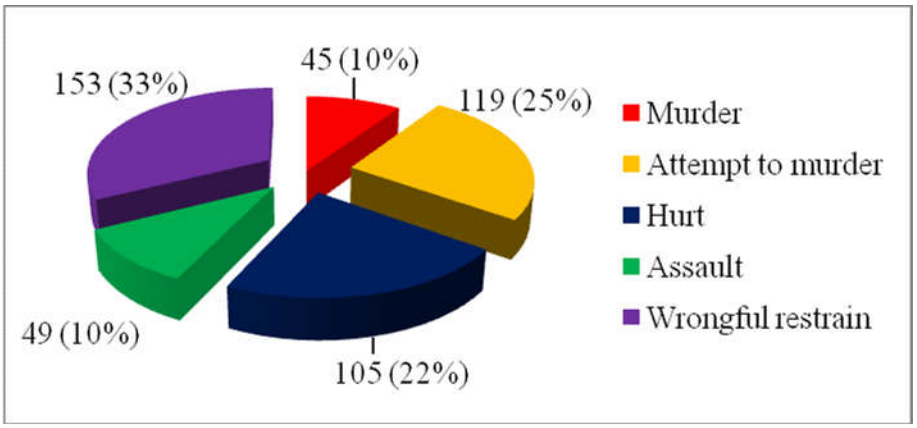

Figure 4.7: Cumulative violent crimes committed by Cases

Table 4.6: Association between age of onset of crime and ACE exposure rate in Cases

ACE exposure / Age of onset of crime	High		Extreme	
	Count	Percentage (%)	Count	Percentage (%)
17 Years	0	0.0	2	10.5
18 Years	0	0.0	3	15.8
19 Years	0	0.0	4	21.1
20 Years	1	6.3	6	31.6
21 Years	1	6.3	1	5.3
22 Years	5	31.3	1	5.3
23 Years	4	25.0	2	10.5
24 Years	5	31.3	0	0.0

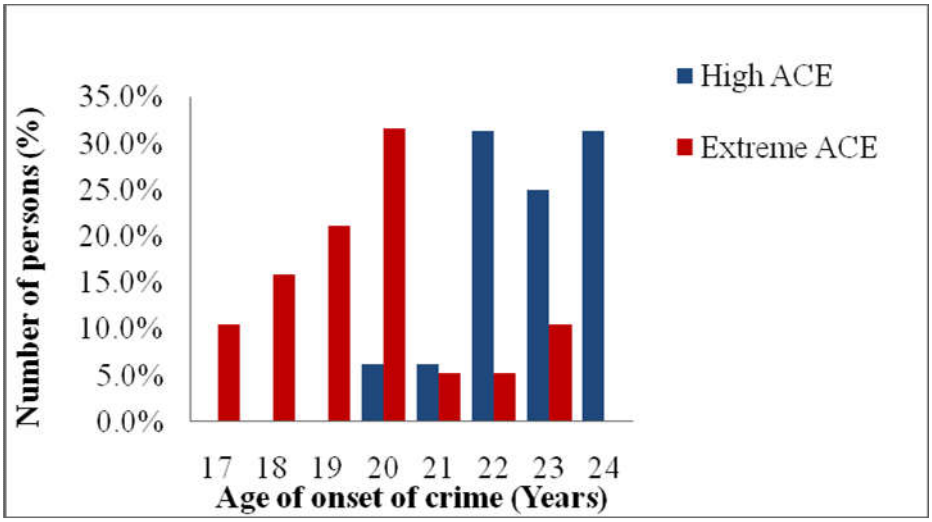

Figure 4.8: Association between age of onset of crime and ACE score in Cases

A significant association between age of onset of crime depending upon the ACE exposure in cases was found (χ^2 (7, N = 35) = 20.8, $p < 0.05$), which indicates extreme exposure to ACEs favored the early onset of criminality (Table 4.6 and figure 4.8).

4.3.4 Frequency and prevalence of ACEs

This section deals with the results of the chi-square analysis of each category of ACEs respectively in cases and controls. Results are illustrated in tables and discussed.

Hypothesis: There will be significant difference in the frequency and prevalence of ACEs between cases and controls.

As mentioned in Chapter 3, section 3.4.6, modified version of the Adverse Childhood Experiences-International Questionnaire (ACE-IQ) was used to investigate the history of adverse childhood events that the participants experienced during their first 18 years of life. ACE-IQ includes thirteen categories of ACE. Results of the chi-square analysis of the responses to each question in the 13 categories are given in the tables 4.7a, 4.7b, 4.7c, 4.7d, 4.7e, 4.7f, 4.7g, 4.7h, 4.7i, 4.7j, 4.7k, 4.7l, 4.7m and 4.7n.

Table 4.7a: Frequency of Physical Abuse among Controls and Cases

Variables	Group	Many times (%)	A few times (%)	Once (%)	Never (%)	χ^2	*p*
Did a parent, guardian or other household member spank, slap, kick, punch or beat you up?	Controls	0	78.1	21.9	0	25.06	0.000
	Cases	48.6	51.4	0	0		
Did a parent, guardian or other household member hit or cut you with an object, such as a stick (or cane), bottle, club, knife, whip etc?	Controls	0	0	50	50	42.34	0.000
	Cases	14.3	57.1	28.6	0		

Table 4.7a shows the frequency of physical abuse. Two acts of parent, guardian or household member that caused physical pain or injury to a child indicated that the child was physically abused. Nearly half of the cases (48.6 %) reported that during the first 18 years of their life they were spanked, slapped, kicked, punched or beaten up by a parent, guardian or by other household member many times. Also, 57.1% of cases were hit or cut by an object a few times. The association of variables were found to be significant between groups ($p < 0.001$).

Table 4.7b: Frequency of Emotional Abuse among Controls and Cases

Variables	Group	Many times (%)	A few times (%)	Once (%)	Never (%)	χ^2	*p*
Did a parent, guardian or other household member yell, scream or swear at you, insult or humiliate you?	Controls	18.8	50	28.1	3.1	1.72	0.633
	Cases	25.7	45.7	20	8.6		
Did a parent, guardian or other household member threaten to, or actually, abandon you or throw you out of the house?	Controls	0	3.1	21.9	75.0	41.76	0.000
	Cases	22.9	51.4	20	5.7		

Table 4.7b shows the frequency of emotional abuse. Over one-fourth (25.7 %) of cases reported that they were yelled at, screamed at or sworn at, insulted or humiliated many times during the first 18 years of their life. Half of the cases (51.4 %) reported that a parent, guardian

or other household member threatened to abandon them or throw out of the house a few times. The association of second variable was found to be significant between groups ($p < 0.001$).

Table 4.7c: Frequency of Contact Sexual Abuse among Controls and Cases

Variables	Group	Many times (%)	A few times (%)	Once (%)	Never (%)	χ^2	*p*
Did someone touch or fondle you in a sexual way when you did not want them to?	Controls	0	3.1	15.6	81.2	15.20	0.000
	Cases	0	34.3	28.6	37.1		
Did someone make you touch their body in a sexual way when you did not want them to?	Controls	0	3.1	9.4	87.5	2.76	0.25
	Cases	0	14.3	11.4	74.3		
Did someone attempt oral, anal, or vaginal intercourse with you when you did not want them to?	Controls	0	0	9.4	90.6	3.44	0.18
	Cases	0	8.6	14.3	77.1		
Did someone actually have oral, anal, or vaginal intercourse with you when you did not want them to?	Controls	0	0	9.4	90.6	0.08	0.78
	Cases	0	0	11.4	88.6		

Table 4.7c shows the frequency of contact sexual abuse. Unwanted sexual touching or fondling was experienced by 34.3 % of cases, a few times. Also, 14.3 % of cases reported that during the first 18 years of their life, they were forced to touch someone's body in a sexual way a few times; 14.3 % and 11.4 % reported that they had been attempted to and subjected to unwanted intercourse at least one time respectively. The association of first variable was found to be significant between groups ($p < 0.001$).

Table 4.7d: Prevalence of alcohol and/or drug abuser in the household of Controls and Cases

Variable	Group	Yes (%)	No (%)	χ^2	*P*
Did you live with a household member who was a problem drinker or alcoholic, or misused street or prescription drugs?	Controls	18.8	81.2	18.66	0.000
	Cases	71.4	28.6		

Table 4.7d shows the prevalence of alcohol and/or drug abuser in the household. Majority of cases (71.4 %) reported that when they were growing up, they lived with a household member who was a problem drinker or alcoholic or misused street or prescription drugs. The association was found to be significant between groups ($p < 0.001$).

Table 4.7e: Prevalence of incarcerated member in the house of Controls and Cases

Variable	Group	Yes (%)	No (%)	χ^2	*p*
Did you live with a household member who was ever sent to jail or prison?	Controls	0	100	14.75	0.000
	Cases	37.1	62.9		

Table4.7e shows the prevalence of living with a household member who was ever sent to jail or prison. Participants belong to cases (37.1 %) reported that they lived with a household member who was ever sent to jail or prison, whereas no such experiences were reported by control participants. The association was found to be significant between groups ($p < 0.001$).

Table 4.7f: Prevalence of someone chronically depressed, mentally ill, institutionalized or suicidal in the house of Controls and Cases

Variable	Group	Yes (%)	No (%)	χ^2	*p*
Did you live with a household member who was depressed, mentally ill or suicidal?	Controls	0	100	7.15	0.008
	Cases	20	80		

Table 4.7f shows the prevalence of living with someone chronically depressed, mentally ill, institutionalized or suicidal. Only cases (20 %) reported that they lived with a household member who was depressed, mentally ill or suicidal. The association was found to be significant between groups ($p = 0.008$).

Table 4.7g: Frequency of Household member treated violently among Controls and Cases

Variables	Group	Many times (%)	A few times (%)	Once (%)	Never (%)	χ^2	*p*
Did you see or hear a parent or household member in your home being yelled at, screamed at, sworn at, insulted or humiliated?	Controls	0	37.5	43.8	18.8	34.50	0.000
	Cases	28.6	71.4	0	0		
Did you see or hear a parent or household member in your home being slapped, kicked, punched or beaten up?	Controls	0	9.4	12.5	78.1	46.09	0.000
	Cases	28.6	48.6	22.9	0		
Did you see or hear a parent or household member in your home being hit or cut with an object, such as a stick (or cane), bottle, club, knife, whip etc.?	Controls	0	12.5	6.2	81.2	46.63	0.000
	Cases	2.9	57.1	40	0		

Table 4.7g shows the frequency of household members treated violently. Whole of the participants belonging to cases reported that during the first 18 years of their life, they had seen or heard that a parent or household member in their home being yelled at, screamed at, sworn at, insulted or humiliated many times (28.6 %)and a few times (71.4 %). About 28.6 % of cases reported that they heard a parent or household member in their home being slapped, kicked, punched or beaten up many times and 57.1 % had seen or heard a parent or household member in their home being hit or cut with an object a few times. The association of variables were found to be significant between groups ($p < 0.001$).

Table 4.7h: Prevalence of one or no parents, parental separation or divorce among Controls and Cases

Variables	Group	Yes (%)	No (%)	χ^2	*p*
Were your parents ever separated or divorced?	Controls	3.1	96.9	24.52	0.000
	Cases	60	40		
Did your mother, father or guardian die?	Controls	18.8	81.2	0.47	0.495
	Cases	25.7	74.3		

Table 4.7h shows the prevalence one or no parents, parental separation or divorce. More than half of the cases (60 %) reported that their parents were separated or divorced and 25.7 % of cases reported that when they were growing up, their mother, father or guardian died. The association of first variable was found to be significant between groups ($p < 0.001$).

Table 4.7i: Frequency of Emotional Neglect among Controls and Cases

Variables	Group	Always (%)	Sometimes (%)	Rarely (%)	Never (%)	χ^2	*p*
Did your parents/guardians understand your problems and worries?	Controls	6.2	40.6	53.1	0	21.12	0.000
	Cases	0	0	68.6	31.4		
Did your parents/ guardians really know what you were doing with your free time when you were not at school or work?	Controls	3.1	43.8	53.1	0	22.06	0.495
	Cases	0	2.9	74.3	22.9		

Table 4.7i shows frequency of emotional neglect. Unfortunately, it was reported by the cases (68.6 %) that their parents/ guardians rarely understand their problems and worries. It was reported by 74.3 % of cases that their parents/ guardians rarely knows what they were doing with their free time when they were not at school or work and 22.9 % of cases reported that their parents never know these. The association of variables were found to be significant between groups ($p < 0.001$).

Table 4.7j: Frequency of Physical Neglect among Controls and Cases

Variables	Group	Many times (%)	A few times (%)	Once (%)	Never (%)	χ^2	*p*
How often did your parents/guardians not give you enough food even when they could easily have done so?	Controls	0	0	0	100	4.94	0.176
	Cases	2.9	8.6	2.9	85.7		
Were your parents/guardians too drunk or intoxicated by drugs to take care of you?	Controls	0	18.8	12.5	68.8	20.97	0.000
	Cases	28.6	42.9	5.7	22.9		
How often did your parents/guardians not send you to school even when it was available?	Controls	0	9.4	6.2	84.4	37.59	0.000
	Cases	14.3	65.7	8.6	11.4		

Table 4.7j shows frequency of physical neglect. Only 2.9 % of the cases reported that many times their parents/ guardians did not give them enough food even when options were available and majority of cases (85.7 %) never experienced any such adversity during the first 18 years of their life. Lack of care due to parent's/guardian's alcoholism or drug intoxication were reported many times (28.6 %) and a few times (42.9 %) by the cases. More than half of the participants belong to cases (65.7 %) experienced lack of education. Except the first variable, all other variables showed significant association between groups ($p < 0.001$).

Table 4.7k: Frequency of Bullying and Physical fights among Controls and Cases

Variables	Group	Many times (%)	A few times (%)	Once (%)	Never (%)	χ^2	*p*
How often were you bullied?	Controls	3.1	28.1	37.5	31.2	38.16	0.000
	Cases	60	37.1	2.9	0		
How often were you in a physical fight?	Controls	3.1	18.8	37.5	40.6	38.34	0.000
	Cases	42.9	51.4	5.7	0		

Table 4.7k shows the frequency of bullying and Physical fights. The number of cases who reported being bullied many times during the first 18 years of their life was about 60 %. Nearly half of the cases (51.4 %) reported that they involved in physical fight a few times and 42.9 % reported the involvement in physical fights many times. The associations of variables were found to be significant between groups ($p < 0.001$).

Table 4.7l: Types of Bullying experienced by Controls and Cases

How were you bullied most often?	Controls	Cases	Overall
I was hit, kicked, pushed, shoved around, or locked indoors	90.90 %	48.60 %	64.90 %
I was made fun of because of my religion	00 %	2.90 %	1.80 %
I was made fun of with sexual jokes, comments, or gestures	00 %	5.70 %	3.50 %
I was left out of activities on purpose or completely ignored	00 %	11.40 %	7.00 %
I was made fun of because of how my body or face looked	00 %	8.60 %	5.30 %
I was bullied in some other way	9.10 %	22.90 %	17.50 %

Table 4.7l shows the types of bullying. Even though most of the controls (90.90 %) experienced one type of bullying during the first 18 years of their life, the cases reported that they were bullied in several ways.

Table 4.7m: Frequency of Community violence among Controls and Cases

Variables	Group	Many times (%)	A few times (%)	Once (%)	Never (%)	χ^2	*p*
Did you see or hear someone being beaten up in real life?	Controls	0	34.4	62.5	3.1	12.73	0.005
	Cases	11.4	62.9	25.7	0		
Did you see or hear someone being stabbed or shot in real life?	Controls	0	9.4	25.0	65.6	16.48	0.000
	Cases	0	28.6	54.3	17.1		
Did you see or hear someone being threatened with a knife or gun in real life?	Controls	0	0	6.2	93.8	59.93	0.000
	Cases	2.9	54.3	42.9	0		

Table 4.7m shows the frequency of Community violence. More than half of the cases (62.9 %) reported that they saw or heard someone being beaten up in real life a few times and at least one time (54.3 %) they have seen or heard someone being stabbed or shot in real life. Nearly half of the cases (54.3 %) reported that during the first 18 years of their life they saw or heard someone being threatened with a knife or gun in real life a few times. The association of variables were found to be significant between groups ($p < 0.001$).

Table 4.7n: Frequency of Collective violence among Controls and Cases

Variables	Group	Many times (%)	A few times (%)	Once (%)	Never (%)	χ^2	*p*
Were you forced to go and live in another place due to any of these events?	Controls	0	0	0	100	56.01	0.000
	Cases	2.9	45.7	42.9	8.6		
Did you experience the deliberate destruction of your home due to any of these events?	Controls	0	0	0	100	49.67	0.000
	Cases	0	14.3	71.4	14.3		
Were you beaten up by soldiers, police, militia, or gangs?	Controls	0	6.2	18.8	75.0	45.20	0.000
	Cases	8.6	65.7	25.7	0		
Was a family member or friend killed or beaten up by soldiers, police, militia, or gangs?	Controls	0	0	0	100	67.00	0.000
	Cases	28.6	34.3	37.1	0		

Table 4.7n shows the frequency of collective violence. Due to organized violent crimes such as gang warfare, 45.7 % of the cases were forced to go and live in another place a few times and 42.9 % reported that they were forced to go and live in another place due to these adverse events at least once. Deliberate destruction of the house due to organized violent crimes was experienced at least once by 71.4 % of cases. More than half of the cases (65.7 %) were beaten up by

police and gangs. Family member and friends of the all the cases were either many times (28.6 %), a few times (34.3 %) or once (37.1 %) beaten up by police and gangs. The association of variables were found to be significant between groups ($p < 0.001$).

4.3.5 Prevalence of ACE categories in Controls and Cases

Based on the significant association of ACEs among the groups, the intensity of ACEs in both the groups were examined. The prevalence of ACEs among the controls and cases are presented in table 4.8 and in figure 4.9. When ACEs of cases (M = 72.14, SD = 6.80, N = 35) were compared with that of controls (M = 44.91, SD = 5.39, N = 32), it was found that cases experienced significantly more ACEs (t (65) = -17.30, $p < 0.001$) and hence the hypothesis is accepted.

For five ACE categories (Household member treated violently, physical neglect, bullying and physical fight, community violence and collective violence), the cases that experienced this trauma were nearly double the rate of the controls. The ACEs, incarcerated household members (M = 1.00, SD = 00, N = 32) and household mental illness (M = 1.00, SD = 00, N = 32) were not experienced by the controls.

Among both the groups, the most frequent ACE reported by the participants in this study was physical abuse and none of the participants reported that they have never subjected to physically abuse during childhood. This was similar to the findings of Felitti and colleagues (1998) and contrasting to the published study done in Kerala among youth (Damodaran & Paul, 2017).

Table 4.8: Prevalence of ACE categories in Control and Cases

ACE categories		Controls (Mean±SD)	Cases (Mean±SD)	t value	df	*p*
ACE 1	Physical Abuse	4.28 (0.63)	6.34 (0.99)	-9.98	65	0.000**
ACE 2	Emotional Abuse	4.13 (0.94)	5.80 (1.59)	-5.19	65	0.000**
ACE 3	Contact Sexual Abuse	4.56 (1.37)	5.80 (2.04)	-2.89	65	0.005*
ACE 4	Household alcohol/substance abuse	1.19 (0.40)	1.71 (0.46)	-5.01	65	0.000**
ACE 5	Incarcerated household member	1.00 (0.00)	1.37 (0.49)	-4.28	65	0.000**
ACE 6	Household mental illness	1.00 (0.00)	1.20 (0.41)	-2.79	65	0.006*
ACE 7	Household violence	4.81 (1.55)	8.97 (1.25)	-12.13	65	0.000**
ACE 8	Parental separation or divorce	2.22 (0.42)	2.86 (0.73)	-4.32	65	0.000**
ACE 9	Emotional neglect	4.97 (1.12)	6.51 (0.82)	-6.49	65	0.000**
ACE 10	Physical neglect	3.75 (1.24)	6.89 (2.10)	-7.35	65	0.000**
ACE 11	Bullying and physical fight	3.88 (1.64)	6.94 (0.99)	-9.33	65	0.000**
ACE 12	Community violence	4.81 (1.12)	7.57 (1.27)	-9.41	65	0.000**
ACE 13	Collective Violence	4.31 (0.59)	10.17 (1.82)	-17.35	65	0.000**
Total ACE Score		**44.91 (5.39)**	**72.14 (6.80)**	**-18.06**	**65**	**0.000****

Controls (N = 32), Cases (N = 35), Values are expressed as Mean ± SD. Asterisks (*) and (**) denotes significance at $p < 0.05$ and $p < 0.001$ respectively against controls.

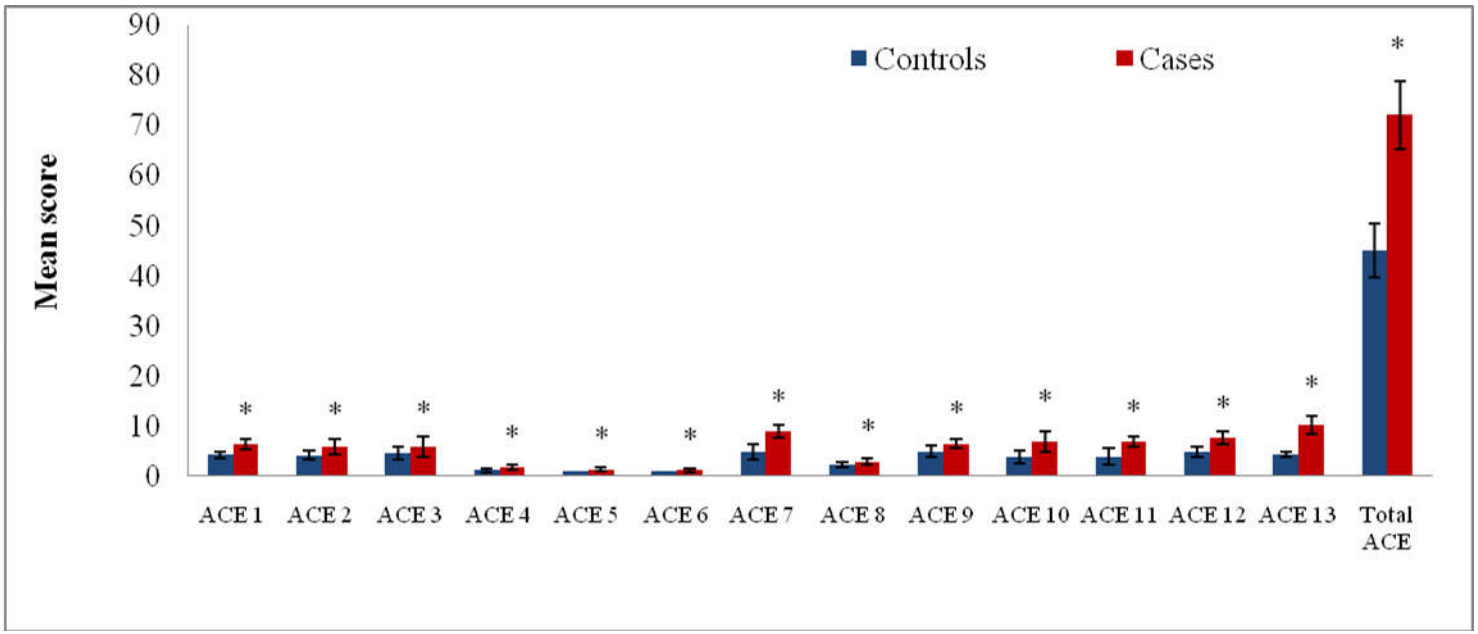

Figure 4.9: Prevalence of ACE categories in the Controls and Cases. ACE 1- Physical Abuse, ACE 2- Emotional Abuse, ACE 3- Contact Sexual Abuse, ACE 4- Household alcohol/substance abuse, ACE 5- Incarcerated household member, ACE 6- Household mental illness, ACE 7- Household violence, ACE 8- Parental separation or divorce, ACE 9- Emotional neglect, ACE 10- Physical neglect, ACE 11- Bullying and physical fight, ACE 12- Community violence, ACE 13- Collective Violence,Controls (N = 32), Cases (N = 35),Values are expressed as Mean ± SD. Asterisk (*) denotes significance at $p < 0.05$ against controls.

As reported by the participants, the frequency of exposure to sexual abuse was least among them and similar to the prevalence already reported in past investigations done in Kerala among adolescents and youths (Nair & Devika, 2014; Damodaran & Paul, 2017). The response rates were found to be less for the questions regarding sexual abuse in several studies due to the inherent nature of this social problem (Gorey & Leslie, 1997; Damodaran & Paul, 2017).

As the mean individual score of 13 categories of ACEs and total ACE score differed significantly among the cases and controls in this study, the impact of ACEs on cases were specifically analyzed. This helped to understand the impact that ACEs have on serious, violent and chronic criminal behaviour.

Results indicated that when the intensity of ACEs increased there was an early onset of violent crimes among offenders. This finding was similar to those reported by more than 20 longitudinal studies (Krohn *et al*., 2001; Howell, 2008; Baglivio *et al*., 2015; Howell & Griffiths 2018). On examining the official crime records of the offenders with highest ACE score, it was understood that the frequency, serious offences and gang involvement were more among these offenders (Tolan, 1987; Tolan & Thomas, 1995; Loeber & Farrington, 1998; Howell, 2008; DeLisi & Piquero, 2011; Howell, 2012).

Among the recidivist violent offenders in this study, the number of incidents, longevity or the severities of the exposure to individual ACEs were significantly higher, which were supported by past

research (Bellis *et al*., 2013; Barrett *et al*., 2014). The major adverse experiences to which the offenders exposed were witnessing household, community and collective violence; physical neglect; bullying and involvement in physical fights, which were double the rate when compared to the control population, which indicated the "double whammy" or trigger compounding effect on violent behaviour in recidivist violent offenders (Herrenkohl *et al*., 2008).

The mean score of the ACE category, bullying and physical fight, was significantly high in offenders when compared to the controls. This showed that the offenders were subjected to frequent bullying and involved in physical fights when they were children. This might have resulted in peer adjustment problem, associating with deviant peers and peer conflict resolution difficulties (Margolin & Gordis, 2004; Chapple *et al*., 2005).

Even though the offenders in this study were convicted several times and presumably corrected, the rate of violent criminal recidivism were high in this group. This showed the similar effect of ACEs in offenders as reported in several other investigations (Cicchetti & Manly, 2001; Chen *et al*., 2011; Yu-Ling Chiu *et al*., 2011; Baglivio *et al*., 2013; Barrett *et al*., 2014). Household incarceration and mental illness were reported only by the offender population in this study and the same adversity during childhood could have contributed in developing the violent criminal recidivism in them (Thomas *et al*., 1995; Wildeman, 2009).

4.4 Section- II: Relationship between Adverse Childhood Experiences (ACEs) and violent criminality

4.4.1 Interrelationship of ACEs

In this section the results of bivariate correlation of ACE categories among the participants are illustrated.

Hypothesis: There will be significant correlation between variables of ACEs in the participants.

Bivariate correlations were performed to demonstrate the relationship between the ACE categories and results are found in table 4.9. The correlation coefficients range from $r = 0.079$ to $r = 0.813$. Between the ACE items, the largest correlation was found between the presence of collective violence and household violence ($r = 0.813$). Whereas, between household mental illness and incarcerated household member, very weak correlation was found ($r = 0.079$). Most of the ACE categories were positively correlated to one another and hence the hypothesis is accepted.

Fourteen correlations were higher than 0.60. This result indicated that the 13 categories of ACE were correlated each other indicating the co-occurrence and interrelationship of ACEs in Indian population (Baglivio & Epps, 2015; Damodaran & Paul, 2017). In the land marking study conducted by Felitti and colleagues (1998) it was reported that 67 % of the participants experienced one or more ACEs together.

Table 4.9: Bivariate correlations showing co-occurrence of ACEs

ACE Categories	ACE 1	ACE 2	ACE 3	ACE 4	ACE 5	ACE 6	ACE 7	ACE 8	ACE 9	ACE 10	ACE 11	ACE 12	ACE 13
ACE 1:Physical Abuse	1												
ACE 2:Emotional Abuse	0.591**	1											
ACE 3:Contact Sexual Abuse	0.320**	0.2	1										
ACE 4:Householdalcohol/ substance abuse	0.473**	0.407**	0.09	1									
ACE 5:Incarcerated household member	0.466**	0.269*	0.212	0.302*	1								
ACE 6:Household mental illness	0.350**	0.505**	0.254*	0.270*	0.079	1							
ACE 7:Household violence	0.752**	0.503**	0.265*	0.570**	0.472**	0.237	1						
ACE 8:Parental separation or divorce	0.396**	0.229	0.389**	0.394**	0.325**	0.299*	0.412**	1					
ACE 9:Emotional neglect	0.479**	0.376**	0.199	0.460**	0.304*	0.260*	0.601**	0.292*	1				
ACE 10:Physical neglect	0.547**	0.536**	0.345**	0.450**	0.275*	0.489**	0.627**	0.396**	0.609**	1			
ACE 11:Bullying & physical fight	0.604**	0.444**	0.315**	0.491**	0.499**	0.257*	0.654**	0.375**	0.564**	0.573**	1		
ACE 12:Community violence	0.633**	0.341**	0.186	0.398**	0.472**	0.194	0.723**	0.385**	0.520**	0.500**	0.735**	1	
ACE 13:Collective Violence	0.744**	0.539**	0.358**	0.598**	0.493**	0.323**	0.813**	0.515**	0.592**	0.635**	0.795**	0.748**	1

N = 67, Asteriks (*) and (**) denotes significant correlations at $p < 0.05$ and $p < 0.001$ respectively.

4.4.2 Correlation between ACEs and violent criminality in Cases

In this section the results of bivariate correlation of ACEs and violent criminality in cases are given in table 4.10. Violent criminality is determined by calculating the total number of violent crimes as mentioned in the Chapter 3, section 3.4.6b.

Hypothesis: There will be significant correlation between variables of ACEs and violent criminality in cases.

Table 4.10 represents the bivariate correlation analysis of relationship between ACE categories and violent criminality (in terms of total number of violent crimes committed). Emotional neglect and community violence did not show any significant correlation with violent criminality, whereas all the other ACE categories showed a significant correlation with violent criminality ($r > 0.30$). The highly significant correlation of total ACE score and criminality proved that adverse childhood experiences influence violent criminal behaviour ($r(35) = 0.927$, $p < 0.001$) and hence the hypothesis is accepted.

The significant correlation of total ACE score and violent criminality among offenders indicated the developmental perspective of pathological aggression (Sroufe & Rutter, 1984; Toth & Cicchetti, 2013). Predictions of Moffit's developmental taxonomy is also supported by the findings of this study, as the offenders subjected to

the most significant developmental risk factors and multiple adverse childhood experiences, became serious, violent and chronic offenders. It could be assumed that the recidivist violent offenders in this study belong to the Moffit's LCP offending group. Also, the criminal trajectory of these offenders showed that these offenders belong to the SVC offenders who may continue to commit violence and serious crimes all through the life-course similar to the LCP offenders (Fox *et al.,* 2015).

Table 4.10: Bivariate correlations of ACEs and criminality in Cases

		r
	Criminality	1
ACE 1	Physical Abuse	0.418*
ACE 2	Emotional Abuse	0.468**
ACE 3	Contact Sexual Abuse	0.495**
ACE 4	Household alcohol/substance abuse	0.313*
ACE 5	Incarcerated household member	0.370*
ACE 6	Household mental illness	0.355*
ACE 7	Household violence	0.393*
ACE 8	Parental separation or divorce	0.415*
ACE 9	Emotional neglect	0.059
ACE 10	Physical neglect	0.375*
ACE 11	Bullying & physical fight	0.586**
ACE 12	Community violence	0.222
ACE 13	Collective Violence	0.641**
ACE Total		**0.927****

Cases (N = 35), Asterisks (*) and (**) denotes significant correlations at $p < 0.05$ and $p < 0.001$ respectively, r = correlation coefficient.

4.4.3 ACEs as predictors of violent criminality

Based on significant differences in ACE scores between controls and cases, followed by a significant correlation between total ACE score with violent criminality in cases, a series of linear and multiple regression analysis were performed to demonstrate whether scores of each ACE categories, total ACE and collective ACEs can successfully used as predictors for violent criminality. The results are presented in table 4.11 and 4.12

Hypothesis: ACEs can significantly predict violent criminality in cases.

The thirteen items that make up the total ACE score were individually and collectively evaluated for their impact on violent criminality (Table 4.11 & 4.12). Except for household alcohol/ substance abuse and emotional neglect a marginally significant regression equation was found between categories of ACE scores and violent criminality as the dependent variable. Using total ACE score as the predictor variable, 86.0 % of the variability in the violent criminality as the dependent variable is accounted for by the regression (F (1, 33) = 202.71, $p < 0.001$) and the predictor variability of each of the 13 categories are presented in table 4.11. Also, in multiple regression analysis (Table 4.12), collective ACEs significantly predicted violent criminality (F (13, 21) = 34.693, $p < 0.001$, $R^2 = 0.956$). Hence the hypothesis is accepted.

Table 4.11: Categories of ACEs predicting violent criminality

ACE variables		Un-stanardized Coefficients		Standardized Coefficients (β)	R^2	*P*
		Constant (B) (SE)	Variable (B) (SE)			
ACE 1	Physical Abuse	4.270(6.111)	2.52 (0.952)	0.418	0.175	0.012*
ACE 2	Emotional Abuse	9.957(3.501)	1.771 (5.583)	0.468	0.219	0.005*
ACE 3	Contact Sexual Abuse	11.766(2.734)	1.459 (0.445)	0.495	0.245	0.002*
ACE 4	Household alcohol/ substance abuse	13.200(3.844)	4.1 (2.168)	0.313	0.098	0.067
ACE 5	Incarcerated household member	14.014(2.883)	4.531 (1.983)	0.37	0.137	0.029*
ACE 6	Household mental illness	13.929(3.049)	5.25 (2.411)	0.355	0.126	0.037*
ACE 7	Household violence	3.254(6.980)	1.892 (0.771)	0.393	0.154	0.020*
ACE 8	Parental separation or divorce	10.519(3.826)	3.398 (1.298)	0.415	0.172	0.013*
ACE 9	Emotional neglect	17.397(8.382)	0.435 (1.277)	0.059	0.003	0.736
ACE 10	Physical neglect	12.819(3.324)	1.076 (0.462)	0.375	0.141	0.026*
ACE 11	Bullying and physical fight	-4.274(5.957)	3.525 (0.850)	0.586	0.343	0.000**
ACE 12	Community violence	12.261(6.179)	1.052 (0.805)	0.222	0.049	0.2
ACE 13	Collective Violence	-1.256(4.552)	2.112 (0.441)	0.641	0.41	0.000**
Total ACE Score		**-38.939(4.174)**	**0.820 (0.058)**	**0.927**	**0.86**	**0.000****

Un-standardized Coefficients (B), Standard error (SE), Standardized coefficients (β), Coefficient of determination (R^2) and significance levels (*p*) for individual ACE categories predicting violent criminality of Cases (N = 35), ACE = Adverse childhood experience; Asterisks (*) and (**) denotes significant correlations at $p < 0.05$ and $p < 0.001$ respectively.

Table 4.12: Multiple regression showing ACEs collectively predicting violent criminality

ACE variables		Unstandardized Coefficients		
		B	SE	*p*
	(Constant)	-35.719	4.090	0.000**
ACE 1	Physical Abuse	0.575	0.335	0.101
ACE 2	Emotional Abuse	1.196	0.252	0.000**
ACE 3	Contact Sexual Abuse	1.189	0.158	0.000**
ACE 4	Household alcohol/substance abuse	2.303	0.732	0.005*
ACE 5	Incarcerated household member	0.497	0.680	0.473
ACE 6	Household mental illness	0.595	0.861	0.497
ACE 7	Household violence	0.530	0.266	0.060
ACE 8	Parental separation or divorce	0.692	0.438	0.129
ACE 9	Emotional neglect	-0.659	0.386	0.102
ACE 10	Physical neglect	0.366	0.163	0.036*
ACE 11	Bullying	1.364	0.374	0.002*
ACE 12	Community violence	1.297	0.266	0.000**
ACE 13	Collective Violence	0.873	0.191	0.000**

Un-standardized Coefficients (B), Standard error (SE), Standardized coefficients (β) and significance levels (*p*) for collective ACEs predicting violent criminality of Cases (N = 35), ACE = Adverse childhood experience. Asteriks (*) and (**) denotes significance at $p < 0.05$ and $p < 0.001$ respectively.

Thirteen ACE categories were grouped under four main subcategories- abuse, neglect, violence and household challenges. Multipe regressions were performed in these subcategories to know whether these subcategories differ in strength as predictors for violent criminality.

Abuse

The three abuse categories (Physical, Emotional and Contact Sexual Abuses) were found to be significant, but were weak predictors of violent criminality (Table 4.11). A significant regression equation was found when subcategories of Abuse of ACEs were collectively considered to predict criminality, (F (3, 31) = 9.994, $p < 0.001$, R^2 = 0.492) (Table 4.13). In another study, self-reported violence, emotional and psychological abuse had shown to be predictive of violent behaviour (Song *et al.,* 1998). Mass and colleagues (2008) reported that physical abuse as a consistent predictor for the perpetration of chronic violence. This view was opposed by Yun and colleagues (2011) and they concluded that for chronic violence, physical abuse alone was unrelated, but instead sexual abuse and childhood neglect were each independently predictive.

Table 4.13: Abuse category of ACEs predicting violent criminality

ACE variables of abuse subcategory	Unstandardized Coefficients		*p*
	B	SE	
(Constant)	-5.381	5.413	0.328
Physical Abuse	1.695	0.810	0.045*
Emotional Abuse	1.271	0.511	0.018*
Contact Sexual Abuse	1.290	0.380	0.002*

Un-standardized Coefficients (B), Standard error (SE) and significance levels (p) for abuse subcategory of ACEs predicting violent criminality of Cases (N = 35). Asteriks (*) and (**) denotes significance at $p < 0.05$ and $p < 0.001$ respectively.

Neglect

Since a no significant regression equation was found for emotional neglect with a very weak predictor variable, this ACE category cannot be used as a good predictor for violent criminality (F (1, 33) = 0.116, p = 0.736, R^2 = 0.003) (Table 4.11). Whereas, physical neglect showed a significant but weak regression equation (F (1, 33) = 5.416, p = 0.026, R^2 = 0.141). When these two categories were collectively considered as predictor, a non-significant equation was found (F (2, 32) = 2.64, p = 0.087, R^2 = 0.142) (Table 4.14).

Table 4.14: Neglect category of ACEs predicting violent criminality

ACE variables of neglect subcategory	Unstandardized Coefficients		*p*
	B	SE	
(Constant)	13.959	8.044	0.092
Emotional neglect	-0.193	1.235	0.877
Physical neglect	1.093	0.482	0.030*

Un-standardized Coefficients (B), Standard error (SE) and significance levels (p) for neglect subcategory of ACEs predicting violent criminality of Cases (N = 35). Asteriks (*) and (**) denotes significance at $p < 0.05$ and $p < 0.001$ respectively.

Violence

Under violence subcategory, the bullying, community violence and collective violence were considered. Multiple regression equation of exposure to violence predicting criminality was found to be highly significant (F (3, 31) = 11.017, $p < 0.001$, R^2 = 0.516) (Table 4.15). A series of linear regression found that only bullying and collective violence significantly predicted criminality (Table 4.11).

Table 4.15: Violence category of ACEs predicting violent criminality

ACE variables of violence subcategory	Unstandardized Coefficients		*p*
	B	SE	
(Constant)	-12.063	6.417	0.070
Bullying	2.074	0.891	0.027*
Community violence	0.281	0.623	0.655
Collective Violence	1.550	0.468	0.002*

Un-standardized Coefficients (B), Standard error (SE) and significance levels (*p*) for violence subcategory of ACEs predicting violent criminality of Cases (N = 35). Asteriks (*) and (**) denotes significance at $p < 0.05$ and $p < 0.001$ respectively.

Household challenges

Multiple regression was performed to predict the Household challenges category of ACE and a significant regression equation was found between household challenges and criminality (F (5, 29) = 5.028, p = 0.002, R^2 = 0.464) (Table 4.16). Incarcerated household member, household mental illness, household violence and parental separation or divorce significantly predicted criminality (Table 4.11). In a study conducted by Baglivio and colleagues (2017) it was found that among early onset juvenile offenders who have committed homicide and attempted homicide, there have a link between these offences and ACE such as living with household mental illness. Household alcohol/ substance abuse failed to predict criminality. Among the four ACEs parental separation or divorce predicted (R^2 = 0.172) criminality over and above the other three (Incarcerated household member, household mental illness and household violence).

Table 4.16: Category of household challenges of ACEs predicting violent criminality

ACE variables of household challenges subcategory	Unstandardized Coefficients		*p*
	B	SE	
(Constant)	-10.678	6.963	0.136
Household alcohol/substance abuse	1.956	1.855	0.300
Incarcerated household member	3.497	1.740	0.054
Household mental illness	5.157	2.113	0.021*
Household violence	1.354	0.712	0.067
Parental separation or divorce	1.548	1.217	0.214

Un-standardized Coefficients (B), Standard error (SE) and significance levels (*p*) for household challenges subcategory of ACEs predicting violent criminality of Cases (N = 35). Asteriks (*) and (**) denotes significance at $p < 0.05$ and $p < 0.001$ respectively.

4.5 Section III: Prevalence and relationship of Health Risk Behaviours (HRBs) with violent criminality and ACEs

Health risk behaviours (HRBs) included three categories, tobacco use, alcohol use and street drug/ substance abuse. As mentioned in the Chapter 3, section 3.3.2 (2), six questions from the male version of Family Health History Questionnaire of the CDC-Kaiser Permanente Adverse Childhood Experiences (ACE) Study were used to investigate the history of HRBs in participants during their first 18 years of life (The Centers for Disease Control and Prevention, 2014). Overall HRB score ranges from 3– 6. The lowest score 3 means the participant did not have any HRBs during first 18 years of life. The highest score 6 means the participant had all the HRBs during first 18 years of life.

Hypothesis: There will be significant difference in the prevalence of HRBs between cases and controls.

4.5.1 Occurrence and prevalence of HRBs based on age of onset and ACE exposure in participants

Chi-square and percentage analysis of HRBs, age of onset HRBs and trend of association between exposures to ACEs are described in this section. This is followed by the result of t- test done for analyzing the prevalence of HRBs in cases and controls.

Table 4.17 and figure 10 shows the habit of HRBs, tobacco use, alcohol use and street drug/ substance abuse in all the participants. Most of the cases reported that they have these habits. All HRBs showed a significant association between controls and cases ($p < 0.001$).

Table 4.17: Occurrence of HRBs in participants

HRB	Occurrence	Controls		Cases		Total		$\chi 2$	df	p
		N	%	N	%	N	%			
Tobacco Use	No	22	68.8	1	2.9	23	34.3	32.2	1	0.000**
	Yes	10	31.2	34	97.1	44	65.7			
Alcohol Use	No	18	56.2	2	5.7	20	29.9	20.39	1	0.000**
	Yes	14	43.8	33	94.3	47	70.1			
Street drug/ Substance Abuse	No	23	71.9	8	22.9	31	46.3	16.156	1	0.000**
	Yes	9	28.1	27	77.1	36	53.7			

Controls (N = 32), Cases (N = 35). Asterisks (**) denotes significance at $p < 0.001$.

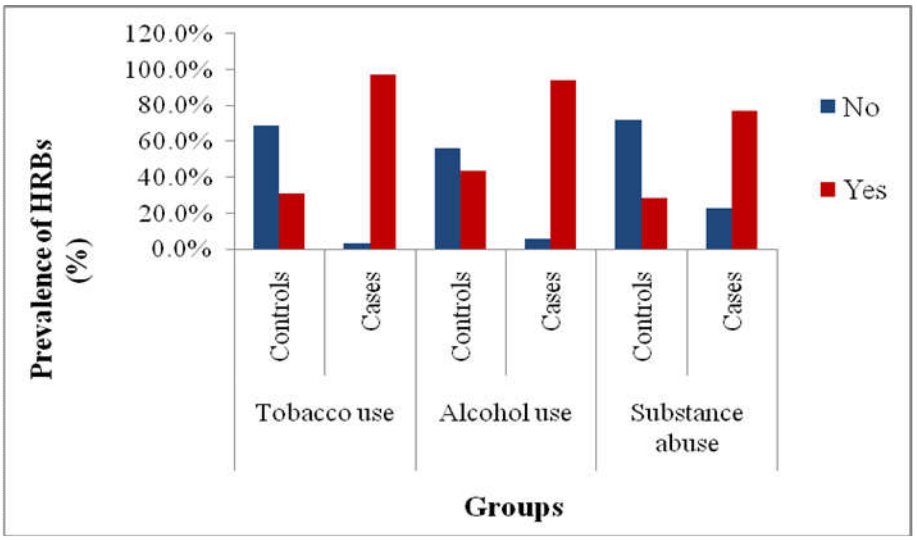

Figure 4.10: Prevalence of HRBs in groups

Table 4.18 shows the age of onset of HRBs in the participants. In controls all the three habits started in the age of 16 or after. Whereas, onset of HRB habits in cases were found to begin from age 12 and 14. The data are pictorially represented in figure 4.11a, 4.11b and 4.11c.

Table 4.18: Age of onset of HRBs

Age of onset of HRBs		Tobacco use		Alcohol use		Street drugs/ substance abuse	
		Controls	Cases	Controls	Cases	Controls	Cases
12 years	Count	0	1	0	0	0	0
	Percentage (%)	0.00	3.00	0.00	0.00	0.00	0.00
13 years	Count	0	3	0	0	0	0
	Percentage (%)	0.00	9.10	0.00	0.00	0.00	0.00
14 years	Count	0	4	0	3	0	6
	Percentage (%)	0.00	12.10	0.00	9.10	0.00	22.20
15 years	Count	0	6	0	8	0	5
	Percentage (%)	0.00	18.20	0.00	24.20	0.00	18.50
16 years	Count	2	12	0	14	1	11
	Percentage (%)	20.00	36.40	0.00	42.40	11.10	40.70
17 years	Count	4	4	3	6	3	4
	Percentage (%)	40.00	12.10	21.40	18.20	33.30	14.80
18 years	Count	1	3	3	1	2	1
	Percentage (%)	10.00	9.10	21.40	3.00	22.20	3.70
19 years	Count	1	0	2	0	0	0
	Percentage (%)	10.00	0.00	14.30	0.00	0.00	0.00
20 years	Count	0	0	2	1	1	0
	Percentage (%)	0.00	0.00	14.30	3.00	11.10	0.00
22 years	Count	2	0	2	0	0	0
	Percentage (%)	20.00	0.00	14.30	0.00	0.00	0.00
24 years	Count	0	0	1	0	2	0
	Percentage (%)	0.00	0.00	7.10	0.00	22.20	0.00
26 years	Count	0	0	1	0	0	0
	Percentage (%)	0.00	0.00	7.10	0.00	0.00	0.00

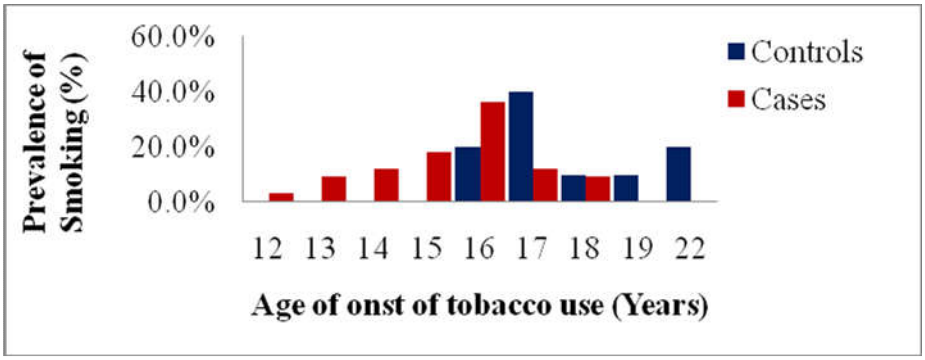

Figure 4.11a: Age of onset of tobacco use in participants

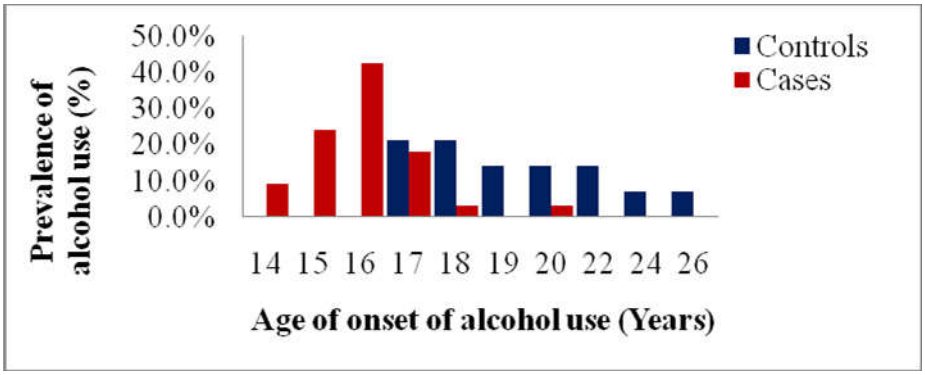

Figure 4.11b: Age of onset of alcohol use in participants

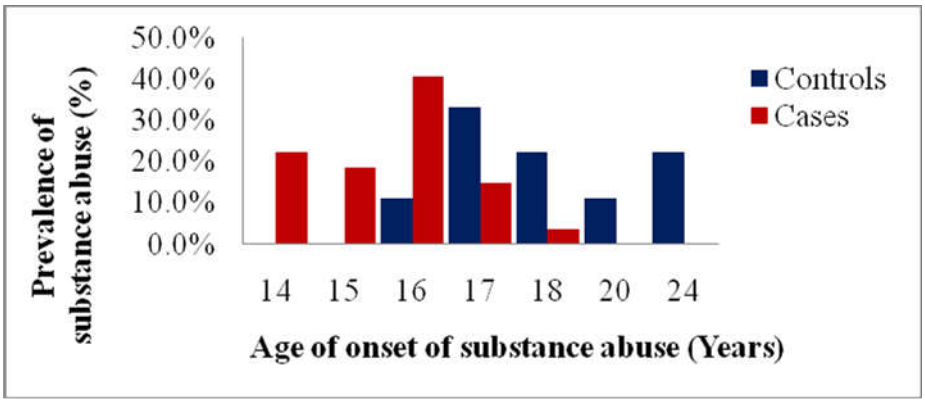

Figure 4.11c: Age of onset of street drugs/ substance abuse in participants

Table 4.19: Occurrence of HRBs based on ACE exposure in participants

ACE Exposure / Intensity of HRB		Low		Moderate		Medium		High		Extreme	
		Count	**%**	**Count**	**%**	**Count**	**%**	**Count**	**%**	**Count**	**%**
Controls											
Tobacco use	No	5	100.0	16	69.6	1	33.3	0	0.0	0	0.0
	Yes	0	0.0	7	0.0	2	0.0	1	6.2	0	0.0
Alcohol use	No	4	100.0	13	52.0	1	33.3	18	56.3	0	0.0
	Yes	0	0.0	12	48.0	2	66.7	14	43.8	0	0.0
Street drug/substance abuse	No	5	100.0	17	73.9	1	33.3	0	0.0	0	0.0
	Yes	0	0.0	6	26.1	2	66.7	1	100.0	0	0.0
Cases											
Tobacco use	No	0	0.0	0	0.0	0	0.0	1	6.2	0	0.0
	Yes	0	0.0	0	0.0	0	0.0	15	93.8	19	100.0
Alcohol use	No	0	0.0	0	0.0	0	0.0	1	62.0	1	5.3
	Yes	0	0.0	0	0.0	0	0.0	15	93.8	18	94.7
Street drug/substance abuse	No	0	0.0	0	0.0	0	0.0	5	31.2	3	15.8
	Yes	0	0.0	0	0.0	0	0.0	11	68.8	16	84.2

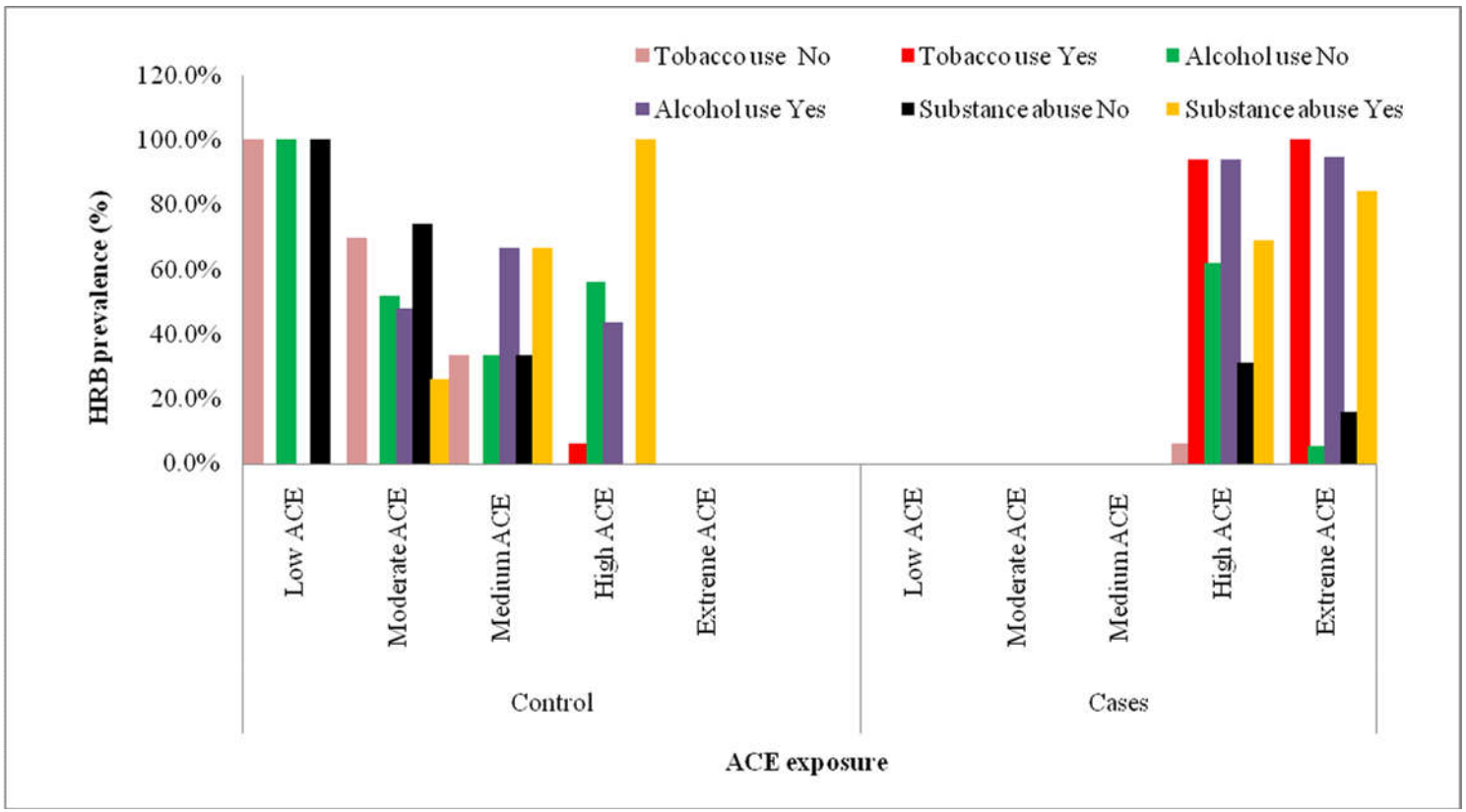

Figure 4.12: Occurrence of HRBs based on ACE exposure in participants

When ACEs influence on occurrence of HRBs were analysed in participants (Table 4.19 and figure 4.12), it was found that depending upon the intensity of ACE exposure, the number HRBs also increased.

4.5.2 Mean HRBs in Controls and Cases

Based on the significant association between HRBs in controls and cases, an independent sample t- test in both the groups was performed (Table 4.20) and demonstrated significant differences in the mean score of HRBs for tobacco use (t (65) = -7.754, $p < 0.001$), alcohol use (t (65) = -5.332, $p < 0.001$), substance abuse (t(65) = -4.545, $p < 0.001$) and also overall HRBs (t(65) = -6.687, $p < 0.001$). Hence the hypothesis is accepted. Out of the three HRBs, tobacco risk behaviour seemed to have more prevalent in cases (M = 1.97, SD = 0.17, N = 35) than controls (M = 1.31, SD = 0.47, N = 32).

Table 4.20: Mean HRBs in Controls and Cases

Types of HRBs	Controls (Mean ± SD)	Cases (Mean ± SD)	t-value	df	*p*
Tobacco use	1.31(0.47)	1.97(0.17)	-7.754	65	0.000**
Alcohol use	1.44(0.50)	1.94(0.24)	-5.332	65	0.000**
Street drugs/ Substance Abuse	1.28(0.46)	1.77(0.43)	-4.545	65	0.000**
Overall HRB	4.03(1.31)	5.69(0.63)	-6.687	65	0.000**

Controls (N = 32), Cases (N = 35), Asterisks (**) denotes significance at $p < 0.001$.

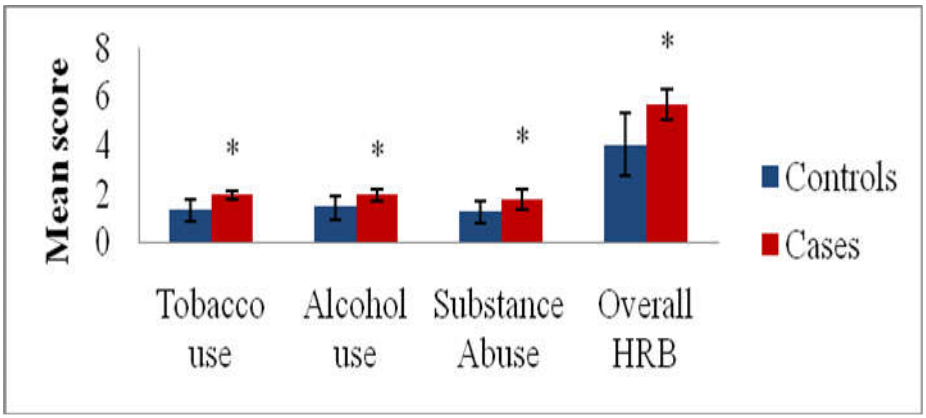

Figure 4.13: Mean HRBs in Controls and Cases. Controls (N = 32), Cases (N = 35). Asterisk (*) denotes significance ($p < 0.001$) over the respective controls.

The mean prevalence of HRBs was significantly more in offenders when compared with the control population (Rosenfeld & White, 2012). HRBs in offenders elevated the chances of criminal recidivism and violent behaviour in several other studies (Cottle *et al*., 2001; Dowden & Brown, 2002; Loeber & Farrington, 2012). Approximately one-third of the violent crimes, like homicide, sexual assault, domestic violence etc., in U.S are linked to alcohol use and similar results were obtained in this study also with high prevalence of alcohol use among recidivist violent offenders (Horvath & LeBoutillier, 2014).

4.5.3 Correlation between ACEs and HRBs

Results of the bivariate correlation between ACEs and HRBs are presented and discussed in this section.

Hypothesis: There will be significant correlation between ACEs and HRBs in the participants.

Table 4.21: Relationship between HRBs and total ACE score among participants

HRBs	1	2	3	4	5
1. Tobacco use	1				
2. Alcohol use	0.834**	1			
3. Street drugs/ Substance Abuse	0.716**	0.638**	1		
4. Total ACE Score	0.724**	0.575**	0.559**	1	
5. Overall HRB	0.937**	0.905**	0.873**	0.684**	1

N = 67, HRBs = Health risk behaviours, ACE = Adverse childhood experience, Asterisks (**) denotes significance at $p < 0.001$.

Table 4.21 represents the bivariate correlation analysis of relationship between HRBs and total ACE score of the participants. Using a series of correlations, significant relations were found between total ACE scores and tobacco use (r (67) = 0.724, $p < 0.001$), alcohol use (r (67) = 0·575, $p < 0.001$) and substance abuse (r (67) = 0.559, $p < 0.001$); all were higher than 0.50. Overall HRB and total ACE score were also found to be significantly correlated (r (67) = 0.684, $p < 0.001$) and hence the hypothesis is accepted. These relations revealed that when ACE scores increased HRBs also increased.

4.5.4 Correlation between HRBs and violent criminality in Cases

The results of bivariate correlation of ACEs and violent criminality in cases are given in table 4.22. Violent criminality is determined by calculating the total number of crimes as mentioned in the Chapter 3, section 3.4.6b.

Hypothesis: There will be significant correlation between HRBs and violent criminality in cases.

Table 4.22: Relationship between HRBs and Criminality in Cases

HRBs	1	2	3	4	5
1. Tobacco use	1				
2. Alcohol use	0.697**	1			
3. Street drugs/ Substance Abuse	0.315*	0.159	1		
4. Overall HRB	0.740**	0.667**	0.819**		
5. Total crimes	0.209	0.03	0.09	0.128	1

N = 35, HRBs = Health risk behaviours, Asterisks (*) and (**) denotes significance at $p < 0.05$ and $p < 0.001$ respectively.

Table 4.22 represents the bivariate correlation analysis of relationship between HRBs, overall HRB and violent criminality in cases. No significant relations were found between violent criminality and tobacco use (r (35) = 0.209, p = 0.114), alcohol use (r (35) = 0.030, p = 0.431), substance abuse (r (35) = 0.090, p = 0.304) and overall HRB (r (35) = 0.128, p = 0.232) and hence the hypothesis is rejected.

Relationship between HRBs and violent criminality were not found in this study and hence the hypothesis is rejected. This result is against the finding of many other researches where significant relationship was found between substance abuse and criminal recidivism (Cottle *et al*., 2001; Dowden & Brown, 2002).

4.5.5 ACEs as predictors for developing HRBs

Based on significant differences in individual and overall HRBs between controls and cases and significant correlation between ACEs

and HRBs, a series of linear regression analysis were done to check whether ACE scores could be used as predictors for developing HRBs.

Hypothesis: ACEs can significantly predict HRBs in the participants.

Table 4.23 represents the linear regression results showing the impact of ACEs on HRBs. A highly significant regression equation was found between total ACE scores and tobacco use (F (1, 65) = 71.56, $p < 0.001$, $R^2 = 0.524$), alcohol use (F (1, 65) = 32.12, $p < 0.001$, $R^2 = 0.331$), substance abuse (F (1, 65) =29.53, $p < 0.001$, $R^2 = 0.312$) and overall HRB (F (1, 65) = 57.18, $p < 0.001$, $R^2 = 0.468$). Hence the hypothesis is accepted.

Table 4.23: ACEs predicting HRBs

HRBs	Un-standardized Coefficients		R^2	p
	Constant (B) (SE)	Variable (B) (SE)		
Tobacco use	0.292 (0.166)	0.023 (0.003)	0.524	0.000**
Alcohol use	0.657 (0.190)	0.018 (0.003)	0.331	0.000**
Street drugs/Substance Abuse	0.431 (0.210)	0.019 (0.003)	0.312	0.000**
Overall HRB	1.381 (0.479)	0.059 (0.008)	0.468	0.000**

Un-standardized Coefficients (B), Standard error (SE), Coefficient of determination (R^2) and significance levels (p) for total ACE score predicting HRBs; N = 67, ACE = Adverse childhood experience, HRBs = Health risk behaviours, Asterisks (**) denotes significance at $p < 0.001$.

Most of the participants reported that during the first 18 years of life they had started the health risk behaviours like smoking, consumption of alcohol and usage of street drugs. In the whole population smoking was common, followed by alcohol use and substance abuse. Early onsets of HRBs were seen in both the groups. In the participants, when the frequency of ACE exposure increased the number of HRBs also increased. Also, significant positive correlations were found between total ACE score and score of each category of HRBs and total HRB. These findings were supported by several previous researches which demonstrated a graded relationship between ACEs and HRBs (Harrison *et al*., 1997; Dube *et al*., 2003; Rothman *et al*., 2008). As reported in this study, ACEs also could be used as predictors for HRBs (Harrison *et al*., 1997; Anda *et al*., 2002).

PART III

CANDIDATE GENE- ENVIRONMENT INTERACTION (cGxE)

This part illustrates the results of the interaction between allelic variants (3.5R and 4.5R) of *MAOA*-uVNTR polymorphism and adverse childhood experiences (ACEs) in all the participants.

4.6 Interaction between allelic variants of *MAOA*-uVNTR polymorphism and ACEs in Cases and Controls

Hypothesis: There will be significant difference in the interaction of allelic variants of *MAOA*-uVNTR polymorphism and ACEs in cases and controls.

One-way ANOVA was performed to understand the interaction of *MAOA* genotype (3.5R and 4.5R of *MAOA*-uVNTR) and ACEs (environment) of controls and cases. This showed a significant difference in ACEs (environment) faced by participants with different *MAOA* genotype. In the presence of a significant difference, multiple comparisons were performed using the Scheffe procedure at $p < 0.05$ and 0.001 significance level. Leven's test was done to confirm the homogeneity of variance (Table 4.24 and figure 4.14).

These results clearly indicates that cases with 3.5R of *MAOA*-uVNTR experienced significantly more ACEs (M = 72.14, SD = 6.80) than controls with 3.5R of *MAOA*-uVNTR (M = 43.78, SD = 4.47) and controls with 4.5R of *MAOA*-uVNTR (M = 51.00, SD = 6.36) (F (2, 64) = 179.42, $p < 0.001$). Also, the mean total ACE score of the controls with 3.5R and 4.5R *MAOA*-uVNTR were found to be slightly significant ($p = 0.051$) between each other. Hence the hypothesis is accepted.

In the abuse category, post hoc testing for physical and emotional abuse revealed that the mean ACE score for the cases with 3.5R *MAOA*-uVNTR was significantly higher than that for the controls with 3.5R and 4.5R of *MAOA*-uVNTR ($p < 0.001$). Also, mean ACE score for the controls with 3.5R and 4.5R *MAOA*-uVNTR were not found to be significantly different between each other in the ACE categories of physical abuse and emotional abuse ($p = 0.658$ and $p = 0.974$ respectively). In the case of sexual abuse, the mean ACE score for the controls with 3.5R *MAOA*-uVNTR were found to be significantly low between controls with 4.5R *MAOA*-uVNTR ($p = 0.001$) and cases with 3.5R *MAOA*-uVNTR ($p < 0.001$).

Under household challenges (Household alcohol/substance abuse, incarcerated household member, household mental illness, household violence and parental separation or divorce) post hoc test revealed that mean ACE score for these sub categories were significantly different between cases with 3.5R *MAOA*-uVNTR and controls with 3.5R *MAOA*-uVNTR ($p < 0.05$). Also, significance was noted between cases with 3.5R *MAOA*-uVNTR and controls with 4.5R *MAOA*-uVNTR in the ACE category of household violence ($p < 0.001$).

Table 4.24: ACE prevalence in Cases and Controls based on *MAOA*-uVNTR genotype (3.5R and 4.5R alleles) showing candidate Gene-Environment interaction

ACE categories		3.5R Cases (Mean ± SD)	4.5R Controls (Mean ± SD)	3.5R Controls (Mean ± SD)	*F*	df	*p*
ACE 1	Physical abuse	6.34 (1.00)	4.60 (0.55)	4.22 (0.64)	50.11	2, 64	0.000**
ACE 2	Emotional abuse	5.80 (1.59)	4.00 (1.58)	4.15 (0.82)	13.31	2, 64	0.000**
ACE 3	Contact Sexual abuse	5.80 (2.04)	7.20 (1.92)	4.07 (0.27)	13.51	2, 64	0.000**
ACE 4	Household alcohol or drug abuse	1.71 (0.46)	1.40 (0.55)	1.15 (0.36)	13.36	2, 64	0.000**
ACE 5	Incarcerated household member	1.37 (0.49)	1.00 (0.00)	1.00 (0.00)	9.03	2, 64	0.000**
ACE 6	Household mental illness	1.20 (0.41)	1.00 (0.00)	1.00 (0.00)	3.82	2, 64	0.027*
ACE 7	Household violence	8.97 (1.25)	4.60 (1.95)	4.85(1.51)	72.59	2, 64	0.000**
ACE 8	Parental separation or divorce	2.86 (0.73)	2.40 (0.55)	2.19 (0.39)	9.52	2, 64	0.000**
ACE 9	Emotional neglect	6.51 (0.82)	5.20 (1.30)	4.93 (1.11)	20.98	2, 64	0.000**
ACE 10	Physical neglect	6.89 (2.10)	4.00 (1.41)	3.70 (1.24)	26.74	2, 64	0.000**
ACE 11	Bullying	6.94 (1.00)	5.40 (1.67)	3.59 (1.50)	52.85	2, 64	0.000**
ACE 12	Community violence	7.57 (1.27)	5.20 (1.64)	4.74 (1.02)	44.3	2, 64	0.000**
ACE 13	Collective violence	10.17(1.82)	5.00 (0.71)	4.19 (0.48)	152.44	2,64	0.000**
Total ACE score		**72.14 (6.80)**	**51.00 (6.36)**	**43.78 (4.47)**	**179.42**	**2, 64**	**0.000****

3.5R Cases (N = 35, 3.5 repeat allele of *MAOA*-uVNTR found in cases), 4.5R Controls (N = 5, 4.5 repeat allele of *MAOA*-uVNTR found in controls), 3.5R Controls (N = 27, 3.5 repeat allele of *MAOA*-uVNTR found in controls); Asterisks (*) and (**) denotes significance at $p < 0.05$ and $p < 0.001$ respectively.

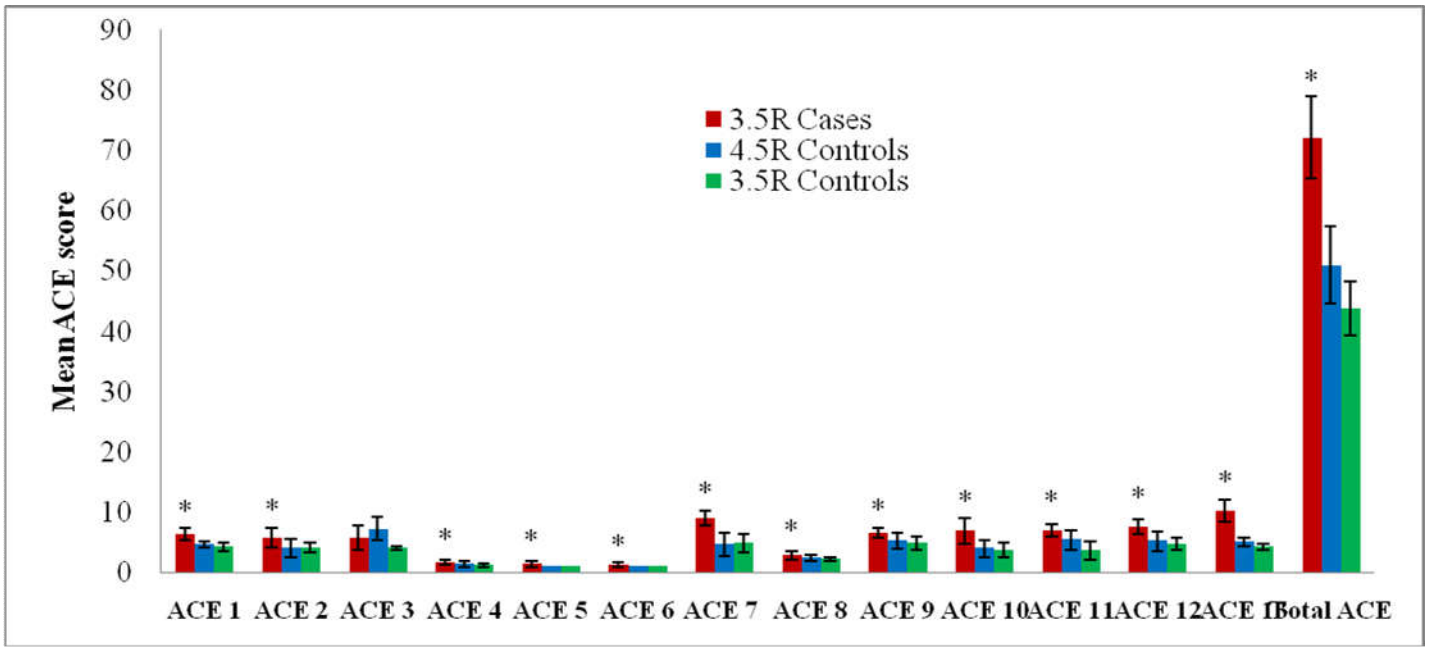

Figure 4.14: *MAOA*-uVNTR genotype and ACE prevalence in Cases and Controls. ACE 1- Physical Abuse, ACE 2- Emotional Abuse, ACE 3- Contact Sexual Abuse, ACE 4- Household alcohol/substance abuse, ACE 5- Incarcerated household member, ACE 6- Household mental illness, ACE 7- Household violence, ACE 8- Parental separation or divorce, ACE 9- Emotional neglect, ACE 10- Physical neglect, ACE 11- Bullying and physical fight, ACE 12- Community violence, ACE 13- Collective Violence. Values are expressed as Mean ± SD. Asteriks (*) denotes significance at $p < 0.05$ against two controls (3.5R and 4.5R).

When post hoc was done for neglect category, mean ACE scores for emotional neglect and physical neglect of cases with 3.5R *MAOA*-uVNTR were significantly higher than controls with 3.5R and 4.5R *MAOA*-uVNTR ($p < 0.05$).

In the ACE category of violence (bullying, community violence and collective violence), mean ACE scores for these sub categories were found to be significantly increased in cases with 3.5R *MAOA*-uVNTR and controls with 3.5R and 4.5R *MAOA*-uVNTR ($p < 0.001$).

For the first time in India, this study is reporting the prevalence of 3.5 repeat allele of *MAOA*-uVNTR in offender population and the interaction between this allele and multiple ACEs. Also, according to the available data, no such studies have reported such interaction in any countries. In this study, all the recidivist violent offenders belonging to the group of cases carried short allele (3.5R) of *MAOA*-uVNTR and participants of control group had both short allele (3.5 repeat) and long allele (4.5R). The high frequency of this short allele in offenders is similar to the reports that documented the association of short allele with an increased risk to commit violent acts (Reif *et al*., 2007).

Furthermore, this result is in line with the evidence highlighting short allele of *MAOA*-uVNTR as a vulnerability genetic factor for the tendency to violence among members of criminal gangs (Beaver *et al*. 2010). At the same time it is worth noting that the gene - environment interaction result of this study strongly suggest the implication of *MAOA* in the genetic bases of the disposition to criminal violence, only

in relation to the recidivist violent offenders and not to the general population (Stetler *et al*., 2014).

In this study, there was a significant difference in the ACEs faced by the participants belonging to cases and controls, with different genotype (i.e. 3.5R and 4.5R) of *MAOA*-uVNTR. As the recidivist violent offenders in the present study possessed short allele of *MAOA*-uVNTR which predisposed them to criminal violence by interacting with ACEs linked to a higher predisposition to commit crimes repeatedly. Also, it's worth noting that in the present study, in the control group, five participants possessed long allele (4.5R) of *MAOA*-uVNTR and also the total ACE score of these participants were slightly higher ($p = 0.051$) than the other participants with short allele (3.5R).

Even though the aggressiveness and antisocial behaviour in these five participants with long allele were not measured in this study, they were acceptable as controls as they did not exhibit any specified aggressive behaviour or antisocial acts according to the information from the community and Police Station in their locality. This finding was in line with the moderating effect of high activity allele of *MAOA*-uVNTR on the impact of childhood maltreatment in the development of antisocial behaviour in males as reported in the seminal study conducted by Caspi and colleagues (2002) and replicated in many further studies (Huang *et al*., 2004; Kim-Cohen *et al*., 2006; Widom *et al*., 2006; Nilsson *et al*., 2006; Reif *et al*., 2007; Enoch *et al*., 2010). Also, the moderating effect of high activity alleles of *MAOA*-uVNTR could be viewed similar to the resiliency factor that resist maladaptive and antisocial behaviour, that was explained in the ecological-

transactional model of child development (Cicchetti & Rogosch, 1997).

The results showed that the offenders belonging to cases with short allele (3.5R) were antisocial and repeatedly involved in violent crimes. Also, they had faced significantly high ACEs than the twenty seven and five participants in the control group with the short allele (3.5R) and long alleles (4.5R) respectively. Moreover, multiple regressions was performed and found as the best-fitting model, with a predictive power of 95.6 %, revealed independent effects of ACEs on violent criminality (Reif *et al.,* 2007). This showed a significant candidate gene- environment (cGxE) interaction in the genesis of antisocial social behaviour and violent criminal recidivism in the offenders in this study (Reif *et al.,* 2007; Tikkanen *et al.,* 2011; Fergusson *et al.,* 2011; Sadeh *et al.,* 2013; Kolla *et al.,* 2014; Byrd & Manuck, 2014; Ficks & Waldman, 2014). Born with the short allele (3.5R) *MAOA*-uVNTR genotype and growing up by facing frequent high ACEs before the age of 18, have developed pathological aggression and related problem behaviour leading to antisocial behaviour, violence and criminality in these offenders. These findings could be viewed in line with the Moffit's developmental taxonomy theory and LCP offending group (Moffitt, 1993).

In the early regulation of aggression circuitry, the functioning of serotonin, dopamine and norepinephrine neurotransmission are important and the role of *MAOA* in violence reflects neurodevelopmental alterations in the homeostatic regulation of these neurotransmissions (Bortolato & Shih, 2011). Several studies have shown that low expressing or activity allele of *MAOA* do not confer as an inherent predisposition to aggression (Fowler *et al.*, 2007). The

influences of these alleles of *MAOA* on aggressive traits are likely mediated by interactions with early exposure to traumatic or adverse childhood experiences (Caspi *et al.*, 2002; Kim-Cohen *et al.,* 2006; Williams *et al.,* 2009; Fergusson *et al.,* 2011; Beaver *et al.,* 2011; Pickles *et al.,* 2013). Likewise, the interaction between short allele (3.5R) of *MAOA* and ACEs in recidivist violent offenders in this study could be viewed in connection to the epigenetic mechanism (Palumbo *et al.,* 2018).

Recent studies have indicated that gene expression is modulated by the adverse events experienced, by introducing constant changes to DNA without modifying its sequence through a mechanism known as "epigenetics" (Kramer, 2005; Weinhold, 2006; Nestler, 2012; Landecker & Panofsky, 2013, Prasad, *et al.,* 2016; Palumbo *et al.,* 2018). Prenatal life, infancy and early adolescence are the periods of maximal sensitivity to environment and experiencing adversities during these periods of growth may introduce lasting epigenetic marks or scars in genes (Roberts *et al.,* 2018). As a result, the maturational processes in brain are affected which favors the development of dysfunctional behaviours, including exaggerate aggression in adulthood (Palumbo *et al.,* 2018). In the incarcerated antisocial population, the discovery of elevated levels of *MAOA* promoter methylation supports the suggestion of epigenetic mechanisms of *MAOA* dysregulation in offenders (Checknita *et al.,* 2015). Hence further investigations are warranted among large samples of cohorts of recidivist violent offenders in Indian population in order to understand the molecular mechanism that underlies the relationship among genes, brain, environment and behaviour.

SIVA PRASAD M. S. “EFFECT OF ADVERSE CHILDHOOD EXPERIENCES AND OCCURRENCE OF UVNTR POLYMORPHISM IN MONOAMINE OXIDASE A GENE IN RECIDIVIST VIOLENT OFFENDERS: FORENSIC IMPLICATIONS”. THESIS. DEPARTMENT OF ZOOLOGY, UNIVERSITY OF CALICUT, 2018.

Chapter 5
RESUME OF THE STUDY

Summary

Conclusions of the study

Practical and policy implications

Strengths and limitations

Suggestions for further research

5.1 Summary

The primary aim of this study was to identify whether there was any interaction between allelic variants of *MAOA*-uVNTR polymorphism and adverse childhood experience (ACEs) in recidivist violent offenders. This study was conducted in two parts since both the *MAOA*-uVNTR genotype and ACEs of the participants need to be analyzed with same importance. Each part had two phases of which the 1st phase is the pilot study and 2nd phase is the main study. First phase of the part-I standardized the various laboratory protocols for genotyping the DNA samples for 30 bp *MAOA*-uVNTR polymorphism and found out buccal swab as the best biological sample for obtaining genomic DNA from the participants in the field conditions. Second phase of the part-I focused on the identification of the recidivist violent offenders and control population, along with the genotyping of these participants for identifying the allelic variants of *MAOA*-uVNTR polymorphism and its association with violent criminality.

First phase of the part-II standardized the data collection tools, Adverse Childhood Experiences International Questionnaire (ACE-IQ) and Health Risk Behaviour questions (HRBs). In the second phase, with the help of these data collection tools, face-to-face interview was conducted with the participants. The violent crime history of the offenders was collected from the official records in the police stations. Chi-square test was conducted to check the association of ACE and HRB categories in participants. Independent t-test was performed to find out the mean difference in the ACEs and HRBs of recidivist

violent offenders and control population. Bivariate correlations were done to check the interrelationship between 13 ACE categories; relationship between ACEs and violent criminality; HRBs and total ACE score; HRBs and violent criminality. Linear and multiple regressions were performed for predicting violent criminality and HRBs using ACEs. One-way ANOVA was performed to know the mean prevalence of ACEs in the recidivist violent offenders and comparing population, grouped on the basis of allelic variants of *MAOA*-uVNTR polymorphism.

5.2 Conclusions of the study

In conclusion, the present findings indicate that:

1. Occurrence of 3.5R allele of *MAOA*-uVNTR polymorphism is more in recidivist violent offenders.

2. The frequency and prevalence of adverse childhood experiences (ACEs) in recidivist violent offenders is high.

3. There is an interrelationship between the occurrences of various dimensions of adverse childhood experiences (ACEs).

4. Exposure to extreme adverse childhood experiences (ACEs) results in early onset of criminality.

5. Prevalence of health risk behaviours (HRBs) is high in recidivist violent offenders.

6. There is early onset of health risk behaviours (HRBs) in participants with extreme adverse childhood experiences (ACEs).

7. Prevalence of health risk behaviours (HRBs) is more in participants who faced more adverse childhood experiences (ACEs).

8. Total adverse childhood experience (ACE) score predicts violent criminality in offenders.

9. Health risk behaviours (HRBs) failed to predict violent criminality in offenders.

10. Total adverse childhood experience (ACE) score predicts health risk behaviours (HRBs).

11. Individuals with 3.5R allele of 30 bp *MAOA*-uVNTR polymorphism develop violent criminal behaviour when exposed to extreme adverse childhood experiences (ACEs) during the early ages of life.

12. In individuals with 4.5R allele of 30 bp *MAOA*-uVNTR polymorphism and exposed to adverse childhood experiences (ACEs) during early ages of life, this allele ameliorates the development of violent criminal behaviour.

5.3 Policy and Practical implications

From the results of this study, a number of practical interventions can be drawn. The results can be used to support the

development of specific programs to prevent the paths toward SVC delinquency in juveniles who lacks proper attention. Prevention of the onset of childhood adverse experiences should be the primary goal of the intervention based on the result of this study. Since adverse childhood experiences (ACEs) was a salient and significant predictor of both health risk behaviours (HRBs) and criminality in this study, the ability to prevent ACEs from occurring should be of the utmost importance. Theoretically, by preventing the variety of ACEs early in the lives of children, they should be able to develop in more adaptive and positive ways, which equips them to avoid more problematic, maladaptive and violent behavioural outcomes.

5.3.1 Prevention of ACEs

Developing programs to prevent children from ACEs is principally endorsed by the findings of this study. Cost-effective practices like early life interventions which provide improved prenatal assistance and parental care have improved the family life of those at-risk of trauma and subsequent adverse outcomes (Zigler & Hall, 1989; Cohen *et al.,* 2010). Parental and family training programs have found a robust effect on the lives of the children, showing a reduction on the antisocial behaviour and subsequent delinquency over time (Piquero *et al.,* 2009).

Visiting the home of high-risk families were designed to prevent ACEs and family conflicts, which enhanced the care giving abilities of parent's at risk, educated them on the effects of child maltreatment and teach positive problem-solving behaviours (Zigler &

Hall, 1989). These interventions also found that nurse home visit during pregnancy of women in these families reduced child maltreatment and reduced consequent adverse behaviour, delinquency, arrests, convictions and running away (Olds *et al.,* 1997; Olds *et al.,* 1998; Poole *et al.,* 2014). It was found that the parent management training (PMT) and functional family therapy (FFT) improved family dynamics reduced family dysfunction and enhanced the overall development of the child (Wasserman & Miller 1998). Evaluation of such programs conducted by Bugental & colleagues (2010) found that the prevalence of child physical abuse was reduced significantly in such families. Hence, these findings suggest the effectiveness of more comprehensive program in preventing the ACEs will preclude children from a path that can lead to antisocial behaviour and criminality.

Preventions programs on sexual abuse was best implemented at the school level which included role playing, lectures, behavioural training, multimedia, doll or puppet shows in order to teach children about what constitutes sexual abuse. At school, sexual abuse prevention programs were found to be effective and the effectiveness increased with more sessions (Davis & Gidycz, 2000). In the case of incarcerated parents those who re-entering the family household after incarceration, several support programs like behavioural or attitude training were introduced and found effective in preventing multiple ACEs (Hairson & Lockett, 1985). The children experienced trauma of losing a parent during their formative years, when their parents were incarcerated for lengthy sentences. In such situations, in U.S some prisons have started ‘Parents in Prison’ program which improved the

parental skills, enhanced family relationships and thus prevented child maltreatment (Hairson & Lockett, 1985; Gilhuly & Taylor-Penn, 2018).

5.3.2 Prevention of HRBs

Beyond the above mentioned programs to reduce ACEs, interventions are also recommended to address HRBs in those who experienced ACEs. Also, SVC delinquency was directly related to substance abuse and hence their prevention and decrease would reduce the development of serious violent behaviours (Perez *et al.,* 2018). "Drug Abuse Resistance Education" (D.A.R.E.) program implemented in U.S was found to be largely ineffective (Perez, 2016). Science-based substance abuse prevention program for at-risk high school youth, "Project Towards No Drug Abuse" (Project TND), were able to be successful in preventing the onset of substance abuse (Gorman, 2003). This program was based on education about the consequences of drug abuse, motivation enhancement and coping skills management (Sussman *et al.,* 2012). Hence such scientifically evaluated interventions should be done among the children who have faced ACEs and at-risk of substance abuse. Such scientific interventions may be included in the similar programs like 'Clean Campus Safe Campus' implemented in the schools of Kerala as a joint initiative of Home, Education and Health Departments of Government of Kerala (Government of Kerala, 2018).

5.3.3 Prevention of violent criminal recidivism

Based on the results of this study when a youth comes in contact with the juvenile justice system, coordinated primary interventions for assessing their ACEs, HRBs and the mediating factors related to their behaviour and personality should be done and considered for determining their risk for serious, violent and chronic delinquency later in life. "Positive Achievement Change Tool" (PACT), a program developed by Florida Department of Juvenile Justice, screens the youth's overall risk for recidivism (Perez, 2016). Based on the PACT assessment and recommendations, these juveniles are directed for community-based correctional services or intensive attention and treatment (Vincent *et al.,* 2012). Another program outside of the juvenile justice system, specifically for children who faces ACEs, known as "Childhaven", which is an ecological-model therapeutic child caring intervention also found to be affective (Moore *et al*., 1998). "The Incredible Years Parent, Teacher, and Child" training series which targets children who display early indications of conduct problems, have also been shown to reduce the chances of violent offending later in adolescence (Piquero *et al.,* 2009). In order to reduce the SVC delinquency in youths whom this behaviour has already manifested, tertiary prevention program known as multisystemic therapy (MST) was implanted and found as a strong tool in the reduction and cessation of SVC delinquency (Henggeler *et al.,* 1992; Borduin *et al.,* 1995; Curtis *et al.,* 2004). This intervention was individualized and highly flexible, addresses intrapersonal (cognitive) and systemic factors (family, peer, school) factors that were known to

be related with adolescent antisocial behaviour (Borduin *et al.,* 1995). Results of present study also support the use of ACE assessment as an efficient predictor for violent criminal recidivism in juvenile delinquents. Also, the ACEs could be used as mitigating factors in the sentencing of youthful and non-youthful offenders (Trapassi, 2017).

5.4 Strengths and limitations

Several scholarly researches have discussed independently the harmful effects of childhood traumas and HRBs on criminality, association between ACEs and HRBs, interactions of these traumas with the allelic variants of *MAOA*-uVNTR polymorphism in different populations. Majority of these studies examined only one or two ACEs or its interaction with *MAOA* gene. By including thirteen categories of ACEs in the ACE score, three HRBs and the interaction of these thirteen ACEs with allelic variants of *MAOA*-uVNTR polymorphism in recidivist violent offenders, the present study was unique to its breath. Also, frequency of the ACEs was measured in this study, which most of the studies has not yet demonstrated (Perez, 2016). More over this was the first behavioural genetic study conducted in Indian population which demonstrated candidate gene-environment (cGxE) interaction in the development of violent criminality in offenders.

In the interpretation of the results of this study, several limitations should be considered. First of all the limitation of the present study was the relatively small sample size. Nevertheless, it should be noted that for research purposes, recruitment of prison inmates or prison released offenders were extremely problematic due to the ethical limitations and significant legal hurdles (Gostin *et al.,* 2007). From this perspective, the sample size of this study was

comparable with other genetic investigations in offender population (Nilsson *et al.,* 2006; Garcia *et al.,*2010; Tikkanen *et al.,* 2010; Aluja *et al.,* 2011; Kolla *et al.,* 2014; Stetler *et al.,* 2014). However, owing to this relatively small sample size these findings need to be considered as preliminary only.

Antisocial personality disorder (ASPD), aggression and impulsivity in the offenders and control population were not measured which might raise issue on selection of the subjects. The recidivist violent offenders were selected upon verifying their official criminal records, categorization done by the concerned Government departments and adopted by expert's consensus as done in other similar studies (Kolla *et al.,* 2014).

Another issue might be regarding the retrospective assessment of ACEs and HRBs of the subjects using questionnaire. Many large-scale studies in these areas have relayed upon self-complete questionnaires, but in this study face-to-face interview were conducted for each participants to know their ACEs and HRBs, a method having evidence of good validity (Jaffee *et al.,* 2004; Kim-Cohen *et al.,* 2006; Moffit & Caspi, 2014; Newbury *et al*., 2018).

Results of this study provide the first demonstration of an association between 3.5 repeat allele of *MAOA*-uVNTR and criminal violence in offenders. This evidence is in support of the genetic components in violent crime. Though this observed association was statistically significant, they tended to be limited in magnitude since the expression or activity of 3.5 repeat allele of *MAOA*-uVNTR was not done in this study. Conversely, 3.5 repeat allele of *MAOA*-uVNTR was referred as 3 repeat allele or low repeat allele in many other

studies depending upon the frequency of various alleles of *MAOA*-uVNTR in the population under study (Jorm *et al.,* 2000; Das *et al.,* 2006; Melas *et al.,* 2013; Lim *et al.,* 2018). Hence the possible effect of population stratification cannot be ignored (Huang *et al.,* 2004). Thus it could be suggested that depending on the small magnitude of the interaction of 3.5 repeat allele of *MAOA*-uVNTR and ACEs, the contribution of these interactions on violent criminality, over and above the main effect of ACEs, might be somewhat limited. These findings should be demonstrated in future studies on larger cohorts of recidivist violent offenders and non-violent control individuals in Indian population, in order to make clear the role of *MAOA* polymorphism in the predisposition to violence. As the genetic evidence of *MAOA*-uVNTR variants was used to justify sentence reductions in criminal cases in U.S and Italy, extreme caution is necessary in the application of the findings in this study to the Criminal Justice ystem (Baum, 2013; McSwiggan *et al.,* 2017).

5.5 Suggestions for further studies

Today, in many communities all over the world, violence has become common, resulting in drastic outcomes in society such as an increase in drive by homicides, rapes, gang related activities etc.,. Hence more work is needed in understanding genetic and environment underpinnings of aggression and violence. The nature of the association between *MAOA*-uVNTR polymorphism and aggression in recidivist violent offenders need to be studied in consideration to several other environmental factors also in large sample size. This will help in developing better psychological and psychiatric interventions in

the rehabilitation and reintegration of violent offenders. Similarly understanding the allelic variants, its frequency, sequence variations and epigenetic modifications in the promoter region of *MAOA* gene will help to understand the aetiology of pathological aggression and various psychiatric disorders which is currently a growing requirement.

Molecular genetics findings offer promise of development of biochemical approaches to control or modulation of aggressive behaviour. Obviously, the physiology of aggression is complex and many factors impinge on the biochemical system in the brain. It remains to be seen whether specific biochemical interventions can be devised to affect aggressiveness without causing problems in other behavioural areas. One of the advantages of the molecular biology approach is the ability to distinguish the effect of a specific gene on the spectrum of aggressive behaviour, which people as well as animals manifest. This perspective could result in a biologically based classification of aggressive behaviours that could rationally guide both biochemical and behavioural approaches to aggressiveness management.

Other gene polymorphisms, especially in the serotonin transporter gene (5-HTT) have been found significant in contributing to violent behaviour. Interactions of serotonin transporter promoter repeat length polymorphism (5-HTTLPR) and *MAOA*-uVNTR polymorphism may be studied for the better understanding of violent behaviour. Landmark based shape analysis of the face of offenders using advance technique, Geometric Morphometric (GMM) may be done for measuring symmetry, asymmetry and fluctuating asymmetry

so as to visualize and compare the dysmorphogenesis due to developmental and environmental noise.

Impulsivity, aggressiveness and associated violent behaviour are common traits, the expression of which must be carefully modulated to ensure the success of individuals, small groups and large societies, especially within the current framework of globalization. From behavioural genetics, molecular genetics and evolutionary models of violence, we may more fully understand which individuals are at greatest risk of extreme violence. We can then begin to examine the interaction not only between genes and environmental catalysts for violence, but also interaction between genes and treatment and prevention efforts for violence. This is the promise that behavioural genetics and molecular genetics may hold as it's ultimately turns from treatment to outcome research.

SIVA PRASAD M. S. “EFFECT OF ADVERSE CHILDHOOD EXPERIENCES AND OCCURRENCE OF UVNTR POLYMORPHISM IN MONOAMINE OXIDASE A GENE IN RECIDIVIST VIOLENT OFFENDERS: FORENSIC IMPLICATIONS”. THESIS. DEPARTMENT OF ZOOLOGY, UNIVERSITY OF CALICUT, 2018.

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APPENDICES

APPENDIX 1

Protocol: DNA Purification from Buccal Swabs (Spin Protocol)

This protocol is for purification of total (genomic, mitochondrial, and viral) DNA from buccal swabs using a microcentrifuge. For total DNA purification using a vacuum manifold, see "Protocol: DNA Purification from Buccal Swabs (Vacuum Protocol)" on page 39.

Buccal Swab Spin Protocol

Important points before starting

- Due to the increased volume of Buffer AL that is required for the buccal swab protocol, fewer preparations can be performed. Additional Buffer AL can be purchased separately (see ordering information on page 70).
- This protocol is recommended for the following swab types: C.E.P. (Omni Swabs from Whatman Bioscience, www.whatman.com), cotton, and DACRON (Daigger, Puritan® applicators with plastic stick and cotton or DACRON tip from Hardwood Products Company, www.hwppuritan.com, or from Hain Diagnostika, www.hain-diagnostika.de).
- To collect a sample, scrape the swab firmly against the inside of each cheek 6 times. Air-dry the swab for at least 2 h after collection. Ensure that the person providing the sample has not consumed any food or drink in the 30 min prior to sample collection.
- All centrifugation steps are carried out at room temperature (15–25°C).

Things to do before starting

- Prepare a 56°C water bath for use in step 3.
- Equilibrate Buffer AE or distilled water to room temperature (15–25°C) for elution in step 10.
- Ensure that Buffer AW1, Buffer AW2, and QIAGEN Protease have been prepared according to the instructions on page 16.
- If a precipitate has formed in Buffer AL, dissolve by incubating at 56°C.

Procedure

1. **Place buccal swab in a 2 ml microcentrifuge tube. Add 400 µl (cotton and DACRON swab) or 600 µl (Omni Swab) PBS to the sample.**

   The Omni Swab is ejected into the microcentrifuge tube by pressing the stem end towards the swab. Cotton or DACRON swabs are separated from the stick by hand or with scissors.

   QIAamp Mini spin columns copurify RNA and DNA in parallel when both are present in the sample. RNA may inhibit some downstream enzymatic reactions, but not PCR. If RNA-free genomic DNA is required, 4 µl of an RNase A stock solution (100 mg/ml) should be added to the sample prior to the addition of Buffer AL.

2. **Add 20 µl QIAGEN Protease stock solution (or proteinase K) and 400 µl (cotton or DACRON swab) or 600 µl (Omni Swab) Buffer AL to the sample. Mix immediately by vortexing for 15 s.**

   To ensure efficient lysis, it is essential that the sample and Buffer AL are mixed immediately and thoroughly.

   Note: Do not add QIAGEN Protease or proteinase K directly to Buffer AL.

3. **Incubate at 56°C for 10 min. Briefly centrifuge to remove drops from inside the lid.**
4. **Add 400 µl (cotton or DACRON swab) or 600 µl (Omni Swab) ethanol (96–100%) to the sample, and mix again by vortexing. Briefly centrifuge to remove drops from inside the lid.**
5. **Carefully apply 700 µl of the mixture from step 4 to the QIAamp Mini spin column (in a 2 ml collection tube) without wetting the rim. Close the cap, and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Place the QIAamp Mini spin column in a clean 2 ml collection tube (provided), and discard the tube containing the filtrate.***

   Close each spin column to avoid aerosol formation during centrifugation.

6. **Repeat step 5 by applying up to 700 µl of the remaining mixture from step 4 to the QIAamp Mini spin column.**
7. **Carefully open the QIAamp Mini spin column and add 500 µl Buffer AW1 without wetting the rim. Close the cap and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Place the QIAamp Mini spin column in a clean 2 ml collection tube (provided), and discard the collection tube containing the filtrate.***
8. **Carefully open the QIAamp Mini spin column and add 500 µl Buffer AW2 without wetting the rim. Close the cap and centrifuge at full speed (20,000 x *g*; 14,000 rpm) for 3 min.**

* Flow-through contains Buffer AL or Buffer AW1 and is therefore not compatible with bleach. See page 6 for safety information.

9. **Recommended: Place the QIAamp Mini spin column in a new 2 ml collection tube (not provided) and discard the old collection tube with the filtrate. Centrifuge at full speed for 1 min.**

   This step helps to eliminate the chance of possible Buffer AW2 carryover.

10. **Place the QIAamp Mini spin column in a clean 1.5 ml microcentrifuge tube (not provided), and discard the collection tube containing the filtrate. Carefully open the QIAamp Mini spin column and add 150 µl Buffer AE or distilled water. Incubate at room temperature (15–25°C) for 1 min, and then centrifuge at 6000 x *g* (8000 rpm) for 1 min.**

    Elution with 100 µl buffer increases the final DNA concentration in the eluate significantly, but may slightly reduce the overall DNA yield. Elution with volumes of less than 100 µl is not recommended as the overall DNA yield decreases dramatically.

    A second elution step with the same 150 µl eluate containing the DNA will increase yield significantly. However this is not recommended when using the eluate for PCR.

    For long-term storage of DNA, eluting in Buffer AE and placing at –30 to –15°C is recommended.

    One buccal swab typically yields 0.5–3.5 µg of DNA in 150 µl of buffer (3–23 ng/µl), with A_{260}/A_{280} ratios of 1.7–1.9 (measured in water).

Buccal Swab Spin Protocol

APPENDIX 2

prepIT®·L2P

Laboratory protocol for manual purification of DNA from whole sample

Ethanol precipitation protocol and prepIT®•L2P reagent for the purification of genomic DNA from Oragene® products and ORAcollect® formats OC-175, OCD-100 and OCR-100. Not for use with OCD-100A.

Visit our website at www.dnagenotek.com for any additional languages and protocols.

Note: This protocol requires the use of a centrifuge capable of generating at least 3,500 × *g* to obtain optimal results.

The procedure is described for purifying DNA from the entire collected sample (approximately 1 mL to 4 mL total volume). The volumes shown should be adjusted for the actual collected volume.

Reagents included

- prepIT•L2P (catalog #: PT-L2P)

Equipment and reagents

- Centrifuge that accommodates 15 mL tubes, and is capable of generating at least 3,500 × *g* (see Table 2)
- 15 mL conical polypropylene tubes (e.g., BD Falcon #352196)
- Microcentrifuge capable of running at 15,000 × *g* (optional)
- 1.5 mL microtubes (e.g., Axygen #MCT-150-C)
- Air or water incubator at 50°C
- Ethanol (95% to 100%) at room temperature
- Ethanol (70%) at room temperature
- DNA storage buffer: TE (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) or similar solution

Pre-purification check

Weigh the sample to estimate the amount of saliva provided by the donor (see Table 1; not required for ORAcollect products). The amount of saliva collected is directly proportional to the amount of DNA recovered. As an example, if a donor has provided less than 2 mL of saliva, you should expect to recover a lower total yield from this sample. Correspondingly, a donor providing more than 2 mL of saliva should result in higher total yield.

Weight of kit (without sample)

Once a sample arrives at the lab, we suggest weighing the sample to estimate if the right amount of saliva was provided by the donor. You can expect some variability across donors as determined by weight of triggered kit with sample. The average weight of an empty kit is provided (Table 1). To calculate the amount of sample collected (assuming 1 g/mL), perform the following subtraction:

Weight of kit containing sample - Weight of kit without sample = Amount of sample collected

Table 1

Product #	Weight of kit without sample
OG-250/OGR-250	14.15 g
OG-500/OGD-500/OGR-500/OG-600*/ OGD-600*/OGR-600*	6.81 g
OG-510/OGD-510/OG-610*/OGD-610*	5.83 g
OG-520	5.41 g
OG-575/OGD-575/OGR-575/OG-675*/ OGD-675*/OGR-675*	5.66 g
ON-500/ON-600*	6.47 g

* 2D bottom barcoded kits are 1 g heavier than the respective non-barcoded kit.

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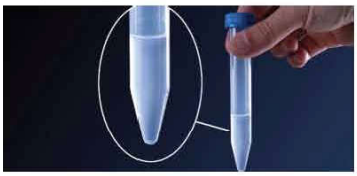
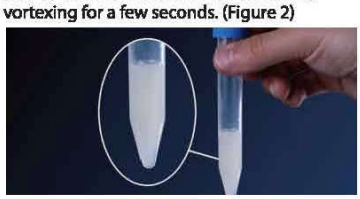
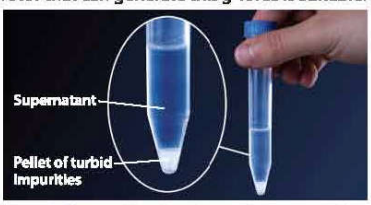
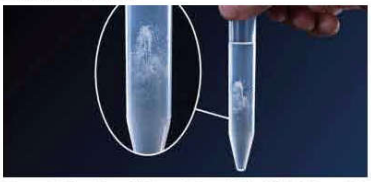
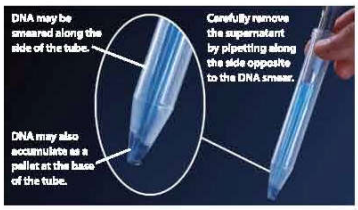
DNA genotek

Procedure

Purification steps	Notes
1. Mix the sample in the DNA Genotek kit by inversion and gentle shaking for a few seconds.	• This is to ensure that viscous samples are properly mixed.
2. Incubate the sample at 50°C in a water incubator for a minimum of 1 hour or in an air incubator for a minimum of 2 hours. **Note:** The use of an air incubator may be preferable since the sample tubes may float in a water bath. If a water bath must be used, ensure the sample-containing portion of the tube remains immersed in water.	• This heat-treatment step is essential to maximize DNA yield and ensure that nucleases are permanently inactivated. • This must be done in the original collection tube. • The sample may be incubated at 50°C overnight if more convenient. • This incubation step may be performed at any time after sample is collected and before DNA is purified. • A longer time is required in an air incubator because temperature equilibration is slower than in a water incubator.
3. Transfer the entire sample to a 15 mL centrifuge tube (Figure 1). Note the volume of the sample.  ***Figure 1:** Before proceeding to Step 4, ensure that the entire sample has been incubated and transferred to a fresh 15 mL centrifuge tube, as shown.*	• Transfer can be carried out either by pouring or by pipetting with a glass or plastic pipette.
4. Add 1/25th volume of PT-L2P and mix by vortexing for a few seconds. (Figure 2)  ***Figure 2:** After adding the PT-L2P and incubating on ice for 10 minutes, the sample will no longer look clear, but rather a cloudy solution.*	• e.g., 160 µL for 4 mL of sample to each tube. • The sample will become turbid as impurities and inhibitors are precipitated.
5. Incubate on ice for 10 minutes.	• Room temperature incubation can be substituted but will be less effective at removing impurities.

Purification steps	Notes
6. Centrifuge at room temperature for 10 minutes at as high a speed as is possible. Minimum 3,500 x *g*. Any centrifuge, either swing-out bucket or angle rotor that can generate this *g*-force is suitable.  ***Figure 3:** After centrifugation, there will be an accumulation of turbid material at the base of the tube. The supernatant should be visibly clear.*	• Higher centrifugal force minimizes the amount of turbid material that will be carried over into the purified DNA (Figure 3). • Before proceeding, you should verify with the tube manufacturer that the 15 mL centrifuge tubes can withstand the centrifugal force. • A longer period of centrifugation (up to 20 minutes) can be carried out where reducing the turbidity of the final DNA solution is considered to be important.
7. Carefully transfer the majority of the clear supernatant with a pipette to a fresh 15 mL centrifuge tube. **Discard the pellet.**	• Leave a small volume of the supernatant behind to avoid disturbing the pellet. The pellet contains turbid impurities.
8. Add 1.2x volume of room temperature 95% to 100% ethanol to the clear supernatant. Mix gently by inversion 10 times.  ***Figure 4:** After the addition of ethanol, the DNA will precipitate which may result in a visible clot of fibres.*	• During mixing with ethanol, the DNA will be precipitated. Precipitated DNA may appear as a clot of DNA fibres (Figure 4). Even if no clot is seen, DNA will be recovered in the following steps.
9. Let the sample stand at room temperature for 10 minutes to allow the DNA to fully precipitate.	• Incubation at -20°C is not recommended because impurities may co-precipitate with the DNA.
10. Centrifuge at room temperature for 10 minutes at as high a speed as is possible. Minimum 3,500 × *g*.	• A minimum centrifuge speed of 3,500 × *g* (see Table 2) is required. Any centrifuge, either swinging-bucket or angle rotor that can generate this *g*-force is suitable.

Purification steps	Notes
11. Carefully remove the supernatant with a glass or plastic pipette and discard it. Take care to avoid disturbing the DNA pellet.  ***Figure 5:** Using a pipette tip to gently scratch along the inside of the tube may reveal the presence of a DNA smear.*	• The supernatant may contain impurities and should be removed as completely as possible. • Precipitated DNA will be found as a pellet at the bottom of the tube and possibly as a smear down the side of the tube (Figures 5). • The DNA smear may be located on the side of the tube facing away from the centre of the centrifuge. • A smear can be located using the "scratch" test. You can check for the presence of a DNA smear by scratching the inside of the tube using a P1000 tip. A smear, as shown in figure 5, may be visible.
12. Ethanol wash: Carefully add 1 mL of 70% ethanol to the tube without disturbing the smear or the pellet. Let it stand at room temperature for 1 minute. Gently swirl and **completely remove the ethanol, being careful not to disturb the pellet and the smear.**	• **It is important to remove all ethanol from the sample. Carryover of ethanol may impact the performance of the assay.** • Take care not to disturb the DNA pellet or the smear. • A short centrifugation (less than 1 minute) can be performed to facilitate complete removal of the supernatant. • Should the pellet detach after the ethanol wash step, centrifuge the sample for 5 minutes at as high a speed as is possible. Minimum 3,500 x *g*.
13. Rehydrate the DNA by adding 0.2 – 1 mL of TE solution and by vortexing the sample for 30 seconds. For ORAcollect products, rehydrate the DNA by adding 0.2 mL of TE solution and vortex the sample for 30 seconds.  ***Figure 6:** Vortexing the sample for 30 seconds will allow you to recover DNA smeared on the side of the tube. The DNA will remain high molecular weight.*	• If a higher concentration of DNA is desired, the volume of TE may be reduced. A minimum of 200 µL TE solution should be used. • Excessive drying of the pellet (> 10 minutes) and using less than 500 µL of TE solution can make it difficult to rehydrate (dissolve) the DNA and may decrease the yield or make quantification difficult. • Precipitated DNA will be found as a pellet at the bottom of the tube and possibly as a smear down the side of the tube. • To ensure maximum DNA recovery, the sample must be vortexed after the addition of DNA solvent (TE solution). Vortexing will ensure that the DNA smeared on the side of the tube is recovered (Figure 6). • Do not hesitate to vortex the sample as the DNA will remain high molecular weight.

Purification steps	Notes
14. To ensure complete rehydration of the DNA (pellet and smear) incubate at room temperature overnight followed by vortexing or at 50°C for 1 hour with occasional vortexing.	• Incomplete rehydration of the DNA is a cause of inaccuracy in estimating DNA concentration and potential failure of downstream applications such as PCR.
15. Transfer the rehydrated DNA to a 1.5 mL microcentrifuge tube for storage.	
Optional step: a) Centrifuge the rehydrated DNA at room temperature for 15 minutes at 15,000 x *g*. b) Transfer the supernatant to a fresh 1.5 mL microcentrifuge tube without disturbing the pellet.	Note that the pellet contains insoluble, turbid material. • To maximize DNA recovery, ensure that the DNA is completely rehydrated (step 14) prior to performing this centrifugation step. • This centrifugation step ensures that any remaining turbid material is removed from the DNA sample. • Care should be taken not to disturb the pellet when transferring the clear supernatant to a fresh tube.
16. Options for storage of the fully rehydrated DNA: a) **Recommended** in TE, in aliquots at -20°C for long-term storage, or b) In TE at 4°C for up to 2 months.	• Freezing of purified DNA in TE may cause the DNA to precipitate. When thawing frozen purified DNA, pay careful attention to rehydration, as discussed in step 14.

Quantification of DNA

By fluorescence method

Assays that use fluorescent dyes are more specific than absorbance at 260 nm for quantifying the amount of double-stranded DNA (dsDNA) in a DNA sample. We recommend using fluorescent dyes such as PicoGreen® or SYBR® Green I to quantify dsDNA since there is less interference by contaminating RNA. An inexpensive protocol using SYBR Green I is described in PD-PR-075, *DNA quantification using SYBR Green I Dye and a micro-plate reader*[1]. Alternatively, commercially available kits such as Invitrogen's Quant-iT™ PicoGreen dsDNA Assay Kit (Cat. No. Q-33130) can be used. For either protocol, we recommend that the purified DNA be diluted 1:50 with TE solution and that 5 µL be used in the quantification assay.

APPENDIX 3

Protocol: DNA Purification from Dried Blood Spots (QIAamp DNA Mini Kit)

This protocol is for purification of total (genomic, mitochondrial, and viral) DNA from blood, both untreated and treated with anticoagulants, which has been spotted and dried on filter paper (Schleicher and Schuell 903).

Important point before starting

- All centrifugation steps are carried out at room temperature (15–25°C).

Things to do before starting

- Prepare an 85°C water bath for use in step 2, a 56°C water bath for use in step 3, and a 70°C water bath for use in step 4.
- Equilibrate Buffer AE or distilled water to room temperature (15–25°C) for elution in step 10.
- Ensure that Buffer AW1 and Buffer AW2 have been prepared according to the instructions on page 16.
- If a precipitate has formed in Buffer AL or Buffer ATL, dissolve by incubating at 56°C.

Procedure

1. **Place 3 punched-out circles from a dried blood spot into a 1.5 ml microcentrifuge tube and add 180 µl of Buffer ATL.**

   Cut 3 mm (1/8 inch) diameter punches from a dried blood spot with a single-hole paper puncher.

2. **Incubate at 85°C for 10 min. Briefly centrifuge to remove drops from inside the lid.**
3. **Add 20 µl proteinase K stock solution. Mix by vortexing, and incubate at 56°C for 1 h. Briefly centrifuge to remove drops from inside the lid.**

   Note: The addition of proteinase K is essential.

4. **Add 200 µl Buffer AL to the sample. Mix thoroughly by vortexing, and incubate at 70°C for 10 min. Briefly centrifuge to remove drops from inside the lid.**

   To ensure efficient lysis, it is essential that the sample and Buffer AL are mixed immediately and thoroughly.

   Note: Do not add proteinase K directly to Buffer AL.

   A white precipitate may form when Buffer AL is added to the sample. In most cases, the precipitate will dissolve during incubation. The precipitate does not interfere with the QIAamp procedure or with any subsequent application.

Dried Blood Spots

5. **Add 200 µl ethanol (96–100%) to the sample, and mix thoroughly by vortexing. Briefly centrifuge to remove drops from inside the lid.**

   It is essential that the sample and ethanol are mixed thoroughly.

6. **Carefully apply the mixture from step 5 to the QIAamp Mini spin column (in a 2 ml collection tube) without wetting the rim. Close the cap, and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Place the QIAamp Mini spin column in a clean 2 ml collection tube (provided), and discard the tube containing the filtrate.***

   Close each QIAamp Mini spin column to avoid aerosol formation during centrifugation.

7. **Carefully open the QIAamp Mini spin column and add 500 µl Buffer AW1 without wetting the rim. Close the cap and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Place the QIAamp Mini spin column in a clean 2 ml collection tube (provided), and discard the collection tube containing the filtrate.***

8. **Carefully open the QIAamp Mini spin column and add 500 µl Buffer AW2 without wetting the rim. Close the cap and centrifuge at full speed (20,000 x *g*; 14,000 rpm) for 3 min.**

9. **Recommended: Place the QIAamp Mini spin column in a new 2 ml collection tube (not provided) and discard the old collection tube with the filtrate. Centrifuge at full speed for 1 min.**

   This step helps to eliminate the chance of possible Buffer AW2 carryover.

10. **Place the QIAamp Mini spin column in a clean 1.5 ml microcentrifuge tube (not provided), and discard the collection tube containing the filtrate. Carefully open the QIAamp Mini spin column and add 150 µl Buffer AE or distilled water. Incubate at room temperature (15–25°C) for 1 min, and then centrifuge at 6000 x *g* (8000 rpm) for 1 min.**

    Three punched-out circles (3 mm diameter) typically yield 150 ng and 75 ng of DNA from anticoagulated and untreated blood, respectively. If the yield from untreated blood is not sufficient, use 6 circles per prep instead of 3.

    The volume of the DNA eluate used in a PCR assay should not exceed 10%; for example, for a 50 µl PCR, add no more than 5 µl of eluate.

* Flow-through contains Buffer AL or Buffer AW1 and is therefore not compatible with bleach. See page 6 for safety information.

APPENDIX 4

Participant Identification Number:

Modified Adverse Childhood Experiences International Questionnaire (ACE-IQ)

0	DEMOGRAPHIC INFORMATION	
0.1 [C1]	How old are you?	
0.2 [C2]	What is your religion?	
0.3 [C3]	What is the highest level of education you have completed?	No formal schooling Less than primary school Primary school completed Secondary/High school completed College/University completed Post graduate degree
0.4 [C4]	Which of the following best describes your main work status over the last 12 months?	Government employee Non-government employee Self-employed Non-paid Student Homemaker Retired Unemployed (able to work) Unemployed (unable to work)
0.5 [C5]	What is your civic status?	Married Living as couple Divorced or separated Single Widowed Other

Participant Identification Number:

1	RELATIONSHIP WITH PARENTS/GUARDIANS	
	When you were growing up, during the first 18 years of your life . . .	
1.1 [P1]	Did your parents/guardians understand your problems and worries?	Always Sometimes Rarely Never
1.2 [P2]	Did your parents/ guardians **really** know what you were doing with your free time when you were not at school orwork?	Always Sometimes Rarely Never
2		
2.1 [P3]	How often did your parents/guardians **not** give you enough food even when they could easily have done so?	Many times A few times Once Never
2.2 [P4]	Were your parents/guardians too drunk or intoxicated by drugs to take care of you?	Many times A few times Once Never
2.3 [P5]	How often did your parents/guardians **not** send you to school even when it was available?	Many times A few times Once Never
3	FAMILY ENVIRONMENT	
	When you were growing up, during the first 18 years of your life . . .	
3.1 [F1]	Did you live with a household member who was a problem drinker or alcoholic, or misused street or prescription drugs?	Yes No
3.2 [F2]	Did you live with a household member who was depressed, mentally ill or suicidal?	Yes No
3.3 [F3]	Did you live with a household member who was ever sent to jail or prison?	Yes No
3.4 [F4]	Were your parents ever separated or divorced?	Yes No Not applicable
3.5 [F5]	Did your mother, father or guardian die?	Yes No Don't know / Not sure

These next questions are about certain things you may actually have heard or seen IN YOUR HOME. These are things that may have been done to another household member but not necessarily toyou.

Participant Identification Number:

When you were growing up, during the first 18 years of your life . . .		
3.6 [F6]	Did you see or hear a parent or household member in your home being yelled at, screamed at, sworn at, insulted or humiliated?	Many times A few times Once Never
3.7 [F7]	Did you see or hear a parent or household member in your home being slapped, kicked, punched or beaten up?	Many times A few times Once Never
3.8 [F8]	Did you see or hear a parent or household member in your home being hit or cut with an object, such as a stick (or cane), bottle, club, knife, whip etc.?	Many times A few times Once Never
These next questions are about certain things YOU may have experienced. When you were growing up, during the first 18 years of your life...		
4		
4.1 [A1]	Did a parent, guardian or other household member yell, scream or swear at you, insult or humiliate you?	Many times A few times Once Never
4.2 [A2]	Did a parent, guardian or other household member threaten to, or actually, abandon you or throw you out of the house?	Many times A few times Once Never
4.3 [A3]	Did a parent, guardian or other household member spank, slap, kick, punch or beat you up?	Many times A few times Once Never
4.4 [A4]	Did a parent, guardian or other household member hit or cut you with an object, such as a stick (or cane), bottle, club, knife, whip etc?	Many times A few times Once Never
4.5 [A5]	Did someone touch or fondle you in a sexual way when you did not want them to?	Many times A few times Once Never
4.6 [A6]	Did someone make you touch their body in a sexual way when you did not want them to?	Many times A few times Once Never
4.7 [A7]	Did someone attempt oral, anal, or vaginal intercourse with you when you did not want them to?	Many times A few times Once Never

Participant Identification Number:

4.8 [A8]	Did someone actually have oral, anal, or vaginal intercourse with you when you did not want them to?	Manytimes A fewtimes Once Never
5	**PEER VIOLENCE**	
	These next questions are about BEING BULLIED when you were growing up. Bullying is when a young person or group of young people say or do bad and unpleasant things to another young person. It is also bullying when a young person is teased a lot in an unpleasant way or when a young person is left out of things on purpose. It is not bullying when two young people of about the same strength or power argue or fight or when teasing is done in a friendly and funway. **When you were growing up, during the first 18 years of your life . . .**	
5.1 [V1]	How often were you bullied?	Many times A few time Once Never
5.2 [V2]	How were you bullied most often?	I was hit, kicked, pushed, shoved around, or locked indoors I was made fun of because of my race, nationality or colour I was made fun of because of my religion I was made fun of with sexual jokes, comments, or gestures I was left out of activities on purpose or completely ignored I was made fun of because of how my body or face looked I was bullied in some other way
	This next question is about PHYSICAL FIGHTS. A physical fight occurs when two young people of about the same strength or power choose to fight each other. **When you were growing up, during the first 18 years of your life . . .**	
5.3 [V3]	How often were you in a physical fight?	Many times A few times Once Never
6	**WITNESSING COMMUNITY VIOLENCE**	
	These next questions are about how often, when you were a child, YOU may have seen or heard certain things in your NEIGHBOURHOOD OR COMMUNITY (not in your home or on TV, movies, or the radio). **When you were growing up, during the first 18 years of your life . . .**	
6.1 [V4]	Did you see or hear someone being beaten up in real life?	Many times A few times Once Never
6.2	Did you see or hear someone being stabbed	Many times

Participant Identification Number:

[V5]	or shot in real life?	A few times Once Never
6.3 [V6]	Did you see or hear someone being threatened with a knife or gun in real life?	Manytimes A fewtimes Once Never
7	**EXPOSURE TO WAR/COLLECTIVE VIOLENCE**	
	These questions are about whether YOU did or did not experience any of the following events when you were a child. The events are all to do with collective violence, including wars, terrorism, political or ethnic conflicts, genocide, repression, disappearances, torture and organized violent crime such as banditry and gang warfare. **When you were growing up, during the first 18 years of your life . . .**	
7.1 [V7]	Were you forced to go and live in another place due to any of these events?	Manytimes A fewtimes Once Never
7.2 [V8]	Did you experience the deliberate destruction of your home due to any of these events?	Many times A few times Once Never
7.3 [V9]	Were you beaten up by soldiers, police, militia, or gangs?	Many times A few times Once Never
7.4 [V10]	Was a family member or friend killed or beaten up by soldiers, police, militia or gangs?	Many times A few times Once Never

APPENDIX 4.1

ബാല്യകാലത്തെ പ്രതികൂല അനുഭവങ്ങളെക്കുറിച്ചുള്ള വിവരങ്ങൾ ശേഖരിക്കുന്നതിനുള്ള ചോദ്യാവലി

0.1 (C1)	വയസ്സ്	
0.2 (C2)	താങ്കൾ ഏതു മതത്തിൽപെടുന്നു?	
0.3 (C3)	താങ്കൾ പൂർത്തിയാക്കിയ ഏറ്റവും ഉയർന്ന വിദ്യാഭ്യാസയോഗ്യത ഏതാണ്?	സ്ക്കൂളിൽ പോയിട്ടില്ല പ്രൈമറി വിദ്യാഭ്യാസത്തിനു താഴെ പ്രൈമിറ വിദ്യാഭ്യാസം പൂർത്തിയാക്കി ഹൈസ്ക്കുൾ/+2 വിദ്യാഭ്യാസം പൂർത്തിയാക്കി ബിരുദം പൂർത്തിയാക്കി ബിരുദാനന്തരബിരുദം പൂർത്തിയാക്കി
0.4 (C4)	ഇവിടെ കാണിച്ചിരിക്കുന്നതിൽ ഏതു തൊഴിൽ ആണ് താങ്കളുടെ കഴിഞ്ഞ 12 മാസക്കാലത്തെ പ്രധാനതൊഴിലായി രേഖപ്പെടുത്താൻ പറ്റുന്നത്?	സർക്കാർ ജോലിക്കാരൻ സർക്കാർ ഇതരജോലിക്കാരൻ സ്വയം തൊഴിൽ വേതനം ഇല്ലാത്ത തൊഴിൽ വിദ്യാർത്ഥി കുടുംബിനി ജോലിയിൽ നിന്ന് വിരമിച്ചത് തൊഴിൽ രഹിതൻ (ജോലി ചെയ്യാൻ ശേഷിയുണ്ട്) തൊഴിൽ രഹിതൻ (ജോലി ചെയ്യാൻ ശേഷിയില്ല)
0.5 (C5)	താങ്കളുടെ വൈവാഹിക ജീവിതത്തെപ്പറ്റിയുള്ള വിവരങ്ങൾ	വിവാഹിതൻ വിവാഹിതരാകാതെ ഒരുമിച്ചു താമസിക്കുന്നു വിവാഹമോചിതൻ/ അകന്നു താമസിക്കുന്നു അവിവാഹിതൻ വിഭാര്യൻ മറ്റുള്ളവ
1	**മാതാപിതാക്കൾ/രക്ഷിതാക്കളുമായുള്ള ബന്ധം** **താങ്കളുടെ വളർച്ചയിൽ ആദ്യത്തെ 18 വർഷ കാലഘട്ടത്തിൽ . . .**	
1.1 (P1)	താങ്കളുടെ പ്രശ്നങ്ങളും സങ്കടങ്ങളും മാതാപിതാക്കൾ/ രക്ഷിതാക്കൾ മനസ്സിലാക്കിയിരുന്നോ?	എപ്പോഴും മിക്കവാറും വല്ലപ്പോഴും ഒരിക്കലുമില്ല
1.2 (P 2)	(സ്കൂൾ/ തൊഴിൽ ഇടങ്ങളിൽ അല്ലാതെ) ഒഴിവു സമയങ്ങളിൽ താങ്കൾ എന്താണ് ചെയ്തിരുന്നതെന്ന് താങ്കളുടെ മാതാപിതാക്കൾ/ രക്ഷി	എപ്പോഴും മിക്കവാറും വല്ലപ്പോഴും

	താക്കൾക്കോ യഥാർത്ഥത്തിൽ അിറയുമായിരുന്നോ?	ഒരിക്കലുമില്ല
2		
2.1 (P 3)	ഭക്ഷണം നൽകാൻ താങ്കളുടെ മാതാപിതാക്കൾ/ രക്ഷിതാക്കൾക്ക് കഴിവുണ്ടായിരുന്നിട്ടും താങ്കൾക്ക് വേണ്ടത്ര ഭക്ഷണം എത്ര തവണ നൽകാതിരുന്നിട്ടുണ്ട്?	പലതവണ വളരെ കുറച്ചുതവണ ഒരിക്കൽ ഒരിക്കലും ഇല്ല
2.2 (P4)	താങ്കളെ സംരക്ഷിക്കാനാകാത്തവിധം മാതാപിതാക്കൾ/ രക്ഷിതാക്കൾ വളരെയധികം മദ്യം/ലഹരിവസ്തുക്കൾ ഉപയോഗിച്ചിരുന്നോ?	പലതവണ വളരെകുറച്ചു തവണ ഒരിക്കൽ ഒരിക്കലും ഇല്ല
2.3 (P 5)	സ്ക്കൂൾ ദിനങ്ങളിൽ എത്ര തവണ മാതാപിതാക്കൾ/രക്ഷിതാക്കൾ താങ്കളെ പഠനത്തിനയക്കാതിരുന്നിട്ടുണ്ട്?	പലതവണ വളരെകുറച്ചു തവണ ഒരിക്കൽ ഒരിക്കലും ഇല്ല
3	**കുടുംബസാഹചര്യം** **താങ്കളുടെ വളർച്ചയിൽ ആദ്യത്തെ 18 വർഷ കാലഘട്ടം...**	
3.1 (F1)	സ്ഥിരം മദ്യപാനികളോ/ മദ്യപിച്ച് വഴക്കുണ്ടാക്കുന്നതോ/ലഹരി വസ്തുക്കളോ/ മരുന്നുകളോ ദുരുപയോഗം ചെയ്യുന്ന കുടുംബാംഗവുമായി സഹവസിക്കേണ്ടി വന്നിട്ടുണ്ടോ?	ഉണ്ട് ഇല്ല
3.2 (F2)	വിഷാദരോഗമോ/മാനസികാസ്വസ്ഥമോ/ആത്മഹത്യാപ്രവണതയോ ഉള്ള കുടുംബാഗവുമായി സഹവസിക്കേണ്ടി വന്നിട്ടുണ്ടോ?	ഉണ്ട് ഇല്ല
3.3 (F3)	എപ്പോഴെങ്കിലും ജയിൽവാസം അനുഭവിച്ച കുടുംബാംഗവുമായി സഹവസിക്കേണ്ടി വന്നിട്ടുണ്ടോ?	ഉണ്ട് ഇല്ല
3.4 (F4)	താങ്കളുടെ മാതാപിതാക്കൽ എപ്പോഴെങ്കിലും അകന്നു താമസിക്കുകയോ അല്ലെങ്കിൽ വിവാഹമോചിതരോ ആണോ?	അതെ അല്ല പ്രസക്തമല്ല
3.5 (F5)	താങ്കളുടെ അമ്മ, അച്ഛൻ അല്ലെങ്കിൽ രക്ഷിതാവ് മരിച്ചതാണോ?	അതെ അല്ല അിറയില്ല/ ഉറപ്പില്ല
	അടുത്ത ചോദ്യങ്ങൾ താങ്കളുടെ കുടുംബത്തിൽ താങ്കൾ യഥാർത്ഥത്തിൽ കേൾക്കുകയോ കാണുകയോ ചെയ്ത ചില കാര്യങ്ങളെ പറ്റിയാണ്. ഇത് താങ്കൾ അനുഭവിച്ച കാര്യങ്ങൾ ആകണമെന്നില്ല, മറ്റു കുടുംബാഗങ്ങൾക്ക് നേരിട്ടതാകാം. **താങ്കളുടെ വളർച്ചയിൽ ആദ്യത്തെ 18 വർഷകാലഘട്ടത്തിൽ . . .**	
3.6 (F6)	താങ്കളുടെ വീട്ടിൽ വച്ച് താങ്കളുടെ മാതാപിതാക്കളിൽ ആർക്കെങ്കിലും നേരെയോ മറ്റ് കുടുംബാഗത്തിനു നേരെയോ ഉച്ചത്തിൽ ആക്രോശിക്കുകയോ ശാപവാക്കുകൾ പറയുകയോ അധിക്ഷേപിക്കുകയോ, അപമാനിക്കുകയോ ചെയ്യുന്നതായി കാണുകയോ കേൾക്കുകയോ ചെയ്തിട്ടുണ്ടോ?	പലതവണ വളരെകുറച്ചു തവണ ഒരിക്കൽ ഒരിക്കലും ഇല്ല
3.7 (F7)	താങ്കളുടെ വീട്ടിൽ വച്ച് താങ്കളുടെ മാതാപിതാക്കളിൽ ആർക്കെങ്കിലും നേരെയോ മറ്റു കുടുബാംഗത്തിനെയോ കൈകൊണ്ട് അടിക്കുകയോ, തൊഴിക്കുകയോ, ഇടിക്കുകയോ തുടർച്ചയായി ദേഹോപദ്രവം ഏൽപ്പിക്കുകയോ ചെയ്യുന്നതായി കാണുകയോ കേൾക്കുകയോ ചെയ്തിട്ടുണ്ടോ?	പല തവണ വളരെ കുറച്ചു തവണ ഒരിക്കൽ ഒരിക്കലും ഇല്ല

3.8 (F8)	താങ്കളുടെ വീട്ടിൽ വച്ച് താങ്കളുടെ മാതാപിതാക്കൾ ആർക്കെങ്കിലും നേരെയോ മറ്റു കുടുംബാഗത്തിനെയോ വടി, ചൂരൽ, കുപ്പി, കത്തി മുതലായ വസ്തുക്കൾ കൊണ്ട് അടിക്കുകയോ മുറിവേൽപ്പിക്കുകയോ ചെയ്യുന്നതായി കാണുകയോ കേൾക്കുകയോ ചെയ്തിട്ടുണ്ടോ?	പലതവണ വളരെ കുറച്ചു തവണ ഒരിക്കൽ ഒരിക്കലും ഇല്ല
	അടുത്ത ചോദ്യങ്ങൾ താങ്കൾ അഭിമുഖീകരിക്കാൻ സാധ്യതയുള്ളതാണ് ചില കാര്യങ്ങളെ പറ്റിയാണ്. **താങ്കളുടെ വളർച്ചയിൽ ആദ്യത്തെ 18 വർഷകാലഘട്ടത്തിൽ . . .**	
4		
4.1 (A1)	മാതാപിതാക്കൾ ആരെങ്കിലും, രക്ഷിതാവ് അല്ലെങ്കിൽ മറ്റൊരു കുടുംബാഗം താങ്കൾക്കു നേരെ ഉച്ചത്തിൽ ആക്രോശിക്കുകയോ ശാപവാക്കുകൾ പറയുകയോ, അധിക്ഷേപിക്കുകയോ, അപമാനിക്കുകയോ ചെയ്തിട്ടുണ്ടോ?	പലതവണ വളരെ കുറച്ചുതവണ ഒരിക്കൽ ഒരിക്കലും ഇല്ല
4.2 (A2)	മാതാപിതാക്കളിൽ ആരെങ്കിലും, രക്ഷിതാവ് അല്ലെങ്കിൽ മറ്റൊരു കുടുംബാംഗം താങ്കളെ ഉപേക്ഷിക്കും അല്ലെങ്കിൽ വീട്ടിൽ നിന്നു പുറത്താക്കും എന്ന് ഭീഷണിപ്പെടുത്തുകയോ അല്ലെങ്കിൽ യഥാർത്ഥത്തിൽ അങ്ങനെ ചെയ്തിട്ടുണ്ടോ?	പലതവണ വളരെ കുറച്ചു തവണ ഒരിക്കൽ ഒരിക്കലും ഇല്ല
4.3 (A3)	മാതാപിതാക്കൾ ആരെങ്കിലും, രക്ഷിതാവ് അല്ലെങ്കിൽ മറ്റൊരു കുടുംബാംഗം താങ്കളെ കൈകൊണ്ട് അടിക്കുകയോ, ചവിട്ടുകോ, തൊഴിക്കുകയോ, ഇടിക്കുകയോ അല്ലെങ്കിൽ തുടർച്ചയായി ദേഹോപദ്രവം ഏൽപ്പിക്കുകയോ ചെയ്തിട്ടുണ്ടോ?	പലതവണ വളരെകുറച്ചു തവണ ഒരിക്കൽ ഒരിക്കവും ഇല്ല
4.4 (A4)	മാതാപിതാക്കളിൽ ആരെങ്കിലും, രക്ഷിതാവ്, അല്ലെങ്കിൽ മറ്റൊരു കുടുംബാംഗം താങ്കളെ വടി, ചൂരൽ, കുപ്പി, കത്തി മുതലായ വസ്തുക്കൾ കൊണ്ട് അടിക്കുകയോ മുറിവേൽപ്പിക്കുകയോ ചെയ്തിട്ടുണ്ടോ?	പലതവണ വളരെകുറച്ചു തവണ ഒരിക്കൽ ഒരിക്കലും ഇല്ല വിസമ്മതിച്ചു
4.5 (A5)	താങ്കൾക്ക് വേണ്ടാതിരുന്നിട്ടും ആരെങ്കിലും താങ്കളെ ലൈംഗികോദ്ദേശത്തോടെ സ്പർശിക്കുകയോ ലാളിക്കുകയോ ചെയ്തിട്ടുണ്ടോ?	പലതവണ വളരെകുറച്ചു തവണ ഒരിക്കൽ ഒരിക്കലും ഇല്ല
4.6 (A6)	താങ്കൾക്ക് വേണ്ടാതിരിന്നിട്ടും ആരെങ്കിലും താങ്കളെക്കൊണ്ട് അവരുടെ ശരീരത്തിൽ ലൈംഗികോദ്ദേശത്തോടെ സ്പർശിച്ചിട്ടുണ്ടോ?	പലതവണ വളരെകുറച്ചു തവണ ഒരിക്കൽ ഒരിക്കലും ഇല്ല
4.7 (A7)	താങ്കൾക്ക് വേണ്ടാതിരിന്നിട്ടും ആരെങ്കിലും താങ്കളുമായി ഏതെങ്കിലും തരത്തിലുള്ള ലൈംഗികബന്ധത്തിന് ശ്രമിച്ചിട്ടുണ്ടോ?	പലതവണ വളരെകുറച്ചു തവണ ഒരിക്കൽ ഒരിക്കലും ഇല്ല
4.8 (A8)	താങ്കൾക്ക് വേണ്ടാതിരുന്നിട്ടും ആരെങ്കിലും താങ്കളുമായി യഥാർത്ഥ്യത്തിൽ ഏതെങ്കിലും തരത്തിലുള്ള ലൈംഗിക ബന്ധത്തിൽ ഏർപ്പെട്ടിട്ടുണ്ടോ?	പലതവണ വളരെകുറച്ചു തവണ ഒരിക്കൽ ഒരിക്കലും ഇല്ല
5	**സമപ്രായക്കാർക്കിടയിലുള്ള അക്രമം**	
	അടുത്ത ചോദ്യങ്ങൾ താങ്കൾ വളർന്നു വരുമ്പോൾ ബുള്ളിയിങ്ങിന് വിധേയൻ ആയിട്ടുണ്ടോ എന്നറിയുവാൻ വേണ്ടിയാണ്. ചെറുപ്രായത്തിലുള്ള വ്യക്തിയോ വ്യക്തികളോ സമപ്രായക്കാരനായ വ്യക്തിയെ ചീത്തവിളിക്കുകയോ, പരിഹസിക്കുകയോ, മനപ്പൂർവ്വം മാറ്റി നിർത്തുകയോ ചെയ്യുന്നതാണ്	

	ബുള്ളിയിങ്ങ്. സമപ്രായക്കാർക്കിടയിൽ സൗഹൃദപൂർവ്വം ഇത്തരത്തിൽ നടക്കുന്നത് ബുള്ളിയിങ്ങിൽപ്പെടില്ല. **താങ്കളുടെ വളർച്ചയിൽ ആദ്യത്തെ 18 വർഷകാലഘട്ടത്തിൽ . . .**	
5.1 (V1)	താങ്കൾ എത്ര തവണ ബുള്ളിയിങ്ങിനു വിധേയനായിട്ടുണ്ട്?	പലതവണ വളരെകുറച്ചു തവണ ഒരിക്കൽ ഒരിക്കലും ഇല്ല
5.2 (V2)	പലതവണ താങ്കൾ ഏതു രീതിയിലാണ് ബുള്ളിയിങ്ങിനു വിധേയനായിട്ടുള്ളത്?	എന്നെ അടിക്കുകയും, തൊഴിക്കുകയും, ഉന്തുകയും, തള്ളിമാറ്റുകയോ അല്ലെങ്കിൽ മുറിയിൽ പൂട്ടി ഇടുകയോ ചെയ്തിട്ടുണ്ട്. എന്റെ ജാതിയുടെയും, രാജ്യത്തിന്റെയോ അല്ലെങ്കിൽ നിറത്തിന്റെയോ പേരിൽ കളിയാക്കിയിട്ടുണ്ട്. എന്റെ മതത്തിന്റെ പേരിൽ എന്നെ കളിയാക്കിയിട്ടുണ്ട്. ലൈംഗികചുവയുള്ള തമാശകൾ വിമർശനങ്ങൾ പറഞ്ഞോ അല്ലെങ്കിൽ ചേഷ്ടകൾ കാണിച്ചോ എന്നെ കളിയാക്കിയിട്ടുണ്ട്. എന്നെ മനപ്പൂർവ്വം പ്രവർത്തികളിൽ നിന്ന് മാറ്റി നിർത്തുകയോ അല്ലെങ്കിൽ പൂർണ്ണമായി അവഗണിക്കുകയോ ചെയ്തിട്ടുണ്ട്. എന്റെ ശരീരമോ മുഖമോ എങ്ങനെ കാണപ്പെടുന്നു എന്നതിൽ എന്നെ കളിയാക്കിയിട്ടുണ്ട്. മറ്റുതരത്തിൽ എന്നെ ഭീഷണിപ്പെടുത്തിയിട്ടുണ്ട്.
	അടുത്ത ചോദ്യങ്ങൾ അടിപിടികളെ പറ്റിയാണ്, തുല്യബലമുള്ള ചെറുപ്രായത്തിലുള്ള വ്യക്തികൾ തമ്മിലുള്ള ശാരീരകമായ അടിപിടിയാണ് ഉദ്ദേശിക്കുന്നത്. **താങ്കളുടെ വളർച്ചയിൽ ആദ്യത്തെ 18 വർഷ കാലഘട്ടത്തിൽ . . .**	
5.3 (V3)	താങ്കൾ എത്ര തവണ അടിപിടികളിൽ ഏർപ്പെട്ടിട്ടുണ്ട്?	പലതവണ വളരെകുറച്ചുതവണ ഒരിക്കൽ ഒരിക്കലും ഇല്ല
6	**സമൂഹത്തിലെ അക്രമങ്ങൾക്ക് സാക്ഷിയാകൽ**	
	അടുത്ത ചോദ്യങ്ങൾ താങ്കൾ കുട്ടിയായിരിക്കുമ്പോൾ എത്ര തവണ അയൽപക്കത്തോ സമൂഹത്തിലോ കണ്ടയോ കേട്ടതോ ആയ കാര്യങ്ങളോ പറ്റിയാണ് (വീട്ടിലോ, ടി.വിയിലോ, പല ചിത്രങ്ങളിലോ, റേഡിയോയിലോ അല്ല) **താങ്കളുടെ വളർച്ചയിൽ ആദ്യത്തെ 18 വർഷകാലഘട്ടത്തിൽ . . .**	
6.1 (V4)	യഥാർത്ഥ ജീവിതത്തിൽ ആരെങ്കിലും മർദ്ദിക്കപ്പെടുന്നത് താങ്കൾ കാണുകയോ മർദ്ദിക്കപ്പെട്ടയായി കേൾക്കുയയോ ചെയ്തിട്ടുണ്ടോ?	പലതവണ വളരെകുറച്ചുതന്നെ ഒരിക്കൽ ഒരിക്കലും ഇല്ല

6.2 (V5)	യഥാർത്ഥ ജീവീതത്തിൽ ആർക്കെങ്കിലും കുത്തേൽക്കുന്നതോ, വെടിയേൽക്കുന്നതോ താങ്കൾ കാണുകയോ അതിനെപ്പറ്റി കേൾക്കുകയോ ചെയ്തിട്ടുണ്ടോ?	പലതവണ വളരെകുറച്ചുതവണ ഒരിക്കൽ ഒരിക്കലും ഇല്ല
6.3 (V6)	യഥാർത്ഥത്തിൽ ജീവിതത്തിൽ ആരെങ്കിലും കത്തിയുമായോ, തോക്കുമായോ ഭീഷണിപ്പെടുത്തുന്നത് താങ്കൾ കാണുകയോ അതിനെപ്പറ്റി കേൾക്കുകയോ ചെയ്തിട്ടുണ്ടോ?	പലതവണ വളരെകുറച്ചുതവണ ഒരിക്കൽ ഒരിക്കലും ഇല്ല
7	**യുദ്ധം/ സംഘടിത/ആൾക്കൂട്ട അക്രമത്തിനെ അഭിമുഖീകരിച്ചത്**	
	അടുത്ത ചോദ്യങ്ങൾ താങ്കൾ കുട്ടിയായിരിക്കുമ്പോൾ അഭിമുഖീകരിച്ചതോ അല്ലാത്തതോ ആയ സംഭവങ്ങളെപ്പറ്റിയാണ്. യുദ്ധം, ഭീകരപ്രവർത്തനം, രാഷ്ട്രീയ/വംശീയ സംഘർഷങ്ങൾ, കൂട്ടക്കൊല, അടിച്ചമർത്തൽ, തിരോധാനം, പീഡനം, സംഘടിതമയാ അക്രമസ്വഭാവമുള്ള കുറ്റകൃത്യങ്ങൾ, ഗുണ്ടാസംഘങ്ങൾ തമ്മിലുള്ള അക്രമം എന്നിവ. **താങ്കളുടെ വളർച്ചയിൽ ആദ്യത്തെ 18 വർഷകാലഘട്ടത്തിൽ . . .**	
7.1 (V7)	ഇത്തരത്തിൽ ഏരെങ്കിലും സംഭവങ്ങൾ മൂലം താങ്കൾ മറ്റൊരിടത്തേക്ക് താമസം മാറ്റാൻ നിർബന്ധിതനായിട്ടുണ്ടോ?	പലതവണ വളരെകുറച്ചുതവണ ഒരിക്കൽ ഒരിക്കലും ഇല്ല
7.2 (V8)	ഇത്തരത്തിൽ ഏതെങ്കിലും സംഭവങ്ങൾ മൂലം താങ്കളുടെ വീട് കരുതിക്കൂട്ടി നശിപ്പിക്കപ്പെടുന്ന അനുഭവം താങ്കൾക്കുണ്ടായിട്ടുണ്ടോ?	പലതവണ വളരെകുറച്ചുതവണ ഒരിക്കൽ ഒരിക്കലും ഇല്ല
7.3 (V9)	താങ്കളെ സൈനികരോ, പോലീസുകാരോ, പൗരനസേനക്കാരോ ഗുണ്ടകളോ മർദ്ദിച്ചിട്ടുണ്ടോ?	പലതവണ വളരെകുറച്ചുതവണ ഒരിക്കൽ ഒരിക്കലും ഇല്ല
7.4 (V10)	താങ്കളുടെ കുടുംബാംഗമോ സുഹൃത്തോ, സൈനികരായോ, പോലീസുകാരാലോ പൗരസേനക്കാരാലോ ഗുണ്ടകളാലോ കൊല്ലപ്പെടുകയോ മർദ്ദിക്കുകയോ ചെയ്തിട്ടുണ്ടോ?	പലതവണ വളരെകുറച്ചുതവണ ഒരിക്കൽ ഒരിക്കലും ഇല്ല

APPENDIX 5 & 5.1

Health Risk Behavior questions (HRBs)

Question number	Question	Response category
During the first 18 years of your life…		
1.	Do you have a habit of smoking cigarette?	Yes No
1a.	How old were you when you began to smoke cigarettes fairly regularly?	
2.	Have you ever drunk alcohol?	Yes No
2a.	How old were you when you had first drink of alcohol other than few sips?	
3.	Have you ever used street drugs?	Yes No
3a.	How old were you the first time you used street drugs?	

ആരോഗ്യത്തിന് ഹാനികരമായ സ്വഭാവങ്ങൾ – അറിയുവാനുള്ള ചോദ്യാവലി

ചോദ്യ നമ്പർ	ചോദ്യം	മറുപടി
താങ്കളുടെ വളർച്ചയുടെ ആദ്യത്തെ 18 വർഷങ്ങളിൽ		
1.	പുകവലിശീലം ഉണ്ടായിരുന്നോ?	ഉണ്ട് ഇല്ല
1.a	എത്രവയസ്സുമുതൽ പതിവായി പുകവലിക്കാൻ തുടങ്ങി	
2.	മദ്യം കഴിച്ചിട്ടുണ്ടോ?	ഉണ്ട് ഇല്ല
2.a	എത്രവയസ്സുമുതൽ മദ്യം സ്ഥിരമായി കഴിക്കാൻ തുടങ്ങി	
3.	ലഹരിപദാർത്ഥങ്ങൾ ഉപയോഗിച്ചിട്ടുണ്ടോ?	ഉണ്ട് ഇല്ല
3.a	എത്രാമത്തെ വയസ്സിലാണ് ആദ്യമായി ലഹരിപദാർത്ഥങ്ങൾ ഉപയോഗിച്ചത്	

APPENDIX 6

CALICUT UNIVERSITY HUMAN ETHICAL COMMITTEE

University of Calicut, Malappuram - 673 635, Kerala, India

Chairman :
Sri. P. N. Vijayakumar,
Former District & Sessions Judge, Thrissur, Kerala, India;
Former Kerala State Information Commissioner;
Chairperson, Kerala State Commission for Scheduled Castes and Scheduled Tribes.

Member Secretary :
Head of the Department,
Department of Zoology,
University of Calicut,
Malappuram, Kerala, India

Members :
Dean,
Faculty of Science,
University of Calicut,
Malappuram, Kerala, India

Director,
Directorate of Research,
University of Calicut,
Malappuram, Kerala, India

Dr. S. P. Shaji Prabha,
Junior Scientific Officer,
Chemical Examiner's Laboratory,
Thiruvananthapuram, Kerala, India

Dr. P. B. Gujaral,
District Police Surgeon,
District Hospital, Palakkad,
Kerala, India

Dr. V. V. Unnikrishnan,
Additional Professor of Physiology,
Government Medical College,
Thrissur, Kerala, India

Dr. Ajith Kumar,
Additional Professor of Dermatology,
Government Medical College,
Thrissur, Kerala, India

Sri. Jayesh K. Joseph,
Criminologist,
Kerala Police Academy,
Thrissur, Kerala, India

Smt. Jayasree,
Assistant Professor,
Department of Philosophy,
University of Calicut,
Malappuram, Kerala, India

Sri. P. Aboobacker,
Pookat House, Pallikkal,
Malappuram, Kerala, India

003/CUEC/CR/2013-14-CU — 25/04/2014

Ref. No: — *Date*

CERTIFICATE OF ETHICS CLEARANCE TO INVOLVE HUMAN PARTICIPANTS IN RESEARCH

CUEC Application No: 002/CUEC/2013-14-CU

~~Research~~ Project/Ph.D topic:
Study on the Forensic aspects of uVNTR polymorphism of MAOA gene in Recidivist Offenders: Adverse Childhood Environment

~~Principal Investigator~~/Research Scholar:
Mr. Siva Prasad M.S

Name & Address of Institution:
Department of Zoology, University of Calicut, Malappuram- 673635, Kerala, India

[ ] New review [√] Revised review [ ] Expedited review

Date of review (D/M/Y): **25/04/2014**

Date of previous review, if revised application: **07/02/2014**

Decision of the CUEC:

[ ] Recommended [√] Recommended with suggestions
[ ] Revision [ ] Rejected

Suggestions/ ~~Reasons~~/ Remarks (If any):

- Questionnaire should be validated, modifications may be included.
- Data should not be disclosed to other authorities like Police department & Prison department.
- The Applicant itself is educationally & technically qualified to collect blood & buccal samples from the Human subjects.
- Occupational offences may incorporate.

Recommended for a period of : **Five years or till the submission of Ph.D Thesis**

Reporting frequency : **Yearly**

Number of Samples : **300 (100 from each sample groups)**

Please note *

- **Inform CUEC immediately in case of any Adverse events and Serious adverse events.**
- **Inform CUEC in case of any change of study procedure, site and investigator.**
- **This permission is only for period mentioned above. Annual report to be submitted to CUEC.**
- **Members of CUEC have right to monitor the trial with prior intimation.**

Signature of the Chairperson
Date: 25/4/2014

Signature of Member Secretary
Date: 25/4/14

Dr. KANNAN V.M., Ph.D.
ASSOCIATE PROFESSOR
DEPARTMENT OF ZOOLOGY
UNIVERSITY OF CALICUT
KERALA 673 635, INDIA

Calicut University Human Ethical Committee ★ University of Calicut ★ Date: 25/04/14

Dept. of Zoology 673 635 Dt. 25/4 University of Calicut

Address for Communication :
Head of the Department, Department of Zoology, University of Calicut, Calicut University P.O., Malappuram - 673 635, Kerala, India | Telephone No: 0494 2407420 | e-mail: zoohod@uoc.ac.in

APPENDIX 6.1

Control Group
Sample No.

സ്ഥിരം കുറ്റവാളികളിലെ മോണോ അമൈൻ ഓക്സിഡേസ്-എ (*MAOA*) എന്ന ജീനിന്റെ ഘടനാ വ്യതിയാനങ്ങളും ബാല്യകാലത്തെ പ്രതികൂല ചുറ്റുപാടുകളും: ഒരു ഫോറൻസിക് സാധ്യതാപഠനം

പരീക്ഷണത്തിൽ പങ്കെടുക്കുന്നതിനുള്ള സമ്മത പത്രം

പങ്കെടുക്കുന്ന വ്യക്തിയുടെ പേരും വിലാസവും:

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1. ഈ ഗവേഷണം എന്തിനെപ്പറ്റിയാണ്?

കേരളത്തിലെ കോഴിക്കോട് സർവ്വകലാശാലയിലെ സുവോളജി പഠനവിഭാഗത്തിൽ Ph.D പഠനത്തിന്റെ ഭാഗമായി ശ്രീ. ശിവപ്രസാദ് എം. എസ്. നടത്തുന്ന ഗവേഷണമാണിത്. സ്ഥിരം കുറ്റവാളികളുടെ ശാസ്ത്രീയമായ പുനരധിവാസത്തിന്റെ സാധ്യതകൾ കണ്ടെത്തുകയാണ് ഈ ഗവേഷണത്തിന്റെ പരമമായ ലക്ഷ്യം. ഒരു വ്യക്തിയിൽ ബാല്യകാലത്തെ പ്രതികൂല ചുറ്റുപാടുകൾ/സാഹചര്യങ്ങൾ, ശാരീരികവും മാനസികവും ലൈംഗികവുമായ പീഡനങ്ങൾ, മോണോ അമൈൻ ഓക്സിഡേസ്-എ (*MAOA*) എന്ന ജീനിന്റെ ഘടനാവ്യതിയാനങ്ങൾ എന്നിവ ആ വ്യക്തിയിൽ സഹജമായ അക്രമവാസന ഉണ്ടാക്കുന്നതിൽ എന്തെങ്കിലും പങ്കുവഹിക്കുന്നുണ്ടോ എന്നറിയുന്നതിനുള്ള പഠനമാണിത്.

നിലവിൽ താങ്കൾ ഇന്ത്യൻ ശിക്ഷാനിയമപ്രകാരം ശിക്ഷിക്കപ്പെട്ടിട്ടില്ലെന്നും താങ്കൾക്കെതിരെ ക്രിമിനൽ കേസുകൾ യാതൊന്നും രജിസ്റ്റർചെയ്യപ്പെട്ടിട്ടില്ലെന്നും മനസ്സിലാക്കുവാൻ കഴിഞ്ഞു. ആയതിനാൽ ഈ ഗവേഷണത്തിൽ പങ്കെടുക്കാൻ താങ്കളോട് അഭ്യർത്ഥിക്കുന്നു. താങ്കൾ ഈ ഗവേഷണത്തിലെ നിയന്ത്രണ വിഭാഗത്തിൽ (Control group) പെടുന്നതായിരിക്കും. ഈ ഗവേഷണം മുഖാന്തിരം ലഭിക്കുന്ന അറിവ് സ്ഥിരം കുറ്റവാളികളുടെ ശാസ്ത്രീയമായ പുനരധിവാസത്തിനും നിലവിലുള്ള പുനരധിവാസ പദ്ധതികൾ മെച്ചപ്പെടുത്തുന്നതിനും സമൂഹത്തിന് സ്ഥിരം കുറ്റവാളികളോടുള്ള കാഴ്ച്ചപ്പാടുകൾ മാറ്റുന്നതിനും ഉപകരിച്ചേക്കും. ഈ ഗവേഷണത്തിന്റെ ഉത്തരവാദിത്വം ശ്രീ. ശിവപ്രസാദ് എം. എസിനായിരിക്കും. ഈ ഗവേഷണത്തിനു വേണ്ടി താങ്കളുടെ

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ബാല്യകാലത്തെ ജീവിത രീതി, ജീവിത സാഹചര്യങ്ങൾ, ദുരനുഭവങ്ങൾ ഉണ്ടെങ്കിൽ അതിന്റെ വിശദാംശങ്ങൾ, ശാരീരികമായോ മാനസികമായോ ലൈംഗികമായോ ഏറ്റ പീഡനങ്ങൾ ഉണ്ടെങ്കിൽ അതിന്റെ വിശദാംശങ്ങൾ, കുടുംബാംഗങ്ങളുടെ വിവരങ്ങൾ മുതലായവ ആവശ്യമാണ്. കൂടാതെ താങ്കളുടെ രക്തത്തിന്റേയോ വായിലെ കോശങ്ങളുടെയോ സാമ്പിളുകളും ആവശ്യമുണ്ട്.

2. ഈ ഗവേഷണത്തിന്റെ നടപടിക്രമം എങ്ങനെയാണ്?

ഈ ഗവേഷണത്തിൽ താങ്കളും പങ്കുചേരുവാൻ അനുമതി തരികയാണെങ്കിൽ:

i) അതാതു സർക്കാർ വകുപ്പുകളുടെ അനുമതി ലഭിച്ചതിനുശേഷം ശ്രീ. ശിവപ്രസാദ് എം. എസ്. എന്ന വ്യക്തി താങ്കളുടെ അനുവാദത്തോടെ മുൻകൂട്ടി നിശ്ചയിച്ചിട്ടുള്ള ചോദ്യാവലി അനുസരിച്ച് താങ്കളുമായി അഭിമുഖ സംഭാഷണം നടത്തുന്നതായിരിക്കും. ചോദ്യാവലിയിൽ താങ്കളുടെ ബാല്യകാലത്തെ ജീവിതരീതി, ജീവിത സാഹചര്യം, ദുരനുഭവങ്ങൾ, ശാരീരികവും മാനസികവും ലൈംഗികവുമായി ഏറ്റ പീഡനങ്ങൾ, കുടുംബാംഗങ്ങളുടെ വിവരങ്ങൾ എന്നിവയെപ്പറ്റിയുള്ള ചോദ്യങ്ങൾ ഉണ്ടായിരിക്കും. ചോദ്യാവലി ശ്രീ. ശിവപ്രസാദ് എം. എസിന്റെ സഹായത്തോടെ പൂരിപ്പിച്ച് തരേണ്ടതായിരിക്കും.

ii) താങ്കളുടെ അനുവാദത്തോടെ ചെറിയ സൂചി (ലാൻസെറ്റ്) ഉപയോഗിച്ച് താങ്കളുടെ വിരൽത്തുമ്പിൽ നിന്നും രണ്ടോ മൂന്നോ തുള്ളി രക്തമോ പഞ്ഞി ഉപയോഗിച്ച് വായിലെ കോശങ്ങളോ എടുക്കുന്നതായിരിക്കും. ഈ പ്രക്രിയകളെയെല്ലാം താങ്കളുടെ ആരോഗ്യത്തെ ഒരു തരത്തിലും ഹാനികരമായി ബാധിക്കാത്ത തരത്തിൽ എല്ലാ മുൻകരുതലുകളും ശുചിത്വവും പാലിച്ചായിരിക്കും നടത്തുക.

3. ഈ ഗവേഷണത്തിൽ താങ്കളെ വേദനിപ്പിക്കുന്ന എന്തെങ്കിലും സംഭവിക്കുമോ?

ചോദ്യാവലിയിൽ താങ്കളുടെ ബാല്യകാലത്തെ ദുരനുഭവങ്ങൾ, അനുഭവിക്കേിവന്ന പീഡനങ്ങൾ എന്നിവയെപ്പറ്റിയുള്ള ചോദ്യങ്ങളുണ്ട്. അത് താങ്കൾക്ക് ചിലപ്പോൾ വേദനയുണർത്തുന്ന ഓർമ്മകൾ ഉണ്ടാക്കിയേക്കാം. താങ്കൾക്ക് മറുപടി തരാൻ താൽപര്യമില്ലാത്തതോ വേദനിപ്പിക്കുന്നതോ ആയ ചോദ്യങ്ങൾക്ക് മറുപടി തരേണ്ടതില്ല. വിരൽത്തുമ്പിൽ നിന്നും രക്തമെടുക്കുമ്പോൾ ചെറിയ വേദന (രക്തത്തിലെ ഗ്ലൂക്കോസ് അളക്കാനുള്ള പരിശോധനക്ക് സമാനം) അനുഭവപ്പെടും. അഭിമുഖത്തിനിടക്ക് അൽപനേരം വിശ്രമം വേണമെങ്കിലോ അഭിമുഖം പിന്നീടൊരു സമയത്തേക്ക് മാറ്റിവെയ്ക്കാൻ താൽപ്പര്യപ്പെടാനോ താങ്കൾക്ക് എല്ലാ സ്വാതന്ത്ര്യവുമുണ്ട്.

4. ഈ ഗവേഷണം എപ്രകാരം സഹായിക്കും?

ഈ ഗവേഷണത്തിൽ പങ്കെടുക്കുന്നതുകൊണ്ട് താങ്കൾക്ക് വ്യക്തിപരമായ ലാഭങ്ങളൊന്നുമില്ല. താങ്കളുടേയും സ്ഥിരം കുറ്റവാളികളുടേയുംബാല്യകാലത്തെ പ്രതികൂല സാഹചര്യങ്ങളും ജനിതക വ്യതിയാനങ്ങളും തമ്മിലുള്ള താരതമ്യപഠനം ആയിരിക്കും ഈ ഗവേഷണത്തിൽ നടത്തുക. താങ്കൾ തരുന്ന വിവരങ്ങൾ ഒരു പക്ഷേ വരും തലമുറയെ പ്രതികൂല സാഹചര്യങ്ങളിൽ നിന്നും പീഡനങ്ങളിൽ നിന്നും സംരക്ഷിക്കുവാൻ വേണ്ട പദ്ധതികൾ വിഭാവനം ചെയ്യാൻ അധികാരികളെ പ്രേരിപ്പിച്ചേക്കാം. മോണോ അമൈൻ ഓക്സിഡേസ്-എ (*MAOA*) ജീനിന്റെ ഘടനാവ്യതിയാനങ്ങൾ അക്രമവാസനയെ സാധൂകരിക്കുന്നു എന്ന് ഈ

ഗവേഷണത്തിലൂടെ മനസ്സിലാക്കുവാൻ സാധിച്ചാൽ ഇത്തരം ജനിതകവ്യതിയാനങ്ങൾ ഉള്ളവരെ ശാസ്ത്രീയമായി പുനരധിവസിപ്പിക്കുന്നതിൽ ഉചിതമായ തീരുമാനങ്ങൾ എടുക്കുവാൻ വൈദ്യശാസ്ത്രത്തെയും ശിക്ഷാനിയമത്തെയും സഹായിച്ചേക്കാം.

5. താങ്കളുടെ സ്വകാര്യത എങ്ങനെ പരിരക്ഷിക്കപ്പെടും?

താങ്കളിൽ നിന്നു ലഭിക്കുന്ന എല്ലാ വിവരങ്ങളും (ജനിതക വിവരങ്ങൾ ഉൾപ്പെടെ) ഇന്ത്യാഗവൺമെന്റിന്റെ സ്വകാര്യത നിയമങ്ങൾക്കനുസരിച്ച് സ്വകാര്യമായി വെക്കുന്നതായിരിക്കും. ഈ ഗവേഷണം നടത്തുന്ന ശ്രീ. ശിവപ്രസാദ് എം. എസ്, ഗവേഷണ പ്രവർത്തനങ്ങൾക്കു മേൽ നോട്ടം വഹിക്കുന്ന ഡോ. ഷിബു വർദ്ധനൻ, അസോസിയേറ്റ് പ്രൊഫസർ, സുവോളജി പഠനവിഭാഗം, കാലിക്കറ്റ് സർവ്വകലാശാല എന്നിവർക്കും, കാലിക്കറ്റ് സർവ്വകലാശാലയിൽ ഈ ഗവേഷണവുമായി ബന്ധമുള്ളമറ്റുള്ളവർക്കും താങ്കൾ നൽകുന്ന വിവരങ്ങൾ, താങ്കളുടെ ജനിതക വിവരങ്ങൾ എന്നിവ പങ്കുവയ്ക്കുവാൻ ഈ സമ്മതപത്രപ്രകാരം അനുവാദം താങ്കൾ തരുന്നതായി അനുമാനിക്കും.

6. താങ്കളുടെ വിവരങ്ങൾ ലഭ്യമാകാവുന്ന മറ്റു വ്യക്തികൾ:

1) കാലിക്കറ്റ് സർവ്വകലാശാലയിലെ ഗവേഷണങ്ങളുടെ ധാർമ്മികത പരിശോധിച്ച് ഗവേഷണങ്ങൾക്ക് അനുമതി നൽകുന്ന ഹ്യൂമൺ എത്തിക്കൽ സമിതി അംഗങ്ങൾ

ഈ സമ്മതപത്രം താങ്കൾ ഒപ്പ് വയ്ക്കുന്നതുകൊണ്ട് ഈ ഗവേഷണം മുഖാന്തിരം ശേഖരിച്ച താങ്കളുടെ വിവരങ്ങൾ മേൽപറഞ്ഞ വ്യക്തികളുമായി പങ്കുവെയ്ക്കാൻ അനുവാദം തന്നതായി അനുമാനിക്കും. താങ്കളിൽ നിന്ന് ഒരു തവണയേ അഭിമുഖം നടത്തി വിവരങ്ങൾ, രക്തം അല്ലെങ്കിൽ വായിലെ കോശങ്ങളുടെ സാമ്പിൾ എന്നിവ ശേഖരിക്കുകയുള്ളൂ. ഈ ഗവേഷണത്തിൽ താങ്കൾ പങ്കെടുക്കുന്നത് തികച്ചും സ്വന്തം തീരുമാനപ്രകാരമായിരിക്കും. എന്നിരുന്നാലും, ഈ സമ്മതപത്രത്തിൽ ഒപ്പുവയ്ക്കാതെ ഈ ഗവേഷണത്തിന്റെ ഭാഗമാകുവാൻ സാധ്യമല്ല.

7. ഈ ഗവേഷണത്തിൽ പങ്കെടുത്താൽ പ്രതിഫലം ലഭിക്കുമോ?

ഈ ഗവേഷണത്തിൽ പങ്കെടുക്കുന്നതിന്റെ പ്രതിഫലമായി താങ്കൾക്ക് യാതൊന്നും ലഭിക്കുന്നതല്ല.

8. ഞാൻ ഈ ഗവേഷണത്തിൽ പങ്കെടുക്കേതുണ്ടോ?

താങ്കൾക്ക് താത്പര്യമില്ലെങ്കിലോ, മറ്റ് എന്തെങ്കിലും ബുദ്ധിമുട്ടുകൾ ഉണ്ടെങ്കിലോ ഈ ഗവേഷണത്തിൽ പങ്കെടുക്കേതില്ല. അത് തികച്ചും താങ്കളുടെ തീരുമാനമാണ്. ഈ ഗവേഷണത്തിൽ പങ്കുചേരാതിരിക്കുന്നതുകൊണ്ട് താങ്കൾക്ക് ഒരു വിധത്തിലുമുള്ള ബുദ്ധിമുട്ടുകൾ ആരിൽ നിന്നും അനുഭവിക്കേണ്ടതായി വരില്ല.

9. ഈ ഗവേഷണത്തിൽ പങ്കുചേരുന്നില്ലെങ്കിൽ വേറെ എന്ത് ചെയ്യാൻ പറ്റും?

ഈ ഗവേഷണത്തിൽ പങ്കുചേരുകയല്ലാതെ മറ്റൊരു സഹായവും താങ്കൾക്ക് ചെയ്യുവാൻ സാധിക്കില്ല.

10. ഈ ഗവേഷണത്തിൽ എന്റെ അവകാശങ്ങളെക്കുറിച്ച് കൂടുതൽ അറിയണമെങ്കിൽ ഞാൻ ആരെ സമീപിക്കണം?

ഈ സമ്മതപത്രത്തിൽ ഒപ്പുവയ്ക്കുന്നതിനുമുമ്പ് താങ്കൾക്ക് സംശയങ്ങൾ ചോദിക്കാം. കൂടുതൽ സംശയങ്ങൾ ഉണ്ടെങ്കിൽ താഴെകാണുന്ന വിലാസത്തിൽ ബന്ധപ്പെടാം:

Shri. P. M. Vijayakumar,
(Rtd. District Judge, Thrissur)
(Chairman – Calicut University Human Ethical Committee),
Chairperson,
Kerala State Commission for Scheduled Caste & Scheduled Tribes,
Vellayambalam, Thiruvananthapuram - 3
Mob: 9447767520

Head of the Department & Member Secretary,
Calicut University Human Ethical Committee,
Department of Zoology,
University of Calicut,
Thenjipalam – 673635,
Malappuram, Kerala, India.
Phone No. 04942407420.

11. ഈ ഗവേഷണത്തെക്കുറിച്ച് എന്തെങ്കിലും ചോദ്യങ്ങളുണ്ടെങ്കിൽ ആരെ സമീപിക്കണം?

Dr. Y. Shibu Vardhanan,
Associate Professor,
Department of Zoology,
University of Calicut,
Malappuram, Kerala, India.
Mob:- 9447108980.

Mr. Siva Prasad M. S,
Ph.D Research Scholar,
Department of Zoology,
Universtiy of Calicut,
Malappuram, Kerala, India.
Mob:- 9895086515.

കൈയ്യൊപ്പുകൾ

ഈ ഗവേഷണത്തെക്കുറിച്ച് താങ്കൾക്ക് എന്തെങ്കിലും ചോദ്യങ്ങൾ ഉണ്ടെങ്കിൽ ദയവായി ചോദിക്കുക. കൈയ്യൊപ്പു തരുന്നതിന് മുമ്പ് ഈ ഗവേഷണത്തെപ്പറ്റി നന്നായി മനസ്സിലാക്കി എന്ന് ഉറപ്പ് വരുത്തുവാൻ അപേക്ഷിക്കുന്നു.

ഈ പരീക്ഷണത്തിൽ പങ്കെടുക്കുവാൻ പൂർണ്ണസമ്മതം തന്നുകൊണ്ടുള്ള കൈയ്യൊപ്പ് അല്ലെങ്കിൽ വിരലടയാളം.

ഒപ്പ്/ വിരലടയാളം	പേര്	തീയ്യതി

പങ്കെടുക്കുന്നവരിൽ നിന്ന് കൈയ്യൊപ്പ് വാങ്ങുന്ന വ്യക്തി പൂരിപ്പിക്കേണ്ടത്:

താഴെ കൈയ്യൊപ്പുവയ്ക്കുന്നതുകൊണ്ട് ഈ ഗവേഷണത്തെക്കുറിച്ച് പങ്കെടുക്കുവാൻ താൽപര്യമുള്ള വ്യക്തിക്ക് മുഴുവനായി വിശദീകരിച്ച് കൊടുത്തെന്നും ഈ സമ്മതപത്രം മുഴുവനായി വായിച്ചുകൊടുത്തെന്നും അദ്ദേഹത്തിന്റെ എല്ലാ ചോദ്യങ്ങൾക്കും മറുപടി കൊടുത്തെന്നും സമ്മതിക്കുന്നു.

സമ്മതപത്രം ഒപ്പിട്ടു വാങ്ങുന്ന വ്യക്തിയുടെ ഒപ്പ്	പേര്	തീയ്യതി
ഗവേഷണത്തിന്റെ മേൽനോട്ടം വഹിക്കുന്ന വ്യക്തിയുടെ ഒപ്പ്	പേര്	തീയ്യതി

Experiment Group
Sample No.

APPENDIX 6. 2

സ്ഥിരംകുറ്റവാളികളിലെമോണോ അമൈൻ ഓക്സിഡേസ്-എ (*MAOA*) എന്ന ജീനിന്റെ ഘടനാ വ്യതിയാനങ്ങളുംബാല്യകാലത്തെ പ്രതികൂലചുറ്റുപാടുകളും: ഒരു ഫോറൻസിക് സാധ്യതാപഠനം

പരീക്ഷണത്തിൽപങ്കെടുക്കുന്നതിനുള്ള സമ്മത പത്രം

പങ്കെടുക്കുന്ന വ്യക്തിയുടെ പേരുംവിലാസവും:

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1. ഈ ഗവേഷണംഎന്തിനെപ്പറ്റിയാണ്?

കേരളത്തിലെ കോഴിക്കോട് സർവ്വകലാശാലയിലെ സുവോളജി പഠനവിഭാഗത്തിൽ Ph.D പഠനത്തിന്റെ ഭാഗമായി ശ്രീ. ശിവപ്രസാദ്എം. എസ്. നടത്തുന്ന ഗവേഷണമാണിത്. സ്ഥിരംകുറ്റവാളികളുടെ ശാസ്ത്രീയമായ പുനരധിവാസത്തിന്റെ സാധ്യതകൾ കണ്ടെത്തുകയാണ് ഈ ഗവേഷണത്തിന്റെ പരമായലക്ഷ്യം. ഒരുവ്യക്തിയിൽ ബാല്യകാലത്തെ പ്രതികൂലചുറ്റുപാടുകൾസാഹചര്യങ്ങൾ, ശാരീരികവും മാനസികവും ലൈംഗികവുമായ പീഡനങ്ങൾ, മോണോ അമൈൻ ഓക്സിഡേസ്-എ (*MAOA*) എന്ന ജീനിന്റെ ഘടനാവ്യതിയാനങ്ങൾ എന്നിവ ആവ്യക്തിയിൽ സഹജമായ അക്രമവാസന ഉണ്ടാക്കുന്നതിൽ എന്തെങ്കിലും പങ്കുവഹിക്കുന്നുണ്ടോ എന്നറിയുന്നതിനുള്ള പഠനമാണിത്.

താങ്കൾ ചെയ്ത കുറ്റകൃത്യങ്ങളുടെ വിശദാംശങ്ങൾ, ശിക്ഷിക്കപ്പെട്ടതിന്റെ രേഖകൾ എന്നിവ പരിശോധിച്ചതിൽ നിന്നും താങ്കൾ കേരളാ പോലീസിന്റെ സ്ഥിരംകുറ്റവാളികളുടെ പട്ടികയിൽപ്പെടുന്നുവെന്ന് മനസ്സിലാക്കാൻ കഴിഞ്ഞു. ആയതിനാൽ ഈ ഗവേഷണത്തിൽ പങ്കെടുക്കാൻ താങ്കളോട്അഭ്യർത്ഥിക്കുന്നു. ഈ ഗവേഷണം മുഖാന്തിരം ലഭിക്കുന്ന അറിവ് സ്ഥിരം കുറ്റവാളികളുടെ ശാസ്ത്രീയമായ പുനരധിവാസത്തിനും നിലവിലുള്ള പുനരധിവാസ പദ്ധതികൾ മെച്ചപ്പെടുത്തുന്നതിനും സമൂഹത്തിന് സ്ഥിരം കുറ്റവാളികളോടുള്ള കാഴ്ച്ചപ്പാടുകൾ മാറ്റുന്നതിനും ഉപകരിച്ചേക്കും. ഈ ഗവേഷണത്തിന്റെ ഉത്തരവാദിത്വം ശ്രീ. ശിവപ്രസാദ് എം. എസിനായിരിക്കും. ഈ ഗവേഷണത്തിനു വേണ്ടി താങ്കളുടെ ബാല്യകാലത്തെ ജീവിത രീതി, ജീവിത സാഹചര്യങ്ങൾ, ദുരനുഭവങ്ങൾ ഉണ്ടെങ്കിൽ അതിന്റെ വിശദാംശങ്ങൾ, ശാരീരികമായോ മാനസികമായോ ലൈംഗികമായോ ഏറ്റ പീഡനങ്ങൾ ഉണ്ടെങ്കിൽ അതിന്റെ വിശദാംശങ്ങൾ, കുടുംബാംഗങ്ങ

ളുടെ വിവരങ്ങൾ മുതലായവ ആവശ്യമാണ്. കൂടാതെ താങ്കളുടെ രക്തത്തി ന്റേയോ വായിലെ കോശങ്ങളുടെയോ സാമ്പിളുകളും ആവശ്യമുണ്ട്.

2. ഈ ഗവേഷണത്തിന്റെ നടപടിക്രമം എങ്ങനെയാണ്?

ഈ ഗവേഷണത്തിൽതാങ്കളും പങ്കുചേരുവാൻ അനുമതിതരികയാണെ ങ്കിൽ:

i) അതാതു സർക്കാർ വകുപ്പുകളുടെ അനുമതി ലഭിച്ചതിനുശേഷം ശ്രീ. ശിവപ്ര സാദ്എം. എസ്. എന്ന വ്യക്തി താങ്കളുടെ അനുവാദത്തോടെ മുൻകൂട്ടി നിശ്ചയി ച്ചിട്ടുള്ള ചോദ്യാവലി അനുസരിച്ച് താങ്കളുമായി അഭിമുഖസംഭാഷണം നടത്തുന്ന തായിരിക്കും. ചോദ്യാവലിയിൽ താങ്കളുടെ ബാല്യകാലത്തെ ജീവിതരീതി, ജീവിതസാഹചര്യം, ദുരനുഭവങ്ങൾ, ശാരീരികവും മാനസികവും ലൈംഗികവു മായി ഏറ്റ പീഡനങ്ങൾ, കുടുംബാംഗങ്ങളുടെ വിവരങ്ങൾ എന്നിവയെപ്പറ്റിയുള്ള ചോദ്യങ്ങൾ ഉണ്ടായിരിക്കും. ചോദ്യാവലി ശ്രീ. ശിവപ്രസാദ്എം. എസിന്റെ സഹാ യത്തോടെ പൂരിപ്പിച്ച് തരേണ്ടതായിരിക്കും.

ii) താങ്കളുടെ അനുവാദത്തോടെ ചെറിയസൂചി (ലാൻസെറ്റ്) ഉപയോഗിച്ച് താങ്ക ളുടെ വിരൽത്തുമ്പിൽ നിന്നും രണ്ടോമൂന്നോ തുള്ളി രക്തമോ പഞ്ഞി ഉപയോ ഗിച്ച് വായിലെ കോശങ്ങളോ എടുക്കുന്നതായിരിക്കും. ഈ പ്രക്രിയകളെയെല്ലാം താങ്കളുടെ ആരോഗ്യത്തെ ഒരുതരത്തിലും ഹാനികരമായി ബാധിക്കാത്ത തര ത്തിൽഎല്ലാ മുൻകരുതലുകളും ശുചിത്വവും പാലിച്ചായിരിക്കും നടത്തുക.

3. ഈ ഗവേഷണത്തിൽതാങ്കളെവേദനിപ്പിക്കുന്ന എന്തെങ്കിലും സംഭവി ക്കുമോ?

ചോദ്യാവലിയിൽ താങ്കളുടെ ബാല്യകാലത്തെ ദുരനുഭവങ്ങൾ, അനുഭവി ക്കേണ്ടിവന്ന പീഡനങ്ങൾ എന്നിവയെപ്പറ്റിയുള്ള ചോദ്യങ്ങളുണ്ട്. അത് താങ്കൾക്ക് ചിലപ്പോൾ വേദനയുണർത്തുന്ന ഓർമ്മകൾ ഉണ്ടാക്കിയേക്കാം. താങ്കൾക്ക് മറു പടി തരാൻ താൽപര്യമില്ലാത്തതോ വേദനിപ്പിക്കുന്നതോ ആയ ചോദ്യങ്ങൾക്ക്മറു പടിതരേണ്ടതില്ല. വിരൽത്തുമ്പിൽ നിന്നുംരക്തമെടുക്കുമ്പോൾ ചെറിയ വേദന (രക്തത്തിലെ ഗ്ലൂക്കോസ്അളക്കാനുള്ള പരിശോധനക്ക് സമാനം) അനുഭവപ്പെടും. അഭിമുഖത്തിനിടക്ക് അൽപനേരം വിശ്രമം വേണമെങ്കിലോ അഭിമുഖം പിന്നീ ടൊരു സമയത്തേക്ക് മാറ്റിവെയ്ക്കാൻ താൽപ്പര്യപ്പെടാനോ താങ്കൾക്ക് എല്ലാ സ്വാതന്ത്ര്യവുമുണ്ട്.

4. ഈ ഗവേഷണം എപ്രകാരം സഹായിക്കും?

ഈ ഗവേഷണത്തിൽ പങ്കെടുക്കുന്നതുകൊണ്ട് താങ്കൾക്ക് വ്യക്തിപര മായ ലാഭങ്ങളൊന്നുമില്ല. പക്ഷേ, താങ്കളുടെ ബാല്യകാലത്തെ പ്രതികൂല ജീവിതസാഹചര്യങ്ങളും ജീവിതരീതിയും പീഡനാനുഭവങ്ങളും മോണോ അമൈൻ ഓക്സിഡേസ്-എ (*MAOA*) ജീനിന്റെ ഘടനാ വ്യതിയാനങ്ങളും ചേർന്ന അവസ്ഥയാണോ താങ്കളുടെ അക്രമവാസനയ്ക്ക് കാരണമെന്നതു മനസ്സി ലാക്കാൻ സാധിച്ചേക്കാം. താങ്കൾ തരുന്ന വിവരങ്ങൾ ഒരു പക്ഷേ വരും തലമു റയെ പ്രതികൂലസാഹചര്യങ്ങളിൽ നിന്നും പീഡനങ്ങളിൽ നിന്നും സംരക്ഷിക്കു വാൻ വേണ്ട പദ്ധതികൾ വിഭാവനം ചെയ്യാൻ അധികാരികളെ പ്രേരിപ്പിച്ചേക്കാം. മോണോ അമൈൻ ഓക്സിഡേസ്-എ (*MAOA*) ജീനിന്റെ ഘടനാവ്യതിയാനങ്ങൾ അക്രമവാസനയെ സാധൂകരിക്കുന്നു എന്ന് ഈ ഗവേഷണത്തിലൂടെ മനസ്സിലാ ക്കുവാൻ സാധിച്ചാൽ ഇത്തരം ജനിതക വ്യതിയാനങ്ങൾ ഉള്ളവരെ ശാസ്ത്രീയ

മായി പുനരധിവസിപ്പിക്കുന്നതിൽ ഉചിതമായ തീരുമാനങ്ങൾ എടുക്കുവാൻ വൈദ്യശാസ്ത്രത്തെയും ശിക്ഷാനിയമത്തെയും സഹായിച്ചേക്കാം.

5. താങ്കളുടെ സ്വകാര്യത എങ്ങനെ പരിരക്ഷിക്കപ്പെടും?

താങ്കളിൽ നിന്നുലഭിക്കുന്ന എല്ലാ വിവരങ്ങളും, ജനിതക വിവരങ്ങൾ ഉൾപ്പെടെ ഇന്ത്യാഗവൺമെന്റിന്റെ സ്വകാര്യത നിയമങ്ങൾക്കനുസരിച്ച് സ്വകാര്യ മായിവെക്കുന്നതായിരിക്കും. ഈ ഗവേഷണം നടത്തുന്ന ശ്രീ. ശിവപ്രസാദ് എം. എസ്, ഗവേഷണ പ്രവർത്തനങ്ങൾ മേൽനോട്ടംവഹിക്കുന്ന ഡോ. ഷിബു വർദ്ധ നൻ, അസോസിയേറ്റ് പ്രൊഫസർ, സുവോളജി പഠനവിഭാഗം, കാലിക്കറ്റ്സർവ്വക ലാശാല എന്നിവർക്കും, കാലിക്കറ്റ് സർവ്വകലാശാലയിൽ ഈ ഗവേഷണവുമായി ബന്ധമുള്ളമറ്റുള്ളവർക്കും താങ്കൾ നൽകുന്ന വിവരങ്ങൾ, താങ്കളുടെ ജനിതക വിവരങ്ങൾ എന്നിവപങ്കുവയ്ക്കുവാൻ ഈ സമ്മതപത്രപ്രകാരം താങ്കൾ അനു വാദംതരുന്നതായി അനുമാനിക്കും.

6. താങ്കളുടെവിവരങ്ങൾ ലഭ്യമാകാവുന്ന മറ്റുവ്യക്തികൾ:

1) കാലിക്കറ്റ സർവ്വകലാശാലയിലെ ഗവേഷണങ്ങളുടെ ധാർമ്മികത പരി ശോധിച്ച് ഗവേഷണങ്ങൾക്ക് അനുമതി നൽകുന്ന ഹ്യൂമൺ എത്തിക്കൽ സമിതിഅംഗങ്ങൾ.

ഈ സമ്മതപത്രം താങ്കൾ ഒപ്പ് വയ്ക്കുന്നതുകൊണ്ട് ഈ ഗവേഷണം മുഖാ ന്തിരം ശേഖരിച്ച താങ്കളുടെവിവരങ്ങൾ മേൽപറഞ്ഞ വ്യക്തികളുമായി പങ്കുവെ യ്ക്കാൻ അനുവാദം തന്നതായി അനുമാനിക്കും. താങ്കളിൽ നിന്ന് ഒരുതവണയേ അഭിമുഖം നടത്തിവിവരങ്ങൾ, രക്തം അല്ലെങ്കിൽ വായിലെ കോശങ്ങളുടെ സാമ്പിൾ എന്നിവ ശേഖരിക്കുകയുള്ളൂ. ഈ ഗവേഷണത്തിൽ താങ്കൾ പങ്കെടു ക്കുന്നത് തികച്ചും സ്വന്തം തീരുമാനപ്രകാരമായിരിക്കും. എന്നിരുന്നാലും, ഈ സമ്മതപത്രത്തിൽ ഒപ്പുവയ്ക്കാതെ ഈ ഗവേഷണത്തിന്റെ ഭാഗമാകുവാൻ സാധ്യ മല്ല.

7. ഈ ഗവേഷണത്തിൽ പങ്കെടുത്താൽ പ്രതിഫലംലഭിക്കുമോ?

ഈ ഗവേഷണത്തിൽ പങ്കെടുക്കുന്നതിന്റെ പ്രതിഫല മായിതാങ്കൾക്ക്യാതൊന്നുംലഭിക്കില്ല.

8. ഞാൻ ഈ ഗവേഷണത്തിൽ പങ്കെടുക്കേണ്ടതുണ്ടോ?

താങ്കൾക്ക് താത്പര്യമില്ലെങ്കിലോ, മറ്റ് എന്തെങ്കിലും ബുദ്ധിമുട്ടുകൾ ഉണ്ടെങ്കിലോ ഈ ഗവേഷണത്തിൽ പങ്കെടുക്കേണ്ടതില്ല. അത്തികച്ചും താങ്ക ളുടെതീരുമാനമാണ്. ഈ ഗവേഷണത്തിൽ പങ്കു ചേരാതിരിക്കുന്നതുകൊണ്ട് താങ്കൾക്ക് ഒരുവിധത്തിലുമുള്ള ബുദ്ധിമുട്ടുകൾ ആരിൽ നിന്നും അനുഭവിക്കേണ്ട തായിവരില്ല.

9. ഈ ഗവേഷണത്തിൽ പങ്കുചേരുന്നില്ലെങ്കിൽ വേറെ എന്ത് ചെയ്യാൻ പറ്റും?

ഈ ഗവേഷണത്തിൽ പങ്കുചേരുകയല്ലാതെ മറ്റൊരു സഹായവും താങ്കൾക്ക് ചെയ്യുവാൻ സാധിക്കില്ല.

10. ഈ ഗവേഷണത്തിൽ എന്റെ അവകാശങ്ങളെക്കുറിച്ച് കൂടുതൽ അറിയണമെങ്കിൽ ഞാൻ ആരെസമീപിക്കണം?

ഈ സമ്മതപത്രത്തിൽ ഒപ്പുവയ്ക്കുന്നതിനുമുമ്പ് താങ്കൾക്ക് സംശയങ്ങൾ ചോദിക്കാം. കൂടുതൽ സംശയങ്ങൾ ഉണ്ടെങ്കിൽ താഴെകാണുന്ന വിലാസത്തിൽ ബന്ധപ്പെടാം:

Shri. P. M.Vijayakumar,
(Rtd. District Judge, Thrissur),
(Chairman – Calicut University Human Ethical Committee),
Chairperson,
Kerala State Commission for Scheduled Caste & Scheduled Tribes, Vellayambalam, Thiruvananthapuram – 3, Kerala, India.
Mob: 9447767520

Head of the Department & Member Secretary,
Calicut University Human Ethical Committee,
Department of Zoology,
University of Calicut
Thenjipalam – 673635
Malappuram, Kerala, India.
Phone No. 04942407420.

11. ഈ ഗവേഷണത്തെക്കുറിച്ച് എന്തെങ്കിലും ചോദ്യങ്ങളുണ്ടെങ്കിൽ ആരെ സമീപിക്കണം?

Dr. Y. ShibuVardhanan,
Associate Professor,
Department of Zoology,
University of Calicut, Thenhipalam - 673635,
Malappuram, Kerala, India.
Mob:- 9447108980.

Mr. Siva Prasad M. S,
Ph.D Research Scholar,
Department of Zoology,
Universtiy of Calicut, Thenhipalam - 673635,
Malappuram, Kerala, India.
Mob:- 9895086515.

കൈയ്യൊപ്പുകൾ:

ഈ ഗവേഷണത്തെക്കുറിച്ച് താങ്കൾക്ക് എന്തെങ്കിലും ചോദ്യങ്ങൾ ഉണ്ടെങ്കിൽ ദയവായി ചോദിക്കുക.കൈയ്യൊപ്പുതരുന്നതിന് മുമ്പ് ഈ ഗവേഷണത്തെപ്പറ്റി നന്നായി മനസ്സിലാക്കിഎന്ന്ഉറപ്പ്വരുത്തുവാൻ അപേക്ഷിക്കുന്നു.

ഈ പരീക്ഷണത്തിൽ പങ്കെടുക്കുവാൻ പൂർണ്ണസമ്മതം തന്നുകൊണ്ടുള്ളകൈയ്യൊപ്പ്അല്ലെങ്കിൽവിരലടയാളം.

_____	_____	_____
ഒപ്പ്/വിരലടയാളം	പേര്	തീയ്യതി

പങ്കെടുക്കുന്നവരിൽ നിന്ന്കൈയ്യൊപ്പ് വാങ്ങുന്ന വ്യക്തി പൂരിപ്പിക്കേണ്ടത്:
താഴെകൈയ്യൊപ്പുവയ്ക്കുന്നതുകൊണ്ട് ഈ ഗവേഷണത്തെക്കുറിച്ച് പങ്കെടുക്കുവാൻ താൽപര്യമുള്ളവ്യക്തിക്ക്മുഴുവനായിവിശദീകരിച്ച്കൊടുത്തെന്നും ഈ സമ്മതപത്രംമുഴുവനായിവായിച്ചുകൊടുത്തെന്നും അദ്ദേഹത്തിന്റെഎല്ലാചോദ്യങ്ങളൾക്കുംമറുപടികൊടുത്തെന്നുംസമ്മതിക്കുന്നു.

_____	_____	_____
സമ്മതപത്രംഒപ്പിട്ടു വാങ്ങുന്ന വ്യക്തിയുടെ ഒപ്പ്	പേര്	തീയ്യതി

_____	_____	_____
ഗവേഷണത്തിന്റെ മേൽനോട്ടംവഹിക്കുന്ന വ്യക്തിയുടെ ഒപ്പ്	പേര്	തീയ്യതി

APPENDIX 6. 3

Government of Kerala

URGENT

No. 71726/B1/2014/Home

Home [B] Department
Thiruvananthapuram,
Dated: 27.11.2014.

From

The Additional Chief Secretary to Government

To

The Registrar,
University of Calicut,
Calicut University (P.O.),
Malappuram, Pin – 673 635.

Sir,

Sub:- Home Department - Prisons – Request for permission to visit all prisons for sample collection - reg.

Ref:- 1) Your letter No. 84866/ZOO-ASST-1/2014/Admin. Dated 29.7.2014.
2) Representation dated 20.9.2014 from Sri.M.S.Sivaprasad.

I am to invite your attention to the letter cited and to inform you that sample collection from Prisoners is not allowed. I am also to inform that Sri.Sivaprasad can contact released prisoners to conduct the study. However he is permitted to interview prisoners for the study if he desires. Necessary direction in this regard have already been given to Director General of Police (Prisons).

Yours faithfully,
P.VIJAYACHANDRAN,
Deputy Secretary,
For Additional Chief Secretary to Government.

Approved for issue,

Section Officer

Prisons Headquarters,
Thiruvananthapuram

Endt. No.G2-18954/2014/Ldis. Dated: 01/12/2014

Copy of the letter is communicated to the Superintendent of all Jails for further action.

Sd/-
For DIRECTOR GENERAL OF PRISONS & CORRECTIONAL SERVICES

To

The Superintendent of All Jails.

Copy to : - Sri. Siva Prasad M.S.
Ph.D Research Scholar
Department of Zoology
University of Calicut
Malappuram District
Pin: 673635

File.

Approved for Issue,

Superintendent.

R.V.02/12/2014

Government of Kerala

No.71726/B1/2014/Home

25098

Home (B) Department
Thiruvananthapuram,
Dated,25.11.2014.

From

The Additional Chief Secretary to Government

To

The Director General of Police (Prisons),
Thiruvananthapuram.

Sir,

Sub: Home Department - Prisons – Request for permission to visit and collect data from Prisons - Reg

Ref: 1. Representation dated 20.9.2014 from Mr.Sivaprasad.M.S.
2.Letter no:84866/ZOO-Asst/2014/Admin dated:29.07.2014 from Registrar University of Calicut.
3. Your letter No. G2 18954/14 dated 20.9.2014 and 15.10.14.

I am to invite your attention to the reference cited and to inform you that the consent of someone in custody is no consent at all. Therefore prisoners cannot be allowed to be used for study purpose. The permission applicable to Sri:Sivaprasad is limited to interview only and is subject to the following ~~certain limits.~~ conditions

1) The data collected from the prisoners shall be kept confidential and not used for any publication.
2) If interview schedule is used for collection of data, a copy of the same will be submitted to the Superintendent of Jail for approval before the collections of data.
3) Interview of Prisoners shall be conducted in the presence of Jail officials only.
4) Prisoners shall not be interviewed with out their consent and their convict number and name shall not be recorded.
5) A Copy of the research report shall be submitted to the office of the Director General of Police (Prisons), for reference.

Yours Faithfully,
P.VIJAYACHANDRAN,
Deputy Secretary
for Additional Chief Secretary to Government.

Approved for issue

Section Officer

File No.HOME-B1/14/2017-HOME

GOVERNMENT OF KERALA

No:B1/14/2017-HOME

Home(B)Department
Thiruvananthapuram,
Dated:20/11/2017

The Additional Chief Secretary to Government

Sri. Sivaprasad M.S,
Research Scholar (Forensic Science)
Zoology Department, University of Calicut,
Malappuram-673 635

Sir,

Sub: Home Department-Prisons- Request for granting permission to conduct research study in Prisons -reg;

Ref: 1) your request dated 20.4.2017
2) Letter No. G2-18954/14/PrHQ dated 8.6.2017 of Director General of Prisons and Correctional Services

I am to invite your attention to the reference cited and to inform you that your request for granting permission to conduct research study in prisons is rejected as it adversely affects the normal functioning of the prisons and human rights of the inmates.

Yours Faithfully,
SUBHASH.R
UNDER SECRETARY
For Secretary to Government.

Approved for Issue,

Section Officer.

APPENDIX 7

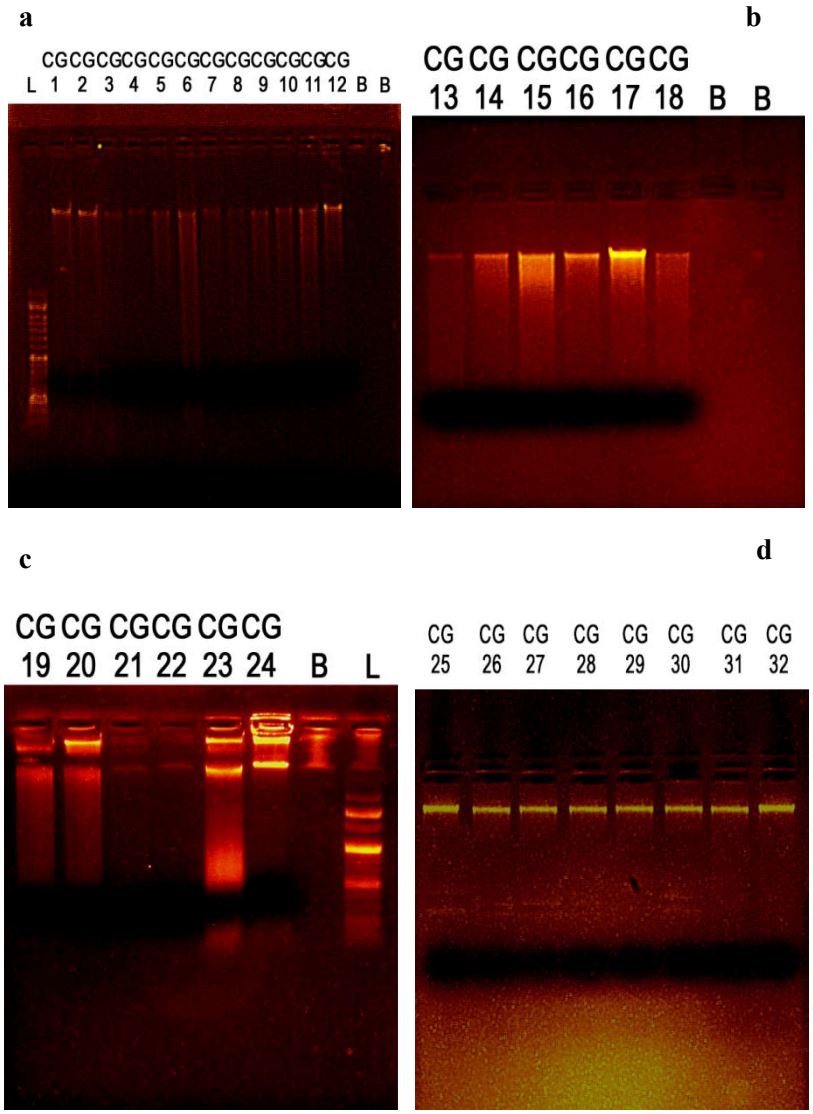


Figure (a), (b), (c), (d): Agarose gel (0.9 %) of genomic DNA extracted from buccal swabs of 32 participants. CG = Controls, L = DNA ladder, B = Blank.

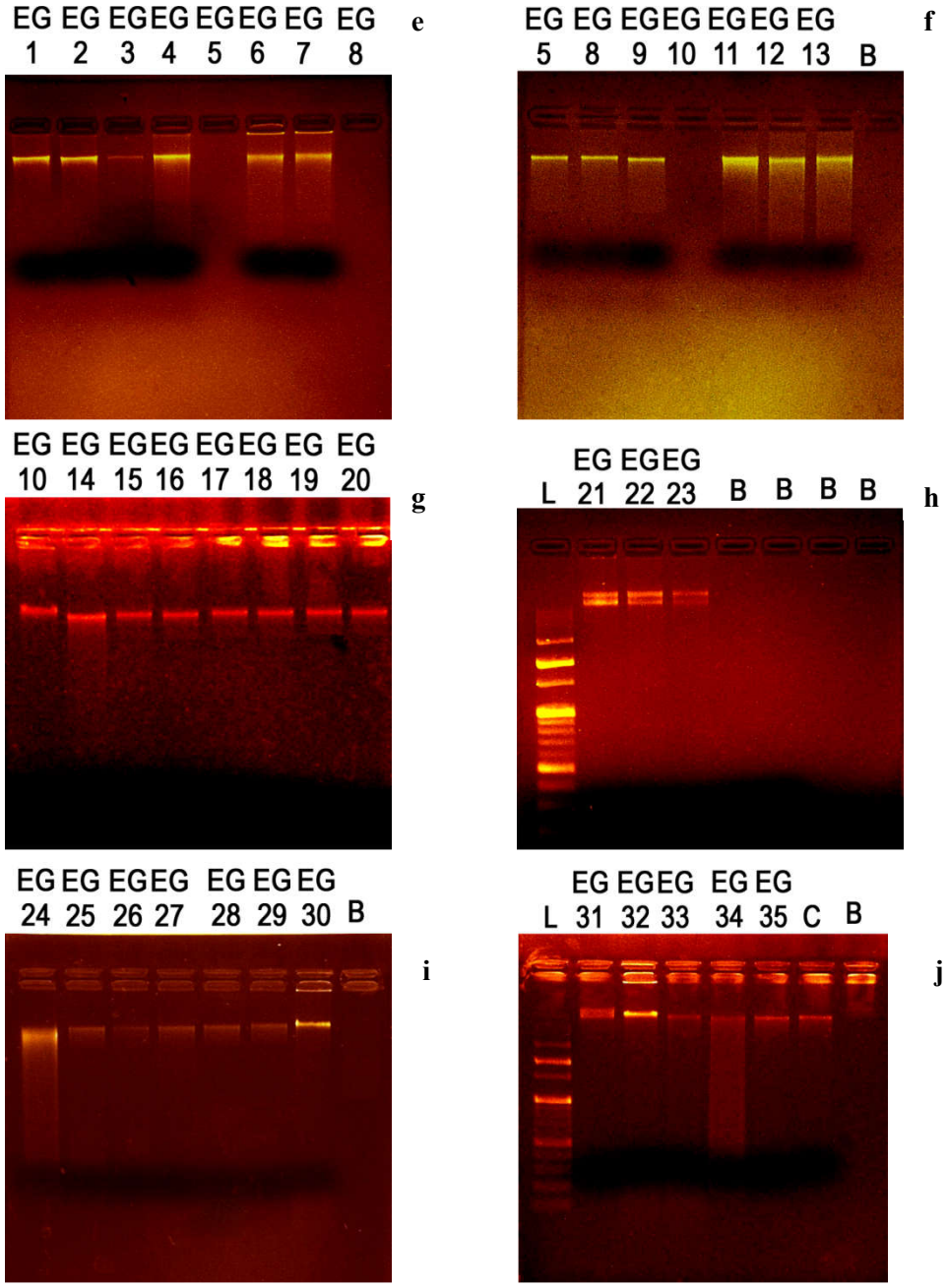


Figure (e), (f), (g), (h): Agarose gel (0.9 %) of genomic DNA extracted from buccal swabs of 35 participants. EG = Cases, L = DNA ladder, B = Blank, C = Positive control.

APPENDIX 8

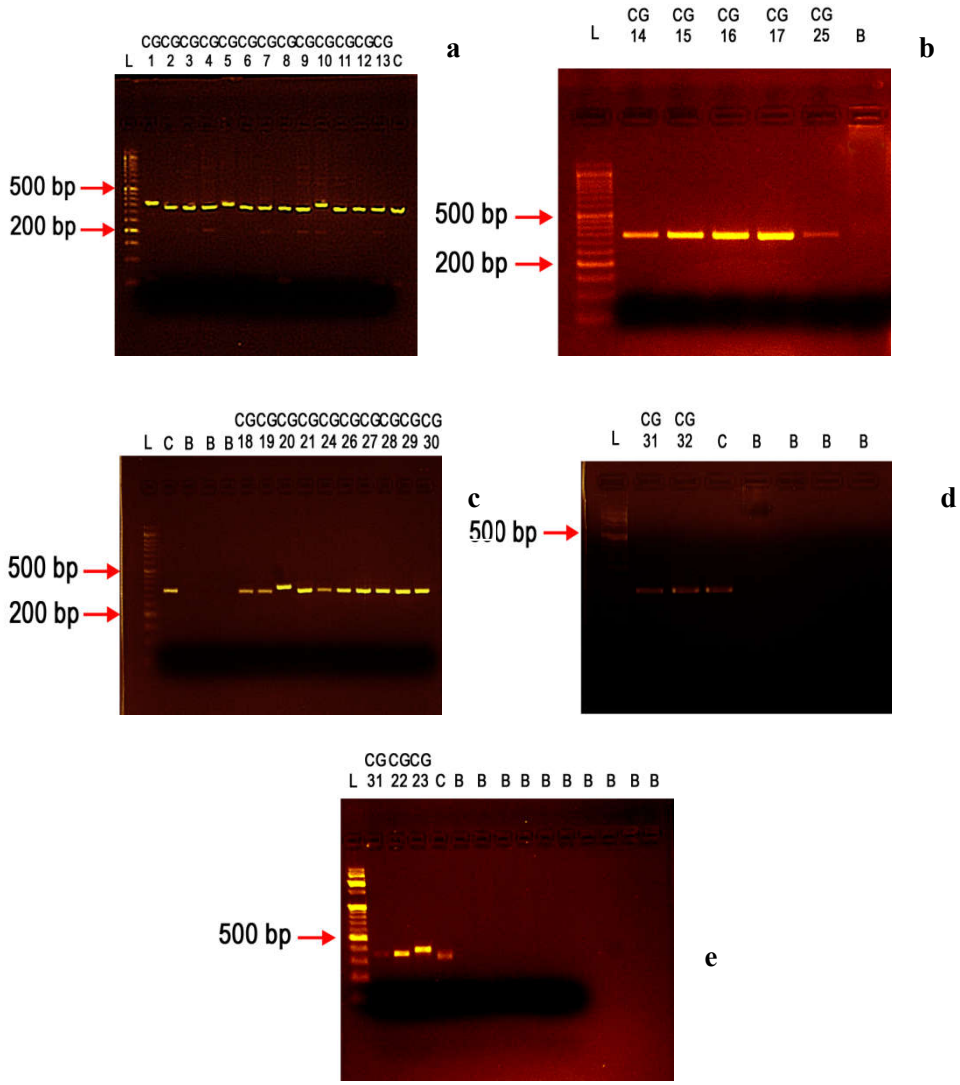


Figure (a), (b), (c), (d), (e): Agarose gel (2 %) of PCR products from 32 participants. CG = Controls, L = DNA ladder, B = Blank, C = Positive control.

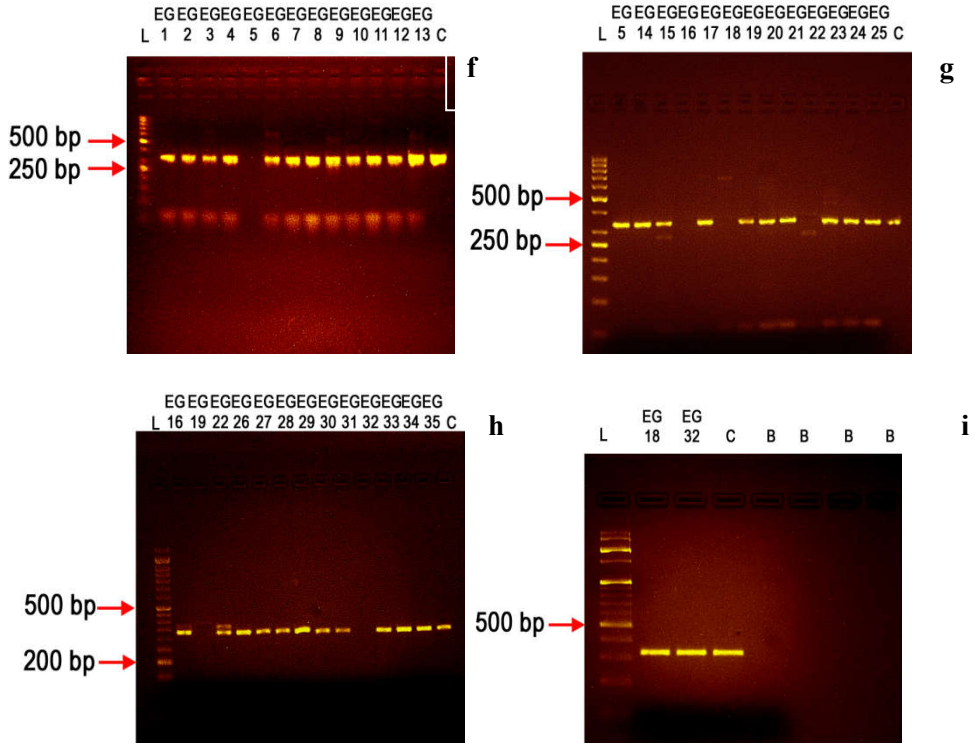


Figure (f), (g), (h), (i): Agarose gel (2 %) of PCR products from 35 participants. CG = Controls, L = DNA ladder, B = Blank, C = Positive control.

APPENDIX 9

```
Tandem Repeats Finder Program written by:

                Gary Benson
      Program in Bioinformatics
          Boston University

Version 4.09

Sequence: CG2

Parameters: 2 7 7 80 10 50 500

Pmatch=0.80,Pindel=0.10
tuple sizes 0,4,5,7
tuple distances 0, 29, 159, 200

Length: 242
ACGTcount: A:0.23, C:0.44, G:0.27, T:0.06


Found at i:85 original size:6 final size:6

Alignment explanation


    Indices: 76--185  Score: 85
    Period size: 6  Copynumber: 18.3  Consensus size: 6

         66 CCTTCCCCGG

                                  * *    *      * *
         76 CGGCAC CGGCAC CGGCAC CAGTAC CCGCAC CAGTAC CGGCAC CGGCAC
          1 CGGCAC CGGCAC CGGCAC CGGCAC CGGCAC CGGCAC CGGCAC CGGCAC

             * *    *      * *                  * *    *      * *
        124 CAGTAC CCGCAC CAGTAC CGGCAC CGGCAC CAGTAC CCGCAC CAGTAC
          1 CGGCAC CGGCAC CGGCAC CGGCAC CGGCAC CGGCAC CGGCAC CGGCAC


        172 CGGCAC CGGCAC CG
          1 CGGCAC CGGCAC CG

        186 AGCGCAAGGC


Statistics
Matches: 80,  Mismatches: 24, Indels: 0
        0.77            0.23        0.00

Matches are distributed among these distances:
   6   80  1.00

ACGTcount: A:0.22, C:0.47, G:0.25, T:0.05


Consensus pattern (6 bp):
CGGCAC

Found at i:114 original size:30 final size:30

Alignment explanation


    Indices: 80--184  Score: 210
```

http://tandem.bu.edu/trf/output/12zdwSniHEL3Q.s1.2.7.7.80.10.50.500.1.txt.html#80--184,30,3.5,30.3

Cont...

```
    Period size: 30  Copynumber: 3.5  Consensus size: 30

         70 CCCCGGCGGC


         80 ACCGGCACCGGCACCAGTACCCGCACCAGT
          1 ACCGGCACCGGCACCAGTACCCGCACCAGT


        110 ACCGGCACCGGCACCAGTACCCGCACCAGT
          1 ACCGGCACCGGCACCAGTACCCGCACCAGT


        140 ACCGGCACCGGCACCAGTACCCGCACCAGT
          1 ACCGGCACCGGCACCAGTACCCGCACCAGT


        170 ACCGGCACCGGCACC
          1 ACCGGCACCGGCACC

        185 GAGCGCAAGG


Statistics
Matches: 75,  Mismatches: 0, Indels: 0
        1.00            0.00        0.00

Matches are distributed among these distances:
  30   75  1.00

ACGTcount: A:0.23, C:0.48, G:0.24, T:0.06


Consensus pattern (30 bp):
ACCGGCACCGGCACCAGTACCCGCACCAGT
Done.
```

```
Tandem Repeats Finder Program written by:

                Gary Benson
      Program in Bioinformatics
          Boston University

Version 4.09

Sequence: CG1

Parameters: 2 7 7 80 10 50 500

Pmatch=0.80,Pindel=0.10
tuple sizes 0,4,5,7
tuple distances 0, 29, 159, 200

Length: 297
ACGTcount: A:0.23, C:0.44, G:0.27, T:0.07


Found at i:93 original size:12 final size:12

Alignment explanation


    Indices: 76--215  Score: 100
    Period size: 12  Copynumber: 11.7  Consensus size: 12

         66 CCTTCCCCGG


         76 CGGCACCGGCAC
          1 CGGCACCGGCAC

                  * *
         88 CGGCACCAGTAC
          1 CGGCACCGGCAC

             *     * *
        100 CCGCACCAGTAC
          1 CGGCACCGGCAC


        112 CGGCACCGGCAC
          1 CGGCACCGGCAC

             * *   *
        124 CAGTACCCGCAC
          1 CGGCACCGGCAC

             * *
        136 CAGTACCGGCAC
          1 CGGCACCGGCAC

                  * *
        148 CGGCACCAGTAC
          1 CGGCACCGGCAC

             *     * *
        160 CCGCACCAGTAC
          1 CGGCACCGGCAC


        172 CGGCACCGGCAC
```

http://tandem.bu.edu/trf/output/29A5WHmys6OOQ.s1.2.7.7.80.10.50.500.1.txt.html#80--214,30,4.5,30,2

Cont...

```
  1 CGGCACCGGCAC

     * *   *
184 CAGTACCCGCAC
  1 CGGCACCGGCAC

     * *
196 CAGTACCGGCAC
  1 CGGCACCGGCAC


208 CGGCACCG
  1 CGGCACCG

216 AGCGCAAGGC


Statistics
Matches: 104,  Mismatches: 24, Indels: 0
       0.81            0.19       0.00

Matches are distributed among these distances:
  12  104  1.00

ACGTcount: A:0.22, C:0.47, G:0.25, T:0.06


Consensus pattern (12 bp):
CGGCACCGGCAC

Found at i:114 original size:30 final size:30

Alignment explanation


    Indices: 80--214  Score: 270
    Period size: 30  Copynumber: 4.5  Consensus size: 30

         70 CCCCGGCGGC


         80 ACCGGCACCGGCACCAGTACCCGCACCAGT
          1 ACCGGCACCGGCACCAGTACCCGCACCAGT


        110 ACCGGCACCGGCACCAGTACCCGCACCAGT
          1 ACCGGCACCGGCACCAGTACCCGCACCAGT


        140 ACCGGCACCGGCACCAGTACCCGCACCAGT
          1 ACCGGCACCGGCACCAGTACCCGCACCAGT


        170 ACCGGCACCGGCACCAGTACCCGCACCAGT
          1 ACCGGCACCGGCACCAGTACCCGCACCAGT


        200 ACCGGCACCGGCACC
          1 ACCGGCACCGGCACC

        215 GAGCGCAAGG


Statistics
Matches: 105,  Mismatches: 0, Indels: 0
```

http://tandem.bu.edu/trf/output/29A5WHmys6OOQ.s1.2.7.7.80.10.50.500.1.txt.html#80--214,30,4.5,30,2

Cont...

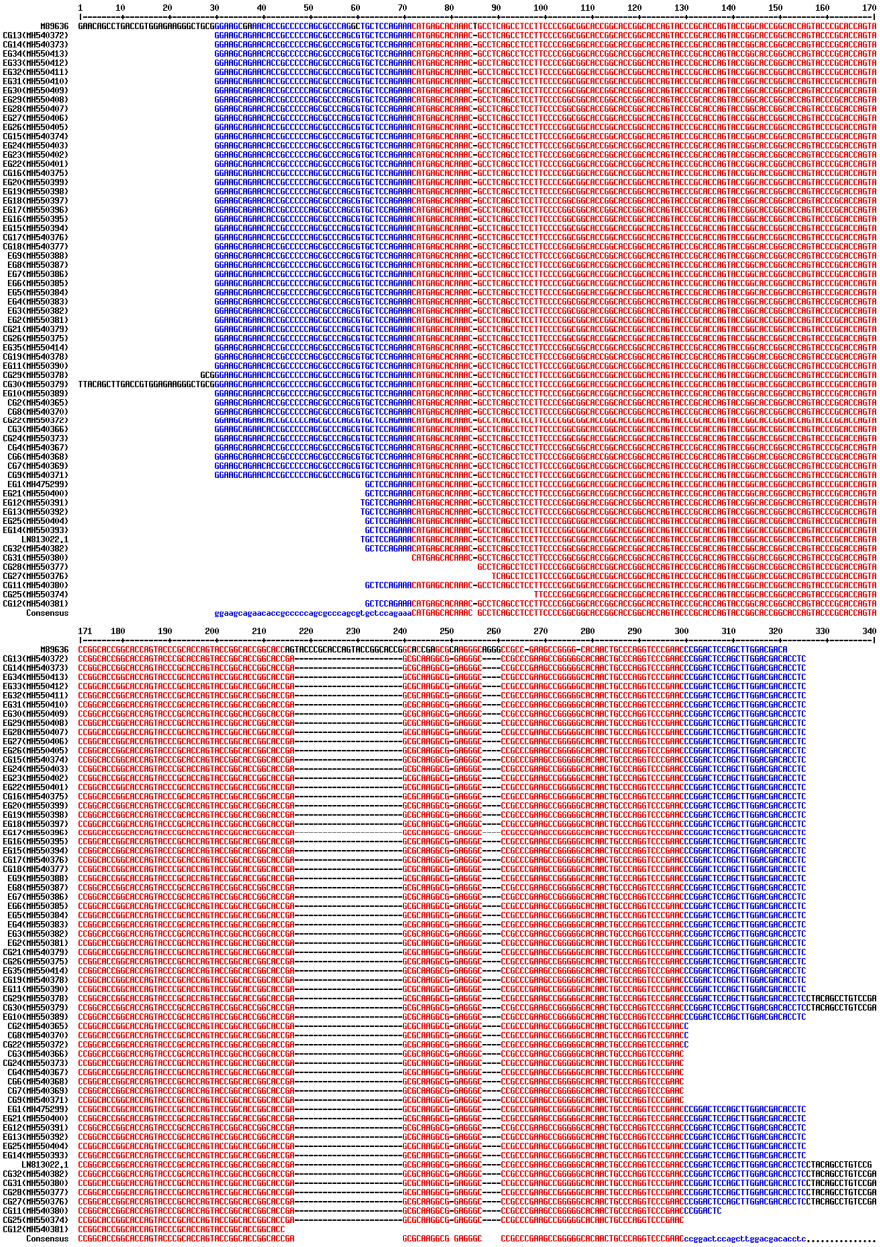
```
        1.00            0.00        0.00

Matches are distributed among these distances:
  30  105  1.00

ACGTcount: A:0.23, C:0.47, G:0.24, T:0.06


Consensus pattern (30 bp):
ACCGGCACCGGCACCAGTACCCGCACCAGT

Done.
```

APPENDIX 10

Sample code*	GenBank ® Accession number	Sample code*	GenBank ® Accession number
CG1	MH540360	EG3	MH550382
CG2	MH540365	EG4	MH550383
CG3	MH540366	EG5	MH550384
CG4	MH540367	EG6	MH550385
CG5	MH540361	EG7	MH550386
CG6	MH540368	EG8	MH550387
CG7	MH540369	EG9	MH550388
CG8	MH540370	EG10	MH550389
CG9	MH540371	EG11	MH550390
CG10	MH540362	EG12	MH550391
CG11	MH540380	EG13	MH550392
CG12	MH540381	EG14	MH550393
CG13	MH540372	EG15	MH550394
CG14	MH540373	EG16	MH550395
CG15	MH540374	EG17	MH550396
CG16	MH540375	EG18	MH550397
CG17	MH540376	EG19	MH550398
CG18	MH540377	EG20	MH550399
CG19	MH540378	EG21	MH550400
CG20	MH540363	EG22	MH550401
CG21	MH540379	EG23	MH550402
CG22	MH550372	EG24	MH550403
CG23	MH540364	EG25	MH550404
CG24	MH550373	EG26	MH550405
CG25	MH550374	EG27	MH550406
CG26	MH550375	EG28	MH550407
CG27	MH550376	EG29	MH550408
CG28	MH550377	EG30	MH550409
CG29	MH550378	EG31	MH550410
CG30	MH550379	EG32	MH550411
CG31	MH550380	EG33	MH550412
CG32	MH540382	EG34	MH550413
EG1	MH475299	EG35	MH550414
EG2	MH550381		

*Sample code, CG= Controls; EG= Cases;
CG1, CG5, CG10, CG20, CG23 belongs to 4.5 repeat allele;
Rest all sample belongs to 3.5 repeat allele.

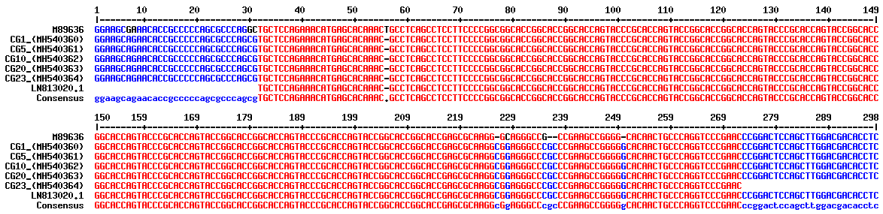
APPENDIX 11



Multiple alignment of 3.5R allele of MAOA-uVNTR (MH550379) of 62 samples of present study and reference sequences available with GenBank; M89636.1, Author: Zhu *et al*., 1992; LN813022.1, Author: Erasmus *et al*., 2015. Alignment was done by using MultAlin software (Corpet, 1988).

Effect of adverse childhood experiences and occurrence of uVNTR polymorphism in monoamine oxidase A gene in recidivist violent offenders: Forensic implications- Siva Prasad. M. S.

APPENDIX 12



Multiple alignment of 4.5R allele of *MAOA*-uVNTR of 5 samples of present study and reference sequences available with GenBank; M89636.1, Author: Zhu *et al*., 1992; LN813020.1, Author: Erasmus *et al*., 2015. Alignment was done by using MultAlin software (Corpet, 1988).

APPENDIX 13
Details of crimes committed by cases

Case	Murder	Attempt to murder	Hurt	Assault	Wrongful restrain	Total offences against human body	Other crimes	Total crimes committed
1.	5	7	4	2	6	24	6	30
2.	4	5	5	3	2	19	9	28
3.	0	3	3	1	6	13	7	20
4.	1	4	4	2	4	15	9	24
5.	1	2	2	2	4	11	6	17
6.	3	4	4	1	7	19	8	27
7.	0	4	2	1	5	12	7	19
8.	0	2	2	0	3	7	6	13
9.	2	4	2	2	8	18	9	27
10.	1	2	3	1	4	11	8	19
11.	0	3	4	1	3	11	7	18
12.	1	2	3	0	4	10	6	16
13.	3	4	3	1	6	17	9	26
14.	0	3	2	2	3	10	5	15
15.	4	5	4	2	6	21	8	29
16.	1	3	3	1	4	12	6	18
17.	0	3	3	2	4	12	8	20
18.	2	4	4	2	8	20	5	25
19.	1	3	5	0	2	11	6	17
20.	0	2	2	1	2	7	6	13
21.	0	4	3	1	4	12	9	21
22.	0	3	2	0	2	7	4	11
23.	1	5	3	3	7	19	8	27
24.	1	2	2	1	2	8	3	11
25.	0	2	1	1	2	6	4	10
26.	2	5	2	2	5	16	8	24
27.	0	1	1	0	3	5	5	10
28.	2	4	3	2	3	14	11	25
29.	0	3	1	3	5	12	8	20
30.	3	5	5	3	7	23	5	28
31.	3	6	4	2	7	22	4	26
32.	1	2	3	1	2	9	3	12
33.	1	3	3	1	5	13	8	21
34.	0	2	3	2	4	11	6	17
35.	2	3	5	0	4	14	10	24