The Association between Polymorphism in the ABCB1 Gene and Opioid Use Disorder in an Egyptian Sample

A Thesis

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List of Abbreviations

Abb.	Full term
6-MAM	*6 monoacetylmorphine*
A.D	*Anno Domini*
ABC	*Human ATP binding cassette genes*
AC	*Anterior commissure*
ACA	*Anterior cingulate*
ADH	*Alcohol dehydrogenase*
ALDH	*Aldehyde dehydrogenase*
AMG	*Amygdala*
APA	*American psychiatric association*
ARC	*Arcuate nucleus*
BBB	*Blood Brain Barrier*
BNDF	*Brain derived neurotrophic factor*
BNST	*Bed nucleus of the stria terminalis*
CD	*Caudate Nucleus*
Cer	*Cerebellum*
CP	*Caudate putamen*
DA	*Dopamine*
DMT	*Dorsomedial thalamus*
DNA	*Deoxyribonucleic acid*
DSM-5	*Diagnostic and Statistical Manual of Mental Disorder 5th Edition*
ECDD	*The WHO Expert Committee on Drug Dependence*
ENT	*Entorhinal cortex*
FC	*Frontal cortex*
GABA	*γ-aminobutyric acid*
GHB	*γ-hydroxy butyrate*
GP	*Globus pallidus*
GPCRs	*G protein–coupled receptors*

List of Abbreviations (Cont...)

Abb.	Full term

GWAS ..... *Genome Wide Association Studies*
Hippo ..... *Hippocampus*
HIV ..... *Human Immuno-deficiency virus*
ICD- 10 ..... *International Classification of Diseases, 10th Revision*
IF ..... *Inferior colliculus*
ITG ..... *Inferior temporal gyrus*
IV ..... *Intravenous*
LC ..... *Locus coeruleus*
LH ..... *Lateral hypothalamus*
LSD ..... *Lysergic acid diethylamide*
M3G *Morphine-3-glucuronide*
MAOIs ..... *Monoamine oxidase inhibitors*
MDMA ..... *Methylenedioxymetamphetamine*
MDR1 ..... *Multidrug Resistance gene 1*
MGL ..... *Methionine gamma-lyase*
mPFC ..... *Medial prefrontal cortex*
NA ..... *Nucleus accumbens*
NIDA ..... *National Institute on Drug Abuse*
ODT ..... *O-desmethyl metabolite of tramadol*
OFC ..... *Orbitofrontal cortex*
OPRD1 ..... *Delta-opioid receptor*
OPRK1 ..... *kappa-opioid receptor*
OPRL1 ..... *Nociceptin/orphanin FQ receptor*
OPRM1 ..... *Mu-opioid receptor*
OT ..... *Olfactory tract*
PAG ..... *Periaqueductal gray*
PENK ..... *Proenkephalin*

List of Abbreviations (Cont...)

Abb.	Full term
P-gp	*P Glycoproteins*
POMC	*Pro-Opiomelanocortin*
POR	*Proove Opioid Risk*
Pos	*Prescription opioids*
RPn	*Reticular pontine nucleus*
RR	*Respiratory rate*
SAMHSA	*Substance Abuse and Mental Health Services Administration*
SC	*Superior colliculus*
SNP	*Single nucleotide polymorphism*
SNr	*Substantia nigra pars reticulata*
SNRIs	*Selective serotonin / norepinephrine reuptake inhibitors*
SSRIs	*Selective serotonin reuptake inhibitors*
STG	*Superior temporal gyrus*
STR	*Short tandem repeat*
Thal	*Thalamus*
VP	*Ventral pallidum*
VS	*Ventral striatum*
VTA	*Ventral tegmental area*

INTRODUCTION

Addiction is a chronic, relapsing disease that changes the brain's reward circuit and hence leads to compulsive drug seeking and other behavioral changes. On the long term, there are biological effects of repeated drug exposure, which cause adverse effects throughout the body. Despite these catastrophic consequences, prevalence rate remains high. A study in 2016, done by the national addiction research program in Egypt revealed that about one fifth (19.1%) of the studied sample was regularly using an addicted substance regardless tobacco smoking. As regards the used substances, opioids were the 3rd common substance of use in Egypt after Cannabis and alcohol respectively, except in Upper Egypt where the opioids were commoner than alcohol ***(Hamdi et al., 2016).***

Opioids are a category of drugs which promote rewarding and anti-nociception effects by acting at opioid receptors in the central and peripheral nervous systems ***(Henderson et al., 2016; Fornasari, 2012).*** Their actions are mediated through binding to opioids receptors Mu(μ), Kappa(κ) and delta (δ) ***(Narita et al., 2001).*** They are found throughout the brain and are varied in concentration according to their classification, however they all are much distributed in the ventral tegmental area, amygdala, nucleus accumbens, caudate and putamen nuclei ***(Hancock et al., 2018; Mansour et al., 1987).*** Endogenous opioid peptides, including enkephalins, β-

endorphins, endomorphins and dynorphins, are mediated by their binding to opioid receptors, modulating mood and regulating stress Responses ***(Contet et al., 2004).***

Tramadol which is a synthetic opioid, is a centrally acting analgesic with a multimode of action. It acts on serotonergic and noradrenergic nociception, while its metabolite O-desmethyltramadol acts on the μ-opioid receptor. Its analgesic potency is claimed to be about one tenth that of morphine. Tramadol is used to treat both acute and chronic pain of moderate to moderately severe intensity ***(Grond & Sablotzki, 2004).***

Abuse of tramadol is increasing in some African and West Asian nations. Abuse of tramadol is grown in Egypt, Gaza, Jordan, Lebanon, Libya, Mauritius, Saudi Arabia and Togo. Because of expanding rate of abuse, Egypt has up-scheduled tramadol in 2009 ***(36th ECDD, 2014).***

According to an Egyptian study of opioid dependence carried out on 700 male university students, they found that out of 100 students who were opioid users, 88 students used tramadol and 12 used heroin ***(Mahgoub et al., 2016).*** Also an Egyptian study carried out on 204 school students, among them, 18 (8.8%) were using tramadol as shown by urine screen ***(Bassiony et al., 2015).***

Dopamine, which is the primary neurotransmitter responsible for eliciting feelings of euphoria and pleasure, is the main component of the mechanism of dependence. It works in conjunction with the opioid peptides and receptors to stimulate the dopaminergic pathway that is required for dopamine transmission ***(Kalivas, 2009).*** The release of GABA, an inhibitory neurotransmitter also identified in this pathway, is decreased when opioid agonists bind to presynaptic mu-opioid receptors of (GABA)-ergic interneurons ***(Contet et al., 2009).*** The inhibition of GABA-ergic neurons via activation of the mu-opioid receptor allows dopaminergic neurons to release more dopamine into the reward pathway, creating a positive reinforcement of pleasurable feelings ***(Kalivas, 2009).***

Approximately 40-60% of the vulnerability to addiction of opioids is attributed to genetic factors ***(Farah et al., 2017; Kreek et al., 2012; Bikash et al., 2016)***. The mu-opioid receptor (MOPr) is the primary site of action of several of the endogenous opioid peptides. This receptor is also the major target for clinically important opioid, as well as a major site of action for tramadol ***(Nurnberger and Berrettini, 2012; Henderson et al., 2016; Zadina et al., 1999).*** The opioid receptor μ 1 (OPRM1) gene, located on chromosome 6 ***(Kapur et al., 2007),*** is responsible for encoding the μ opioid receptor, which has been implicated in respiration, gastrointestinal motility, physical dependence, euphoria, and analgesia. It was shown to be involved in substance addiction, including

alcoholism and opioid addiction ***(Miller, 2013; Bond et al., 1998).***

Genome wide association studies (GWAS) have identified several single nucleotide polymorphisms (SNP) that are associated with opioids dependence ***(Hancock et al., 2018).*** There were two SNPs of high allelic frequency, each of which alters an amino acid in the N-terminus of the receptor. The 17T (rs1799972) variant results in an amino acid change of alanine to valine at position 6 (A6V) (overall allelic frequency 6.6%), and the A118G, variant, with allelic frequency range around 2% in African-Americans to over 40% in Japanese ***(Kreek et al., 2012)***. The most studied polymorphism of OPRM1 is the functional 118A>G (rs1799971) which is present in 5-30% of the general population with some racial variation ***(Tan et al., 2003)*** encoding for a substitution of aspartic acid for an asparagine Asn40Asp that results in the removal of a potential N-glycosylation site from the N terminal extracellular domain of the receptor ***(Bond et al., 1998).***

The ABCB1 gene (formerly called *MDR1*) is located on chromosome 7. it is highly polymorphic and codes for the production of an ATP-dependent drug efflux pump for xenobiotic compounds with broad substrate specificity. It is responsible for decreased drug accumulation in multidrug resistant cells ***(Salagacka, 2011)***. One of the most extensively studied of the *ABCB1* polymorphisms is the silent single nucleotide polymorphism (SNP) C3435T (rs1045642), located

in the middle of exon 26, the consequence of which is the alteration of the nucleotide, from cytosine to thymine. It is important to emphasize that the T allele of this polymorphism occurs with a high prevalence in the Caucasian population (about 50%) and its frequency is influenced by ethnicity ***(Jelen et al., 2015).*** An Austrian study published in 2013 had indicated a potential contribution of *ABCB1* SNPs to the development of opioid addiction in the European population ***(Beer et al., 2013).***

Our study will evaluate the possible role of ABCB1 gene polymorphisms and its association with opioid (heroin & tramadol) use disorder as there are no studies in the literature cover this field of research.