Transgenerational epigenetics in substance abuse: exploring the inheritable DNA methylation underlying the agressive behaviour and altered stress response.

Specifically on:

Mechanisms underlying aggression and anxiety up to three generations due to transgenerational paternal heroin addiction

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DECEMBER 2016

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There are growing evidences that epigenetics are the critical molecular processes that regulate gene expression and determine health risks and complications that are passed from one generation to the next contributing to phenotypic diversity. This is of particular interest in drug addiction as behavioral (e.g. aggressive behaviors, suicidal attempts and stress responsitivity) and structural changes have been associated with persistent alterations in gene expression and are seen in unexposed offsprings. These epigenetic changes are hypothesized to be imprinted in DNA and inheritable (**Transgeneration Epigenetics**). The involvement of transgenerational epigenetic inheritance in substance abuse especially heroin is however not well studied but could lead to significant advances in our understanding of vulnerability to heroin use disorders and help resolve the challenges in treatment. In this study, we will explore transgenerational epigenetic phenotypes (aggressive, suicidal and differential stress responsitivity) that are passed on to the next generations and the number of generations affected using animal model. A total of 156 rats will be used in this study. First batch of male rats (n=6) will be chronically exposed to heroin to produce addictive behavior. The addictive behaviour will be monitored using animal behavioural and aggression test. Metabolomics analysis will be performed to monitor the metabolomics changes as a consequence of change in epigenetics. The rats will be mated with heroin-naïve female rats before being sacrificed. Brain tissues will be obtained to investigate the DNA methylation profile using array which target globally the genes affected by methylation as a result of heroin addiction. Offsprings up to 6 generations will be produced and the epigenetic, metabolomics and behavioural changes will be studied and compared. By understanding the underlying epigenetic mechanisms in addictive behaviour, new therapeutics such as epigenetic modifier can be designed in conjunction with behavioral therapy which may aid in reversing this process. This is in line with the government vision and mission towards building a high income and healthy society.

4.2 Enhanced Executive Summary

There are growing evidences that epigenetics are the critical molecular processes that regulate gene expression and determine health risks and complications that are passed from one generation to the next contributing to phenotypic diversity not explained by the genomics variation. In this study, we explored the transgenerational phenotypes (aggressive, suicidal and differential stress responsitivity) that are passed on to the next generations from the paternal rather than maternal and the number of generations which were affected. Male rats (n=6) were chronically exposed to heroin to produce addictive behavior. The addictive behaviour was monitored using animal behavioural and aggression test. Metabolomics analysis was performed to profile the metabolomics changes as a consequence of change in epigenetics. The rats were mated with heroin-naïve female rats before being sacrificed. We observed significant differences in non social activities, social activities and aggresion behaviors up to 3 generations of offsprings. Our results indicated that heroin addicted male rats were more anxious in both the OF box and EPM tasks which are in accordance with other studies using female addicted rats. Current findings demonstrate significant transgenerational effects of male rats addicted with heroin. Interestingly, two biomarkers of epigenetics which are the thymidine and deoxycytidine were found during the heroin introduction phase and heroin withdrawal phase. These two biomarkers denote an increase DNA methylation patterns in the paternal and their offsprings. We derived that the DNA methylation changes due to the paternal heroin exposure were paased down to two generations of the offsprings causing the behavioral traits which are anxiety and aggresiveness to be observed in the offsprings. Significant elevated levels of neurosteroids which are pregnolone sulfate and allopregnolone were also observed in the serum metabolites analysis of the offsprings of the heroin addicted male rats. Neurosteroids are steroids synthesized within the brain and modulate neuronal excitability by rapid non-genomic actions. Leukotrine A4 was increased in the offspring of the heroin addicted rats, which indicates that neuroinflammation might be accouring. However, Prostaglandins and leukotrine was not found to be significantly different in the F2 and F3 generations. This is the first time study integrating trangenerational behaviroal changes due to paternal addictions and the metabolomics changes is reported. By understanding the underlying mechanisms in addictive behaviour, new therapeutics such as epigenetic modifier can be designed in conjunction with behavioral therapy which may aid in reversing this process. This is in line with the government vision and mission towards building a high income and healthy society.

4.3 Introduction

Addiction is defined as a chronic, relapsing disease that is characterized by (1) compulsive behaviour to seek and take drug, (2) loss of control due to limited intake (3) emergence of negative emotional states (e.g., dysphoria, anxiety, irritability) when access to the drug is prevented (Koob and Le Moal, 1997). Addictive behaviours include a complex variety of symptoms, including loss of control over its use, compulsive use, continued use despite negative consequences, and drug cravings.

Of the 20.5 million Americans 12 or older that had a substance use disorder in 2015, 2 million had a substance use disorder involving prescription pain relievers and 591,000 had a substance use disorder involving heroin (Center for Behavioral Health Statistics and Quality, 2016). It is estimated that 23% of individuals who use heroin develop opioid addiction (National Institute on Drug Abuse, 2014; Centers for Disease Control and Prevention, 2016). It is also a chronic relapsing disorder characterized by cycles of escalating drug exposure, intermittent episodes of withdrawal with or without maintained abstinence, and acute or chronic relapse to drug use. Heroin and morphine are more widely used than any other illicit opioids with 13,852 users in Malaysia alone. The profile of drug users detected in 2015 recorded that 97.97% of the addicts are male (Statistics of Drug Users in Malaysia, 2015).

Psychiatric comorbidity is commonly found among individuals with addictive disorders. The addicted patients often suffer from anxiety disorders. Hodgson and colleagues (2016) has confirmed the shared genetic underpinnings of addiction and anxiety. Genomic loci that involved in the etiology of the comorbid disorders were found to be heritable. However, a phenotypical study on this inheritance was not yet studied. The association between heroin use and crime has been widely documented. Researchers have consistently found that a large proportion of the heroin-dependent population regularly engage in criminal activity (Inciardi and Chambers, 1972; Voss and Stephens, 1973). Kokkevi et al. (1993), for example, reported that 79% of a small community of heroin-dependent individuals had been arrested and 60% had been convicted for a criminal offence. Recurrent cycles of heroin use and abstinence are thought to cause neurobiological changes in brain regions associated with reward, motivation, stress, learning and executive function (Jentsch and Taylor 1999; Koob and Le Moal 2008; Kreek et al. 2009a,b; Le Merrer et al. 2009; Winstanley et al. 2010). Such changes are thought to persist across extended drug-free periods to alter an individual's response to drug re-exposure and contribute to subsequent escalation of drug use (i.e., relapse-like behaviour) (Dalley et al. 2005).