

# **CNS Immune Signalling And Drug Addiction: The Role Of Interleukin-1 beta**

A thesis submitted for the degree of

**Doctor of Philosophy**

by

**Liang Liu**

Discipline of Pharmacology  
School of Medical Sciences,  
Faculty of Health Sciences  
The University of Adelaide

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*of* ADELAIDE

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# *Abstract*

Opioid and alcohol dependencies are significant public health problems worldwide. The causes of drug dependence are complex, comprising both genetic and environmental factors. Recent evidence from animal models has shown that opioid-/alcohol-induced proinflammation within the central nervous system (CNS) plays a contributing role in the development of dependence. Proinflammatory cytokines, such as interleukin-1 beta (IL-1 $\beta$ ), may be involved in the development of opioid/alcohol dependence. Thus, genetic polymorphisms in the proinflammatory cytokine genes that alter their expression and/or function may alter the risk of dependence in humans. Previously, genetic polymorphisms in two genes, *IL-1B* and *IL-1RN*, which encode for IL-1 $\beta$  and IL-1 receptor antagonist (IL-1Ra), respectively, were shown to be associated with altered risk of alcohol dependence. However, this has yet to be examined in an opioid dependent population. Therefore, the first major aim of this thesis was to examine the possible association between genetic variability of *IL-1B* and *IL-1RN* in opioid dependent and healthy control populations. In order to confirm the previous study within the Australian context, this thesis also examined the variability of the *IL-1B* and *IL-1RN* genetic polymorphisms in an Australian alcohol dependent population. Genomic DNA from opioid dependent subjects (n = 60), alcohol dependent subjects (n = 99), and healthy non-dependent subjects (n = 60) were genotyped for the *IL-1B* -31T>C, -511C>T, and 3954C>T single nucleotide polymorphisms (SNPs) using PCR-RFLP assays, and *IL-1RN* VNTR polymorphism using a PCR assay. Significant linkage disequilibrium was observed between *IL-1B* -31 and -511 SNPs. *IL-1B* -511C and -31T alleles were more frequent in both

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the opioid and alcohol dependent patients compared to the control group: odds ratio (OR, 95% confidence interval) = 1.91 (1.14 to 3.20),  $P = 0.014$  and 1.89 (1.19 to 2.99),  $P = 0.007$ , respectively, for *IL-1B* -511C>T; and OR = 1.74 (1.02 to 2.97),  $P = 0.044$  and 1.80 (1.13 to 2.87),  $P = 0.017$ , respectively, for *IL-1B* -31T>C. In contrast, no association was observed between: the *IL-1B* 3954C>T SNP and opioid dependence; and the *IL-1RN* VNTR polymorphism and opioid or alcohol dependence. This study has shown at least two susceptibility loci, -31T and -511C, in the *IL-1B* gene for opioid dependence, and have also confirmed their involvement in alcohol dependence.

With the findings of the significant association between *IL-1B* SNPs and opioid dependence, one important question raised is how drug-induced brain IL-1 $\beta$  expression affects the actions of these drugs, and thereby contributes to dependence. In an attempt to answer this question, I designed my second main study using different strains of mice. The primary aim of this study was to investigate whether the differences in opioid-induced brain IL-1 $\beta$  expression contribute to the differences in opioid tolerance and withdrawal behaviours. In addition, opioid-induced CNS glial activation is reported to occur via non-classical opioid mechanisms, engaging the toll-like receptor 4 (TLR4) and a series of downstream signalling pathways including MyD88-dependent pathways. However, the behavioural consequence of TLR4 and MyD88-dependent signalling pathways in opioid tolerance and withdrawal has yet to be examined. Therefore, this study also aimed to examine the role of TLR4 and MyD88-dependent pathways in opioid actions. Chronic morphine-induced tolerance and dependence were assessed in 3 inbred wild-type mouse strains (Balb/c, CBA, and C57BL/6) and 2 knockout strains (TLR4 and MyD88). Analysis of brain nuclei

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(medial prefrontal cortex, cortex, brain stem, hippocampus, and midbrain and diencephalon regions combined) revealed that, of inbred wild-type mice, there were significant main effects of morphine treatment on IL-1 $\beta$  expression in the brain regions analysed ( $P < 0.02$  for all regions analysed). A significant increase in hippocampal IL-1 $\beta$  expression was found in C57BL/6 mice after morphine treatment, whilst, a significant decrease was found in the medial prefrontal cortex region of wild-type Balb/c mice. Furthermore, the results of wild-type inbred strains demonstrated that the elevated hippocampal IL-1 $\beta$  expression was associated with withdrawal jumping behavior. Interestingly, knockout of TLR4, but not MyD88 protected against the development of analgesic tolerance. All these data support the involvement of opioid-induced brain IL-1 $\beta$  expression in dependence development.

Together, this thesis provides the first human evidence for the contribution of IL-1 $\beta$  release in opioid dependence, and animal data supporting the involvement of CNS immune signalling in the development of opioid dependence, and confirmed the *IL-1B* genetic findings in alcohol dependence.

# *Declaration*

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Liang Liu and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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**Liu L**, Hutchinson MR, White JM, Somogyi AA, Collier JK. Association of *IL-1B* genetic polymorphisms with an increased risk of opioid and alcohol dependence. (2009) *Pharmacogenetics and Genomics* 19:869–876

**Liu L**, Collier JK, Watkins LR, Somogyi AA, Hutchinson MR. Naloxone-precipitated morphine withdrawal behavior and brain IL-1 $\beta$  expression: Comparison of different mouse strains. (2011) *Brain Behavior and Immunity* Epub Apr 3

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Mr Liu had a major input in the experimental design, performed genetic analysis on all samples, statistical analysis and graphical presentation of the data collected, and prepared the manuscript of submission.

Signed \_\_\_\_\_ Date \_\_\_\_\_

Dr Hutchinson was involved in the experimental design, contributed to the data interpretation and preparation of the manuscript.

Signed \_\_\_\_\_ Date \_\_\_\_\_

Prof. White was involved in the experimental design, contributed to the data interpretation and preparation of the manuscript.

Signed \_\_\_\_\_ Date \_\_\_\_\_

Prof. Somogyi was involved in the experimental design, contributed to the data interpretation and preparation of the manuscript.

Signed \_\_\_\_\_ Date \_\_\_\_\_

Dr Collier was involved in the experimental design, assisted with statistical analyses, contributed to the data interpretation and preparation of the manuscript.

Signed \_\_\_\_\_ Date \_\_\_\_\_

## **Statement of Authorship**

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**Liu L**, Hutchinson MR, Somogyi AA, Collier JK Lack of association between *IL-1RN* VNTR polymorphism and risk of opioid dependence and alcohol dependence. *Text in manuscript*.

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Signed \_\_\_\_\_ Date \_\_\_\_\_

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Signed \_\_\_\_\_ Date \_\_\_\_\_

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Signed \_\_\_\_\_ Date \_\_\_\_\_

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Signed \_\_\_\_\_ Date \_\_\_\_\_

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Mr Liu had a major input in the experimental design, conducted all experimental procedures, statistical analysis and graphical presentation of the data collected, and prepared the manuscript of submission.

Signed \_\_\_\_\_ Date \_\_\_\_\_

Dr Coller was involved in the experimental design, contributed to the data interpretation and preparation of the manuscript.

Signed \_\_\_\_\_ Date \_\_\_\_\_

Prof. Watkins was involved in the experimental design, contributed to the data interpretation and preparation of the manuscript.

Signed \_\_\_\_\_ Date 31/05/11

Prof. Somogyi was involved in the experimental design, assisted with statistical analysis, contributed to the data interpretation and preparation of the manuscript.

Signed \_\_\_\_\_ Date \_\_\_\_\_

Dr Hutchinson was involved in the experimental design, assisted with behavioural testing, tissue collection and statistical analysis, contributed to the data interpretation and preparation of the manuscript.

Signed \_\_\_\_\_ Date \_\_\_\_\_

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## ***Statement of Authorship***

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# ***Abbreviations***

5-HT	Serotonin
AD <sub>50</sub>	Half-maximal morphine analgesic dose
ADH	Alcohol dehydrogenase
ALDH	Aldehyde dehydrogenase
ATP	Adenosine triphosphate
AV411	Ibudilast
bp	Base pair
CGRP	Calcitonin gene-related peptide
CI	Confidence interval
CNS	Central nervous system
COMT	Catechol-O-methyl transferase
CPP	Conditioned place preference
CSF	Cerebrospinal fluid
CYPs	Cytochrome p450 family enzymes
DA	Dopamine
DBH	Dopamine $\beta$ hydroxylase
DNA	Deoxyribonucleic acid
DSM-IV	Diagnostic and statistical manual of mental disorders, fourth edition
EMSA	Electrophoretic mobility shift assay
ERK	Extracellular signal-regulated kinase

## **Abbreviations**

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GABA	$\gamma$ -aminobutyric acid
GABA <sub>A</sub>	GABA receptor type A
GABA <sub>B</sub>	GABA receptor type B
GFAP	Glial fibrillary acidic protein
GWAS	Genome-wide association studies
IFN	Interferon
IL-1	Interleukin-1
IL-6	Interleukin-6
IL-10	Interleukin-10
IL-1 $\beta$	Interleukin-1 beta
<i>IL-1B</i>	Interleukin-1 beta gene
IL-1R	IL-1 receptor
IL-1Ra	IL-1 receptor antagonist
<i>IL-1RN</i>	IL-1 receptor antagonist gene
iNOS	Inducible nitric-oxide synthase
IRF3	Interferon-regulatory factor
IRAK	IL-1R-associated kinase
LPS	Lipopolysaccharide
M-3-G	Morphine-3-glucuronide
M-6-G	Morphine-6-glucuronide
MAP	Mitogen-activated protein
MAPK	p38 mitogen-activated protein kinase
% MPE	Maximum possible analgesic effect
MyD88	Myeloid differentiation primary-response protein 88
NF- $\kappa$ B	Nuclear factor- $\kappa$ B

## **Abbreviations**

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NMDA	N-methyl-D-aspartate glutamate receptor
OR	Odds ratio
mRNA	Messenger Ribonucleic acid
SEM	The standard error of the mean
siRNA	Small interfering RNA
SNPs	Single nucleotide polymorphisms
sTNFR	Soluble TNF receptor
TRIF	Toll/IL-1 receptor-domain-containing adapter-inducing interferon- $\beta$
TLR2	Toll-like receptor 2
TLR4	Toll-like receptor 4
TLR4 KO	TLR4 knockout
TNF- $\alpha$	Tumour necrosis factor alpha
UGTs	Uridine Diphosphate Glucuronosyltransferases
VNTRs	Variable number of tandem repeats