PHARMACOGENOMICS OF *ABCB1* IN MAINTENANCE PHARMACOTHERAPIES FOR OPIOID DEPENDENCE

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Table of contents

Abstract ____ *xix*

Declaration ____ *xxi*

Acknowledgements ____ *xxii*

Publications in support of this thesis ____ *xxiv*

Abbreviations ____ *xxv*

Chapter 1. Introduction ____ *1*

1.1. Opioid pharmacology ____ **2**

1.1.1. Mechanisms of action ____ 2

1.1.1.1. Opioid analgesia ____ 3

1.1.1.2. Opioid reward ____ 3

1.1.1.3. Side effects ____ 4

1.1.2. Endogenous opioids ____ 5

1.1.3. Therapeutic use of opioids ____ 5

1.1.4. Illicit opioid use ____ 5

1.1.5. Summary ____ 6

1.2. Opioid dependence ____ **6**

1.2.1. The path to opioid addiction ____ 6

1.2.1.1. Opioid tolerance ____ 7

1.2.1.2. Physical dependence and withdrawal ____ 8

1.2.1.3. Psychological dependence, craving and relapse ____ 9

1.2.1.4. Summary ____ 10

1.2.2. Burden of opioid dependence ____ 10

1.2.2.1. Prevalence and demographics of opioid dependence ____ 10

1.2.2.2. Costs of opioid dependence ____ 11

1.2.2.2.1. Morbidity and mortality ____ 11

1.2.2.2.2. Economic costs ____ 11

1.2.3. Summary ____ 12

1.3. Opioid substitution therapy ____ **12**

1.3.1. Treatment goals and outcomes ____ 12

1.3.2. Methadone maintenance treatment ____ 13

1.3.2.1. Methadone pharmacology ____ 13

1.3.2.2. General treatment protocol ____ 15

1.3.2.3. Treatment effectiveness 15
1.3.3. Buprenorphine maintenance treatment 16
1.3.3.1. Buprenorphine pharmacology 16
1.3.3.2. General treatment protocol 16
1.3.3.3. Treatment effectiveness 17
1.3.4. Barriers to effective opioid substitution therapy 17

1.4. Variability in opioid response 20
1.4.1. Variability in response to heroin 20
1.4.2. Variability in response to methadone 23
1.4.2.1. Variability in methadone pharmacokinetics 23
1.4.2.2. Variability in methadone plasma PK/PD relationship 26
1.4.2.3. Genetic variability influencing methadone response 27
1.4.3. Variability in response to buprenorphine 28
1.4.4. Summary 29

1.5. P-glycoprotein efflux transporter 30
1.5.1. P-glycoprotein structure 30
1.5.2. Expression and function 31
1.5.2.1. Intestinal absorption 33
1.5.2.2. Brain distribution 34
1.5.2.3. Elimination 35
1.5.2.4. Summary 36
1.5.3. P-glycoprotein transport of opioids 36
1.5.3.1. *In vitro* and animal studies 36
1.5.3.2. Human studies 39
1.5.3.2.1. Methadone intestinal absorption 39
1.5.3.2.2. Opioid brain distribution 39
1.5.3.2.3. Opioid elimination 42
1.5.3.3. Summary 42
1.5.4. Variability in P-glycoprotein expression and function 43
1.5.4.1. Expression 43
1.5.4.2. Function (drug-drug interactions) 44

1.6. *ABCB1* genetic variability 46
1.6.1. *ABCB1* gene structure 46
1.6.2. Single nucleotide polymorphisms 46
1.6.3. *ABCB1* haplotypes 47
1.6.4. Functional consequences of *ABCB1* genetic variability 48

1.6.4.1. *In vitro* expression and function 49
1.6.4.1.1. Haplotypes 51
1.6.4.2. *Ex vivo* expression and function 53
1.6.4.3. *In vivo* function and clinical significance 55
1.6.4.3.1. Function in healthy subjects 55
1.6.4.3.2. Functional effects on opioids 56

1.7. Summary, aims and hypotheses 59

Chapter 2. Determination of ABCB1 genotypes and haplotypes 62

2.1. Genotyping 62
2.1.1. Introduction 62
2.1.1.1. Polymerase chain reaction 63
2.1.1.2. Restriction fragment length polymorphism analysis 64
2.1.2. Methods 64
2.1.2.1. Materials 64
2.1.2.2. Genomic DNA isolation, purification and quantification 65
2.1.2.3. General genotyping protocols 65
2.1.2.3.1. Polymerase chain reaction setup 65
2.1.2.3.2. Restriction enzyme digest setup 66
2.1.2.3.3. Agarose gel electrophoresis 68
2.1.2.4. C1236T PCR-RFLP assay development 69
2.1.2.4.1. Polymerase chain reaction 69
2.1.2.4.2. Restriction enzyme digest 69
2.1.2.5. Assay quality control. 70
2.1.3. Results 71
2.1.3.1. A61G, G1199A, G2677T, C3435T 72
2.1.3.2. C1236T 72
2.1.4. Discussion 73
2.1.5. Conclusion 73

2.2. Estimation of haplotypes and linkage disequilibrium 74
2.2.1. Introduction 74
2.2.2. Methods 76
2.2.2.1. Haplotype estimations 76
2.2.2.1.1. Validation of haplotype estimations 77
2.2.2.2. Linkage disequilibrium 78
2.2.3. Results 79
2.2.3.1. Haplotype estimations 79

2.2.3.2. Linkage disequilibrium 81
2.2.4. Discussion 81
2.2.5. Conclusion 82
2.3. Summary 82
Chapter 3. ABCB1 pharmacogenetics in standard dose opioid substitution treatment 84
3.1. Introduction 84
3.2. Aims 85
3.3. Materials and methods 85
3.3.1. Subjects 85
3.3.2. *ABCB1* genotyping 86
3.3.3. Haplotype prediction and linkage disequilibrium 86
3.3.4. Subject data 87
3.3.4.1. Opioid withdrawal and adverse effects 88
3.3.4.2. Treatment outcome 89
3.3.5. Statistical methods 90
3.3.5.1. *ABCB1* genetic variability and opioid dependence 90
3.3.5.2. *ABCB1* genetic variability and opioid substitution treatment 91
3.4. Results 93
3.4.1. *ABCB1* genetic variability and opioid dependence 93
3.4.1.1. Subject demographics 93
3.4.1.2. *ABCB1* genotypes 94
3.4.1.3. *ABCB1* haplotypes 96
3.4.1.3.1. Validation check 96
3.4.1.3.1.1. Linkage disequilibrium 96
3.4.1.3.2. Haplotype frequencies 97
3.4.1.4. *ABCB1* genetic variability and pre-treatment heroin use 99
3.4.2. *ABCB1* genetic variability and opioid substitution treatment 100
3.4.2.1. Subject demographics 100
3.4.2.2. Methadone maintenance treatment 102
3.4.2.2.1. Dose requirements 102
3.4.2.2.1.1. Covariates 104
3.4.2.2.2. Trough plasma (R)-methadone concentrations 105
3.4.2.2.2.1. Covariates 108
3.4.2.2.3. Methadone pharmacokinetics 109
3.4.2.2.3.1. Covariates 109

3.4.2.2.4. Methadone maintenance treatment response 111
3.4.2.2.4.1. Successful versus poor treatment outcome 111
3.4.2.2.4.2. In-treatment withdrawal and opioid side-effects 112
3.4.2.2.5. Summary 113
3.4.2.3. Buprenorphine maintenance treatment 114
3.4.2.3.1. Dose requirements 114
3.4.2.3.1.1. Covariates 115
3.4.2.3.2. Trough plasma concentrations 116
3.4.2.3.3. Buprenorphine pharmacokinetics 116
3.4.2.3.4. Buprenorphine maintenance treatment response 119
3.4.2.3.5. Summary 119
3.5. Discussion 119
3.5.1. *ABCB1* genetic variability and opioid dependence 120
3.5.1.1. Validation of haplotype predictions 121
3.5.1.2. *ABCB1* haplotypes and opioid dependence 122
3.5.2. *ABCB1* genetic variability and opioid substitution treatment 123
3.5.2.1. Methadone maintenance treatment 124
3.5.2.1.1. Methadone requirements and pharmacokinetics 124
3.5.2.1.1.1. Covariates 127
3.5.2.1.2. Methadone maintenance treatment response 129
3.5.2.1.3. Comparisons with other literature 130
3.5.2.2. Buprenorphine maintenance treatment 135
3.5.2.2.1. Buprenorphine requirements and pharmacokinetics 135
3.5.2.2.2. Treatment outcome 136
3.5.2.2.3. Comparisons with other literature 136
3.5.3. Study limitations 137
3.6. Conclusions 138
Chapter 4. ABCB1 pharmacogenetics in high dose methadone maintenance treatment 140
4.1. Introduction 140
4.2. Materials and methods 141
4.2.1. Subjects 141
4.2.2. Demographics, methadone requirements and pharmacokinetic data 141
4.2.3. *ABCB1* genotyping and haplotyping 142
4.2.4. Data analysis 142
4.3. Results 144

4.3.1. Subject demographics 144
4.3.2. *ABCB1* genetic variability and opioid dependence 144
4.3.3. *ABCB1* genetic variability and methadone requirements 149
4.3.4. *ABCB1* genetic variability and methadone pharmacokinetics 151
4.3.5. Summary 154
4.3.6. *ABCB1* haplotype effects when ND and HD MMT subjects are combined 154

4.4. Discussion 155

Chapter 5. Ex vivo expression and function of P-glycoprotein 165

5.1. Introduction 165

5.2. Method development and validation 166
5.2.1. Introduction 166
5.2.2. Materials 167
5.2.3. Isolation of $CD4^+$, $CD56^+$ and $CD8^+$ lymphocytes 169
5.2.3.1. Methods 169
5.2.3.1.1. Isolation of peripheral blood mononuclear cells 169
5.2.3.1.2. Magnetic positive selection 170
5.2.3.1.2.1. Basic protocol 170
5.2.3.1.2.2. Validation of cell selection by flow cytometry 172
5.2.3.1.2.3. Optimised protocol 172
5.2.3.1.2.4. Processing of positive fractions 173
5.2.3.1.3. Qualitative detection of CD4, CD56 and CD8 expression 175
5.2.3.2. Results 176
5.2.3.2.1. Isolation of PBMCs 176
5.2.3.2.2. Magnetic positive selection 177
5.2.3.2.2.1. Basic protocol 177
5.2.3.2.2.2. Optimised protocol 178
5.2.3.2.3. Qualitative detection of CD4, CD56 and CD8 expression 178
5.2.3.3. Discussion 179
5.2.3.4. Conclusion 180
5.2.4. *ABCB1* mRNA expression by qRT-PCR 181
5.2.4.1. Methods 181
5.2.4.1.1. mRNA isolation 182
5.2.4.1.2. cDNA synthesis 182
5.2.4.1.2.1. Protocol development 182
5.2.4.1.2.2. DNase treatment and cDNA purification 184
5.2.4.1.2.3. Optimised protocol for cDNA synthesis 185

5.2.4.1.3. Quantitative real-time PCR 185
5.2.4.1.3.1. General protocol 186
5.2.4.1.3.2. Data analysis 187
5.2.4.1.4. Validation experiments 189
5.2.4.2. Results 191
5.2.4.3. Discussion 195
5.2.4.3.1. mRNA isolation and cDNA synthesis 195
5.2.4.3.2. Quantitative real-time PCR 195
5.2.4.4. Conclusion 196
5.2.5. P-gp protein expression by Western blot 196
5.2.5.1. Methods 198
5.2.5.1.1. Protein isolation and quantification 198
5.2.5.1.2. SDS-PAGE 200
5.2.5.1.2.1. Gel preparation 200
5.2.5.1.2.2. Sample preparation 200
5.2.5.1.2.3. Gel electrophoresis 200
5.2.5.1.3. Gel transfer 201
5.2.5.1.4. Western blot 201
5.2.5.1.4.1. Membrane treatment 202
5.2.5.1.4.2. Chemiluminescence imaging and data analysis 203
5.2.5.1.5. Dot blot optimization of antibody dilutions 203
5.2.5.1.5.1. P-glycoprotein dot blot 203
5.2.5.1.5.2. Calnexin dot blot 204
5.2.5.1.6. Additional Western blot trial experiments 204
5.2.5.2. Results 206
5.2.5.2.1. Dot blot optimization 206
5.2.5.2.2. Trial experiment 1: Further optimisation of calnexin detection 207
5.2.5.2.3. Trial experiment 2: Differences between lymphocyte populations 208
5.2.5.2.4. Trial experiment 3: Effects of lysate preparation 209
5.2.5.2.5. Trial experiment 4: Positive control for P-gp detection 211
5.2.5.3. Discussion 211
5.2.5.3.1. Sensitivity and specificity 212
5.2.5.3.2. Quantitative validity 213
5.2.5.4. Conclusion 213
5.2.6. P-gp function by rhodamine efflux assay 214
5.2.6.1. Methods 214
5.2.6.1.1. Lymphocyte preparation 215

5.2.6.1.2. Step 1: Substrate loading 216
5.2.6.1.3. Step 2: Quantifying substrate accumulation 217
5.2.6.1.4. Step 3: Substrate efflux 218
5.2.6.1.5. Step 4: Quantifying substrate efflux 218
5.2.6.1.6. Rhodamine quantification 218
5.2.6.1.6.1. Extracellular rhodamine quantification 219
5.2.6.1.6.2. Intracellular rhodamine quantification 221
5.2.6.1.6.2.1. Normalisation to protein content 222
5.2.6.1.7. Quantifying cell loss 223
5.2.6.1.8. Data analysis 223
5.2.6.2. Results 223
5.2.6.2.1. Substrate loading 223
5.2.6.2.2. Substrate efflux 224
5.2.6.2.3. Cell loss 225
5.2.6.3. Discussion 225
5.2.6.4. Conclusion 227
5.2.7. Summary 227

5.3. Pilot study 227

5.3.1. Introduction 227
5.3.2. Methods 228
5.3.2.1. qRT-PCR 230
5.3.2.2. Western blot 230
5.3.2.3. Functional assay 231
5.3.2.4. Data analysis 231
5.3.3. Results 231
5.3.3.1. Subject demographics & genetic variability 231
5.3.3.2. Lymphocyte isolation 232
5.3.3.3. *ABCB1* mRNA expression 233
5.3.3.4. P-glycoprotein protein expression 236
5.3.4. Discussion 238
5.3.4.1. Protocol performance in opioid-dependent subjects 238
5.3.4.2. Pilot study findings 240

5.4. Conclusions 241

Chapter 6. In vitro P-glycoprotein transport of opioids 242

6.1. Introduction 242

6.2. Transport assay development and validation 243

6.2.1. Introduction 243
6.2.2. Methods 246
6.2.2.1. Materials 246
6.2.2.2. Cell culture 247
6.2.2.3. Balimane and Chong (2005) method 247
6.2.2.4. „Classical" method 248
6.2.2.4.1. Original protocol 248
6.2.2.4.2. Optimised protocol 249
6.2.2.5. Quantification of radiolabelled [H^3]-digoxin 250
6.2.2.6. Quantification of FITC-inulin 250
6.2.2.7. Data analysis 251
6.2.3. Results 252
6.2.3.1. Cell culture 252
6.2.3.2. Balimane and Chong (2005) method 253
6.2.3.3. Classical method 253
6.2.4. Discussion 257

6.3. *In vitro* transport of opioids 258
6.3.1. Methods 258
6.3.1.1. (R)-methadone quantification 259
6.3.1.2. Buprenorphine quantification 259
6.3.2. Results 261
6.3.3. Discussion 263

6.4. Conclusion 266

Chapter 7. Discussion 267

7.1. New methods 267

7.2. *ABCB1* genetic variability as a determinant of substitution opioid requirements 268
7.2.1. Confounding factors 270

7.3. Secondary findings 271
7.3.1. *ABCB1* genetic variability and maintenance treatment response 271
7.3.2. Methadone requirements/exposure and *ex vivo* P-gp expression 272
7.3.3. *ABCB1* genetic variability and opioid dependence 272

7.4. Summary 273

7.5. Conclusion 274

Chapter 8. References *275*

Appendix A: Supplementary tables *296*

Additional references for Appendix A **311**

Appendix B: Supplementary figures *317*

Appendix C: Genomic locations, primer recognition sites and PCR product sequences for ABCB1 SNPs *319*

Appendix D: Publications in support of this thesis *321*

List of in-text tables

Table 1-1. Summary of opioid P-gp substrates. 37

Table 1-2. Clinically relevant drug-drug interactions due to P-glycoprotein inhibition. 45

Table 1-3. Common *ABCB1* single nucleotide polymorphisms found in Caucasians. 47

Table 2-1. Primer sequences and expected product size for polymerase chain reaction amplification. 67

Table 2-2. Optimal polymerase chain reaction conditions. 71

Table 2-3. Restriction digest enzymes for PCR-RFLP analysis. 71

Table 3-1. Criteria for treatment outcome classification. 89

Table 3-2. Pre-treatment alcohol, tobacco and illicit drug use demographics of opioid-dependent subjects. 94

Table 3-3. *ABCB1* SNP variant allele and genotype frequencies in control and opioid-dependent subjects. 95

Table 3-4. Linkage disequilibrium between pairs of *ABCB1* SNP variant loci. 97

Table 3-5. *ABCB1* haplotype frequencies in control and opioid-dependent subjects. 98

Table 3-6. *ABCB1* diplotype frequencies in control and opioid-dependent subjects. 99

Table 3-7. Demographics, drug use and treatment parameters of methadone and buprenorphine maintenance subjects included in the analysis of *ABCB1* genetic variability in opioid maintenance treatment. 100

Table 3-8. Relationships between daily methadone maintenance dose requirements and *ABCB1* genotypes. 102

Table 3-9. Relationships between daily methadone maintenance dose requirements and *ABCB1* haplotypes not displayed in Figure 3-1. 103

Table 3-10. Relationship between (R)-methadone C_{trough} requirements and *ABCB1* genotypes not displayed in Figure 3-3. 106

Table 3-11. Relationship between (R)-methadone C_{trough} requirements and *ABCB1* haplotypes not displayed in Figure 3-4. 107

Table 3-12. Summary table of results from Fisher‟s Exact Tests comparing the frequency of *ABCB1* variant alleles between successful and poor MMT outcome subjects. 111

Table 3-13. Comparison of *ABCB1* haplotype frequencies between male MMT subjects with successful or poor treatment outcome. 112

Table 3-14. Summary table of results from Fisher‟s Exact Tests comparing the frequency of *ABCB1* genotypes between subjects who did or did not experience withdrawal or opioid side-effects. 113

Table 3-15. Summary of major findings in MMT subjects. 113

Table 3-16. Associations between daily buprenorphine maintenance dose requirements and *ABCB1* genotypes. 114

Table 3-17. Associations between daily buprenorphine maintenance dose requirements and *ABCB1* variant haplotypes. 114

Table 3-18. Sex differences in the relationships between dose-adjusted trough plasma buprenorphine concentrations and *ABCB1* genotypes/haplotypes. 117

Table 3-19. Sex differences in the relationships between dose-adjusted trough plasma norbuprenorphine concentrations and *ABCB1* genotypes/haplotypes. 118

Table 3-20. Summary of major findings in MMT subjects. 119

Table 4-1. Demographics of high dose methadone maintenance subjects. 144

Table 4-2. *ABCB1* SNP variant allele and genotype frequencies in high dose methadone maintenance, standard dose methadone maintenance and non-opioid-dependent control subjects. 145

Table 4-3. *ABCB1* haplotype frequencies in high dose methadone maintenance, standard dose methadone maintenance and non-opioid-dependent control subjects. 146

Table 4-4. *ABCB1* diplotype frequencies in high dose methadone maintenance, standard dose methadone maintenance and non-opioid-dependent control subjects. 147

Table 4-5. *ABCB1* 3-locus (C1236T, G2677T, C3435T) haplotype frequencies in high dose methadone maintenance, normal dose methadone maintenance and non-opioid-dependent control subjects. 148

Table 4-6. *ABCB1* 3-locus (C1236T, G2677T, C3435T) diplotype frequencies in high dose methadone maintenance, normal dose methadone maintenance and non-opioid-dependent control subjects. 149

Table 4-7. Relationships between daily methadone maintenance dose or (R)-methadone C_{trough} requirements and *ABCB1* genotypes. 150

Table 4-8. Relationships between daily methadone maintenance dose or (R)-methadone C_{trough} requirements and *ABCB1* haplotypes. 150

Table 4-9. Relationship between plasma (R)- and (S)-methadone pharmacokinetics and *ABCB1* genotypes. 152

Table 4-10. Relationship between plasma (R)- and (S)-methadone pharmacokinetics and *ABCB1* haplotypes. 153

Table 5-1. List of antibodies (and their combinations) used for flow cytometry to test cell surface antigen expression in whole human PBMCs, magnetically isolated $CD4^+$, $CD56^+$ and $CD8^+$ lymphocytes, and the magnetic isolation negative fraction. 172

Table 5-2. PCR conditions trialled for the qualitative detection of CD4, CD56 and CD8 cDNA. 176

Table 5-3. Flow cytometric analysis of human whole PBMCs and lymphocyte cell fractions isolated by the basic magnetic separation procedure. 177

Table 5-4. Optimised CD4, CD56 and CD8 PCR conditions. 179

Table 5-5. Subject demographics, treatment history and *ABCB1* genetic variability. 232

Table 5-6. *ABCB1* mRNA expression in $CD4^+$, $CD56^+$ and $CD8^+$ lymphocytes of each MMT subject relative to $CD4^+$ lymphocytes of a non-opioid-dependent healthy control. 234

Table A-1. Clinically confirmed cytochrome P450-mediated drug-drug interactions affecting methadone pharmacokinetics. 296

Table A-2. Summary of *in vitro* studies investigating the functional effects of *ABCB1* SNPs and haplotypes on P-glycoprotein expression and function. 297

Table A-3. Summary of the relationships between *ABCB1* genetic variability and P-gp *ex vivo* expression and function in healthy volunteers. 301

Table A-4. Summary of *in vivo* clinical studies examining the impact of *ABCB1* genetic variants on probe substrate pharmacokinetics and pharmacodynamics in healthy volunteers. 302

Table A-5. Sex effects on relationships between MMT dose requirements and *ABCB1* genotypes and haplotypes. 303

Table A-6. Treatment outcome effects on relationships between MMT dose requirements and *ABCB1* genotypes and haplotypes. 304

Table A-7. Sex effects on relationships between (R)-methadone C_{trough} requirements and *ABCB1* genotypes and haplotypes. 304

Table A-8. Treatment outcome effects on relationships between (R)-methadone C_{trough} requirements and *ABCB1* genotypes and haplotypes. 305

Table A-9. Relationships between (R)-methadone C_{trough}/dose and *ABCB1* genotypes and haplotypes. 306

Table A-10. Sex effects on relationships between (R)-methadone C_{trough}/dose and *ABCB1* genotypes and haplotypes. 307

Table A-11. Treatment outcome effects on relationships between BMT dose requirements and *ABCB1* genotypes. 307

Table A-12. Sex effects on relationships between BMT dose requirements and *ABCB1* genotypes and haplotypes. 307

Table A-13. Relationship between buprenorphine and norbuprenorphine C_{trough} and *ABCB1* genotypes. 308

Table A-14. Relationship between buprenorphine and norbuprenorphine C_{trough} and *ABCB1* haplotypes. 308

Table A-15. Relationship between buprenorphine and norbuprenorphine C_{trough}/dose and *ABCB1* genotypes/haplotypes. 309

Table A-16. Summary table of results from Fisher‟s Exact Tests comparing the frequency of *ABCB1* variant alleles between successful and poor BMT outcome subjects. 310

Table A-17. Summary table of results from Fisher‟s Exact Tests comparing the frequency of *ABCB1* variant alleles between BMT subjects who did or did not experience withdrawal. 310

Table A-18. Primer sequences for the qualitative PCR detection of CD4, CD56 and CD8 cDNA. 310

List of in-text figures

Figure 1-1. Heroin metabolism. 22

Figure 1-2. P-glycoprotein sites of action important for opioid pharmacokinetics. 32

Figure 1-3. Functionally significant regions of the P-glycoprotein efflux transporter and the location of common *ABCB1* non-synonymous mutations. 49

Figure 1-4. Mfold predictions displaying how *ABCB1* genetic variants can significantly alter the folding and secondary structure of *ABCB1* mRNA. 52

Figure 1-5. The proposed multiple mechanisms by which *ABCB1* genetic variability, affecting P-gp transport, could influence and individual"s risk of opioid dependence, severity of addiction and substitution opioid treatment response. 58

Figure 2-1. Restriction fragment patterns for A61G, G1199A, G2677T and C3435T SNP genotypes. 72

Figure 2-2. Optimised *Eco*0109I restriction fragment patterns for C1236T genotypes. 72

Figure 2-3. Examples of possible haplotype pairs (diplotypes) formed from unambiguous and ambiguous genotype combinations. 74

Figure 2-4. Example input file for PHASE version 2.1. 77

Figure 2-5. Example input file for Arlequin version 3.11. 78

Figure 2-6. Confidence probabilities of ambiguous phase calls made by PHASE. 80

Figure 3-1. Associations of wild-type AGCGC and variant AGCTT haplotypes of *ABCB1* with daily methadone maintenance dose requirements. 103

Figure 3-2. Sex differences in the relationship between the wild-type *ABCB1* haplotype (AGCGC) and MMT dose requirements. 104

Figure 3-3. Association between *ABCB1* C1236T genotypes and trough plasma (R)-methadone concentrations. 105

Figure 3-4. Association between *ABCB1* haplotypes and trough plasma (R)-methadone concentrations 107

Figure 3-5. Sex differences in *ABCB1* genotype-C_{trough} relationships for (R)-methadone in MMT 108

Figure 3-6. Association between the *ABCB1* C1236T SNP and AGTTT haplotype variants and (S)-methadone C_{trough}/dose ratios 110

Figure 3-7. Potential genotype (*ABCB1* G1199A)-sex interaction influencing (R)-methadone dose-adjusted C_{trough}. 110

Figure 3-8. The influence of treatment outcome on *ABCB1* wild-type (AGCGC) haplotype-dose relationship in buprenorphine maintenance treatment. 115

Figure 4-1. Correlation between HD subjects‟ time in treatment and MMT dose requirements. 151

Figure 4-2. Relationship between the AGCGC and AGCTT haplotypes of *ABCB1* and methadone dose and trough plasma (R)-methadone concentrations of all MMT subjects. 154

Figure 4-3. Summary of the multiple factors potentially influencing MMT dose requirements and response. 163

Figure 5-1. Basic protocol for magnetic bead positive selection and isolation of lymphocyte subsets. 171

Figure 5-2. Optimised protocol for magnetic bead positive selection and isolation of lymphocyte subsets. 174

Figure 5-3. Detection of both cDNA and DNA in $CD4^+$, $CD56^+$ and $CD8^+$ lymphocyte reverse transcription products. 183

Figure 5-4. DNase treatment components inhibit PCR amplification, but purification of DNase-treated mRNA is effective in removing these PCR-inhibitory DNase components, revealing that DNase treatment is effective in removing DNA contamination in mRNA. 185

Figure 5-5. Examples of *ABCB1* and *GAPDH* real-time PCR data graphs generated by the Rotor-Gene 6000 software and the designation of a fluorescence threshold. 188

Figure 5-6. Association between template concentrations and linearised C_T values from validation experiments. 193

Figure 5-7. Association between total template concentration and ΔC_T for quantification of *ABCB1* in lymphocytes. 194

Figure 5-8. Example of a BSA standard curve produced using the 10 µL microplate BCA protocol. 199

Figure 5-9. Dot blot experiment identifying optimal primary and secondary antibody dilutions for detecting P-gp. 207

Figure 5-10. Dot blot experiment identifying significant background staining for all primary and secondary antibody dilutions tested for detection of calnexin. 207

Figure 5-11. Additional dilution of secondary antibody from 1:10,000 to 1:50,000 produces reduction in background membrane staining, whilst maintaining adequate quantitative detection of calnexin by Western blot. 208

Figure 5-12. Western blot detection of P-gp in $CD4^+$ and $CD8^+$ lymphocytes but not whole PBMC cell lysate. 209

Figure 5-13. Influence of protein sample preparation on detection of P-gp and calnexin in $CD4^+$ lymphocytes. 209

Figure 5-14. Influence of protein sample preparation on correlations between BCA protein quantification and loading control (calnexin) band volume and peak height, and between loading control and P-gp band volumes and peak heights. 210

Figure 5-15. Confirmation of P-gp detection in $CD4^+$ lymphocytes using overexpressing *MDR1*-transfected LLC-PK1 cells as a positive control. 211

Figure 5-16. Outline of proposed functional assay procedure. 215

Figure 5-17. Rhodamine fluorescence standard curves in the presence and absence of 100 μM verapamil. 220

Figure 5-18. Standard curves for quantification of intracellular rhodamine concentrations. . 222

Figure 5-19. Time-course of rhodamine efflux in the presence and absence of 100 μM verapamil. 224

Figure 5-20. Lack of significant correlations between $CD4^+$ and $CD56^+$, $CD4^+$ and $CD8^+$, or $CD56^+$ and $CD8^+$ lymphocyte *ABCB1* expression in MMT subjects. 234

Figure 5-21. Relationship between *ABCB1* diplotypes and *ex vivo* $CD4^+$, $CD56^+$ and $CD8^+$ lymphocyte *ABCB1* mRNA expression in MMT subjects. 235

Figure 5-22. Relationship between MMT dose requirements and relative *ABCB1* mRNA expression in $CD4^+$, $CD56^+$ and $CD8^+$ lymphocytes of MMT subjects. 235

Figure 5-23. Relationship between (R)-methadone (MD) C_{trough} and relative *ABCB1* mRNA expression in $CD4^+$ and $CD8^+$ lymphocytes of MMT subjects. 236

Figure 5-24. Western blot detection of P-gp in $CD4^+$ lymphocytes of MMT subjects. 237

Figure 5-25. Relationship between *ABCB1* mRNA and P-gp expression in $CD4^+$ lymphocytes of MMT subjects. 237

Figure 5-26. Relationship between $CD4^+$ lymphocyte P-gp expression and MMT dose requirements and (R)-methadone C_{trough}. 238

Figure 6-1. Example of P-gp apical expression in a confluent monolayer of polarised epithelial/endothelial cells. 244

Figure 6-2. Example of typical results from a „classical" cell monolayer transport study. 244

Figure 6-3. Outline of the combined P-gp substrate and inhibitor assay described by Balimane and Chong (2005). 245

Figure 6-4. Example of a [H^3]-digoxin standard curve determined by liquid scintillation counting. 250

Figure 6-5. Example standard curve of FITC-inulin fluorescence. 251

Figure 6-6. Average basal-to-apical and apical-to-basal digoxin permeability alone and in the presence of 10 μM or 100 μM verapamil using the basic protocol. 254

Figure 6-7. Example of significant variability in digoxin B>A and A>B permeability within treatment groups over the course of a 4 hour experiment using the basic protocol. 255

Figure 6-8. Average basal-to-apical and apical-to-basal digoxin permeability alone and in the presence of 100 μM verapamil using the optimised protocol. 256

Figure 6-9. Digoxin basal-to-apical and apical-to-basal permeability alone and in the presence of 100 μM verapamil over the course of a 4-hour experiment when using the optimised protocol. 256

Figure 6-10. Example of HPLC chromatograms for detection of buprenorphine. 260

Figure 6-11. Example standard curve of buprenorphine by HPLC detection. 261

Figure 6-12. (R)-methadone apparent permeability across Caco-2 cell monolayers in the absence and presence of 100 μM verapamil. 262

Figure 6-13. Buprenorphine apparent permeability across Caco-2 cell monolayers in the absence and presence of 100 μM verapamil. 263

Figure 7-1. Revised summary of the mechanisms behind the impact of *ABCB1* genetic variability on opioid substitution treatment based on thesis findings. 273

Figure B-1. Confidence probabilities of ambiguous phase calls made by PHASE. 317

Figure B-2. Linear relationships between $\log_2$(mRNA concentration) and *GAPDH* C_T values over the range of 0.6 to 28 mRNA units. 318

Abstract

Opioid dependence is a significant public health problem. Whilst long-term opioid maintenance is the most cost-effective approach for treating opioid dependence, the safe and effective use of substitution opioids like methadone and buprenorphine is complicated by their narrow therapeutic indices and a considerable, as yet unexplained, interindividual variability in their dose-effect relationships. Since there is evidence that the P-glycoprotein efflux transporter may influence the plasma pharmacokinetics and CNS distribution of opioids, it was hypothesised that genetic variability in the *ABCB1* gene (encoding P-glycoprotein) could play a major role in the interindividual variability in opioid maintenance treatment response. Therefore, the primary aim of this thesis was to investigate *ABCB1* genetic variability as a determinant of opioid requirements during maintenance therapy, as well as treatment outcome. This thesis also set out to identify the relationship between *ABCB1* genetic variability and the risk of illicit opioid use and dependence, as well as develop new methods for investigating the dynamic interactions between *ABCB1* genetic variability, P-glycoprotein expression/function and opioid exposure.

For the first major study of this thesis, opioid-dependent methadone maintenance treatment (MMT, n = 78) and buprenorphine maintenance treatment (BMT, n = 30) subjects, as well as non-opioid-dependent healthy controls (n = 98), were retrospectively genotyped and haplotyped for 5 common single nucleotide polymorphisms (SNPs) of *ABCB1* (A61G, G1199A, C1236T, G2677T and C3435T). Whilst no link was observed between *ABCB1* genetic variability and the risk of opioid dependence, the wild-type AGCGC (61A-1199G-1236C-2677G-3435C) haplotype was associated with significantly higher maintenance opioid requirements among both MMT and BMT subjects. In addition, MMT subjects carrying one of the variant haplotypes, AGCTT, required significantly less methadone, presumably due to a decreased P-gp activity at the blood-brain-barrier. Interestingly, a second retrospective study of a specific cohort of 21 (very) high-dose (≥180 mg/day) MMT subjects could not replicate

these findings, suggesting that dose range and/or clinic policy may be important factors influencing the clinical significance of *ABCB1* genetic variability.

The third major study of this thesis incorporated the development and validation of new methods for quantifying *ex vivo* P-glycoprotein expression (mRNA and protein) and function in specific lymphocyte subsets ($CD4^+$, $CD56^+$ and $CD8^+$) of healthy and opioid-dependent subjects, with the aim of determining the combined effects of *ABCB1* genetic variability and opioid exposure on P-glycoprotein function. Applying these new methods in a pilot study of 6 MMT subjects, $CD4^+$ lymphocyte *ABCB1* mRNA and P-glycoprotein expression were found to be positively associated with methadone requirements, and were lowest in the only subject homozygous for the AGCTT haplotype (providing potential mechanistic support for the link between AGCTT haplotypes and low MMT dose requirements).

Therefore, this thesis provides the first evidence that *ABCB1* haplotypes contribute to variability in substitution opioid requirements. However, *ABCB1* genetic variability should not be considered alone, and a combined interpretation of multiple genetic and environmental factors will be required to provide a more complete picture of the factors governing the successful treatment of opioid dependence.

Declaration

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Daniel T Barratt

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Somogyi AA, Barratt DT, Coller JK. (2007) Pharmacogenetics of opioids. *Clin Pharmacol Ther* **81**:429-444.

6-MAM	6-monoacetylmorphine
A>B	Apical-to-basal permeability
A_{260}	Absorbance at 260 nm
A_{280}	Absorbance at 280 nm
AAG	α_1-acid glycoprotein
AUC	Area under the concentration-time curve
B>A	Basal-to-apical permeability
BBB	Blood-brain-barrier
BCA	Bicinchoninic acid
BMT	Buprenorphine maintenance treatment
bp	Base pairs
BSA	Bovine serum albumin
cDNA	Complementary DNA
CI	Confidence interval
CL/F	Oral clearance
CL_R	Renal clearance
C_{max}	Maximum plasma concentration
CNS	Central nervous system
CSF	Cerebrospinal fluid
C_{trough}	Trough plasma concentration
C_{trough}/dose	Dose-adjusted trough plasma concentration
CV	Coefficient of variation
DADLE	[D-Ala^2,D-Leu^5]-enkephalin
DAMGO	[D-Ala^2,N-Me-Phe^4,Gly^5-ol]-enkephalin
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleoside triphosphate
DPDE	[D-Pen^{2,5}]-enkephalin
DPM	Disintegrations per minute
EDTA	Ethylenediaminetetraacetic acid
FCS	Fetal bovine serum
HBSS	Hank‟s buffered salt solution
HD	High dose
HPLC	High performance liquid chromatography
IC_{50}	50% inhibitory concentration
IDRS	Australian Illicit Drug Reporting System
IDU	Injecting drug users
kb	kilobases
LAAM	Levo-alpha-acetyl-methadol
LD	Linkage disequilibrium
M-6-G	Morphine-6-glucuronide

MEM	Minimal essential medium with Earl‟s salts
MMT	Methadone maintenance treatment
mRNA	Messenger RNA
NBD	Nucleotide binding domain
ND	Normal dose
OR	Odds Ratio
P_{app}	Apparent permeability
PBMC	Peripheral blood mononuclear cell
PCR	Polymerase chain reaction
PCR-RFLP	PCR - restriction fragment length polymorphism
P-gp	P-glycoprotein
PK/PD	Pharmacokinetic/pharmacodynamic
Pop-PK	Population-pharmacokinetic
qRT-PCR	Quantitative real time - PCR
RNA	Ribonucleic acid
SD	Standard deviation
SDS-PAGE	Sodium dodecyl sulphate – polyacrylamide gel electrophoresis
SNP	Single nucleotide polymorphism
$T_{1/2}$	Half-life
TDM	Therapeutic drug monitoring
TEER	Transepithelial electrical resistance
T_{max}	Time to maximum plasma concentration
TMD	Transmembrane domain
V	Variant allele or digest fragment
V_d	Volume of distribution
Wt	Wild-type allele or digest fragment