Molecular interactions between alcohol, hepatitis C virus and interferon

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# Table of Contents

List of Figures and Tables.............................................................................. x

Abstract ........................................................................................................ xv

Declaration ................................................................................................... xviii

Acknowledgements ..................................................................................... xix

Publications Arising During PhD ................................................................. xx

Awards Received During PhD .................................................................... xx

Presentations Arising From PhD ................................................................. xxi

Materials Providers ...................................................................................... xxiii

Abbreviations Used ..................................................................................... xxv

Chapter 1 ...................................................................................................... 1

Introduction .................................................................................................. 1

1.1 Hepatitis C Virus ................................................................................... 1

1.1.1 Epidemiology .................................................................................... 1

1.1.2 Transmission ..................................................................................... 2

1.1.3 Pathogenesis .................................................................................... 2

1.1.4 Treatment ......................................................................................... 4

1.1.5 The HCV genome ............................................................................ 5

1.1.6 Classification of genotypes ................................................................. 6

1.1.7 HCV proteins .................................................................................. 6

1.1.8 HCV life cycle ................................................................................ 9

1.1.9 HCV model systems ....................................................................... 10

1.1.9.1 Animal models .......................................................................... 11

1.1.9.2 Cell culture systems .................................................................. 11

1.1.9.3 Infectious cell culture model ...................................................... 12

1.2 Alcohol and HCV ................................................................................. 12
1.3 Alcohol Metabolism ................................................................. 15
  1.3.1 Cytochrome P4502E1 ............................................................ 16
1.4 Oxidative Stress ................................................................. 17
  1.4.1 Oxidative stress and alcohol .................................................. 17
  1.4.2 Oxidative stress and HCV ...................................................... 18
1.5 ROS Induced Liver Damage ................................................... 19
1.6 Interferon ............................................................................ 22
  1.6.1 Effect of alcohol on IFN-α efficacy ......................................... 23
  1.6.2 Effect of HCV and alcohol on IFN signaling ............................ 25
1.7 Cellular Factors Involved In HCV Life Cycle ......................... 27
  1.7.1 STAT3 ............................................................................... 27
  1.7.2 Oxidative stress and STAT3 .................................................. 29
1.8 Hypothesis and Aims ............................................................ 29

Chapter 2 ................................................................................. 31

Materials and Methods .......................................................... 31

2.1 General Reagents ................................................................. 31
  2.1.1 Transient transfection of plasmid DNA ................................. 31
  2.1.2 Stable transfection of plasmid DNA to generate over-expressing cell lines .... 31
  2.1.3 Transient transfection of Stealth™ siRNA oligonucleotides .............. 32

2.2 Tissue Culture Techniques .................................................. 32
  2.2.1 Tissue culture medium ......................................................... 32
  2.2.2 Maintenance of cell lines ...................................................... 33
  2.2.3 Cryopreservation of cultured cells ....................................... 33
  2.2.4 Resuscitation of frozen cells ................................................. 34
  2.2.5 Trypan blue exclusion ......................................................... 34
  2.2.6 CellTiter-Blue® cell viability assay ...................................... 34
  2.2.7 CellTiter 96® non-radioactive cell proliferation assay (MTT) .......... 34

2.3 Cultured Cell Lines .............................................................. 35
2.3.1 Huh-7 ......................................................................................................... 35
2.3.2 NNeoC-5B (RG) .......................................................................................... 35
2.3.3 NNeo3-5B (RG) .......................................................................................... 36
2.3.4 HCV Genomic Replicon + CYP2E1 .............................................................. 36
2.3.5 Huh-7 (EG) + CYP2E1 ................................................................................. 36
2.3.6 Huh-7.5 ........................................................................................................ 36
2.3.7 Huh-7.5 + CYP2E1 ..................................................................................... 37

2.4 HCVcc Infectious System .............................................................................. 37
2.4.1 Generation of HCVcc viral stock ................................................................. 37
2.4.1.1 Preparation of HCV RNA ........................................................................... 37
2.4.1.2 HCV RNA transfection ............................................................................. 37
2.4.1.3 Concentration of HCV viral stocks (PEG precipitation) ......................... 38
2.4.1.4 Titration of infectious HCV ....................................................................... 38
2.4.1.5 Amplification of HCV viral stocks (‘up-scale’) ............................................ 39
2.4.2 General infection protocol for HCVcc ......................................................... 40

2.5 General Molecular Biology Methods ............................................................ 40
2.5.1 Synthetic oligonucleotides .......................................................................... 40
2.5.2 Bacterial transformation .............................................................................. 41
2.5.3 Mini-preparation (small scale) of plasmid DNA .......................................... 41
2.5.4 Maxi-preparation (large scale) of plasmid DNA .......................................... 42
2.5.5 Restriction endonuclease digestion ............................................................ 42
2.5.6 Agarose gel electrophoresis ........................................................................ 43
2.5.7 DNA ligation ................................................................................................ 43
2.5.8 Gel purification ............................................................................................. 43
2.5.9 DNA sequencing .......................................................................................... 44
2.5.10 Extraction of total RNA ............................................................................ 45
2.5.11 DNAseI treatment of RNA samples .......................................................... 45
2.5.12 Nucleic acid quantification ..................................................................... 46
2.5.13 cDNA preparation ..................................................................................... 46
2.5.14 Polymerase Chain Reaction ................................................................. 46
2.5.15 Real-Time Quantitative PCR ............................................................... 47
2.5.16 Extraction of cellular protein ................................................................. 47
2.5.17 Protein quantification ........................................................................... 48
2.5.18 SDS PAGE and protein transfer ............................................................. 48
2.5.19 Western blotting .................................................................................... 49
2.5.20 Dual Renilla luciferase assay ................................................................. 50
2.5.21 Measurement of ROS ........................................................................... 51
2.5.22 Acetaminophen assay ........................................................................... 51
2.5.23 Treatment of cells .................................................................................. 52
2.5.24 Immunofluorescence microscopy ........................................................... 54
  2.5.24.1 HCV antigen staining ...................................................................... 54
  2.5.24.2 STAT3-C-fLAG staining .................................................................. 54
  2.5.24.3 α-tubulin staining ............................................................................ 55
2.6 Data Analysis ............................................................................................... 55

Chapter 3 ........................................................................................................... 56

An in vitro Model System to Study the Effects of Alcohol Metabolism on HCV Replication ............................................................................................................. 56

3.1 Introduction .................................................................................................. 56
  3.1.1 Generation of stable CYP2E1 HCV replicon cell lines ......................... 57
  3.1.2 Generation of stable CYP2E1 Huh-7 cell lines ..................................... 58
  3.1.3 CYP2E1 stable cell lines harbour replicon RNA and are permissive for HCV JFH-1 infection ......................................................................................... 59

3.2 Characterisation of Stable CYP2E1 Cell Lines ............................................. 60
  3.2.1 Determination of growth rates for stable CYP2E1 cell lines ................. 60
  3.2.2 CYP2E1 is metabolically active in the stable cell lines ......................... 61
  3.2.3 Is CYP2E1 mediated metabolism of ethanol toxic to cells? ................. 61

3.3 Discussion ..................................................................................................... 62
Chapter 4 .......................................................................................................................... 67

The Effect of Alcohol Metabolism on HCV Replication ................................. 67

4.1 Introduction ............................................................................................................. 67

4.2 The Effect of Ethanol metabolism on HCV Replication .................. 67

4.2.1 Ethanol metabolism by CYP2E1 increases HCV replication in replicon cells ..... 67

4.3 Establishing a Molecular Mechanism For the Ethanol Induced Increase in HCV Replication ......................................................................................................................... 69

4.3.1 Ethanol metabolism increases oxidative stress in HCV replicon cells ........ 69

4.3.2 Anti-oxidant treatment decreases HCV replication .................................. 70

4.3.3 Acetaldehyde does not modulate HCV replication ................................. 71

4.3.4 Ethanol metabolism does not modulate HCV IRES activity ................. 71

4.3.5 Exogenous H2O2 decreases HCV replication .............................................. 72

4.4 The Effect of Ethanol Metabolism on HCVcc .............................................. 73

4.4.1 Ethanol metabolism increases JFH-1 replication ................................. 73

4.4.2 Pre treatment with ethanol is required to increase HCV JFH-1 replication ........ 73

4.4.3 Exogenous H2O2 decreases HCV JFH-1 replication .......................... 74

4.4.4 NAC treatment decreases HCV JFH-1 replication ................................ 75

4.5 The Oxidative Stress Sensitive Transcription Factor STAT3 ................. 75

4.5.1 Rationale for investigating the involvement of STAT3 in the ethanol induced increase in HCV replication ................................................................. 75

4.5.2 The oxidative stress sensitive transcription factor STAT3 plays a role in the ethanol induced increase in HCV replication ........................................ 76

4.5.2.1 Ethanol metabolism increases STAT3 activation .............................. 76

4.5.2.2 Ethanol metabolism increases STAT3 promoter activity .................. 77

4.6 Discussion ............................................................................................................ 78

Chapter 5 .................................................................................................................... 85

The Effect of Ethanol Metabolism on IFN-α Signaling ........................................ 85

5.1 Introduction ........................................................................................................... 85
5.2 Ethanol Metabolism Decreases the Efficacy of IFN-α ........................................85
  5.2.1 The effect of ethanol metabolism on the anti-viral efficacy of IFN-α ...............85

5.3 CYP2E1 Mediated Ethanol Metabolism Modulates the JAK/STAT Signaling
  Cascade .................................................................................................................86
  5.3.1 The phosphorylation status of signal transduction molecules in the JAK/STAT
  signaling pathway in the presence of ethanol metabolism ......................................86
  5.3.2 Decreased STAT1-Y701 phosphorylation is dependent on CYP2E1 mediated
  metabolism of ethanol ..........................................................................................88
  5.3.3 The ethanol induced decrease in STAT1-Y701 phosphorylation is independent of
  HCV replication .................................................................................................88

5.4 The Effect of Ethanol Metabolism on HCVcc and IFN-α ........................................89
  5.4.1 The effect of ethanol metabolism on the efficacy of IFN-α against HCVcc ........89
  5.4.2 Ethanol metabolism disturbs the JAK/STAT signaling pathway in the presence of
  HCV JFH-1 ..............................................................................................................90

5.5 Ethanol Metabolism Decreases ISRE Promoter Activity ....................................90
  5.5.1 Ethanol metabolism alters ISG expression .....................................................91

5.6 Discussion ...........................................................................................................92

Chapter 6 .................................................................................................................99

  The role of STAT3 in HCV replication .................................................................99
  6.1 Introduction .......................................................................................................99

  6.2 HCV Replication Activates STAT3 .................................................................100
    6.2.1 STAT3 is constitutively activated in HCV genomic replicon cells ...............100
    6.2.2 STAT3 mRNA is increased during HCV JFH-1 infection ...........................100
    6.2.3 HCV JFH-1 replication constitutively activates STAT3 ..............................101
    6.2.4 HCV JFH-1 activates the STAT3 promoter .................................................102

  6.3 Characterisation of a Constitutively Active STAT3 (STAT3-C) .......................102
    6.3.1 STAT3-C is functionally active .................................................................102
    6.3.2 STAT3-C expression in Huh-7.5 cells .......................................................103
6.3.3 Transient expression of STAT3-C increases HCV JFH-1 replication .......... 104

6.4 Characterisation of Huh-7.5 Cells Stably Expressing a Constitutively Active Form of STAT3 (STAT3-C) ................................................................. 104
  6.4.1 Detection of STAT3-C positive clones ............................................. 104
  6.4.2 STAT3-C stable cell lines maintain permissiveness for JFH-1 infection ....... 105

6.5 The Effect of STAT3-C Expression on HCV Replication ......................... 106
  6.5.1 Stable expression of STAT3-C increases HCV JFH-1 replication ........... 106

6.6 Can Leukemia inhibitory factor (LIF) increase HCV replication? .......... 106

6.7 The Effect of STAT3 Inhibition on HCV Replication ............................ 107
  6.7.1 siRNA knockdown of STAT3 decreases HCV JFH-1 replication ............. 107
  6.7.2 Chemical Inhibition of STAT3 decreases HCV replication .................... 108
    6.7.2.1 AG490 and STA-21 decrease HCV replication in genomic replicon cells ....... 108
    6.7.2.2 Chemical inhibition of STAT3 decreases HCV JFH-1 replication ............. 109

6.8 Inhibition of STAT3 Prevents HCV Establishing a Productive Infection .... 110

6.9 STA-21 Inhibits Microtubule Polymerization .................................... 111

6.10 Discussion ....................................................................................... 112

Chapter 7 ................................................................................................. 118

Conclusions and Future Directions .......................................................... 118

  7.1 Proposed Model of Interactions Between HCV and Alcohol ................. 125

Appendices ............................................................................................... 127

  Appendix I. General Solutions and Buffers ............................................. 127
  Appendix II. Infectious HCV Constructs. ................................................. 130
  Appendix III. pcDNA6/V5-His ................................................................. 131
  Appendix IV. pcDNA-2E1 .................................................................. 132
  Appendix V. pcDNA-2E1-AS (CYP2E1 Anti-Sense) ................................. 133
  Appendix VI. PRL-HL .................................................................. 134
  Appendix VII. pSTAT3-Luc ................................................................. 135
  Appendix VIII. pRL-TK .................................................................. 136
Appendix IX. pISRE-Luc...............................................................137
Appendix X. PRc/CMV-STAT3-C ........................................138
Appendix XI. pRc/CMV............................................................139
Appendix XII. Publications....................................................140
References..............................................................................141
# List of Figures and Tables

<table>
<thead>
<tr>
<th>Figure Number</th>
<th>On page:</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chapter 1</strong></td>
<td></td>
</tr>
<tr>
<td>Figure 1.1</td>
<td>Clinical spectrum of HCV infection</td>
</tr>
<tr>
<td>Figure 1.2</td>
<td>Progression of HCV induced liver disease</td>
</tr>
<tr>
<td>Figure 1.3</td>
<td>HCV genome and polyprotein processing</td>
</tr>
<tr>
<td>Figure 1.4</td>
<td>Global HCV genotype distribution</td>
</tr>
<tr>
<td>Figure 1.5</td>
<td>Model of HCV entry</td>
</tr>
<tr>
<td>Figure 1.6</td>
<td>Life cycle of HCV</td>
</tr>
<tr>
<td>Figure 1.7</td>
<td>HCV model systems</td>
</tr>
<tr>
<td>Figure 1.8</td>
<td>Construction of HCV genomic replicon</td>
</tr>
<tr>
<td>Figure 1.9</td>
<td>Pathways of alcohol metabolism</td>
</tr>
<tr>
<td>Table 1.1</td>
<td>The interferon family members</td>
</tr>
<tr>
<td>Figure 1.10</td>
<td>IFN-α signal transduction</td>
</tr>
<tr>
<td>Figure 1.11</td>
<td>Potential and known host factors involved in the complete life cycle of HCV</td>
</tr>
<tr>
<td>Figure 1.12</td>
<td>STAT3 signal transduction</td>
</tr>
<tr>
<td><strong>Chapter 2</strong></td>
<td></td>
</tr>
<tr>
<td>Table 2.1</td>
<td>Cell lines and culture conditions used in this study</td>
</tr>
<tr>
<td>Table 2.2</td>
<td>Primer sequence table</td>
</tr>
</tbody>
</table>
Table 2.3  Antibody concentration  50

**Chapter 3**

Figure 3.1  Detection of CYP2E1 expression in HCV sub-genomic replicon cell lines +/-CYP2E1  57

Figure 3.2  Detection of CYP2E1 expression in HCV genomic replicon cell lines +/-CYP2E1  58

Figure 3.3  Characterisation of Huh-7 cells expressing CYP2E1  58

Figure 3.4  Detection of HCV antigens in CYP2E1 stable cell lines  59

Figure 3.5  Comparison of growth rates in parental replicon cells versus replicon cells expressing CYP2E1  60

Figure 3.6  CYP2E1 is metabolically active via acetaminophen toxicity assay  61

Figure 3.7  CYP2E1 metabolism of ethanol is not toxic to replicon cells  62

**Chapter 4**

Figure 4.1  Ethanol modulates HCV replication in the presence of CYP2E1 mediated metabolism  68

Figure 4.2  In the absence of CYP2E1 ethanol does not modulate HCV replication  68

Figure 4.3  The ethanol induced increase in HCV replication is dependent on CYP2E1 mediated metabolism of ethanol  69

Figure 4.4  Metabolism of ethanol by CYP2E1 increases oxidative stress in HCV replication cells  70

Figure 4.5  Anti-oxidants decrease HCV replication  70

Figure 4.6  Acetaldehyde does not modulate HCV replication  71
Figure 4.7 Ethanol metabolism does not modulate HCV IRES activity 72
Figure 4.8 H$_2$O$_2$ decreases HCV replication 72
Figure 4.9 Ethanol metabolism increases HCV JFH-1 replication 73
Figure 4.10 Pre-treatment of ethanol is required to enhance HCV JFH-1 replication via ethanol metabolism 74
Figure 4.11 H$_2$O$_2$ decreases HCV JFH-1 replication 74
Figure 4.12 The anti-oxidant NAC decreases HCV JFH-1 replication 75
Figure 4.13 Ethanol metabolism increases STAT3-Y705 phosphorylation 76
Figure 4.14 Ethanol metabolism increases STAT3-S727 phosphorylation 77
Figure 4.15 Ethanol metabolism increases STAT3 promoter activity 77
Figure 4.16 Possible role of STAT3 and oxidative stress in HCV replication 82

Chapter 5
Figure 5.1 IFN-α signal transduction pathway 85
Figure 5.2 Ethanol metabolism decreases the anti-HCV efficacy of IFN-α 86
Figure 5.3 Ethanol metabolism by CYP2E1 results in decreased STAT1 phosphorylation at tyrosine residue 701 87
Figure 5.4 STAT1-Y701 phosphorylation is decreased by ethanol metabolism 87
Figure 5.5 The ethanol induced decrease in STAT1-Y701 phosphorylation is dependent on CYP2E1 expression 88
Figure 5.6 The ethanol induced decrease in STAT1-Y701 phosphorylation is independent of HCV replication 88
Figure 5.7  Ethanol metabolism decreases the anti-HCV JFH-1 efficacy of IFN-α  

Figure 5.8  Ethanol metabolism by CYP2E1 decreases STAT1-Y701 phosphorylation in the presence of JFH-1  

Figure 5.9  Ethanol metabolism decreases ISRE promoter activity  

Figure 5.10  Ethanol metabolism reduces anti-viral ISG expression  

Figure 5.11  Alcohol metabolism decreases IFN-α efficacy via perturbation of the JAK/STAT signaling cascade  

Figure 5.12  Possible mechanism for the inhibition of STAT1-Y701 phosphorylation in the presence of ethanol metabolism via SHP-2 or SOCS3  

Chapter 6  

Figure 6.1  STAT3 activation is increased in HCV genomic replicon cells  

Figure 6.2  Signaling pathway generated from microarray data showing STAT3 mRNA up-regulated 2-fold in JFH-1 Huh-7 cells  

Figure 6.3  STAT3 phosphorylation is increased in the presence of HCV JFH-1  

Figure 6.4  STAT3 promoter activity is increased in the presence of HCV  

Figure 6.5  The STAT3-C construct is functionally active  

Figure 6.6  STAT3-C expression in Huh-7.5 cells  

Figure 6.7  Expression of STAT3-C in HCV JFH-1 infected Huh-7.5 cells  

Figure 6.8  Transient expression of STAT3-C increases HCV JFH-1 replication
Figure 6.9  Characterisation of Huh-7.5 cell lines stably expressing STAT3-C

Figure 6.10  STAT3-C stable cell lines are permissive for HCV JFH-1 infection

Figure 6.11  Stable expression of STAT3-C increases HCV JFH-1 replication

Figure 6.12  LIF activates STAT3 and enhances HCV JFH-1 replication

Figure 6.13  Knockdown of STAT3 with siRNA decreases HCV JFH-1 replication

Figure 6.14  Action of STAT3 inhibitors

Figure 6.15  Inhibition of STAT3 modestly decreases HCV replication in genomic replicon cells

Figure 6.16  Inhibition of STAT3 decreases HCV JFH-1 replication

Figure 6.17  Inhibition of STAT3 decreases the susceptibility of Huh-7.5 cells to HCV JFH-1 infection

Figure 6.18  Inhibition of STAT3 decreases the susceptibility of Huh-7.5 cells to HCV JFH-1 infection

Figure 6.19  Model of STAT3 interaction with STMN1

Figure 6.20  Inhibition of STAT3 with STA-21 inhibits α-tubulin polymerization

Chapter 7

Figure 7.1  Proposed model of interactions between HCV, alcohol and hepatocytes
Hepatitis C virus (HCV) is a significant human pathogen that in many cases, establishes a chronic life long infection of the liver, resulting in progressive liver disease that culminates in the development of cirrhosis and hepatocellular carcinoma (HCC). The only treatment option available for HCV infection is a combination therapy of Interferon-α (IFN-α) and Ribavirin. However, it is only successful in a limited number of patients. There are a number of co-factors that accelerate liver disease in chronic hepatitis C (CHC) and one of the most significant factors is alcohol consumption. Furthermore, alcohol consumption has been shown to reduce the efficacy of IFN-α treatment. Despite these clinical observations, the molecular mechanisms by which alcohol exerts these effects are unknown and remain relatively unexplored. This is largely due to the lack of an appropriate model system to enable studies into the interaction between the HCV life cycle, alcohol metabolism and IFN.

To overcome this limitation, we have developed an in vitro cell culture model system that enables Huh-7 cells to metabolise alcohol (ethanol), via the enzyme cytochrome P4502E1 (CYP2E1), while also supporting HCV replication directed from both the HCV replicon and infectious HCV model systems. As such, this model system has been used in this thesis to extensively investigate the interactions between alcohol metabolism, HCV and IFN.

It is known clinically that HCV infected persons who consume alcohol, have exacerbated liver disease and in some cases increased serum of HCV. One postulated mechanism for this effect is that alcohol consumption increases HCV replication, which in turn leads to increased viral burden in the liver and associated pathogenic effects. We have shown that CYP2E1 mediated metabolism of alcohol increases HCV RNA replication in vitro, in both the replicon and infectious HCV model systems. Furthermore, we have demonstrated that this process is mediated via the oxidative
stress produced by alcohol metabolism, as the anti-oxidant NAC blocked this alcohol-induced increase in HCV RNA replication. These observations correlate with what is noted clinically and suggest a potential mechanism whereby alcohol consumption in chronically infected HCV individuals, leads to accelerated rates of liver disease progression. These findings form a rationale to clinically investigate the use of anti-oxidant therapy in CHC patients consuming alcohol.

In this thesis we present a molecular mechanism for the reduced response rates to IFN-α therapy in HCV infected individuals consuming alcohol. Specifically we have shown that alcohol metabolism attenuates the anti-HCV activity of IFN-α via perturbation of the JAK/STAT signaling cascade and subsequently decreases the expression of anti-viral ISGs, which are the effector molecules of an IFN response. Thus alcohol metabolism seems to be able to blunt the anti-viral effects of IFN and this has implications for anti-viral directed therapy and the innate immune response to HCV infection in the liver.

Also arising from this thesis was the novel observation that levels of the oxidative stress sensitive transcription factor signal transducer and activator of transcription 3 (STAT3) were increased in the context of HCV replication and alcohol metabolism. From these observations we hypothesized that STAT3 could be a potential pro-viral host factor. We have presented strong evidence in this thesis to suggest that STAT3 is working at multiple levels to assist HCV replication. Firstly, we have shown that STAT3 is activated in the presence of replicating HCV, and we believe STAT3 may be facilitating HCV replication via the production of specific STAT3 dependent genes. Secondly, we have presented significant data in this thesis to suggest that STAT3 may be assisting HCV entry into hepatocytes via the control of microtubule dynamics. These studies emphasize the need for further investigations into the role of STAT3 in the life cycle of HCV and suggest a role for therapies directed against STAT3 in patients with CHC, in order to limit disease progression. Furthermore, the
ability of HCV to activate STAT3 and the oncogenic nature of STAT3 suggest that STAT3 could be playing a mechanistic role in the development of HCC in individuals infected with HCV.

In summary we have developed an *in vitro* model system to simultaneously evaluate the impact of HCV replication, alcohol metabolism and IFN, on each other. We have shown that alcohol metabolism increases HCV replication via an oxidative stress related mechanism and that the anti-viral action of IFN is severely attenuated in the presence of alcohol metabolism. Moreover, we have also identified STAT3 as a pro-viral host factor that may exert its effect at multiple stages of the HCV life cycle. While all of the experiments in this thesis were conducted *in vitro*, the knowledge gained from this work will aid in the design of future studies to be performed when a small animal model of HCV pathogenesis becomes available. We believe we have significantly added to our understanding of the interplay between HCV and alcohol metabolism and that in the long term these findings will aid in therapeutic responses and management of patients chronically infected with HCV.
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 Erin Marie McCartney 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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Erin Marie McCartney

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2009 Australian Centre for Hepatitis and HIV Annual Meeting, Terrigal, ACH2 Young Investigator Travel Award for oral presentation - $5000
*National award - one awarded per year for travel to international HCV meeting.*

2008 Adelaide University Health Sciences Travel Fellowship - $2000

2008 School of Molecular and Biomedical Science PhD student poster award - $200

2007 Australian Centre for Hepatitis and HIV Annual Meeting, Barossa Valley, ACH2 PhD Student Oral Presentation Award – $500

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National


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McCartney EM, L Semendric, KJ Helbig, MR Beard. CYP2E1 metabolism of alcohol suppresses the anti-HCV action of interferon. Australian Centre for Hepatitis Virology and HIV virology interest group inaugural workshop, Terrigal, Australia, 2005. (oral presentation)
## Materials Providers

<table>
<thead>
<tr>
<th>Provider</th>
<th>Location</th>
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</thead>
<tbody>
<tr>
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<td>Cambridge, UK</td>
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<td>Texas, USA</td>
</tr>
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<td>Birminghamshire, UK</td>
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<td>Boronia, VIC, Australia</td>
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<td>Ontario, Canada</td>
</tr>
<tr>
<td>Applied Biosystems</td>
<td>Warrington, UK</td>
</tr>
<tr>
<td>Becton Dickson Labware</td>
<td>New Jersey, USA</td>
</tr>
<tr>
<td>Biomol</td>
<td>New Jersey, USA</td>
</tr>
<tr>
<td>BioRad Laboratories</td>
<td>California, USA</td>
</tr>
<tr>
<td>Cell Signaling</td>
<td>Massachusetts, USA</td>
</tr>
<tr>
<td>Chemicon International</td>
<td>Massachusetts, USA</td>
</tr>
<tr>
<td>Cohu</td>
<td>California, USA</td>
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### Abbreviations Used

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<td>A</td>
<td>adenosine</td>
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<td>base pairs</td>
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<tr>
<td>BSA</td>
<td>bovine serum albumin</td>
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<td>BVDV</td>
<td>bovine viral diarrhoea virus</td>
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<td>C</td>
<td>cytosine</td>
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<td>° C</td>
<td>degrees Celsius</td>
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<td>cDNA</td>
<td>complimentary deoxyribonucleic acid</td>
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<td>dCTP</td>
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<td>GAPDH</td>
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N/A not applicable
nt nucleotide
ORF open reading frame
PAGE polyacrylamide gel electrophoresis
PBS phosphate buffered saline; 0.15M NaCl, 6M K$_2$HPO$_4$, 2mM KH$_2$PO$_4$ (pH 7)
PCR polymerase chain reaction
pg picograms
pmol picomolar
RNA ribonucleic acid
rpm revolutions per minute
RT room temperature
RT-PCR reverse transcriptase polymerase chain reaction
sd standard deviation
SDS sodium dodecyl sulfate
sec second(s)
ss single stranded
STAT signal transducer and activator of transcription
STMN1 Stathmin
T thymidine
TAE 0.04M Tris (pH 8), 0.04M Acetic Acid, 1mM EDTA
TEMED N,N,N’,N’-tetramethylethylenediamine
Tris 3,3,5,5-tetramethylbenzidine
TYK2 tyrosine kinase 2
U units
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<td>untranslated region</td>
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<tr>
<td>V</td>
<td>volts</td>
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<tr>
<td>w/v</td>
<td>weight per volume</td>
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