The role of human cytomegalovirus in stillbirth

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Research funded by the Stillbirth Foundation Australia investigating viral causes of stillbirth has revealed findings that shed new light on how infections during pregnancy may result in stillbirth. This research was primarily carried out by Stuart Hamilton, who received the inaugural Stillbirth Foundation Australia PhD Scholarship in 2010, and led by Professor William Rawlinson at the Virology Division, SEALS Microbiology, Prince of Wales Hospital and the University of New South Wales.

Almost half of all stillbirths are of unknown cause, even after autopsy examination. We know some viral and bacterial infections during pregnancy cause stillbirth. However, in many cases the exact mechanisms by which infection leads to stillbirth remain unclear. One such infection is a common virus known as human cytomegalovirus (CMV). CMV can cause a number of serious birth defects to the developing baby including mental disability, hearing and vision loss and in the most severe cases result in fetal death. Fetal injury is usually caused directly by the virus infecting the baby *in utero*; however, recent evidence suggests CMV may indirectly cause fetal injury via infection of the placenta.

This research investigated the immune environment within placental tissue from stillborn babies infected with the CMV virus and then used a novel *in vitro* model of placental infection to confirm these findings (Hamilton *et al*, 2012, PLOS ONE). The research found the placenta from stillborn babies naturally infected with CMV had a shift from a balanced immune environment towards a more pro-inflammatory state. A similar immune shift was observed in artificially-infected placental tissues cultured in the laboratory that were donated from women undergoing elective Caesarean sections. Furthermore, infection of cell cultures *in vitro* showed this pro-inflammatory shift was most likely a direct cellular response to CMV replication within infected cells of the placenta (Hamilton *et al*, 2013, Journal of General Virology).

A pro-inflammatory shift within the placenta has been associated with a number of adverse pregnancy outcomes, and can negatively affect many aspects of placental development and function. Placental dysfunction would in turn lead to the developing baby not receiving enough nutrients and oxygen and could lead to fetal injury and death *in utero*. This research suggests that monitoring the immune environment of pregnant women at high risk of certain infections may be used as an indicator of at risk pregnancies of stillbirth and could give rise to new therapeutic treatments.

A systematic review of the scientific literature showed there are limited therapeutic options available to prevent or treat CMV infection during pregnancy (Hamilton *et al.* 2014, Reviews in Medical Virology). This is mainly due to significant drug toxicity and limited clinical evidence for drug efficacy. Therefore, this research also worked on the development of novel antiviral therapeutics for safe use during pregnancy, one of which utilises small interfering RNA (siRNA) molecules (Hamilton *et al*, 2014, PLOS ONE). These siRNA molecules bind to the viruses' genomic material and prevents generation of essential proteins needed for the virus to replicate, thus inhibiting viral growth and spread.

Current investigations are now focused on using the novel placental explant model developed in this study to test the efficacy and safety profiles of various established and novel anti-CMV therapeutics for use during pregnancy. These studies will better inform decision making on future clinical trials and help lead the way in the prevention of infectious causes of stillbirth and other virus-associated adverse pregnancy outcomes.

PUBLICATIONS AND PRESENTATIONS

Throughout the course of this candidature, the following manuscripts were published:

- **Hamilton ST**, Scott GM, Naing Z, Iwasenko J, Hall B, Graf N, Arbuckle N, Craig ME, Rawlinson WD. (2012). *Human Cytomegalovirus-Induces Cytokine Changes in the Placenta with Implications for Adverse Pregnancy Outcomes.* PLOS ONE 7(12): e52899.

- **Hamilton ST**, Scott GM, Naing Z, Rawlinson WD. (2013). *Human Cytomegalovirus Directly Modulates Expression of Chemokine CCL2 (MCP-1) During Viral Replication.* J Gen Virol. 94 (Pt 11):2495-503.

- **Hamilton ST**, Milbradt J, Marschall M, Rawlinson WD. (2014). *Human Cytomegalovirus Replication is Strictly Inhibited by siRNAs Targeting UL54, UL97 or UL122/123 Gene Transcripts.* PLOS ONE 9(6): e97231

- **Hamilton ST**, van Zuylen W, Shand A, Scott GM, Naing Z, Hall B, Craig ME, Rawlinson WD. (2014). *Prevention of Congenital Cytomegalovirus Complications by Maternal and Neonatal Treatments: A systematic review.* Reviews in Medical Virology. 24(6), 420-433.

- van Zuylen W, **Hamilton ST**, Naing Z, Hall B, Shand A, Rawlinson WD. (2014). *Congenital Cytomegalovirus Infection: Clinical Presentation, Epidemiology, Diagnosis and Prevention.* Obstetric Medicine. 7(4) 140-146.

- Graf L, Webel R, Wagner S, **Hamilton ST**, Rawlinson WD, Sticht H, Marschall M. (2013). *The Cyclin-Dependent Kinase Ortholog pUL97 of Human Cytomegalovirus Interacts with Cyclins.* Viruses. 5(12), 3213-3230.

- Milbradt J, Kraut A, Hutterer C, Sonntag E, Schmeiser C, Ferro M, Wagner S, Lenac T, Claus C, Pinkert S, **Hamilton ST**, Rawlinson WD, Sticht H, Coute´ Y, and Marschall M. (2014). *Proteomic Analysis of the Multimeric Nuclear Egress Complex of Human Cytomegalovirus.* Mol Cell Proteomics. 13(8):2132-46.

Presentations arising from this candidature were as follows:

- Milbradt J, Kraut A, Hutterer C, Sonntag E, Schmeiser C, Ferro M, Wagner S, Lenac T, Claus C, Pinkert S, **Hamilton ST**, Rawlinson WD, Sticht H, Coute´ Y, and Marschall M. (2014). Proteomic Analysis of the Multimeric Nuclear Egress Complex of Human Cytomegalovirus. *International Herpesvirus Workshop*, Kobe, Japan 25-29th July 2014.

- Graf L, Webel R, **Hamilton ST**, *et al.* (2013). Direct interaction between cyclin T1 and the cyclin-dependent kinase ortholog pUL97 of human cytomegalovirus. *5th European Congress of Virology*, Lyon, France. 11-14th September 2013.

- **Hamilton ST**, *et al.* 2012. Small Interfering RNA (siRNA) Molecules as a Novel Therapeutic Strategy in the Prevention of Congenital CMV. *The 14th International CMV/Betaherpesvirus Workshop and Congenital Cytomegalovirus Conference Combined Meeting.* San Francisco. 29th October – 2nd November, 2012.

- **Hamilton ST**, *et al.* 2012. The Role of CMV Infection in Adverse Outcomes of Pregnancy and Potential Therapeutic Treatments. *Institute for Clinical and Molecular Virology.* University of Erlangen-Nürnberg (Oral presentation). December 2012.

- **Hamilton ST**, *et al.* 2012 Human Cytomegalovirus Immune Modulation within the Placenta and the Implications for Placental Function and Pregnancy Outcome. *The Australian Health and Medical Research Congress.* Adelaide. 25-28th November 2012.

- Naing Z, **Hamilton ST**, *et al.* 2012. Cytomegalovirus UL130 variants have altered replication in trophoblast cells and differential effects on cytokine expression. *The 14th International CMV/Betaherpesvirus Workshop and Congenital Cytomegalovirus Conference Combined Meeting.* San Francisco. (Oral presentation). 29th October – 2nd November, 2012.

- Naing Z, **Hamilton ST**, *et al.* 2012. Cytomegalovirus gene important for infection of placental trophoblasts and infection-dependent dysregulation of cytokine expression. *The Australian Health and Medical Research Congress.* Adelaide (Oral presentation). 25-28th November 2012.

- Scott GM, **Hamilton ST**, *et al.* 2012. Cytomegalovirus (CMV) Genes Important for Placental Infection and Viral Replication: Novel Therapeutic Targets for Prevention of Congenital CMV Transmission? *The Australian Society for Medical Research NSW Annual Scientific Meeting 2012.* Sydney, Australia. June, 2012

- Scott GM, **Hamilton ST**, *et al.* 2012. Cytomegalovirus (CMV) Genes Important for Placental Infection and Viral Replication: Novel Therapeutic Targets for Prevention of Congenital CMV Transmission? *The Australian Society for Microbiology Annual Meeting.* Brisbane, Australia. 1-4th July, 2012. (Oral Presentation)

- Rawlinson W, **Hamilton ST**, *et al.* 2012. Congenital CMV pathogenesis studies in placental explant models – research to reduce virus transmission. *The 14th International CMV/Betaherpesvirus Workshop and Congenital Cytomegalovirus Conference Combined Meeting.* San Francisco. (Oral presentation). 29th October – 2nd November, 2012.

- Graf L, **Hamilton ST**, *et al.* 2012 Interaction between human cytomegalovirus pUL69 and cylin T1 is generally detectable and independent from virus strains and host cell types. *22nd Annual Meeting of the Society of Virology.* Essen, Germany. 14-17th March, 2012.

- **Hamilton ST**, *et al.* 2011. CMV-induced Cytokine Changes within the Placenta and the Implications for Pregnancy Outome. *The 13th International CMV/Betaherpesvirus Workshop.* Nuremberg, Germany. 14-17th May, 2011. Abstract 2.04 (Oral presentation).

- **Hamilton ST**, *et al.* 2011. Human Cytomegalovirus Infection During Pregnancy and the Implications for Pregnancy Outcome. *The Coast Association Tow Research Awards.* Sydney. 7th September, 2011. Abstract 4.06 (Oral presentation).

- **Hamilton ST**, *et al.* 2011. Human Cytomegalovirus-Induced Cytokine Changes within the Placenta and the Implications for Pregnancy Outcome. *The 6th Australasian Virology Society Meeting,* Kingscliff. 4-8th December, 2011. Abstract 143.

- **Hamilton ST**, *et al.* 2010. The Role of Human Cytomegalovirus in Stillbirth and Other Adverse Outcomes of Pregnancy. *The International Stillbirth Alliance (ISA)/International Society for the Study and Prevention of Perinatal and Infant Deaths (ISPID) Joint Conference,* Sydney. 8-10th October 2010. Abstract (Oral presentation).