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Australian Respiratory Council Ann Woolcock Research Fellowship 2005-2009
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Genetic influences on causal pathways of acute lower respiratory tract infections (ALRIs) in highly susceptible infants.

Acute lower respiratory infections (ALRI) are the leading cause of death in children under 5 years of age, excluding neonatal causes (those occurring in the first 4 weeks of life)[1]. Infants from the highlands of Papua New Guinea (PNG) experience early onset of dense upper respiratory tract bacterial carriage leading to one of the highest rates of childhood ALRI mortality in the world[2], with an average incidence of 4.3 episodes in the first 18 months of life, with approximately one third of those being moderate or severe[3]. This fellowship project comprised a systematic investigation of the contribution of genetic factors to the development of immune responses and susceptibility to pneumonia in this population.

The PNG Institute of Medical Research, the Telethon Institute for Child Health Research and the School of Paediatrics and Child Health, University of Western Australia have completed a randomised controlled trial of the 7-valent pneumococcal-CRM197 conjugate vaccine (PCV - Prevnar®) in a population of infants from PNG (NPCV study). Vaccination with PCV that began at birth (Neonatal group - 0, 1 and 2 months) or soon after birth (Infant group - 1, 2 and 3 months) was compared with no PCV (Control group). It should be noted that infants from all three groups received the 23-valent pneumococcal polysaccharide vaccine at nine months of age. The children were followed to 18mths of age to determine the number and severity of each episode of ALRI as well as their development of bacterial carriage and innate and adaptive immunity.

Recruitment of 318 infants was completed in September 2007 and the 18mths of follow-up finished in March 2009. Measurements of antibody levels to each of the seven vaccine antigens at birth, 2, 3 and 4mths of age are complete. Assays to determine T cell responses in cord blood and at 3 months have been completed[4] and the results from samples collected at 9mths are now also available. Follow-up was completed in 253 children with 441 ALRI episodes recorded of which 192 were moderate or severe and 8 children died. There were no striking differences in the total number of ALRIs between vaccination groups. However, infants in the neonatal or infant vaccination groups were on average older when they had their first moderate or severe ALRI compared with infants who did not receive PCV. The immune

and vaccine response components of the study will further elucidate the mechanisms by which PCV may have helped to protect the study children from moderate or severe ALRIs.

RESEARCH TRAINING AND SITE VISITS

The ARC Ann Woolcock Research Fellowship began in March 2005. The travel component of the fellowship funded 3 visits to Goroka, PNG. These were for the purposes of attending both the 2005 PNG Medical Symposium and the PNG Institute of Medical Research 40th Anniversary Colloquium, meeting with the local NPCV study team members and completing DNA extractions on blood samples collected from study children. These visits provided training on the clinical, social and environmental aetiology of lower respiratory infections in a developing country, allowed me to meet with local and international researchers studying the genetic susceptibility of other infectious diseases common in PNG (malaria and lymphatic filariasis) and helped me to establish and maintain the collaborative ties necessary for the success of this fellowship project. Thanks to the assistance of researchers at the PNGIMR and a UWA small grant, my visits to the PNG IMR also produced 283 DNA samples from the 318 study children for genotyping. This was the maximum number of DNA samples that would be available for the project, as a number of infants and their families had withdrawn from the study prior to the 3-month follow-up visit, which included the first blood sample collection.

The ARC also funded attendance at the 2005 European Respiratory Society Annual Congress in Copenhagen where I attended two postgraduate workshops on respiratory infections; the 5th International Symposium on Pneumococci and Pneumococcal Diseases in Alice Springs; a workshop on Consultation with Indigenous Communities in Perth; the 2007 American Thoracic Society International Conference in San Francisco; and partially funded 3 months of training with Prof. Fernando Martinez and his research team at the Arizona Respiratory Center. These training opportunities supported my work on the fellowship project - providing insight into the burden of ALRI in developed countries and further developing my skills in the statistical analysis of longitudinal data, multiple genetic variations/haplotypes and causal pathways.

Since completion of my ARC Ann Woolcock Research Fellowship, I have been awarded the Peter Phelan Paediatric Travel Grant by the Thoracic Society of Australia and New Zealand to present my fellowship results at the 2009 PNG Medical Symposium and the 2009 European Respiratory Society Annual Congress. Presentation in PNG was to inform the community that had supported my project, including community health workers, researchers and PNG Health Department representatives, of the results of my study. My presentation was given at the main body of the symposium rather than in a concurrent session or the more

specialised Biological Sciences Meeting and concluded that human genetic factors are likely to play a significant role in a child's susceptibility to ALRI in PNG. Presentation at the European Respiratory Society meeting was to gain critical review of my fellowship project results from experts in respiratory disease research. This travel award also partially funded another short visit with Prof. Fernando Martinez in order to discuss the ongoing analysis of my fellowship results. I have also recently presented my fellowship results at the 2010 Thoracic Society of Australia and New Zealand Annual Scientific Meeting. Two abstracts were selected by the Respiratory Infectious Diseases special interest group, for presentation in a poster discussion session and one for an oral presentation. The poster entitled "Adaptive immune gene polymorphisms are associated with earlier and increased incidence of ALRI in children from Papua New Guinea (PNG)" was selected as the best poster presentation for the special interest group.

RESULTS

Genotyping of 36 gene sequence variations known to be involved in the development of innate or adaptive immune responses was completed on the 283 DNA samples. As most children had had at least one ALRI, the first phase of statistical analysis assessed the potential contribution of each genetic variation to the age at which they had their first ALRI. Where some evidence of a relationship between a gene and the age of first ALRI was identified, the total number of ALRIs over the follow-up period was also investigated.

Somewhat surprisingly, in the first 66 subjects genotyped, 3 genetic variations involved in the adaptive immune response were not identified and in the whole cohort, another 8 genetic variations involved in innate immune responses were not found or were extremely rare. A previous study in PNG found that another 5 innate immune gene variants were also absent/very rare. Previous investigation of some of these innate immune gene variations in other studies suggest that the gene sequence found in people from PNG may initiate lower immune responses to infections than if the gene variants were present[5]. You might think that having a vigorous immune response would help to protect PNG children from infections and it has been shown that some gene variants that initiate a stronger immune response, are likely to protect people from severe malaria[6]. However, other studies have suggested that gene variants that initiate a stronger innate immune response are associated with sepsis and death from bacterial infection[7]. Therefore it is possible that people from PNG have a particular pattern of immune gene variants that helps to protect them from more severe respiratory infections.

The genotyping results did find 9 innate immune and 19 adaptive immune genetic variants in the study children. The results of the preliminary statistical analyses showed that variants in 3 innate immune genes and 5 adaptive immune genes had evidence of association with an earlier age of first ALRI and 3 of those were associated with an increased total number of ALRIs. Vaccination group did not affect these results. This is the first immunogenetic analysis of a prospective study of childhood ALRI completed in a population from a developing country. These results show that particular genetic factors, in addition to environmental and socio-economic factors, may play a role in the high incidence of ALRI in Papua New Guinea. Furthermore, almost without exception the polymorphisms that show evidence of contributing to increased ALRI susceptibility are more prevalent in African-American and Sub-Sahara African populations than in either Caucasian and PNG populations. This suggests that these polymorphisms may also contribute to the high rates of ALRI found in populations of African origin. The next phase of the statistical analyses will investigate the contribution of these polymorphisms to measures of innate and adaptive immunity in study children at 3 and 9mths of age. Although the genetic analyses have been completed, final data analysis and publication is awaiting morbidity database curation and completion of bacterial carriage, vaccine response and immune development analyses for the 18 months of follow-up, which have experienced significant delays (primarily due to the necessarily large number of assays) and are nearing completion.

In addition to the genetic analysis completed for this fellowship, I have recently completed an investigation of the role of respiratory viruses in the aetiology of ALRI in the NPCV study children, in collaboration with the NPCV study investigators and colleagues at Pathwest Laboratory Medicine. Nasal swab samples from 135 episodes of ALRI and 274 routine visits were analysed by multiplex PCR for the presence of rhinoviruses, respiratory syncytial virus A & B, adenoviruses (Groups B, C, D, E), influenza A-C, parainfluenza virus types 1-4, coronaviruses (229E, HKU1, NL63, OC43), human metapneumovirus, enterovirus, bocavirus, and polyomavirus. Two samples collected during routine visits were matched to each ALRI sample on the basis of age, gender, vaccine group, season of birth and date of collection. Several viruses were more often present in children when sick with an ALRI than in the matched control samples and a large number and variety of rhinoviruses were found in these samples. Interactions between the viruses detected and the bacteria cultured from these samples will be investigated in the future.

Work will continue on the results obtained from this project. However, my Australian Respiratory Council Ann Woolcock Research Fellowship finished last year. I would like to say what an honour it was to be the first recipient of this fellowship and what a pleasure it has

been to work with members of the ARC staff, Research Committee and Board over the last 5 years, particularly Bruce Ramage, David Macintosh, Judy Begnell and more recently Amanda Christensen and Kerrie Shaw.

ACHIEVEMENTS DURING THE TENURE OF THE AUSTRALIAN RESPIRATORY COUNCIL ANN WOOLCOCK RESEARCH FELLOWSHIP

Invited presentations

Australian Respiratory Council launch, Sydney, NSW, March 2006
Prevention

Arizona Respiratory Center, Tucson, Arizona, USA, 14th June 2007
Are children in developing countries more genetically susceptible to ALRI's than children in developed countries?

18th Australasian Society of Clinical Immunology & Allergy Annual Scientific Meeting/Australasian & South East Asian Tissue Typing Association 31st Annual Scientific Meeting, Fremantle, WA, 16th November 2007
Genetics of asthma and allergy – are we getting anywhere?

Australian Respiratory Council, Sydney, NSW, 10th December 2008
The contribution of immune gene variations to the age of first pneumonia in children from the highlands of Papua New Guinea

Japanese Respiratory Society, Tokyo, Japan, 12th June 2009
TLR8 up-regulation during acute asthma in children, identified by micro-array and confirmed by qRT-PCR, is associated with TLR8 genotypes

Arizona Respiratory Center, Tucson, Arizona, USA, 29th October 2009
Pneumonia amongst Papua New Guinean Infants

Publications

ARC acknowledged as a source of support.

Book chapter:

Laing IA and PN Le Souef, Association Studies in Asthma, in *Genetics of Asthma and COPD* Ed. Dirkje Postma and Scott T Weiss, 2007, Informa Healthcare USA, Inc.

Peer reviewed journal articles:

1. Tulic MK, Hurrelbrink RJ, Prêle CM, **Laing IA**, Upham JW, Le Souef PN, Sly PD, Holt PG. TLR4 polymorphisms mediate impaired responses to respiratory syncytial virus and lipopolysaccharide. *Journal of Immunology* 2007; 179:132-40.
2. Hales BJ, **Laing IA**, Hazell LA, Pearce LJ, Mills KL, Chua KY, Thornton RB, Richmond P, Musk AW, James AL, LeSouef PN, Thomas WR. Distinctive immunoglobulin E anti-house dust allergen-binding specificities in a tropical

Australian Aboriginal community. *Clinical and Experimental Allergy* 2007; 37:1357-63.

3. **Laing IA**, de Klerk NH, Turner SW, Judge PK, Hayden CM, Landau LI, Goldblatt J, Le Souëf PN. Cross-sectional and longitudinal association of the secretoglobin 1A1 gene A38G polymorphism with asthma phenotype in the Perth Infant Asthma Follow-up (PIAF) Cohort. *Clin Exp Allergy* 2009; 39:62-71. (editorial comment Clin Exp Allergy 2009; 39:8-11).
4. Hales B, Martin AC, Pearce LJ, Rueter K, Zhang G, Khoo SK, Hayden CM, Bizzintino J, McMinn P, Geelhoed GC, Goldblatt J, Lee W-M, **Laing IA**, LeSouëf PN, Thomas WR. Anti-bacterial IgE in the antibody responses of house dust mite allergic children convalescent from asthma exacerbation. *Clinical and Experimental Allergy* 2009; 39:1170-8.
5. Subrata LS, Bizzintino J, Mamessier E, Bosco A, McKenna KL, Wikström ME, Goldblatt J, Sly PD, Hales BJ, Thomas WR, **Laing IA**, Le Souëf PN, Holt PG. Interactions between innate antiviral and atopic immunoinflammatory pathways precipitate and sustain asthma exacerbations in children. *The J Immunol* 2009; 183:2793-800.

Publications on work that preceded support from the ARC include:

1. Martin AC, **Laing IA**, Khoo SK, Zhang G, Rueter K, Teoh L, Taheri S, Hayden CM, Geelhoed GC, Goldblatt J and Peter N Le Souef. Acute asthma in children: relationship between CD14 and CC16 genotype, plasma levels and severity. *American Journal of Respiratory and Critical Care Medicine* 2006; 173:617-22.
2. Hales BJ, Martin AC, Pearce LJ, **Laing IA**, Hayden CM, Goldblatt J, Le Souëf PN and WR Thomas. IgE and IgG anti-house dust mite specificities in allergic disease. *Journal of Allergy and Clinical Immunology* 2006; 118:361-7.
3. Wiertsema SP, Khoo SK, Baynam G, Veenhoven RH, **Laing IA**, Zielhuis GA, Rijkers GT, Goldblatt J, LeSouëf PN and EAM Sanders. Association of the CD14 promoter polymorphism with otitis media and pneumococcal vaccine responses. *Clinical and Vaccine Immunology* 2006; 13:892-7
4. Zhang G, Hayden CM, Khoo SK, **Laing IA**, Turner S, Landau L, Goldblatt J and Le Souëf PN. Association of haplotypes of ß2-adrenoceptor polymorphisms with lung function and airway responsiveness in a paediatric cohort. *Pediatric Pulmonology* 2006; 41:1233-41.
5. Wiertsema SP, Baynam G, Khoo SK, Veenhoven RH, van Heerbeek N, Zhang B, **Laing IA**, Rijkers GT, Goldblatt J, Sanders EAM, LeSouef PN, Association of

genetic variants in IL-4, IL-4RA and IL-13 with the anti-pneumococcal antibody response. *Vaccine* 2007; 25:306-13.

6. Baynam G, Khoo SK, Rowe J, Zhang G, **Laing I**, Hayden C, Kusel M, de Klerk N, Sly P, Goldblatt J, Holt P, Le Souef P. Parental smoking impairs vaccine responses in children with atopic genotypes *Journal of Allergy and Clinical Immunology* 2007; 119:366-74.
7. Zhang G, Hayden CM, Khoo SK, Candelaria PV, **Laing IA**, Turner S, Franklin P, Stick S, Landau L, Goldblatt J, Le Souëf PN. Beta2-Adrenoceptor polymorphisms and asthma phenotypes: interactions with passive smoking. *European Respiratory Journal* 2007; 30:48-55.
8. Martin AC, Zhang G, Rueter K, Khoo S-K, Bizzintino J, Hayden CM, Geelhoed GC, Goldblatt J, **Laing IA**, Le Souëf PN. β2-adrenoceptor polymorphisms predict response to β2-agonists in children with acute asthma *J Asthma* 2008; 45:383-8.
9. Ali M, Zhang G, Thomas WR, McLean CJ, Bizzintino JA, **Laing IA**, Martin AC, Goldblatt J, Le Souëf PN, Hayden CM. Investigations into the role of ST2 in acute asthma in children. *Tissue Antigens* 2009; 73:206-12.
10. Bizzintino JA, Khoo S-K, Zhang G, Martin AC, Rueter K, Geelhoed GC, Goldblatt J, **Laing IA**, Le Souëf PN and Hayden CM. Leukotriene pathway polymorphisms are associated with altered cysteinyl leukotriene production in children with acute asthma. *Prostaglandins, Leukot Essent Fatty Acids* 2009; 81:9-15.
11. Candelaria PV, Backer V, Khoo S-K, Bizzintino JA, Hayden CM, Baynam G, **Laing IA**, Zhang G, Porsbjerg C, Goldblatt J, LeSouëf PN. The Importance of Environment on Respiratory Genotype/Phenotype Relationships in the Inuit. Allergy – accepted 11th June 2009

Student supervision

Chris Chan

Summer student (November 2006 – January 2007)

recipient of a Human Genetics Society of Australasia Summer Vacation Scholarship

Offered an Asthma Foundation of WA Summer Scholarship

Offered a Faculty of Medicine and Dentistry Summer Scholarship

Joelene Bizzintino

PhD student (Feb 2007 – present)

recipient of an Australian Postgraduate Award.

Ashraf Sharafi

Higher degree by research preliminary project (Feb – July 2007)

Sing Ching Lee

BMedSci (Feb – Nov 2008)

recipient of the Jean Rogerson Undergraduate Studentship, awarded 1st Class Honours.

Prizes and grants

2005 UWA Research Grants Scheme "Genetic influences on early bacterial colonisation and infection in infants from PNG with high rates of pneumonia"; $21 633; Ingrid Laing.

2007 Certificate of high commendation, Qantas New Investigator Award, Telethon Institute for Child Health Research

2007 UWA supplementary travel grant for new academic staff

2007 Friends of the Telethon Institute for Child Health Research Travel Grant

2007 Best presentation (joint winner), Respiratory Molecular Genetics Special Interest Group, TSANZ Annual Scientific Meeting

2008 NH&MRC Equipment Grant; "Applied Biosystems 7900HT Fast Real-Time PCR System with 384-well, 96-well and micro-fluidic array adapter and automation unit for plate loading"; $100 000; Jenefer Blackwell, Ursula Kees, Wayne Thomas, Susan Prescott, Peter Richmond, Prue Hart, Peter Dallas, Sarra Jamieson, David Burgner, Anita van den Biggelaar, Selma Wiertsema and Ingrid Laing.

Invitations to serve on committees

1. Epigenetics 2007 – Australian Scientific Conference Organising Committee
   June 2006 – November 2007
2. Asthma Foundation of Western Australia Research Committee
   October 2007 – present
3. Thoracic Society of Australia and New Zealand WA Branch Executive Committee
   October 2008 – present

REFERENCES

1. Rudan I, Tomaskovic L, Boschi-Pinto C, Campbell H. Global estimate of the incidence of clinical pneumonia among children under five years of age. *Bull World Health Organ* 2004; 82:895-903.

2. Coakley K, Lehmann D, Smith D. The Asaro valley surveillance unit of the Papua New Guinea Institute for Medical Research: methodology, demography and mortality report. *Garoka: Papua New Guinea Institute for Medical Research*, 1993.
3. Smith TA, Lehmann D, Coakley C, Spooner V, Alpers MP. Relationships between growth and acute lower-respiratory infections in children aged less than 5 y in a highland population of Papua New Guinea. *Am J Clin Nutr* 1991; 53:963-70.
4. van den Biggelaar AHJ, Richmond PC, Pomat WS, Phuanukoonnon S, Nadal-Sims MA, Devitt CJ, Siba PM, Lehmann D, Holt PG, Neontatal pneumococcal conjugate vaccine immunization primes T cells for preferential Th2 cytokine expression: A randomised controlled trial in Papua New Guinea. *Vaccine* 2009; 27:1340-7.
5. Kormann MSD, Depner M, Hartl D, Klopp N, Illig T, Adamski J, Vogelberg C, Weiland SK, von Mutius E, Kabesh M, Toll-like receptor heterodimer variants protect from childhood asthma. *J Allergy Clin Immunol* 2008; 122: 86-92.
6. Mockenhaupt FP, Cramer JP, Hamann L, Stegemann MS, Eckert J, Oh NR, Otchwemah RN, Dietz E, Ehrhardt S, Schroder NWJ, Bienzle U, Schumann RR, Toll-like receptor (TLR) polymorphisms in African children: Common TLR-4 variants predispose to severe malaria. *PNAS* 2006; 103: 177-82.
7. Wurfel MM, Gordon AC, Holden TD, Radella F, Strout J, Kijikawa O, Ruzinski JT, Rona G, Black RA, Stratton S, Jarvik GP, Hajjar AM, Nickerson DA, Reider M, Sevransky J, Maloney JP, Moss M, Martin G, Shanholtz C, Garcia JGN, Gao L, Brower R, Barnes KC, Walley KR, Russell JA, Martin TR, Toll-like receptor 1 polymorphisms affect innate immune responses and outcomes in sepsis. *Am J Respir Crit Care Med* 2008; 178:710-20.

Photo captions

1. NPCV study team 2005.
2. NPCV study team 2009.