

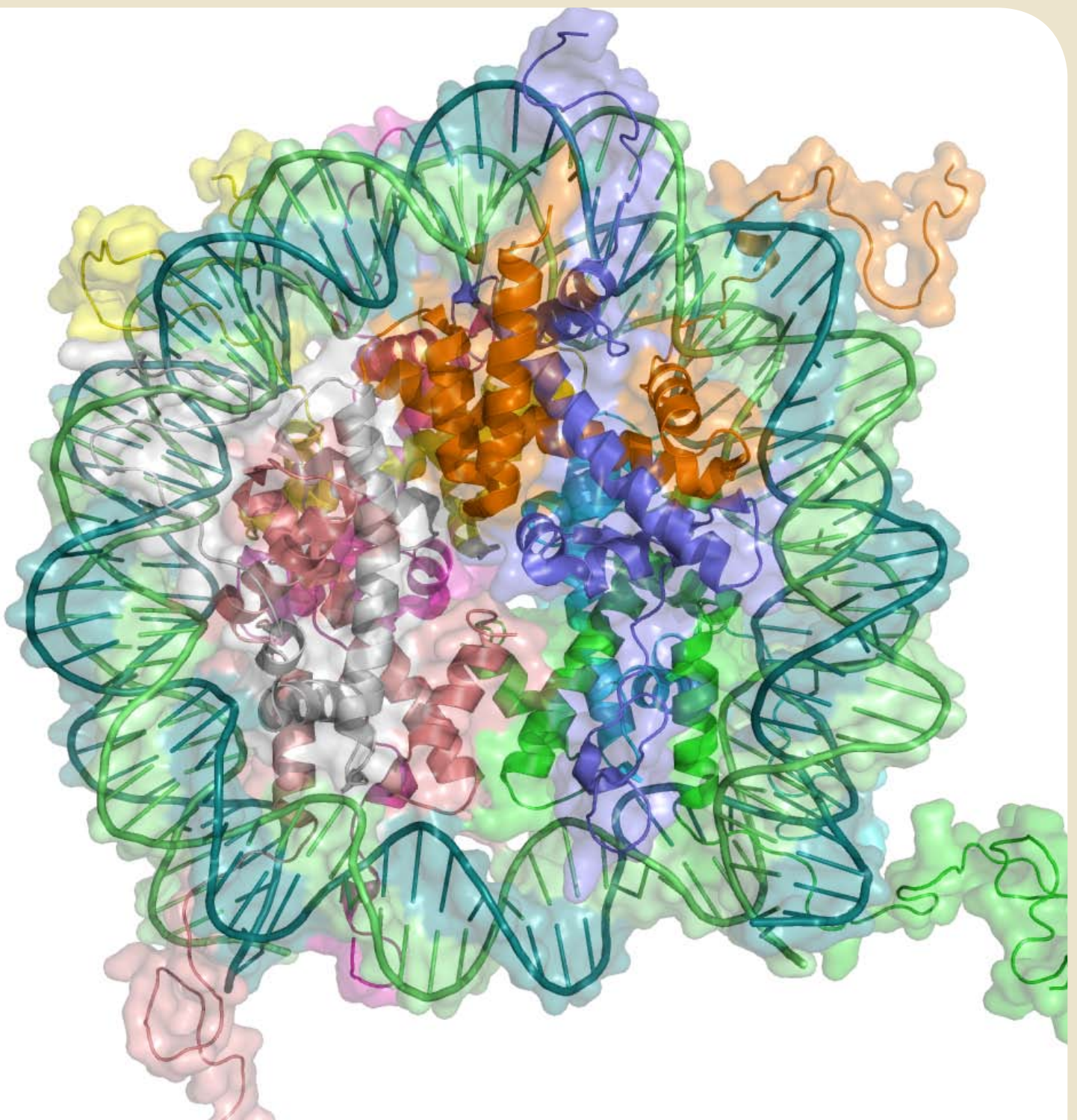


THE UNIVERSITY OF  
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# Epigenetics and Human Health

20 -21 August 2009, University Club, UWA

Convenor: David Ravine, Professor of Medical Genetics,  
Western Australian Institute for Medical Research, The University of Western Australia



# Epigenetics and Human Health

## PROGRAM

Thursday 20 August

6.00pm **Keynote Public Lecture: Epigenetics Explained**  
Professor Emma Whitelaw, NHMRC Australia Fellow, Queensland Institute of Medical Research  
*University Club Theatre Auditorium*

Friday 21 August - Symposium

8.30am Coffee and Registration

9.00am **Welcome and Overview**  
Professor David Ravine, Convenor

9.15am–10.00am **The Pre-Implantation Environment of the Mouse Embryo affects Epigenetic Reprogramming and Post-Natal Gene Expression**  
Hugh Morgan, Sydney Centre for Developmental and Regenerative Medicine, The Kolling Institute, The University of Sydney, Royal North Shore Hospital

10.00am–10.45am **Epigenetics and Birth Defects**  
Jane Halliday, Head of Public Health Genetics, Murdoch Childrens Research Institute, Melbourne

10.45am–11.15am Morning tea

11.15am–Noon **Germline Epimutation as a Basis for Human Disease**  
Catherine Suter, Head, Epigenetics Laboratory, Victor Chang Cardiac Research Institute

Noon–12.45pm **Rett Syndrome – Towards Decrypting the Epigenetics of Autism**  
David Ravine, Professor of Medical Genetics, School of Pathology and Laboratory Medicine, Western Australian Institute for Medical Research, The University of Western Australia

12.45pm–1.30pm Lunch

1.30pm–2.15pm **Epigenetic Inheritance – Sorting Fact from Fiction**  
Emma Whitelaw, NHMRC Australia Fellow, Queensland Institute of Medical Research

2.15pm–3.00pm **Prospects for Epigenetic Therapy**  
Ricky Johnstone, Group Leader, Gene Regulation Laboratory, Cancer Therapeutics Program, The Peter MacCallum Cancer Institute, Melbourne

3.00pm–3.30pm Afternoon tea

3.30pm–4.15pm **Epigenetic Silencing of Domains in Cancer**  
Susan Clark, Principal Research Fellow; Group Leader, Epigenetics Research Group, Cancer Research Program, Garvan Institute of Medical Research; NHMRC Principal Research Fellow; Professor, Faculty of Medicine, The University of New South Wales.

4.15pm–5.00pm **Epigenetic Analysis in Twins**  
Jeff Craig, Co-Head, Developmental Epigenetics Group, Murdoch Childrens Research Institute, Melbourne

5.00pm–5.30pm **Opportunities for Epigenetic Research in Western Australia**  
Panel discussion

5.30pm Close and Sundowner

## Abstracts

### **The Pre-Implantation Environment of the Mouse Embryo affects Epigenetic Reprogramming and Post-Natal Gene Expression**

Hugh Morgan, Sydney Centre for Developmental and Regenerative Medicine, The Kolling Institute, The University of Sydney, Royal North Shore Hospital, St Leonards, New South Wales

The growth environment of the foetus is known to influence postnatal homeostasis. This Centre has previously shown that the pre-implantation environment of the embryo affects short- and long-term gene expression that can persist for two generations. The mechanism for the persistence of this affect is not known. Epigenetic modification of the genome could explain persistently altered gene expression because epigenetic modifications can be maintained through mitosis and affect gene expression.

We have recently shown that the pre-implantation environment of the embryo affects the expression of an epigenetically regulated gene. We used the Avy metastable epiallele, which is sensitive to epigenetic perturbations. We altered the pre-implantation environment of the embryos using embryo culture, transferred the embryos to recipient mothers and analysed the coat colour of the resulting pups. We saw that embryo culture and transfer changes the expression and epigenetic modifications of the Avy allele in these pups.

We have examined epigenetic modifications in pre-implantation embryos using immunofluorescence. We have described for the first time the loss of DNA methylation of the maternal and paternal genomes in zygotes that develop in vivo. We find that the environment of the zygote can reduce the loss of DNA methylation in the zygote. We also find that changing the epigenetic reprogramming in the pre-implantation embryo affects the establishment of the pluripotent inner cell mass of the blastocyst.

These show that the environment of the embryo can alter epigenetic reprogramming in the embryo, and expression of epigenetically regulated genes in the resulting offspring.

### **Epigenetics and Birth Defects**

Jane Halliday, Head of Public Health Genetics, Murdoch Children's Research Institute, Parkville, Victoria

Most birth defects are thought to result from a mixture of genetic and environmental factors, although the underlying pathogenesis remains unknown. Recent evidence suggests that epigenetic effects may help to explain the mechanisms by which many birth defects are arising, including gene-environment interactions. Some examples of epigenetic effects on development of birth defects will be provided:

- 1) Nutritional deprivation of co-factors required for re-establishment and maintenance of the genomic methylation pattern (folic acid) has been shown to increase the risk of birth defects, in particular neural tube defects and most recently congenital heart defects.
- 2) Mutations in genes that produce proteins involved in maintaining and modifying DNA methylation (e.g. MECP2 and ATRX on the X chromosome) can cause Rett Syndrome and alpha thalassemia respectively
- 3) De novo DNA methylation of
  - a) imprinted genes (e.g. H19/IGF2 on chromosome 11) can lead to birth defects such as Beckwith Wiedemann syndrome.
  - b) the repeat sequence in the FMR1 gene leading to fragile X mental retardation

These de novo methylation alterations may be due to some critical environmental exposure, such as those associated with assisted reproductive technologies.

There may in fact be many environmental determinants of birth defects that act through an influence on the timing and pattern of re-methylation after conception and with advances in technology and understanding of the epigenome, that may in time be increasingly recognised.

## Germline Epimutation as a Basis for Human Disease

Catherine M Suter, Head, Epigenetics Laboratory, Victor Chang Cardiac Research Institute, Darlinghurst, New South Wales

Aberrant silencing of a normally active gene, termed “epimutation”, can result from errors in the complex system of epigenetic gene regulation typical of higher eukaryotes. We sought germline epimutations in humans as a test of the hypothesis that this constitutively active system of epigenetic silencing will sometimes result in abnormal silencing of a gene in the germline. In testing this hypothesis we identified individuals in whom one allele of the gene encoding the DNA mismatch repair protein MLH1 is epigenetically silenced throughout the soma (implying a germline event). There are now several dozen documented cases of MLH1 germline epimutation: these individuals fit clinical criteria for hereditary nonpolyposis colorectal cancer, which is usually produced by germline mutation of MLH1, yet none of the affected individuals have any genetic abnormality that would explain the epimutation, or their phenotype. In most instances the epimutation has affected only one individual within a kindred, but at least two reported cases exist in which apparent inheritance has occurred. Thus an epimutation can occur (and be maintained) in the germline, but inheritance appears to be weak. We believe that germline epimutation is not necessarily the result of anything other than chance. Epigenetic phenomena tend to be stochastic, reversible, and mosaic, so it is no surprise that epimutations are proving to have rules completely different from those of Mendelian genetics. The unique nature of germline epimutation in humans and the broader biological implications of this phenomenon will be discussed.

## Rett Syndrome - Towards Decrypting the Epigenetics of Autism

David Ravine, Professor of Medical Genetics, School of Pathology and Laboratory Medicine, Western Australian Institute for Medical Research, The University of Western Australia

Autism is typically characterized by early childhood onset of impaired reciprocal communication and speech, social withdrawal and repetitive hyperfocused behaviors. Over the past decade there has been a 10-fold increase in the rate of autism diagnosis, although it remains uncertain whether autism is truly increasing in frequency. The estimated heritability of autism, based on 4 twin studies, is >90%. From this remarkably high value, autism has been regarded as one of the most hereditary disorders in psychiatry. In this setting it is notable that the search for susceptibility genes has so far proven to be relatively unrewarding.

From the work of Whitelaw and colleagues it is now appreciated that heritability embraces both genetic and epigenetic inheritance. This awareness coincides with the progressive emergence of evidence supportive of epigenetic changes contributing to the occurrence of autism. Cases arising from duplications of the Prader-Willi/ Angelman domain on chromosome 15 reveal that abnormalities of genomic imprinting contribute to the occurrence of autism. There is also evidence of brain specific epigenetic abnormalities involving the *UBE3A* and *MECP2* genes, both of which are well known to clinical geneticists and paediatricians as harbouring mutations responsible for the severe neurodevelopmental disorders Angelman syndrome and Rett syndrome.

Recent findings of reduced MeCP2 expression in up to 80% of autism brain samples, as well as in brains from Prader-Willi and Angelman syndrome patients, has prompted some to suggest that Rett syndrome is akin to a “Rosetta Stone” for understanding autism. Curiously, MeCP2 has a central place in epigenetic biology, as it contributes to the molecular mechanism responsible for DNA methylation-induced histone changes altering the structure of chromatin. In fact, multiple pathways lead to MeCP2 in the aetiology of autism spectrum-associated neurodevelopmental disorders, including recent findings of aberrant MeCP2 promoter hypermethylation in frontal cortex tissue from autistic juveniles. MeCP2 is also involved in the regulation of several imprinted genes linked to autism, including *UBE3A*. There are also reports of reduced methylation capacity arising from abnormal methionine metabolism in autistic children and also, surprisingly, in their parents. Studies are now underway to determine whether the abnormal profile in parents reflects linked genetic polymorphisms in these pathways or, alternatively, the chronic stress of coping with an autistic child.

Although Rett syndrome and related disorders are relatively rare, it is becoming increasingly apparent that recent advances in understanding the altered neurobiology of these serious neurodevelopmental disorders are serving also to offer important new insights into the biological basis of autism, particularly epigenetic abnormalities that have the potential to be reversible.

## Epigenetic Inheritance - Sorting Fact from Fiction

Neil Youngson, Suyinn Chong, Nadia Whitelaw, Alyson Ashe, Marnie Blewitt, Nicola Vickaryous and [Emma Whitelaw](#),  
Queensland Institute of Medical Research

It is well recognised that there is a surprising degree of phenotypic variation among genetically identical individuals even when the environmental influences, in the strict sense of the word, are controlled. Genetic textbooks acknowledge this fact and use different terms such as “intangible variation” or “developmental noise” to describe it. We believe that this intangible variation results from the stochastic establishment of epigenetic modifications to the DNA nucleotide sequence. These modifications, which involve cytosine methylation and chromatin remodelling, result in alterations in gene expression which, in turn, affects the phenotype of the organism. Recent evidence, from our work and that of others, suggests that these epigenetic modifications, which in the past were thought to be cleared and reset on passage through the germline, may sometimes be inherited to the next generation. This is termed epigenetic inheritance, and while this process has been well recognised in plants, the recent findings in mice force us to consider the implications of this type of inheritance in mammals. We have recently identified some of the proteins involved in this process in the mouse and these findings will be presented. At this stage, we do not know how extensive this phenomenon is in humans but it may turn out to be the explanation for some diseases which appear to be sporadic or show only weak genetic linkage.

## Prospects for Epigenetic Therapy

Dr Ricky Johnstone, Group Leader, Gene Regulation Laboratory, Cancer Therapeutics Program, The Peter MacCallum Cancer Institute, Smorgon Family Building, St Andrews Place, East Melbourne, Victoria

Epigenetic changes including histone acetylation, histone methylation, and DNA methylation are now thought to play important roles in the onset and progression of cancer in numerous tumor types. Indeed dysregulated epigenetic modifications, especially in early neoplastic development, may be just as significant as genetic mutations in driving cancer development and growth. The reversal of aberrant epigenetic changes has therefore emerged as a potential strategy for the treatment of cancer.

A number of compounds targeting enzymes that regulate histone acetylation, histone methylation and DNA methylation have been developed as epigenetic therapies, with some demonstrating efficacy in hematological malignancies and solid tumors. Histone deacetylase inhibitors (HDACi) are emerging as important and efficacious anti-cancer agents and although their mechanisms of action remain to be fully defined, these agents can clearly induce tumor cell death (apoptosis) and inhibit tumor cell proliferation. We have utilised genetically engineered mouse models of cancer to identify the genes and molecular pathways necessary for HDACi-mediated apoptosis and subsequent therapeutic efficacy. Using this information, we have rationally designed combination therapy regimens that demonstrate superior anti-tumor activity compared to single agents.

## Epigenetic Silencing of Domains in Cancer

Susan J. Clark, Principal Research Fellow; Group Leader, Epigenetics Research Group, Cancer Research Program, Garvan Institute of Medical Research; NHMRC Principal Research Fellow; Professor, Faculty of Medicine, The University of New South Wales

Despite the completion of the Human Genome Project we are still far from understanding the molecular events underlying epigenetic *change* in cancer<sup>1</sup>. Even though it is now accepted that tumour suppressor genes, with CpG island-associated promoters, are commonly hypermethylated and silenced in cancer, we do not understand what triggers this process or when it occurs during carcinogenesis. Epigenetic gene silencing has always been envisaged as a local event silencing discrete genes, but recent data indicates that large regions of chromosomes also can be co-coordinately suppressed; a process we termed long range epigenetic silencing (LRES)<sup>2,3</sup>. LRES can span contiguous DNA regions and involves broad heterochromatin formation accompanied by hypermethylation of clusters of contiguous CpG islands within the region. To determine the propensity of LRES in cancer cells, we have integrated in vivo gene expression profiles from clinical samples and epigenome tiling arrays to map DNA methylation and histone modifications. We have now identified 47 LRES regions in prostate cancer, each spanning ~ 2Mb. We find that adjacent genes are commonly changed to the same epigenetic silencing state, by replacement and reinforcement of repressive histone and DNA methylation marks, implicating a deregulation of the epigenome in domains that span multiple genes.

Our results show for the first time that in cancer both long range epigenetic silencing and loss of heterozygosity combine to reduce plasticity by an overall reduction of the accessible genome.

References:

1. Jones P et al (2008) Moving AHEAD with an international human epigenome project. *Nature* 454: 711-715.
2. Frigola J, Song J, Stirzaker C, Hinshelwood RA, Peinado MA and Clark SJ (2006). Epigenetic remodeling in colorectal cancer results in coordinate gene suppression across an entire chromosome band. *Nat Genet* 38: 540-549.
3. Clark SJ (2007) Action at a distance: Epigenetic silencing of large chromosomal regions in carcinogenesis. *Hum Mol Genet* 16: R88-R95.

### **Epigenetic Analysis in Twins**

Jeffrey M Craig, Ruth Morley, Eric Joo, Miina Ollikainen, Roberta Andronikos, Lavinia Gordon, Katherine Smith, Gordon Smyth, Alicia Oshlak, Richard Saffery. Developmental Epigenetics Group, Murdoch Childrens Research Institute, Parkville, Victoria

Twins have proved invaluable for investigating contributions of nature and nurture to phenotype. Only recently has epigenetics entered the equation. We already knew that monozygotic (MZ) twins, derived from a single zygote and assumed to have identical DNA, can be discordant for simple and complex disease. The study of Esteller and colleagues in 2005 began to lift the lid of epigenetic differences between MZ twins. They found that older twins exhibited more epigenetic differences than younger twins, suggesting that “epigenetic drift” occurs during the life course. Other studies have looked at epigenetic variation (mainly DNA methylation) at specific genes, such as imprinted genes, which may be more responsive to the environment. Recently, the Petronis group performed a genome-wide search for methylation differences between teenage MZ and DZ twin pairs and made some controversial conclusions about the role of epigenetics in the twinning process itself. Our main aim is to look specifically for epigenetic differences within twin pairs at birth – focusing on those changes that have accumulated in utero.

Studying MZ twin pairs controls largely for genotype and enables us to investigate the extent of microenvironment-induced and stochastic epigenetic changes in genetically identical newborns. We hypothesise that epigenetic differences begin to emerge from conception and accumulate throughout the life course. Studying dizygotic twins enables us to examine potential contribution of genetic variation in phenotypic and epigenetic variation, controlling largely for environment. For the former comparison, we are focusing on low birth weight and in the latter, on the folate pathway. We are also asking twin-independent questions about the influence of maternal environment on epigenetic state in all newborns (focusing on global methylation levels). We are close to finishing recruiting 250 mothers pregnant with twins, have collected extensive environmental data and maternal blood at 28 weeks gestation, and collected multiple biological samples from newborn twins. A follow up at 18 months is also in progress to assess epigenetic change over time in two tissues. Preliminary data obtained from expression and methylation arrays will be presented in addition to a parallel focus on imprinted genes.

## Speaker Biographies

**Professor Susan Clark is a Principal Research Fellow; Group Leader, Epigenetics Research Group, Cancer Research Program at the Garvan Institute of Medical Research; She is also NHMRC Principal Research Fellow and a Professor in the Faculty of Medicine, The University of New South Wales.**

Professor Susan Clark's research over the last 15 years working has contributed significantly to the field of DNA methylation in development and cancer, both with the advancement of new technologies for methylation detection, as well as in the understanding of the biological processes that lead to abnormal methylation patterns in cancer. Sue Clark's group was responsible for developing the bisulphite sequencing protocol that was pioneered in Dr Marianne Frommer's lab in the early 1990s and this technique is now well established internationally as the best method to detect methylated cytosines in a DNA sequence. More recently, Professor Clark's research interest has extended to include histone modifications associated with gene silencing.

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**Dr Jeff Craig is co-head (with Dr Richard Saffery) of the Developmental Epigenetics Group at the Murdoch Childrens Research Institute.**

Having established a range of technologies to characterise DNA methylation changes and histone modifications that contribute to human disease, Jeff and Richard have developed strong collaborations with groups working on depression, schizophrenia, and childhood leukaemia. They have also established their own mothers and twins birth cohort, with plans afoot for a prematurity and very low birth weight cohort.

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**Associate Professor Jane Halliday is head of Public Health Genetics at the Murdoch Childrens Research Institute and the consultant epidemiologist to the Victorian Birth Defects Register.**

Jane Halliday's epidemiological research has made major contributions to the monitoring and surveillance of birth defects, including imprinting disorders and other birth defects arising early in embryonic development following assisted reproduction. The role of epigenetic mechanisms is unknown at this stage, but continued research into environmental exposures is warranted to understand aetiology of these birth defects.

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**Associate Professor Ricky W. Johnstone is Group Leader, Gene Regulation Laboratory, Cancer Therapeutics Program, The Peter MacCallum Cancer Institute.**

Dr Johnstone has made seminal contributions to the understanding of how tumours become resistant to chemotherapeutic drugs. He is also interested in the molecular events underpinning drug-induced cancer cell death (apoptosis). Dr Johnstone's search for compounds that can overcome cancer cell's resistance to apoptosis has led to his current work with histone deacetylase inhibitors (HDACi), which regulate gene transcription through chromatin remodelling. He has made key discoveries defining novel molecular mechanisms of action of structurally diverse HDACi and with clinical colleagues at the Peter MacCallum Cancer Centre has initiated clinical trials with two HDACi for the treatment of T cell lymphoma. He is also interested in the therapeutic potential of drugs that alter other epigenetic mechanisms, including DNA methylation of promoter regions of tumor-suppressor genes.

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**Dr Hugh Morgan is a Post-Doctoral Scientist with the Human Reproduction Unit, Royal North Shore Hospital, University of Sydney, NSW.**

Dr Morgan's research is focussed on understanding the expression, organisation and function of imprinted genes. He is particularly interested in factors that disrupt normal epigenetic programming in pre-implantation embryos, research that is vital to understanding the epigenetic abnormalities that sometimes arise with use of human assisted reproduction technologies (ART) such as IVF.

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**Dr David Ravine is Professor of Medical Genetics, School of Pathology and Laboratory Medicine, Western Australian Institute for Medical Research, The University of Western Australia**

After graduating from University of Western Australia, Dr Ravine trained in paediatrics and then in medical genetics at the Royal Children's Hospital, Melbourne and at the Institute of Medical Genetics, University Hospital of Wales, Cardiff in the United Kingdom. Since returning to Perth, his research focus has been on Rett syndrome and the related disorder autism. There is increasing evidence that clinical features in both disorders occur as a result of disturbance to normal epigenetic processes within the brain. David's work is conducted within the Western Australian Institute for Medical Research, and he also collaborates with colleagues within the Telethon Institute for Child Health Research and the Children's Hospital at Westmead in Sydney.

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**Dr Catherine Suter is head of the Epigenetics Laboratory at the Victor Chang Cardiac Research Institute in Sydney.**

With a long-standing interest in the role that epigenetics plays in phenotypic variation and human disease, Catherine Suter's recent work has focused on epimutations. Catherine's group is also interested in the role of nutrition and in utero environment in epigenetic variation and inheritance and have recently found that vitamin supplementation in genetically identical mice can have a health effect that is mediated by epigenetic changes and persists for generations.

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**Emma Whitelaw is an NHMRC Australia Fellow at the Queensland Institute of Medical Research.**

After completing her undergraduate degree at the Australian National University, she obtained a D.Phil at the University of Oxford and remained working in London and Oxford for the next fifteen years, moving back to Australia in 1991. She was offered a Senior Lectureship at the University of Sydney and carried out both teaching and research. She has focused her research on eukaryotic transcription using the mouse as a model organism. Her most notable research achievements are in the area of epigenetics. In particular, her studies on the transgenerational inheritance of epigenetic marks have stimulated a great deal of interest from the wider scientific community. In 2008 she was awarded an Australia Fellowship, the most prestigious fellowship in medical research in Australia.

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