John Christodoulou ORCID iD: 0000-0002-8431-0641

Wendy Gold ORCID iD: 0000-0003-1808-0646

Genome-wide transcriptomic and proteomic studies of Rett syndrome mouse models identify common signalling pathways and cellular functions as potential therapeutic targets.

Rahul Krishnaraj¹, Florencia Haase³, Bronte Coorey³, Edward J Luca², Ingar Wong³, Alexandra Boyling³, Carolyn Ellaway^{1,4,5}, John Christodoulou^{*1,4,5,6} and Wendy A Gold^{*1,3,4}

¹ Genetic Metabolic Disorders Research Unit, Western Sydney Genetics Program, The Children's Hospital at Westmead, Sydney, NSW, Australia

² The University of Sydney, University Library, Sydney, NSW, Australia

³ Molecular Neurobiology Research Lab, Kids Research, Sydney Children's Hospitals Network, Westmead, Australia

⁴ Discipline of Child and Adolescent Health, The University of Sydney, Sydney, NSW Australia

⁵ Genetic Medicine, Sydney Medical School, The University of Sydney, Sydney, NSW Australia

⁶ Brain and Mitochondrial Research Group, Murdoch Children's Research Institute, and Department of Paediatrics, Melbourne Medical School, University of Melbourne, Melbourne, Australia

* Co-last authors

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Correspondence to Wendy Gold

Molecular Neurobiology Research Lab

Kids Research

The Children's Hospital at Westmead,

Locked Bag 4001

Sydney, NSW, Australia

Email: wendy.gold@sydney.edu.au

ABSTRACT

The discovery that Rett syndrome is caused by mutations in the *MECP2* gene has provided a major breakthrough in our understanding of the disorder. However, despite this, there is still limited understanding of the underlying pathophysiology of the disorder hampering the development of curative treatments. Over the years, a number of animal models have been developed contributing to our knowledge on the role of *MECP2* in development and improving our understanding of how subtle expression levels affect brain morphology and function. Transcriptomic and proteomic studies of animal models are useful in identifying perturbations in functional pathways and providing avenues for novel areas of research into disease. This review focuses on published transcriptomic and proteomic studies of mouse models of Rett syndrome with the aim of providing a summary of all the studies, the reported dysregulated genes and functional pathways that are found to be perturbed. The 36 articles identified highlighted a number of dysfunctional pathways as well as perturbed biological networks and cellular functions including synaptic dysfunction and neuronal transmission, inflammation and mitochondrial dysfunction. These data

reveal biological insights that contribute to the disease process which may be targeted to investigate curative treatments.

KEYWORDS:

Rett syndrome, *MECP2*, transcriptomic, proteomic, mouse model, pathways

INTRODUCTION

Rett syndrome (Rett syndrome; MIM# 312750) is a severe X-linked neurological disorder which mostly affects females and is the second most common cause of severe intellectual disability in females after Down syndrome (Ellaway and Christodoulou, 2001). Rett syndrome is recognised as a disorder of neuronal plasticity characterized by the loss of intellectual functioning, fine and gross motor skills, communication skills, deceleration of head growth and the development of stereotypic hand movements occurring after a period of apparently normal development (Neul et al., 2010). The disorder is mostly caused by mutations in the gene encoding Methyl CpG binding protein 2 (*MECP2*) (Amir et al., 1999), a multifunctional protein which is highly expressed in the brain. The *MECP2* gene is located on the long arm of the X chromosome at the Xq28 locus (Liyanage and Rastegar, 2014) and due to alternative splice sites, gives rise to 2 distinct isoforms, MeCP2e1 and MeCP2e2 (Kriaucionis and Bird, 2004, Mnatzakanian et al., 2004). MeCP2 was first purified in 1992, where it was identified as a chromatin associated nuclear protein capable of binding methylated CpG sites (Lewis et al., 1992). The protein is ubiquitously expressed but found to be most abundantly expressed in mature neurons (Kriaucionis and Bird, 2004). Initially, MeCP2 was believed to function as a transcriptional repressor, given its ability to bind methylated DNA and recruit chromatin remodelling complexes containing histone deacetylase (HDAC) enzymes (Jones et al., 1998, Nan et al.,

1998). However, more recent studies have demonstrated a MeCP2-mediated mechanism for activation of multiple genes, including Brain Derived Neurotrophic Factor (BDNF) and Glucose Transporter 3 (GLUT3) (Chahrour et al., 2008, Chen et al., 2013). MeCP2 also regulates microRNA processing (Cheng et al., 2014) and is also considered an epigenetic regulator (McGraw et al., 2011). It is now widely accepted that expression of MeCP2 is critical for maintaining normal neuronal and synaptic function. Many studies have been conducted on murine and patient samples (e.g. blood and post-mortem brain) to better understand the disorder and identify potential therapies. Following the identification of *MECP2*, these studies have led to the discovery of additional disease genes, such as *Cyclin-dependent kinase-like 5* (*CDKL5*), *Forkhead box protein G1* (*FOXG1*), *Myocyte-specific enhancer factor 2C* (*MEF2C*) and *Transcription factor 4* (*TCF4*) (Armani et al., 2012, Evans et al., 2005, Philippe et al., 2010). Pathogenic variants in these genes, albeit to a lesser degree, are also associated with Rett syndrome, and display clinical phenotypes that overlap with this disorder.

Recent estimates recognize that around 95% of classical Rett syndrome cases and 75% of atypical Rett syndrome cases have mutations in *MECP2* (Krishnaraj et al., 2017). To date, over 900 unique variants have been identified within the *MECP2* gene with about 500 being pathogenic or likely pathogenic and the rest being benign, likely benign, or VOUS (variants of unknown significance) variants (Krishnaraj et al., 2017). Before the ground-breaking discovery in 1999 that pathogenic variants in *MECP2* caused Rett syndrome, the diagnosis of Rett syndrome was based purely on clinical diagnostic criteria as no useful biomarkers of the disorder or genes had been identified. Despite this discovery, due to the complex nature of the disorder, the diagnosis of Rett syndrome still relies largely on the clinical diagnostic criteria and

the exclusion of differential diagnoses (Gold et al., 2018). In addition, effective treatments remain to be identified and treatment is still largely symptomatic. A better understanding of the underlying biological, genetic, transcriptomic and proteomic architecture of the disorder and the functional pathways would significantly improve prospects for novel targeted therapies for Rett syndrome patients.

Transcriptomic and proteomic studies have been successful in identifying new candidate genes and proteins, providing a better understanding of the underlying pathophysiology, identifying perturbations in functional pathways, and providing avenues for novel areas of research. In addition to the data generated for a specific research question, there lies a wealth of additional untapped information potentially available within these “omic” studies. This then raises the question as to whether we can learn anything new from previously conducted studies through comparing existing data sets and ultimately extracting valuable information by conducting meta-analysis. A notable example of utilizing omic data within the Rett syndrome research field is the recent study by Shovlin and Tropea that delineated transcriptomic data from human Rett syndrome patient samples, to present trends in differential gene expression for investigating broader research questions (Shovlin and Tropea, 2018).

A compelling approach to unravel novel molecular insights into the role of MeCP2 and the pathophysiology of Rett syndrome is genome-wide expression (transcriptomic) profiling and proteomic profiling. The objectives of transcriptomic profiling, whether it is using microarrays or RNA sequencing (RNA-Seq), and proteomic profiling, are to quantitate and compare gene and protein expression profiles of two or more samples in order to detect differentially expressed genes and proteins. In addition to single genes and proteins, identifying dysregulated and

overrepresented functional pathways, biological networks and cellular functions may also reveal novel biological insights that contribute to the disease process.

In this review, we focus on published transcriptomic and proteomic studies that were performed in mouse models of Rett syndrome, highlight the main findings and discuss where future research efforts in this field should be directed. The aim of this review is to provide a summary of all the transcriptomic and proteomic studies conducted in mouse models of Rett syndrome studies, to highlight common dysregulated genes and perturbed functional pathways, biological networks and cellular functions salient to Rett syndrome.

Study Characteristics

A comprehensive list of all the studies are outlined in Table 1. Studies were conducted on a range of tissue samples, primarily in the brain given the predominant neurological nature of Rett syndrome. Transcriptomic studies were conducted on various *Mecp2*-deficient mouse models. Most studies were conducted in males, with two exclusively in females (Bedogni et al., 2016, Zhao et al., 2017), and one study using both genders (Johnson et al., 2017). The studies were mostly carried out using knock out mice (null), with a small proportion in *Mecp2*-knock in mice (*Mecp2*^{R270X}, *Mecp2*^{G273X}, *Mecp2*³⁰⁸, *Mecp2*^{T158A-Tavi}, *Mecp2*^{R106W-Tavi}) (Baker et al., 2013, Delepine et al., 2015, Gabel et al., 2015, Johnson et al., 2017), and *Mecp2* overexpressing mice (Tg) (Ben-Shachar et al., 2009, Chahrour et al., 2008, Chen et al., 2015, Samaco et al., 2012).

In an attempt to identify region specific gene expression, studies were conducted on individual brain regions and cells isolated from these regions. These included the cortex (Tudor et al., 2002, Urduingio et al., 2008, Kishi et al., 2016, Pacheco et al.,

2017), visual cortex (Gabel et al., 2015, Renthall et al., 2018), the cerebellum (Ben-Shachar et al., 2009, Gabel et al., 2015, Jordan et al., 2007, Mellen et al., 2012, Mellen et al., 2017, Sanfeliu et al., 2019, Urdinguio et al., 2008), whole brain (Nuber et al., 2005, Kriaucionis et al., 2006, Guo et al., 2014), the whole hippocampus (Baker et al., 2013, Tudor et al., 2002) and the amygdala (Samaco et al., 2012). Studies were also conducted on cell sources originated from distinct regions of the brain including forebrain (Tudor et al., 2002), hypothalamus (Chahrour et al., 2008, Chen et al., 2015), midbrain (Urdinguio et al., 2008) and striatum (Zhao et al., 2013), granule cells of the dentate gyrus hippocampal region (Smrt et al., 2007), embryonic cortical neurons (Bedogni et al., 2016, Vacca et al., 2016), and excitatory and inhibitory cortical neurons (Johnson et al., 2017). Two studies used a combination of four distinct neuronal cell populations including thick tufted pyramidal neurons from the motor cortex, fast-spiking parvalbumin-positive interneurons from the motor cortex, noradrenergic locus coeruleus neurons and cerebellar Purkinje cells (Ehrhart et al., 2016, Sugino et al., 2014). Other studies used astrocytes (Delepine et al., 2015, Yasui et al., 2013) and microglia and peritoneal macrophages (Cronk et al., 2015), whilst others used cardiovascular progenitor cells (Hara et al., 2015) and cortical callosal projection neurons (Kishi et al., 2016). Studies using cell sources not originating from brain regions include the use of fibroblasts (Orlic-Milacic et al., 2014), skeletal muscle (Gold et al., 2014) and blood (Sanfeliu et al., 2019). Sample sizes of the studies varied greatly with the highest sample size being 205 (Sanfeliu et al., 2019) and the others varying between 1 and 100 samples. The age of mice used in the studies varied between embryonic, juvenile and mature with the youngest being E15 (Vacca et al., 2016, Bedogni et al., 2016) and the eldest 25 weeks (Tudor et al., 2002).

The proteomic studies identified were conducted on three different *Mecp2* mouse models: *Mecp2*-null, *Mecp2*³⁰⁸ and *Mecp2*^{Jae} mice (Cortelazzo et al., 2017, Matarazzo and Ronnett, 2004, Pacheco et al., 2017). The ages of mice ranged from 2 weeks to 12 months old. Two studies were conducted on male mice (Matarazzo and Ronnett, 2004, Pacheco et al., 2017) with one study on female mice (Cortelazzo et al., 2017). Two studies were conducted on brain regions (Matarazzo and Ronnett, 2004, Pacheco et al., 2017) and one on plasma (Cortelazzo et al., 2017).

Analysis platforms used:

Oligonucleotide microarrays were the most extensively used method for the transcriptomic studies, especially the Affymetrix GeneChip and Mouse Exon subfamilies of microarrays. Other platforms such as the Affymetrix Mu11k and MGU74A arrays, Affymetrix U430 arrays, ADDER, cDNA microarrays, Illumina, and Agilent were also used. Interestingly, in experiments carried out after 2012, next generation sequencing technologies started becoming a more predominant platform of choice. Data analysis approaches varied in stringency between experiments, with cut-off values ranging from $p < 0.05$ to $p < 0.005$ and fold change (fc) from > 1.2 (< 0.67) to > 2 (< 0.5). These different methodologies are of great consequence to the interpretation of data as subtle changes caused by *Mecp2* deficiency may be reported to be important in the less stringent tests which do not reflect the real function of *Mecp2* (Raman et al., 2018). The opposite can also happen, as too few genes may be reported in more stringent tests, hence ‘loosing’ critical data. One would expect to find a larger list of potential dysregulated genes in the less stringent tests ($p < 0.05$; $fc > 1.2$) than the more stringent ones ($p < 0.005$; $fc > 2$), however this is not always the case which may be a reflection of the technical and design differences between the

studies such as sample size, statistical cut-offs, mouse models, statistical power, ages, sex and anatomical regions studied.

The three proteomic studies used slightly different protein analysis and quantification methods, with Matarazzo and Ronnett using 2D gel electrophoresis and Q-T of mass spectrometry (Matarazzo and Ronnett, 2004). Cortelazzo *et al* used 2D and MALDI-*ToF/ToF* (Cortelazzo et al., 2017), whilst Pacheco *et al* used an in gel tryptic digest and LC-MS/MS (Pacheco et al., 2017).

Common differentially expressed genes and pathways

The most striking evidence emanating from this review is the lack of concordance in the dysregulated gene lists between the different studies. This may be attributed to a number of aspects including the fact that no two studies conducted gene expression profiling in the same mouse model, in the same tissue and at the same age. It would be expected that studies using the same, or similar sources of material would have a higher degree of concordance in their differential gene expression lists, however this is not the case, which highlights the complexity of the disorder and how delicately *Mecp2* regulates gene expression both spatially and temporally. A collation of the highlighted differentially expressed genes in these studies did reveal a few genes that showed altered expression across three or more studies which include *Irak1*, *Efna5*, *Fabp7*, *Fkbp5*, *Plagl1*, *Fgf11*, *Homer2*, *Nsdhl* and *Sgk1*. However, as no meta-analysis have been conducted on the raw data from all, or a subset of these studies, it is difficult to determine whether these genes are an under-represented list or indeed a true reflection of commonly dysregulated genes.

Owing to such a small number of commonly dysregulated genes amongst these studies, another approach to better understand the aetiology of Rett syndrome is to

interrogate groups of dysregulated genes that fall into distinct functional signalling pathways and biological networks. A comparison of altered pathways as opposed to individual genes may overcome the variability observed between each experiment. These variabilities may be due to biological sample differences, experimental noise and differences in experimental and analysis approaches, or they could equally be valid discrepancies due to the tight temporal and spatial gene regulation of *Mecp2*. Either way, grouping differentially expressed genes that fall into the same functional molecular pathways may provide more insight into the biological pathways, providing new directions for therapeutic interventions.

Not all the studies ascribed groups of genes to specific biological networks, pathways or cellular functions and mechanisms. However, those that did map the differential gene expression to pathways and cellular functions highlighted the diversity of the pathways, attesting to the great complexity of the disease. Several studies reported dysregulation in the NF- κ B, TNF and TLR signalling pathways. In addition, perturbations in biological mechanisms and specific cellular functions associated with neuronal migration, immune response, stress response, mitochondrial dysfunction, lipid metabolism, neuronal maturation, imprinting, and those relating to synaptic function, specifically synaptogenesis, plasticity, function and transmission were also highlighted.

Dysregulated NF- κ B, TLR and TNF signalling pathways:

Several studies reported an upregulation in the expression of the *Interleukin-1 receptor-associated kinase (Irak1)* gene (Jordan et al., 2007, Kishi et al., 2016, Urduingio et al., 2008) implicating the NF- κ B, TNF and TLR signalling pathways. The transcription factor NF- κ B controls the expression of many genes encoding

cytokines involved in inflammation, TNF is a pro-inflammatory cytokine involved in a number of biological processes including the immune response, and the TLR signalling pathway can lead to pro-inflammatory cytokine genes being activated and transcribed. This inflammatory response most likely mediates neuro-inflammation and may contribute to neuronal dysfunction in the brains of individuals with Rett syndrome.

This concordance in *Irak1* expression was despite different brain regions and age of mice being investigated (cortical callosal projection neurons in 2 week old *Mecp2*^{-/-Y} mice (Kishi et al., 2016), cortex, midbrain and cerebellum in *Mecp2*^{-/-Y} mice aged between 6 and 10 weeks (Urduingio et al., 2008), and cerebellum of male *Mecp2*^{tm1.1Jae/Y} and *Mecp2*^{tm1.1Bird/Y} mice (Jordan et al., 2007)) and suggests the activation of the signalling pathways is a pervasive feature of Rett syndrome and not restricted to brain region or mouse age. *Irak1* is a serine/threonine kinase that is activated by receptors IL1R and TLRs resulting in the translocation of NF-κB into the nucleus and the transcription of pro-inflammatory cytokines (Janssens and Beyaert, 2003). More recently, *IRAK1* has been shown to play a key role in the NF-κB signalling pathway, a pathway which is closely associated with neuronal development and its consequent dysregulation can result in neurodegenerative diseases (Gutierrez and Davies, 2011). In addition, and pertinent to Rett syndrome, Kishi *et al* reported that alleviating the abnormal NF-κB signalling pathway extends the life span of *Mecp2*-null mice (Kishi et al., 2016), confirming the importance of this pathway in the disorder. As *Irak1* expression is central to the functioning of this pathway, and the abnormally increased expression of *Irak1* is associated with *Mecp2* loss of function, significant interest is gathering in the investigation of the possible role of *IRAK1* regulation in the NF-κB pathway and the pathogenesis of Rett syndrome. A

dysregulated immune-inflammatory response implicating the NF- κ B pathway has more recently been observed by Sanfeliu *et al* in brains and blood of mice (Sanfeliu *et al.*, 2019). However, although the authors did not specifically mention *Irak1*, the overall findings confirm the immune-inflammatory response in Rett syndrome mouse models. Finally, it must be noted that the *Irak1* locus lies adjacent to the *Mecp2* locus on the X chromosome and the apparent increase in expression has been proposed hold a ‘bystander’ effect of *Mecp2* deficiency.

Studies also reported a significant dysregulation in genes enriched in the TNF-mediated signalling pathway. Interestingly, these studies conducted by Vacca *et al* and Cronk *et al* used different cells of the brain, with Vacca *et al* testing embryonic cortical cells from male *Mecp2*^{-Y} mice and Cronk *et al* testing microglia and peritoneal macrophages from *Mecp2*^{Tm1.1Bird} mice, suggesting again a pervasive response (Cronk *et al.*, 2015, Vacca *et al.*, 2016).

Dysregulated biological networks and cellular functions:

Mitochondrial function:

The role of mitochondrial dysfunction in Rett syndrome is well established and strongly supported by these studies as well as from human studies using blood samples (Shulyakova *et al.*, 2017). In these transcriptomic murine studies, despite using different tissues, both Gold *et al* who tested skeletal muscle from 6 and 12 week old male *Mecp2*^{-Y} mice and Kriaucionis *et al* who tested whole brains from P70 male *Mecp2*^{-Y} mice, identified dysregulation in the Ubiquinol-cytochrome c reductase core protein 1 (*Uqcrc1*) (Kriaucionis *et al.*, 2006) and *cardiolipin synthase 1* (*Crls1*) (Gold *et al.*, 2014) genes encoding mitochondrial proteins. *Uqcrc1* is a nuclear gene encoding a subunit of mitochondrial respiratory complex III and was found to be

overexpressed in mice displaying overt neurological symptoms (P70) (Kriaucionis et al., 2006) whereas a downregulation of *cardiolipin synthase 1* (*Crls1*) in symptomatic mice (12 weeks) compared to wild type mice was observed (Gold et al., 2014). *Crls1* is involved in the synthesis of the mitochondrial-localised phospholipid cardiolipin (Schlame and Haldar, 1993) which is known to interact with various complexes of the respiratory chain and stabilize the higher order organization of the super-complexes (COI/III/IV and COII/III/IV) (Schagger, 2002).

Synaptic function and neurotransmission:

Prominent functional networks that are consistently altered in these studies relate to synaptic function and neurotransmission. Dysregulated genes involved in synaptic plasticity and function, neuronal migration, synaptic transmission, learning and behaviour modulation, and hippocampal dendrite development were reported in the cerebellum of male *Mecp2*^{tm1.1Jae/Y} and *Mecp2*^{tm1.1Bird/Y} mice (Jordan et al., 2007). Genes enriched in cellular adhesion and communication were found to be perturbed in a number of different neuronal cell types isolated from male *Mecp2*^{- /Y} mice (Sugino et al., 2014) and later confirmed by Ehrhart *et al* whose data was generated by reanalysing the data from Sugino *et al* (Ehrhart et al., 2016). Ehrhart *et al* also highlighted dysregulated genes involved in neuronal connectivity and communication particularly synaptic function, glutamate and glutathione metabolism as well as abnormal neuronal excitatory and inhibitory activity (Ehrhart et al., 2016). Differentially regulated genes including several immediate early and late response genes that are induced by neuronal activity and modulate signalling pathways associated with synaptic plasticity were also reported in excitatory and inhibitory

cortical neurons from 18 week female and 6 week male *Mecp2*^{T158M-Tavi} and *Mecp2*^{R106W-Tavi} mice (Johnson et al., 2017).

Cortex development and maturation:

Genes involved in the development and maturation of the cortex were also found to be dysregulated. Specifically genes involved in ionic channels, glutamatergic receptors, cerebral cortex development and pathways delaying the maturation of the cortex were identified in embryonic cortical neurons from female *Mecp2*-null mice (Bedogni et al., 2016).

Glial cells:

Although *MECP2* is expressed in a wide range of tissues, Rett syndrome is principally caused by the deficiency of MeCP2 in the cells of the brain (Chen et al., 2001) and thus apart from four studies (Gold et al., 2014, Hara et al., 2015, Orlic-Milacic et al., 2014, Sanfeliu et al., 2019), all transcriptional murine studies were confined to the brain. A handful of studies focused on the transcriptome of the whole brain (Guo et al., 2014, Kriaucionis et al., 2006, Nuber et al., 2005) however these studies revealed only subtle changes in gene expression which can be attributed to the brain being a heterogeneous organ consisting of many distinct functional regions that may individually be affected by the lack of *Mecp2*. As such, profiling studies of discrete regions of the brain and subtypes of neurons have ensued, uncovering more dramatic gene effects resulting from the loss of *Mecp2*, and overcoming the dilution issue associated with assaying complex tissues. Although the Rett phenotype is predominantly due to neuronal *Mecp2* deficiency, astrocytes and microglia play a significant role in the pathogenesis of Rett syndrome (Sharma et al., 2018). Transcriptomic profiling of astrocytes cultured from early post-natal pups identified

differentially expressed genes enriched in astrocyte signalling, neuronal support and function (Yasui et al., 2013), as well as glutamate receptor signalling and cytokine signalling (Delepine et al., 2015). In addition, microglia cultured from whole brains of female heterozygous *Mecp2*-null mice at 5 weeks old with no phenotypic symptoms, and 24 weeks with apparent phenotypic symptoms, revealed many dysregulated genes involved in the activation of macrophages, heat shock protein family genes and genes regulated by NK- κ B in response to TNF (Zhao et al., 2017). This activation of microglia was supported by another study which showed increased expression of TNF as well as glucocorticoid and hypoxia induced gene transcripts (Cronk et al., 2015).

MECP2 overexpression:

The overexpression of *MECP2* is the cause of a clinically recognizable disorder that leads to a severe phenotype of intellectual disability and autistic features in males (Ramocki et al., 2010, Van Esch, 2012). Hence there has been considerable interest in understanding the effects of *MECP2* overexpression and its genotype-phenotype correlation. Our search identified four transcriptomic studies that investigated the overexpression of *Mecp2* in mice, two in the hypothalamus (Chahrour et al., 2008, Chen et al., 2015), one in the cerebellum (Ben-Shachar et al., 2009), and one in the amygdala (Samaco et al., 2012). These studies demonstrated that overexpression of *Mecp2* leads to an increased expression of many genes, thus supporting the role of *MECP2* as a transcription activator. Of interest, the Fatty acid binding protein 7 (*Fabp7*) gene was found to be upregulated in the overexpression mice in three of these studies (Ben-Shachar et al., 2009, Chahrour et al., 2008, Samaco et al., 2012) with Chen *et al* not recording the expression. FABP7 is a brain specific fatty acid binding protein part of the fatty acid binding proteins (FABPs) family which are

small, highly conserved, cytoplasmic proteins that bind long-chain fatty acids and other hydrophobic ligands. *FABP7* is expressed during development in radial glia, progenitor cells that are responsible for producing all of the neurons in the cerebral cortex.

The reciprocal relationship between differentially expressed genes in the knockout mouse studies and the overexpression mouse studies is of great interest as those genes that are dysregulated in both studies may be valid target genes of interest and worthy of further scrutiny. In order to shed further light on the molecular functions of MeCP2, these studies also compared the gene expression profiles of both overexpressing mice and knockout mice to tease out dysregulated genes resulting from loss or gain of MeCP2 expression. Within each study, the authors found a number of common genes that were upregulated in the overexpressing mice and downregulated in the knockout mice, supporting the role for MeCP2 as a modulator that can both increase and decrease gene expression. Of interest, in these comparisons, *Fabp7* was upregulated in the overexpression mice but also downregulated in the knockout mice (Ben-Shachar et al., 2009, Chahrour et al., 2008, Samaco et al., 2012). Together with supporting evidence of *Fabp7* being downregulated in a number of the other knockout studies (Pacheco et al., 2017, Urduingio et al., 2008, Zhao et al., 2013) strongly suggests that *Fabp7* could be a valid target gene of interest.

Proteomic studies:

Proteomic studies are a powerful approach to study disease in that they reveal changes in protein regulation during the progression of disease. Validating transcriptomic with proteomic data can be extremely powerful, but technically challenging. Most genome-

wide expression studies on mouse models of Rett syndrome lack integration of multi-omics datasets with one exception by Pacheco *et al.*, who compared the transcriptome and proteome of symptomatic *Mecp2*-null male mice brain samples (Pacheco et al., 2017). This study identified 35 gene-protein matches with significant expression changes. One of these was FKBP Prolyl Isomerase 5 (*FKBP5*), a gene implicated in human stress and mood disorders through its actions in modulating glucocorticoid sensitivity (Klengel and Binder, 2015). The *Fkbp5* gene was also found to be dysregulated in a number of transcriptomic studies (Ben-Shachar et al., 2009, Pacheco et al., 2017, Zhao et al., 2013) which suggests its potentially functional significance in Rett syndrome. Further, the study by Pacheco *et al* revealed a dysregulation in a number of signalling pathways and cellular networks including RNA metabolism, proteostasis, monoamine metabolism, and cholesterol synthesis in protein lysates from the whole cortex of symptomatic male *Mecp2*^{Ja^e/y} mice at P60 (Pacheco et al., 2017). As this was the only study that conducted both transcriptomic and proteomic studies, the authors were able to find common ‘hits’ in both datasets, strengthening the analysis. Disrupted pathways associated with synaptic function and neurotransmission, neuronal morphology, and development were observed, indicating disturbed conduction and neuronal structure and organization. A disruption in glia markers such as myelination processes, astrocyte morphology, reactivation, and apoptosis were also observed. In addition the authors also observed a dysregulation in unique pathways associated with inflammation and the vasculature, cellular metabolism, calcium signalling, protein stability, DNA binding, and cytoskeletal cell structure (Pacheco et al., 2017). The study by Matarazzo and Ronnett, who studied protein expression in male *Mecp2*-null mice at 2 weeks and 4 weeks of age, also revealed aberrant protein expression enriched in cytoskeletal arrangement as well as

in other biological functional networks such as chromatin modelling, energy metabolism, cell signalling, and neuroprotection (Matarazzo and Ronnett, 2004). In support of the transcriptomic studies, evidence of inflammation was observed by Cortelazzo *et al*, who demonstrated differentially expressed proteins involved in the acute phase response (APR) and inflammation in the plasma of symptomatic *Mecp2*³⁰⁸ female mice (Cortelazzo et al., 2017).

DISCUSSION

The main aim of this review was to provide a summary of all the studies and highlight the reported dysregulated genes and functional pathways that are found to be perturbed in Rett syndrome mouse models. This data can be further analysed, using meta-analysis for the purpose of identifying new gene targets that may lead to therapeutics for the treatment of Rett syndrome or for unraveling novel biological insights that contribute to our understanding of the underlying pathophysiology of the disorder.

With the advent of new technologies such as RNA-seq, it is now possible to rapidly and effectively sequence and quantitate the gene expression of transcriptomes of interest in a way that has not been achieved before. However, despite this technology, the complete molecular picture for Rett syndrome is still unclear and by combining multiple omics studies, there is the potential to identify new pathways, reveal multi-gene interactions and establish the basis for disease progression.

Mouse models have been shown to recapitulate some of the features of the phenotype observed in patients (Calfa et al., 2011) and have provided a wealth of information on the underlying pathophysiology of the disorder. In addition to reproducing the genetic architecture of Rett syndrome in humans, a number of the biochemical abnormalities

are also well represented in these mouse models (Deng et al., 2007). Despite considerable research in identifying the role of *MECP2* and other Rett syndrome-associated genes (Ariani et al., 2008, Weaving et al., 2004) in the pathophysiology of the disease, there is still a lack of clarity in the factors leading to disease progression.

There has been considerable success in utilizing mouse models to develop therapeutic strategies for Rett syndrome. A number of promising clinical trials including insulin-like growth factor (IGF-1), sarizotan, trofinetide, glatiramer acetate, dextromethorphan, desipramine, fingolimod, triheptanoin, ketamine, and lovastatin have been produced from murine preclinical studies (Gold et al., 2018). However, to date, no therapeutic strategy has been translated into the clinic. This may not be due to the lack of efficacy of these treatments, but rather to the confounding and complex nature of the disorder and a general lack of a deep understanding of the functions of MeCP2. Patients display a broad range of phenotypic features, each manifesting at varying time points throughout development. Therefore, further scrutiny of the mouse model omic studies may uncover more information that could support future clinical trials. Although care must be taken when extrapolating the preclinical results to potential human clinical trials, studies in mouse models are critical for identifying convergent molecular pathways in Rett syndrome.

In this review, we have identified published transcriptomic and proteomic studies conducted on Rett syndrome mouse models and highlighted the signalling pathways and cellular networks that were identified to be aberrantly regulated. Although many genes have been investigated for their involvement in the pathogenesis of the disorder, a set of commonly dysregulated genes has yet to be identified across all (or most) studies. This may be achieved using meta-analysis, however, caution should be

exercised and careful consideration needs to be taken with the design of the analysis. One of the confounding factors that may result in a lack of concordant dysregulated genes between studies, amongst others, is the heterogeneous cell populations of the brain which may dilute the effect of *Mecp2*-deficiency. As gene expression profiles can vary significantly between different cell types, this could result in failure to identify a common list of differentially expressed genes. In addition, the subtle changes in the differential expression of many genes could contribute to the diversity of gene expression encountered, yet may miss being detected by transcriptomic and proteomic analysis because the expression differences were too subtle. The variations in mouse models, age and sex of the mice used could also contribute to discordant results between studies. This is highlighted by the transcriptomic study by Jordan *et al.*, who compared the *Mecp2*^{tm1.1Jae} and *Mecp2*^{tm1.1Bird} mouse models at three different disease progression stages using the same transcriptomic platform, yet they were unable to find more than a handful of commonly differentially expressed genes between the models (Jordan et al., 2007).

Limitations:

A major limitation of conducting transcriptomic studies of whole brains or selected brain regions is the heterogeneous cell population consisting of many different cell types including a variety of neuronal cells, microglia, astrocytes and oligodendrocytes as well as endothelial and fibroblast cells. It is widely accepted that different cell types confer different gene expressions and thus it is expected that the gene expression profiles will differ considerably among this heterogeneous cell population. Further, isolating specific brain regions of interest, especially small regions such as the hippocampus, is technically challenging, and therefore, the differentially

expressed genes may be representative of the surrounding cells and not only of that particular region. Another limitation is that some samples may be pooled to meet the large RNA requirements for expression profiling. In this case, we should consider the possible effects of inter-mouse variation, where the gene expression of individual mice are so different that they dilute out any significant values.

Cultured cells also pose a limitation in that they no longer display the inherent phenotype and thus gene expression profiles that they would *in situ*. Microglia are particularly sensitive, as there are no *in vitro* methods that recapitulates all characteristics of adult homeostatic glia (Timmerman et al., 2018). Data showing that RNA transcript profiles of *ex vivo* microglia differ considerably from those of *in vitro* microglia which has, to a certain degree, been attributed to culturing in the presence of serum (Bohlen et al., 2017).

Differences in study design and utilizing different measurement platforms is a major issue, introducing platform based inconsistencies, making it difficult to conduct meaningful and accurate comparisons between studies. Other inherent problems that introduce study bias include small sample size, different statistical cut-offs (p-value and fold-change), genetic background of transgenic mouse models, animal environment, data quality, statistical power, different mouse models, ages, sex and anatomical regions studied. Lastly, despite Rett syndrome being a predominantly female-based disorder, the majority of these studies in this meta-analysis used male mice. Sexual dimorphism has been recognised as a major potential confounder in mouse studies (Karp et al., 2017).

Future directions:

Despite all these studies, no meta-analysis has been conducted using the raw data in an attempt to tease out any converging genes or signalling pathways with the idea of identifying novel curative treatments for Rett syndrome. There may be a number of reasons for this, which include, but not limited to, the raw data of some of the studies not being publically accessible. Moving forward, raw data from every study should be deposited in publically accessible online repositories where meta-analysis, using standardised analysis approaches, may be conducted. As such, statistical parameters such as p-values, corrections for multiple testing, sample pooling and in the case of RNA-seq read-depth, read length and single/paired end reads, may be applied universally across all data sets. Furthermore combining transcriptomics with proteomics will enrich the investigations which may provide more reliable 'druggable' targets.

Conclusion

The importance of omics studies in driving discoveries in human genetics, especially in a complex neurodevelopmental disorder such as Rett syndrome is undeniable. The data generated from genome-wide studies supported by proteomics will systematically build an improved understanding of the key biological aberrations associated with *MECP2* deficiency. We cannot also exclude the possibility of identification of "false positives" and inconclusive "leads" while we search for answers, however some of these leads may indeed open new avenues of research, bridging the gap between the known and the unknown. As a resource to quickly and comprehensively identify transcriptomic studies conducted in mouse models in Rett

syndrome, we hope this review will assist researchers working towards tackling this devastating disorder.

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Table 1. Summary of transcriptomics and proteomics studies on samples from mice models associated with RTT

No	Reference	Year	Transgenic Model & Genetic Background	Gender	Cell source	Age when studied	No of samples	DE at statistical cut-off	IE	RE	SIGNIFICANT GENES
1	Tudor et al. PMID:12432090	2002	(a) <i>Mecp2</i> ^{fl} _{ox/Y} (b) <i>Mecp2</i> -conditional knockout 129/SVJae, BALB/c, C57BL/6	male	forebrain, cortex, hippocampus	(a) P24, P35, P56, P63 (b) P135-P180	100 (total)	No significant gene expression changes reported p< 0.05			
2	Nuber et al. PMID:16002417	2005	<i>Mecp2</i> ^{-/-} C57BL/6J	male	whole brain	P74	1	11 p<0.001	8	3	<i>SGK1, FKBP5</i>
3	Kriaucionis et al. PMID:16782889	2006	<i>Mecp2</i> ^{-/-} C57BL/6	male	whole brain	P30, P55, P70	3	12 (at P70) p< 0.05	7	4	<i>UQCRC1, CDON</i>
4	Jordan et al. PMID:17584923	2007	(a) <i>Mecp2</i> ^{tm1.1Jae/Y} (<i>Mecp2</i> -mutant) (b) <i>Mecp2</i> ^{tm1.1Bird/Y} BALB/cJ, C57BL/6J	male	cerebellum	2, 4, 8 weeks	>20	(a) 840 (8 weeks) (b) 849 (8 weeks)† p< 0.05	441 615	399 234	<i>IRAK1, MEG3, FXR1, RELN</i>

5	Smrt et al. PMID:17532643	2007	<i>Mecp2</i> ^{m1.1Jae} (<i>Mecp2</i> -mutant) ICR	male	granule cells of dentate gyrus (hippocampus)	8 weeks	4	13 p< 0.05	12	1	<i>PFDN5</i> , <i>UCHL1</i> , <i>SFXN3</i> , <i>OSBPL9</i> , <i>ZSCAN21</i> , <i>ARP</i> <i>C3</i> , <i>SDC2</i> , <i>UQCR10</i> <i>HMGB1</i>
6	Chahrouh et al. PMID:18511691	2008	(a) <i>Mecp2</i> ^{-/-} (b) <i>MECP2-Tg</i> (<i>MECP2</i> -overexpressed) C57BL/6J	male	hypothalamus	6 weeks	4	a) 369(b) 1187± p< 0.05	85 940	284 247	<i>CREB1</i> , <i>BDNF</i>
7	Urduingui et al. PMID:18989361	2008	<i>Mecp2</i> ^{-/-} C57BL/6J	male	cortex, midbrain, cerebellum	6-10 weeks	4	53	29	24	<i>FKBP5</i> , <i>MOBP</i> , <i>PLAGL1</i> , <i>DDC</i> , <i>MLLT2H</i> , <i>EYA2</i> , AND <i>S100A9</i> <i>IRAK1</i> , <i>PRODH</i> AND <i>DLK1</i>
8	Ben-Shachar et al. PMID:19369296	2009	(a) <i>Mecp2</i> ^{-/-} (b) <i>MECP2-Tg</i> (<i>MECP2</i> -overexpressed) C57BL/6J	male	cerebellum	6 weeks	5	a)1102(b) 1180 p< 0.05	286 755	816 425	<i>PRLF2</i> , <i>RCOR2</i> , <i>GPR26</i> , <i>LRP1B</i>
9	Samaco et al. PMID:22231481	2012	a) <i>Mecp2</i> ^{-/-} (b) <i>MECP2-Tg</i> (<i>MECP2</i> -overexpressed)	male	amygdala	6 weeks	4	1060 (genes altered in opposite directions in <i>Mecp2</i> -null and <i>Mecp2</i> -overexpressed) FDR ≤ 0.05			<i>CRH</i> , <i>OPRM1</i>

10	Mellén et al. PMID: 23260135	2012	<i>Mecp2</i> ^{1m1.1Bird} C57BL/6J	male	cerebellum	7-11 weeks	4		37	262	<i>PRKAB1, CCD C63, MYBPC3, CPNE6, BMF</i>
11	Baker et al. PMID: 23452848	2013	<i>Mecp2</i> ^{-/-} <i>Mecp2</i> ^{R270X/Y} <i>Mecp2</i> ^{G273X/Y} ; F1 FVB;129SvEv	male	hippocampus	(a) 4 weeks (b) 9 weeks	4	a) 2778± b) 3082 p < 0.05	n/s		<i>BDNF, SST, TAK1, OPRK1</i> <i>MEF2C AND GRIN2</i>
12	Yasui et al. PMID: 23351786	2013	<i>Mecp2</i> -null C57BL/6J	n/s	astrocyte cells from cortex	P1 + 2-4 weeks (DIV)	n/s	118 p < 0.05	55	63	<i>AARD1, SLC38A1, ARMC3, CDON</i>
13	Zhao et al. PMID: 23948639	2013	(a) <i>Mecp2</i> -null (b) <i>Mecp2</i> ^{2lox/y} ; Dlx5/6-cre C57BL/6J	male	striatum	(a) P60 (b) P7, P90	n/s	(a) 127 (b) 21 (at P90) FDR ≤ 0.05	68 12	599	<i>EXPH5, ROBO3, DRD3, SATB1, DSG1C, AND DLK1</i>
14	Gold et al. PMID: 24613463	2014	<i>Mecp2</i> ^{1m1Tam}	male	skeletal muscle	(a) 6 weeks (b) 12 weeks	3	(a) 23 (b) 22 p < 0.05	19 14	48	<i>CRLS1, MTCO1, CILP2, TINAG</i>
15	Guo et al. PMID: 24968028	2014	<i>Mecp2</i> ^{1m1.1Jae/Y} (<i>Mecp2</i> mutant) + saline ICR	male	whole brain	8 weeks	3	>300	n/s		<i>CNTN1, ZKSCAN1, FOXP1</i>

16	Orlic-Milacic et al. PMID:24699272	2014	(a) <i>Mecp2</i> ^{-/-} + <i>MECP2_e1</i> rescue (b) <i>Mecp2</i> ^{-/-} + <i>MECP2_e2</i> rescue	male	fibroblasts	DIV7	3	Varies between transcript p < 0.05	(a) 807 (b) 94	(a) 236 (b) 140	<i>SRPX2, NAV3, NPY1R, SYN3, AND SEMA3D</i> <i>DOCK8, GABRA2, KCNA1, FOXG1, UNC5C AND RPH3A</i>
17	Sugino et al. PMID:25232122	2014	<i>Mecp2</i> ^{-/-} C57BL/6J	male	4 distinct neuronal cell types	P37-P55 P22-P25	3	822 Q < 0.005	462	350	<i>CD99L2, RAB39B</i>
18	Chen et al. PMID:25870282	2015	(a) <i>Mecp2</i> ^{Tm1.1Bird} (b) <i>MECP2-Tg</i> (<i>MECP2</i> -overexpressed)	male	hypothalamus	7 weeks		Q < 0.005			
19	Cronk et al. PMID:25902482	2015	<i>Mecp2</i> ^{Tm1.1Bird} C57BL/6J	n/s	(a) microglia (b) peritoneal macrophages	n/s	Varies between groups	Only glucocorticoid signature genes studied. ‡			<i>FKBP5, IL6, TNF, CXCL2, CXCL3, AND CSF3</i>
20	Delépine et al. PMID:26208914	2015	<i>Mecp2</i> ^{308/y} C57BL/6J	male	astrocytes from cortex	P0 + DIV7	4	257 p < 0.05	132	125	<i>ADCY8, CDON, CHGB, HTR5B AND MYOC</i> <i>CCL2, GAD1, LCN2,</i> <i>NR2F2 AND SHH</i>

2 1	Gabel et al. PMID:25762136	20 15	(a) <i>Mecp2^{tm1.1Bird}</i> (b) <i>Mecp2^{R306C/y}</i>	male	(a) visual cortex(b) cerebellum	8-10 week s	(a) 3(b) 4			n/s	<i>CAMK2D, EPH A7, SDK1 AND CNTN4</i>
2 2	Hara et al. PMID:26073556	20 15	<i>Mecp2^{-/-}</i> C57BL/6 FVB/N × C57BL/6	male	cardio-vascular progenitor cells	DIV4		2296	123 6	106 0	<i>TBX5, MYH7, CACNA1G, AND HCN</i>

2 3	Kishi et al. PMID:26821816	20 16	<i>Mecp2^{-/-}</i> C57BL/6	male	cortical callosal projection neurons	P14	3	37 p < 0.001, p < 0.005	18	19	<i>KIF1B, MCF2 AND GSN IRAK1</i>
2 4	Bedogni et al. PMID:25979088	20 16	<i>Mecp2</i> -null CD1	female	embryonic cortical neurons	E15.5	3	135 p < 0.005			
2 5	Ehrhart et al. PMID:27517371	20 16	<i>Mecp2^{-/-}</i> C57BL/6	male	data from Sugino et al used ‡						
2 6	Vacca et al. PMID:26821710	20 16	<i>Mecp2^{-/-}</i> C57BL6/J	male	embryonic cortical neurons	E15+DIV3	3	490 q ≤ 0.005	39 4	96	<i>GFAP, ALDH1L1, MEF2C AND TIAM1</i>

27	Johnson et al. PMID:28920956	2017	(a) <i>Mecp2^{T158M-Tavi}</i> (<i>Mecp2</i> knock-in) (b) <i>Mecp2^{R106W-Tavi}</i> (<i>Mecp2</i> knock-in) C57BL6/J	male & female	(i) excitatory & inhibitory cortical neurons (ii) excitatory cortical neurons	(i) 6 weeks (male) (ii) 18 weeks (female)	(i) 4 (ii) ≥ 2	Differs between groups ‡ FDR < 0.05	
28	Pacheco et al. PMID:29090078	2017	<i>Mecp2^{Jae/Y}</i> C57BL6/J	male	cortex	P60	4	391 q < 0.05 132 259	ARHGDIG, CACNB3, CALB1, FABP7, FKBP5, ITM2A, MSMO1, PLAGL1, PVALB, SGK1
29	Zhao et al. PMID:28367307	2017	<i>Mecp2^{+/-}</i> C57BL/6	female	microglia from whole brain	(a) 5 weeks (b) 24 weeks	6	(a) 464 (b) 79 p < 0.05 (a) 78 (b) 42 (a) 386 (b) 37	A) SEMA3B, NOV, DNAJA1, VCAM1, MGP CBLN3, RETNLA, RNF17. CASS4, IFIT2, B) NFKBID, RIAN, AND LSP1
30	Mellén et al. PMID:28847947	2017	<i>Mecp2^{tm1.1Bird}</i> C57BL/6J	male	cerebellum	7-12 weeks	4	n/s	
31	Osenberg et al. PMID:29769330	2018	a) <i>Mecp2^{tm1.1Bird}</i> b) <i>Mecp2^{-/-}</i> C57/BL6	male	a) cortical neurons b) hippocampus	a) P(0-1)+10 days b) 7 weeks	3	a) 27 b) 80 q-value < 0.05 a) 8 b) 48 a) 19 b) 32	HAPLN1, STUM, GFRA1, IQGAP3, BRCA1, UHRF1, CDC20, FBLN2, STEAP4 SERPINB2, GPRC5A, GM20594, GM5778, MREG, RBPM S

32	Renthal et al. PMID: 30455458	2018	<i>Mecp2^{tm1.1Bird}</i>	a)Female b)Male	Visual cortex	a) 12-20 weeks b) 8 weeks	5	a) 734 b) >1000	a) 366 b) 105	a) 368 b) 100	
33	Sanfeliu et al. PMID: 31110484	2019	<i>Mecp2^{tm1.1Bird}</i> <i>C57/BL6</i>	male	a) brain b) blood	7 weeks	3	a) 81 b) 205 p<0.05	a) 44 b) 105	a) 37 b) 100	<i>UBE2V1, SERPIN1</i>

Proteomic studies

No	Reference	Year	Transgenic Model & Genetic Background	Gender	Cell source	Age when studied	No of samples	DE at statistical cut-off	IE	RE
1	Matarazzo & Ronnett PMID: 15128950	2004	<i>Mecp2</i> -null	male	a) olfactory epithelium b) olfactory bulb	(i) 2 weeks (ii) 4 weeks	3	27(a)(i), 67(b)(i) 6(a)(ii), 1(b)(i)	13(a)(i), 64(b)(i) 1(a)(ii)	14(a)(i), 3(b)(i) 5(a)(ii), 1(b)(ii)
2	Cortellazzo et al. PMID: 28592917	2017	<i>Mecp2</i> 308 B6.129S <i>MeCP2^{tm1H}zo/J</i>	female	plasma	10-12 months	3	10 p<0.05	6	4

3	Pache co et al. PMID: 290900 78	201 7	Mecp2 Jae/y C57BL6/J	male	cortex	P60	4	465 p < 0.1	299	166
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Footnotes :

P = Post-natal; E = Embryonic; DIV = days in vitro; PMID = Pubmed identifier; n=Mecp2 – null;

DE = Differential expression; IE = Increased expression; RE = Reduced Expression

n/a – data not available

n/s - not specified

‡- more data available in paper

§- 4 neuronal cell types: (1) layer 5 thick tufted pyramidal neurons in motor cortex; (2) fast-spiking

parvalbumin-positive interneurons in motor cortex; (3) noradrenergic locus ceruleus neurons; (4) cerebellar purkinje

cells

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Author/s:

Krishnaraj, R;Haase, F;Coorey, B;Luca, EJ;Wong, I;Boyling, A;Ellaway, C;Christodoulou, J;Gold, WA

Title:

Genome-wide transcriptomic and proteomic studies of Rett syndrome mouse models identify common signaling pathways and cellular functions as potential therapeutic targets

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