Transcriptome data of temporal and cingulate cortex in the Rett syndrome brain

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Rett syndrome is an X-linked neurodevelopmental disorder caused by mutation in the methyl-CpG-binding protein 2 gene (MECP2) in the majority of cases. We describe an RNA sequencing dataset of postmortem brain tissue samples from four females clinically diagnosed with Rett syndrome and four age-matched female donors. The dataset contains 16 transcriptomes, including two brain regions, temporal and cingulate cortex, for each individual. We compared our dataset with published transcriptomic analyses of postmortem brain tissue from Rett syndrome and found consistent gene expression alterations among regions of the cerebral cortex. Our data provide a valuable resource to explore the biology of the human brain in Rett syndrome.

Background & Summary

Rett syndrome (RTT) is an X-linked neurodevelopmental disorder mostly caused by heterozygous de novo mutation in the methyl-CpG-binding protein 2 gene (MECP2) and predominantly affecting females1. MECP2 duplications have been identified in males with developmental encephalopathy, seizures, autistic features, and recurrent infection2. These clinical disorders illustrate the critical requirement for proper MECP2 expression in human brain development, though how MeCP2 dysfunction leads to the RTT phenotype is unclear.

MeCP2 acts as a global transcriptional regulator by recruiting chromatin-remodeling complexes or regulating higher-order chromatin structures3–8. Thus, MeCP2 may be required for fine-tuning the gene expression for a network of protein-coding genes through both direct and indirect mechanisms. Consistent with this hypothesis, small magnitude changes in gene expression have been detected in brain tissue from either human postmortem RTT samples or mouse Mecp2-mutants9–12. However, most transcriptional studies of postmortem RTT brain have used microarray platforms with small numbers and a lack of age-matched control samples, which impact the sensitivity for detecting transcriptional changes. One study used both microarrays and RNA sequencing (RNA-seq) to examine frontal and temporal cortex from individuals with RTT compared to controls and identified over 200 differentially expressed genes after normalizing data for neuron versus glia composition of samples13. Another larger study used RNA-seq to examine motor cortex and cerebellum and identified over 2,000 differentially expressed genes with a global increase in expression14.

We generated RNA-seq data using brain samples for two distinct brain regions, temporal cortex and cingulate cortex, from four female RTT and four age-matched female donors. Reduced volume and dendritic branching of neurons in the temporal cortex and reduced connectivity of the cingulate cortex have been reported in RTT, indicating the importance of these brain regions in the disorder15–18. We also compared our data with the

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transcriptomic profiles of RTT brain samples from published RNA-seq datasets \(^\text{13,14}\). The composite analysis will be useful to facilitate interpretation and further understanding of MECP2-mediated changes in human brain.

**Methods**

**Brain samples.** Postmortem brain tissue samples were obtained from the Harvard Brain Bank (http://hbtrc.mclean.harvard.edu/) and the National Institutes of Health (NIH) NeuroBioBank (https://neurobiobank.nih.gov), with approval from the coordinating foundation (https://www.rettsyndrome.org). Consent was obtained from next of kin and tissue was collected with approval from the Partners Human Research Committee for the Harvard Brain Bank and from The University of Maryland Institutional Review Board (IRB) and The Maryland Department of Health and Mental Hygiene IRB for the NeuroBioBank. Work was approved by the University of Southern California and is compliant with all ethical regulations. Frozen temporal (BA36/38) and cingulate cortex samples were obtained from four RTT and four control (CTL) brain donors that were matched in age (Fig. 1). The Harvard Brain Bank sequenced MECP2 coding exons and reported intragenic mutations in two of the four brains. Brain donor characteristics are described in Table 1.

**MECP2 variant confirmation.** Genomic DNA was isolated from brain samples for 7773 and 7783 using the PureLink Genomic DNA Kit (LifeTechnologies) according to the manufacturer’s protocol. We performed Sanger sequencing of MECP2 to verify the reported variants (Table 1). Chromatograms were aligned to MECP2 (ENSG00000169057) using MAFFT v7\(^\text{17}\). No additional genes were screened.

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| Brain | Group | Gender | Age | PMI | Source | MECP2 |
|-------|-------|--------|-----|-----|--------|-------|
| 1038  | CTL   | F      | 24  | 7   | NBB    | NA    |
| 1614  | CTL   | F      | 27  | 18  | NBB    | NA    |
| 4724  | CTL   | F      | 16  | 15  | NBB    | NA    |
| 4725  | CTL   | F      | 32  | 17  | NBB    | NA    |
| 6355  | RTT   | F      | 16  | 28  | HBTRC  | NR    |
| 7773  | RTT   | F      | 24  | 25  | HBTRC  | c.473C>T p.Thr158Met |
| 7783  | RTT   | F      | 26  | 39  | HBTRC  | Exon Del |
| 87992 | RTT   | F      | 31  | 30  | HBTRC  | NR    |

**Table 1.** Brain Donor Characteristics. Abbreviations: CTL, control; F, female; HBTRC, Harvard Brain Tissue Resource Center; NA, not applicable; NBB, NIH NeuroBioBank; NR, no mutation in MECP2 reported; PMI, postmortem interval in hours; RTT, Rett syndrome.
RNa sample and library preparation. Total RNA was previously isolated using the Qiagen RNeasy Kit according to the manufacturer’s instructions. Double stranded cDNA fragments were synthesized from mRNA, ligated with adapters, and size-selected for library construction according to the TruSeq Sample Preparation v2 protocol using 0.5–1.5 μg of total RNA (Table 2). ERCC RNA spike-in controls were not included in this experiment. Library quality was measured using an Agilent 2100 Bioanalyzer and concentration was assessed by PicoGreen incorporation. Barcoded libraries were pooled and sequenced in two lanes using an Illumina HiSeq 2000 sequencer.

RNA-Seq data analysis. Single-end reads (100 bp) were aligned to the Human reference genome (NCBI build 37/hg19) using STAR v2.5.3a (see Code Availability 1). Aligned reads mapping to the exons of a gene were summarized into gene counts using featureCounts v1.6 (see Code Availability 2). Picard CollectRNASeqMetrics was used to measure the 3′ bias of genes in the RNA-seq data (see Code Availability 3). Gene-level differential expression was analyzed using DESeq2 specifying ~ region + group + bias as the experimental design (see Code Availability 4). Aligned reads mapping to MECP2 isoforms were also summarized using featureCounts v1.6 (see Code Availability 2) by substituting isoforms for gene name.

Data Records
Count matrix and normalized count matrix were submitted to the NCBI Gene Expression Omnibus (GEO) under accession number GSE12838024. The raw FASTQ files can be downloaded from the Sequence Read Archive (SRA) under accession number SRP18855525.

Technical Validation
MECP2 variant confirmation. We verified the presence of the MECP2 c.473 C > T (p.Thr158Met) intragenic variant using DNA isolated from brain 7773 (Supplemental Fig. 1). No MECP2 variants were detected in exons 2–4 of brain 7783. Since we were unable to amplify exon 1 in 7783, we infer exon 1 is likely to be the deleted exon. We also examined RNA-seq data for presence of MECP2 variants (Supplemental Fig. 2). The MECP2 c.473 C > T (p.Thr158Met) intragenic variant was also detected in RNA-seq data from CCTX and TCTX for brain 7773. MECP2 variants were not detected in RNA-seq data for other RTT brain samples, possibly due to low sequencing read depth of MECP2 (Supplemental Fig. 3), or because causal variants are present in another gene.

RNA and data quality. RNA quality was determined using the Agilent 2100 Bioanalyzer and the RNA 6000 Pico Kit and high-quality RNA was obtained from all samples (RNA integrity number [RIN] > 8.0; median RIN = 9.4 [Table 2]). At the time the experiment was performed, the TruSeq RNA Sample Prep v2 protocol (Part # 15026495 Rev.C, May 2012) was optimized for 0.1–4 μg of total RNA. Although the quantity of RNA input varied among the samples in our experiment, it was equivalent within each age- and tissue- matched case-control sample pair, and all samples were within the optimized range. On average, RNA-seq generated 21.9 million high-quality reads per sample, 70.3% of which mapped uniquely to the Human reference genome (NCBI build 37/hg19) (Table 3). RIN and RNA quantity were each correlated with the number of uniquely mapped reads (Fig. 2). Cook’s distance was calculated to test for outliers, with none detected (Fig. 3a). The first principal component explained over 50% of the variance (Fig. 3b). A correlation matrix based on the gene expression data indicated that samples mostly cluster by individual and diagnostic group, but also by 3′ bias (Fig. 3c).

Table 2. RNA Sample Characteristics. Abbreviations: BA, Brodmann area; CCTX, cingulate cortex; CTL, control; RIN, RNA integrity number; RNA, quantity of total RNA used as input for library preparation; RTT, Rett syndrome; TCTX, temporal cortex.
MECP2 and MET differential expression. We previously used quantitative reverse transcription PCR to compare expression of MECP2_e1 (NM_004992.3), MECP2_e2 (NM_001110792.1), and MET (NM_000245.3) in the temporal cortex between RTT and CTL brains. Consistent with our previous results, the RNA-seq data showed no significant difference in MECP2 expression between RTT and CTL brains (FDR adjusted p-value = 0.16 and 0.59, respectively), while MET expression was significantly reduced in RTT brains (FDR = 1.07 \times 10^{-05}; Fig. 4).

Compatibility with published transcriptional profiles. Two RNA-seq datasets of postmortem brain from females with RTT compared to controls have been published (Table 4). The first dataset examined pooled frontal and temporal cortex (FTTX) for each of three individuals with RTT compared to three CTL and is available from the Sequence Read Archive under accession number PRJNA302685. The second larger dataset examined motor cortex (Motor) and cerebellum (Cblm) for nine females and six females with RTT, respectively, compared to eight CTL of each tissue, but the primary data were not accessible. We downloaded the FASTQ files for the available dataset, aligned reads using salmon, summarized the aligned reads into gene counts using tximport, and retained genes with \( \geq 10 \) counts in \( \geq 3 \) samples. Count data were converted to logCPM to adjust for the total counts per sample using limma. Our meta-analysis identified 1,455 genes that were significantly differentially expressed (FDR < 0.05) between brain samples from control individuals and those with RTT.

| Brain | Group | Region | Total Reads | % Bases \( \geq Q30 \) | Mean Base Quality | Uniquely Mapped Reads | Mapping Rate | Median 5' to 3' Bias |
|-------|-------|--------|-------------|---------------------|------------------|-----------------------|--------------|----------------------|
| 1038  | CTL   | CCTX   | 22,666,185  | 77.09               | 31.71            | 18,508,346            | 81.66        | 2.16                 |
| 1614  | CTL   | CCTX   | 20,406,028  | 76.18               | 31.42            | 13,966,751            | 68.44        | 0.76                 |
| 4724  | CTL   | CCTX   | 17,775,778  | 76.21               | 31.45            | 12,903,748            | 72.59        | 0.51                 |
| 4725  | CTL   | CCTX   | 17,152,400  | 76.58               | 31.57            | 13,787,313            | 80.38        | 1.36                 |
| 6355  | RTT   | CCTX   | 16,799,774  | 76.38               | 31.49            | 11,754,027            | 69.96        | 0.71                 |
| 7773  | RTT   | CCTX   | 22,119,416  | 77.01               | 31.70            | 17,627,391            | 79.69        | 1.36                 |
| 7783  | RTT   | CCTX   | 32,207,239  | 77.16               | 31.72            | 22,511,241            | 69.89        | 0.43                 |
| 87992 | RTT   | CCTX   | 21,882,359  | 76.58               | 31.56            | 17,064,617            | 77.91        | 1.09                 |
| 1038  | CTL   | CCTX (BA38) | 23,689,678 | 77.72             | 31.88           | 16,671,042            | 70.37        | 1.34                 |
| 1614  | CTL   | CCTX (BA36) | 17,569,738 | 78.01             | 31.97           | 11,367,085            | 64.70        | 0.44                 |
| 4724  | CTL   | CCTX (BA36) | 22,429,527 | 77.84             | 31.93           | 15,519,596            | 69.19        | 0.98                 |
| 4725  | RTL   | TCTX (BA38) | 24,262,545 | 78.03             | 31.97           | 16,322,635            | 67.28        | 1.04                 |
| 6355  | RTT   | TCTX   | 25,330,544  | 76.95               | 31.64            | 14,294,799            | 56.43        | 0.36                 |
| 7773  | RTT   | TCTX   | 22,557,491  | 77.85               | 31.92            | 15,330,591            | 67.96        | 0.97                 |
| 7783  | RTT   | TCTX   | 17,060,381  | 77.82               | 31.92            | 11,301,790            | 66.25        | 0.82                 |
| 87992 | RTT   | TCTX   | 26,211,057  | 77.11               | 31.70            | 16,434,103            | 62.70        | 1.01                 |
To verify these results, we compared the results from our meta-analysis with differential gene expression results from previous RTT RNA-seq analyses\(^\text{13,14}\) (Fig. 5). We compared the Z-score for each of the significantly differentially expressed genes from our meta-analysis with the log2 fold change from our previous analysis (GEO DESeq2).
and from each of the three published RNA-seq datasets (Lin et al., Gogliotti et al. Motor, and Gogliotti et al. Cblm; Fig. 5a). We found strong concordance among RTT transcriptional profiles from regions within the cerebral cortex, while RTT transcriptional profiles from the cerebellum were least correlated with the regions from the cerebral cortex (Fig. 5b). We aggregated the gene-wise correlation coefficients among datasets and found an overall positive correlation for 63% of the comparisons among datasets, indicating an overall agreement among the differential gene expression per dataset (Fig. 5c). Not only do our data represent an independent technical and biological replication of molecular alterations in RTT brain, but our meta-analysis demonstrates the power of combining datasets to maximize detectable results among several smaller studies.

**Code availability**

We used the following software and versions to process our dataset as described in the text:

1. STAR v2.5.3a was used for mapping reads to the Human reference genome NCBI build 37/hg19: https://github.com/alexdobin/STAR
2. featureCounts v1.6 was used to summarize gene counts: http://bioinf.wehi.edu.au/featureCounts/
3. Picard v2.15.0 was used to measure 5′ to 3′ bias: http://broadinstitute.github.io/picard
4. DESeq2 v1.20.0 was used for differential expression analysis: https://bioconductor.org/packages/release/bioc/html/DESeq2.html.
5. IGV v2.8.2 was used to visualize MECP2 coding regions for sequence variation: http://software.broadinstitute.org/software/igv/
6. Salmon was used to align reads to the Human GRCh38 reference transcriptome and estimate counts for each transcript: https://combine-lab.github.io/salmon/
7. tximport v1.12.1 was used to summarize gene counts: https://bioconductor.org/packages/release/bioc/html/tximport.html
8. limma v3.40.2 was used to convert count data to log counts per million (logCPM) and to estimate weights: https://bioconductor.org/packages/release/bioc/html/limma.html
9. GeneMeta v1.56.0 was used to perform a random effects meta-analysis: https://www.bioconductor.org/packages/release/bioc/html/GeneMeta.html
K.A.A. conceived the project, performed experiments with guidance from O.V., analyzed data, and wrote the manuscript. A.E.T., J.W.M. and J.S.H. analyzed data. H.M. performed experiments. T.K.B., J.A.K. and P.L. supervised the work. All authors reviewed and accepted the final version of the manuscript.

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Author contributions
K.A.A. conceived the project, performed experiments with guidance from O.V.E., analyzed data, and wrote the manuscript. A.E.T., J.W.M. and J.S.H. analyzed data. H.M. performed experiments. T.K.B., J.A.K. and P.L. supervised the work. All authors reviewed and accepted the final version of the manuscript.

References
1. Amir, R. E. et al. Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. Nat Genet 23, 185–188, https://doi.org/10.1038/13810 (1999).
2. Ramocki, M. B., Tayevo, Y. J. & Peters, S. U. The MECP2 duplication syndrome. Am J Med Genet A 152A, 1079–1088, https://doi.org/10.1002/ajmg.a.33184 (2010).
3. Baker, S. A. et al. An AT-hook domain in MeCP2 determines the clinical course of Rett syndrome and related disorders. Cell 152, 984–996, https://doi.org/10.1016/j.cell.2013.01.038 (2013).
4. Ho, K. L. et al. MeCP2 binding to DNA depends upon hydration at methyl-CpG. Mol Cell 29, 525–531, https://doi.org/10.1016/j.molcel.2007.12.028 (2008).
5. Lewis, J. D. et al. Purification, sequence, and cellular localization of a novel chromosomal protein that binds to methylated DNA. Cell 69, 905–914 (1992).
6. Nan, X., Campoy, F. J. & Bird, A. MeCP2 is a transcriptional repressor with abundant binding sites in genomic chromatin. Cell 88, 471–481 (1997).
7. Nan, X. et al. Transcriptional repression by the methyl-CpG-binding protein MeCP2 involves a histone deacetylase complex. Nature 393, 386–389, https://doi.org/10.1038/30764 (1998).
8. Skene, P. J. et al. Neuronal MeCP2 is expressed at near histone-octamer levels and globally alters the chromatin state. Mol Cell 37, 457–468, https://doi.org/10.1016/j.molcel.2010.01.030 (2010).
9. Chahroul, M. et al. MeCP2, a key contributor to neurological disease, activates and represses transcription. Science 320, 1224–1229, https://doi.org/10.1126/science.1152525 (2008).
10. Colantuoni, C. et al. Gene expression profiling in postmortem Rett Syndrome brain: differential gene expression and patient classification. Neurobiol Dis 8, 847–865, https://doi.org/10.1006/nbd.2001.0428 (2001).
11. Deng, W. et al. FXPD1 is an MeCP2 target gene overexpressed in the brains of Rett syndrome patients and Mecp2-null mice. Hum Mol Genet 16, 640–650, https://doi.org/10.1093/hmg/ddm007 (2007).
12. Gibson, J. H. et al. Downstream targets of methyl CpG binding protein 2 and their abnormal expression in the frontal cortex of the human Rett syndrome brain. BMC Neurosci 11, 53, https://doi.org/10.1186/1471-2202-11-53 (2010).
13. Lin, P. et al. Transcriptome analysis of human brain tissue identifies reduced expression of complement complex C1Q Genes in Rett syndrome. BMC Genomics 17, 427, https://doi.org/10.1186/s12864-016-2746-7 (2016).
14. Gogliotti, R. G. et al. Total RNA Sequencing of Rett Syndrome Autopsy Samples Identifies the M4 Muscarinic Receptor as a Novel Therapeutic Target. J Pharmacol Exp Ther 365, 291–300, https://doi.org/10.1124/jpet.117.246991 (2016).
15. Armstrong, D. D., Dunn, K. & Antalffy, B. Decreased dendritic branching in frontal, motor and limbic cortex in Rett syndrome compared with trisomy 21. J Neurophysiol Exp Neurol 57, 1013–1017, https://doi.org/10.1007/978-0-387-95003-0_3 (1998).
16. Mahmood, A. et al. White matter impairment in Rett syndrome: diffusion tensor imaging study with clinical correlations. AJNR Am J Neuroradiol 31, 295–299, https://doi.org/10.3174/ajnr.A1792 (2010).
17. Khong, P. L., Lam, C. W., Ooi, C. G., Ko, C. H. & Wong, V. C. Magnetic resonance spectroscopy and analysis of MECP2 in Rett syndrome. Pediatr Neuro 26, 205–209, https://doi.org/10.1101/sf0887-8994(01)00385-x (2002).
18. Subramaniam, B., Naidu, S. S. & Reiss, A. L. Neuroanatomy in Rett syndrome: cerebral cortex and posterior fossa. Neurology 48, 399–407, https://doi.org/10.1212/wnl.48.2.399 (1997).
19. Kozak, K. & Standley, D. M. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol 30, 772–780, https://doi.org/10.1093/molbev/msu010 (2013).
20. Aldinger, K. A., Plummer, J. T. & Levitt, P. Comparative DNA methylation among females with neurodevelopmental disorders and seizures identifies TACC as a MeCP2 target gene. J Neurodev Disord 5, 15, https://doi.org/10.1186/s12011-015-0135-3 (2013).
21. Dobin, A. et al. STAR: ultrarapid universal RNA-seq aligner. Bioinformatics 29, 15–21, https://doi.org/10.1093/bioinformatics/btt635 (2013).
22. Liao, Y., Smyth, G. K. & Shi, W. featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. Bioinformatics 30, 923–930, https://doi.org/10.1093/bioinformatics/btt654 (2014).
23. Love, M. I., Huber, W. & Anders, S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol 15, 550, https://doi.org/10.1186/s13059-014-0550-8 (2014).
24. Aldinger, K. A. Transcriptome data of temporal and circulate cortex in the Rett syndrome brain. Gene Expression Omnibus https://identifiers.org/geo:GSE128380 (2019).
25. NCBI Sequence Read Archive https://identifiers.org/ncbi:insdc:sra:SRP185555 (2019).
26. Sajjan, S. A. et al. Enrichment of mutations in chromatin regulators in people with Rett syndrome lacking mutations in MECP2. Genet Med 19, 13–19, https://doi.org/10.1038/gim.2016.42 (2017).
27. Wang, J. et al. Rett and Rett-like syndrome: Expanding the genetic spectrum to KIF1A and GRIN1 gene. Mol Genet Genomic Med 7, e968, https://doi.org/10.1002/mgg3.968 (2019).
28. Plummer, J. T. et al. Transcriptional regulation of the MET receptor tyrosine kinase gene by MeCP2 and sex-specific expression in autism and Rett syndrome. Transl Psychiatry 3, e316, https://doi.org/10.1038/tp.2013.91 (2013).
29. NCBI Sequence Read Archive https://identifiers.org/ncbi:insdc:sra:SRP066394 (2015).
30. Patro, R., Duggal, G., Love, M. I., Irizarry, R. A. & Kingsford, C. Salmon provides fast and bias-aware quantification of transcript expression. Nat Methods 14, 417–419, https://doi.org/10.1038/nmeth.4197 (2017).
31. Soneson, C., Love, M. I. & Robinson, M. D. Differential analyses for RNA-seq: transcript-level estimates improve gene-level inferences. F1000Res 4, 1521, https://doi.org/10.12688/f1000research.7563.2 (2015).
32. Smyth, G. K. Linear models and empirical bayes methods for assessing differential expression in microarray experiments. Stat Appl Genet Mol Biol 3, Article3, https://doi.org/10.2202/1544-6155.1027 (2004).
33. Law, C. W., Chen, Y., Shi, W. & Smyth, G. K. voom: Precision weights unlock linear model analysis tools for RNA-seq read counts. Genome Biol 15, R29, https://doi.org/10.1186/gb-2014-15-2-r29 (2014).
34. Choi, J. K., Yu, U., Kim, S. & Yoo, O. J. Combining multiple microarray studies and modeling interstudy variation. Bioinformatics 19(Suppl 1), i84–90, https://doi.org/10.1093/bioinformatics/btg1010 (2003).
Competing interests
The authors declare no competing interests.

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