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Diurnal salivary cortisol and regression status in *MECP2* Duplication syndrome

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Abstract

MECP2 duplication syndrome is an X-linked genomic disorder that is characterized by infantile hypotonia, intellectual disability, and recurrent respiratory infections. Regression affects a subset of individuals, and the etiology of regression has yet to be examined. In this study, alterations in the hypothalamus-pituitary-adrenal axis, including diurnal patterns in salivary cortisol, were examined in four males with *MECP2* duplication syndrome who had regression, and four males with the same syndrome without regression (ages 3–22 years). Individuals who had experienced regression do not exhibit typical diurnal cortisol rhythms, and their profiles were flatter throughout the day. In contrast, individuals with *MECP2* duplication syndrome who had not experienced regression showed more typical patterns of higher cortisol levels in the morning with linear decreases throughout the day. This study is the first to suggest a link between atypical diurnal cortisol rhythms and regression status in *MECP2* duplication syndrome, and may have implications for treatment.

Keywords

MECP2; cortisol; regression

Introduction

MECP2 duplication syndrome is an X-linked genomic disorder that is characterized by infantile hypotonia, intellectual disability (ID), autism, progressive neurological decline, and

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Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author Contributions

SUP, BJB, and FJS developed the study idea. SUP was responsible for patient enrollment. SUP and BJB assessed all patients clinically, and wrote the draft of the article. BJB and FJS carried out data analysis and interpretation of salivary cortisol data. FJS edited the manuscript.

Ethical approval

This study was approved by the Institutional Review Boards of Vanderbilt University, and the University of Minnesota. All parents gave consent for their children to participate.

recurrent respiratory infections^{1–3}. Duplications of Xq28 underlie this disorder, which accounts for 1–2% of all cases of X-linked ID⁴, and primarily affects males. The common genomic region involved includes *Methyl-CpG-binding protein 2 (MECP2)*, the gene mutated in Rett Syndrome (RTT). *MECP2* duplication syndrome is a relatively newly described disorder, and many elements of the behavioral phenotype have yet to be characterized. One of the most challenging consequences of *MECP2* disorders is the phenomenon of developmental regression. Regression is defined as a partial or complete loss of skills related to language, socialization, self-help skills, and/or motor skills. Although regression has been well defined in Rett Syndrome (RTT) and is a requirement for clinical diagnosis, the onset and associated features of regression in *MECP2* duplication syndrome is just beginning to be characterized.⁵ It is unclear whether regression occurs directly due to changes in the central nervous system (CNS), or whether peripheral factors (i.e. immune function) also play a role. Recent work suggests that at least 50% of individuals with *MECP2* duplication syndrome exhibit losses of language and/or motor skills; increased respiratory infections, lower cognition, seizures, and social withdrawal are associated factors⁵. When regression occurs, the loss of skills is often dramatic (e.g. loss of mobility, head control, self-help skills, and/or language, and hand use), and in some cases associated with the onset or worsening of seizures and viral illnesses/hospitalizations.

The underlying etiology associated with regression has yet to be investigated in *MECP2* duplication syndrome. Immune and hypothalamus-pituitary-adrenal axis activity components have been considered to contribute to disease fluctuations of chronic inflammatory diseases. For example, two autoimmune conditions associated with *MeCP2* overexpression^{6, 7}, systemic lupus erythematosus and Sjögren's syndrome, have been associated with neurological changes/declines^{8, 9} and hypo-activity of the hypothalamus-pituitary-adrenal axis¹⁰. The enhanced inflammatory susceptibility in some autoimmune conditions can be related to impairments at the level of hypothalamic corticotrophin releasing hormone (CRH)^{11, 12}. CRH causes release of adrenocorticotrophic hormone from the pituitary gland, which in turn travels through the bloodstream to the adrenal glands, where it causes the release of cortisol. Recent studies of RTT and *MECP2* duplication animal models demonstrate that the *crh* gene is a “bona-fide” target of *MECP2*¹³, and doubling the expression of *MECP2* leads to anxiety and social withdrawal. Social withdrawal has been associated with “sickness” behavior, increased susceptibility to infections, and persistent inflammation¹⁴. Abnormalities in *MECP2* are associated with abnormalities in acute stress response,¹⁵ but the long term consequences of alterations in *MECP2* to hypothalamus-pituitary-adrenal axis activity have not been examined.

Levels of cortisol are usually highest early in the morning and gradually decline during the day. Blunted cortisol (low levels in the morning) has been noted in rheumatoid arthritis, lupus, and Sjögren's syndrome^{16, 17}. Behaviorally, blunted cortisol has been associated with elevations in anxiety and depression¹⁸. Some studies in other populations^{19, 20} suggest that HPA-axis hypoactivity may contribute to increased frequency and/or severity of infections, including pneumonias. Given the immunocompromised state of many patients with *MECP2* duplication syndrome, and the possible association with regression, the purpose of this preliminary study was to investigate whether alterations in HPA-axis activity, including

abnormal diurnal patterns in salivary cortisol, were associated with regression in *MECP2* duplication syndrome.

Methods

Participants

In total, 8 males with *MECP2* duplication syndrome, aged 3 to 22 years, participated in the current study (see Table 1 for participant characteristics). All participants had a confirmed diagnosis of *MECP2* duplication by array comparative genome hybridization (aCGH). No participants were on immunotherapy prior to or during their participation in the study.

Regression measurement

To assess regression, we used the Autism Diagnostic Interview – Revised (ADI-R) questions regarding regression, early milestones, and symptom onset. Since the ADI-R questions regarding language-regression require the minimum use of at least five different words (other than “mama” and “dada”) on a daily basis for at least three months, a research-based regression interview was designed as an extension to capture sub-threshold losses of skills as separated into language (e.g. loss of cooing, babbling, vocal imitation, communicative intent), and non-language skills (hand use, motor skills, self-help skills, social interest, and curiosity)^{21, 22}. This interview utilizes the same coding and similar questions to the ADI-R, and was utilized as part of the Simons Simplex Collection (SSC). Information regarding the age of the loss, duration of the loss, as well as any association with physical illness is captured. To be defined as “regression” we used the convention that the loss must have been present for the duration of at least 3 months. Regression was also defined as a loss of non-language (i.e. motor) skills because losses of these skills are very dramatic and occur without recovery of functioning. Losses within this domain include loss of hand use, posture, gait, coordination, and self-help skills. Loss of language skills alone is more transient and consists of babbling as opposed to the loss of words since our prior studies show that most patients never acquire the fluent use of five or more words on a daily basis⁵.

Behavioral Measurement

All parents were interviewed using the Vineland Adaptive Behavior Scales, Interview Edition. The Vineland has been commonly used in studies of individuals with *MECP2* duplication syndrome¹.

Saliva Collection

Participating families were provided with a saliva collection kit that included Children’s Swabs (Salimetrics Pennsylvania, USA), syringes, and cryovials to collect and store saliva samples. An instruction sheet with directions regarding collection and storage of the samples, as well as a diary to be filled out with information regarding meals, sleep, and medications during each collection day were also included.

Participating caregivers were instructed to collect eight saliva samples with a minimum volume of 0.5 mL over the course of two days at four pre-specified time points: approximately 30 minutes after the individual woke up in the morning, mid-morning, mid-

afternoon, and approximately 30 minutes before the individual went to bed in the evening. Caregivers were instructed to avoid collecting samples within 30 minutes of eating or drinking, and to select sampling days that were typical for their child, meaning that there were no pre-planned occurrences of non-routine events (e.g., doctors' appointments or parties/celebrations), and when their child was in good health. Samples were returned to the university in uncooled padded mailers, but this was not expected to affect cortisol concentration levels. Because cortisol is a relatively stable hormone, it may remain at room temperature for several weeks without degrading (Gunnar & White, 2001). At the university, they were stored in a -20°C freezer until all samples were returned.

Cortisol Assay

The saliva samples were processed using the Cortisol (Saliva) ELISA kit (ALPCO Diagnostics, Salem, NH) at the Cytokine Reference Laboratory at the University of Minnesota. The sensitivity of the assay was 1.0 ng/mL. All measurements were performed in duplicate and averaged to attain concentration values for each sample. The duplicate correlation value was .99, and the inter-assay coefficient of variation was 3.7%.

Missing Data and Tests of Assumptions

Only one sample was returned with insufficient volume to assay. Resulting values were evaluated to identify potential outliers, or for values below the detection threshold of the assay. One participants' cortisol concentrations were well outside of the range of any of the other participants, which was likely due to a recent course of steroid medications for a respiratory infection. As a result, his data were excluded from further analyses. No other outliers were identified. Cortisol concentrations are typically highly skewed, therefore a logarithmic transformation was applied prior to subsequent analyses. The resulting data were approximately normally distributed. Values and patterns of cortisol concentrations were relatively stable across the two days, so values were averaged across the two days to simplify the analyses.

Data analysis

The diurnal cortisol slope for each participant was calculated by regressing log-transformed cortisol concentrations on sample collection time within a multi-level regression model²⁰. Cortisol results were compared using t-tests and a repeated-measures ANOVA with age as a covariate.

Results

There were four participants each in the regression and no-regression groups. In all four cases of regression, there were losses of motor skills (i.e. hand use, gait, coordination, feeding themselves) in addition to sub-threshold language skills (i.e. babbling). In each case, the onset of regression was associated with a physical illness (e.g. adenovirus, pneumonia, RSV, etc.) and/or seizure onset without subsequent recovery of functioning. Most of these participants previously had some language use (at least babbling vowel-consonant sounds), were able to walk, engage in functional play, and had developed some self-help skills (e.g. feeding themselves) prior to regression. Three of the four participants in the regression

group had lower adaptive behavior skills as compared to their peers in the non-regression group. In addition, three of the four participants in the regression group had a prior diagnosis of an autism spectrum disorder. No children within the non-regression group had a prior diagnosis of autism, although all exhibited stereotypic behaviors such as hand-flapping. These differences were primarily attributable to a loss of mobility (i.e. walking) and hand use, in addition to social withdrawal and diminished babbling and communicative intent. Further details of this cohort as related to regression can be found in a previous publication⁵. When the primary caregiver of each participant was asked to estimate the number of respiratory infections in the previous 12 months, estimates ranged from 0 to 4 (see Table 1). There were no relationships between infection history and age in this sample.

Diurnal patterns for each of the participants are presented in Figure 1. In the no-regression group, all participants had negative cortisol slope values ($M = -.085$), indicating a typical pattern of decline throughout the day. In the regression group, however, three of the four participants showed positive slopes ($M = -.005$), indicating no diurnal rhythm in cortisol production. The difference in slopes between the two groups was statistically significant ($t(6) = -3.923, p = .008, d = 3.23$). The repeated measures indicated a significant time by group interaction ($F(3,15) = 7.935, p = .002$, partial $\eta^2 = .613$), with post-hoc analyses indicating that differences in the change in cortisol concentrations from the first sample to the last sample was responsible for this interaction ($F(1,5) = 2.993, p = .002$, partial $\eta^2 = .810$), as can be seen in Figure 2. There were no relationships between any cortisol measures and frequency of respiratory infections in this sample.

Discussion

The purpose of this preliminary study was to investigate whether alterations in HPA-axis activity, including abnormal diurnal patterns in salivary cortisol, were associated with regression in *MECP2* duplication syndrome. Results suggest that individuals who had experienced regression do not exhibit typical diurnal cortisol rhythms, and their profiles were flatter through the day. In contrast, individuals with *MECP2* duplication syndrome who had not experienced regression were more likely to show typical patterns of higher cortisol levels in the morning with linear decreases throughout the day. As far as we know, this is the first study to document differences in HPA-axis function in *MECP2* duplication syndrome based on regression status. Because no typically developing comparison sample was included in the study, it is impossible to know whether the absolute values of cortisol measured in the non-regression group were typical, higher, or lower than would be expected, and additional research is needed to clarify this point.

In some populations (multiple sclerosis, sepsis)^{19, 20} atypical diurnal rhythms and blunted profiles are related to disease severity. Therefore, the finding that the number of respiratory infections reported in the previous 12 months was not significantly related to cortisol levels or patterns in this sample was unexpected, especially given previous findings indicating a potential link between regression and immune issues in *MECP2* duplication syndrome⁵. There are several possible reasons for this result. First, the very small sample may have obscured an effect that would have been apparent in a larger sample. Second, it is possible that the frequency of infections is less important than the severity and/or type of infection,

number of hospitalizations, length of illness, and/or the number of antibiotic courses. Third, three of the four individuals in the regression group were homebound from school to prevent further infections, and this may have affected the frequency of respiratory infections by limiting exposure.

This study is clearly preliminary and should be interpreted with caution given its methodological limitations. In particular, the very small, non-matched samples of individuals with and without regression limit the generalizability of the findings and the ability to control for many potentially important factors in more complex statistical analyses. Future studies with larger samples should investigate the potential relationships between immune function, regression status, and cortisol with many other health and behavioral issues, including sleep problems, seizures, medications, and behavioral features such as social withdrawal and other features of autism, particularly since three of four children within the regression group had a prior diagnosis of autism. In addition, C-reactive protein values were not measured at the same time as salivary cortisol sampling, so the possibility that inflammation may have impacted the results cannot be ruled out. Additionally, the cross-sectional nature of these data preclude interpretations regarding the timing and sequence of changes in diurnal cortisol patterns with regard to regression in this population. Longitudinal studies are needed to examine whether such changes predict or follow regression. In spite of these limitations, the findings are novel and suggest several important avenues for additional research to further investigate the potential links between immune functioning, regression, and cortisol patterns in *MECP2* duplication syndrome. If confirmed, these links could have important implications for predicting prognosis of individuals with the syndrome, and for improving health and quality of life for affected individuals. It may also have important implications for potential therapies to mitigate disease severity and/or course.

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References

1. Ramocki MB, Peters SU, Tavyev YJ, Zhang F, Carvalho CM, Schaaf CP, et al. Autism and other neuropsychiatric symptoms are prevalent in individuals with MeCP2 duplication syndrome. *Ann Neurol*. 2009; 66(6):771–82. [PubMed: 20035514]
2. Van Esch H. MECP2 Duplication Syndrome. *Mol Syndromol*. 2012; 2(3–5):128–36. [PubMed: 22679399]
3. Friez MJ, Jones JR, Clarkson K, Lubs H, Abuelo D, Bier JA, et al. Recurrent infections, hypotonia, and mental retardation caused by duplication of MECP2 and adjacent region in Xq28. *Pediatrics*. 2006; 118(6):e1687–95. [PubMed: 17088400]
4. Amir RE, Van den Veyver IB, Wan M, Tran CQ, Francke U, Zoghbi HY. Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. *Nat Genet*. 1999; 23(2): 185–8. [PubMed: 10508514]

5. Peters SU, Hundley RJ, Wilson AK, Carvalho CM, Lupski JR, Ramocki MB. Brief Report: Regression Timing and Associated Features in MECP2 Duplication Syndrome. *J Autism Dev Disord.* 2013
6. Cobb BL, Fei Y, Jonsson R, Bolstad AI, Brun JG, Rischmueller M, et al. Genetic association between methyl-CpG binding protein 2 (MECP2) and primary Sjogren's syndrome. *Annals of the rheumatic diseases.* 2010; 69(9):1731–2. [PubMed: 20215141]
7. Sawalha AH, Webb R, Han S, Kelly JA, Kaufman KM, Kimberly RP, et al. Common variants within MECP2 confer risk of systemic lupus erythematosus. *PLoS One.* 2008; 3(3):e1727. [PubMed: 18320046]
8. Baizabal-Carvallo JF, Bonnet C, Jankovic J. Movement disorders in systemic lupus erythematosus and the antiphospholipid syndrome. *Journal of neural transmission (Vienna, Austria : 1996).* 2013
9. Baizabal-Carvallo JF, Jankovic J. Movement disorders in autoimmune diseases. *Mov Disord.* 2012; 27(8):935–46. [PubMed: 22555904]
10. Tzioufas AG, Tsonis J, Moutsopoulos HM. Neuroendocrine dysfunction in Sjogren's syndrome. *Neuroimmunomodulation.* 2008; 15(1):37–45. [PubMed: 18667798]
11. Straub RH, Buttgereit F, Cutolo M. Alterations of the hypothalamic-pituitary-adrenal axis in systemic immune diseases - a role for misguided energy regulation. *Clinical and experimental rheumatology.* 2011; 29(5 Suppl 68):S23–31. [PubMed: 22018180]
12. DeSantis AS, DiezRoux AV, Hajat A, Aiello AE, Golden SH, Jenny NS, et al. Associations of salivary cortisol levels with inflammatory markers: the Multi-Ethnic Study of Atherosclerosis. *Psychoneuroendocrinology.* 2012; 37(7):1009–18. [PubMed: 22178583]
13. Samaco RC, Mandel-Brehm C, McGraw CM, Shaw CA, McGill BE, Zoghbi HY. Crh and Oprm1 mediate anxiety-related behavior and social approach in a mouse model of MECP2 duplication syndrome. *Nat Genet.* 2012; 44(2):206–11. [PubMed: 22231481]
14. Dantzer R, Kelley KW. Twenty years of research on cytokine-induced sickness behavior. *Brain, behavior, and immunity.* 2007; 21(2):153–60.
15. Fyffe SL, Neul JL, Samaco RC, Chao HT, Ben-Shachar S, Moretti P, et al. Deletion of Mecp2 in Sim1-expressing neurons reveals a critical role for Mecp2 in feeding behavior, aggression, and the response to stress. *Neuron.* 2008; 59(6):947–58. [PubMed: 18817733]
16. van der Goes MC, Bossema ER, Hartkamp A, Godaert GL, Jacobs JW, Kruize AA, et al. Cortisol during the day in patients with systemic lupus erythematosus or primary Sjogren's syndrome. *The Journal of rheumatology.* 2011; 38(2):285–8. [PubMed: 21159832]
17. Pool AJ, Whipp BJ, Skasick AJ, Alavi A, Bland JM, Axford JS. Serum cortisol reduction and abnormal prolactin and CD4+/CD8+ T-cell response as a result of controlled exercise in patients with rheumatoid arthritis and systemic lupus erythematosus despite unaltered muscle energetics. *Rheumatology (Oxford, England).* 2004; 43(1):43–8.
18. Roozendaal B, Kim S, Wolf OT, Kim MS, Sung KK, Lee S. The cortisol awakening response in amyotrophic lateral sclerosis is blunted and correlates with clinical status and depressive mood. *Psychoneuroendocrinology.* 2012; 37(1):20–6. [PubMed: 21616601]
19. Melief J, de Wit SJ, van Eden CG, Teunissen C, Hamann J, Uitdehaag BM, et al. HPA axis activity in multiple sclerosis correlates with disease severity, lesion type and gene expression in normal-appearing white matter. *Acta neuropathologica.* 2013; 126(2):237–49. [PubMed: 23812288]
20. Schroeder S, Wichers M, Klingmuller D, Hofer M, Lehmann LE, von Spiegel T, et al. The hypothalamic-pituitary-adrenal axis of patients with severe sepsis: altered response to corticotropin-releasing hormone. *Critical care medicine.* 2001; 29(2):310–6. [PubMed: 11246311]
21. Parr JR, Le Couteur A, Baird G, Rutter M, Pickles A, Fombonne E, et al. Early developmental regression in autism spectrum disorder: evidence from an international multiplex sample. *J Autism Dev Disord.* 2011; 41(3):332–40. [PubMed: 20711649]
22. Luyster R, Richler J, Risi S, Hsu WL, Dawson G, Bernier R, et al. Early regression in social communication in autism spectrum disorders: a CPEA Study. *Dev Neuropsychol.* 2005; 27(3): 311–36. [PubMed: 15843100]

Figure 1.
Individual level cortisol concentration across the four sampling times.

Figure 2.

Estimated mean cortisol concentrations in regression and no regression groups at all four sampling times controlling for age.

Table 1

Participant characteristics

Participant	Age	Regression	Cortisol Slope	Vineland composite score	Respiratory infections (12 months)	Homebound from school
1	10	Y	0.009	36	1	Y
2	22	Y	-0.063	20	4+	Y
3	7	Y	0.022	60	1	N
4	20	Y	0.014	24	4+	Y
5	9	N	-0.071	46	0	N
6	4	N	-0.087	52	4+	N
7	10	N	-0.084	48	1	N
8	10	N	-0.099	45	2	N