



Published in final edited form as:

*Pediatr Neurol.* 2018 March ; 80: 88–89. doi:10.1016/j.pediatrneurol.2017.11.005.

## Initial Observations of Salivary BDNF Levels in Rett Syndrome

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### Keywords

Rett syndrome; Brain-Derived Neurotrophic Factor (BDNF); saliva; biomarker

Rett syndrome (RTT) is a neurodevelopmental disorder affecting primarily females, caused by mutations in the methyl-CpG-binding protein (*MECP2*) 2 gene.<sup>1</sup> Alterations to the expression of brain-derived neurotrophic factor (BDNF) expression have been implicated in the pathogenesis of RTT.<sup>2</sup> Clinical evidence of altered BDNF concentrations in central and peripheral samples in humans has been equivocal.<sup>3</sup> BDNF is detectable in human saliva,<sup>4</sup> but there has been no work investigating salivary BDNF in RTT. In the work described below, we report on the first use of saliva sampling to characterize peripheral BDNF levels in RTT.

### Methods

#### Sample

A clinical convenience sample of 16 female, Caucasian patients (mean age = 12.4 years, range = 4.9–29.8 years) with clinical diagnosis of RTT participated. All but one (94%) had confirmed pathogenic mutations of *MECP2*. One patient had a clinical diagnosis only (RTT is still a behaviorally-defined syndrome; if patients meet diagnostic criteria they are considered as having RTT with or without evidence of mutation). All but one participant (94%) were taking anticonvulsant medications at the time of participation. Three participants (19%) could walk without support, and six (38%) had nutritional needs met via gastrostomy tubes.

#### Specimen collection & processing

Saliva was collected during a routine clinic visit. Approximately 3 ml of unstimulated saliva was procured between 7:00–10:00 am using toothette oral swabs. Samples were centrifuged immediately at 3,000 rpm for 5 min then aliquoted into cryovials (500 ul) and frozen at –80 C. Assays were performed by the Cytokine Reference Laboratory in the University of

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Minnesota according to manufacturers' instructions. See Table 1 for details of both assay kits.

## Results

None of the patients had detectable salivary BDNF levels using the first assay method. We had observed salivary BDNF using the same assay in other clinical samples (patients with cerebral palsy; mean age = 9.5 years old, mean salivary BDNF level = 3.4 pg/ml, sd = 3.8, range = 1.3–14.1 pg/ml<sup>5</sup>). The ELISA assay was evaluated on a subgroup for which we had additional available saliva specimen from the original sample (N=10, mean age = 11yr/7mo, range = 4yr11 mo – 18yr7mo). Using this approach, salivary BDNF was detectable (mean = 6.99 pg/ml, median = 3.7, sd = 11.9, range = 0.7–42.1 pg/ml). The highest concentration (17 yr-old with T158M mutation) appeared to be an outlier, as all others fell below 5 pg/mL. Two of the values were below the lower limit of quantification (LLOQ = 1.0), and all but one of the values were outside the assay range. For descriptive purposes, these values were kept in the summary statistics (mean, median value reported above).

## Discussion

The results of the current study suggest that additional work is needed to determine the utility of commercial assays for qualification of salivary BDNF in clinical samples. The wide range of concentrations is consistent with observations from normative samples.<sup>4</sup> To our knowledge, no studies have reported values in healthy, typically-developing children.

Understanding the sources of variability will be paramount if salivary BDNF is to become useful as an outcome measure. It remains unclear if commercially available kits can reliably detect BDNF levels in saliva<sup>5</sup>. Our observations also suggest caution in adopting salivary BDNF as an endpoint until our understanding improves.

## Acknowledgments

We give our sincere thanks to the families who participated in the study, and the Midwest Rett Syndrome Foundation for their continued support.

### Funding

Supported, in part, by The National Institutes of Health [Grant Nos 44763, 69985, UL1TR000114], The Mayday Fund, and RettSyndrome.org. The content is solely the responsibility of the authors and does not necessarily represent the official views of the funding agencies.

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**Table 1**

	<b>Kit 1</b>	<b>Kit 2</b>
Analyte	Human BDNF	Total Human BDNF
Manufacturer	EMD Millipore	R&D Systems
Kit Type	MILLIPLEX MAP	Quantikine ELISA
Cat #	HPTP2MAG-66K	DBNT00
Sensitivity	2.45 pg/mL	0.997 pg/mL
Detection Range	12 – 50,000 pg/mL	15.6 – 1000 pg/mL
Intra-Assay Variation	< 10 %	2.4 – 3.2 %
Inter-Assay Variation	< 15 %	4.3 – 7.2 %

Sensitivity and detection ranges for both kits used in the study, as provided by the manufacturers.

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