

The effects of the MeCP2 gene on neurons of the enteric nervous system and the central nervous system

Anika Mukherjee, Dr. William Staines, Dr. David Grynspan, George Wahba
University of Ottawa, CHEORI



uOttawa

Introduction/ Context

Rett syndrome is caused by a mutation in the MeCP2 gene on the X chromosome. The incidence of Rett syndrome is approximately 1 in 10 000, which means that it has a low priority for funding compared to similar disorders. It is significant to do both basic and clinical research on this neurological disorder because new discoveries may lead to alleviation of the symptoms, which consequently may permit a more comfortable lifestyle for sufferers of this disease.

Some typical symptoms include severe digestive problems and serious neurological defects. During the first portion of this study, the neurons of the gastro-intestinal tract (enteric neurons) in appendix tissue were examined to see if there was a correlation between the expression of the MeCP2 gene and the degree of inflammation of the appendix. During the second portion of the study, a more quantitative approach was taken in order to determine how much the gene was expressed in hypothalamic central nervous system neurons and in enteric neurons of the appendix.

Methodology

Part 1 (qualitative analysis):

- Sections of appendix were de-paraffinized
- Immunoperoxidase staining used to stain both normal and inflamed appendix tissue
- Tissue sections were viewed under a light microscope
- Expression of MeCP2 was analyzed in the normal and inflamed appendix tissue with the use of an excel spreadsheet
- Number of neurons expressing the gene per slide were compared to the degree of inflammation in the section

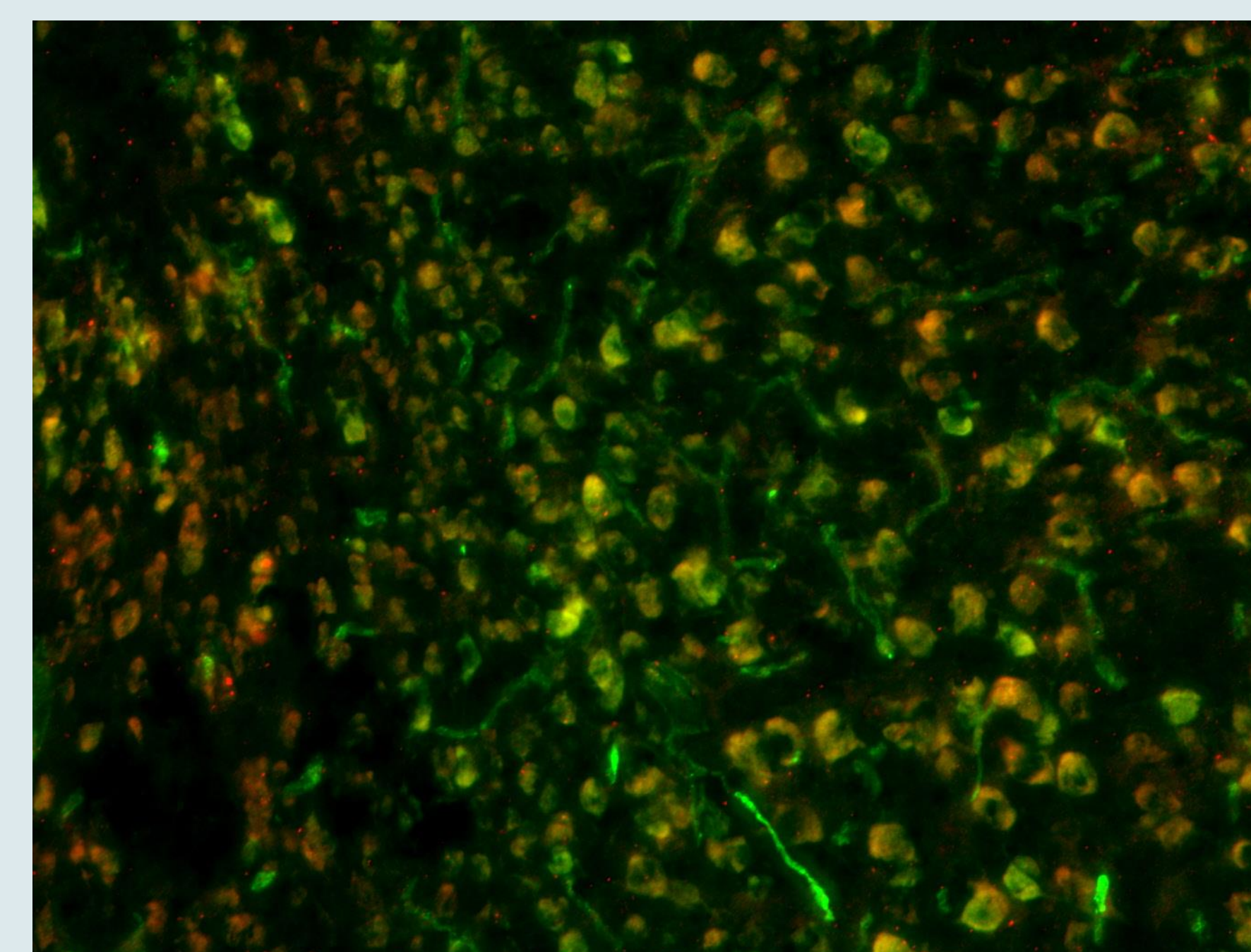


Part 2 (quantitative analysis)

- Mouse hypothalamus sections were taken using a cryostat
- Sections of appendix were de-paraffinized
- Tissue was double stained with primary and secondary antibodies for the gene in question, MeCP2, and for the neuronal protein marker HuC
- Images of central nervous system neurons and gut neurons were taken with a fluorescent microscope and analyzed with Image J

Results

After a qualitative analysis of the degrees of inflammation in human appendix tissue against the expression of the MeCP2 neuron in the same tissue, it was found that there was no correlation between the two. To confirm this finding, new slides of appendix tissue and central nervous system tissue were fluorescently stained because the MeCP2 expressing neurons can only be seen in the infrared spectrum. The images (samples below) were then analyzed using quantitative image analysis methods to quantitatively assess whether or not the expression of the gene in the gut neurons is a causal factor in the degree of inflammation of the tissue. Expected result of image analysis based on results from qualitative analysis: there is no measurable difference in MeCP2 expression in inflamed appendices compared to normal ones.



Mouse CNS stained for MeCP2 and neuronal protein marker HuC

Conclusion

The results of the qualitative analysis were effective in proving the null hypothesis: there is no observable difference in inflammation of appendix tissue depending on the degree of MeCP2 expression in the tissue.

These results are significant because a new question arises: if varying levels of MeCP2 expression in gut neurons does not cause inflammation in the appendix tissue, what causes the gastrointestinal defects in patients with Rett syndrome? Next steps may include formulating a new hypothesis around whether or not the gastrointestinal issues observed in these patients have a genetic basis or not.

References

WA Staines et. al. (1988): Three-color immunofluorescence histochemistry allowing triple labeling within a single section. *J Histochem Cytochem* 36:145 .

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