

## PEER-REVIEW REPORT

**Name of journal:** World Journal of Gastrointestinal Pathophysiology

**Manuscript NO:** 31448

**Title:** Multiple endocrine neoplasia 2B: Differential increase in enteric nerve subgroups in muscle and mucosa.

**Reviewer's code:** 00092753

**Reviewer's country:** Germany

**Science editor:** Yuan Qi

**Date sent for review:** 2016-11-22

**Date reviewed:** 2016-11-26

CLASSIFICATION	LANGUAGE EVALUATION	SCIENTIFIC MISCONDUCT	CONCLUSION
<input type="checkbox"/> Grade A: Excellent	<input type="checkbox"/> Grade A: Priority publishing	Google Search:	<input type="checkbox"/> Accept
<input checked="" type="checkbox"/> Grade B: Very good	<input checked="" type="checkbox"/> Grade B: Minor language polishing	<input type="checkbox"/> The same title	<input type="checkbox"/> High priority for publication
<input type="checkbox"/> Grade C: Good		<input type="checkbox"/> Duplicate publication	
<input type="checkbox"/> Grade D: Fair	<input type="checkbox"/> Grade C: A great deal of language polishing	<input type="checkbox"/> Plagiarism	<input type="checkbox"/> Rejection
<input type="checkbox"/> Grade E: Poor		<input checked="" type="checkbox"/> No	<input type="checkbox"/> Minor revision
	<input type="checkbox"/> Grade D: Rejected	BPG Search:	<input checked="" type="checkbox"/> Major revision
		<input type="checkbox"/> The same title	
		<input type="checkbox"/> Duplicate publication	
		<input type="checkbox"/> Plagiarism	
		<input checked="" type="checkbox"/> No	

## COMMENTS TO AUTHORS

In their manuscript, Hutson endeavor to characterize the morphological and immunohistochemical changes of MEN 2B-associated intestinal ganglioneuromatosis in a 4-year-old boy, as compared to normal mucosa from a 4-year-old child with Hirschsprung's disease and a 12-year-old child with familial adenomatous polyposis. Based on morphological and immunohistochemical analyses, the authors found that M918T-induced RET activation led to a massive increase in intrinsic nerve fibers in the myenteric and submucosal ganglia, specifically NOS (nitric oxide synthase)-IR (immunoreactive?) fibres in the circular and VIP (vasoactive intestinal peptide) in the submucosal ganglia and mucosa. In view of the paucity of data on MEN 2B-induced intestinal ganglioneuromatosis, it would be great if the authors would kindly clarify the following points, which would greatly enhance the clinical relevance of their manuscript:

1. The natural comparator for MEN 2B-induced intestinal ganglioneuromatosis, causing constipation (also dubbed pseudo-Hirschsprung's

disease), is Hirschsprung's disease (in which only the second, but not the first, efferent cholinergic neuron is affected, and which progresses in severity from proximally to distally). Hirschsprung's disease also may affect a subset of MEN 2A patients, i.e., carriers of RET germline mutations in exon 10, codons 609, 611, 618, or 620, which may be worthwhile mentioning.

2. The present manuscript would be clinically more meaningful if the authors provided quantitative information on whether the increases in ganglionic growth in number and thickness and immunohistochemical subgroups of ganglia differed: By location (terminal ileum vs. proximal/ascending colon vs. distal/descending colon): - in total - broken down by submucosal vs. myenteric ganglia - broken down by longitudinal vs. circular myenteric layers

3. To better convey that point, box and whisker diagrams, detailing the number of counted neurons per stratum with a legend clarifying all abbreviations used, would be greatly appreciated. These box and whisker diagrams could replace most, if not all, of the present numerous black and white immunostains which are much less informative.

4. A rationale is necessary for the (convenience?) antibody panel the authors chose to use in their investigation, specifically for not using VACHT (vesicular acetyl choline) or high-affinity CHT (choline transporter) antibodies (Discussion, p. 9). For example, the choice of NOS (nitric oxide synthase) antibodies is intuitive giving the known dilative properties of nitric oxide on smooth muscle. Yet the authors do not discuss how more and larger, intrinsic NOS-synthesizing nerve fibers in the myenteric and submucosal ganglia may give rise to constipation, the leading symptom of MEN 2B-associated intestinal ganglioneuromatosis, and muscular hypertrophy/thickening (reactive?).

5. The clinical references are dated and do not include relevant clinical literature on MEN 2B, specifically Brauckhoff et al., Ann Surg 2014; Brauckhoff et al., Surgery 2008.

a. In MEN 2B patients, intestinal ganglioneuromatosis develops in an age-dependent fashion (not mentioned by the authors), not all of whom develop severe constipation necessitating gastrointestinal surgery. Hence, the wisdom to use tissue from a 12-year-old child as a control for tissue from a 4-year-old MEN 2B carrier is debatable and requires a comment.

b. As detailed by Brauckhoff (Ann Surg 2014), the most important clinical consequence of a positive RET gene test (also not mentioned by the authors) is pre-emptive thyroidectomy, with or without central node dissection depending on the child's serum calcitonin level. Pre-emptive neck surgery in children 4 years and younger in expert hands clears most, if not all, malignant C-cell disease before it spreads beyond the thyroid gland, resulting in biochemical cure (postoperative normalization of serum calcitonin)

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**Manuscript NO:** 31448

**Title:** Multiple endocrine neoplasia 2B: Differential increase in enteric nerve subgroups in muscle and mucosa.

**Reviewer's code:** 00000652

**Reviewer's country:** Germany

**Science editor:** Yuan Qi

**Date sent for review:** 2016-11-22

**Date reviewed:** 2016-11-29

CLASSIFICATION	LANGUAGE EVALUATION	SCIENTIFIC MISCONDUCT	CONCLUSION
<input type="checkbox"/> Grade A: Excellent	<input type="checkbox"/> Grade A: Priority publishing	Google Search:	<input type="checkbox"/> Accept
<input type="checkbox"/> Grade B: Very good	<input type="checkbox"/> Grade B: Minor language polishing	<input type="checkbox"/> The same title	<input type="checkbox"/> High priority for publication
<input type="checkbox"/> Grade C: Good	<input type="checkbox"/> Grade C: A great deal of language polishing	<input type="checkbox"/> Duplicate publication	<input type="checkbox"/> Rejection
<input type="checkbox"/> Grade D: Fair	<input type="checkbox"/> Grade D: Rejected	<input type="checkbox"/> Plagiarism	<input type="checkbox"/> Minor revision
<input type="checkbox"/> Grade E: Poor		<input type="checkbox"/> No	<input type="checkbox"/> Major revision
		BPG Search:	
		<input type="checkbox"/> The same title	
		<input type="checkbox"/> Duplicate publication	
		<input type="checkbox"/> Plagiarism	
		<input type="checkbox"/> No	

## COMMENTS TO AUTHORS

In this descriptive case report, the authors investigate the morphology by IHC (confocal IF) in MEN2B-intestinal ganglioneuromatosis of a 4yr-old child. As control, tissues from a 12yr-old child are used. --> I strongly recommend to include age-matched control tissue instead. It would be also helpful to provide some quantative data (= measurements) regarding the observed "massive increases" (see conclusion p. 10). All images (patient vs. control) should include scale bars. Page 22: Images A,C appear to be blurry? MM: I recomend to include a table of all antibodies used. Please also indicate that appropriate negative controls have been used for all antibodies (no primary and isotype IgG).

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**Name of journal:** World Journal of Gastrointestinal Pathophysiology

**Manuscript NO:** 31448

**Title:** Multiple endocrine neoplasia 2B: Differential increase in enteric nerve subgroups in muscle and mucosa.

**Reviewer's code:** 03084420

**Reviewer's country:** France

**Science editor:** Yuan Qi

**Date sent for review:** 2016-11-22

**Date reviewed:** 2016-12-06

CLASSIFICATION	LANGUAGE EVALUATION	SCIENTIFIC MISCONDUCT	CONCLUSION
<input type="checkbox"/> Grade A: Excellent	<input checked="" type="checkbox"/> Grade A: Priority publishing	Google Search:	<input type="checkbox"/> Accept
<input checked="" type="checkbox"/> Grade B: Very good	<input type="checkbox"/> Grade B: Minor language polishing	<input type="checkbox"/> The same title	<input checked="" type="checkbox"/> High priority for publication
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		<input type="checkbox"/> Duplicate publication	
		<input type="checkbox"/> Plagiarism	
		<input checked="" type="checkbox"/> No	

## COMMENTS TO AUTHORS

acceptable