Author’s response to reviews

Title: Vocal and Motor Behaviors as a Possible Expression of Gastrointestinal Problems in Preschoolers with Autism Spectrum Disorder

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Author’s response to reviews:
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Dear Editor,

attached please find the revised version of the manuscript number BPED-D-19-00716 titled: “Vocal and Motor Behaviors as a Possible Expression of Gastrointestinal Problems in Preschoolers with Autism Spectrum Disorder”, by Margherita Prosperi, Elisa Santocchi, Filippo Muratori, Chiara Narducci, Sara Calderoni, Raffaella Tancredi, Maria Aurora Morales, Letizia Guiducci. In the following a response to the reviewers concerns is given, along with a description of the changes that were made.

For the sake of clarity, reviewer's questions and criticisms are reported in italic.

-Reviewer #1
General comments: This manuscript reports a study which investigate the complex behavioral manifestations of gastrointestinal problems in a population of 85 young children with autism spectrum disorders and also evaluate the potential implication of recognition of these behaviors
as clinical markers of GI disorders in ASD children. This topic has good relevance for clinicians managing young children with ASD, especially for nonverbal or minimally verbal children that cannot express pain or discomfort through speech. Although the hypothesis and results of the study is fascinating, there are some queries that should be addressed to help with the clarity and impact of the manuscript.

We agree with the reviewer that the topic of this study has a good relevance for clinicians, especially for those taking care of young patients with ASD and a concomitant relevant delay of verbal abilities. We thank you for your precious advices and we modified the manuscript according to your suggestions, as we have specified in the following responses.

Page 4 - Background

The aims of the study should be better focused on the topic area of your research, which is the identification of specific pattern of behavioral manifestations potentially correlated to the expression of GI problems and the impact of those associated behaviors on the GI disorders diagnosis in ASD children with low communication skills; - at line 23, please change with "investigate the correlation between GI symptoms and presence and type of the AB reported in the Consensus report in an Italian sample of preschoolers with ASD" - at line 24, please change with "to evaluate potential differences in the expression of AB in verbal vs non-verbal ASD subjects with GI problems"

Following referee’s suggestions, we changed the main aims of our study (see lines 1 and 3 page 6 at the end of the “background” section). In this way, not only the aims but also the rational of the study have become more clear.

Page 6 - Methods

Inclusion/exclusion criteria of the study should be more detailed: 1. please clarify why subjects with a diagnosis of organic GI Disorder were excluded: did you expect any difference in frequency or type of GI symptoms or AB in organic versus functional GI disorders? if you decided to study only children with functional gastrointestinal disorders, you should better argue
your choice and also mention data about differences in clinical manifestation in the background session.

We decided to exclude subjects with a diagnosis of organic GI disorder because they are less frequently referred to our ASD Unit than subjects with functional GI symptoms. Their inclusion in the study could determine a numerical imbalance within the studied sample and consequently a bias during statistical analyses. In fact, in literature organic GI disorders in ASD are reported as less frequent than functional GI ones. About differences of GI symptoms or AB in organic versus functional GI disorders, currently there is a scarce body of literature. Thanks to the reviewer, we have now specified these choices in the text (page 6 line 20) and we have added these explanations in the “background” section (page 4 line 20 and 25) and in the “discussion” section as a limit (page 16 line 19).

Page 7 - Methods

In the "Instrument" section (pag 7), authors described the total score of Gastrointestinal Severity Index that they considered clinically significant for the classification of a subject within the GI group. This information should be appropriately placed in the "Procedure" section (pag 9), in order to better describe inclusion criteria of the two subgroups "GI vs NGI" of the study.

As suggested by the reviewer, we inserted the description of how we considered the score of Gastrointestinal Severity Index as a clinical score in the “Procedure” section (page 10 line 1).

Page 7 - Methods

No information about pharmacological treatment for GI symptoms and specifically about prebiotic/probiotic therapy has been provided. Several literature studies reported that patients with ASD and GI disorders often use probiotic supplementation in order to alleviate GI symptoms as well as improve behavioral issues, so this could be an important confounding factor in term of expression or intensity of GI and AB manifestations. Please clarify if you investigated the use of pharmacological/probiotic therapy in your sample or if you decided to exclude treated patients.

We collected data regarding the pharmacological treatments and dietary supplements used by subjects involved in the study in the last three months prior the recruitment. In order to better characterize the sample, it seemed useful to add this information to the text (page 9 line 6). In particular, we are aware that the chronic administration of osmotic laxatives could alleviate GI symptoms in children with chronic constipation, representing a potential confounding factor in terms of expression or severity of GI and AB manifestations. Nevertheless, our aim was to perform a naturalistic study on the correlations between associated behaviors and the current level of GI problems, even during pharmacological treatment for GI symptoms.

Page 7 - Methods

A general description of clinical features of the whole sample has been reported in Table 2. It would be relevant to expand this description in the text and actually discuss if any differences
has been found between the two groups of GI and NGI children. Specifically, differences in symptoms severity (ADOS score), developmental/adaptive level (GMDS and VABS score) and Internalizing, Externalizing and Total Problems (CBCL score) could influence both presence and intensity of AB in the two studied groups; in this view, these variables should be considered and appropriately discussed both in Methods and Results sections.

Following referee’s suggestions, we compared GI versus No-GI subjects using all the clinical variables explored in this study. We did not find significant differences in the clinical variables between the GI group and the No-GI group with the exception of the “Global Score” of the RBS-R, the “Internalizing” and “Total” problems scores of the CBCL (all significantly higher in the GI group than in the No-GI group) and the “Communication” and “Daily Living” adaptive scores of the VABS (significantly higher in the No-GI group than in the GI group). These differences between groups confirmed our previous data on the same topic (Prosperi et al., 2017): ASD subjects with GI problems have a worse clinical functioning than ASD subjects without GI problems, independently from the severity of autistic symptoms. Using the resulted significant variables, a multivariate regression has been performed, confirming the significant correlation between GI symptomatology and AB independently from other clinical scores. For completeness of information, these new results have been added to Table 2 and in the “statistical analysis” (page 9 line 15), “results” (page 10 line 14 and 20) and “discussion” (page 13 line 11) sections.

Page 10 - Results

The results section include general results about the whole sample and then specify differences about two different subgroups: GI vs NGI and verbal vs non verbal ASD children. It could be interesting to know the percentage of GI symptoms in verbal vs non verbal subgroups, in order to identify any specific clinical phenotype or differences in the incidence of GI disorders in children with higher communication skills: it's possible that a diagnosis of GI disorder could be made earlier in verbal individuals due to their ability to express pain/disconfort and consequently influence intensity or frequency of AB manifestation; authors could also used their own results to strengthen data about the association between GI symptoms and verbal skills in subjects with ASD reported in the "Discussion" (pag 13).

Following referee’s suggestions, we added the description of the distribution (expressed as a percentage) of the GI subjects both within the verbal group and within the non-verbal group. No statistically significant differences were found between verbal and non-verbal groups as far as the prevalence of GI subjects, the 6GI scores and the Total GSI scores, as reported in the “Results” section (page 11 line 15) and in Table 4. Considering these results, we added some arguments to the discussion section (page 15 lines 10 and 19).

Page 10 - Results

Pag 10, line 11: please clarify the sentence "GSI total score (mean ±SD) was 3.69 ± 2.76, while the 6-GI score (mean ±SD) was 2.09 ± 2.00.": is GSI total score referred to whole sample or GI Group? If it refers to GI Group, please report also the comparision with NGI Group values.
Thanks for this suggestion. This sentence is unclear and confusing, for this reason it was deleted without compromising the clarity of the remaining text.

Minor revisions: Background: pag 4 grammar: please change "quality of life both of the child and their parents" with "quality of life both of the children and their parents"

Done. Thank you for reporting this error.

-Reviewer #2

General comments, PARTICIPANTS: In regard to the demographic data of the sample of subjects studied, I believe that the age range examined by the authors, namely the pre-school age group with an average age value of 4 years, is actually the most representative for studying gastro-intestinal problems in the Autism Spectrum Disorder. In fact it is precisely the age group in which these problems, also based on my clinical experience, are mostly reported by families. [...] The major contribution this work offers is, in my opinion, the correlation between visceral conditions and atypical ASD related manifestations.

Thank you to remark this aspect of our research, which stems from a clinical need to respond to the concerns of parents in this age group. The relationship between visceral conditions and atypical behaviors in ASD seems an interesting topic that has not yet been deepened, as it emerges from the few researches published on this topic.

Page 7 - Instruments

It might be useful to compare the results obtained in the ABQ questionnaire, and the modality of expressing abdominal discomfort in the sample of ASD subjects, with what would instead occur in another age-sex matched group of controls. In this way a comparison could be possible, not only between the results of ASD with or without gastro-intestinal disorders or between the results of ASD with verbal or non-verbal profile, but also between the group of cases (subjects with diagnosis) and a group of healthy controls (without diagnosis), as is usually the case in case-control studies. In fact, this would allow the reader to understand what would be the expected result in the administration of the ABQ questionnaire to the healthy pre-school general population. However, in the context of this work, it is possible to consider the group of ASD subjects without gastrointestinal disorders and, secondly, the group of verbal ASD subjects, as "control" groups for the group of ASD with gastrointestinal disorders and the group of non-verbal ASD, respectively, and then carry out the research work as a case-control study. Anyway, a reference to the possibility of a future comparison between the obtained results with those obtained from a possible new group of healthy subjects recruited for this purpose and belonging to the same preschool age group could be included in the discussion or within the limits of the study.

Unfortunately, we are not able to quickly recruit a sex and age-matched control sample of children evaluated as required in this study; therefore we added this useful suggestion as a possible future direction of the study at the end of the “discussion” section (page 16 line 22).
Within the context of this work, as suggested by the reviewer, we have considered a control group within subjects with ASD, differentiating subjects with GI symptoms from subjects without GI symptoms and verbal subjects from non-verbal subjects (the “cases” and the “controls”, respectively). Consequently, we can consider this study as a case-control study, as it is now specified in the beginning of the “participants” section (page 6 line 12).

Page 7 - Instruments

To increase specificity in the subdivision method between verbal and non-verbal groups, given the very young age of the subjects and the fact that some of them could develop language skills at an older age, it would be useful to consider the results of additional diagnostic tests such as Griffith’s other than just the ADOS.

We absolutely agree with this criticism of the study. We did not use the Griffiths’ Hearing and Speech scale to assess verbal skills in our subjects because 20 out of 85 children were not evaluable through this tool. Not being evaluable does not necessarily correspond to the presence of a delay in the skills tested (e.g. "dysregulated" children). For this reason, we have used the ADOS standardized test to differentiate verbal from non-verbal children.

Page 10 - Results

Having a number of subjects as equal as possible between each group and a homogeneous sex-age distribution are crucial factors. If, on one hand, the results of this study did not differ significantly in terms of gender of the sample (M or F), on the other hand it cannot be excluded that an important numerical disparity between the two groups could represent a bias during the interpretation of the results. In fact, it tends to emerge when subjects with gastrointestinal problems are compared with subjects without such problems, because in this case the number of subjects in the second group (55) is almost double compared to the first one (30). This does not occur when comparing verbal subjects and non-verbal subjects, as they are more evenly distributed (39 vs 46). With such a high disparity between groups it would be advisable to take them it into account for the purposes of analysis or, at least, to refer to it in the discussion of the data when considering the potential confounding factors that may have excerpted a bias effect on the results obtained.

As rightly pointed out by the referee, in our study there is a consistent numerical difference between children with GI problems and children without them. This numerical difference was predictable, as it was found in our previous research about this topic (Fulceri et al., 2016). Following referee’s suggestions, we reported the presence of this disparity within the limits of the study (at the end of the “discussion” section, page 16 line 18), as a possible confounding factor in the interpretation of the results.

Pages 9 and 12 –Statistical analysis and discussion

CBCL scores were reported to initially describe the sample of enrolled subjects: in my clinical experience, the CBCL scale is a useful clinical tool to better understand the neurofunctional profiles and detect internalizing and externalizing problems. Although the Authors focus on the
atypical manifestations shown by some subjects as compared to those typically observed in ASD, in order to correctly interpret the data it is recommended to consider the influence of having more internalizing or more externalizing problems on the subject's expression of abdominal discomfort. It would therefore be appropriate to either extend statistical analysis to these CBCL scores when discussing the results, or to report this as a limit of the study when possible confounding factors and bias are mentioned.

Following referee’s suggestions, we studied the influence of Internalizing, Externalizing and Total Problems (the summary scales of the CBCL) on the 6GI score. We added these analyses in the “results” section and in the “discussion” session (page 12 line 20 and page 13 line 22, respectively).

Thank you for your accurate and meaningful suggestions. The reviewers’ comments have stimulated changes we feel improved the paper. We hope our revised manuscript will be now considered as moved forward and eligible for publication. Should you find the paper requires further clarification or revision, we most certainly stand ready to do so.

Sincerely,

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