Peer Review File

Article information: https://dx.doi.org/10.21037/tp-2025-573

Reviewer A

General Comment

Comment: There is paucity of data on vibegron in bedwetting for children. Therefore, this real-life large and long-term retrospective and multicenter analysis is interesting. Considerable rewriting is asked and detailed below and mainly critics description of the method section and results sections. The paucity of available data requests that the authors discuss information available with a competitor available since 2013 (mirabegron). I quickly checked PubMed and could find a recent RCT from Mansour I et al in Int Braz J Urol. This RCT has to be included in the references and added to the introduction. I also miss a paragraph on the competitor in the discussion, why there is so little in the literature and perhaps explaining why you did not for example include both drugs to increase the database, especially that also for mirabegron there is a paucity of publications.

Reply: We sincerely appreciate the reviewer for recognizing the importance of our large-scale multicenter study despite the paucity of pediatric data on β3-adrenoceptor agonists for nocturnal enuresis. We agree that a comparison with mirabegron, the other available β3-adrenoceptor agonist, is essential and has substantially expanded our discussion of both agents.

We have now incorporated the recent RCT by Mansour et al. (Into Braz J Urol 2023) into both the Introduction and Discussion sections. This study showed the efficacy of mirabegron in children with non-neurogenic overactive bladder who were resistant to solifenacin, providing valuable comparative context for our findings on vibegron.

The reason why we did not include both drugs to increase the information in the database was that this retrospective study analyzed real-world clinical practice patterns across 12 Japanese institutions from 2018 to 2021. During this period, vibegron became available in Japan (approved in November 2018). Meanwhile, mirabegron was not approved for pediatric use in Japan during our study period. Hence, its prescription was limited. The participating physicians' treatment decisions reflected individual clinical practice and institutional preferences. Therefore, this study represents real-world practice patterns rather than a prospectively designed comparative trial. We acknowledge that future prospective studies comparing vibegron and mirabegron in patients with pediatric nocturnal enuresis would be valuable.

A new paragraph was also added to the Discussion section. It addresses the following: (1) the limited number of studies on both β3-agonists for pediatric nocturnal enuresis; (2) the possible reasons for this paucity, which include regulatory approval timelines, concerns regarding off-label use, and previous studies traditionally focusing on anticholinergics; (3) comparison of the available evidence on both agents; and (4) the need for future comparative studies.

Changes in the text:

1. Introduction - Added reference to Mansour et al. study (Page 5, Lines 82–87):

A recent randomized controlled trial by Mansour et al. demonstrated that mirabegron significantly improved lower urinary tract symptoms in children with non-neurogenic overactive bladder who were resistant to standard urotherapy, showing superior efficacy and better tolerability compared with solifenacin. Although evidence for mirabegron use in nocturnal enuresis remains limited, its favorable safety profile and efficacy in pediatric overactive bladder suggest potential applicability in desmopressin-resistant cases, warranting further investigation (12)."

2. Discussion - Added new subsection on literature paucity and drug comparison (Page 19, Lines 357–367424):

"**4.4 Comparison with mirabegron and literature gaps**

Although vibegron and mirabegron are established treatments for adult overactive bladder, data on their use against pediatric NE remain limited. This reflects regulatory approval delays for pediatric indications, traditional reliance on anticholinergics, and the challenges of conducting large-scale trials in heterogeneous treatment-resistant populations. Our study focused on vibegron because it represented real-world practice after its approval in 2018 in Japan. Mirabegron lacked pediatric approval during our study period. The recent RCT conducted by Mansour et al. (12) provides important comparative evidence, showing the efficacy of mirabegron against desmopressin-resistant enuresis. Future prospective comparative studies would help guide evidence-based therapeutic selection."

Reference:

- Mansour I, Hassan A, Farag F, et al. Efficacy and safety of mirabegron add-on therapy in children with desmopressin-resistant primary monosymptomatic enuresis: a randomized clinical trial. Int Braz J Urol. 2023;49:89-97.

1) Introduction

Comment: Line 12: This multicenter study: please add retrospective

Reply: We have added "retrospective" as recommended.

Changes in the text: Revised in Abstract (Page 1, Line 10):

“This multicenter retrospective study aimed to evaluate the safety and effectiveness of vibegron...”

2) Methods

Comment: Exclusion is well discussed. No lost to follow-up? No inclusion criterium like in example at least 4 or 5 wet nights/week? Primary outcome is >50% improvement in n° wet nights after 1 month: how was this assessed, a diary or by history taking from the file, or mixed, please specify? I understand from the retrospective nature and the multicenter aspect that the evaluation period was not the week before the consultation but just the question how many wet nights they had as asked at the 1 month consultation. I also presume this was not strictly 1 month but 3 weeks to 2 months? This variation is important to know if available. Safety assessment is well written.

Reply: We want to thank the reviewer for these important methodological questions.

Lost to follow-up: Of the 387 patients who were enrolled in this study, one who dropped out during the first visit after treatment initiation was excluded from the safety analysis. In addition, 17 patients who self-discontinued within 1 month were excluded from the efficacy analysis. Finally, 386 patients were included in the safety assessment and 369 in the efficacy evaluation (Figure 1). The reasons for early discontinuation could not be completely documented due to the study's retrospective design. Regarding follow-up, the treatment effect was evaluated at 1 month, and patients without documented follow-up at this time point were excluded from the analysis. Some patients who initially responded were lost to follow-up beyond 1 month. Therefore, long-term outcomes such as relapse and sustained complete response could not be systematically assessed. This limitation was added to the manuscript.

Inclusion criteria: The following inclusion criteria were standardized across centers: children with nocturnal enuresis who did not achieve adequate response (defined as $<50\%$ symptom improvement after ≥ 12 weeks) to first-line active treatments recommended by the ICCS guidelines (desmopressin and/or enuresis alarm therapy) after urotherapy. Treatment resistance to standard therapies was the primary inclusion criterion rather than specifying the minimum wet night frequency. However, the specific decision to initiate vibegron and the choice between combination versus switch strategies was based on the discretion of the attending physician, depending on each patient's clinical status. This is considered a limitation of the retrospective multicenter design, as treatment decisions were not governed by a prospectively defined protocol.

Primary outcome assessment: The primary outcome was reduction in wet nights per 30 days, categorized using the ICCS criteria into complete response (CR), partial response (PR), and no response (NR). This outcome was assessed based on patient/parent reports of wet nights within 30 days prior to follow-up visits. Due to the study's retrospective multicenter design, standardized 30-day voiding diaries were not uniformly collected across all participating centers. Data were primarily obtained via history taking documented in medical records at follow-up visits. The reviewer was correct as the evaluation period was not strictly 30 days and it varied from approximately 3 weeks to 2 months depending on clinic schedules and patient attendance. Values were normalized to 30-day equivalents to account for this variability, which is a limitation of the retrospective design.

Changes in the text:

1. Methods (Pages8, Lines 138-145152–160):

“This study included children aged 5–18 years who received vibegron (50 mg once daily) for refractory NE between November 27, 2018, and December 31, 2021. The following inclusion criteria were standardized: children with NE who did not achieve adequate response (defined as <50% symptom improvement after ≥12 weeks) to the first-line active treatments recommended by the ICCS guidelines (desmopressin and/or enuresis alarm therapy) after urotherapy. Of the 387 patients who were enrolled, 1 who dropped out during the first visit was excluded from the safety analysis, and 17 who self-discontinued within 1 month were excluded from the efficacy analysis. Finally, 386 patients were included in the safety assessment and 369 in the efficacy evaluation (Figure 1).”

2. Methods (Pages 910–11, Lines 155-162171-181):

“The primary outcome was reduction in wet nights per 30 days, categorized using the ICCS criteria into CR (100% reduction or ≤1 wet night/month), partial response (PR: 50%–99% reduction), and no response (NR: <50% reduction). This outcome was assessed based on the patient/parent reports of wet nights within 30 days prior to follow-up visits. Due to the study’s retrospective multicenter design, standardized 30-day voiding diaries were not uniformly collected. Data were primarily obtained via history taking documented in medical records. To account for variability in follow-up intervals (ranging from approximately 3 weeks to 2 months depending on clinic schedules), values were normalized to 30-day equivalents.”

3. Limitations (Page 16-17, Lines 304-317361–374):

“First, the retrospective design prevented standardized objective assessments, including urodynamic studies, maximum voided volume measurements, and postvoid residual volume assessments, which could have provided valuable physiological data to support clinical findings. The study’s retrospective design also limited our ability to ensure standardized 30-day evaluation periods, with actual follow-up intervals ranging from approximately 3 to 8 weeks depending on clinic schedules and patient attendance. Values were normalized to 30-day equivalents. Moreover, the decision to initiate vibegron and the choice between switch and combination strategies were made based on the discretion of attending physicians rather than by a prospectively defined protocol. This might have introduced heterogeneity across centers. In addition, the reasons for early treatment discontinuation could not be completely documented. Further, some patients who initially responded to the treatment were lost to follow-up beyond 1 month. Therefore, long-term outcomes such as relapse rates and sustained CR could not be systematically assessed. Thus, future prospective studies with structured long-term follow-up should be conducted to evaluate treatment durability.”

3) Results

Comment: It is a bit strange to write in the methods that the primary endpoint is change in n° of wet nights but starting with safety in the result section. Only in line 184-185 and table 3 I am informed that you used the number of wet nights per 30 days. This must be better explained in the methods section but than again how did you deal with the variability around this 30 days interval in a retrospective study in several hospitals? 37% had also daytime incontinence: it is late to realize this, please add to the method section a sentence explain that this was not an exclusion criterion. Were the daytime symptoms assessed (is a large group)?

Reply: We appreciate these organizational and methodological concerns.

Results organization: The Results section was reorganized to present efficacy data before safety data, as suggested by the reviewer, which better aligns with standard reporting conventions where primary outcomes are presented first.

Variability in 30-day intervals: As noted in our response to comment 2, values were normalized to 30-day equivalents when actual follow-up periods deviated from 30 days. This methodology is now explicitly described in the Methods section.

Daytime urinary incontinence (DUI): We acknowledge that this point deserved a clearer explanation. In this study, DUI was defined as the involuntary loss of urine during daytime that occurred at least once per week, as documented in medical records based on patient/parent reports. Our study intentionally included both monosymptomatic nocturnal enuresis (MNE) and non-monosymptomatic nocturnal enuresis (NMNE) with daytime symptoms. Children with NMNE frequently exhibit detrusor overactivity. Hence, they are particularly suitable candidates for anticholinergics and β3-adrenoceptor agonists such as vibegron. These patients (37.2% with DUI) were included in the evaluation of the efficacy of vibegron against real-world treatment-resistant NE encompassing both presentations. However, we did not systematically assess the severity or frequency of DUI beyond the once-weekly threshold nor did we systematically evaluate other daytime lower urinary tract symptoms such as urgency and increased daytime frequency. We also did not perform stratified analyses according to DUI status or daytime symptom profiles, which is considered a limitation of this study.

Changes in the text:

1. Methods (Page 8, Lines 145-149):

“This study included monosymptomatic nocturnal enuresis (MNE) and nonmonosymptomatic nocturnal enuresis (NMNE) with daytime urinary incontinence (DUI). Among the enrolled patients, 144 (37.2%) had concurrent DUI, defined as the involuntary loss of urine during daytime that occurred at least once per week, as documented in medical records based on patient/parent reports.”

2. Limitations (Page 7, Lines 326-331):

“Fifth, although our study intentionally included MNE and NMNE (37.2% with DUI), we did not systematically assess DUI severity or frequency beyond the once-weekly threshold nor did we systematically evaluate other daytime lower urinary tract symptoms such as urgency and increased daytime frequency. Stratified analyses according to DUI status or daytime symptom profiles were not performed, and this might have provided additional insights into differential treatment responses.”

4) Discussion

Comment: There is a paragraph "4.3 Comparison with similar research" but I miss comparison with mirabegron, what is published about bedwetting in children treated with mirabegron?

Reply: We appreciate this valuable comment and have substantially expanded our discussion about β3-adrenoceptor agonists, including mirabegron, in the context of nocturnal enuresis treatment.

Changes in the text: Revised and expanded in Discussion Section 4.3 (Page 18-19, Lines 4341-367):

"Previous studies have examined the efficacy of vibegron in pediatric lower urinary tract dysfunction and TR-NE. Kitta et al. reported that vibegron improved bladder capacity and compliance without adversely affecting voiding function in children and adolescents with overactive bladder (14). Fujinaga and Onuki found that vibegron, when combined with desmopressin, solifenacin, and a wireless alarm therapy, reduced enuretic episodes in children with refractory NE (18). Our findings are consistent with these reports and expand the evidence base by providing the largest multicenter dataset to date, quantifying comparative outcomes across treatment strategies, and confirming the superior efficacy of triple therapy. The use of mirabegron, which is another β3-adrenoceptor agonist, primarily for overactive bladder and neurogenic detrusor overactivity in pediatric populations has been evaluated. Soliman et al. reported that mirabegron was comparable to solifenacin in terms of efficacy and had a better tolerability in patients with pediatric idiopathic overactive bladder (11). Another report has shown that combination therapy with mirabegron and anticholinergics produces synergistic therapeutic effects. This finding supports the hypothesis that β3-receptor agonists and anticholinergics improve bladder function via distinct pathways (19). Our findings are in accordance with this concept, demonstrating that the application of vibegron as an add-on therapy to existing anticholinergic regimens achieved significant improvements in treatment-resistant cases.

4.4 Comparison with mirabegron and literature gaps

Although vibegron and mirabegron are established treatments for adult overactive bladder, data on their use against pediatric NE remain limited. This reflects regulatory approval delays for pediatric indications, traditional reliance on anticholinergics, and the challenges of conducting large-scale trials in heterogeneous treatment-resistant populations. Our study focused on vibegron because it represented real-world practice after its approval in 2018 in Japan. Mirabegron lacked pediatric approval during our study period. The recent RCT conducted by Mansour et al. (12) provides important comparative evidence, showing the efficacy of mirabegron against desmopressin-resistant enuresis. Future prospective comparative studies would help guide evidence-based therapeutic selection."

5) Conclusion

Comment: Conclusion is well done

Reply: We appreciate the reviewer's positive evaluation.

Reviewer B

Comment 1: Please define the abbreviations for the first use of the terms as exemplified below and use this abbreviation throughout the manuscript, which will increase the paper's readability further.

"Nocturnal enuresis (NE) is a common"

"treatment-resistant nocturnal enuresis"

"contribute to treatment-resistant nocturnal enuresis. The"

Reply 1: The manuscript was revised to define each abbreviation at its first mention and to use the abbreviated form consistently thereafter. For example, the revised Introduction section is as follows: "Nocturnal enuresis (NE) is a common condition..." and "treatment-resistant nocturnal enuresis (TR-NE)," with subsequent mentions using the abbreviated forms. The other abbreviations—International Children's Continence Society (ICCS), complete response (CR), partial response (PR), no response (NR), anticholinergics (AC), and desmopressin (DDAVP)—are likewise defined at first use. A list of abbreviations was also provided for consistency.

Changes in the text:

1. Abbreviations defined at first use throughout manuscript

2. Abbreviations list added before References section (Page 23, Lines 5443-454)

Comment 2: Please provide the organisational outline of the manuscript at the end of the introduction section.

Comment 3: Please place the contribution of the manuscript with bullet points before the organisation of the manuscript in the introduction section.

Responses 2, 3: We implemented both suggestions at the end of the Introduction section, listing the manuscript's contributions first, followed by a brief organizational outline.

Changes in the text: Added to Introduction (Pages7, Lines 119-136108-124):

"**1.5 Study contributions and organization**

The current study can make the following contributions to the literature:

• Is the largest multicenter retrospective cohort research evaluating the safety and efficacy of vibegron in TR-NE (387 patients across 12 institutions)

• Evaluates multiple treatment strategies including switching from anticholinergics, add-on therapy, dual therapy, and triple therapy combinations

• Shows that combination therapy targeting multiple pathophysiological mechanisms significantly outperforms monotherapy switching

• Provides real-world safety and efficacy data with detailed subgroup analyses, offering practical insights for clinical decision-making

The remainder of this manuscript is organized as follows: Section 2 describes the Methods section, which included study design, patient selection, treatment protocols, and statistical analyses. Section 3 presents the study results, which included safety outcomes, overall treatment efficacy, and subgroup comparison findings across treatment strategies. Section 4 discusses key findings, compares the results of the current study with those of previous research, explains the mechanisms underlying the observed treatment effects, and acknowledges study limitations. Section 5 presents the conclusion of the study with clinical implications and recommendations for future research."

Comment 4: Please incorporate the following treatment concepts into the manuscript appropriately with references, considering the objective of the manuscript, “compare different treatment strategies”. These concepts would increase the significance of the research (“The lower-than-expected response rates with vibegron + desmopressin further emphasize the critical importance of behavioral interventions in treatment-resistant cases.”).

“Behavioral interventions” as an initial approach to treating NE, developing appropriate urination and defecation habits.

“Imipramine”

“herbal medicine, acupuncture, and tuina”

Reply: We want to thank the reviewer for the suggestion to acknowledge the broader landscape of nocturnal enuresis treatment approaches. We have expanded the Introduction section to incorporate the discussion about these treatment modalities with appropriate references, thereby emphasizing that urotherapy (behavioral interventions) remains the foundation of treatment as recommended by the ICCS guidelines. We have discussed the efficacy and safety profile of imipramine, emphasizing recent evidence showing that desmopressin + imipramine had superior outcomes compared with desmopressin + oxybutynin. We have also cited a systematic review regarding complementary approaches.

Changes in the text: Revised Introduction Section 1.1 (Pages 4-5–5, Lines 59-73):

“Alternative treatments for refractory cases have been explored. Imipramine, a tricyclic antidepressant, was traditionally considered the primary pharmacological treatment. However, it has been relegated to third-line therapy due to concerns about potential cardiac adverse effects (tachycardia, QTc prolongation) that require monitoring in pediatric populations. Recent evidence has shown that desmopressin + imipramine has significantly superior complete response (CR) rates compared with desmopressin + oxybutynin (68% vs 5%, OR: 42.5, $p < 0.001$). This finding is attributed to imipramine’s central noradrenergic mechanism, which enhances arousal, rather than to its peripheral anticholinergic effects (6). The use of complementary approaches including acupuncture, herbal medicine, and tuina (Chinese therapeutic massage), particularly in Asian populations, has been investigated. Based on a systematic review of acupuncture modalities (7), moxibustion (RR: 1.47, $p = 0.004$), acupoint injection (RR: 1.45, $p = 0.020$), and laser acupuncture (SMD: −0.69, $p = 0.001$) led to improvements compared with control interventions. However, all data obtained were rated as having very-low quality. These nonpharmacological options may be interesting for families seeking alternative treatments. Nevertheless, high-quality evidence regarding their efficacy against treatment-resistant nocturnal enuresis (TR-NE) remains limited."

References:

Shain S, et al. Management of the refractory nocturnal enuresis patient to desmopressin in a pediatric population. J Pediatr Urol. 2024; 20:603.e1-e8.

Kannan P, Bello UM. The efficacy of different forms of acupuncture for the treatment of nocturnal enuresis in children. Explore. 2022; 18:488-497.

Comment 5: It is not clear enough how this work bridges the research gaps (challenges, research novelty) in the research community, considering recent literature. Please clarify the research gap and research question more clearly concerning recent similar papers in the literature, so that readers can understand what this research aims to address. The problem space should be defined better.

Reply: We appreciate the reviewer's concern regarding the clarification of research gaps and the study's novelty. We have substantially revised the Introduction section to better present the specific gaps that our study aims to address and to validate our research contributions relative to recent literature. The revised Introduction section now explicitly identifies three critical research gaps and emphasizes that this represents the largest multicenter retrospective analysis of vibegron in treatment-resistant pediatric nocturnal enuresis to date.

Changes in the text: Added new subsection to Introduction (Page 6, Lines 96-104):

"**1.3 Research gap and study rationale**

Recent studies have revealed the efficacy of β3-adrenoceptor agonists for pediatric lower urinary tract dysfunction. However, there are still several critical research gaps in the literature. First, existing studies on vibegron (13,14) in children have been limited to small, single-center cohorts. Hence, they lack the statistical power to detect significant differences between treatment approaches or identify optimal patient selection criteria. Second, no large-scale studies have systematically compared different vibegron treatment strategies, specifically switching from failed therapies versus add-on approaches versus dual or triple combination regimens to guide evidence-based clinical decision-making. Finally, larger-scale real-world safety data from multicenter studies are required to establish evidence-based clinical guidelines.”

Comment 6: In the discussion section, please explain how this study is different from many other similar studies in the literature regarding novelty. In the discussion section, mention the other technological developments, and research in the literature, such as the use of pre-void alerting-based wearable electronics for Nocturnal enuresis (NE) combined with Artificial Intelligence and discuss how they can change the game when an effective pre-void solution is deployed.

“enuresis alarms and desmopressin are considered the first-line treatment modalities”

“The alarm addition subgroup demonstrated the highest efficacy, with wet nights decreasing from 14.7 +-7.5 to 4.4 +- 4.2 days per 30 days, representing a 71.6% reduction.”

“Although this study did not directly assess relapse rates after therapy cessation, the inclusion of alarm therapy—known to reduce relapse compared with desmopressin monotherapy—may contribute to greater long-term treatment persistence and sustainability.”

“This finding indicates that addressing arousal dysfunction through alarm therapy may be essential to optimize treatment outcomes when pharmacological interventions prove insufficient.”

Reply: We appreciate the reviewer’s interest in contextualizing our findings within emerging technological developments. We have substantially revised the Discussion section to validate our study’s novelty and to discuss future technological directions. Our study differs from existing literature as it had the largest multicenter cohort (n = 387), systematically compared multiple treatment strategies, and demonstrated that alarm therapy combined with pharmacotherapy achieved superior outcomes. We have added a discussion about promising technological developments, including AI-based prevoid alerting systems and wireless alarm technology, thereby acknowledging that future research should explore the integration of these novel devices with pharmacological approaches.

Changes in the text: Added to Discussion Section 4.3 (Page 20, Lines 378-387:

“4.6 Emerging technologies and future directions

Recent technological advancements represent promising future directions for the management of NE. Prevoid alerting systems, such as the MyPAD device, use AI-based algorithms (Bi-LSTM-RNN) to detect bladder activity before voiding occurs, achieving a sensitivity of 99% and a specificity of 99.5% (21). This represents a paradigm shift from traditional postvoid alarms to predictive alerting. In addition, wireless alarm devices have demonstrated superior outcomes compared with wired systems, with significantly lower dropout rates (6.1% vs 20.0%) and higher CR rates (72.9% vs 39.7%) (22). Our study evaluated established alarm and pharmacological therapies. However, future research should explore the integration of these AI-based prevoid alerting systems with pharmacological approaches such as vibegron, which can potentially enhance treatment outcomes in refractory cases.”

References:

Kuru K, et al. Treatment of nocturnal enuresis using miniaturised smart mechatronics with artificial intelligence. IEEE J Transl Eng Health Med. 2024;12:204-214.

Watanabe T, et al. Comparison of wireless and wired alarm devices for nocturnal enuresis treatment. Pediatr Int. 2022;64(1):e15328.

Comment 7: What are the key outcomes of the research (e.g. the quality of life of children and parents with nocturnal enuresis based on the aforementioned treatment approaches)? Please explain how this research can be exploited by the research community in the conclusion section (e.g. some parents do not seek treatment for their children because they are unaware or misinformed about NE). The manuscript should reveal the contribution of the research, considering the research gap in the conclusion section (e.g. effective treatment to avoid the punishment of children by families leading to depression or other psychological problems).

Reply: We appreciate the reviewer’s suggestion to expand the Conclusion section by adding information on broader clinical implications and psychosocial impact. Treatment-resistant nocturnal enuresis significantly impairs children’s quality of life. Research has shown elevated parental stress levels and reduced quality of life in enuretic children. Previous studies have shown that prolonged treatment failure can lead to inappropriate disciplinary responses, exacerbating children’s anxiety and depression. Our response rates—particularly the 71.6%

reduction achieved with the addition of alarm therapy—could provide evidence-based tools to disrupt this cycle early. The revised Conclusion section now shows how the research community can utilize these findings for establishing treatment protocols, facilitating patient counseling, and selecting future study design.

Changes in the text: Incorporated into revised Conclusion (see Comment 9 below)

References:

- Roccella M, et al. Parental stress and parental ratings of behavioral problems of enuretic children. Front Neurol. 2019;10:1054.

- Al-Zaben FN, Sehlo MG. Punishment for bedwetting is associated with child depression. Child Abuse Negl. 2015;43:132-138.

- Butler RJ. Childhood nocturnal enuresis: developing a conceptual framework. Clin Psychol Rev. 2004;24(8):909-931.

Comment 8: The conclusion section does not represent the scope, objectives and achievements of the manuscript sufficiently. Please explain briefly how this research contributes to the general knowledge body of current research in the literature regarding novelty. The significance, originality, and contribution of the study should be emphasised in the conclusion section better and strongly, considering the research gap and your research question. Please mention how the findings in your study may direct the stakeholders/research community.

Reply: The Conclusion section was comprehensively revised to clearly articulate the following: (1) the study's scope, objectives, and key achievements; (2) how our findings addressed the identified research gaps; (3) the study's originality, which lies in its largest multicenter analysis; (4) clinical and psychosocial significance; and (5) actionable guidance for stakeholders and future research directions.

Changes in the text: Completely revised Section 5 "Conclusion" (Pages 22-23, Lines 4422-441):

“**5. Conclusions**

This multicenter study provides the largest real-world evidence base for vibegron in TR-NE (n = 387 across 12 institutions), addressing critical gaps in the literature. We showed favorable safety (mild adverse events with no serious adverse events: 1.0% [4/386]) and meaningful efficacy (overall response rate: 52.8%), with the addition of alarm therapy achieving superior outcomes (reduction in wet nights: 71.6%) compared with switching monotherapy (40.1%). Based on our systematic comparison of treatment strategies, combination approaches targeting multiple pathophysiological mechanisms significantly outperformed monotherapy switching, providing evidence-based guidance for decisions regarding treatment escalation.

These findings address an important clinical need, effective treatment not only reduces bedwetting symptoms but also prevents psychosocial sequelae, including family conflict and inappropriate disciplinary responses arising from parental frustration, which exacerbate children’s anxiety and depression (23, 24, 25). For the research community, this study provides the following: (1) comparative efficacy data that guide treatment protocols, (2) comprehensive subgroup analyses identifying optimal strategies for specific patient populations, and (3) a framework for integrating behavioral and pharmacological approaches. Clinicians can utilize these findings to counsel families about realistic expectations and timely intervention options when first-line therapies are not successful.

Nevertheless, future prospective trials incorporating quality of life assessments, facilitating longer follow-ups for relapse evaluation, and integrating emerging technologies (AI-based alerting systems) should be performed to validate and extend these findings.”

Comment 9: Overall, while the study explores an interesting concept, moderate revisions are needed to improve the clarity, rigour, and support for its claims. Refining the discussion and addressing the issues mentioned above would significantly enhance the quality of the manuscript.

Reply: We sincerely appreciate the reviewer’s constructive and thoughtful feedback. We have carefully revised the manuscript to improve its clarity, rigor, and the strength of evidence supporting our claims. Specifically, we refined the discussion section to provide clearer interpretations of the findings and incorporated additional references to support our arguments. We believe that these revisions have substantially enhanced the overall quality of the manuscript.

Reviewer C

We sincerely appreciate the reviewer for performing a comprehensive evaluation and providing constructive comments. We have addressed all points that were raised and believe that these revisions have improved the manuscript quality.

Comment 1: Please update Line 105 and 106: the members and approval no.

Reply: As requested, the institutional affiliation and approval number were corrected.

Changes in the text: Revised in Methods (Page 7-8, Lines 126-137):

"This retrospective observational study was conducted across 12 hospitals and clinics affiliated with the members of the Japanese Society on Enuresis and Incontinence. The study protocol was approved by the Ethics Committees of all participating institutions: Showa University Northern Yokohama Hospital (approval no. 21-105-B), Juntendo University Urayasu Hospital (approval no. P21-0012-U01), Kansai Medical University Hospital (approval no. 2021370), Fussa Municipal Hospital (approval no. 2021-41), Sasaki Clinic (approval no. 0001), Teikyo University Hospital/Teikyo University Medical Research Ethics Committee (approval no. 22-003), Showa University Fujigaoka Hospital (approval no. 21-105-B), Ehime University Hospital (approval no. 2205005), Kitano Hospital (approval no. P220300700), Juntendo University Nerima Hospital (approval no. E22-0062), Jin Children's Clinic (approval no. 0001), Saitama Children's Medical Center (approval no. 2022-01-015), and Funabashi Municipal Hospital (approval no. 4-27). The current study was retrospective in nature, and the medical records of the patients were reviewed while maintaining the privacy of each individual. Hence, informed consent was not required."

Comment 2: Methods: Is there a standardized indication for using vibegron in refractory NE in the different centers in the study?

Reply: We appreciate this important methodological question. The following inclusion criteria were standardized across all participating centers: children with nocturnal enuresis who did not achieve adequate response (defined as <50% symptom improvement after ≥12 weeks) to first-line active treatments recommended by the ICCS guidelines (desmopressin and/or enuresis alarm therapy) after urotherapy. However, the specific decision to initiate vibegron and the choice of combination versus switch strategies were made according to the discretion of the attending physician based on each patient's clinical status. This represents a limitation of the retrospective multicenter study design, as treatment decisions were not governed by a prospectively defined protocol.

Changes in the text:

Clarified in Methods (Pages 9, Lines 165-169):

"Patients were retrospectively classified based on insufficient response to prior therapy into an add-on group in which vibegron was combined with existing therapy (e.g., desmopressin, enuresis alarm, or anticholinergics), and a switch group in which patients were transitioned from prior anticholinergic agents to vibegron. The treatment strategy was selected based on the discretion of the attending physician."

The following statement was added to the Limitations section (Page 17, lines 310-312:

"Moreover, the decision to initiate vibegron and the choice between switch and combination strategies were made based on the discretion of attending physicians rather than by a prospectively defined protocol. This might have introduced heterogeneity across centers "

Comment 3: How is constipation defined in this study? And is it only assessed at start of vibegron?

Reply: Constipation was defined as fewer than three bowel movements per week, as documented in medical records based on the physician's discretion. Constipation status was assessed at baseline (start of vibegron administration). Due to the retrospective design of the study, systematic follow-up of bowel management during vibegron therapy was not standardized across participating centers.

Changes in the text:

Added to Methods (Page 8, Lines 149-150):

"Constipation was defined as fewer than three bowel movements per week, as documented in medical records based on the physician's discretion."

Added to Limitations (Page 17, Lines 317-321):

"Second, although constipation status was documented at baseline (20.2%), we did not systematically implement or monitor bowel management protocols, nor did we perform stratified analyses according to constipation status. This might have influenced treatment outcomes."

Comment 4: How is the compliance of medication ensured? Is the change of other treatment (e.g., stop of using enuresis alarm during the FU) being documented? This may need to be mentioned in the limitation part.

Reply: We appreciate this important question. Medication compliance was assessed during each follow-up visit by reviewing patient/parent reports and prescribing records. Among the 369 patients, 366 had available compliance data. Three patients were excluded due to missing compliance records. The adherence rates were as follows: 100% in 239 (65.3%) patients, 90%–99% in 63 (17.2%), 50%–89% in 63 (17.2%), and < 50% in 3 (0.8%). Overall, 82.5% of the patients had a medication adherence rate of ≥90%, thereby supporting the reliability of our efficacy outcomes. However, changes in concomitant treatments (e.g., discontinuation of alarm therapy) during follow-up were not systematically documented. This limitation was acknowledged in the revised manuscript.

Changes in the text:

Added to Methods (Page 10, Lines 180-182):

“Medication compliance, calculated as the percentage of prescribed doses taken over 30 days, was assessed during follow-up visits based on patient/parent reports and via a review of prescribing records.”

Added to Results Section 3.1 (Page 11, Lines 206-209):

“In total, 366/369 patients had available data on medication compliance, and three patients with missing compliance records were excluded from the analysis. The adherence rates were as follows: 100% in 239 (65.3%) patients, 90%–99% in 63 (17.2%), 50%–89% in 63 (17.2%), and <50% in 3 (0.8%). Overall, 302 (82.5%) patients had an adherence rate of ≥90%.”

Added to Limitations (Page 17, Lines 320-321):

“Third, changes in concomitant treatments (e.g., discontinuation of alarm therapy) during follow-up were not systematically documented, which might have influenced treatment outcomes.”

Comment 5: Line 83: What is the duration of vibegron use before achieving CR?

Reply: We sincerely want to thank the reviewer for raising this point. We were able to collect data on the start and end dates of vibegron treatment, enabling us to calculate treatment duration (median: 245 days, IQR: 126–484). However, due to the retrospective design of the study, we did not systematically document treatment response (CR, PR, or NR) at the end of vibegron therapy for all patients. Our efficacy analysis was based on response at approximately 1 month after initiation. However, we could not determine whether patients who achieved CR at 1 month maintained this response throughout their whole treatment course, or at what point CR was first achieved during treatment. This represents an important limitation for understanding the time course of treatment response.

Changes in the text: Added to Limitations (Page 17, Lines 321-326):

“Fourth, although the treatment duration of vibegron (median: 245 days) was documented, we did not systematically assess treatment response at the end of therapy. Treatment efficacy was evaluated at approximately 1 month. However, we could not determine whether the initial responders maintained their response or when CR was first achieved during the treatment course. Therefore, future prospective studies should incorporate the systematic documentation of treatment milestones throughout the whole treatment period.”

Reviewer D

Comment 1: I don't see much mention of the role of constipation in nocturnal enuresis and its effect on bladder overactivity, it's worth mentioning in detail.

Reply: We appreciate this important observation and agree that the role of constipation in the pathophysiology of NE deserves greater emphasis. Approximately 20.2% of our patients presented with constipation at baseline. However, we did not systematically address bowel management or perform stratified analyses according to constipation status. We have added a discussion about the effects of constipation on bladder overactivity, and this was acknowledged as a study limitation.

Changes in the text:

1. Added to Discussion Section 4.7 "Explanations of findings" (Pages 21, Lines 397-408):

“Role of constipation and bowel dysfunction: Constipation plays an essential role in the pathophysiology of NE. The ICCS standardization document (2) emphasizes that urotherapy, including bowel management, should be the foundation of treatment prior to pharmacological interventions. Constipation increases bladder overactivity via rectal distension and altered pelvic floor dynamics, and successful treatment of constipation has been shown to improve daytime lower urinary tract symptoms and NE. In our study, 20.2% of patients presented with constipation at baseline. However, systematic bowel management protocols and follow-up of constipation status during vibegron therapy were not standardized across participating centers. In addition, we did not perform subgroup analyses stratified by constipation status to evaluate whether bowel dysfunction modified treatment responsiveness. Hence, future prospective studies should systematically address constipation management and evaluate its impact on pharmacological and combination therapy outcomes.”

2. Added to Limitations (Page 17, Lines 317-319):

“Second, although constipation status was documented at baseline (20.2%), we did not systematically implement or monitor bowel management protocols, nor did we perform stratified analyses according to constipation status.”

Comment 2: I see triple therapy as desmopressin, anticholinergic and beta agonists, which some of the patients were on, it would be interesting to discuss those patients and any results as a unique subset if possible.

Reply: We agree that a detailed analysis of this pharmacological combination deserves specific attention. Upon a retrospective re-analysis of our data, the “dual therapy addition” group (originally n = 50) was subdivided into two distinct subgroups: (1) desmopressin + anticholinergic + vibegron group (n = 33) and (2) desmopressin + alarm + vibegron group (n = 17).

In the DDAVP + anticholinergic + vibegron (dual therapy addition) subgroup, shown in Table 3 as “add dual (DDAVP + AC)” (n = 33), the mean wet nights decreased from 17.7 ± 8.3 at baseline to 8.4 ± 8.5 after treatment, representing a 58.3% reduction, with CR, PR, and NR rates of 27%, 36%, and 36%, respectively. In the DDAVP + alarm + vibegron (dual therapy addition) subgroup, shown as “add dual (alarm + AC)” (n = 17), the number of episodes decreased from 12.7 ± 6.6 to 4.6 ± 6.2 (63.8% reduction), with CR, PR, and NR rates of 42%, 29%, and 29%, respectively. Although the sample sizes were limited, both regimens had clinically significant efficacy in treatment-resistant cases. These findings support the hypothesis that β3-adrenoceptor agonists exert synergistic effects when combined with desmopressin and/or anticholinergics. This finding is consistent with that of previous pediatric studies showing the additive benefits of β3-agonist and antimuscarinic combinations on bladder function.

Changes in the text:

1. Revised Table 3 to present dual therapy addition subgroups separately (DDAVP + AC + vibegron and DDAVP + alarm + AC + vibegron), and removed redundant footnote that duplicated the AC abbreviation definition.

2. Results Section 3.3 (Page 12, Lines 219-222):

“Among the dual therapy combinations, the DDAVP + anticholinergic + vibegron subgroup (n = 33) achieved a 58.3% reduction in wet nights with 27% CR. Meanwhile, the DDAVP + alarm + vibegron subgroup (n = 17) exhibited a 63.8% reduction rate and the highest CR rate (42%).”

3. Added to Discussion Section 4.1.2 (Pages 15, Lines 269-281):

“Most notably, the combination approaches had superior outcomes compared with monotherapy. The alarm addition subgroup achieved the highest reduction in wet nights (71.6%), and the DDAVP + alarm + vibegron combination had the highest CR rate (42%). Triple therapy (vibegron + desmopressin + alarm + anticholinergic), which represents a comprehensive four-drug regimen, achieved a reduction rate of 61.6% and a CR rate of 26%. These findings support the hypothesis that β3-adrenoceptor agonists and anticholinergics can improve bladder function via distinct pathways, creating synergistic therapeutic effects. The combination of desmopressin (which addresses nocturnal polyuria), anticholinergics (which reduces detrusor overactivity), and vibegron (which facilitates β3-mediated bladder relaxation) targets multiple pathophysiological mechanisms simultaneously. This may explain the favorable outcomes observed in treatment-resistant cases. Notably, the superior performance of DDAVP + alarm + vibegron over DDAVP

+ anticholinergic + vibegron underscores the importance of behavioral interventions via alarm therapy in addition to pharmacological interventions. .”