HHS Public Access

Author manuscript

Neurourol Urodyn. Author manuscript; available in PMC 2020 May 12.

Published in final edited form as:

Neurourol Urodyn. 2019 June ; 38(5): 1261–1265. doi:10.1002/nau.24006.

Role of β-3 Adrenergic Receptor Polymorphism in Overactive Bladder

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Abstract

Aims: Women with overactive bladder (OAB) have a higher frequency of a single nucleotide polymorphism (SNP) at codon 64 of the β-3 adrenergic receptor gene (*ADRB3*). Since the SNP results in an amino acid substitution that could theoretically alter receptor protein function, we hypothesized that those with the SNP would display greater OAB symptom severity. Therefore we aimed to compare OAB severity between women with this SNP and women with the wild type genotype.

Methods: A retrospective cohort study was performed in women with bothersome OAB from two academic institutions. Banked blood samples were tested for the codon 64 SNP. Women were divided into two groups based on genotype: wild type (WT) and heterozygous (HZ). We compared mean OAB Symptom Severity questionnaire (OAB-q) scores between groups using t-tests. Linear regression was performed to control for potential confounders.

Results: Of the 303 women with OAB, 254 (83.8%) had the WT genotype, and 49 (16.2%) the HZ genotype. There were no homozygous women for the rare allele. The majority were Caucasian (86%) and non-Hispanic (97%). There were no significant differences in mean OAB-q symptom severity scores (WT 21.2 ±7 v. HZ 22.0 ±6.6; p=0.49) and quality of life scores (WT 39.6 ±15.5 v. HZ 39.1 ±16.6; p=0.83) between groups. These remained non-significant in a linear regression model.

Conclusions: In a predominantly non-Hispanic, Caucasian population of women with bothersome OAB, symptom severity was not related to *ADRB3* codon 64 SNP genotype.

Keywords

Bladder function; symptom severity; *ADRB3*

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Introduction

The pathophysiology of overactive bladder (OAB) is multifaceted and not entirely understood.[1, 2] Bladder filling and storage is a complex process with the parasympathetic system promoting emptying through detrusor smooth muscle contraction and urethral relaxation, and the sympathetic system promoting storage via contraction of the urethral skeletal muscle and inhibition of parasympathetic activity. Acetylcholine release from parasympathetic neurons leads to activation of cholinergic receptors and ultimately detrusor contraction. Catecholamines, released from sympathetic neurons in the detrusor muscle, activate G protein-coupled β-3 adrenergic receptors leading to bladder relaxation.[1]

Since the β-3 adrenergic receptor plays an integral role in bladder storage, altered receptor function has been proposed as a pathophysiologic mechanism for OAB. The *ADRB3* gene, which encodes this receptor, is a 3.975 kilobase gene that contains multiple single nucleotide polymorphisms (SNPs). One of these SNPs (ID rs4994) is specifically located at codon 64, leading to a missense mutation resulting in an amino acid change from tryptophan to arginine. In a study using human omental fat tissue, the presence of this SNP was associated with a 10-fold decrease in receptor responsiveness in vitro. [3,4] Therefore, preclinical studies suggest that the variant genotypes at the codon 64 SNP and the resulting protein mutation may result in reduced receptor function. In the bladder, decreased receptor responsiveness would theoretically translate to increased OAB severity or reduced responsiveness to medications. Though prior human studies have shown that the variant genotypes are more common in patients with OAB compared to individuals with normal bladder function, [5–7] there are no specific studies assessing whether codon 64 SNP status is associated with OAB severity or response to therapy.

An *in vitro* study using human bladder tissue demonstrated that the β-3 receptor is implicated in inhibition of cholinergic nerve signals by decreasing acetylcholine at parasympathetic nerve terminals. [8] Therefore, the β-3 adrenergic receptor may influence bladder contractility and response to anticholinergic medications, which are commonly used as second-line treatment for OAB. [9] Due to the multiple possible mechanisms for how the β-3 receptor could impact OAB, we sought to explore the relationship between presence of the *ADRB3* codon 64 SNP and OAB severity.

For the primary outcome, we hypothesized that *ADRB3* codon 64 SNP variant genotypes would be associated with more severe bladder symptoms in women with OAB. Additionally, for the secondary outcome, we hypothesized that women with the variant genotypes would be less responsive to anticholinergic medications when compared to wild type individuals. Therefore, we performed a retrospective cohort study to compare OAB severity and medication response in those with variant versus wild type genotypes.

Material and Methods

This study used banked specimens from women with pelvic floor disorders who were previously enrolled in biorepository studies through the Division of Urogynecology & Reconstructive Pelvic Surgery at Duke University and the Division of Urogynecology at the

University of North Carolina at Chapel Hill. For these initial biorepository studies, women were approached and provided informed consent for storage of biospecimens and associated clinical data with validated questionnaires after IRB approval at each institution. Participants were specifically consented for their blood samples to be used for future studies including genotyping. The current analysis underwent additional review and IRB approval at Duke University.

At the time of consent, all women were 18 years or older, English literate, and had capacity to provide informed consent. All women had 30 mL of peripheral whole blood collected and completed the following validated questionnaires to assess urinary symptoms and quality of life: Short Form 12 (SF-12), the Pelvic Floor Distress Inventory Short Form – 20 (PFDI-20), and the Pelvic Floor Impact Questionnaire Short Form – 7 (PFDI-7). Question #16 of the PFDI-20 asks if patients "usually experience urine leakage with a feeling of urgency." If the participant answers yes, they are prompted to rate their level of bother. Women who responded that they were "somewhat", "moderately", or "quite a bit" bothered by urgency-associated leakage were then asked to complete the OAB-q Short Form for Symptom Bother (OAB-q). For the current analysis, we included all women with available OAB-q data.

OAB medication information was recorded for a subset of patients enrolled in the original biorepository studies. We defined medication failure as a decision to discontinue a particular drug or to switch to a different drug, whether due to ineffective symptom relief or side effects. The number of different medications reportedly tried was assessed.

DNA extraction and sequencing:

In humans, the *ADR3B* codon 64 SNP (ID rs4994) can be found on one or both alleles leading to three possible genotypes: wild type (WT), and two variant genotypes, heterozygous (HZ) and homozygous individuals. Whole blood samples were thawed and DNA extraction was performed using Qiagen QIAamp DNA Blood Mini Kit (cat# 51106) per manufacturer's protocol. The rs4994 SNP genotype was then identified using pyrosequencing on a PyroMark Q96MD following PCR with the following primers: ADRB3-SNP2-F1: 5'-GTG GGA GGC AAC CTG C-3' and ADRB3-SNP2-PS-R1* 5'-(Btn)GCC AGC GAA GTC ACG AAC-3', pyrosequencing was performed with primer ADRB3-SNP2-Seq2: 5'-CAT CGT GGC CAT CGC-3'. All samples were run in duplicate.

Of note, we also examined repeat polymorphism, rs149590171, located upstream of the 5'UTR of *ADBR3* but found that the length of the repeat was highly variable (data not presented). We were therefore unable to determine potential associations of OAB severity and genotype for this specific location.

Data analysis:

Subjects were divided into two groups: 1) WT and 2) variant (including HZ and homozygous) genotypes. Student's *t* tests and χ^2 were used to compare continuous and categorical variables, respectively among groups. Linear regression was performed to control for potential confounders. Variables included in this model included age, race, ethnicity, and BMI. Variable selection was based on known relationships between the variables and the presence of OAB and/or the SNP genotype. Wilcoxon rank sum test was

used to compare the number of anticholinergic medication failures between groups and Poisson regression was used to control for potential confounders. Statistical analysis was performed using Statistical Package for Social Sciences software (IBM SPSS Statistics Version 25; 2017).

Power calculation:

A power analysis was performed based on the estimated number of 275 subjects who would meet inclusion criteria and was calculated to account for various variant genotype frequencies in order to detect a 10-point clinically meaningful important difference (MID) on the OAB-q. With a significance level of 0.05 and assuming a standard deviation (SD) of 20 with OAB-q scores, we would have at least 80% power to detect a MID, even with variant genotype frequency of 15%.

Results

The study included 303 women with bothersome OAB. The mean age and BMI of subjects were 61.7 years (±11.9) and 30 kg/m^2 (±6.4), respectively (Table 1). The majority were Caucasian (86%) and non-Hispanic (97%). There were no differences in baseline characteristics between the two cohorts. There were 254 (83.8%) women with the WT genotype, and 49 (16.2%) with the HZ genotype. As expected, there were no individuals with the homozygous variant genotype. The overall variant allele frequency in this population was therefore 9.6%. These data are consistent with Hardy-Weinberg Equilibrium ($\chi 2$=2.34).

Caucasian women had lower incidence of the HZ genotype as compared to non-Caucasian women, but this was not statistically significant (14.8% vs. 25.0% respectively, p= 0.10).

There were no significant differences in mean OAB-q summary scores for symptom severity (WT 21.2 ±7 v. HZ 22.0 ±6.6; p=0.49) and quality of life (WT 39.6 ±15.5 v. HZ 39.1 ±16.6; p=0.83) (Table 2). These remained non-significant after controlling for age, race, ethnicity, and BMI in a linear regression model.

Of the 303 subjects, there were anticholinergic medication data available for only 98 (32%). Of these, 81 subjects had the WT genotype and 17 had the HZ genotype. Of these women, 88% failed at least one medication. The median number of anticholinergic medication failures was the same in both groups, 1 (IQR 1–2) and was therefore not significantly different in a Wilcoxon rank sum analysis (p=0.54). There remained no significant difference after controlling for age, race, ethnicity, and BMI in a Poisson regression model (β=0.07 [95% CI: −0.26, 0.41], p=0.75).

Discussion

Our results suggest there is no relationship between the presence of the *ARDB3* rs4994 SNP and severity of OAB symptoms based on OAB-q summary scores. In addition, we did not find an association between the presence of the SNP and number of anticholinergic medication failures. Given the complexity of bladder physiology, it is possible that the

interaction between the adrenergic and cholinergic systems makes it challenging to identify one genetic marker that explains the constellation of symptoms that constitute OAB.

An *in vitro* study showed that β-3 receptor function varies based on the presence of the *ADRB3* rs4994 SNP, such that receptors with the variant allele were less responsive to stimulation.[5] Translating this information to bladder function, we hypothesized that if a patient has decreased β-3 receptor activity and a normal sympathetic pathway, the bladder may have a decreased ability to fill and therefore be more likely to manifest with urgency symptoms.

Two studies concluded that the SNP is more common in those with OAB. [5,6] A meta-analysis investigating the role of the β-3 receptor SNP and OAB suggests that there is an association between OAB and the SNP, but concluded that this association was not well-characterized with regards to the relationship between presence of the polymorphic allele and severity of symptoms or response to medication therapy. [7] Ferriera *et al.* performed a case-control study in a Brazilian population including 49 women with OAB and 169 controls. Genotyping of the rs4994 SNP revealed that at least one polymorphic allele was present in nearly 50% of the OAB group, as compared to only 24% of the control group.[5] Similarly, Honda *et al.* conducted a case-control study in Japan with 100 women with OAB and 101 age-matched controls; this revealed that 47% of the cases were either heterozygous or homozygous for the rs4994 SNP, as compared to only 23% of controls. [6] Notably, our population of women with OAB was found to have a lower frequency of the variant genotype than women in these prior studies. [5,6] However, the majority of subjects included in the present study were Caucasian and non-Hispanic, which is a group that has been shown to have a lower baseline variant genotype frequency. [3] Given this, our results are actually consistent with the above studies, in that the variant genotype frequency was appropriately double in OAB patients (16.2% in the current study) as compared to the general population (8–10%).

Our study did not find differences between genotypes and anticholinergic medication failure. As mentioned previously, the β-3 receptor is implicated in inhibition of cholinergic nerve signals *in vitro* and more recently this was suggested by work *in vivo*. [8, 13] Gurocak and colleagues studied 34 children with lower urinary tract symptoms. As compared to those with the wild type genotype, those with the variant genotype of the rs4994 SNP were less likely to improve with anticholinergic medication therapy. [13] Our study was not powered for this outcome and only a small percentage of subjects had data available on medication use; this could account for not observing a relationship between rs4994 genotype and medication failures.

This study had multiple strengths. The sample size was large and the population was well characterized. Furthermore, the patient reported outcome measures used, PFDI-20 and OAB-q, are established and validated tools for assessing OAB symptoms. [11, 12] This study is limited by the lack of a non-OAB control group, which was a result of the retrospective nature of the study using samples from a biorepository. There was limited medication data for this population. Available data did not include mirabegron treatment

information given samples were collected prior to FDA approval in 2012. Ideally, we would have liked to evaluate response to mirabegron therapy based on rs4994 SNP genotype.

Conclusions

Based on these findings, OAB severity, as indicated by OAB-q summary scores, is not related to the *ADRB3* codon 64 variant genotype, in our sample population. Given the complexity of bladder physiology, more work is needed to explore the relationship between *ADRB3* gene function, potential genetic risk factors, and OAB severity and medication response. In the present study, there was not a significant relationship between genotype and response to anticholinergic medications but the small number of subjects with available medication data limits these conclusions. In the future, it will be useful to investigate if response to OAB therapy, including both anticholinergic and beta agonist medications, varies by genotype at this site. Understanding who will respond to various medications could help target therapies to decrease patient exposure to ineffective medications with significant side effects.

Acknowledgments

Funding

1. Charles B Hammond, MD, Research Fund, Duke University School of Medicine

2. Dr. Siddiqui is supported by grant # K23-DK110417 from the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)

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Table 1.

Demographic information for all subjects and by group.

	All Subjects N=303	Wild type (Trp64Trp) n=254	Heterozygous (Trg64Arg) n=49	p-value*
Age	61.8 (±11.9)	61.9 (±12.0)	61.1 (±11.2)	0.65
BMI	30.1 (±6.7)	29.8 (±6.5)	31.2 (±7.3)	0.23
Non-Hispanic	294 (97.7%)	246 (96.9%)	48 (98%)	0.99
Caucasian	263 (86.8%)	224 (88.2%)	39 (79.6%)	0.10

*Comparing Wild type to Heterozygous. Data presented as Mean (± standard deviation) or Count (% of column).

Table 2.

Mean OABq summary scores for Wild-type and Heterozygous subjects.

	Wild type (T/T) n=254	Heterozygous (T/C) n=49	p-value
OAB-q Summary Score			
Symptom Severity	21.2 (±7)	22 (±6.6)	0.49
Quality of Life	39.6 (±15.5)	39.1 (±16.6)	0.86

Data presented as Mean (± standard deviation)