



Association between polymorphism of β 3-adrenoceptor gene and overactive bladder: a meta-analysis

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ABSTRACT. Genetic variations in the human β 3-adrenoceptor (β 3-AR) gene are known to be involved in insufficient relaxation of the bladder muscle during urine storage. The Trp64Arg polymorphism in the β 3-AR gene has been found to be an important regulator of the development of overactive bladder (OAB). However, the association between this polymorphism and OAB remains controversial. Therefore, we conducted a meta-analysis to explore the association between the Trp64Arg polymorphism and OAB risk. We examined 2 case-control studies, including a total of 149 OAB cases and 270 healthy controls. The meta-analysis results showed that the Arg allele of the β 3-AR gene is positively associated with OAB susceptibility, while Arg allele carriers (Trp64Arg + Arg64Arg) showed positive associations with OAB. These results also demonstrated that the

Trp64Arg polymorphism in the β 3-AR gene is involved in the pathogenesis of OAB.

Key words: β 3-adrenoceptor genes; Genetic polymorphism; Meta-analysis; Overactive bladder risk

INTRODUCTION

Overactive bladder (OAB) is a symptomatic diagnosis based on the presence of urgency, with or without incontinence, and is typically accompanied by frequency and nocturia. OAB is prevalent in the adult population (Irwin et al., 2006). The urinary bladder is innervated by both the sympathetic and parasympathetic nervous systems. It contributes to urine storage by activating the sympathetic nerves (Andersson, 1993), and the lumbosacral parasympathetic outflow provides an excitatory motor input to the urinary bladder detrusor (de Groat, 1997).

β -adrenoceptors (ARs) are classified into the β 1-, β 2-, and β 3-subtypes (Bylund et al., 1994). Of these 3 subtypes, β 3-AR mRNA is the most predominantly expressed by human detrusor tissue (Yamaguchi, 2002; Nomiya and Yamaguchi, 2003). When sympathetic nerves are activated, β 3-AR mediates bladder smooth muscle relaxation through adrenergic stimulation of the human detrusor, contributing to urine storage (Andersson, 1993; Takeda et al., 1999; Igawa et al., 1999; Michel and Vrydag, 2006; Yamaguchi and Chapple, 2007).

The human β 3-AR gene is polymorphic; a mutation in codon 64 of the β 3-AR gene changes tryptophan (Trp) to arginine (Arg) (Trp64Arg) (Clément et al., 1995; Leineweber et al., 2004). Because activation of β 3-AR regulates detrusor muscle relaxation, a polymorphism in the β 3-AR gene may result in insufficient relaxation of the bladder muscle during urine storage, leading to detrusor over-activity. Therefore, we hypothesized that the Trp64Arg polymorphism is a risk factor for OAB syndrome. To examine this hypothesis, we performed a meta-analysis that included recent and relevant articles.

MATERIAL AND METHODS

Literature search

We performed an extensive electronic search of the PubMed, Cochrane library, Embase, Web of Science, Springer Link, and CBM databases to identify relevant studies available through December 10, 2013. The search terms included ["overactive bladder" or "OAB" (Mesh)] and ["SNPs" or "SNP" (Mesh)] and ["genetic polymorphism" (Mesh)]. References in eligible studies or textbooks were also reviewed through a manual search to identify other potentially eligible studies.

Inclusion and exclusion criteria

The studies included met the following criteria: i) the type of study was a case-control study; ii) these case-control studies focused on the association between the Trp64Arg polymorphism in the β 3-AR gene and OAB symptoms; iii) all patients were diagnosed with clini-

cal OAB symptoms (confirmed by a 3-day voiding diary indicating a frequency of 8 episodes of urgency or 1 or more episodes of urgency incontinence per day); iv) all patients and healthy controls were free from chronic neurologic or muscular diseases, urinary tract disease, and treatment history of OAB; and v) the publication was in English. Studies were excluded if they reported incomplete, non-useful, or overlapping data or if they were meta-analyses, letters, reviews, or editorial articles.

Data extraction

Using a standardized form, data from published studies were independently extracted by 2 reviewers (H.C. Qu and W. Zhang) to collect the necessary information. The following information was extracted from each article: first author, year of publication, language, study design, source of cases and controls, number of cases and controls, mean age, sample, clinical symptom, diagnostic criteria, genotype methods, polymorphism genotype frequency, and evidence of Hardy-Weinberg equilibrium (HWE) in controls. In case of conflicting evaluations, agreement was reached following discussion with a third reviewer (P. Wang).

Quality assessment of the studies included

Two reviewers (HC Qu and W Zhang) independently assessed the quality of the studies according to modified STROBE quality score systems (von Elm et al., 2007; Zhang et al., 2011). Forty assessment items related to quality appraisal were used in this meta-analysis, with scores ranging from 0 to 40. Scores of 0-20, 20-30, and 30-40 were defined as low, moderate, and high quality, respectively. Disagreement was resolved by discussion.

Statistical analysis

The odds ratio (OR) and 95% confidence interval (95%CI) were calculated using Review Manager Version 5.1.6 [provided by Cochrane Collaboration; <http://ims.cochrane.org/revman/download> (accessed August 9, 2012)] and STATA Version 12.0 (Stata Corp., College Station, TX, USA). Between-study variation and heterogeneity were estimated using Cochran's Q-statistic (Higgins and Thompson, 2002; Zintzaras and Ioannidis, 2005). $P \leq 0.05$ was considered to be a manifestation of statistically significant heterogeneity. We also quantified the effect of heterogeneity using the I^2 test, which ranged from 0-100% and represented the proportion of inter-study variability attributed to heterogeneity rather than to chance. When a significant Q-test ($P < 0.05$) or an I^2 of $>50\%$ indicated that heterogeneity among studies existed, the random-effect model was used for the meta-analysis. Otherwise, the fixed-effect model was used. We examined whether genotype frequencies of controls were in HWE using the chi-square test. Sensitivity analysis was mainly performed through sequential omission of individual studies. Funnel plots were used to detect publication bias. All P values were two-sided. To ensure the reliability and accuracy of the results, 2 reviewers (H.C. Qu and W. Zhang) entered the data in the statistical software programs independently and obtained the same results.

RESULTS

Characteristics of included studies

The search strategy resulted in the retrieval of 80 potentially relevant studies. According to the inclusion criteria, 2 studies (Ferreira et al., 2011; Honda et al., 2013) were included in the meta-analysis and 78 were excluded. The flow chart of study selection is shown in Figure 1. The 2 case-control studies selected included 149 OAB cases and 270 healthy controls, which evaluated the relationship between the Trp64Arg polymorphism of the β 3-AR gene and OAB risk. The publication years of the 2 involved studies were 2011 and 2013. All patients fulfilled the diagnostic criteria for clinical OAB symptoms (urgency or urgency incontinence), confirmed by a 3-day voiding diary (a frequency of 8 episodes of urgency or 1 or more episodes of urgency incontinence per day). The source of controls was a healthy population. The HWE test was performed on the genotype distribution of the controls in all studies included and all were in HWE ($P > 0.05$). All quality scores of studies included were >20 (moderate-high quality). The characteristics and methodological quality of the studies included are summarized in Table 1.

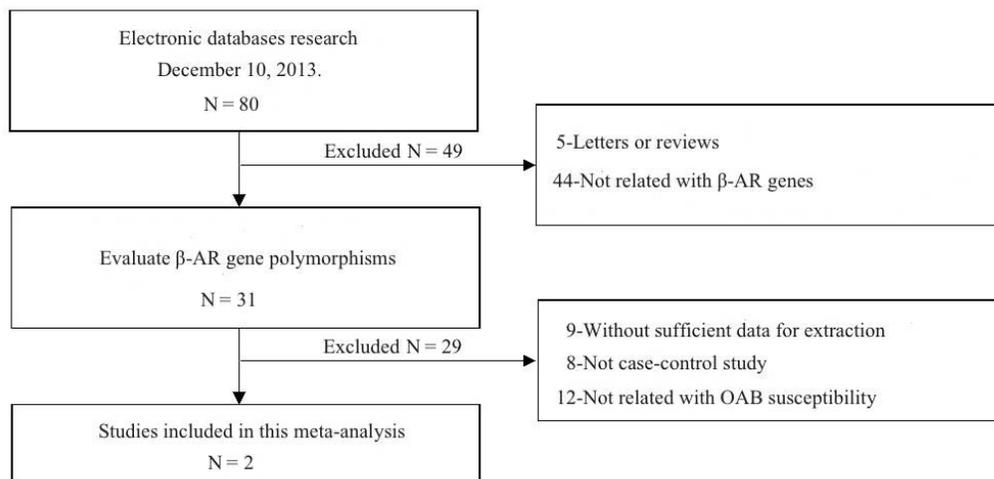


Figure 1. Flow chart shows the study selection procedure.

Table 1. Characteristics of individual studies in this meta-analysis.

Author	Ferreira	Honda
Year	2011	2013
Case number	49	100
Control number	169	101
Sample	Blood	Blood
Genotype method	PCR-RFLP	PCR-RFLP
Quality score	28	32

PCR = polymerase chain reaction; RFLP = restriction fragment length polymorphism.

Association between β3-AR gene polymorphisms and OAB risk

A summary of the meta-analysis findings of the association between β3-AR gene polymorphisms and OAB risk is shown in Figure 2. The Arg allele of β3-AR genes showed a positive association with OAB susceptibility (OR = 2.46, 95%CI = 1.67-3.6, P < 0.00001). In addition, Arg allele carriers (the heterozygous 64Arg variant and the homozygous 64Arg variant, or Trp64Arg + Arg64Arg) also showed positive associations with OAB risk (OR = 3.11, 95%CI = 1.99-4.87, P < 0.00001) compared to the Arg allele non-carriers (wild-type gene, or Trp64Trp). The significance of the pooled OR in all individual analyses was not excessively influenced by omitting a single study.

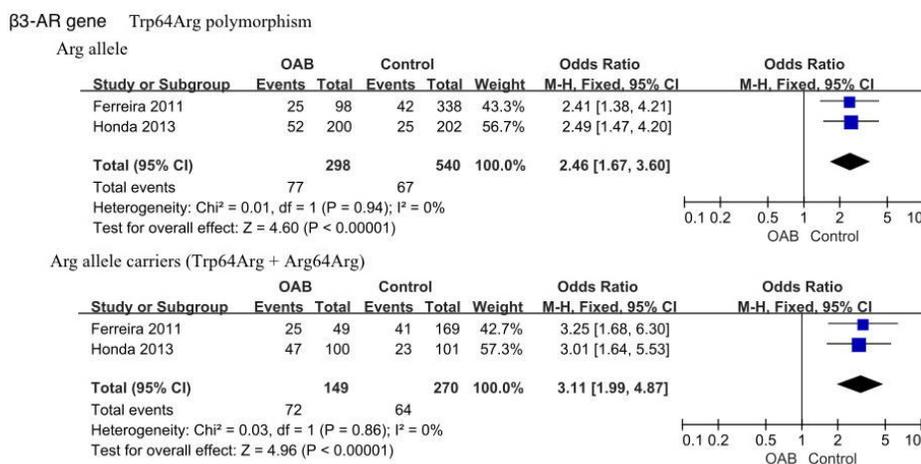


Figure 2. Meta-analysis of the association between β3-AR gene polymorphisms and OAB risk.

Publication bias

Publication bias was assessed by funnel plot. The funnel plots of the studies included appeared to be symmetrical (Figure 3).

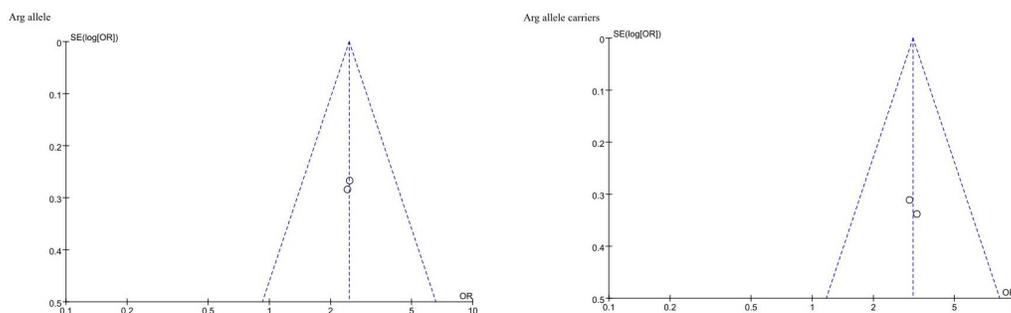


Figure 3. Begg’s funnel plot of publication bias.

DISCUSSION

OAB is a symptomatic diagnosis based on the presence of urgency, with or without incontinence, which afflicts both men and women at similar rates and an estimated overall prevalence of nearly 12%, which increases with age (Irwin et al., 2006). Although the pathophysiology of OAB is not fully understood, either neurogenic or myogenic dysfunction can produce OAB symptoms (Miller and Hoffman, 2006). Recent studies showed that activation of β 3-AR caused relaxation of the detrusor muscle during bladder filling. Thus, it was hypothesized that dysfunction of the β 3-AR gene contributes to impaired relaxation of the bladder muscle during urine storage, leading to OAB symptoms (Andersson, 1993; Takeda et al., 1999; Igawa et al., 1999; Michel and Vrydag, 2006; Yamaguchi and Chapple, 2007).

A single nucleotide polymorphism (SNP) is the most abundant type of DNA sequence variation in the human genome (Skorupski et al., 2006; Rodrigues et al., 2008). The gene encoding human β 3-AR is polymorphic; the first SNP reported in the β 3-AR gene was an exchange of the amino acid tryptophan at position 64 of the receptor protein with arginine, referred to as the Trp64Arg polymorphism (Clément et al., 1995). This mutation occurs with an approximate frequency of 8-10% in the Caucasian population, 20% in the Japanese population, and 40% in Alaskan Eskimos (Arner and Hoffstedt, 1999). Recently, Teitsma et al. (2013) analyzed the association between urodynamic characteristics and genotype in the β 3-AR gene in more than 1000 male patients with lower urinary tract symptoms. These findings suggest that a relationship exists between the Trp64Arg polymorphism and human micturition symptoms. Thus, the Trp64Arg polymorphism is important for the pathophysiological conditions of OAB syndrome.

In this meta-analysis, we included a total of 149 OAB cases and 270 healthy controls from 2 independent studies. We examined the association between the Trp64Arg polymorphism of the β 3-AR gene and OAB risk. Similarly to the results of previous studies, our meta-analysis demonstrated that the Arg allele may increase the risk of OAB (OR = 2.46, 95%CI = 1.67-3.6, $P < 0.00001$). In addition, we identified that Arg allele carriers (heterozygous 64Arg variant and homozygous 64Arg variant, or Trp64Trp + Arg64Arg) were positively associated with OAB (OR = 3.11, 95%CI = 1.99-4.87, $P < 0.00001$). In conclusion, our results showed that the Trp64Arg polymorphism was associated with OAB, at least in part.

There were some limitations to our study. First, the eligible number of studies in this meta-analysis and the sample size of this meta-analysis were small. In addition, some relevant studies could not be included in our analysis because of incomplete raw data. Third, although all cases and controls of each study were well defined with similar inclusion criteria, there may be factors that were not taken into account, which could have influenced our results. Fourth, meta-analysis is a retrospective method that is subject to methodological limitations. Most importantly, our meta-analysis was based on unadjusted OR estimates because not all published studies presented adjusted ORs or presented ORs that were not adjusted by the same potential confounders, including age, gender, ethnicity, and environmental exposure. Based on these results, additional studies are necessary and our conclusions should be interpreted with caution.

In conclusion, this meta-analysis of 2 case-control studies demonstrated that the Trp64Arg polymorphism in the β 3-AR gene is involved in the pathogenesis of OAB syndrome. The Arg allele and the Arg allele carrier (heterozygous 64Arg variant and homozygous 64Arg variant, or Trp64Trp + Arg64Arg) may be positive factors for OAB risk. Because few studies have been conducted in this field, the current evidence remains limited. Therefore,

large studies with adequate methodological quality and proper control of confounding factors are necessary to validate our results.

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