Published in final edited form as:

Prostate. 2017 August ; 77(11): 1213–1220. doi:10.1002/pros.23380.

A genetic variant near *GATA3* implicated in inherited susceptibility and etiology of benign prostatic hyperplasia (BPH) and lower urinary tract symptoms (LUTS)

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Abstract

Background—Benign prostatic hyperplasia (BPH) and associated lower urinary tract symptoms (LUTS) are common conditions. Little is known about their etiologies except that studies have suggested a substantial heritable component. Our objective is to provide a comprehensive, genome-wide evaluation of inherited risks and possible mechanisms of etiology in BPH.

Methods—We performed a three-stage, genome-wide association study (GWAS) of men from three independent populations, the REDuction by DUtasteride of prostate Cancer Events (REDUCE) trial, the CLUE II cohort, and a Finnish hospital-based population. DNA samples were genotyped using the Illumina HumanOmniExpress BeadChip in REDUCE and CLUE II, and using the Sequenom iPLEX system for the confirmation stage in the Finnish population. A logistic regression model was used to evaluate the association between each SNP and BPH/LUTS.

Results—Fourteen SNPs reached $P < 5.0 \times 10^{-4}$ in the meta-analysis of the two GWASs (CLUE II and REDUCE). A total of 773 SNPs were chosen for the confirmation step in the Finnish cohort. Only one SNP (rs17144046) located ~489kb downstream of *GATA3* remained significant after

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Conflict of interest and Disclosure Statement

No known conflict of interest is related to the study

correction for multiple testing ($P < 6.5 \times 10^{-5}$). This SNP marginally reached the GWAS significance level after performing a meta-analysis of the three stages ($P_{\text{meta}} = 8.89 \times 10^{-7}$). Expression quantitative trait loci (eQTL) analyses showed that the risk allele (G) of rs17144046 was significantly associated with increased expression of *GATA3* ($P = 0.017$). Reported studies indicated a close correlation between *GATA3* and BPH pathogenesis and progression.

Conclusions—Rs17144046 located near *GATA3* was significantly associated with BPH/LUTS in three independent populations, but did not reach a stringent GWAS significance level. Genetic variants of *GATA3* may play a role in the inherited susceptibility and etiology of BPH/LUTS. Further research in this area is needed.

Keywords

BPH; LUTS; Genome-wide association study

Background

Benign prostatic hyperplasia (BPH) is common in the aging human male with a histopathological prevalence in 80% of men by age 80¹. BPH contributes to bladder outlet obstruction, leading to bothersome lower urinary tract symptoms (LUTS). BPH is associated with complications such as urinary tract infections, acute urinary retention, hematuria, and stones. One third of men with BPH ultimately undergo medical and/or surgical interventions. In addition, BPH has been associated with increased risks of mortality, depression and diminished health-related quality-of-life, as well as with billions of U.S. dollars in annual health expenditures². These societal and financial burdens are becoming exacerbated with the shift towards an older age distribution of the population in economically developed countries.

The etiology of BPH is not well understood. Increasing age and the extended presence of testicular androgens are consistent risk factors. BPH has long been considered a product of androgen and estrogen action upon an aging prostate³. However, it is now clear that multiple mechanisms contribute to the disease⁴. Comorbidities such as obesity and type 2 diabetes mellitus appear to contribute to disease progression, potentially contributing to cell proliferation and resistance to current therapies⁵. Metabolic syndrome is associated with BPH, LUTS and prostate growth⁶. In addition, inflammatory conditions are also associated with increased incidence of BPH^{7–9}.

In addition to environmental risks, family and twin studies have provided strong evidence for genetics as an important risk factor for developing BPH. One study reported a 66% lifetime risk of requiring surgical intervention for BPH among first degree male relatives of case probands, which was four-fold higher than those without a similar family history¹⁰. A twin study indicating that the relative risk for BPH for monozygotic twins was 3.3¹¹. More recently, a 37% heritability of the International Prostate Symptom Score (IPSS, which indicates LUTS) was estimated in a twin study¹². In addition, studies have suggested that single nucleotide polymorphisms (SNPs) in certain genes and pathways are associated with BPH/LUTS^{13,14}, providing additional evidence for the heritability of the disease. However, as a more comprehensive and unbiased way to investigate the association between genetic

variants and diseases, a genome-wide association study (GWAS) of BPH has never been reported.

Here, we describe the first genome-wide analyses of BPH/LUTS with two GWAS studies of independent populations, the REDuction by DUtasteride of prostate Cancer Events (REDUCE) trial and CLUE II cohort, together with a third cohort from a Finnish population for validation/confirmation. Our objective is to provide a better understanding of inherited risks and possible mechanisms of etiology in BPH.

Methods

Study populations

REDUCE is a 4-year, randomized, double-blind, placebo-controlled study evaluating the efficacy and safety of once-daily 0.5-mg dutasteride in reducing the risk of biopsy-detectable prostate cancer in men at increased risk of developing prostate cancer¹⁵. Inclusion and exclusion criteria for REDUCE were described in a previous study¹⁶. The definitions of BPH/LUTS were: (a) IPSS score ≥ 8 (moderate symptoms, ≥ 25 were excluded due to the exclusion criteria of REDUCE) at study entry, or (b) prostate volume ≥ 30 mL. A total of 3,103 men of European descent who consented for genetic studies with genotype data were included. It should be noted that only baseline data was used, and the effects of dutasteride on LUTS were not investigated.

The characteristics of the study population from CLUE II study were described in a previous publication¹⁷. Briefly, this is a community cohort with 32,898 volunteers who were recruited in Washington County, MD, USA from May through October 1989. Participants were asked to update their exposure histories on mailed questionnaires in 1996, 1998, and 2000. To be included in the present study, CLUE II study subjects had to respond to the year 2000 follow-up questionnaire, did not have a cancer diagnosis prior to 2000, and did not have undergone a transurethral resection of the prostate (TURP) or other prostatectomy prior to 1989. The definitions of BPH/LUTS were: (a) history of TURP, or (b) medication treatment of BPH, or (c) modified AUA Symptom Index ≥ 15 (high-moderate to severe symptoms). A total of 568 men met these criteria, as well as a group of age- and race-paired control men ($n=568$) who were also included.

Subjects were obtained for the confirmation stage from a hospital-based population¹⁸. Briefly, 485 Finnish men with BPH (confirmed without prostate cancer or high-grade prostate intraepithelial neoplasia after years of follow-up) were recruited from June 1992 to April 2003. The diagnosis of BPH was based on lower urinary tract symptoms, free uroflowmetry, and evidence by palpation or transrectal ultrasound of prostates of increased size. An age-paired control population ($n=475$) was recruited from the Finnish population-based prostate cancer screening trial^{19,20}.

All study protocols were approved by the institutional review board at each research site, and all participants provided written informed consent.

SNP genotyping and quality control (QC) in the GWAS stage

SNPs were genotyped using the Illumina HumanOmniExpress BeadChip for REDUCE and CLUE II populations at the Center for Cancer Genomics at Wake Forest University School of Medicine including 730,525 SNPs across the genome. All 3,103 samples in REDUCE (14 samples were excluded due to poor DNA quality) and 1,136 samples in CLUE II were genotyped. Subjects were removed from subsequent analyses if they met any of the following criteria: (1) overall genotype call rate <90%; (2) duplicates or familial relationship ($PI_HAT > 0.025$). SNPs were excluded if they had: (1) overall genotype call <95%; (2) minor allele frequency (MAF): <0.05 for CLUE II and <0.01 for REDUCE; or (3) $P < 1 \times 10^{-3}$ from a Hardy-Weinberg Equilibrium (HWE) test among controls. Genotypes of SNPs that were not genotyped were imputed by IMPUTE 2.2.2 using the combined data of the 1000 Genomes project and HapMap3 data as reference haplotype maps²¹. A posterior probability of >0.90 was applied to call genotypes during imputation and the same QC procedure for excluding genotyped SNPs was applied to imputed SNPs.

SNP genotyping and QC in confirmation stages

Selected SNPs were genotyped using the Sequenom iPLEX system (San Diego, CA). All plates contained an asymmetric distribution of control samples (CEPH for positive control and water for negative control). Two percent of the samples were randomly selected to be duplicated. The concordance rates for all SNPs were calculated among duplicates.

Statistical analyses and eQTL analyses

A logistic regression model was used to evaluate the association between each SNP and BPH/LUTS performing by using Plink 1.07. The meta-analysis of the two GWAS datasets (CLUE II and REDUCE) was performed using GWAMA²². Fixed-effect modeling was used if the heterogeneity was not significant (P for Cochran's Q statistic >0.05), otherwise the random-effect model was used. At the GWAS stage, a P -value < 5×10^{-8} was considered as genome-wide significant, and at the confirmation stage, a P -value < 6.5×10^{-5} (0.05/772) was considered significant after Bonferroni correction. Manhattan Plots were performed using R3.3.1. The SNPs regional plots were performed by LocusZoom (<http://locuszoom.sph.umich.edu/>). *Cis*-expression quantitative trait loci (eQTL) analyses were performed using the Genotype-Tissue Expression (GTEx) project portal in normal prostate tissue (n=87) (data download from: <http://www.gtexportal.org>).

Results

The demographic information of the study populations is shown in Table 1. The detailed characteristics of the CLUE II study population and Finnish population have been described in previous studies^{17,19,20}.

To initially identify candidate genetic variants in the form of SNPs that may be associated with BPH/LUTS, we performed GWAS analysis separately in two populations (CLUE II and REDUCE) using ~700,000 SNPs distributed across the genome on the Illumina HumanOmniExpress BeadChip. After quality control of genotyping results, 642,461 SNPs in 1,419 cases and 1,684 controls in REDUCE, as well as 624,353 SNPs in 498 cases and

518 controls in the CLUE II study, were used for imputation. After quality control of imputed SNPs, 7,852,412 SNPs in REDUCE and 15,866,381 SNPs in CLUE II remained for further analyses. Quantile-quantile plots (Q-Q plots) analysis of the association results showed an inflation factor (λ) of 1.037 in REDUCE and 1.013 in CLUE II (Supplementary Figure 1). The $-\log_{10}P$ -values by chromosome location for BPH/LUTS-associated SNPs are shown in Figure 1a (REDUCE), Figure 1b (CLUE II) and Figure 1c (meta-analysis of the two GWASs). None of the SNPs reached a genome-wide significance level in the two original GWASs or the meta-analysis of the two studies. Fourteen SNPs reaching $P < 5.0 \times 10^{-4}$ in the meta-analysis of the two GWASs are presented in Table 2.

From the combined GWAS data, a total of 772 SNPs were selected for confirmation in the Finnish BPH/LUTS population based on three strategies (Supplementary Table 1): first, 638 independent SNPs (CLUMP in Plink, $r^2=0.2$) with $P < 0.05$ from the CLUE II and REDUCE GWAS; second, the top 116 independent SNPs (CLUMP, $r^2=0.2$) based on the CLUE II GWAS ($P < 0.001$) or sorted by meta P -value of CLUE II and REDUCE studies; and third, 14 reported candidate SNPs in genes implicated in BPH/LUTS (e.g. *SRD5A2* and *IL10*)^{23,24}. After genotyping, all samples passed the quality control with an average call rate of 99.7%. For 772 SNPs, the average call frequency, average GenTrain Score and average Cluster Sep were 99.7%, 71.3% and 57.4%, respectively.

Table 3 shows the SNPs that were significantly associated with BPH/LUTS at the confirmation stage after Bonferroni correction for multiple testing ($< 0.05/772 = 6.5 \times 10^{-5}$). Results from the meta-analysis of the two GWASs and the confirmation study of these two SNPs are also shown in Table 3 and Figure 2. Rs17144046 is located in an intergenic region that is ~488.9kb downstream of the closest gene, *GATA3*. This SNP was significantly associated with BPH/LUTS in the confirmation study (odds ratio, OR=1.55, 95% confidence interval, 95% CI: 1.27–1.90, $P = 1.62 \times 10^{-5}$). Although rs17144046 failed to reach the GWAS significance level in the GWAS stage, its association with BPH/LUTS was consistent in both REDUCE (OR=1.13, 95% CI: 1.01–1.26, $P = 0.030$) and CLUE II (OR=1.30, 95% CI: 1.08–1.57, $P = 0.0067$). After a meta-analysis of the results from the three studies, the association between rs17144046 and the disease reached a GWAS marginally-significant level (OR=1.41, $P_{\text{meta}} = 8.89 \times 10^{-7}$). A regional plot shows that rs17144046 has very mild LD with SNPs located at *GATA3* or other nearby genes (Figure 3).

Another SNP, rs943587, was observed to be significantly associated with BPH/LUTS in the confirmation study (OR=1.63, 95% CI: 1.30–2.03, $P = 1.60 \times 10^{-5}$). However, the locus was no longer significant or marginally significant after meta-analysis (OR=1.11, $P_{\text{meta}} = 0.80$) due to the different directions of ORs in REDUCE and CLUE II (Table 3 and Figure 2).

To assess the possible biologic mechanism, *cis*-eQTL analyses were used to assess any association between rs17144046 and the expression of genes located within 1Mbp in normal prostate tissue. The risk allele (G) of rs17144046 was significantly associated with increased expression of *GATA3* ($P = 0.017$), but not significantly associated with the expression of other nearby genes. Immunohistochemistry of normal prostate tissue is showing expression of *GATA3* is shown in Supplementary Figure 2, indicating that *GATA3* expresses in the nuclei of luminal epithelial cells.

Discussion

The etiology of BPH/LUTS is complex. Many processes likely participate in the processes of prostate overgrowth and development of urinary symptoms that characterize BPH/LUTS, including smooth muscle growth, neuromuscular and hormonal function, bladder urodynamics, etc. This complexity has rendered mechanistic investigations of BPH/LUTS a challenge to perform and results of such studies difficult to consolidate and integrate. To address this and to elucidate potentially important cellular pathways and contributing genes, we utilized the unbiased approach of GWAS to uncover genetic factors associated with BPH/LUTS pathology.

In this study, we report a three-phase GWAS study of BPH/LUTS involving nearly 5,000 men. To our knowledge, this is the first GWAS study reported to-date of this common condition. Given the complexity of BPH/LUTS, no single SNP reached genome wide significance in our study. This does not necessarily indicate that genetic factors are not important in the development of BPH/LUTS, but rather, etiologic heterogeneity may limit signal strength from individual SNPs, precluding identification by our approach. Contributing to this problem, there are multiple and complex phenotypes present in cases and controls. For example, men with large prostates elect to undergo surgical or medical intervention because of associated bothersome LUTS, while men with similar prostate volumes may not experience bothersome LUTS. Ideally populations should be selected based on a stringent, uniform measure of BPH, such as prostate volume, as opposed to a surrogate such as LUTS. This study did not have the ability to conduct a reanalysis to make clearer distinctions between phenotypes due to limitations of available phenotype data. Further studies are required to fully answer this question.

We did observe a SNP near *GATA3* that was marginally statistically significant, and have thus nominated this gene as an interesting candidate gene worthy of further study. *GATA3* belongs to the GATA family of transcription factors that contain two GATA-type zinc fingers. *GATA3* is expressed in a range of human tissues, including cells from all three embryonic germ layers. Deletion of the gene is embryonic lethal in mice and abnormalities are an underlying cause of hypoparathyroidism, sensorineural deafness and renal anomalies (HDR) syndrome in humans^{25,26}. *GATA3* has a broad range of described interactions with other transcription factors. It has been reported to interact with androgen receptors as well as with estrogen receptors- α and - β , all of which have been implicated in BPH pathogenesis and progression^{27,28}. *GATA3* also interacts with nuclear factor-kappaB (NF- κ B) to regulate inflammation. This transcription factor is thus known to be a key player in the major pathways implicated in BPH, providing a strong rationale to suspect that this GWAS finding has a biological basis²⁹.

GATA2 and *GATA3* are expressed in the mouse prostate, localizing to the nuclei of luminal epithelial cells³⁰. The human protein atlas (<http://www.proteinatlas.org>) shows nuclear staining in both luminal and basal cells of normal prostate areas, as well as in cancer glands, with frequent positive stromal cells. Prostate luminal epithelial-specific knockout (using Pb-cre) of either *GATA2* or *GATA3* alone had no phenotypic effects on the prostate, whereas double knockout mice exhibited PIN (prostatic intraepithelial neoplasia) lesions, decreased

prostate-to-body weight ratio, altered AR target gene expression, and expansion of p63-positive basal cells³⁰. There have been few studies assessing the role of *GATA3* in normal and pathologic prostate biology, particularly BPH/LUTS.

There are several important limitations to our study. In spite of including nearly 5000 men, the sample size remains relatively small, limiting our ability to detect more subtle genetic associations, particularly in subsets of affected men. As discussed above, a particularly important limitation is the variability of the criteria used to determine affected status among the various study populations. Such variability in phenotype likely reduced our power to detect significant associations substantially. In addition, our study population was primarily of European background, thus reducing the generalizability of our results to men of other ancestries. In the future, larger studies with more precise and uniform criteria for affected status will be needed to fully elucidate the role of genetic variation in BPH/LUTS etiology. In the meantime, our novel results provide an important basis for additional studies of the role of *GATA3* in normal and pathobiology of the prostate.

Conclusions

Rs17144046, located near *GATA3*, was consistently significantly associated with BPH/LUTS in three independent populations, though it did not reach a stringent GWAS significance level. Genetic variants of *GATA3* may play a role in the inherited susceptibility and etiology of BPH/LUTS.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors thank the patients enrolled in REDUCE who provided the consent and genetic samples that enabled this study, and the clinicians who contributed their expertise in recruiting study patients for the REDUCE clinical study. Dave Pulford, Jennifer Aponte, Jon Charnecki, and Mary Ellyn Volk participated in consent reconciliation and sample management to enable genetic sample selection for inclusion and genotype determination. Karen King provided data management support for this project. We thank Silviu-Alin Bacanu and Matt Nelson for multiple discussions on power determination, method limitations, and results. We appreciate the assistance of Lauren Marmor in coordinating the support of the Avodart Collaborative Research Team. The assistance of Dr. Tamara Lotan is gratefully acknowledged.

Funding

This study was supported by Obrien Center Funding (P50-DK082998) from NIDDK, NIH to William B. Isaacs. In addition, this study is partially supported by a National Cancer Institute RC2 grant (CA148463) and a research contract by GlaxoSmithKline to Jianfeng Xu.

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Figure 1. Manhattan plot: the $-\log_{10}P$ -values by chromosome location for BPH/LUTS-associated SNPs in (a) REDUCE trial, (b) CLUE II and (c) meta-analysis of CLUE II and REDUCE

a**rs17144046****Study Name**

CLUE II

REDUCE

Finnish (Confirmation)

Overall: $P=8.89E-07$, $I^2=38.1$ **b****rs943587****Study Name**

CLUE II

REDUCE

Finnish (Confirmation)

Overall: $P(\text{random})=0.80$, $I^2=95.6$ **Figure 2.**

Forest plot: the results from the meta-analysis of the two GWASs and the confirmation study of these two SNPs

Figure 3.
Regional plot (LocusZoom plot) of rs17144046

Table 1

Baseline demographic information in study populations

Variables	Case	Control	P
REDUCE study			
No.	1419	1684	–
Age (Mean±SD)	63.2±5.8	62.3±6.1	3.04×10 ⁻⁵
Prostate volume (Median, range)	50.38(30.02–257.17)	39.21(3.66–256.83)	<2.20×10 ⁻¹⁶
IPSS (Median, range)	12 (8–24)	5 (0–23)	<2.20×10 ⁻¹⁶
Total PSA levels (Median, range)	5.8(1.9–13.8)	5.5(1.8–15.6)	1.37×10 ⁻⁵
CLUE II study			
No.	568	568	–
Age (Mean±SD)	55.6±10.6	55.6±10.5	Matched
Finish Population (Confirmation study)			
No.	485	475	–
Age (Mean±SD)	66.0±7.5	65.6±3.1	0.31

Table 2

SNPs reached $P < 5.0 \times 10^{-4}$ in the meta-analysis of the two GWASs.

CHR	SNP	Related Gene	Position	A1	A2	CLUE II		REDUCE		Meta	
						P	OR	P	OR	P	OR
1	rs6537704	<i>ADORA3</i>	Intron	G	A	0.004	1.46	6.82×10^{-4}	1.3	1.11×10^{-5}	1.34
1	rs10915971	<i>PARP1</i>	Intergenic	A	G	0.007	1.3	0.001	1.19	3.85×10^{-5}	1.21
2	rs10202096	<i>FOSL2</i>	Intergenic	A	G	0.011	0.78	3.25×10^{-4}	0.81	1.12×10^{-5}	0.81
3	rs12490648	<i>EOMES</i>	Intergenic	G	A	0.006	1.63	3.58×10^{-4}	1.43	7.59×10^{-6}	1.48
3	rs7630221	<i>LRRHIP2</i>	Intron	A	G	0.003	1.31	0.001	1.19	1.44×10^{-5}	1.22
3	rs3922844	<i>SCN5A</i>	Intron	A	G	0.044	1.21	1.54×10^{-4}	1.23	1.82×10^{-5}	1.23
5	rs2676245	<i>DNAH5</i>	Intergenic	A	G	0.004	0.77	4.07×10^{-4}	0.82	7.29×10^{-6}	0.81
10	rs7911634	<i>PCDH15</i>	Intron	A	C	0.033	0.68	1.67×10^{-4}	0.66	1.53×10^{-5}	0.67
11	rs428215	<i>RAG2</i>	Intergenic	G	A	0.032	0.74	4.84×10^{-4}	0.76	4.20×10^{-5}	0.76
11	rs10894022	<i>BARX2</i>	Intergenic	A	G	0.006	0.74	0.001	0.81	2.59×10^{-5}	0.79
12	rs11613206	<i>LRRK2</i>	Intergenic	A	G	1.67×10^{-5}	2.54	0.020	1.3	4.88×10^{-5}	1.5
12	rs6538357	<i>PLEKHG7</i>	Intron	C	A	1.54×10^{-5}	0.68	0.027	0.89	4.86×10^{-5}	0.84
13	rs9302078	<i>CLDN10</i>	Intron	A	G	0.008	0.78	0.001	0.84	3.02×10^{-5}	0.83
14	rs7152796	<i>FOXP3</i>	Intron	A	G	0.005	1.68	0.002	1.39	3.95×10^{-5}	1.46

Table 3

SNPs reached Bonferroni corrected significant P-value ($<6.5 \times 10^{-5}$) in confirmation study and the meta-analysis results of the three studies.

CHR	SNP	Related Gene	Position	Risk allele/non-risk allele	CLUE II			REDUCE			Confirmation			Meta	
					P	OR (95%CI)		P	OR (95%CI)		P	OR (95%CI)		P	OR
10	rs17144046	<i>GATA3</i>	Intergenic	G/A	0.007	1.30 (1.08–1.57)		0.030	1.13 (1.01–1.26)		1.62×10^{-5}	1.55 (1.27–1.90)		8.89×10^{-7}	1.41
14	rs943587	<i>VRK1</i>	Intergenic	G/A	0.014	0.75 (0.60–0.94)		0.049	0.88 (0.77–1.00)		1.60×10^{-5}	1.63 (1.30–2.03)		0.80	1.11