Hum Genet (2013) 132:1361–1369
DOI 10.1007/s00439-013-1340-1

ORIGINAL INVESTIGATION

Variants in *BET1L* and *TNRC6B* associate with increasing fibroid volume and fibroid type among European Americans

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Received: 29 May 2013 / Accepted: 14 July 2013 / Published online: 28 July 2013

Abstract Uterine fibroids (UFs) affect 77 % of women by menopause and account for $9.4 billion in yearly healthcare costs. We recently replicated findings from the first UF genome-wide association study (GWAS), conducted in the Japanese. Here we tested these GWAS-discovered SNPs for association with UF characteristics to further assess whether risk varies by sub-phenotypes of UFs. Women were enrolled in *Right from the Start* (RFTS) and the BioVU DNA repository (BioVU). UF status was determined by pelvic imaging. We tested the top GWAS-associated SNPs for association with UF characteristics (RFTS: type, number, volume; BioVU: type) using covariate adjusted logistic and linear regression. We also combined association results of UF type using meta-analysis. 456 European American (EA) cases and 1,549 controls were examined. Trinucleotide repeat containing 6B (*TNRC6B*) rs12484776 associated with volume in RFTS ($\beta = 0.40$, 95 % CI 0.05–0.75, $p = 0.024$). RFTS analyses evaluating stratified quartiles of volume showed the strongest OR at rs12484776 for the largest volume (16.6–179.1 cc, odds ratio (OR) = 2.19, 95 % confidence interval (CI) 1.07–4.46, $p = 0.031$). Meta-analysis showed a strong association at blocked early in transport 1 homolog (*BET1L*) rs2280543 for intramural UFs (meta-OR = 0.51, standard error (SE) = 0.14, $Q = 0.590$, $I = 0$, $p = 2.48 \times 10^{-6}$), which is stronger than the overall association with UF risk. This study is the first to evaluate these SNPs for association with UF characteristics and suggests these genes associate with increasing UF volume and protection from intramural UF in EAs.

Electronic supplementary material The online version of this article (doi:10.1007/s00439-013-1340-1) contains supplementary material, which is available to authorized users.

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Introduction

Uterine leiomyomata, or fibroids (UFs), are the most common female pelvic tumor. Prevalence estimates range from 20 to 77 %, increasing with age up to menopause (Cramer and Patel 1990; Marshall et al. 1997; Vollenhoven 1998). UF account for $9.4 billion in healthcare costs each year, due in large part to surgeries resulting from symptomatic fibroids. (Cardozo et al. 2012) Symptomatic or severe UFs are most often determined by UF location in the uterus [most common type: submucous (beneath endometrium of uterus), intramural (within wall of uterus), and subserous (underneath mucosal layer of the uterus)], size, and/or number of UFs present. Known risk factors for UFs include African American (AA) race (Baird et al. 2003; Cramer and Patel 1990; Faerstein et al. 2001; Marshall et al. 1997; Ojeda 1979), early age-at-menarche, (Dragomir et al. 2010;

Faerstein et al. 2001; Lumbiganon et al. 1996; Marshall et al. 1998; Samadi et al. 1996; Wise et al. 2004) high body mass index (BMI) (Moore et al. 2008; Takeda et al. 2008), and increased age (Baird et al. 2003). In addition, a protective effect for UFs has also been observed with higher parity, likely due to pregnancy-related hormonal and physical changes including postpartum uterine involution (Baird and Dunson 2003; Laughlin et al. 2010a, 2011).

Multiple lines of evidence have shown that UFs are influenced by genetic risk factors. First, UFs are highly heritable with evidence from twin-pair and familial aggregation studies (Luoto et al. 2000; Treloar et al. 1992). Heritability studies of UFs in several European populations have observed that between 26 and 69 % of UF risk is due to genetic factors (Kurbanova et al. 1989; Luoto et al. 2000; Snieder et al. 1998). Further supporting a genetic contribution to risk are the observed racial disparities in UF age of onset, number, size, and lifetime incidence by menopause (Baird et al. 2003). Genetic epidemiology studies to date have been largely limited to small-scale or single marker studies of steroid hormones, particularly estrogen, as it is potentially the most critical regulator of fibroid growth (Flake et al. 2003). Also other growth factors (Sozen and Arici 2002), reproductive factors (Parazzini et al. 1996), dysregulation of microRNAs (Marsh et al. 2008), shortening of telomeres (Bonatz et al. 1998), excessive production of disorganized extracellular matrix (Malik et al. 2010; Sozen and Arici 2002), and acquired chromosomal aberrations have been noted in UF studies (El-Gharib and Elsobky 2010).

Our group recently replicated, using a US European American (EA) population of all imaged subjects (Edwards et al. 2013), associations observed in a recent a genome-wide association study (GWAS) of UFs among a clinical population of Japanese women (Cha et al. 2011). The single-nucleotide polymorphisms (SNPs) associated in the prior GWAS mapped to three chromosomal regions (10q24.33, 11p15.5, and 22q13.1) that included SNPs on or nearby the genes STE20-like kinase (*SLK*), trinucleotide repeat containing 6B (*TNRC6B*), and blocked early in transport 1 homolog (*BET1L*). Although our group was able to replicate these associations, recent studies by other groups using populations of AAs and European ancestry (US and Australia) subjects with self-reported UF status were unable to replicate association at these SNPs (Eggert et al. 2012; Wise et al. 2012). The inability to replicate these findings may be due to our EA population and the prior GWAS population representing clinical cohorts and these SNPs being more associated with sub-phenotypes of UFs. In efforts to further understand these associations we examined the three previously associated SNPs for association with UF characteristics, including UF type (submucous, intramural, and subserous), UF volume, and UF number. To conduct this analysis we used two cohorts of EA women, all of whom had pelvic imaging performed to detect the presence of UFs. Imaging is critical, because many women with UFs are asymptomatic and without imaging, studies may misclassify as many as 51 % of women.(Baird et al. 2003; Myers et al. 2012) The primary goal of this study was to determine if gene variants within the previously associated gene regions associate with UF characteristics or UF sub-phenotypes in EA US populations.

Materials and methods

Study populations

Right from the Start (RFTS)

RFTS is a community-based pregnancy cohort that enrolled study participants between 2001 and 2012. RFTS enrolled participants from Galveston, Texas; Memphis, Nashville, Knoxville, and Chattanooga, Tennessee; and the Research Triangle region (Raleigh, Durham, and Chapel Hill) in North Carolina. These analyses included RFTS participants who were 18 years or older and non-Hispanic EAs. As a part of participation, consent was obtained to review study participant medical records. Direct marketing and recruitment strategies have been previously described (Promislow et al. 2004). The Institutional Review Board (IRB) of Vanderbilt University, Nashville, Tennessee, approved this study.

At enrollment, a research transvaginal ultrasound was conducted to assess embryonic development for the study pregnancy and to systematically examine the uterus for presence of UFs. The UF measurement protocol required three separate sets of measurements for each UF, with assessment of three perpendicular diameters: length, width, and depth. RFTS includes UFs as small as 0.5 cm in maximum diameter (Laughlin et al. 2009). Multiple still images of each UF with caliper markings of each diameter were recorded and a UF map was completed indicating the location and type (most common types submucous, intramural, and subserous) of all UF(s).

Participants completed an intake interview at enrollment and a computer-assisted telephone interview at the end of the first trimester. The intake and first trimester interviews provided information on reproductive history and candidate confounders. DNA samples were obtained either in person or by mail during follow-up using Oragene saliva DNA kits (DNA Genotek Inc., Ontario, Canada).

The BioVU DNA Repository

The BioVU Repository (2007–present) is located at Vanderbilt University, Nashville, TN, and was designed to link

clinical data available from de-identified electronic medical records to DNA specimens (Pulley et al. 2010). The BioVU repository consists of de-identified blood samples obtained from patients at The Vanderbilt University Medical Center Hospital, including all clinics that are part of the hospital system. De-identified data from multiple sources are available within BioVU, including diagnostic and procedure codes, basic demographics, discharge summaries, nursing notes, progress notes, health history, multidisciplinary assessments, laboratory values, echocardiogram diagnoses, imaging reports, electronically derived data, and inpatient medication orders. All subjects (both UF cases and controls) selected from BioVU had diagnostic imaging with ultrasound, magnetic resonance imaging (MRI), or computed tomography (CT). The majority of both cases and controls ($\sim$80 %) had ultrasound imaging for confirmation of UF status. Included as UF cases were women who had diagnostic imaging and either a diagnosis of a UF, as indicated by physician diagnosis of UFs or a surgical procedure for UF removal. For controls, two or more instances of pelvic imaging on separate dates were required. Initial chart review of a small subset of controls suggests that a large proportion of imaging information comes from prior pregnancy ultrasounds. Women with hysterectomy, myomectomy, or other procedures for UFs were excluded as controls. Controls were density matched to UF cases based on date of diagnostic imaging, where controls' second imaging date had to be within a three to 5 year window of those cases. Both cases and controls were 18–65 years of age. We did not limit controls for age, but did perform secondary analyses limiting controls to those greater than 50 years of age to reduce the possibility that some women might develop a UF after imaging was performed. Our sampling algorithm to define UF cases and controls is informed by a published UF algorithm by Hartmann and colleagues using electronic medical records (Hartmann et al. 2006). Covariate data and UF type information were abstracted using natural language processing algorithms of study participant electronic medical records (EMR), as well as from diagnostic and procedure codes. The IRB of Vanderbilt University, Nashville, TN, approved this study.

DNA extraction and genotyping

Genotyping BioVU

BioVU DNA samples were isolated from whole blood using the Autopure LS system (QIAGEN Inc., Valencia, CA). In BioVU we genotyped the top three associated SNPs from the previously published GWAS (rs7913069, rs2280543, and rs12484776) using a TaqMan allelic discrimination assay (Cha et al. 2011).

RFTS genotyping

DNA for RFTS saliva samples was extracted using Oragene DNA (Genotek Inc., Ontario, Canada) manufacturer-recommended DNA extraction procedures. The three GWAS index SNPs were genotyped using the Sequenom MassARRAY genotyping platform (Sequenom Inc., San Diego, CA). All SNPs in BioVU and RFTS had genotyping call rates of 95 % or better (mean call rates of 98 %) and QC sample match rates of 100 %.

Statistical analysis

Tests for deviations from Hardy–Weinberg equilibrium (HWE) were performed using PLINK statistical software (Purcell et al. 2007). Statistical significance for these analyses was determined using *p* values from Fisher's exact tests. Descriptive statistics of demographic data were expressed as means and standard deviations for continuous covariates and as frequencies and proportions for categorical data, and compared between cases and controls (women with and without UFs) with unadjusted linear or logistic regression using STATA 11.0 statistical software (College Station, TX).

Single locus tests of association with UF characteristics utilizing UF cases and controls were performed using linear, ordinal, and logistic regression assuming an additive genotypic model [0 (homozygous major allele) versus 1 (heterozygous) versus 2 (homozygous minor allele)]. All sub-phenotype analyses in both BioVU and RFTS were performed using subjects with no UF controls and stratified categories of UF characteristics as cases. Characteristics examined as outcomes (dependent variable) in RFTS included UF type (any submucous UF, any intramural UF, and any subserous UF), UF number analyzed with ordinal and logistic regression (one UF, two or more UF), total UF volume stratified by quartiles analyzed both with ordinal and logistic regression [units in cubic centimeters (cc), top quartile volume (0.003–0.7), second quartile volume (0.7–3.6), third quartile volume (3.6–13.3), and fourth quartile (16.6–179.1)], and log transformed total UF volume as a continuous outcome (limiting to fibroid cases). BioVU data were analyzed only for UF type with logistic regression using no UFs as controls and stratified categories of UF type (any submucous UF, any intramural UF, any subserous UF) as outcomes. Independent variables included in statistical models were SNP, age, and BMI. Beta's or odds ratios (ORs) and confidence intervals (CI) were reported for SNPs from all statistical models. We reported results from regression models adjusted for potential confounders: age (continuous) and BMI (continuous). Unadjusted models are presented in Supplemental Table 1 and 2. STATA statistical software (College

Station, TX) was used to perform single locus tests of association.

Single locus association analyses of UF type in RFTS and BioVU were further analyzed together with fixed-effects meta-analyses using PLINK, as well as METAL (Purcell et al. 2007; Willer et al. 2010). We only considered the fixed effects results among EAs from RFTS and BioVU. Thereby, we sought out only loci with consistent evidence between the two populations using this approach.

Results

RFTS

Fourteen percent of women from RFTS had UFs ($n = 89$). Age greater than or equal to 30 years was associated with increased risk for UFs (Table 1A). The majority of the participants with UFs had one and/or intramural UFs. None of the SNPs deviated from HWE. Single SNP associations with UF risk in RFTS and BioVU at these three SNPs have been previously reported (Edwards et al. 2013). Several associations were observed for *TNFRC6B* rs12484776 for UF type as outcome [any submucous UF odds ratio (OR) = 2.05, 95 % confidence interval (CI) 1.00–4.20, $p = 0.049$; any subserous UF OR = 1.95, 95 % CI 1.14–3.33, $p = 0.015$], UF number (one UF OR = 1.74, 95 % CI 1.14–2.64, $p = 0.010$) and increasing UF total volume (ordinal UF by quartiles, OR = 1.66, 95 % CI 1.13–2.44, $p = 0.009$; continuous UF volume, $\beta = 1.97$, 95 % CI 0.21–3.74, $p = 0.028$). Further evaluation of the association between this SNP and total UF volume by individual quartiles showed the strongest evidence for association for the largest volume (16.6–179.1 cc, OR = 2.19, 95 % CI 1.07–4.46, $p = 0.031$) with effect sizes across quartiles showing an increasing risk with increasing total UF volume. *TNFRC6B* rs12484776 SNP also associated with UF characteristics in unadjusted analyses (Supplemental Table 1A).

BioVU

BioVU participants were on average older than RFTS study participants (Table 1B). Similar to women from RFTS, older age was associated with increased risk for UFs (Table 1). Greater proportions of women from BioVU had

Table 1 Demographic characteristics and their associations with UFs in the *Right from the Start* (2001–2012) and BioVU (2007–present) cohorts

A. RFTS cohort						
Characteristics	*n*	No UFs ($n = 552$) %/mean (SD)	UFs ($n = 89$) %/mean (SD)	OR	95 % CI	
					Lower	Upper
Age mean (SD)	641	29.95 (4)	32.65 (5)	1.14	1.08	1.20
Age categories						
<25	58	10	2	1.00	Reference	
25–29	230	38	21	2.52	0.57	11.15
30–34	239	37	42	5.12	1.20	21.94
35–49	114	15	35	10.46	2.41	45.46
≥50	0	0	0	–	–	–
Body mass index mean (SD)	634	25.36 (6)	26.15 (6)	1.03	0.99	1.06
Body mass index categories						
Underweight (<20)	68	11	8	0.74	0.32	1.74
Normal weight (20–24.9)	307	48	46	1.00	Reference	
Overweight (25–29.9)	152	24	24	1.04	0.59	1.83
Obese (≥30)	114	17	22	1.38	0.77	2.48
Study site						
North Carolina	195	28	54	2.20	1.39	3.47
Tennessee	444	72	46	1.00	Reference	
Texas	2	<1	0	–	–	–
UF type[1]						
Any submucous	20	–	17	–	–	–
Any intramural	58	–	49	–	–	–
Any subserous	41	–	34	–	–	–

Table 1 continued

A. RFTS cohort						
Characteristics	*n*	No UFs ($n = 552$) %/mean (SD)	UFs ($n = 89$) %/mean (SD)	OR	95 % CI	
					Lower	Upper
UF number						
One	63	–	71	–	–	–
Two or more	26	–	29	–	–	–
Total UF volume						
Top quartile (0.003–0.7)	25	–	0.33 (0.23)	–	–	–
2nd quartile (0.7–3.6)	21	–	1.47 (0.71)	–	–	–
3rd quartile (3.6–13.3)	26	–	7.33 (2.66)	–	–	–
4th quartile (16.6–179.1)	17	–	65.38 (48.64)	–	–	–

B BioVU cohort						
Characteristics	*n*	No UFs ($n = 997$) %/mean (SD)	UFs ($n = 367$) %/mean (SD)	OR	95 % CI	
					Lower	Upper
Age mean (SD)	1,364	57.45 (18)	53.58 (11)	0.99	0.98	0.99
Age categories						
<25	15	1	<1	1.00	Reference	
25–29	54	5	<1	0.26	0.02	4.49
30–34	84	8	3	1.68	0.20	14.33
35–49	334	21	37	9.26	1.20	71.28
50–64	433	28	43	7.96	1.04	61.14
≥65	411	36	17	2.39	0.31	18.54
Body mass index mean (SD)	1,285	27.80 (8)	28.03 (7)	1.00	0.99	1.02
Body mass index categories						
Underweight (<20)	82	7	4	0.54	0.29	0.98
Normal weight (20–24.9)	374	29	31	1.00	Reference	
Overweight (25–29.9)	379	29	31	0.98	0.72	1.34
Obese (≥30)	450	35	34	0.90	0.67	1.22
UF type[1]						
Any submucous	150	–	31	–	–	–
Any intramural	212	–	44	–	–	–
Any subserous	115	–	24	–	–	–

n, Number; OR, odds ratio from logistic regression; CI, confidence interval; mean (SD), mean (standard deviation)

[1] Fibroid types do not sum to total number of fibroids because subjects may have had more than one fibroid type observed

higher BMIs or were older than women in RFTS; this reflects RFTS samples coming from a younger cohort while BioVU represents a clinical population. The majority of women with UFs had an intramural UF and had a subsequent reported hysterectomy in the EMR.

None of the SNPs examined significantly deviated from HWE. Among the three index SNPs examined for association with UF type, two showed strong evidence for association with intramural UF (*BET1L* rs2280543 OR = 0.50, 95 % CI 0.27–0.91, $p = 0.023$; *TNRC6B* rs12484776 OR = 1.44, 95 % CI 1.12–1.86, $p = 0.004$) (Table 2). The association of these SNPs with intramural UFs is stronger than the overall association with UF risk in our prior studies, and the effect sizes observed for intramural UFs are not within the CIs of the overall associations with UF risk. The unadjusted association analysis with UF type is shown in Supplemental Table 1B.

RFTS BioVU meta-analyses

Meta-analyses across RFTS and BioVU samples at these SNPs for UF type showed the strongest evidence of association with intramural UFs at *BET1L* rs2280543 (meta OR = 0.51, $SE = 0.14, Q = 0.593, I = 0, p = 2.48 \times 10^{-6}$), which was

Table 2 RFTS and BioVU associations of fibroid characteristics (outcome) with GWAS index SNPs among European Americans adjusted for age (continuous) and BMI (continuous)

A. RFTS Cohort

	rs7913069					rs2280543					rs12484776				
	(*T*) AF	OR	95 % CI		*p*	(*T*) AF	OR	95 % CI		*p*	(*G*) AF	OR	95 % CI		*p*
			Lower	Upper				Lower	Upper				Lower	Upper	
UF type															
No UFs	0.01					0.04					0.20				
Any submucous	0.00	ND	ND	ND	ND	0.00	ND	ND	ND	ND	0.32	2.05	1.00	4.20	0.049
Any intramural	0.01	0.69	0.09	5.54	0.730	0.03	0.62	0.19	2.04	0.429	0.23	1.30	0.81	2.09	0.277
Any subserous	0.00	ND	ND	ND	ND	0.04	0.95	0.28	3.17	0.931	0.29	1.95	1.14	3.33	0.015
UF number (ordinal)		0.43	0.05	3.44	0.428		0.54	0.19	1.54	0.252		1.54	1.06	2.24	0.025
UF number															
No UFs	0.01					0.04					0.20				
One	0.01	0.64	0.08	5.09	0.677	0.02	0.37	0.09	1.53	0.168	0.30	1.74	1.14	2.64	0.010
Two or more		ND	ND	ND	ND	0.07	1.83	0.41	8.23	0.431	0.21	1.28	0.50	3.27	0.609
Total UF volume (ordinal)		0.43	0.05	3.41	0.425		0.51	0.18	1.44	0.203		1.66	1.13	2.44	0.009
Total UF volume															
No UFs	0.01					0.04					0.20				
1st quartile (smallest, 0.003–0.7)	0.02	1.69	0.21	13.74	0.622	0.02	0.46	0.06	3.37	0.442	0.22	1.15	0.58	2.67	0.694
2nd quartile (0.7–3.6)		ND	ND	ND	ND	0.05	1.33	0.30	5.89	0.711	0.23	1.48	0.67	3.26	0.334
3rd quartile (3.6–13.3)		ND	ND	ND	ND		ND	ND	ND	ND	0.31	1.95	1.02	3.72	0.044
4th quartile (largest, 16.6–179.1)		ND	ND	ND	ND	0.03	0.71	0.10	5.23	0.735	0.35	2.19	1.07	4.46	0.031

		Beta	95 % CI		*p*		Beta	95 % CI		*p*		Beta	95 % CI		*p*
			Lower	Upper				Lower	Upper				Lower	Upper	
Total UF volume (continuous)	<0.01	−1.00	−2.89	0.89	0.296	0.04	−0.64	−1.59	0.32	0.188	0.21	0.40	0.05	0.75	0.024

B BioVU cohort

	rs7913069					rs2280543					rs12484776				
	(*T*) AF	OR	95 % CI		*p*	(*T*) AF	OR	95 % CI		*p*	(*G*) AF	OR	95 % CI		*p*
			Lower	Upper				Lower	Upper				Lower	Upper	
UF Type															
No UFs	0.01					0.05					0.20				
Any submucous	0.02	1.32	0.55	3.16	0.530	0.05	0.43	0.20	0.93	0.031	0.26	1.45	1.08	1.94	0.012
Any intramural	0.02	1.05	0.46	2.40	0.900	0.06	0.50	0.27	0.91	0.023	0.27	1.44	1.12	1.86	0.004
Any subserous	0.03	1.71	0.71	4.11	0.232	0.06	0.55	0.26	1.19	0.130	0.25	1.34	0.96	1.86	0.085

European Americans are all non-Hispanic

AF-allele frequency, in () is the minor allele/risk allele, OR, odds ratio; CI, confidence interval; ND, no or insufficient data

Table 3 GWAS index SNP and fibroid type associations from meta-analyses across *Right from the Start* and BioVU cohorts adjusted for age (continuous) and BMI (continuous)

Fibroid type	N_{case}	$N_{control}$	rs7913069						rs2280543						rs12484776					
			Meta OR	SE	Q	I	p		Meta OR	SE	Q	I	p		Meta OR	SE	Q	I	p	
Any submucous	150	1,549	ND	ND	ND	ND	ND		ND	ND	ND	ND	ND		1.49	0.21	0.657	0.00	0.055	++
Any intramural	270	1,549	0.94	0.38	0.626	0.00	0.878	+−	0.51	0.14	0.593	0.00	2.48×10^{-6}	−−	1.41	0.16	0.777	0.00	0.033	++
Any subserous	155	1,549	ND	ND	ND	ND	ND		0.59	0.20	0.386	0.00	0.009	−−	1.43	0.21	0.512	0.00	0.094	++

European Americans are all non-Hispanic

Meta OR, meta-analysis odds ratio; SE, standard error; Q, P value for the Cochrane's Q statistics; I, I^2 heterogeneity index (0–100); p, meta-analysis P; + and −, direction of the effect size in each cohort (+ increasing; − decreasing); ND, no or insufficient data

stronger than the overall RFTS-BioVU meta-analysis results at this SNP for association with UF risk (Table 3). A statistically significant association was also shown for intramural UF at *TNRC6B* rs12484776 (meta OR = 1.41, SE = 0.16, $Q = 0.777, I = 0, p = 0.033$) and for subserous UF at *BET1L* rs2280543 (meta OR = 0.59, SE = 0.20, $Q = 0.386$, $I = 0$, $p = 0.009$) (Table 3). We further evaluated RFTS participants with intramural for the association with rs2280543, to determine if the association was due to intramural fibroids being smaller in size. We did not observe evidence of association for rs2280543 and total fibroid volume when limiting to intramural fibroid cases ($\beta = 0.24$, 95 % CI −0.76–1.24, $p = 0.628$).

Discussion

This study is the first to evaluate UF characteristics for association with the previously observed GWAS index SNPs in EA US cohorts and is enhanced by pelvic imaging for cases and controls. We observed strong evidence of association of *BET1L* rs2280543 with intramural UFs in meta-analyses across RFTS and BioVU and increasing UF volume for *TNRC6B* rs12484776 in RFTS. We again did not observe associations with any UF characteristics at rs7913069 within RFTS or BioVU. This is due to the lack of power to detect an association at this SNP in EA populations, as this SNP had a MAF of 0.01 EAs while among the Japanese population the MAF was between 0.07 and 0.11 (Cha et al. 2011).

Although these GWAS index SNPs did not associate with UF risk in a cohort of AA women from the Black Women's Health Study (BWHS) and were not among the top associations reported in a recent association study using women of European ancestry (US European Americans and Australians), it may be that the inability to replicate is due to population or race specific effects, or these cohorts not coming from comparable clinical populations, as these cohorts relied on self-reported UF status (Eggert et al. 2012; Wise et al. 2012). Misclassification of controls is more common among self-reported UFs and as a result cohorts that use self-report may have reduced power to replicate the associations observed at these SNPs discovered in a clinical cohort. Since clinical populations are more likely to have subjects with symptomatic or severe UFs, we evaluated UF characteristics available from our cohorts that included a prospective cohort with standardized ultrasounds for all participants (RFTS) and another that is a clinical population (BioVU). The association we observed with increasing UF volume for *TNRC6B* rs12484776 in RFTS would suggest that the association at this SNP may be due to UF sub-phenotypes and subsequent detection rather than solely on overall UF risk, as the

analyses showed an increasing effect size with increasing UF volume. We did not have abstracted UF volume for BioVU participants to confirm the association.

Meta-analyses across RFTS and BioVU for UF type strongly supported an association with intramural UFs at *BET1L* rs2280543, with an effect size that was stronger than the association with UF risk alone. Intramural UFs are the most common UF with a prevalence ranging from 65–69 % (Eldar-Geva et al. 1998; Exacoustos and Rosati 1993; Ramzy et al. 1998; Rosati et al. 1989). Although the biological mechanisms underlying the associations between UF volume and *BET1L* and *TNRC6B* and intramural UFs are unclear, it may suggest women with particular anatomical UF characteristics and more severe UFs have distinct genetic risks for UFs. Prior studies have shown that quantitative trait loci within the region of *TNRC6B* associate with age-at-menarche and early age-at-menarche, which is an established risk factor for UF (Dragomir et al. 2010; Faerstein et al. 2001; Guo et al. 2006; Lumbiganon et al. 1996; Marshall et al. 1998; Samadi et al. 1996; Wise et al. 2004). It may be that the same biological mechanisms involved in the association between *TNRC6B* and early age-at-menarche lead to larger UF volume; however, further research is required to further understand this potential relationship.

One strength of our study is that all women were systematically screened for UFs using a standardized protocol and endovaginal ultrasounds for RFTS and various forms of pelvic imaging for BioVU. As a result, misclassification of UFs within our cohorts should be very low. Additionally, although BioVU participants had a higher mean age than RFTS participants who were primarily in their 20s, it may be that women with UFs in the RFTS cohort represent a group with an early onset of the condition because estimates of age-specific cumulative incidence suggest that many women develop UFs later in their reproductive years (Laughlin et al. 2010b). As a result of RFTS having a lower mean age, the overall population prevalence of UF was lower than would be expected for a cohort of older women that included women past reproductive age. Furthermore, because of the nested case–control design used by BioVU it is not possible to assess the overall prevalence of UF in this population.

Few studies have evaluated the associations between UF characteristics and genetic risk for UFs. We evaluated UF characteristics to better understand the role of UF sub-phenotypes in the associations previously observed in the GWAS by Cha and colleagues (Cha et al. 2011). Our findings support that there is interindividual variation in genetic risk for UFs and it is derived, in part, from differences in UF characteristics, including UF volume and type. Meta-analysis results for intramural UFs across cohorts have a level of statistical significance that exceeds the canonical genome-wide threshold for multiple testing, and our association results for total UF volume in RFTS for the *BET1L* show a clear pattern of increasing risk for UFs with increasing volume. Our findings support the associations previously observed at *BET1L* and *TNRC6B* in the prior Japanese GWAS and further suggest that the associations may be driven in part by UF sub-phenotypes; however, further research is necessary to assess the etiology of *BET1L* and *TNRC6B* UF risk.

Acknowledgments The field research for *Right from the Start* was supported by grants from the National Institute of Child and Human Development (R01HD043883 and R01HD049675) and the American Water Works Association Research Foundation (2579). Additional funds were provided by the Building Interdisciplinary Research Careers in Women's Health career development program (2K12 HD043483-11) to DRVE, the Vanderbilt Clinical and Translational Research Scholar Award 5KL2RR024977 to TLE from the National Center for Advancing Translational Sciences, the Vanderbilt CTSA award UL1TR000445 from the National Center for Advancing Translational Sciences, and the BioVU dataset used for the analyses described was obtained from Vanderbilt University Medical Center's BioVU which is supported by institutional funding and by the Vanderbilt CTSA Grant ULTR000445 from NCATS/NIH. The contents of this manuscript are solely the responsibility of the authors and do not necessarily represent official views of the National Center for Advancing Translational Sciences or the National Institutes of Health. No co-authors have any conflicts of interest to disclose and the material submitted for publication has not been previously reported and is not under consideration for publication elsewhere.

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