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Genome wide association study identifies two novel loci associated with female stress and urgency urinary incontinence

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Planned the study: Cartwright, Tikkinen, Khullar, Järvelin, Bennett, Walley
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Background: Genome-wide association studies (GWAS) have not identified replicable genetic risk loci for stress or urgency urinary incontinence

Methods: We carried out a discovery stage case control GWAS in three independent discovery cohorts of European women (n=8,979) for stress incontinence, urgency incontinence, and any incontinence phenotypes. We conducted replication in six additional studies of European ancestry (n=4,069). We collected bladder biopsies from women with incontinence to further investigate bladder expression of implicated genes and pathways (n=50) and used symptom questionnaires for phenotyping. We conducted meta-analyses using inverse variance fixed effects models in METAL, and whole transcriptome analyses using Affymetrix arrays, with replication with TaqMan PCR.

Results: In the discovery stage we identified 16 single nucleotide polymorphisms (SNPs) genotyped or imputed at five loci that reached genome-wide significance ($p<5x10^{-8}$). In replication, rs138724718 on chromosome 2, near the macrophage receptor with collagenous structure (*MARCO*) gene (replication p=0.003) associated with stress incontinence. In addition, rs34998271 on chromosome 6 near the Endothelin 1 (*EDN1*) gene (replication p=0.0008) associated with urgency incontinence. In combined meta-analyses of discovery and replication cohorts, associations with genome-wide significance for these two SNPs were confirmed. Transcriptomics analyses showed differential expression of 7 of 19 genes in the endothelin pathway between stress and urgency incontinence ($p<0.0001$).

Conclusion: We uncovered two new risk loci near the genes Endothelin 1 (*EDN1*), associated with urgency incontinence and Macrophage Receptor with Collagenous Structure (*MARCO*), associated with stress incontinence. These loci are biologically plausible given their roles in smooth muscle contraction and innate host defense respectively.

Background

Stress incontinence is loss of urine associated with physical exertion, sneezing or coughing, typically due to deficient support for the urethra [1]. Urgency incontinence is loss of urine associated with a sudden compelling need to void, with separate neurogenic, myogenic, and urotheliogenic hypotheses explaining the pathogenesis[2]. Current treatments ameliorate symptoms, but are not disease modifying, and no prognostic factors are available to help target interventions[3].

The heritability of incontinence was first recognised more than 150 years ago[4]. Genetic variation contributes around half of population phenotypic variability[5]. No clear mode of inheritance is apparent, consistent with our understanding of both subtypes of incontinence as complex polygenic conditions. Neither previous candidate gene association studies[6], nor the three previous genome-wide association studies (GWAS, n=4,894, 11,526 and 10,931)[7,8,9] have identified replicable genetic variants. Identification of susceptibility genes may help define new subtypes of incontinence, enabling better targeting or repurposing of existing treatments, and identification of new drug targets.

To understand the genetic architecture of stress incontinence and urgency incontinence, we first carried out a discovery stage case control GWAS with meta-analysis of >9 million genetic variants in three populations of women of European ancestry, followed by replication in six further studies. In addition, we conducted confirmatory studies to further investigate bladder expression of implicated genes and pathways, and urinary expression of the endothelin molecule.

Methods

The discovery phase of the GWAS was performed using Northern Finnish Birth Cohort 1966 (NFBC1966), UK Twin Cohort (TwinsUK), and the Avon Longitudinal Study of Parents and Children (ALSPAC). Phenotyping for the three discovery cohorts was conducted using symptom questionnaires (Table 1). Categorical responses were collapsed to dichotomous case definitions providing similar prevalences across cohorts. To maximise power both for variants that were unique to each phenotype, and for variants associated with both phenotypes, we compared stress incontinence with no stress incontinence, urgency incontinence with no urgency incontinence, and created a third merged phenotype, of “any incontinence” (meeting criteria for either stress or urgency incontinence) and compared this with no incontinence. Details for phenotype harmonisation are available in Supplementary Methods.

Based on the 1000 Genomes Phase 1 data, IMPUTE v2 and MiniMac, were used for imputation. Primary analyses were run using MACH2DAT or SNPTEST. Meta-analyses between the three discovery cohorts used dichotomous cases definitions in logistic regression models (adjusted for age, BMI and parity) with the allelic dosage model for

each variant (for up to 9.7 million genotyped or imputed SNPs) in METAL. Quantile-Quantile plots were created using GWAtoolbox to compare the distribution of observed p values in comparison to that predicted by chance, with calculation of the lambda statistic to test for genomic inflation. For the meta-analyses, Manhattan plots were generated using R. Finally, Regional Association Plots were generated using LocusZoom v1.1 to display both the chromosomal position and significance of associated SNPs.

We selected 12 SNPs for replication with p values for association less than 10^{-6}, and with the same direction of effect across all three cohorts. These variants were annotated to indicate their closest genes or genomic features, as well as their predicted consequences, using the POLYPHEN and SIFT algorithms.

For replication we collected DNA at three urogynaecology units (Imperial College London UK, University of Lublin, Poland and University of Radboud, Nijmegen, Netherlands), along with three large existing cohorts (Whitehall II Cohort, The MRC National Study of Health and Development, DCCT-EDIC Diabetes Control and Complication Trial - Epidemiology of Diabetes Intervention and Complications), and using additional DNA samples collected in NFBC1966.

Samples from Imperial, Radboud, Lublin, the NSHD and Whitehall II were genotyped at LGC Genomics with KBiosciences Competitive Allele Specific PCR (KASPar) for the 12 replication SNPs. Where validation assays failed, we picked a proxy known to be in high LD with the top SNP using either SNAP or the 1000 Genomes Browser (browser.1000genomes.org). Further details are in Supplementary Methods. Primary analyses were run separately for the cohorts, for each of the 12 replication SNPs, again adjusted for age, BMI and parity. Meta-analyses were conducted first within the replication cohorts, and subsequently with the discovery cohorts.

We collected bladder biopsies from the dome of the bladder with cold-cup biopsy forceps, including epithelium and smooth muscle, from women with either stress or urgency incontinence undergoing diagnostic cystoscopy, or tension free vaginal tape, or having a first intravesical botulinum toxin injection. The biopsies were handled with sterile forceps, and plunged into cold RNAlater. Homogenization was performed using a QIAGEN TissueLyser LT device, using two 3mm titanium beads in 1ml of TRIzol. An Invitrogen iPrep robot was used for RNA extraction, with quality control using a Nanodrop spectrophotometer, and an Agilent 2100 Bioanalyser. After preparation of cDNA, the samples were hybridized onto Affymetrix GeneChip® Human Genome U133 Plus 2.0 arrays. Probes were matched to GenBank IDs, and normalized mean absolute intensities were derived for each probe for stress incontinence and urgency incontinence. Custom 384 well microfluidic TaqMan PCR arrays (Life Technologies) were chosen for replication of the microarray data. From the microarray results, all protein coding genes (Ensembl release 76), differentially expressed at least 1.5 fold, and with $p<0.005$ were included. From regional association plots produced for each GWAS locus with a top SNP significant at $p<1x10^{-7}$, all genes within the locus were also included. Samples were run on an Applied Biosystems 7900HT Fast Real-Time System, with delta Ct values derived from a mean of three house-keeping genes (18s ribosomal RNA, actin beta, and glyceraldehyde-3-phosphate dehydrogenase). Detailed methods are available in Supplementary Methods.

We performed pathway analysis with iPathwayGuide (Advaita Corp., USA). An enrichment *p* value was calculated, representing the probability of seeing the observed overlap between the gene lists and the genes associated with the process by chance. To assess whether the casual variant at the discovered *EDN1* locus was operating through Endothelin 1 expression in the urinary epithelium (urothelium), we collected urinary supernatant, from a subset of cases and controls enrolling in the GWAS replication cohorts, and measured endothelin-1 protein levels using R&D Systems Endothelin-1 Quantikine ELISA Kits.

Results

In the three discovery cohorts 8,979 women provided genotypes and phenotypes for analysis (see Table 1 for demographics and case prevalences). In meta-analysis of the discovery stage cohorts we identified 16 SNPs genotyped or imputed at five loci that reached genome-wide significance ($p<5x10^{-8}$), and a further 361 SNPs at 88 loci, showing suggestive associations ($p<5x10^{-6}$) with one or more phenotypes (Supplementary Methods, Supplementary Figures s1, and s7a-c). Regional association plots of each locus from the discovery stage GWAS are given in Figures 1 and 2, and Supplementary Figures s2a to s2j. Twelve loci where the lead SNP reached a $p<5x10^{-7}$ (including the five that reached genome-wide significance), with consistent direction of effects in all three discovery cohorts were taken forward for replication (six for urgency incontinence, three for stress incontinence, and three for any incontinence).

Replication of the lead SNP at the twelve loci in six additional studies (n=4,069) (Table 1), showed an association of 2 SNPs after Bonferroni correction (p<0.004) (Table 2): rs138724718 on chromosome 2 (replication p=0.003) associated with stress incontinence and rs34998271 on chromosome 6 (replication p=0.0008) associated with urgency incontinence. In combined meta-analyses of discovery and replication cohorts, these two SNPs reached genome-wide significance ($p<5x10^{-8}$). There was no evidence of heterogeneity for associations of either SNP within or between the discovery and replication cohorts (I^2=0%) (Supplementary Figure s3).

The SNP rs34998271 in chromosome 6 (MAF=0.05 in Europeans) is located 240Kb upstream of the endothelin 1 (*EDN1*) and 190Kb downstream of the phosphatase and actin regulator 1 (*PHACTR1*) genes (Figure 1). The minor A allele of this SNP was associated with urgency incontinence (overall OR=1.63, $p=1.70x10^{-09}$). The SNP rs138724718 in chromosome 2 is a low frequency variant (MAF=0.02 among Europeans) located 10Kb from the engrailed homeobox 1 (*EN1*) gene and 110Kb upstream of the macrophage receptor with collagenous structure (*MARCO*) gene (Figure 2). The minor A allele of this SNP was strongly associated with stress incontinence (combined OR=1.82, $p=3.39x10^{-09}$) (Table 2).

We tested all previously reported genes associated with incontinence from candidate gene studies [6]. None of these variants were replicated (at p<0.05) in our meta-analyses. We also examined the most strongly associated variants from the two previous GWAS carried out in subsets of the Women's Health Initiative Study [7,8]. We found no evidence for replication at the *PRCP* gene [8], but identified a suggestive association close to *ADAMTS16* ($p=5x10^{-5}$) [7] (Supplementary Table s1 and Figure s4).

Demographics of the women undergoing bladder biopsy were similar to participants in our discovery GWAS (mean age 50.3; mean BMI 27.5; median parity 2). Differential expression between samples from participants with urgency incontinence (n=5) and stress incontinence (n=5) yielded 1,115 probes with a fold difference >1.3 (p<0.005) (Supplementary Figures s5 and s6). The top-ranked biological processes in the pathway analyses are presented in Table 3. The top-ranked process "muscle system" (GO:0003012), contained 135 of 334 differentially expressed genes ($p=7.5x10^{-10}$), including genes of interest such as the M3-muscarinic receptor (*CHRM3*, fold difference 4.23, p=0.0007), sulfatase 2 (*SULF2*, fold difference 1.52, p=0.005) involved in the formation of the urothelial glycosaminoglycan lining, and also implicated in our discovery stage GWAS ($p<5x10^{-8}$); and endothelin-1, (*EDN1*, fold difference -1.60, p=0.09). The second-ranked process, "intracellular signal transduction" (GO:0035556; 687 of 2263 differentially expressed genes; $p=2.7x10^{-9}$), also includes *EDN1*, as well as adrenoceptor beta 3 (*ADRB3*, fold difference =2.35, p=0.02). The probes with the strongest evidence of differential expression are shown in Supplementary Table s2. Results for genes within the top 12 GWAS loci are shown in Supplementary Table s3.

We evaluated bladder expression of genes in the endothelin-1 signalling pathway (Table 3). We found expression of endothelin isoforms 1 (*EDN1*) and 2 (*EDN2*), but not 3 (*EDN3*) (Table 3). Overall, there was differential expression, with overall pathway significance of p<0.0001 and significance for 7 of 19 genes within the pathway (p<0.05

for each of *PLCB2, NET1, GPR37, GNAO1, GNAL, GNA11, COX7A1*)(Table 4). TaqMan PCR replicated (biopsies n=40, mean age=27.8, median parity=2) 23 of the top 24 differentially expressed genes, providing good evidence for the validity of the microarray probes (Tables s4 and s5).

In available urine samples (n=263), urinary endothelin-1 was present at a mean of 1.13 pg/ml (range 0-12.4, s.d. 1.30). Urinary endothelin-1 concentrations were inversely associated with urinary urgency severity (five point scale, beta -0.189, p=0.005). At the rs138724718 locus in chromosome 2, expression of the *EN1* gene was not detectable in the bladder biopsies by either microarray or TaqMan assay, while *MARCO* was weakly expressed (Table s3 and s5).

Discussion

We identified and independently replicated two genetic variants robustly associated with incontinence in women at genome-wide levels of significance ($p<5x10^{-8}$) for the first time. The rs34998271 SNP associated with urgency incontinence lies close to the gene *EDN1*, and we demonstrated significant differential bladder expression of multiple genes in the endothelin pathway. In the analysis of urinary endothelin, we lacked power to detect an impact of the lead SNP on protein expression. We demonstrated that Endothelin-1 protein levels in urine differ in association with urgency, further supporting a role of *EDN1* in pathogenesis. Endothelin-1 is a potent vasoconstrictor, but also stimulates contraction of bladder smooth muscle cells[10]. Intravesical injection of endothelin-1 induces detrusor overactivity in rats[11], and both endothelin receptor antagonism[12] and endothelin converting enzyme inhibition[13] suppress rabbit detrusor contraction.

The rs138724718 SNP associated with stress incontinence is situated near the macrophage receptor gene *MARCO*, which we found was expressed in bladder. This SNP is a highly significant GTEX eQTL for *MARCO* in kidney cortex. The *MARCO* receptor has a role in non-specific host defense[14] and macrophages are widely distributed throughout the detrusor[15]. Recent evidence has strongly implicated persistent bacterial colonization in lower urinary tract symptoms[16],[17],[18], suggesting that *MARCO* may play a role in the bladder's response to bacteria. The same SNP lies close to *EN1*, and is known as an eQTL for *EN1* in adipose tissue and the tibial nerve. Although we did not find expression of *EN1* in the bladder, this does not preclude a role for *EN1* in stress incontinence through expression in the pelvic floor or another tissue. Transcriptomics analyses additionally provided support for three additional loci (tagged by SNPs rs139329202, rs1218596 and rs146757102), where each showed strong association in the GWAS discovery phase, but did not formally replicate, but where we found evidence of differential expression for genes within each locus. Our gene expression analyses were limited to the comparison between stress and urgency incontinence, rather than a comparison with normal bladder tissue, which precluded identifying shared differentially expressed genes between incontinence subtypes.

Three US groups have previously reported GWAS studies for incontinence using subsets of the Women's Health Initiative Study, and the Nurse's Health Study, none of which has identified replicated loci. One study used a sample of 8,088 African American and 3,438 Hispanic American women participating in the Women's Health Initiative

SHARe cohort [8] and found a single genome wide significant locus, with the common rs2086297 SNP (MAF=12.9%, intronic for the PRCP gene), being associated with stress incontinence with a modest effect size ($p=4.4x10^{-8}$, OR≈0.8). We found no evidence for any effect at that locus among European participants. Despite the larger sample for discovery, statistical power in this study [8] may have been limited by a decision to consider mixed incontinence as a separate entity, and the use of an ethnically diverse sample for discovery. Our study benefits from the availability of well-characterized, closely quality controlled samples, from women of European descent for the discovery process. A further GWAS used 3,017 women of European descent participating in the Women's Health Initiative GARNET substudy to look specifically for urgency incontinence risk variants [7], but found no genome-wide significant loci overall. Despite a similar overall design, the lack of positive findings in that study may relate to a relatively small sample size and an over inclusive case definition (prevalence 74.3%). A recent GWAS used Nurse's Health Study participants [9] and found 8 SNPs, at two loci, reaching genome wide significance for overall urinary incontinence in unadjusted analyses. However, no significant loci were seen after adjusting for known risks. Some of the difficulties in the previous studies may have been mitigated if they had used a pre-planned meta-analysis. Our study benefits from the availability of separate large cohorts for discovery, with highly replicable findings across cohorts increasing validity and reducing the risk of unmeasured or unaccounted source of bias. Furthermore, the availability of samples from women from distinct regions in Europe increases the generalizability of our findings. Although this is the largest GWAS conducted to date for urinary incontinence, the sample size still confers limited power for rarer variants (see Figure s8). Greater statistical power may have been obtained by additional adjustment for cryptic population stratification.

Conclusions

This work highlights novel molecular targets and signalling pathways with potential roles in incontinence. Further analyses will be necessary to understand their suitability as biomarkers and whether they are associated with treatment outcomes. These findings underscores the importance of both myogenic and urotheliogenic mechanisms in urgency incontinence, and suggests that drugs targeting the endothelin pathway may have promise.

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Legends

Table 1: Demographics of each contributing cohort, with case prevalences. Case definitions for each cohort were based on the following questionnaire items or clinical assessments. See "**Selection of phenotypes**" and "**Genome Wide Association Study of incontinence: replication phase"** in **Supplementary Methods** for further details of standardisation of phenotypes. **ALSPAC**: BFLUTS SUI item: Does urine leak when you are physically active, exert yourself, cough or sneeze?" BFLUTS UUI item: "Does urine leak before you can get to the toilet?", **NFBC**: Dan-PSS SUI item (administered in Finnish) "Do you experience leakage of urine when physically active (e.g. lifting, sneezing, coughing)?". DAN-PSS UUI item (administered in Finnish) "Is the urge to urinate so strong that urine starts to flow before you reach the toilet'?". **TwinsUK**: Stem - "Under what conditions are/were you likely to leak urine?". SUI item: "When coughing, sneezing or laughing ("stress incontinence")"; UUI item: "When rushing to the WC ("urge incontinence")". **Imperial**: ICIQ-FLUTS SUI item "Does urine leak when you are physically active, exert yourself, cough or sneeze?" ICIQ-FLUTS UUI item "Does urine leak before you can get to the toilet?". **Nijmegen**: UDI-6 SUI item (administered in Dutch) "Do you experience...urine leakage related to physical activity?", UDI-6 UUI item (administered in Dutch) "Do you experience...urine leakage related to urgency?. **Lublin**: SUI Urodynamics with positive cough stress test. **NHSD**: BFLUTS SUI item "Do you ever lose any urine when you cough, sneeze, laugh, run or exercise?", BFLUTS UUI item "Do you ever lose any urine before you reach the toilet?". **Whitehall II**: ICIQ-SF stem "When does urine leak?", ICIQ-SF SUI item "Leaks when you cough or sneeze", ICIQ-UUI item "Leaks before you can get to the toilet". **DCCT-EDIC**: SUI item "Have you had episodes of leakage with coughing sneezing?", UUI item "Have you had episodes of leakage associated with rushing to the toilet?".

Table 2: Details of the top SNPs from 12 loci taken forward for replication in GWAS discovery (studies n=3), replication (studies n=7), and combined (studies n=10) meta-analyses (UUI = Urgency Urinary Incontinence, SUI = Stress Urinary Incontinence, Any UI = "Any" Urinary Incontinence)

Table 3: Top ten enriched biological pathways for differentially expressed genes in stress incontinence vs. urgency incontinence
*number of differentially expressed genes / total genes in pathway

Table 4: Microarray bladder expression of genes in endothelin pathway comparing women with stress vs. urgency incontinence (n=10). The p-values in bold are significant at p<0.05

Figure 1 Regional association plot of rs138724718 locus of chromosome 2
This was the top SNP in this region of chromosome 2, with the minor allele strongly associated with increased risk of stress incontinence (p=2.88x10-7). The regional association plot demonstrates the gene macrophage receptor with collagenous structure (MARCO), and the top SNP lies in an ENCODE identified promoter flanking region for this gene, and the SNP is a GTEX eQTL for the MARCO gene expression in the kidney cortex (p=0.0005). The other protein coding gene within the locus is Homeobox protein engrailed-1 (EN1) with rs138724718 also being a known GTEX eQTL for EN1 expression in tibial nerve (p=0.02). The SNP had high imputation quality in each of the three discovery cohorts (>0.9).

Figure 2 Regional association plot of rs34998271 locus of chromosome 6
rs34998271 is an uncommon SNP (MAF=0.05) with no known SNPs in strong LD. The SNP had moderate imputation quality in each of the three discovery cohorts (each >0.6). It is the only strongly associated SNP within the locus, with $p=4.97x10^{-7}$ for association of the minor allele with increased risk of stress incontinence. The closest protein coding genes are endothelin 1 (*EDN1*) and phosphatase and actin regulator 1 (*PHACTR1*). A recombination spike in the middle of the plot separates the top SNP from *EDN1*. However, *EDN1* represents a highly plausible candidate gene for incontinence, as endothelin constricts smooth muscle, including the detrusor.

Cohort	Mean Age (range)	Median Parity (range)	Total Sample	UUI Cases	SUI Cases
ALSPAC	43.0 (29-58)	3 (1-10)	4,898	348 (7.1%)	710 (14.5%)
NFBC (discovery)	46.6 (46-47)	2 (0-17)	2,196	105 (4.8%)	692 (31.5%)
TwinsUK	55.6 (16-82)	2 (0-10)	1,903	417 (21.9%)	670 (35.2%)
NFBC (replication)	46.6 (46-47	2 (0-9)	339	17 (5.0%)	69 (20.4%)
Imperial	48.3 (18-89)	1 (0-9)	870	140 (16.1%)	170 (19.5%)
Nijmegen	50.4 (22-87)	2 (0-9)	407	182 (48.8%)	246 (60.4%)
Lublin	54.7 (25-78)	2 (0-5)	302	N/A	201 (66.5%)
NSHD	53 (53-53)	2 (0-7)	1,066	173 (16.2%)	256 (24.0%)
Whitehall	69.9 (59-84)	1 (0-6)	921	147 (16.0%)	215 (23.3%)
DCCT/EDIC	50.4 (34-67)	2 (0-6)	503	85 (16.9%)	164 (32.6%)

Table 1: Demographics of each contributing cohort, with case prevalences. Case definitions for each cohort were based on the following questionnaire items or clinical assessments. See "**Selection of phenotypes**" and "**Genome Wide Association Study of incontinence: replication phase"** in **Supplementary Methods** for further details of standardisation of phenotypes. **ALSPAC**: BFLUTS SUI item: Does urine leak when you are physically active, exert yourself, cough or sneeze?" BFLUTS UUI item: "Does urine leak before you can get to the toilet?", **NFBC**: Dan-PSS SUI item (administered in Finnish) "Do you experience leakage of urine when physically active (e.g. lifting, sneezing, coughing)?". DAN-PSS UUI item (administered in Finnish) "Is the urge to urinate so strong that urine starts to flow before you reach the toilet'?". **TwinsUK**: Stem - "Under what conditions are/were you likely to leak urine?". SUI item: "When coughing, sneezing or laughing ("stress incontinence")"; UUI item: "When rushing to the WC ("urge incontinence")". **Imperial**: ICIQ-FLUTS SUI item "Does urine leak when you are physically active, exert yourself, cough or sneeze?" ICIQ-FLUTS UUI item "Does urine leak before you can get to the toilet?". **Nijmegen**: UDI-6 SUI item (administered in Dutch) "Do you experience...urine leakage related to physical activity?", UDI-6 UUI item (administered in Dutch) "Do you experience...urine leakage related to urgency?. **Lublin**: SUI Urodynamics with positive cough stress test. **NHSD**: BFLUTS SUI item "Do you ever lose any urine when you cough, sneeze, laugh, run or exercise?", BFLUTS UUI item "Do you ever lose any urine before you reach the toilet?". **Whitehall II**: ICIQ-SF stem "When does urine leak?", ICIQ-SF SUI item "Leaks when you cough or sneeze", ICIQ-UUI item "Leaks before you can get to the toilet". **DCCT-EDIC**: SUI item "Have you had episodes of leakage with coughing sneezing?", UUI item "Have you had episodes of leakage associated with rushing to the toilet?".

SNP	Chr	Nearest Gene(s)	Effect Allele	Other Allele	MAF	Phenotype	Discovery Cohorts (n=8,997)			Replication Cohorts (n=4,069)			Overall (n=13,066)
							OR	95%CI	p	OR	95%CI	p	p
rs139329202	20	SULF2	c	g	0.01	UUI	8.50	4.12-17.55	$8.07x10^{-9}$	1.35	0.82-2.21	0.238	$2.38x10^{-05}$
rs146033157	6	FOXF2	a	t	0.03	UUI	0.33	0.23-0.48	$1.73x10^{-8}$	0.97	0.35-2.71	0.960	n/a**
rs146757102*	14	WDHD1/SOCS4	a	g	0.05	UUI	0.45	0.34-0.60	$1.95x10^{-8}$	1.13	0.87-1.45	0.360	$5.12x10^{-03}$
rs10768519*	11	LRRC4C	a	c	0.26	UUI	0.80	0.74-0.87	$2.26x10^{-7}$	1.00	0.85-1.17	0.954	$1.40x10^{-04}$
rs78878767	7	NSPR-AS1	a	c	0.01	UUI	4.26	2.56-7.10	$3.04x10^{-8}$	0.86	0.51-1.43	0.556	$2.11x10^{-07}$
rs34998271	6	EDN1	a	g	0.05	UUI	1.70	1.40-2.07	$4.97 x10^{-7}$	1.55	1.20-2.01	0.0008	$1.70x10^{-09}$
rs13059018	3	WNT5A	c	g	0.07	SUI	0.70	0.61-0.81	$1.01x10^{-7}$	1.14	1.00-1.29	0.054	$1.40x10^{-06}$
rs72738866	9	CLYC2	t	c	0.26	SUI	0.79	0.71-0.87	$2.55x10^{-7}$	1.01	0.89-1.14	0.895	$5.97x10^{-05}$
rs138724718	2	MARCO/EN1	a	g	0.02	SUI	1.85	1.47-2.35	$2.89x10^{-7}$	1.73	1.20-2.48	0.003	$3.39x10^{-09}$
rs78851245	7	AGK	t	c	0.02	Any UI	3.22	2.13-4.86	$2.92x10^{-8}$	1.46	1.00-21.3	0.051	$2.11x10^{-07}$
rs201363123*	12	TAS2R13	ag	a	0.06	Any UI	0.65	0.56-0.76	$1.03x10^{-7}$	1.14	1.00-1.29	0.053	$5.43x10^{-05}$
rs1218596	1	PMVK	t	c	0.06	Any UI	0.64	0.55-0.75	$1.04x10^{-7}$	0.95	0.75-1.20	0.681	$4.49x10^{-06}$

*proxy SNPs rs4281556 rs79077061 and rs10837192 used in replication cohorts
**monomorphic across 5 of 6 replication cohorts

Table 2: Details of the top SNPs from 12 loci taken forward for replication in GWAS discovery (studies n=3), replication (studies n=6), and combined (studies n=9) meta-analyses (UUI = Urgency Urinary Incontinence, SUI = Stress Urinary Incontinence, Any UI = “Any” Urinary Incontinence)

Biological Process	Differentially Expressed genes*	p
muscle system process	135 / 334	$7.5x10^{-10}$
intracellular signal transduction	687 / 2263	$2.7x10^{-09}$
muscle contraction	116 / 284	$5.7 x10^{-09}$
regulation of signal transduction	679 / 2259	$2.1 x10^{-08}$
cell development	538 / 1751	$3.7 x10^{-08}$
cellular component organization or biogenesis	1373 / 4892	$6.0 x10^{-08}$
regulation of heart contraction	67 / 147	$7.5 x10^{-08}$
positive regulation of protein kinase activity	161 / 443	$1.3 x10^{-07}$
regulation of protein kinase activity	226 / 664	$2.0 x10^{-07}$
positive regulation of kinase activity	167 / 466	$2.2 x10^{-07}$

Table 3: Top ten enriched biological pathways for differentially expressed genes in stress incontinence vs. urgency incontinence
*number of differentially expressed genes / total genes in pathway

Gene Symbol	Stress UI Intensity	Urgency UI Intensity	Fold Difference	p
SOS2	1.66	1.68	1.02	0.811
SOS1	5.80	5.57	-1.04	0.645
PTGIS	7.59	10.86	1.44	0.056
PLCB2	0.30	0.24	-1.27	**0.023**
NET1	5.47	4.27	-1.27	**0.010**
GPR37	0.03	0.09	3.07	**0.018**
GNAQ	3.73	4.22	1.14	0.051
GNAO1	0.41	1.13	2.81	**0.001**
GNAL	0.04	0.10	2.59	**0.002**
GNA11	0.17	0.25	1.48	**0.047**
EDNRB	3.09	2.92	-1.05	0.631
EDNRA	6.65	6.35	-1.04	0.581
EDN3	0.01	0.01	1.16	0.851
EDN2	0.10	0.06	-1.73	0.249
EDN1	0.22	0.14	-1.60	0.093
ECEL1	0.01	0.01	1.23	0.718
ECE2	0.15	0.12	-1.33	0.369
ECE1	0.45	0.56	1.25	0.050
COX7A1	1.83	2.74	1.50	**0.043**

Table 4: Microarray bladder expression of genes in endothelin pathway comparing women with stress vs. urgency incontinence (n=10). The p-values in bold are significant at $p<0.05$