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GENOME WIDE ASSOCIATION STUDY IN 8,997 WOMEN IDENTIFIES NOVEL GENETIC VARIANTS AT FIVE GENOMIC LOCI ASSOCIATED WITH STRESS AND URGENCY URINARY INCONTINENCE

Hypothesis / aims of study

Both twin and family studies have provided convincing evidence for genetic predisposition to stress and urgency incontinence in women, with genetic variation contributing up to half of population phenotypic variability [1]. Candidate gene association studies have not however, identified any genetic variants robustly associated with either condition [2]. Genome wide association studies have revolutionised the search for the genetic contributors to common multifactorial conditions, including diabetes, Alzheimer's, schizophrenia, obesity, and osteoporosis [3]. The insights provided have helped explain the pathogenesis of these complex diseases, offering new drug targets, biomarkers, and preventative strategies. We conducted the first genome wide association study of stress and urgency urinary incontinence in women, aiming to identify genetic variants associated with these conditions, and to provide new insights into the molecular mechanisms and biological pathways associated with them.

Study design, materials and methods

After local ethical approvals, unrelated women of European ancestry, participating in three population-representative cohorts in the UK and Finland, completed postal questionnaires reporting both stress and urgency incontinence, using items from the validated BFLUTS questionnaire, the validated Finnish translation of the DAN-PSS questionnaire, or two unvalidated dichotomous items with wording consistent with standardised definitions. We collapsed responses collected with ordinal scales to dichotomous case definitions, and also conducted analyses for different definitions to test consistency of findings. Participants were genotyped from whole blood for up to 1.2 million Single Nucleotide Polymorphisms (SNPs), using Illumina Human Genotyping arrays. Imputation of non-genotyped SNPs and InDels was conducted with the 1000 Genomes data as a reference panel, in order to improve power to detect low frequency variants, and to enable meta-analysis between cohorts. Variants imputed with a quality metric of <0.5 (RSQR or INFO) or a minor allele frequency of <1% were excluded from analyses, giving a final total of 9.4 million directly genotyped or imputed SNPs for analysis. Primary association analyses were run separately for stress, urgency and "any" incontinence in each cohort adjusted for age, BMI, and parity, and if necessary adjusted for principal components to eliminate residual population stratification, using SNPtest or MACH2DAT. Quality control before and after meta-analysis was performed using the GWAToolbox package in R. Meta-analyses of effect sizes from each cohort were conducted using the inverse-variance weighting method in METAL. Consequences of leading SNPs on genes and regulatory regions were tested using the Ensembl VEP tool. To further explore potential functional consequences of variants in or near genes, we measured expression of the associated gene in bladder biopsies from women with stress or urgency incontinence, and checked for differential expression between conditions, using Affymetrix HGU133+ 2.0 microarrays.

Results

8,997 women were available with both incontinence phenotypes and genotypes. The mean age of participants overall was 45 years, with median parity of 3. Sixteen SNPs at 5 independent loci exceeded the threshold for genome wide significance of $p < 5 \times 10^{-8}$ (Figure 1). Four of the loci showed association with urgency incontinence, while one was associated with both stress and urgency incontinence. Two of the five top variants occur at transcription factor binding sites, one is an upstream variant for a novel microRNA, while two are intronic variants for the *AGK* and *WDHD1* genes (Table). We confirmed expression of both these genes in human bladder (n=10), and identified significant differential expression in urgency incontinence for the *WDHD1* gene ($p=0.01$). We identified 361 SNPs at a further 88 loci, with highly suggestive associations ($p < 5 \times 10^{-6}$) with one or both phenotypes. These suggestive loci include 81 genes, which we will explore further in replication cohorts.

Interpretation of results

Our results indicate the presence both of distinct and shared genetic susceptibility for stress and urgency incontinence, suggesting common pathophysiological mechanisms that may explain their frequent co-occurrence. Genes, transcription factor binding sites, and other regulatory elements in or near the loci identified in this study may have important roles in the aetiology of stress and urgency incontinence in women. The interaction of these genotypes with environmental risk factors for incontinence should be explored. Importantly, results reported here require external replication for confirmation. We are undertaking this replication using samples collected in other large population-based cohorts. Although the loci identified here have large effect sizes, most genome wide significant variants were rare, and even in combination would explain a small proportion of the observed heritability of these conditions, suggesting that larger meta-analyses are required in future.

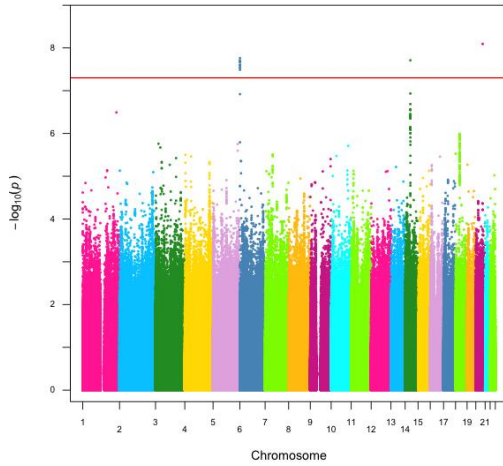


Figure 1: Manhattan plot for urgency incontinence showing genomic distribution of associated variants. Red line indicates threshold for genome wide significance.

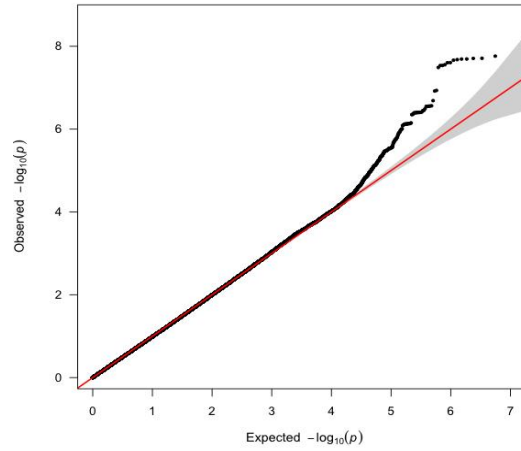


Figure 2: QQ plot for urgency incontinence demonstrating distribution of observed against expected p values. Red line and grey shaded area represent null result.

Locus	MAF	p	OR	95%CI	Phenotypes	Consequence
6p25.3	3.80%	1.73×10^{-8}	0.32	0.22-0.48	UII	Transcription Factor Binding Site
7p14.3	1.30%	3.04×10^{-8}	4.27	2.56-7.14	UII	Upstream Variant for a novel miRNA
7q34	1.07%	2.92×10^{-8}	3.21	2.13-4.86	UII and SUI*	Intron Variant for <i>AGK</i> gene
14q22.3	5.90%	1.95×10^{-8}	0.45	0.34-0.60	UII	Intron Variant for <i>WDHD1</i> gene
20q13.13	1.00%	8.07×10^{-9}	8.48	4.10-17.52	UII	Transcription Factor Binding Site

Table: Top SNPs in each of five genome-wide significant loci ($p < 5 \times 10^{-8}$) OR=odds ratio MAF=minor allele frequency UII=Urgency Urinary Incontinence SUI=Stress Urinary Incontinence *Any UI $p = 2.92 \times 10^{-8}$ UII $p = 1.50 \times 10^{-6}$ SUI $p = 2.88 \times 10^{-7}$

Concluding message

In the first genome wide association study of stress and urgency incontinence in women, we identified 5 novel genomic loci strongly associated with one or both conditions. These loci require external replication, but represent promising targets for future investigation, that may provide new insights into the complex causes of these conditions.

References

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Disclosures

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