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REPLICATION OF PRIOR CANDIDATE SUSCEPTIBILITY GENES FOR STRESS AND URGENCY INCONTINENCE IN A GENOME WIDE ASSOCIATION STUDY

Hypothesis / aims of study

Despite high formal heritability for stress and urgency incontinence, the molecular genetics of these common complex conditions has remained largely elusive. Several putative susceptibility genes have been tested in candidate gene association studies [1], and recent genome-wide association studies (GWAS) have also identified further possible susceptibility loci in women[2,3]. For most putative susceptibility genes, replication studies have not been attempted, and the few available replication studies are substantially underpowered, and typically at high risk of bias. In this study, using the largest currently available GWAS meta-analysis[3], we retested for signals of association with stress and urgency incontinence in and around all previous putative risk genes for incontinence.

Study design, materials and methods

Using data from a recent systematic review [1], we identified all candidate risk genes with at least one genetic variant with a statistically significant association with stress or urgency incontinence, reported in at least one previously published or presented study. We defined a region of 50k base pairs around each candidate gene as a region of interest. Women participating in three large population-representative cohorts in the UK and Finland were genotyped from whole blood using Illumina arrays for up to 1.2 million Single Nucleotide Polymorphisms (SNPs). Respondents completed postal questionnaires including validated items for stress and urgency incontinence. Imputation was conducted with the 1000 Genomes phase 1 release as a reference panel. Across each region of interest around each candidate gene, primary association analyses were run separately for stress, urgency and “any” incontinence in each cohort adjusted for age, BMI, and parity, using SNPtest or MACH2DAT. Meta-analyses of effect sizes from each cohort were conducted using the inverse-variance weighting method in METAL. We plotted each genomic region using LocusZoom, considering $p<1x10^{-4}$ as the threshold for significance.

Results

After quality control, 8,997 women were available for analysis with paired incontinence phenotypes and genotypes. As expected no SNP within the regions analysed here approached the usual threshold for genome-wide significance ($p<5x10^{-8}$). The minimum p values obtained, in the loci around each gene, are shown in the table below. The regional association plot (see figure) demonstrates the pattern of association across a gene (ADAMTS16) with a cluster of downstream variants exceeding our threshold for significance.

Interpretation of results

We found no evidence of associated variants in or around ADRB3, and COL1A1, the only two putative incontinence risk genes with prior evidence of replication from candidate gene association studies, and no evidence in support of all other genes tested in candidate gene studies. We did, however, find one gene (ADAMTS16), identified in a recent GWAS with a near genome-wide significant association, which had a significant cluster of highly associated downstream SNPs, and a further gene (ZNF521) from the same GWAS, with a top SNP at our threshold for significance. Future replication efforts should focus on retesting near-significant association signals from existing GWAS studies, rather than retesting putative variants from candidate gene studies.

Concluding message

In a large scale replication of all putative susceptibiity genes for stress and urgency incontinence we found substantial evidence in favour of ADAMTS16, as a risk gene for urgency incontinence, and suggestive evidence for ZNF521, but no evidence of association across any other gene identified from either previous candidate gene studies or GWAS for incontinence.

Gene	Original Reference	Associated Phenotype	Top SNP	p	Position
Beta-3 adrenoceptor	Honda et al, ICS Meeting 2006	Urgency UI	rs4994	$1x10^{-2}$	Missense
Serotonin Receptor 2a	Noronha et al, J Investig Med 2010	Any UI	rs2246127	$8x10^{-3}$	Intronic
Lysyl oxidase like-1	Ozbek et al, JOG Res 2013	Stress UI	rs12899085	$9x10^{-3}$	28kbp downstream
Collagen Type 1 alpha 1	Skorupski et al, AJOG 2006	Stress UI	rs2075558	$8x10^{-4}$	Intronic
Prolyl carboxypeptidase	Chen et al, ASHG Meeting 2013	Stress UI	rs190663726	$1x10^{-2}$	10kbp downstream
Citron rho-interacting kinase	Richter et al, J Urol 2015	Urgency UI	rs189787272	$5x10^{-4}$	Intronic
Zinc finger protein 521	Richter et al, J Urol 2015	Urgency UI	rs62085804	$1x10^{-4}$	37kbp downstream
ADAM metallopeptidase TS16	Richter et al, J Urol 2015	Urgency UI	rs6883058	$5x10^{-5}$	49kbp downstream
LINC RNA 1516	Richter et al, J Urol 2015	Urgency UI	rs185184464	$7x10^{-3}$	2kbp upstream

Table: Associations of top SNPs in or around each of nine putative susceptibility genes identified in prior candidate gene studies, or GWAS.

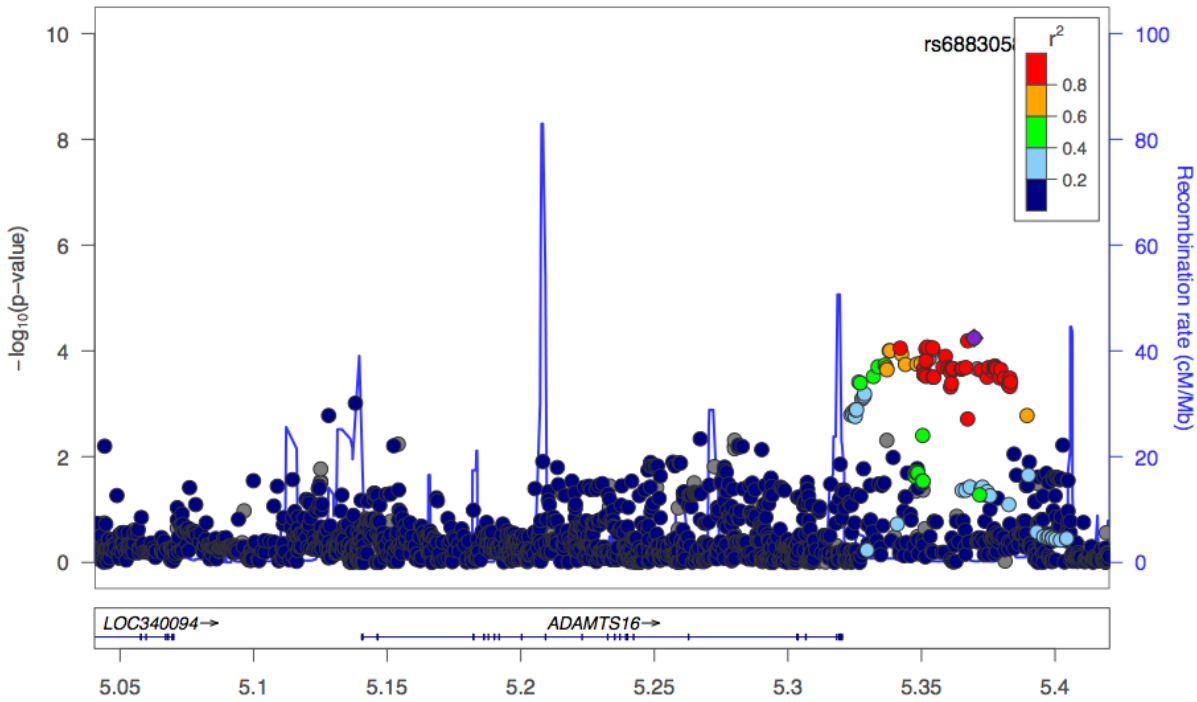

Figure: Regional association plot of SNPs in or near the ADAMTS16 gene on chromosome 5, in association with urgency incontinence. Height of SNP on y axis represents p value, while colour represents linkage disequilibrium with top SNP

References
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Disclosures
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