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Lack of replication of association of *THSD7A* with obesity

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We read with interest the recently published article by Nizamuddin *et al* that reported a novel locus *THSD7A* to be associated with body mass index (BMI) in the Indian population (1). The discovery is important for two reasons; one because obesity as an intermediate trait is a shared risk factor for many disease conditions including type 2 diabetes and cardiovascular disorders and second, *THSD7A* is proposed to be a population-specific and male-specific locus, although the latter is not highlighted in the observations. Several obesity-associated loci such as *FTO, MC4R, TMEM18,* etc. have been identified in European populations and we and others have reported similar association for them in the Indian population (2-4). However, Nizamuddin *et al* failed to replicate them in their study samples and hence, the results need to interpreted with caution (1).

We performed replication analysis using normal adults from two homogeneous populations. Parents of children in the Pune Maternal Nutrition Study (5) [PMNS; Indo-Europeans from Western India; n=1761(829 males/932 females)] and the Parthenon Study (6) [(PS; Dravidians from South India; n=830 (400males/430 females)] were genotyped for rs1526538 in *THSD7A* by Sanger-sequencing using primers described earlier (1) (**Supplementary table 1**). Frequency of the risk allele "T" at rs1526538 was 0.49 in both the cohorts and similar to the reported study (1) and in South Asian Ancestry populations in 1000G data (7). Association analysis using linear regression additive model, adjusted for age and gender failed to detect significant or suggestive association of rs1526538 with BMI in PMNS (β=-0.0007, p=0.996) or PS (β=-0.300, p=0.133) or on meta-analysis (β=-0.091; p=0.407) or any other anthropometric parameters (**Table 1**). Further analysis comparing underweight, normal, overweight and obese groups using standard WHO criteria did not replicate the association of *THSD7A* with obesity(β=-0.042, p=0.136) (8). Gender-specific analysis also showed similar results.

It is intriguing that we could not be replicate *THSD7A* as an Indian population-specific obesity locus, despite having a sample size that was four times larger and >99% powered to detect the association with BMI at same effect size as the original study (beta=-1.0104 for $p=10^{-8}$) (1). We can only speculate the possible reasons which may be related to choice of samples and population stratification leading to probable spurious association. The premise of the reported study was to identify novel BMI-associated loci in South Indians since they are not related to any group outside Indian-subcontinent (1). However, the discovery set comprised of small number of individuals from different geographical regions, which surprisingly did not show population stratification on principal component analysis (PCA), although the original

study demonstrated several clusters (9). This could be attributed to failure to include the known and established samples identified in their earlier study as positive control for PCA (9). The same could be the reason for their failure to replicate association of two strongest obesity-associated loci, *FTO* and *MC4R*.

In conclusion, our findings underline the importance of choosing appropriate samples for association analysis. They also imply that the status of *THSD7A*needs to be validated in another population avoiding any population stratification in a country with huge genetic diversity (10).

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Table 1: Association analysis of *THSD7A* with obesity in Indian population

	N	Male/Female	Beta	SE	L95	U95	p value
PMNS	1761	829/932	-0.001	0.131	-0.257	0.256	0.996
PS	830	400/430	-0.3	0.2	-0.692	0.092	0.133
Meta-analysis*	2591	1229/1362	-0.091	0.11	-0.305	0.124	0.407 (I^2=36.6%)
Meta-analysis**	2591	1229/1362	-0.042	0.03	-0.096	0.013	0.136 (I^2=59.9%)
Meta-analysis* (only males)	1299	1299	-0.006	0.142	-0.285	0.272	0.964 (I^2=28.1%)

PMNS, Pune Maternal Nutrition Study; PS, Parthenon Study; I_2, heterogeneity.

Association analysis by linear regression using additive model of T-allele adjusted for age and sex

Meta-analysis was performed by fixed effect model for the two cohorts.

*indicates meta-analysis for linear regression for BMI.

** indicates meta-analysis for linear regression for four categories of obesity (underweight, normal, overweight and obese)