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Low copy number of the salivary amylase gene predisposes to obesity

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Common multi-allelic copy-number variants (CNVs) appear enriched for phenotypic associations compared to their di-allelic counterparts¹⁻⁵. Here we investigated the influence of gene-dosage effects on adiposity through a CNV association study of gene expression levels in adipose tissue. We identified significant association of a multi-allelic CNV encompassing the salivary amylase gene (*AMY1*) with body mass index and obesity, and replicated this finding in 6,200 subjects. Increased *AMY1* copy-number was positively associated with both amylase gene expression ($P=2.31\times 10^{-14}$) and serum enzyme levels ($P<2.20\times 10^{-16}$), while reduced *AMY1* copy-number was associated with increased BMI (per-estimated-copy: $\beta=-0.15[0.02]$ kg/m²; $P=6.93\times 10^{-10}$) and obesity risk (per-estimated-copy:OR=1.19[1.13-1.26]_{95%CI}; $P=1.46\times 10^{-10}$). The OR of 1.19 per-copy of *AMY1* translates to about an eight-fold difference in risk of obesity between subjects in the top (CN>9) and bottom (CN<4) 10% of the copy-number distribution. Our study provides a first genetic link between carbohydrate metabolism and BMI and demonstrates the power of integrated genomic approaches beyond genome-wide association studies.

We designed a gene-centric association study (GCAS) to identify common CNVs overlapping genes and inducing a dosage effect on gene expression, hypothesising that these might be enriched for physiologically-relevant CNVs. To achieve this, we conducted a family-based association analysis of signal intensity data from DNA arrays (log R ratio and B-allele frequency) using transcriptomic data from adipose tissue using famCNV⁶ in 149 Swedish families ascertained through siblings discordant for obesity (**Table 1;Figure 1;Supplementary Figure 1**). A total of 76 probes located within putative CNVs showed a dosage effect on gene expression at 1% FDR (**Supplementary Table 1**). Of these probes, only cnvi0020639, located within a CNV overlapping the amylase gene cluster (including the *AMY1* salivary and the *AMY2* pancreatic genes expression probeset 208498_s_at; FDR=6.88×10⁻³), was also associated with adiposity [both BMI ($P=3.86\times 10^{-4}$) and fat mass ($P=3.11\times 10^{-4}$)] (**Supplementary Figures 2-4**). Reduced signal intensity at this probe was associated with increased adiposity levels (**Table 2;Figure 2;Supplementary Figures 2-4**).

This inverse association between copy-number in the amylase region and BMI was first replicated using signal intensity data from DNA arrays in 972 subjects from TwinsUK (**Table 1**)⁷. The strongest association was observed at cnvi0022844 ($P=1.13\times 10^{-3}$;**Table 2**), which showed significant association with BMI after Bonferroni correction. When multiple probes were considered through principal component analysis, the BMI association signal actually extended over a region between cnvi0022844 and cnvi0016754 ($P=1.32\times 10^{-3}$), which overlapped the cnvi0020639 probe associated with adiposity in the Swedish discovery families. These results, although supportive of the association in the amylase region, did not permit us to distinguish which of the salivary or pancreatic amylases was driving the

association with adiposity, necessitating use of a non-array-based method of copy-number measurement.

Consequently, we estimated copy-number at *AMY1* and *AMY2* in 481 subjects from the Swedish families (**Table 1**) using quantitative real-time PCR (qPCR). This approach generates a continuous intensity distribution from which integer copy-numbers can be inferred by comparison to a reference sample of known copy-number (**Supplementary Information**). Given the many technical challenges inherent in copy-number measurement at multi-allelic loci^{2,5,8-10}, we treated these discretised measurements as relative estimates or surrogates correlated with the true underlying copy-number state, as opposed to absolute copy-number genotypes.

Only three estimated copy-number states (one to three) were detected for the pancreatic amylase (*AMY2*) gene, and these were not associated with either BMI or fat mass (**Supplementary Table 2**). In contrast, copy-number estimates at *AMY1* ranged from two to fourteen, and showed association with both BMI ($P=8.08\times 10^{-3}$) and fat mass ($P=8.53\times 10^{-3}$) confirming our previous DNA-array based analysis (**Supplementary Table 2**). We found greater correlation between signal intensity at *cnvi0020639* and *AMY1* ($r=0.73$; $P<2.20\times 10^{-16}$) than *AMY2* copy-number ($r=0.35$; $P=1.25\times 10^{-8}$), suggesting that the GCAS association was mainly capturing copy-number variation at *AMY1* as opposed to *AMY2*, justifying follow-up of the former. Furthermore, we validated accuracy of the *AMY1* qPCR assay by using whole-genome shotgun-sequencing data from the 1000 Genomes Project, and observed a correlation of 0.94 ($P<2.20\times 10^{-16}$) between *AMY1* copy-number estimates derived by qPCR and sequencing (**Supplementary Figures 5-8; Supplementary Table 3**). To

further validate the *AMY1* qPCR assay, we also compared the copy-number measured by qPCR in 96 samples from the DESIR cohort with *AMY1* copy-number measured by digital PCR in the same samples, obtaining high correlation between the two methods ($r=0.95$; $P<2.20\times 10^{-16}$; **Supplementary Figure 9**). Analogously, high correlation ($r=0.98\%$; $P<2.20\times 10^{-16}$; **Supplementary Figure 9**) was also observed between copy-numbers measured using our qPCR assay and those obtained using a different qPCR assay on the same 96 DESIR samples.

To replicate the observed association in a larger sample, we next estimated *AMY1* copy-number by qPCR in an additional sample of 1,479 female subjects from TwinsUK and 2,137 male and female subjects from DESIR (**Table 1**). The two population samples showed a similar copy-number distribution (Wilcoxon test $P>0.05$) with estimated median copy-number of six, ranging from one to eighteen (**Supplementary Figure 10; Supplementary Tables 4-5**). Meta-analysis of *AMY1* effects in TwinsUK and DESIR (total $n=3,616$) showed significant association between *AMY1* and BMI (per copy-number $\beta=-0.15[0.02]\text{kg/m}^2$; $P=6.93\times 10^{-10}$; **Table 2; Figure 3; Supplementary Tables 6-9**). Results of associations assessed using both the qPCR intensity signal as a continuous measure, as well as discretised using an unsupervised clustering approach (k-means), were concordant with those generated using integer copy-numbers (**Supplementary Information**).

We then assessed the effect of *AMY1* copy-number on obesity susceptibility by selecting obese cases ($\text{BMI}\geq 30\text{kg/m}^2$) and normal-weight controls ($\text{BMI}<25\text{kg/m}^2$) from TwinsUK and DESIR and by measuring *AMY1* copy-number by qPCR in an additional 205 severely obese cases and 358 age-matched controls from the AOB¹¹

study (**Table 1;Supplementary Information**). In these European samples, subjects with lower estimated *AMY1* copy-number showed significantly increased risk of obesity in each of the three samples (per-estimated *AMY1* copy-number meta-analysis: OR=1.19[1.13-1.26]_{95%CI}; $P=1.46\times 10^{-10}$; **Table 2;Figure 3**). The *AMY1* copy number distribution in our sample ranged from one to eighteen copies, with approximately 10% of subjects carrying fewer than four copies of *AMY1*, and 10% of subjects with an *AMY1* copy number greater than nine (**Table 2**). Given the multi-allelic nature of the *AMY1* CNV, this OR of 1.19 per copy of *AMY1* translates to about an eight-fold difference in risk of obesity between subjects in the top (CN>9) and bottom (CN<4) decile of the estimated *AMY1* copy-number distribution (OR=7.67[3.92-14.99]_{95%CI}; $P=2.52\times 10^{-9}$; **Supplementary Table 10**). Using a multifactorial liability threshold model¹², we estimated the proportion of total variance of obesity explained by estimated *AMY1* copy-number to lie between 1.73-7.94%[95%CI] (**Supplementary Table 11**). Therefore, based on an estimated heritability of 40-70%^{13,14}, copy-number variation at *AMY1* may account for 2.47-19.86% of the total genetic variation of obesity. Analogously, we estimated that between 0.66% and 4.40% of the proportion of genetic variance of BMI could be explained by inferred *AMY1* copy-number in these European samples.

As all the samples included in our analyses were of European origin, we reasoned that replication in a sample of different ethnicity and under differing environmental influences on obesity would provide greater support for its physiological role. We therefore selected a Singaporean Chinese case-control sample from SP2¹⁵. A total of 136 obese and 197 overweight subjects were identified among the 2,431 Chinese subjects included in the SP2 cohort, with 325 matched lean Chinese SP2 normal-

weight controls. *AMY1* copy-number was measured by qPCR in all 658 subjects. Median copy-number in SP2 normal-weight subjects was 6 (ranging from 2 to 16), similar to our French DESIR and UK populations, and in line with previous observations by Perry *et al*¹⁶. Case-control association analysis in the Chinese sample showed reduced *AMY1* estimated copy number to be associated with increased risk of obesity (per copy-number OR=1.17[1.05-1.29]_{95%CI}; $P=3.73\times 10^{-3}$). Extending the case sample to include milder overweight subjects further confirmed the results (per copy-number OR=1.13[1.06-1.21]_{95%CI}; $P=3.52\times 10^{-4}$).

To validate our *AMY1* genomic copy-number data at the protein level, we investigated the effect of copy-number variation at *AMY1* and *AMY2* on serum amylase enzyme levels, and their relationship with BMI using 468 French morbidly obese subjects from the ABOS study (**Table 1; Supplementary Table 12**). On average, salivary and pancreatic amylase proportions were approximately equal in serum (52% and 48%, respectively) and their levels showed close positive association with copy-number variation at their respective genes ($P<2.20\times 10^{-16}$ and $P=1.04\times 10^{-11}$, respectively; **Supplementary Figure 11**). BMI was inversely associated with salivary amylase ($\beta=-0.23[0.04]\text{kg/m}^2$; $P=2.26\times 10^{-7}$; **Supplementary Figure 12**) and to a lesser extent pancreatic amylase ($\beta=-0.23[0.06]\text{kg/m}^2$; $P=2.29\times 10^{-4}$; **Supplementary Figure 12**), likely reflecting the physiological correlation between the levels of the two enzymes ($r=0.21$; $P=4.29\times 10^{-6}$).

Salivary amylase catalyses hydrolysis of the α -1,4-glycosidic bonds of starch, initiating carbohydrate digestion in the oral cavity. While individual salivary amylase levels vary in response to environmental factors including psychological stress¹⁷, they

are genetically influenced by and directly correlated with the highly variable copy-number at *AMY1*^{16,18}. Increased gene copy-numbers at this locus are believed to have evolved in the human lineage as a consequence of a shift to a starch-rich diet¹⁹. Human populations traditionally consuming a high proportion of carbohydrates in their diet show higher copy-numbers and salivary amylase activity than those consuming a low-starch diet^{16,20}. Both the salivary glands and pancreas contribute similarly to determine overall levels of serum amylase²¹, although enzyme activity is also detectable in other organs, including adipose tissue^{22,23}. Indeed amylase was among the 30% most-highly expressed genes in adipose tissue in both our discovery sample and publicly-available data from the general population, thus suggesting that this gene is actively expressed in adipose tissue (**Supplementary Information**). Whether adipose tissue is functionally involved in the link between *AMY1* copy-number and obesity, or whether this link implicates a different tissue in which *AMY1* is also actively transcribed warrants further investigation.

Decreased blood amylase levels have been observed in both obese humans²⁴ and rats²⁵, and have recently been associated with increased risk of metabolic abnormalities^{26,27} and reduced pre-absorptive insulin release²⁸. Furthermore, a recent study in mice fed a high fat/high sugar diet suggested association between the amylase locus and weight gain²⁹. In these mice, this locus was also shown to be associated with the proportion of *Enterobacteriaceae* in murine gut microbiota²⁹, previously correlated with obesity in humans³⁰.

Rare copy-number variants have recently been implicated in highly-penetrant forms of obesity^{31,32} and severe thinness³³, through a gene dosage effect. Common biallelic CNVs have also been associated with BMI³⁴⁻³⁷, however, since most of these are reliably tagged by surrounding SNPs³⁸, they share the same properties of small effect sizes and limited predictive value for obesity risk. In contrast, complex multi-allelic CNVs show decreased linkage disequilibrium with surrounding SNPs (**Supplementary Table 13**) and are consequently less detectable by SNP-based GWAS³⁹. Surprisingly, *FTO* is the most-replicated obesity susceptibility gene identified through GWAS³⁷, yet in our analyses the estimated *AMY1* copy-number appeared to show stronger association with BMI than *FTO* SNPs (**Supplementary Tables 14-15**). It is conceivable that high structural variability in the amylase region and subsequent low SNP coverage (**Supplementary Figures 13-14**) may have hampered previous SNP-based GWAS attempts to detect association between the amylase cluster and adiposity. Indeed, examination of data from the most recent BMI meta-analysis conducted by the GIANT consortium³⁷ revealed a large gap in SNP coverage across the locus encompassing the salivary amylase genes (**Supplementary Figure 14**).

Present DNA high-throughput methods for CNV assessment, including array-, PCR- and sequencing-based approaches, are all affected by a wide number of variables including DNA source, extraction methods, quality and concentration, as well as experimental factors inducing batch effects^{9,10,40}. These factors complicate copy-number measurement at multi-allelic CNVs and hinder pooling of data from multiple centres.

The observed association of *AMY1* with obesity may rekindle interest in the role of multi-allelic CNVs in common disease, driving development of novel technological approaches for accurate and high-throughput measurement of absolute copy-number at such loci. These technological improvements will enable high-quality association analyses at such loci in larger sample sizes similar to those included in SNP association studies, and are mandatory for disease risk-assessment at the individual level, paving the way towards personalized medicine.

Our study provides a first genetic link between carbohydrate metabolism and obesity, with low copy-number at *AMY1* resulting in decreased salivary amylase levels and a higher risk of obesity. This finding provides intriguing insight into some of the biological mechanisms underlying obesity, as well as a novel rationale for the investigation of innovative obesity treatments based on manipulation of digestive enzyme levels.

ONLINE METHODS

Further detailed methods are provided in the **Supplementary Information**.

Associations were assessed using linear mixed effects models, including plate as a random effect and family structure as an additional random effect where appropriate. Age and sex were included as covariates.

Discovery. The discovery sample included 149 Swedish families (342 subjects) ascertained through an obesity-discordant sib-pair (BMI difference $>10\text{kg/m}^2$)⁴¹. Gene expression for 29,546 transcripts (16,563 ENSEMBL genes) was measured in subcutaneous adipose tissue using the Affymetrix Human Genome U133 Plus 2.0 microarray. GWAS signal intensity data from Illumina 610K-Quad arrays were available for 348,150 probes lying within each transcript plus 30kb upstream and downstream to encompass the coding regions and their internal and nearby regulatory regions.

Quantitative real-time PCR (qPCR) was carried out to infer relative copy-number measurements reflecting the underlying copy-number distribution at *AMY1* and *AMY2*, respectively, using the TaqMan assays Hs07226362_cn and Hs04204136_cn on an Applied Biosystems 7900HT Real-Time PCR System. Association analyses were carried out for 481 subjects with complete data on BMI and dual-energy X-ray absorptiometry (DEXA)-derived fat mass.

Replication. *In-silico* replication of the BMI association was conducted using 972 female subjects from the UK adult twin registry (TwinsUK) cohort⁴² using intensity signals from Illumina 610K-Quad arrays⁷. Association with BMI and obesity was analysed in two population samples using qPCR estimates of *AMY1* copy-number for 1,479 female twins from TwinsUK and 2,137 subjects from the French Data from the Epidemiological Study on the Insulin Resistance syndrome (DESIR)⁴³ cohort. Obesity

association with qPCR data was also assessed in an additional case-control sample of 205 obese cases and 358 age-matched controls from the French Adult Obesity study (AOB)¹¹. An additional case-control sample was extracted from the Singapore Prospective Study Program (SP2) cohort, a population-based study including 2,431 adult Chinese Singaporean subjects¹⁵. Cut-offs were defined by the Working Group on Obesity in China⁴⁴ and the WHO expert consultation for Asia⁴⁵, which define obesity in the Chinese population as $BMI \geq 28 \text{ kg/m}^2$ and normal-weight as $BMI < 23 \text{ kg/m}^2$. Obesity in the Chinese population was thus defined as $BMI \geq 28 \text{ kg/m}^2$ and normal-weight as $BMI < 23 \text{ kg/m}^2$. Accordingly, a total of 136 obese and 197 overweight subjects were identified among the 2,431 Chinese subjects of the SP2 cohort, with 325 matched lean SP2 subjects selected as normal-weight controls.

In order to avoid any potential population stratification impacting on our association analyses resulting from the known differences in *AMY1* copy number distribution between populations traditionally consuming high *versus* low starch diets¹⁶, we carried out genotype principal component analysis using genome-wide SNP array data to ensure that samples included in each analysis were of the same ethnicity and genetic background. Furthermore, *AMY1* association analyses were conducted separately in each of the study populations and then combined by meta-analysis using METAL⁴⁶ rather than pooling.

Protein levels. ‘*Atlas Biologique de l’Obésité Sévère*’ (ABOS) is a French cohort comprised of candidates for bariatric surgery. Serum pancreatic and salivary amylase levels for 468 patients were measured by an enzymatic colorimetric assay with an autoanalyzer (CoBAS Icobas® 8000 modular analyser series; kits AMYL2-03183742122 and AMY-P-20766623322, Hoffman-La Roche Ltd).

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Author Contributions

MF conceived the study. MF, PF and TDS directed the project. MF, JSEM and PF wrote the paper. AB, TDS, RS, FPa, HS, PHS, LB, FPe, PT, RDo, PCS and EEE edited the paper. JSEM, PT, FPe, JCA, RDo, MNA, EO, AB, AD and MH performed the laboratory experiments. MF, JSEM, JCA, LB, PHS, EEE, PCS and HS performed the statistical analyses. RWD, AP, RDe, MaM, PGH, JS, MP, RC, VR, EV, SF, BB, MiM, SVS, JW, OP, PJ, LS, CJH, PD, RM, JL, EST, LMSC, AW, FPa, TDS and PF provided samples, data and/or reagents. MF and JSEM are joint-first authors of this study. PT, FPe, AB and JCA are joint-second authors. TDS and PF are joint-last authors. All authors commented on and approved the manuscript.

Figure Legends

Figure 1: Manhattan plot of gene-centric CNV association study (GCAS) association results with gene expression levels in subcutaneous adipose tissue from the Swedish sib-pair dataset. Chromosomal location for each probe is given on the horizontal axis for each of the 22 autosomes, while minus $\log_{10}(P)$ of the association between probe signal intensity and gene expression levels is shown on the vertical axis. The probes tested against the amylase genes transcriptional levels are shown in green.

Figure 2: The amylase region in detail. Top to bottom: famCNV association results between probe signal intensity at probes within 30kb of the amylase cluster and amylase expression levels (probeset 208498_s_at) in adipose tissue (black dots) in the Swedish family discovery study, with chromosomal coordinates given on the horizontal axis and minus $\log_{10}(P)$ on the vertical axis; locations of associated probes: cnvi0020639 (blue; Swedish family discovery study), cnvi0022844 (red; TwinsUK); gene content in the amylase region based on the human reference sequence (hg19; RefSeq), depicting *AMY2B*, *AMY2A* and the *AMY1A/B/C* genes, as well as two high sequence similarity segmental duplications in the region; LD between HapMap markers (release 23) calculated with HaploView⁴⁶ (darker shading corresponds to higher r^2 value). Because of the repetitive nature of this region, which contains six paralogs (including one pseudogenized copy) in the reference genome (**Supplementary Figure 13**), the cnvi0020639 and the cnvi0022844 probes were found to map to two locations within the amylase gene cluster.

Figure 3. Effect of estimated *AMY1* copy-number on obesity and BMI. A: Scatter plots of raw qPCR signal intensity ($\Delta\Delta Ct$) plotted against BMI for the TwinsUK and DESIR samples. B: Boxplots of $\Delta\Delta Ct$ in normal weight ($BMI < 25 \text{ kg/m}^2$) and obese ($BMI \geq 30 \text{ kg/m}^2$) subjects in the TwinsUK, DESIR and AOB samples. For plots A and B, low $\Delta\Delta Ct$ values correspond to high *AMY1* copy-numbers. C: relative copy-number distribution in obese cases ($BMI \geq 30 \text{ kg/m}^2$; black bars) versus normal weight controls ($BMI < 25 \text{ kg/m}^2$; grey bars) in the TwinsUK, DESIR, and AOB studies. Estimated copy-numbers higher than 13 (showing frequency $< 2.5\%$) were collapsed together into a single category. D and E: BMI at different estimated *AMY1* copy-numbers and *AMY1* copy-number estimates by BMI categories in the TwinsUK and DESIR population samples. WHO BMI classification: Underweight (< 18.5); Normal range (18.50 – 24.99); Pre obese (25.00 – 29.99); Obese class I (30.00 – 34.99); Obese class II (35.00 – 39.99). Error bars represent the standard error of the mean. *Association between BMI and qPCR $\Delta\Delta Ct$ intensity signal, corrected for age, sex (DESIR), family (TwinsUK) and genotyping plate. **Wilcoxon rank sum test.

Tables

Table 1: Summary information on subjects included in this study.

Sample	Total	Male	Female	Median age	1st – 3rd quartiles	Median BMI	1st – 3rd quartiles
Swedish	342	98	244	37	(33 - 43)	27.9	(22.6 - 36.5)
TwinsUK	1,479*	-	1,479	53	(45 - 60)	26.0	(22.8 - 28.4)
DESIR	2,137	942	1,195	52	(44 - 61)	24.6	(22.2 - 26.6)
AOB	563	160	403	35	(32 - 39)	-	-
Cases	205	39	166	36	(29 - 41)	46.2	(42.5 - 51.3)
Controls	358	121	237	35	(33 - 38)	21.6	(20.3 - 22.4)
SP2	658	237	421	46	(37 - 52)	-	-
Cases**	333	139	194	47	(40 - 54)	27.1	(25.9 - 28.9)
Controls	325	98	227	44	(34 - 51)	18.4	(17.5 - 19.1)
ABOS	468	122	346	43	(33 - 51)	46.2	(41.7 - 52.3)

*Consisting of 334 dizygotic and 193 monozygotic twin pairs and 425 singletons. **Including 136 obese (BMI \geq 28 kg/m²) and 197 overweight (28 kg/m² > BMI > 23 kg/m²) Singaporean Chinese subjects.

Table 2: Association of relative copy-number in the amylase region with obesity and measures of adiposity. Copy-number estimates used in the association analyses for both the "Population samples" and the "Obesity case-control" samples were derived by qPCR.

DNA Array-based CNV analysis[#]	N	Trait	Associated probe	P		
Swedish families	342	BMI	cnvi0020639	3.86×10 ⁻⁴		
Swedish families	331	Fat mass	cnvi0020639	3.11×10 ⁻⁴		
TwinsUK	972	BMI	cnvi0022844	1.13×10 ⁻³		

Population samples ‡	N	BMI*	Age*	β (SE)[§]	P	
TwinsUK	1,479	26.0 (22.8-28.4)	53 (45-60)	-0.18 (0.05)	5.91×10 ⁻⁴	
DESIR	2,137	24.6 (22.2-26.6)	52 (44-61)	-0.14 (0.03)	2.49×10 ⁻⁷	
	N			β (SE)[§]	P	Het P**
Meta analysis	3,616			-0.15 (0.02)	6.93×10 ⁻¹⁰	0.54

Obesity case-control‡	Cases		Controls		β (SE)[§]	P	OR (95%CI)[§]	Het P**
	N	Age*	N	Age*				
TwinsUK	251	53 (47-60)	711	51 (44-59)	-0.26 (0.09)	3.61×10 ⁻³	1.30 (1.08-1.55)	
DESIR	137	55 (47-64)	1267	51 (42-59)	-0.16 (0.04)	7.47×10 ⁻⁵	1.18 (1.09-1.27)	
AOB	205	36 (29-41)	358	35 (33-38)	-0.17 (0.04)	4.44×10 ⁻⁵	1.19 (1.10-1.29)	
SP2	136	47 (37-54)	325	44 (34-51)	-0.15 (0.05)	3.73×10 ⁻³	1.17 (1.05-1.29)	
	N		N		β (SE)[§]	P	OR (95%CI)[§]	Het P**
Meta analysis***	593		2,336		-0.18 (0.03)	1.46×10 ⁻¹⁰	1.19(1.13-1.26)	0.62

The listed numbers of samples are those which passed quality control and were used in the association analyses. ‡ Obesity case-control analyses in TwinsUK and DESIR were conducted using a subset (subjects with BMI < 25kg/m² and those with BMI ≥ 30kg/m²) of those subjects included in the quantitative trait analysis in the population samples category. #“DNA Array-based” denotes signal intensity data from Illumina SNP genotyping arrays; * median (1st, 3rd quartiles) ; ** Heterogeneity P-value; § Estimates calculated using integer *AMY1* copy-numbers inferred from the underlying continuous distribution. ***Obesity case-control meta-analysis was limited to European samples.