No Evidence for Association of BMI with Salivary Amylase Gene Copy Number in the UK 1958 Birth Cohort

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Objective: In a 2014 publication, evidence was presented supporting the association of BMI with the copy number of the salivary amylase 1 (AMY1) gene, with an unprecedented effect size of −0.15 kg/m² (SE 0.02) per copy of AMY1. Most well-powered attempts to reproduce these findings have not been successful. However, because of different study designs, a significant association may still apply under restricted conditions such as in particular age groups. This study specifically tested the BMI-AMY1 association at different age points in the same individuals using longitudinal BMI information from participants in the UK 1958 Birth Cohort study.

Methods: This study measured the AMY1 copy number by paralogue ratio tests in genomic DNA and by using array comparative genomic hybridization data. BMI data from 1958 Birth Cohort participants were available from eight different age points between 7 and 50 years.

Results: No evidence, even at nominal significance, was found for association of the AMY1 copy number with BMI at any age point in approximately 1,400 members of the 1958 Birth Cohort or in 2,835 people from two disease cohorts from the Wellcome Trust Case Control Consortium.

Conclusions: The results do not support an association between BMI and AMY1 copy number at any age point.

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Author contributions: JALA conceived and designed the study and coordinated access to the 1958 Birth Cohort data and DNA samples. NAAS used paralogue ratio tests to measure the amylase copy number in genomic DNA samples. Both authors analyzed comparative genomic hybridization and paralogue ratio test data and the association with BMI. Both authors contributed to all stages of the manuscript preparation.

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Introduction

Obesity remains a critical public health problem in many populations, and numerous individual factors have been defined that contribute to the heritable component of variation in adiposity (most commonly represented by measurement of BMI). In addition to relatively strong genetic factors, such as the well-characterized variation in the gene FTO (1), genome-wide association studies of very large cohort sizes have defined increasing numbers of single-nucleotide polymorphism (SNP) loci associated with variation in BMI (2).

By contrast with SNP diversity, gene copy number variation (CNV) poses practical difficulties for accurate measurement (3,4) but may have strong functional consequences through gene dosage effects. Furthermore, although most biallelic CNV can be effectively captured in SNP data, SNPs often have very little predictive power for more complex multiallelic CNV (4) so that direct typing of copy number is necessary for effective analysis of association. This extra dimension of variation was explored in an analysis of the association between BMI and the copy number of the AMY1 (salivary amylase 1) gene; array comparative genomic hybridization (CGH) and real-time polymerase chain reaction (PCR) methods were used to analyze the AMY1 copy number in more than 6,000 adult participants, demonstrating a significant association of a low AMY1 copy number with increased BMI (5). The study used adults and drew representative population samples from the TwinsUK (N=1,479; median age 53 years) and data from an epidemiological study on the insulin resistance syndrome (DESIR) (N=2,137; median age 52 years) cohorts. Taken at face value, this effect (β=−0.15 kg/m² per copy of AMY1) would easily be the most substantial heritable factor yet to be discovered in obesity.

In nearly all studies with best power to replicate the core finding of Falchi et al. (5) of population-wide association between BMI and the AMY1 copy number, no significant associations have been observed. However, because different studies have used cohorts with different properties and, in some cases, have also tested different hypotheses, it is unclear whether the proposed association may still apply in some contexts. A very well-powered study in three cohorts of European adults (6) totaling more than 4,000 individuals and including people selected for...
being at the extremes of the BMI distribution strongly excluded a major effect in the general population.

A study of the AMY1 copy number in 4,800 nondiabetic adult participants did not replicate the simple association found by Falchi et al. (5) between the AMY1 copy number and BMI (P = 0.8) (7) but provided evidence of dependence of the relationship between the AMY1 copy number and BMI on dietary starch intake. Plasma enzyme activity was significantly associated both with the AMY1 gene copy number and with BMI in the well-characterized DESIR cohort, but the association between BMI and the AMY1 copy number was only nominally significant (P = 0.023) (8). In the same study, an analysis of two further case-control cohorts for obesity comparing 1,179 affected adults with 2,220 controls and 785 affected children and adolescents with 712 controls revealed a significant association of obesity with the AMY1 copy number (P = 6.8 x 10^{-5}) overall, which was mostly driven by an association (P = 7.1 x 10^{-5}) among the children and adolescents (8).

Studies specifically analyzing obesity in children and young adults have yielded results apparently supporting the association of BMI with the amylase gene copy number. A study comparing 293 Mexican children with obesity with 304 normal weight controls (mean age 9.5 years) revealed a highly significant association (9). Significant associations of AMY1 with BMI were reported in Finnish women (N = 29; P = 0.045), but not in men, among young adults (mean ages 19-20 years) affected by early-onset obesity (10) and in boys (P = 0.033), but not in girls, among 744 Italian children with a mean age of 8.4 years (11). In contrast, no evidence of an association was observed in more than 1,000 Chinese and Malay men aged 18 to 21 in which the AMY1 copy number was convincingly determined by using droplet digital PCR (12).

Taking all these studies together, the general population-wide association claimed by Falchi et al. (5) is not supported by independent replication, but neither is an age-restricted effect decisively excluded by all studies. Any genuine AMY1 association operating in specific restricted contexts could still have great potential power in both understanding etiology and guiding specific interventions in clinical management. An effect of AMY1 variation on metabolism might be strongest at earlier ages because of changes in carbohydrate intake and turnover during an individual's lifetime. We particularly wanted to clarify the possible age dependence of the AMY1-BMI association, and for this purpose, we exploited the potential of the British 1958 Birth Cohort (National Child Development Study) (13) and determined the AMY1 copy number both by direct measurement from genomic DNA and by a custom analysis of existing array CGH data. Samples from the 1958 Birth Cohort made up some of the unaffected control samples used in the Wellcome Trust Case Control Consortium (WTCCC) studies (3,14), from which CGH data were available. The 1958 Birth Cohort is a representative (unselected) population sample, and each 1958 Birth Cohort participant has multiple BMI measurements at intervals from birth to age 50, allowing for longitudinal analysis of the association between the AMY1 copy number and BMI.

DNA samples from 1958 Birth Cohort participants were made available from the Avon Longitudinal Study of Parents and Children Laboratory at the University of Bristol (under the management of Dr. Sue Ring) after application to the Managing Ethico-social and Technical Issues and Administration Data Access Committee (METADAC) Secretariat at Newcastle University (formerly at Bristol University) (https://www.metadac.ac.uk/1958bco/), and after approval from the Access Committee for the Centre for Longitudinal Studies Cohorts (ACCC) (http://www.cls.ioe.ac.uk/).

**BMI data**

Longitudinal BMI data for 1958 Birth Cohort participants were made available after approval from METADAC; BMI (calculated as the mass in kilograms divided by the height in meters squared) measurements had been recorded at ages 7, 11, 16, 23, 33, 42, 44, and 50 years. Participants had a mean of 7.00 BMI measurements, and 90% of participants had at least six of eight possible BMI measurements; all age points had BMI data for at least 1,100 people. BMI values (with corresponding age and sex information) for the WTCCC T2D and CAD cohorts were made available through the relevant WTCCC study leads (Andrew Hattersley [University of Exeter], Mark McCarthy [University of Oxford], and Will Rayner [University of Oxford]) for the T2D cohort and Nilesh Samani [University of Leicester] and Alistair Hall [University of Leeds] for the CAD cohort.

**Array CGH data from WTCCC CNV study (including 1958 Birth Cohort and T2D and CAD cohorts)**

Raw array CGH data generated as part of the WTCCC CNV study (3,15) were made available by the European Genome-Phenome Archive after application to the WTCCC Data Access Committee (cdac@sanger.ac.uk; www.wtccc.org.uk/). Including controls and duplicates, genome-wide array data from 21,058 DNA samples were available for analysis, including 1,448 samples from the 1958 Birth Cohort.

Copy number estimates for the amylase alpha 2B gene (AMY2B) were derived from raw array CGH data by combining ratios of quantile-normalized red to quantile-normalized green for the 10 probes used (15) to define CNVR266.13, followed by probe variance scaling (3) and calibration to the known range of integer copy numbers. The AMY2A copy number was estimated from the ratio of quantile-normalized red to quantile-normalized green for six probes in the region unique to AMY2A, followed by probe variance scaling, correction for cross hybridization from AMY1, and integer calibration. Finally, copy

**Methods**

**Subjects and DNA samples**

The main association analyses described in this study used the subset of the UK 1958 Birth Cohort included as control subjects in the WTCCC CNV analysis (3). The UK 1958 Birth Cohort study initially sampled 17,416 children born in the UK in the same week in 1958, and data sweeps, including interviews and biometric measurements, were conducted at ages 7, 11, 16, 23, 33, 42, 44, and 50 years (13). Loss to follow-up meant that by age 50, there were data from 9,790 participants. DNA was prepared from immortalized cell lines derived from blood taken at age 44, and DNA and array CGH data were available for our study from 1,448 people. The use of this cohort was therefore intended as a representative population sample, with no enrichment of people with extreme values. We also included two disease-cohort samples from the WTCCC CNV study for which BMI, age, and sex data were available as well as raw array CGH data; there were 1,027 samples (425 women) from the type 2 diabetes (T2D) cohort and 1,808 samples (366 women) from the coronary artery disease (CAD) cohort. All samples were from UK individuals; in the WTCCC CNV study, 26 individuals had been removed on the basis of a principal component analysis of genotypes or of self-reported non-European ancestry (3). Recruitment, measurement, and blood sampling of these cohorts were conducted with local ethical approval in the original studies.
number determination for AMY1 used the mean quantile-normalized ratio values for eight probes from the copy-variable AMY1 region; this initial estimate was further refined by a correction to remove the effect of cross hybridization to other similar sequences (represented by seven probes) in the wider amylase gene region that did not bear a simple relationship to the AMY1 copy number. Estimates of the AMY1, AMY2A, and AMY2B copy number derived by these methods showed the known properties of the variation, including predominance of even numbers for AMY1 and the parity for an odd or even copy number between AMY1 and AMY2A (6,16) (Figure 1A).

Paralogue ratio test measurement of copy number in genomic DNA
Measurement of the copy number of AMY1, AMY2A, and AMY2B in 1958 Birth Cohort genomic DNA samples followed the paralogue ratio test (PRT) methods described by Shwan et al. (17); in summary, the AMY1 gene copy number was estimated after measurement of two AMY1A:AMY1C ratios, and copy number estimates for AMY2A and AMY2B were deduced after measurement of the AMY2A/AMY2B duplication sequence combined with measurement of the AMY2A:AMY2B ratio, as described (16,17). Fluorescently labeled PCR products from these assays were combined and separated by electrophoresis in a single ABI3130xl capillary for each sample. Out of the 1,448 samples tested, copy number measures were successfully obtained for AMY1 from 1,308 samples, for AMY2A from 1,300 samples, and for AMY2B from 1,309 samples (Supporting Information Table S1). Amylase gene copy numbers derived from PRT analysis of genomic DNA from 1958 Birth Cohort samples have been submitted (in PLINK generic variant file format) to the central 1958 Birth Cohort database.

Association analysis and power calculations
Analysis of association between BMI and amylase gene copy number was conducted by using linear regression in the context of the PLINK toolset (version 1.07) for genome-wide association studies (18). Amylase gene copy number was encoded in the generic variant file format for multiallelic CNV; sex was included as a covariate, as was age, except in the analysis of single age points for 1958 Birth Cohort data. For analysis in children (ages 7, 11, and 16), we used BMI z scores, relative to the mean and SD of the cohort, to examine association, but at older age points (age 23 and older), BMI was used without transformation. The power of different cohorts was simulated by using Monte Carlo simulations (each with 1 million iterations) and by applying the effect size observed by Falchi et al. (5) to the numbers of samples investigated in each cohort in this work. For the 1958 Birth Cohort samples, different numbers of individuals had both AMY1 copy number information and BMI data at different age points, but the minimum sample size (at age 16, n = 1,107) had 58% power to demonstrate association significant at P = 0.01 and 78% power for P = 0.05; the mean number of informative 1958 Birth Cohort samples across the different age points (n = 1,266) had 60% power to demonstrate association at P = 0.01 and 83% power for P = 0.05. For the WTCCC disease cohorts, 1,808 CAD samples had 92% power, and 1,027 T2D samples had 76% power, to demonstrate association at nominal (P = 0.05) levels of significance. Finally, a combined cohort of 4,237 adult samples had 99.1% power to detect association significant at P = 0.01 and 99.9% power to detect association at nominal (P = 0.05) significance.

Ethics and consent
This study did not undertake any new recruitment of participants but instead used data and DNA collected under existing studies; ethical approvals and informed consent arrangements were confirmed with the relevant original review boards for the original WTCCC and 1958 Birth Cohort studies, and our access to the data and DNA samples was approved (1) for the 1958 Birth Cohort by the METADAC Secretariat (Newcastle University; https://www.metadac.ac.uk/1958bc/) and the ACCC (http://www.cls.ioe.ac.uk/) and (2) for the WTCCC by the Consortium Data Access Committee (cdac@sanger.ac.uk; www.wtccc.org.uk).

Results
In the study by Falchi et al. (5), the authors’ initial demonstration of an association between BMI and AMY1 copy number mostly relied on real-time PCR typing of the amylase gene copy number, and subsequent studies have highlighted the apparently low accuracy of copy number measurement (6,16). In seeking to replicate the association, we therefore paid particular attention to accurate measurement of the gene copy number for AMY1. We started with an analysis of existing array CGH data for 20,158 samples from the WTCCC CNV study, which included 1,448 samples from the 1958 Birth Cohort (3). Variation in the AMY1 gene number is complex at the sequence level, and presumably for this reason, AMY1 variation was not among the copy-variable regions that could be called with high confidence in the WTCCC CNV study (3,15). In this work, customized analysis of raw array CGH data, informed by knowledge of the structural basis of the variation (6,16), allowed us to extract measurements of AMY1 representation that clustered around integer values and matched the known properties of the variation (see Methods). The placement of clusters in Figure 1A demonstrates the known relationship between AMY1 and AMY2A copy number parity; when AMY2A has an odd number of copies, so does AMY1, and when AMY2A has an even number, so does AMY1 (6,16).

In parallel, we obtained independent duplicate copy number measurements by direct analysis of genomic DNA in 1,448 DNA samples from the 1958 Birth Cohort using ratio-based (PRT) methods (17), again demonstrating concordance with previous copy number distributions (Supporting Information Table S1) and the odd-even parity between AMY1 and AMY2A (Figure 1B). The mean copy numbers found in 1958 Birth Cohort samples were 6.71 (range: 2-15) for AMY1, 2.0 (range: 0-5) for AMY2A, and 2.15 (range: 2-5) for AMY2B. Measurements of the AMY1 copy number by array CGH and by PRT showed good agreement, with an r² value of 0.872 and 90% of samples concordant for the inferred integer AMY1 copy number (Figure 1C). Similarly, both array CGH and DNA typing methods were used to derive copy number estimates of the pancreatic amylase genes AMY2A and AMY2B (Figure 2A-2B). Overall, the appearance of integer clusters, with a predominance of even copy numbers for AMY1 (6,16), and the demonstration of known relationships between AMY1 and AMY2A gene copy numbers provided validation of the accuracy of copy number estimates.

We used linear regression with sex as a covariate to examine the association between BMI (or BMI z score for ages 7, 11, and 16) and amylase gene copy number in the 1958 Birth Cohort samples at each of eight age points between ages 7 and 50 (Table 1). Even at nominal levels of significance, no association was observed with the copy number of AMY1, AMY2A, or AMY2B by using either copy numbers measured by direct typing (Table 1) or from array CGH (data not shown). To reduce the effects of error in copy number measurement, which might be most substantial at high copy numbers of AMY1, we also analyzed the association of BMI...
with the *AMY1* gene copy number after rounding to integer values conditional on the *AMY2A* copy number; if the *AMY2A* copy number was even, the measured *AMY1* copy number was rounded to the nearest even number, and likewise for odd numbers. Again, no association of BMI with *AMY1* was observed at any age point, even at nominal significance (data not shown).

No significant association was observed ($P > 0.05$) when specifically testing the childhood data (from ages 7, 11, and 16) for an association between *AMY1* copy number and obesity as a categorical state (defining obesity as a BMI above the 95th percentile, with controls below the 85th percentile) (9). We also examined the trajectory of BMI with age by testing the interaction between *AMY1* copy number, BMI, and age, detecting no significant interaction term ($P = 0.27$).

We tested for associations specific to WTCCC disease cohorts, specifically hypothesizing that T2D might be a state in which handling of dietary starch is more important for overall metabolism. BMI data were available for members of two of the WTCCC disease cohorts (T2D and CAD), and the amylase gene copy number was estimated for these samples by using the array CGH analyses described in this work. By using sex and age as covariates, no significant association was observed between BMI and amylase gene number in participants with T2D or CAD (Supporting Information Table S2). Although use of the
TABLE 1

| Age group, y | AMY2B | AMY2A | AMY1 |
|-------------|-------|-------|------|
| Total       | Total | Total | Total|
| Median BMI (first to third quartiles) | β P | Median BMI (first to third quartiles) | β P | Median BMI (first to third quartiles) | β P |
| 7           | 1,122 | 1,192 | 1,192 |
| 11          | 1,035 | 1,079 | 1,079 |
| 16          | 1,144 | 1,133 | 1,133 |
| 23          | 1,144 | 1,133 | 1,133 |
| 33          | 1,144 | 1,133 | 1,133 |
| 42          | 1,144 | 1,133 | 1,133 |
| 50          | 1,144 | 1,133 | 1,133 |

β is standardized (i.e., measured in SDs per copy); values have unit kilograms/meters squared per copy. 

74, ages 7, 11, and 16; association analysis used z-scores; no values are standardized (i.e., measured in SDs per copy); other ages, BMI used directly, so values have unit kilograms/meters squared per copy.

Discussion

After we attempted to replicate the observations of Falchi et al. (5), our data provide no support, even at nominal levels of significance, for an association between BMI and amylase gene copy number at any age point in participants from the 1958 Birth Cohort or in participants from the WTCCC T2D and CAD cohorts. In this respect, our results are concordant with those of Usher et al. (6) and Yong et al. (12). Although we evaluated copy number directly by typing genomic DNA for only about 1,300 samples from the 1958 Birth Cohort (Supporting Information Table S1), all participants had array CGH data available from the WTCCC CNV study so that in practice, power was limited by the availability of BMI data. In the sample from the UK 1958 Birth Cohort, different age points have different numbers of individuals with BMI data (from n = 1,107 at age 16 to n = 1,402 at age 44) and hence variable power to detect association at any one age point. At the most conservative extreme, if we assume that an association of BMI with amylase gene copy number is only operating at a single age point in the 1958 Birth Cohort, then our study has between 78% and 86% power to detect association at nominal significance, assuming an effect of the magnitude claimed by Falchi et al. (5) (β = −0.15 kg/m² per copy of AMY1). The results of Monte Carlo simulations of power at different individual age points are given in Supporting Information Table S3. These values offer conservative estimates of power, assuming that each individual age point is the only opportunity to detect association. Because, in reality, BMI at different age points for the same individual will not be independent, the overall power to test association in children might be conservatively estimated by using the total number of unique individuals sampled at ages 7, 11, or 16 (n = 1,406), which has more than 85% power to detect the full effect claimed by Falchi et al. (5) at nominal significance (Supporting Information Table S3). If we could assume that the association applies equally and independently at all age points, then, effectively, the longitudinal series of eight age points would create a very powerful resampling, increasing the power to nearly 100% to detect association at nominal significance in at least one of the age points. Furthermore, if the effect is age independent and unaffected by disease status, then our combined analysis of 4,237 independent samples also has close to 100% power to detect an effect of the magnitude reported by Falchi et al. (5) (Supporting Information Table S3).

Given the major role played by pancreatic amylase enzymes in intestinal digestion of dietary starch, it would be natural to predict that variation in the copy-variable pancreatic amylase (AMY2A/AMY2B) genes could have a more substantial influence on carbohydrate metabolism than the salivary amylase genes. As part of these same analyses, we were also able to derive copy number measurements for the pancreatic amylase genes AMY2A and AMY2B; no association was observed between BMI and the copy number of AMY2 genes, even at nominal levels of significance.
The 1,448 samples we studied from the UK 1958 Birth Cohort were used as controls in the WTCCC studies (3,14) and were selected from the 9,790 participants still undergoing follow-up at age 50 from an original birth cohort of 17,406. It is not clear whether any bias may have been introduced alongside these reductions in participant numbers, and if so, what influence such bias might have on our conclusions. By using all age points, our longitudinal analysis allowed us to address the question of whether the AMY1 copy number might be associated with the pattern of BMI change with age, but again, we were unable to demonstrate a significant association. Our study has the obvious limitation that BMI is the only phenotype analyzed, with sex and, in some analyses, age as covariates; we have no information on potentially relevant environmental or lifestyle factors such as nutrition. An association conditional to lifestyle factors not recorded in our study, such as dietary starch intake (7), could therefore escape detection if the association could not be detected without allowance for this covariate. Indeed, the study of 4,800 nondiabetic participants with a mean age of 57 years by Rukh et al. (7) demonstrated no significant association of BMI with AMY1 copy number alone (P≤0.8) but found a significant interaction (P=0.007) conditional upon dietary starch intake. That study used droplet digital PCR to determine the AMY1 copy number, and the excess of samples with even numbers of copies reassures that the accuracy of copy number determination is likely to be high. The use of the same individuals at different ages in our study provides a strong basis for evaluating age-dependent effects of AMY1 copy number on BMI, but suffers from a corresponding lack of constancy for environmental conditions; at earlier time points, the participants were younger, but they were also subject to different environmental conditions relevant to BMI, which have changed considerably during the lifetimes of these individuals.

Conclusion

The original association claimed in the study by Falchi et al. (5), which remains the largest study showing support for a simple association of BMI with amylase gene copy number, had no conditional element other than correcting for age and sex as covariates. As a direct replication of the original findings of Falchi et al. (5), the conclusions of our study remain unambiguously negative. In the analysis of the 1958 Birth Cohort samples with age, we found no evidence to support an association between BMI and AMY1 copy number that is age dependent.

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References

1. Frayling TM, Timpson NJ, Weedon MN, et al. A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. Science 2007;316:889-894.
2. Locke AE, Kathiri B, Berndt SI, et al. Genetic studies of body mass index yield new insights for obesity biology. Nature 2015;518:197-206.
3. Craddock N, Hurles ME, Cardin N, et al; Wellcome Trust Case Control Consortium. Genome-wide association study of CNVs in 16,000 cases of eight common diseases and 3,000 shared controls. Nature 2010;464:713-720.
4. Usher CL, McC Carroll SA. Complex and multi-allelic copy number variation in human disease. Brief Funct Genomics 2015;14:329-338.
5. Falchi M, El-Sayed Moustafa JS, Takouso P, et al. Low copy number of the salivary amylase gene predisposes to obesity. Nat Genet 2014;46:492-497.
6. Usher CL, Handsaker RE, Esko T, et al. Structural forms of the human amylase locus and their relationships to SNPs, haplotypes and obesity. Nat Genet 2015;47:921-925.
7. Rukh G, Ericson U, Andersson-Assarsson J, Ortho-Melander M, Sonestedt E. Dietary starch intake modifies the relation between copy number variation in the salivary amylase gene and BMI. Am J Clinical Nutr 2017;106:256-262.
8. Bonnefond A, Yengo L, Dechaume A, et al. Relationship between salivary/pancreatic amylase and body mass index: a systems biology approach. BMC Med 2017;15:57. doi:10.1186/s12916-017-0784-x.
9. Mejia-Benitez M, Bonnefond A, Yengo L, et al. Beneficial effect of a high number of copies of salivary amylase AMY1 gene on obesity risk in Mexican children. Diabetologia 2015;58:290-294.
10. Viljakainen H, Andersson-Assarsson JC, Armenio M, et al. Low copy number of the AMY1 locus is associated with early-onset female obesity in Finland. PLoS One 2015;10:e0131883. doi:10.1371/journal.pone.0131883.
11. Marcovecchio ML, Florio R, Verginelli F, et al. Low AMY1 gene copy number is associated with increased body mass index in prepubertal boys. PLoS One 2016;11:e0154961. doi:10.1371/journal.pone.0154961.
12. Yong YR, Mustaffa SB, Wasan PS, et al. Complex copy number variation of AMY1 does not associate with obesity in two East Asian cohorts. Hum Mutat 2016;37:669-678.
13. Power C, Elliott J. Cohort profile: 1958 British birth cohort (National Child Development Study). Int J Epidemiol 2006;35:34-41.
14. Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature 2007;447:661-678.
15. Conrad DF, Pinto D, Redon R, et al; Wellcome Trust Case Control Consortium. Origins and functional impact of copy number variation in the human genome. Nature 2010;464:704-712.
16. Carpenter D, Dhar S, Mitchell LM, et al. Obesity, starch digestion and amylase: association between copy number variants at human salivary (AMY1) and pancreatic (AMY2) amylase genes. Hum Mol Genet 2015;24:3472-3480.
17. Shwan NAA, Louzada S, Yang F, Armour JAL. Recurrent rearrangements of human amylase genes create multiple independent CNV series. Hum Mutat 2017;38:532-539.
18. Purcell S, Neale B, Todd-Brown K, et al; PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 2007;81:559-575.