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Smoking status, snus use, and variation at the *CHRNA5-CHRNA3-CHRNA4* locus in relation to obesity: the GLACIER Study

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ABSTRACT

A genetic variant within the *CHRNA5-CHRNA3-CHRN4* region (rs1051730), previously associated with smoking quantity, was recently shown to interact with smoking on obesity predisposition. We attempted to replicate this finding in the Gene-Lifestyle Interactions and Complex Traits Involved in Elevated Disease Risk (GLACIER) Study, a prospective cohort study of adults from northern Sweden ($n = 16,426$). We also investigated whether a similar interaction is apparent between rs1051730 and snus, a type of moist oral tobacco, to determine whether this interaction is driven by factors that cigarettes and snus have in common, such as nicotine. Main effects of smoking, snus, and the rs1051730 variant and pairwise interaction terms (smoking \times rs1051730 and snus \times rs1051730) were tested in relation to body mass index (BMI; calculated as weight (kg)/height (m)²) through the use of multivariate linear models adjusted for age and sex. Smoking status and BMI were inversely related ($\beta = -0.46$ kg/m², standard error (SE) = 0.08; $P < 0.0001$). Snus use and BMI were positively related ($\beta = 0.35$ kg/m², SE = 0.12; $P = 0.003$). The rs1051730 variant was not significantly associated with smoking status or snus use ($P > 0.05$); the T allele was associated with lower BMI in the overall cohort ($\beta = -0.10$ kg/m², SE = 0.05; $P = 0.03$) and with smoking quantity in those in whom this was measured ($n = 5,304$) ($\beta = 0.08$, SE = 0.01; $P < 0.0001$). Neither smoking status ($P_{\text{interaction}} = 0.29$) nor snus use ($P_{\text{interaction}} = 0.89$) modified the association between the rs1051730 variant and BMI.

MeSH Keywords: Body Mass Index; Gene-environment Interaction; Genetic Association Studies; Polymorphism, Single Nucleotide; Smoking; Tobacco, Smokeless.

Abbreviations: BMI, body mass index; *CHRNA3*, nicotinic acetylcholine receptor subunit alpha-3 gene; CI, confidence interval; FFQ, food frequency questionnaire; GWAS, genome-wide association studies; IQR, interquartile range; nAChR, nicotinic acetylcholine receptor; POMC, pro-opiomelanocortin; SD, standard deviation; SE, standard error; SNP, single nucleotide polymorphism; VHU, Västerbotten Health Survey.

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Running head: *Varga et al.; Smoking, snus & rs1051730 in relation to BMI*

Cover letter: TVV, FR, and PWF designed the analysis. TVV undertook the analysis and drafted the manuscript; GH supervised cohort data, and reviewed the manuscript; FBH supervised genotyping and reviewed the manuscript; FR and PWF helped with data analysis, interpretation of the results, and reviewed the manuscript.

Although both obesity and smoking are major preventable causes of disease and premature death (1, 2), smokers are generally leaner than nonsmokers (3, 4), and weight gain is a common unwelcome by-product of smoking cessation (5, 6). The mechanisms by which smoking affects body weight are incompletely understood but are thought to involve the ability of nicotine to block the hypothalamic cannabinoid receptors that are known to be involved in the regulation of appetite and satiation (7). Thus, if smoking and smoking cessation are causally associated with body weight, one would expect other nicotine-containing products to have comparable associations with body weight.

In previously published meta-analyses of genome-wide association studies, an association was identified between a genetic variant (rs1051730) localized to the nicotinic acetylcholine receptor subunit alpha-3 gene (*CHRNA3*) and smoking quantity in smokers (8–15). In white Europeans (www.hapmap.org), the rs1051730 single-nucleotide polymorphism is in strong linkage disequilibrium ($r^2 = 1.0, D' = 1.0$) with the genetic variant rs16969968, encoding an amino acid change previously shown to affect receptor function (16). In a subsequent meta-analysis, Freathy et al. (17) used a heterogeneity test to infer an interaction between the rs1051730 single-nucleotide polymorphism and smoking status on body mass index (BMI), although no formal pairwise test of interaction was performed.

In the present study, we sought to replicate the previously observed associations between rs1051730 and smoking quantity and to examine whether the rs1051730 variant modifies the relationship of smoking with BMI through a formal test of interaction. In secondary analyses, we explored whether another nicotine-containing product, snus (an oral moist tobacco commonly

used in Scandinavian countries), yielded results similar to those for smoking. We examined snus because it contains amounts of nicotine comparable to that in cigarettes and elicits detectable stimulatory effects on the brain (7, 18), yet it might, compared with smoking, be differentially associated with potential confounders such as education, diet, alcohol consumption, and physical activity.

MATERIALS AND METHODS

Study participants

The Gene-Lifestyle Interactions and Complex Traits Involved in Elevated Disease Risk (GLACIER) Study is a prospective, population-based cohort study nested within the Västerbotten Health Survey in northern Sweden (19). Baseline examinations were performed from 1985 through 2004. Participants were invited to attend an examination on their 40th, 50th, and 60th birthdays. Clinical characteristics (age, sex, height, and weight) were collected, and detailed assessments of lifestyle were obtained through the use of a validated questionnaire (19, 20). All participants provided written informed consent as part of the Västerbotten Health Survey, and ethical approval for the GLACIER Study was obtained from the Regional Ethical Review Board in Umeå. From those with data at the first visit ($n = 17,486$), participants not genotyped for rs1051730 ($n = 744$), participants with self-reported diabetes ($n = 312$), and participants for whom we lacked data crucial to the analyses ($n = 4$) were excluded. Thus, the total number of individuals included in our analyses was 16,426.

Clinical measures

Weight (to the nearest 0.1 kg) and height (to the nearest centimeter) were measured with a calibrated balance-beam scale and a wall-mounted stadiometer, respectively. BMI was calculated as weight (kg)/height

(m)². Current smokers were defined as people who reported daily smoking; participants who had never smoked or who smoked occasionally were considered never smokers (21). Participants who reported having quit smoking were considered former smokers. We also refer to a group of ever smokers, composed of current and former smokers. The same classifications were applied to snus use.

In the analysis in which we sought to replicate the main association between rs1051730 and smoking quantity, information on cigarette quantity was available as a categorical variable (1–4, 5–14, 15–24, or >24 cigarettes/day). This variable was available in a subgroup consisting of 5,304 current and former smokers.

Comparison of putative confounders

To determine whether smoking and snus have comparable relationships with putative confounding variables, we conducted Pearson correlation analyses between smoking and snus separately with educational level (6–7 years of compulsory school education, compulsory school plus college, or compulsory school plus college plus university), leisure-time physical activity level (never, occasionally, 1, 2–3, or >3 times per week), alcohol consumption (0, 1–4, 5–10, or >10 g of ethanol/day), and a healthy diet score. The diet score was constructed from intakes of 8 food groups (whole grains, fish, fruits, and vegetables were designated as favorable foods, whereas red and processed meats, sweets, sugared beverages, and fried potatoes were designated as unfavorable), as previously described (22). Intakes for each food group were categorized into quartiles and given ascending values of 0, 1, 2, 3 for favorable foods and descending values of 3, 2, 1, 0 for unfavorable foods. These values were summed to generate a diet score of 0–24, with higher scores indicating a healthier diet (22).

Genotyping

DNA was extracted from peripheral white blood cells, and genomic DNA samples were diluted to 4 ng/μL, as previously described (23, 24). The rs1051730 variant was genotyped with the OpenArray SNP Genotyping

System (BioTrove, Inc., Woburn, Massachusetts) at the Harvard School of Public Health, Boston, Massachusetts. The genotyping success rate was higher than 98%, and within the study sample the genotype frequencies were in Hardy-Weinberg equilibrium ($P = 0.97$). The rs1051730 variant harbors alleles C (major) and T (minor) on the forward strand. The genotyping was done on the reverse strand in GLACIER, but to facilitate interpretations and comparisons of results, we report the corresponding forward strand alleles, in agreement with Freathy et al. (17).

Statistical methods

Statistical analyses were undertaken in SAS, version 9.2, software (SAS Institute, Inc., Cary, North Carolina) and Stata, version 11, software (StataCorp LP, College Station, Texas) (25, 26). Main effects were estimated with generalized linear models by fitting genotype (additive model) as the independent variable and BMI, smoking status, or snus use as the dependent variable. In models constructed to replicate the findings reported by Freathy et al. (17), the exposure of smoking was categorized by strata of never and ever smokers, and separate regression models were run within these strata to generate effect estimates and P values. Power calculations were made in Quanto, version 1.2.4 (written by John Morrison and W. James Gauderman; <http://hydra.usc.edu/gxe>). In the article by Freathy et al. (17), heterogeneity tests (Cochran's Q statistics and I^2 tests) were used to infer interactions, but no formal test of interaction was reported. Therefore, we undertook both tests of heterogeneity and formal pairwise interaction tests, with the caveat noted that the test of heterogeneity is unreliable when few strata are compared. Our heterogeneity test included the comparison of effect estimates and standard errors for the association between rs1051730 and BMI among strata of smoking status. Fixed-effects meta-analysis was performed with the “metan” module in Stata (27). We also assessed heterogeneity in never smokers and ever smokers between the meta-analysis of Freathy et al. (17) and the GLACIER cohort. Finally, we meta-analyzed the results from the GLACIER Study with those from the meta-analysis reported by Freathy et al. (17). We included interaction terms for smoking status and

rs1051730 (smoking status × rs1051730) in linear models to evaluate whether the associations of the single-nucleotide polymorphism on BMI differed in magnitude by strata of smoking status. Finally, we repeated the analyses outlined above with snus use in place of smoking status.

RESULTS

Participant characteristics are shown in Table 1.

Association of tobacco use and BMI

Smoking status and BMI were inversely related ($\beta = -0.46 \text{ kg/m}^2$, standard error (SE) = 0.08, 95% confidence interval (CI): -0.62, -0.31 for current vs. never smokers; $P < 0.0001$). Snus use and BMI were positively related ($\beta = 0.35 \text{ kg/m}^2$, SE = 0.12, 95% CI: 0.12, 0.58 for current vs. never snus users; $P = 0.003$).

Association between rs1051730 and smoking or snus

The rs1051730 variant was positively associated with category of smoking quantity ($\beta = 0.08$, SE = 0.01; $P < 0.0001$). The rs1051730 variant was not associated with smoking status ($P > 0.05$) or with snus use ($P > 0.05$).

Interaction between rs1051730 and smoking on BMI

The BMI means and 95% confidence intervals per strata of genotype, smoking, and snus use are shown in Table 2. In the overall cohort, each minor (T) allele was associated with -0.10 kg/m^2 (SE = 0.05, 95% CI: -0.20, -0.01; $P = 0.03$) lower BMI. In the group of ever smokers, each T allele was associated with -0.16 kg/m^2 (SE = 0.07, 95% CI: -0.31, -0.02; $P = 0.026$) lower BMI, whereas there was no detectable association of rs1051730 among never users ($\beta = -0.06 \text{ kg/m}^2$, SE = 0.06, 95% CI: -0.18, 0.07; $P = 0.37$); nevertheless, the test of interaction was not statistically significant ($P_{\text{interaction}} = 0.29$ for current vs. never and $P_{\text{interaction}} = 0.27$ for ever vs. never users). Additional adjustment for snus use or potential confounders (as listed in Table 3) did not materially affect these results ($P_{\text{interaction}} = 0.60$ and $P_{\text{interaction}} = 0.18$, respectively). Repeating the above analyses

with occasional smokers and former occasional smokers excluded from the group of never smokers did not materially affect the results.

Interaction between rs1051730 and snus on BMI

There was no statistically significant interaction between snus use and the rs1051730 genotype ($P_{\text{interaction}} = 0.89$ for current vs. never and $P_{\text{interaction}} = 0.87$ for ever vs. never users). Additional adjustment for potential confounders (as listed in Table 3) did not materially affect these results ($P_{\text{interaction}} = 0.75$). When we stratified by snus use, the T allele was inversely associated with BMI in the never snus user group ($\beta = -0.11 \text{ kg/m}^2$, $\text{SE} = 0.06$, 95% CI: $-0.22, -0.003$; $P = 0.04$), but despite similar effect sizes, this association was not statistically significant in the ever snus user group ($\beta = -0.09 \text{ kg/m}^2$, $\text{SE} = 0.11$, 95% CI: $-0.30, 0.12$; $P = 0.41$). When the model was additionally adjusted for smoking status, the statistically significant association in the never snus user group disappeared ($P = 0.16$). The per-T allele changes in BMI in strata defined by smoking status or snus use are shown in Table 4.

Heterogeneity tests and meta-analysis

To recapitulate the analysis of Freathy et al. (17), we conducted Cochran's Q test to assess whether genotype modified the association of smoking with BMI. The test of heterogeneity between ever and never smokers was not statistically significant within GLACIER ($P = 0.28$, $I^2 = 13.8\%$).

We conducted heterogeneity tests between the cohort of Freathy et al. (17) and the GLACIER cohort within the never smoker and ever smoker strata. The test of heterogeneity between the 2 cohorts was not statistically significant in never smokers ($P = 0.21$, $I^2 = 35\%$) or in ever smokers ($P = 0.43$, $I^2 = 0\%$). By contrast, significant heterogeneity was observed between never smokers and ever smokers in the meta-analysis ($n = 40,626$) comprising summary statistics from Freathy et al. (17) and the GLACIER cohort ($P < 0.001$, $I^2 = 93\%$).

DISCUSSION

In an attempt to replicate previous reports (8–14, 17), we examined the associations between the *CHRNA5-CHRNA3-CHRNA4* rs1051730 variant and smoking and BMI. In line with previous results, the rs1051730 variant was positively associated with smoking quantity in the GLACIER Study. We also followed up on a prior report of interaction between the rs1051730 variant and smoking status in relation to BMI (17). Consistent with the initial report, the association of the rs1051730 variant with BMI was statistically significant in current and former smokers, with an effect estimate approximately 3-fold larger in magnitude than in never smokers; however, the formal test of interaction was not statistically significant. Thus, we conclude that although there is tentative evidence of an interaction between smoking and the rs1051730 variant in our data on the basis of the smoking stratum-specific results, the formal test of interaction did not confirm that these stratum-specific effect estimates differed in magnitude.

It should be noted that Freathy et al. (17) used a heterogeneity test (I^2) to infer the presence or absence of an interaction effect. Heterogeneity tests of this nature are often more feasible in the context of meta-analyses, where formal cohort-specific interaction test statistics are unavailable, inasmuch as the tests can be performed by pooling and comparing the main effect test statistics from each cohort by strata of the putative effect modifier. In general, it is likely that this approach to inferring interaction effects will yield a P value similar to that of the conventional pairwise test of interaction; indeed, in our study, both approaches yielded comparable P values. However, in some scenarios—for example, where measurement error (a source of heterogeneity) is greater in one stratum than the other—the results from the 2 approaches can differ, with the heterogeneity test result being the least accurate. The conventional pairwise test of interaction also has the advantage of yielding interaction and marginal effect coefficients, which are useful for determining the clinical relevance of an interaction effect and for subsequent power calculations.

A statistically significant interaction might be absent in GLACIER because our study is underpowered to replicate the interaction reported by Freathy et al. (17); however, Freathy et al. did not report an interaction effect estimate in their article, so we could make power calculations only on the basis of a range of plausible effect estimates (see Appendix Table 1). If the interaction effect estimate reported here is accurate, future studies will require a cohort of approximately 72,000 persons with characteristics similar to participants in GLACIER to confirm or refute an interaction of the same magnitude between smoking and the rs1051730 variant on BMI with a power of 80%.

In the present study, we identified an inverse association between smoking and BMI and a positive association between snus use and BMI. These findings are compatible with those reported elsewhere. For example, Albanes et al. (3) were among the first to report that smokers weigh less than nonsmokers (by approximately 2.7 kg on average) in the Second National Health and Nutrition Examination Survey (NHANES II) population-based cohort. Hansson et al. (28) reported that Swedish snus users gained on average 1.9 kg of body weight during 5 years of follow-up, whereas persons who had never used snus gained on average only 0.7 kg. The principal component of cigarettes that is believed to mediate body weight is nicotine (29). Studies in rats have indicated that nicotinic acetylcholine receptor–dependent activation of pro-opiomelanocortin neurons by nicotine regulates melanocortin pathways in the brain (30); the pro-opiomelanocortin and melanocortin circuits are well established as key regulators of appetite and satiety, with loss-of-function mutations in pro-opiomelanocortin (31) and the melanocortin 4 receptor (32) genes harboring some of the most penetrant causes of monogenic obesity. Melanocortin 4 receptor also is implicated in polygenic obesity and type 2 diabetes (24).

As shown in Table 3, the correlation coefficients differ in magnitude and sometimes direction between smoking status or snus use and the putative confounders, which supports our hypothesis that although cigarettes and snus share the factor that is believed to be causally related with obesity (i.e., nicotine), they do not share the same confounding factors in this population. Although it is possible that cigarettes contain

active substances absent from snus that drive the interactions described above, it seems more plausible that it is the obesogenic correlates of snus (i.e., confounders) that underlie the association of snus with obesity, rather than a direct causal effect of snus. Unlike cigarette smoking, which is common in almost all populations, snus use is confined almost exclusively to Sweden and Norway; thus, the standard epidemiologic approach of comparing effect estimates across populations, which differ in the frequency and distribution of potential confounding factors, to infer causal relationships (33) is not possible in epidemiologic studies of snus use.

In conclusion, we found evidence of association between the *CHRNA5-CHRNA3-CHRNA4* rs1051730 variant and smoking quantity but not status. The association of the rs1051730 variant with BMI was statistically significant in the overall study population, and the effect estimate was considerably larger in current and former smokers than in never smokers; however, the pairwise test of interaction between this variant and smoking status or snus use on BMI was not significant in GLACIER. By contrast, the effect estimate was statistically significant only in never snus users, not in current or former snus users, and additional adjustment for smoking status ablated the association. These findings tend to suggest that the observed associations and tentative interaction effect of smoking with BMI are likely to be explained by factors other than nicotine, which is unique to smoking tobacco products or smoking-related behaviors.

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Table 1. Basic Characteristics of the GLACIER Study Participants (N=16,426), GLACIER Study, Sweden, 1985-2004

	n (% male)	Age, years mean (SD)	BMI, kg/m² mean (SD)	BMI, kg/m² median (IQR)
Never smokers	9,096 (35.8)	52.0 (9.1)	25.9 (4.1)	25.4 (23.1, 28.0)
Former smokers	3,709 (49.7)	53.0 (8.0)	26.6 (4.1)	26.2 (23.9, 28.6)
Current smokers	3,621 (37.4)	52.0 (8.3)	25.5 (4.2)	24.9 (22.7, 27.7)
Ever smokers	7,330 (43.6)	52.5 (8.2)	26.0 (4.2)	25.6 (23.2, 28.2)
Never snus users	12,479 (31.2)	52.5 (8.6)	25.9 (4.2)	25.3 (23.0, 28.0)
Former snus users	1,098 (81.8)	50.2 (9.3)	26.3 (3.8)	26.0 (23.9, 28.4)
Current snus users	1,582 (83.3)	49.6 (9.6)	26.2 (3.9)	25.8 (23.7, 28.1)
Ever snus users	2,680 (82.7)	49.9 (9.5)	26.2 (3.8)	25.9 (23.8, 28.3)
No snus data	1,267			
All	16,426	52.3 (8.7)	26.0 (4.2)	25.4 (23.1, 28.1)

Abbreviations: BMI, body mass index; IQR, interquartile range; n, number of participants; SD, standard deviation.

Table 2. BMI Means and 95% Confidence Intervals in GLACIER Participants Stratified by Smoking/Snus Status and Genotype (N=16,426), GLACIER Study, Sweden, 1985-2004

STRATA	n	CC ^a			TC ^a			TT ^a		
		n	BMI mean, kg/m ²	BMI 95% CI, kg/m ²	n	BMI mean, kg/m ²	BMI 95% CI, kg/m ²	n	BMI mean, kg/m ²	BMI 95% CI, kg/m ²
Never smokers	9,096	4,099	26.0	25.9, 26.2	4,002	25.9	25.8, 26.0	995	26.0	25.8, 26.3
Former smokers	3,709	1,626	26.6	26.4, 26.8	1,675	26.4	26.2, 26.5	408	26.5	26.1, 26.9
Current smokers	3,621	1,609	25.6	25.4, 25.8	1,608	25.5	25.3, 25.7	404	25.1	24.7, 25.5
Ever smokers	7,330	3,235	26.1	26.0, 26.3	3,283	25.9	25.8, 26.1	812	25.8	25.5, 26.1
Never snus users	12,479	5,570	26.0	25.9, 26.1	5,523	25.8	25.7, 25.9	1,386	25.8	25.6, 26.1
Former snus users	1,098	499	26.3	26.0, 26.7	476	26.5	26.1, 26.8	123	26.1	25.4, 26.8
Current snus users	1,582	695	26.4	26.1, 26.7	708	26.2	25.8, 26.5	179	26.2	25.6, 26.8
Ever snus users	2,680	1,194	26.3	26.1, 26.6	1,184	26.3	26.0, 26.5	302	26.1	25.7, 26.6
ALL	16,426	7,334 (44.6%)	26.0	26.0, 26.2	7,285 (44.4%)	26.0	25.8, 26.0	1,807 (11%)	25.9	25.7, 26.1

Abbreviations: 95% CI, 95% confidence interval; BMI, body mass index; n, number of participants.

^aCC, TC and TT are allelic variations of the rs1051730 variant.

For compensation of data imbalance, means are adjusted with age and sex. The adjusted means are calculated with multiple regression equations.

Table 3. Correlation Between Smoking/Snus Status, BMI and Potential Confounders (N=16,426), GLACIER Study, Sweden, 1985-2004

	Education correlation coefficient (<i>P</i> value)	Physical activity correlation coefficient (<i>P</i> value)	Alcohol consumption correlation coefficient (<i>P</i> value)	Diet correlation coefficient (<i>P</i> value)
Smoking status (never vs. ever)	-0.10 (<0.0001)	-0.09 (<0.0001)	0.15 (<0.0001)	-0.09 (<0.0001)
Snus status (never vs. ever)	0.01 (0.26)	0.006 (0.45)	0.21 (<0.0001)	-0.19 (<0.0001)
BMI, kg/m ²	-0.14 (<0.0001)	-0.08 (<0.0001)	-0.04 (<0.0001)	0.02 (0.01)

Abbreviations: BMI, body mass index.
P values are based on Pearson correlation analyses.

Table 4. Associations Between BMI and Genotype in GLACIER Participants, Stratified by Smoking/Snus Status (N=16,426), GLACIER Study, Sweden, 1985-2004

STRATA	n	Per-T allele change in BMI, kg/m²	Per-T allele change in BMI, kg/m² (95% CI)	P value
Never smokers	9,096	-0.06	-0.18, 0.07	0.37
Former smokers	3,709	-0.14	-0.34, 0.06	0.16
Current smokers	3,621	-0.19	-0.39, 0.02	0.07
Ever smokers	7,330	-0.16	-0.31, -0.02	0.03
Never snus users	12,479	-0.11	-0.22, 0.00	0.04
Former snus users	1,098	-0.01	-0.34, 0.32	0.94
Current snus users	1,582	-0.14	-0.42, 0.14	0.33
Ever snus users	2,680	-0.09	-0.30, 0.12	0.41
ALL	16,426	-0.10	-0.20, -0.01	0.03

Abbreviations: 95% CI, 95% confidence interval; BMI, body mass index; n, number of participants. P values are based on linear regression models, marginal effects were tested by fitting genotype as the independent variable (coded assuming an additive effect of the modeled allele) with BMI.

Appendix Table 1. Power Calculations to Detect Genetic and Environmental Main Effect and Gene x Environment Interaction (N=16,426), GLACIER Study, Sweden, 1985-2004

β_{GE} , kg/m ²	Power (%)		
	Interaction	Gene	Environment
0.0000	5	54	~100
0.0500	8	71	~100
0.1000	18	84	~100
0.1500	34	93	~100
0.2000	54	97	~100
0.2500	73	99	~100
0.3000	87	~100	~100
0.3500	95	~100	~100
0.4000	99	~100	~100
0.4500	~100	~100	~100

Abbreviations: β_{GE} , beta coefficient for gene x environment interaction.

Power was calculated by using the number of study participants (N=16,426), minor allele (T) frequency at the rs1051730 locus (0.33), smoking prevalence in the cohort (0.45 for ever smokers), population mean and standard deviation for BMI (25.98 ± 4.16 kg/m²) and previously reported β coefficient for the genetic and the environmental main effect (-0.1 kg/m² and -1 kg/m², respectively). Freathy *et al.* did not report an effect size for the gene x environment interaction, so this table presents power calculations for a range of β_{GE} coefficients. Two sided power calculation was used with a 0.05 type I error rate.