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The Genome-wide Influence on Human BMI Depends on Physical Activity, Life-course, and Historical Period

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Abstract

In this analysis, guided by an evolutionary framework, we investigate how the human genome as a whole interacts with historical period, age and physical activity to influence BMI. The genomic influence is estimated by (1) heritability or the proportion of variance in BMI explained by genome-wide genotype data and (2) the random effects or the Best Linear Unbiased Predictors (BLUPs) of GWAS data on BMI (However, we were not doing a traditional GWAS analysis). The hypothesis testing is performed on (2). Data were collected form a New England town in the Framingham Heart Study (FHS) in the United States. The study was initiated in 1948 and the data were collected repeatedly over the decades. The analyses draw analysis samples from a pool of > 8,000 individuals in the FHS and produce three empirical findings. First, the genomic influence on BMI is substantially and significantly larger after the mid-1980s than in the few decades before the mid-1980s within each age group of 21-40, 40-50, 51-60 and >60. Second, the genomic influence on BMI weakens as one ages across the life course or the genome influence on BMI tends to be more important during reproductive ages than after reproductive ages within each of the two historical periods under consideration. Within the age group of 21-50, the genomic influence on BMI among physically active individuals is statistically significantly and noticeably smaller than the influence on those who are not physically active. In summary, this study provides evidence that the influence of human genome as a whole on obesity does depend on historical period, age, and level of physical activity.

INTRODUCTION

Obesity has consequences for morbidity and mortality; it is associated with hypertension, metabolic syndrome, dyslipidemia, type-2 diabetes, coronary heart disease, osteoarthritis, stroke, and several types of cancers (Lewis et al. 2009; NIH-Publication 1998). Currently, more than two thirds of adults in the United States are overweight (defined as body mass index (BMI) of 25-29.9 kg/m^2), and about half of the overweight are obese (BMI ≥ 30) (National Center for Health Statistics. Health 2008).

Is obesity a result of genetic destiny or personal choices about eating and exercise? In the quest for genetic evidence over the past five to six decades, most research depended on bio-metrical methods or family and twin studies based on genetically related individuals (Jou 2014), where genetic effects on obesity are treated as from a blackbox. Family and twin studies based on genetically related individuals suggest that the heritability of BMI ranges from 40 to 70% (Maes, Neale and Eaves 1997; Stunkard, Foch and Hrubec 1986). Recent genome-wide association studies (GWAS) and GWAS consortia studies have confirmed that dozens of genetic loci are associated with obesity (Frayling et al. 2007; Speliotes et al. 2010).

Since the 1980s, the United States saw an increase of almost 200% in obesity prevalence. Why did this obesity epidemic happen so dramatically and quickly while human gene pools could not possibly have altered to such a degree? Extra-genetic factors such as eating and exercise must have played a part. Thus, obesity is a complex health problem that is influenced by both genes and environment, and possibly the interaction between the two.

Gene-environment (GxE) interaction holds that an environment influences how sensitive we are to the effect of a genotype and vice versa. Ignoring GxE interactions forces us to estimate only an average genetic effect (averaged over all environments) or an average environmental effect (averaged over all genotypes), thus potentially missing genetic, environmental or both effects entirely.

The present analysis investigates whether genetic influences at the whole genome level interact with three extra-genetic factors (historical period, life course, and physical activity,) to influence obesity. One main innovation of this gene-environment (GxE) interaction analysis is its consideration of the influences of an entire human genome, using in the analysis hundreds and thousands of genetic variables simultaneously in a single regression model (Yang et al. 2010).

BACKGROUND

The joint effects of human genome and extra-genetic factors such as physical activity and dietary patterns can be understood from a perspective of evolution (Bellisari 2008). The "thrifty genotype" hypothesis proposed several decades ago represents an evolutionary explanation of the current obesity epidemic (Neel 1962). This hypothesis suggests that thrifty genes were selected to give advantages to human populations by storing extra calories as body fat in times when food was abundant. Thrifty genes were advantageous because throughout almost all of human history and all over the world, food is scarce and the level of physical activity is high. However, thrifty genes become disadvantageous in the contemporary developed world where food is plentiful and inexpensive and intense physical activity is typically unnecessary.

The evolutionary theory of obesity has an age dimension. It predicts a number of peaks in fat storage over the life course (Zafon 2007). The first two peaks are an adaptive strategy for reproduction. Fat storage in infancy assists in the transition from placental period to lactation and the transition from lactation to solid food. Fat storage during pregnancy for the mother is considered a safeguard for an infant's lactation period. Humans often experience a third peak in fat deposition at older ages, but apparently it does not have a clear evolutionary significance.

Although difficult to prove (Lazar 2005; Speakman 2008), the hypothesis is plausible and timely, hypothesizing that there exist in human populations genes, certain forms of which are conducive to obesity. Also derived from the hypothesis is how some of extra-genetic factors

may interact with genetic propensities for obesity. The evolutionary theory predicts that obesity genes tend not to be expressed in "normal times" with respect to food and physical activity or the BMIs of human individuals possessing obesity genes tend not to become "overweight" unless food is abundant and inexpensively available and physical activity is low.

Specifically for the current analysis, we hypothesize a smaller genomic influence on BMI among individuals engaged in heavy physical activity than individuals not engaged in such activity. Previous GxE interaction studies targeting one or a few genetic variants report that the effect of *FTO* on obesity among individuals leading a physically active lifestyle is attenuated by about 30% when compared with those who are inactive (Andreasen et al. 2008; Cauchi et al. 2009; Rampersaud et al. 2008). It remains to be seen whether physical activity exerts a similar attenuating effect on the genome-wide susceptibility for obesity.

Consistent with the evolutionary theory, we hypothesize a larger genomic influence on BMI in the current obesity epidemic since the mid 1980s than before the epidemic or before the mid 1980s. The current obesity epidemic is often characterized as an 'obesogenic' environment where unhealthy food is more easily available and exercise is reduced and where obesity genes are expected to be more expressed. The division of the past decades into a pre-obesogenic and an obesogenic environment is based on national surveys of obesity over the same periods. The prevalence of obesity in the United States remained approximately the same from 1960s through 1980s and has increased dramatically since the mid 1980s (Flegal et al. 1998; Flegal et al. 2012; Flegal et al. 2002). Our data from the Framingham Heart Study (FHS) confirm this national trend (Figure 1). The level of BMI before 1970 is similar to that between 1970 and 1985 and a much higher level of BMI is observed for the period after 1985.

Because of a much clearer evolutionary significance for obesity genotype in reproductive ages than after reproductive ages, we hypothesize a larger genome's effect on obesity among females and males in reproductive ages than the effect among those after reproductive ages. The

evolutionary theory predicts such an effect only among females and does not comment on a similar effect among males (Zafon 2007). We extend the prediction to males since the same genotype is likely to be passed onto both females and males.

In this analysis, we investigate whether and how much the level of historical period, life course, and physical activity interact with genome to influence obesity. Equivalently, we seek answers to the following three questions. Does physical activity reduce the genome-wide genetic susceptibility for obesity? Has the genome-wide influence on obesity increased over the past few decades when the prevalence of obesity rose dramatically in the United States? Does the genome-wide genetic susceptibility become smaller after reproductive ages than during reproductive ages?

The investigation uses data from FHS. Since 1948, the FHS has repeatedly collected information on health and health behavior of three cohorts of more than 10,000 individuals. Recently, genome-wide genotype data were collected from about 9,000 of these individuals. By extending a recent mixed-model approach (Yang et al. 2010), our GxE interaction analysis considers the overall impact of the human genome as a whole on obesity in a single regression model.

This analytical approach represents a major methodological shift from the traditional fixed-effects GWAS strategy in genomic data analysis. For example, one of the questions we ask is: Does physical activity reduce the genome-wide genetic susceptibility for obesity? In spite of the generally recognized importance of physical activity and rapid advances in genomic technologies in recent decades, this seemingly straightforward question has not been addressed. The investigation of GxE interaction involving a few genetic variants is uncomplicated, but incorporating genome-wide genotype data into GxE interaction analysis has been a formidable challenge.

A central challenge in working with genomic data is the development of a way to take

advantage of the entire panel of GWAS data simultaneously in one analysis. The current prevailing GWAS strategy estimates the effect of one single nucleotide polymorphism (SNP) at a time. The >500K genetic effects in the fixed-effects regression models are estimated separately. In the fixed-effects framework, it is impossible to include all the SNPs as independent variables in a single regression model because the number of predictors is much larger than the number of observations. The usual strategy of using GWAS for GxE interaction analysis also encounters the intractable difficulty of multiple testing (Boardman et al. 2014). However, once treated as random, the entire panel of genetic polymorphisms can be considered simultaneously. In this random-effects model, we calculated the heritability of BMI or the proportion of the variance in BMI explained by the panel of the SNPs as well as the random effects of this large number of SNPs on BMI.

Using genome-wide genotype data to estimate heritability represents a fundamental development over the traditional twin and other family studies. In the absence of DNA data, family and twin methods had been used to obtain heritability estimates. These twin and family approaches rely on a number of assumptions. These include assumed degrees of genetic relatedness among relatives, the equal environment assumed between monozygotic and dizygotic twins, and the nonexistence of assortative mating. These assumptions cannot be verified by empirical data. In contrast, our approach makes direct use of genome-wide genotype data and does not rely on these assumptions.

DATA and METHODS

Data Source. The Framingham Heart Study (FHS)(FHS 2012) is a community-based, prospective, longitudinal study following three generations of participants. The Original Cohort enrolled in 1948 (N=5,209), the Offspring Cohort enrolled in 1971 (N=5,124) consisting of the children of the Original Cohort and spouses of the children, and the Generation Three Cohort enrolled in 2002 (N=4,095) consisting of the grandchildren of the Original Cohort. These

individuals are of predominantly European origin. About 1% of the subjects self-report as native, black and Asian American. All study subjects undergo physical exams and complete written questionnaires at regular intervals. Weight and height were measured on FHS subjects repeatedly at dozens of medical exams over the decades. These measures at all adult ages and over several decades provide an opportunity for an age-period analysis.

At five times between 1979 and 2008, the FHS asked the study subjects how many hours per day they engaged in activities such as heavy household work, heavy yard work such as stacking or chopping wood, and exercise such as intensive sports (jogging, swimming, etc.). About 85% of the FHS subjects whose BMI and genotype are available responded. A binary variable is constructed that divides the FHS individuals into two groups: those who engaged in heavy activity (N=2,102) and those who did not (N=2,216). Splitting the entire sample into more than two groups encounters the issue of sample size to be discussed in a later section. Our measure of physical activity is merely a proxy for energy expenditure. An accurate measure needs to take into consideration a numerous dimensions such as frequency, intensity, duration, energy cost, efficiency, and locomotory effects (Wells 2006).

Of the 14,428 study subjects in FHS, a total of 9,237 consenting individuals were genotyped including 4,986 women and 4,251 men. Genotyping for FHS participants was performed using the Affymetrix 500K GeneChip array. The Y chromosome was not genotyped. A standard quality control filter is applied to the genotype data. Individuals with 5% or more missing genotype data were excluded from analysis. SNPs that are on X chromosomes, that have a call rate ≤ 99%, or a minor allele frequency ≤0.01 were also eliminated from analysis. The application of the quality control procedures has left 8,738 individuals with 287,525 SNPs from the 500K genotype data. Genotype data were converted to minor allele frequencies for analysis.

A Mixed Model for GxE Interaction Analysis for GWAS Data. Our GxE interaction approach builds upon Yang et al.'s mixed linear model (2009; 2010). These models

are a special class of the general mixed or random effects linear models. A key innovation of Yang et al. is that they recognize an extremely useful feature of these models: by doing a transformation, the models could include, in a single regression model, a million or more genetic variables for each individual. This was accomplished by not estimating fixed effects of the large number of variables. Rather, the large number of genetic variables is assumed to follow a random distribution and the distribution parameters can be estimated. Once the mixed model is estimated, the random effect of each of the large number of genetic SNPs can be derived.

The general mixed model includes both fixed and random effects (Searle 1971; Searle, Casella and McCulloch 1992). In the context of genetic analysis, Yang et al. focus on genetic main effects and treat the effects of a large number of observed SNPs as random. Yang et al.(2010) start with the following general form of mixed model for the purpose of estimating genetic main effects: $Y=X\beta + W\mu + \varepsilon$, with $var(Y)=WW'\sigma^2_\mu+I_\varepsilon\sigma^2_\varepsilon$ (1), where Y is an $n\times1$ vector of the phenotype with n being the number of observations; β is a vector of fixed effects; μ is a vector of SNP effects with $\mu\sim N(0, I_\mu\sigma^2_\mu)$,where I_μ is an $N\times N$ identity matrix with N being the number of SNPs; ε is a vector of residual effects with $\varepsilon \sim N(0, I_\varepsilon\sigma^2_\varepsilon)$, where I_ε is an $n\times n$ identity matrix; W is an n×N standardized genotype matrix with the ij^{th} element $w_{ij} = (s_{ij}-2\rho_j)/\sqrt{[2\rho_j(1-\rho_j)]}$, where s_{ij} is the number of copies of the reference allele for the j^{th} SNP of the i^{th} individual and p_j is the frequency of the reference allele.

Very importantly, Yang et al. (2010) defines $\boldsymbol{A}= WW'/N$ and $\sigma_g^2 = N\sigma_\mu^2$. Then Equation (2) is mathematically equivalent to Equation (1): $Y=X\beta + g + \varepsilon$, with $V = \boldsymbol{A}\,\sigma_g^2+ I_\varepsilon\sigma^2_\varepsilon$ (2), where g is a $n\times1$vector of the total genetic effects of the individuals with $g \sim N(0, \boldsymbol{A}\,\sigma_g^2)$, and $\boldsymbol{A}$ is the genetic relationship matrix (GRM) between individuals. Because N (the dimension of W or the number of SNPs) is reduced to n (the dimension of g or the number of individuals), the entire panel of 500-1,000K SNPs can be incorporated into this single mixed model. Yang et al.'s mixed model based on about 4,000 individuals and approximately 300,000 SNPs shows that 0.45 of

the variance in human height can be explained by common SNPs. In contrast, the prevailing GWAS strategy explains about 10% of the variance in height (Allen et al. 2010).

Our approach expands Yang et al.'s main effect mixed model to a mixed model for GxE interaction analysis. Equation (3) describes a mixed GxE interaction model that considers one multiple-category categorical environmental factor: $Y=X\beta + \sum_{k=1}^{r} G_k \mu_{ek} + \varepsilon_i$ (3), where G_k is a standardized genotype matrix with $G_k = W$ for individuals in the k^{th} environmental category and with $G_k = 0$ for individuals in the other environmental categories; μ_{ek} is a vector of SNP effects for individuals in the k^{th} environmental category, with $\mu_{ek} \sim N(0, I_\mu \sigma^2_{\mu ek})$ where I_μ is an $N \times N$ identity matrix with N being the number of SNPs, and $\sigma^2_{\mu ek}$ can be understood as the total variance explained by the N SNPs for the individuals in the k^{th} environmental category; and r is the number of categories of the environmental factor. All models are estimated with control for sex and the first seven principle components for bio-ancestry (Price et al. 2006).

Our GxE interaction model implemented by the software GCTA (Yang et al. 2011) yields two sets of estimates of genomic influence. The first set amounts to estimating the heritability or the proportion of variance in BMI that is explained by GWAS data in each sub-sample defined by period, age, and/or physical activity. The GCTA models allow $X\beta$ and ε to vary by environmental category k. The second set of gnomic influence is estimated random effects on BMI or the BLUP of μ, where BLUP stands for the Best Linear Unbiased Predictors. As Equations (1) and (2) (*i.e.* $Y=X\beta + W\mu + \varepsilon$ and $Y=X\beta + g + \varepsilon$) are mathematically equivalent, the BLUP of μ can be transformed from the BLUP of g by $\hat{\mu} = W^T A^{-1} \hat{g}/N$ (Yang et al. 2010). Our second set of results include a large number of random effects on BMI for each age-period and age-activity sub-sample. Also estimated is the variance of these random effects of GWAS data in each sub-sample. A large variance of the random effects implies larger proportions of random effects are located away from zero, and thus indicates a larger set of random effects on BMI.

The method of GCTA is built on a number of assumptions. The model is a special case of the general mixed model (Searle et al. 1992). It assumes that the effects of the large number of observed genetic variables follow a normal distribution. This is unusual to those familiar with multilevel or longitudinal models (Goldstein 2011; Guo and Hipp 2004; Raudenbush and Bryk 2002), which are also special cases of the general mixed model, but which assumes that the unobserved effects at level 2 or above follow a normal distribution. The model implies that all genetic variables, which can amount to one or two millions, have an effect on the outcome except for those right on the zero value in the X axis.

Hypothesis Testing. After calculating the parameters of a mixed model for each sub-sample defined by age and period, and by age and level of activity, hypothesis testing needs to be performed to test whether the genomic influences across age-period sub-samples and across age-activity sub-samples are statistically different. However, a direct comparison of the two heritability estimates from a pair of sub-subsamples can't be correctly performed under the current circumstances. For example, we attempted bootstrapping (Efron and Tbshirani 1993). A bootstrapping sample by definition repeatedly samples a portion of observations and this leads to relatedness in each sub-sample, which is not allowed in the GCTA mixed model. Because of this complication, we propose to compare the variance of the random SNP effects on BMI from two sub-samples.

Our hypothesis testing focuses on whether one set of random effects or the BLUPs on BMI in one "environmental" sub-sample is larger than another in a different "environmental" sub-sample. The hypothesis testing was performed using Pitman's test (Howell 1997; Pitman 1939; Snedecor and Cochran 1967). Pitman's test is developed to test the null hypothesis that two correlated samples are drawn from populations with identical variances. Because a pair of sub-samples on which Pitman's test was performed could contain the same or related individuals, the two sub-samples can be correlated. The random effects are paired and

correlated also because the two sets of random effects are based on the same set of SNPs. Even if the individuals are not related, the BLUPs will still be paired and correlated.

Pitman's test involves three steps. The first step is to compute the F ratio of the larger variance to the smaller variance in the pair of sub-samples. Second, it computes $t = \frac{(F-1)\sqrt{n-2}}{2\sqrt{F(1-r^2)}}$, where n is the number of SNPs and r is the correlation between the two sets of BLUPs estimated from two sub-samples, respectively (e.g., one sub-sample consists of individuals aged 21-40 before 1985 and the second sub-sample consists of individuals aged 21-40 after 1985). Finally, t is evaluated on n-2 degrees of freedom. To summarize, we calculated the BLUPs for each age-period and age-physical-activity sub-sample, and employed Pitman's test to compare the distribution of the BLUPs between age groups within each historical period; between historical periods within each age group; and between the physically inactive and the physically active within each age group.

Analytical Samples. When creating samples for mixed model analysis, we must weight two conflicting considerations. First, each mixed-model analysis must be based on a sample of genetically unrelated observations; and second, each analysis sample must be maximized in size to retain statistical power. Including related individuals or observations in the same mixed model would result in biased estimates (Yang et al. 2010). To satisfy this requirement, we could use the information on sibling and parent-child relationships in Framingham and delete one or more individuals in a known genetically-related cluster. However, some individuals could still be genetically-related such as cousins even if they are not siblings or parents and children. Framingham does not provide information on these relationships. The relationship matrix estimated by GCTA can be used to address this issue by setting the cutoff point very close to zero. GCTA's solution is to set the cutoff point at 0.025 (Yang et al. 2010), which assumed to be caused by noise for genetically unrelated individuals. The cutoff point of 0.025 was arrived at in

GCTA by the observation that the maximum negative genome-wide correlation is -0.025. Since related individuals are only correlated positively, the negative genotype correlation is likely to be caused by noise. Assuming that positive noise has a similar magnitude as the negative one, GCTA only deletes one or more individuals in a genetically correlated cluster in which individuals are correlated more than 0.025. We followed a similar logic and found the cutoff point in Framingham to be 0.034. Our analysis samples are drastically reduced because of these procedures.

The GCTA can only use genetically independent observations. Getting rid of correlation among observations due to genetic relatives and repeated measures of the same individual in the Framingham data would leave a small portion (often 20-30%) of the total number of observations for GCTA analysis. This is, indeed, very inefficient, especially when standard random-effects statistical methods can routinely handle complicatedly correlated dataset that include siblings, twins, and cousins (Searle 1971). But the two should not be confused. The GCTA method uses genome-wide genotype data to estimate heritability (Yang et al. 2010) while the standard random effects model can use genetic relatives to estimate heritability in the absence of genotype data (Guo and Wang 2002). When the GCTA method uses genetic relatives and genotype data simultaneously, it has two overlapping sources of genetic information, which result in biases.

Ideally, gene-environment interaction in the current setting is derived by estimating a mixed model for each sub-sample defined by historical period, age range, and level of physical exercise. To compromise because of the available samples, GxE interaction analysis is only performed in age-period sub-samples and age-activity sub-samples, but not in age-period-activity sub-samples, which would result in prohibitively small samples.

In the preparation for the age-period analysis, we grouped all the BMI measures, for which genotype information is available, into eight sub-samples by two historical periods of

before and after 1985, and four age groups of 21-40, 41-50, 51-60, and >60. The cut-off point of 1985 for historical periods is supported the well-known obesity trends in the United States over past decades (NCHS 2010) and confirmed by the Framingham data described in Figure 1.

A wider age range for the 21-40 group is based on two considerations. First, the evolution theory predicts a distinction with regards to effects of genotype on obesity between reproductive ages and after reproductive ages. Second, many fewer independent measures are available at these ages than other age groups. The age group of 21-40 for the period after 1985 has a sample of 799, much smaller than the other age groups in spite of an age range about twice as wide as the age ranges of 41-50 and 51-60. In the age-period analysis, because a separate mixed model is estimated within each age-period sub-sample, genetically related measures from the same individual or related individuals can be used so long as they are included in a separate regression so that the BMI measures in a mixed-model regression remain unrelated. Within each of the eight age-period subsamples, our analysis used the first BMI measure obtained for each individual.

In the preparation of the age-activity analysis, the BMI measures were grouped by age groups of 21-50 and >50 and heavy activity. Other groupings such as those used in the age-period analysis (21-40, 41-50, 51-60, and >60) would produce extremely small samples since a good proportion of respondents did not respond to the question of physical activity. Within each of the 21-50 and >50 groups, we used the BMI measure that was measured at the same time as the first response to the question of physical activity.

Some of our group classifications appear irregular. In the age-period analysis, those aged 21-40 are grouped instead of those aged 21-30 and 31-40. In the analysis of physical activity, those aged 21-50 are grouped instead of those aged 21-40 and 41-50. Nevertheless, the wider groupings are consistent with our theoretical hypotheses, which suggest a split between

reproductive ages and beyond reproductive ages. Thus, a wider age internal of 21-40 or 21-50 still overlaps with reproductive ages and can serve to test the evolutionary hypotheses.

RESULTS

Figure 2 presents the estimated heritability or the proportion of the variance in BMI explained by genome-wide genotype data by age group and historical period. The sample size or the number of individuals used in each sub-sample is also provided. It demonstrates a historical-period effect of genomic influence on BMI within each of the three age groups of 21-40, 41-50, and 51-60. The estimated proportions of BMI variance explained by GWAS for the two periods before and after 1985 are 0.71 vs. 0.42, 0.56 vs. 0.30, and 0.27 vs. 0.10, respectively for the three age groups.

Figure 2 shows that genomic influence measured by heritability on BMI tends to decline as one ages. Before 1985, the estimated genomic influences are 0.42, 0.30, and 0.10 for the age groups of 21-40, 41-50, and 51-60, respectively. After 1985, the estimated genomic influences are 0.71, 0.56, and 0.27, respectively, for the age groups of 21-40, 41-50, and 51-60. Those who aged 60 or older are an exception. Neither the age effect nor the historical effect observed among the younger age groups is present among individuals 60 or older.

Panel 1 of Figure 3 shows that the random effects of SNPs or the BLUPs on BMI are substantially larger after 1985 than before 1985 within each age group in the FHS. In every age group, the variance of the random effects on BMI after 1985 is much larger than that before 1985, especially for age groups of 21-40, 41-50, and 51-60. A larger variance indicates that higher proportions of random effects is located further away from zero and thus represent larger effects.

Panel 2 of Figure 3 shows that the random effects of SNPs or the BLUPs on BMI generally grow smaller with life course or age within each historical period in the FHS. Within the historical period after 1985, the size of the variance of the random effects is correlated

strictly with age. The older the age group is, the smaller the variance, and thus the smaller random effects are on BMI. Within the historical period before 1985, the same pattern emerges with the exception that the variance in 51-60 is larger than that in >60.

Panel 3 of Figure 3 demonstrates that among those aged 21-50, the random effects on BMI are much less among those engaged in heavy physical activity than those not engaged in heavy physical activity. Such an effect is absent among those aged >51.

While Figure 2 describes differences in the effect size of human genome in term of heritability between pairs of sub-samples, Table 1 presents the test results of whether the differences are statistically different. In Table 1, the sample size or the number of individuals used in each sub-sample analysis is described. Panel (1) of Table 1 presenting test results from Pitman's test shows that the random effects of the SNPs (BLUPs) in the period after 1985 are significantly larger than those before 1985 within each age group. Panel (2) of Table 1 shows that the random effects of the SNPs (BLUPs) are generally larger for a younger age group than an older age group within a historical period. The exception is the age group of >60 before 1985, the random effects of which group are statistically smaller than those in the age group of 51-60 before 1985. Panel (3) of Table 1 shows that in the age group of 21-50, the random effects on BMI among those engaged in heavy physical activity are significantly and dramatically smaller than those unengaged in heavy exercise. Although in the age group of >50, the two sets of the random effects between the physically inactive and the physically active are statistically significantly different, the differences in effect size are extremely small (also see Panel 3 of Figure 3). The P values in Table 1 are small and even taking into account multiple testing, these tests are still significant.

DISCUSSION and CONCLUSIONS

Guided by an evolutionary theory of obesity, this study investigates how the human genome as a whole interacts with environment to influence BMI, using data from the

Framingham Heart Study. The FHS collected repeated measures of weight and height over the past six decades from a sample of U.S. residents. The FHS recently obtained genome-wide genotype data.

This analysis found empirical support for three hypotheses concerning genome-wide influence based on the random effects of the mixed model. First, we demonstrate a genome-period interaction on BMI. The genomic influence on BMI is substantially and significantly larger in the current obesity epidemic after the mid 1980s than in the few decades before the mid 1980s within each age group of 21-40, 40-50, 51-60 and >60.

Second, this investigation shows a genomic influence on BMI that weakens as one ages across the life course or as reproduction becomes less important over the life course. This result by and large holds within each of the two historical periods under consideration. Third, within the age group of 21-50, the genomic influence on BMI among physically active individuals is statistically significantly and noticeably smaller than the influence on those who are not physically active.

Among the numerous pieces of empirical evidence from our analysis, two do not support our hypotheses. Before 1985, the genomic influence on BMI in the age group of >60 is larger than that in the age group of 51-60 (not smaller as predicted by our hypothesis). In the age group of >50, the genomic influence on BMI is not related to physical activity. Both exceptions are about older individuals.

Body mass among older individuals develop differently from younger individuals and both males and females start losing lean body mass from about age 50. (Kyle et al. 2003). He and Meng (2008) reported that individuals 70 and older in the United States are prone to weight loss rather than weight gain and that males 70 and older who are engaged in physical activity actually experience less weight loss. The relationship between genomic influence and BMI in the older populations may be different from those for the younger age groups. BMI is an

approximation for excessive adiposity. A difference in BMI may not always indicate a proportional difference in body fat, especially among the elderly population. Older individuals could maintain a constant BMI while simultaneously losing lean body mass and gaining a greater portion of adiposity. One limitation of the current study is that in order to amass reasonably-sized samples, we have to group those older than 60 in the genome-age analysis and group those older than 50 in the genome-activity analysis. Future studies with sufficiently large samples should investigate the age groups of 50s, 60s and >70 separately.

The findings of this analysis are genome-wide. The focus on the overall genomic influence in the mixed-model framework rather than individual genetic loci can be a feasible alternative to the fixed-effect GWAS studies. Investigating whether and how much, for example, physical activity reduces the effects aggregated over the entire panel of GWAS data on obesity will likely yield additional insights to those obtained from investigating whether and how much physical activity reduces the effect of a single or a few genetic variants.

The GxE interaction effect from the physical activity analysis or the period analysis can be quite large. GWAS main-effect studies show that on average, the *FTO* gene allele makes a difference of 1.2 kg in body weight (e.g., Frayling et al. 2007). Activity-*FTO* interaction studies suggest that physical activity attenuates the effect of *FTO* by 30%, which amounts to approximately 0.40 kg. This 0.40 kg is the gene-activity interaction effect based on a single gene. Our estimated gene-activity interactive effects represent a collection of numerous genes throughout the human genome. The finding suggests that a large proportion of genome-wide susceptibility for obesity could be attenuated by physical activity. The genome-by-physical-activity-interaction effect is likely many times larger than 0.4 kg.

The large period effects found in our analysis may help isolate the exact culprits of the current obesity epidemic. These period effects suggest that changes over the past three decades in the United States have induced the human genome to have a larger impact on BMI. Food and

exercise are two most likely candidates. In most human history until very recently, food was scarce and the level of physical activity high (e.g., Bellisari 2008; Swinburn et al. 2011). Health disparity is considered a source of the current obesity epidemic (Braveman 2009). Does the timing of food abundance, sedentary lifestyle, and/or health disparity correspond to the recent increase in genomic influence? A small number of other factors have been considered. An intriguing line of research points to environmental endocrine-disrupting chemicals as a possible source for the development of obesity (Casals-Casas, Feige and Desvergne 2008; Newbold et al. 2007; Wells 2006). A low-grade systematic inflammation has been considered a factor for obesity even though individuals with excessive adiposity do not typically have overt infection (Visser et al. 1999, 2001; Wisse 2004). Our findings suggest looking for endocrine-disrupting chemicals and/or increased low-grade inflammation that appeared in the environment about the same time the obesity epidemic began; these may have altered the genomic susceptibility for obesity.

The estimation of heritability using genome-wide genotype data places heritability estimates firmly on the basis of molecular genetics rather than only genetic relatedness among family members. It also makes possible the use of significance tests like the Pitman test. However, recent work shows that heritability estimates based on genome-wide genotype data are generally smaller than those estimated by twin data (Plomin et al. 2013; Wray et al. 2013). This suggests that the gene-environment interaction effects could be significantly larger than those estimated in this analysis. Our results indicate that the influence of the human genome as a whole may not be a fixed property of a phenotype. Such an influence may depend on environments or other factors external to the human genome. Finally, our approach of assessing how the effects of the human genome as a whole are moderated by environmental factors for a phenotype may be applied to a wide range of health outcomes beyond human obesity.

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Table 1. Pitman's test results showing that the random effects of the SNPs (BLUPs) are significantly different between the two historical periods (before and after 1985) within each age group (1); mostly significantly different between age groups within a historical period (2); and substantially and significantly different between the physically inactive and the physically active within the age group of 21-50 (3). Our three hypotheses are mostly supported. See the estimated effect sizes in Figure 3.

(1) For analysis of genome-period interaction within an age group

Age group	Period	Period	F	r	t	p-value
21-40	after 1985 (N=799) vs	before 1985 (N=1553)	3.09	0.131	321.8	<.00001
41-50	after 1985 (N=1205) vs	before 1985 (N=1760)	4.46	0.224	451.3	<.00001
51-60	after 1985 (N=1504) vs	before 1985 (N=1475)	11.23	0.210	837.5	<.00001
>60	after 1985 (N=1866) vs	before 1985 (N=1071)	3.73	0.403	414.1	<.00001

(2) For analysis of genome-age group interaction within a historical period

Period	Age group	Age group	F	r	t	p-value
Before 1985	21-40 (N=1553) vs	41-50 (N=1760)	1.50	0.516	128.9	<.00001
		51-60 (N=1475)	14.82	0.261	997.2	<.00001
		>61 (N=1071)	5.40	0.238	523.2	<.00001
	41-50 (N=1760) vs	51-60 (N=1475)	9.85	0.572	921.9	<.00001
		>61 (N=1071)	3.59	0.366	394.0	<.00001
	51-60 (N=1475) vs	>61 (N=1071)	0.36	0.560	-340.3	<.00001
After 1985	21-40 (N=799) vs	41-50 (N=1205)	1.04	0.202	11.271	<.00001
		51-60 (N=1504)	4.08	0.157	414.0	<.00001
		>61 (N=1866)	4.48	0.064	442.0	<.00001
	41-50 vs (N=1205)	51-60 (N=1504)	3.91	0.455	443.6	<.00001
		>61 (N=1866)	4.30	0.212	436.7	<.00001
	51-60 (N=1504) vs	>61 (N=1866)	1.09	0.413	27.7	<.00001

(3) For analysis of genome-activity interaction within an age group

Age group			F	r	t	p-value
21-50	physically inactive (N= 1056) vs	physically active (N=1041)	11.30	0.052	822.9	<.00001
> 51	physically inactive (N=1160) vs	physically active (N=1061)	0.88	0.036	-33.7	<.00001

Note: Pitman's t test is used to test the null hypothesis that the variances of two subgroups are equal against the alternative hypothesis that variances of two subgroups are different. Df=287,523.

Figure 1. The average BMI of study subjects by age and historical period in the FHS. The number of measures for the three periods is 9,686, 17,577, and 18,456, respectively.

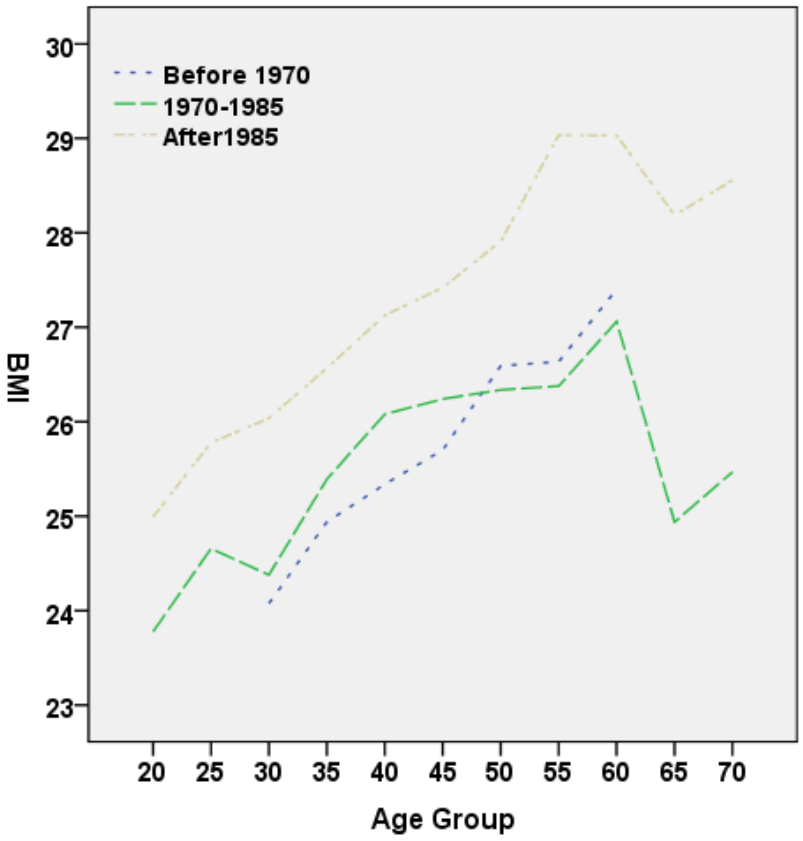


Figure 2. Estimated heritability or the proportion of the variance in BMI explained by genome-wide genotype data by age and historical period in the FHS.

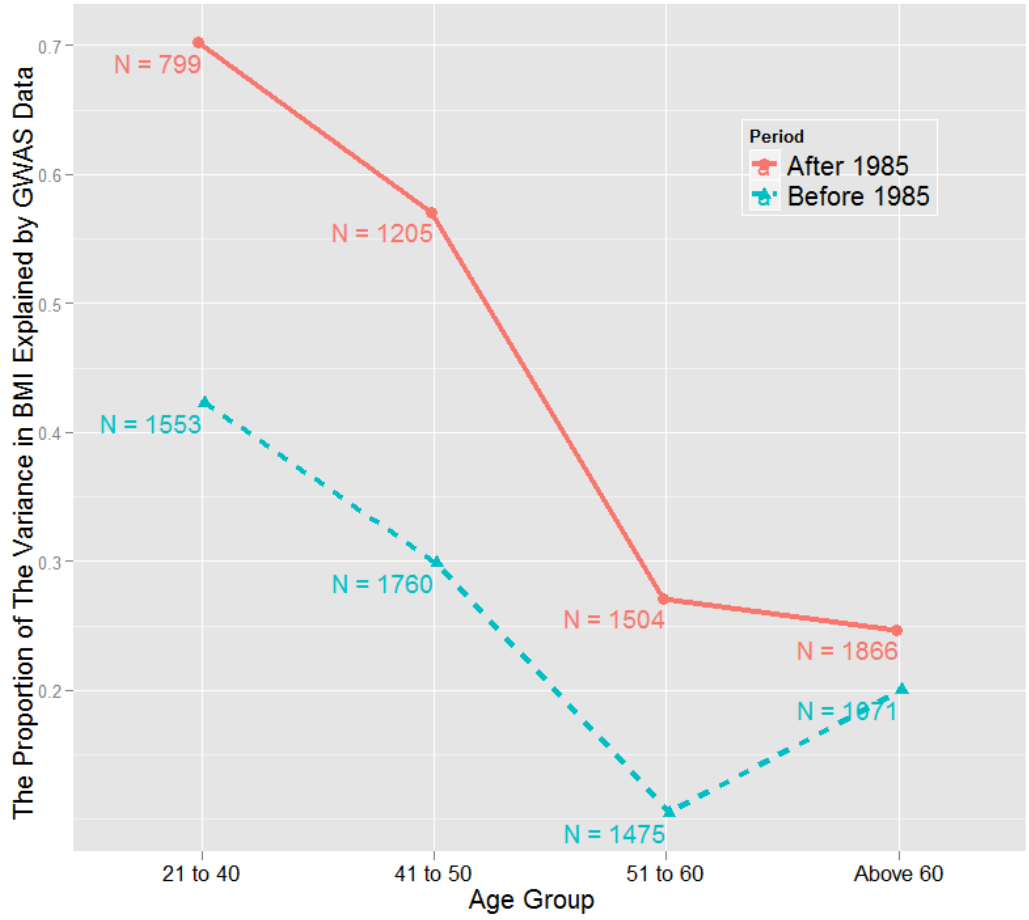
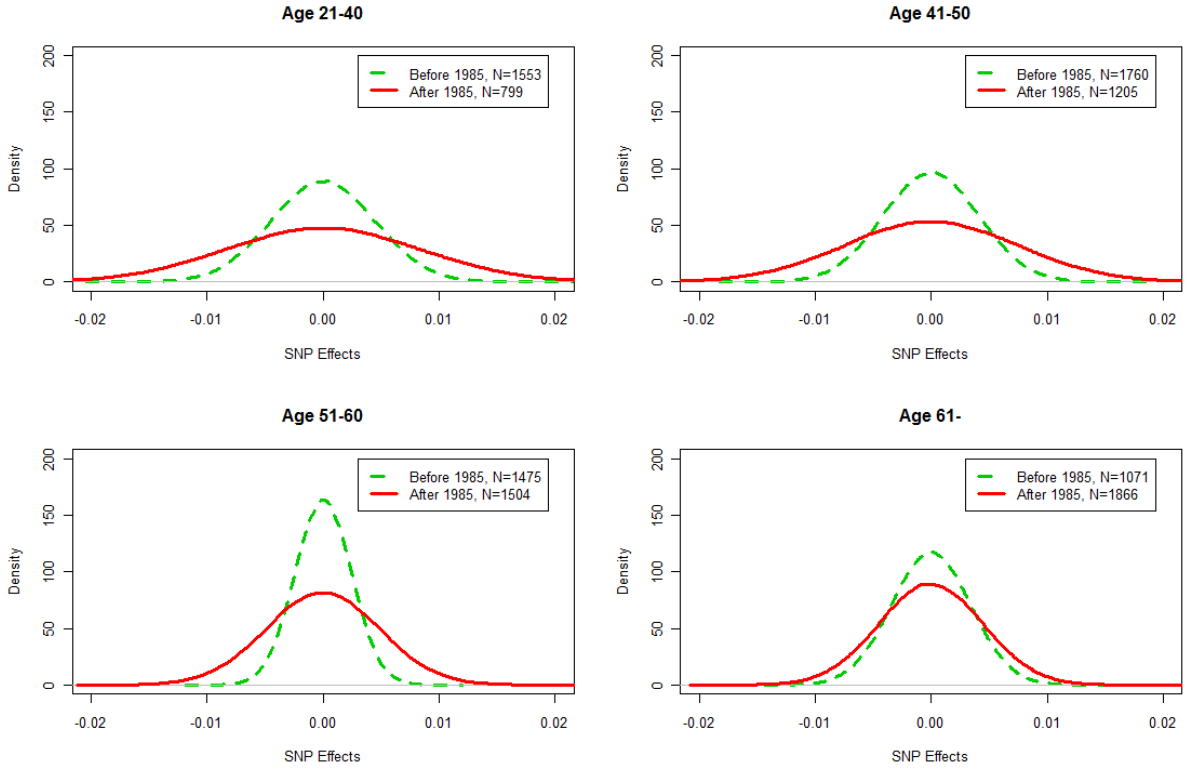
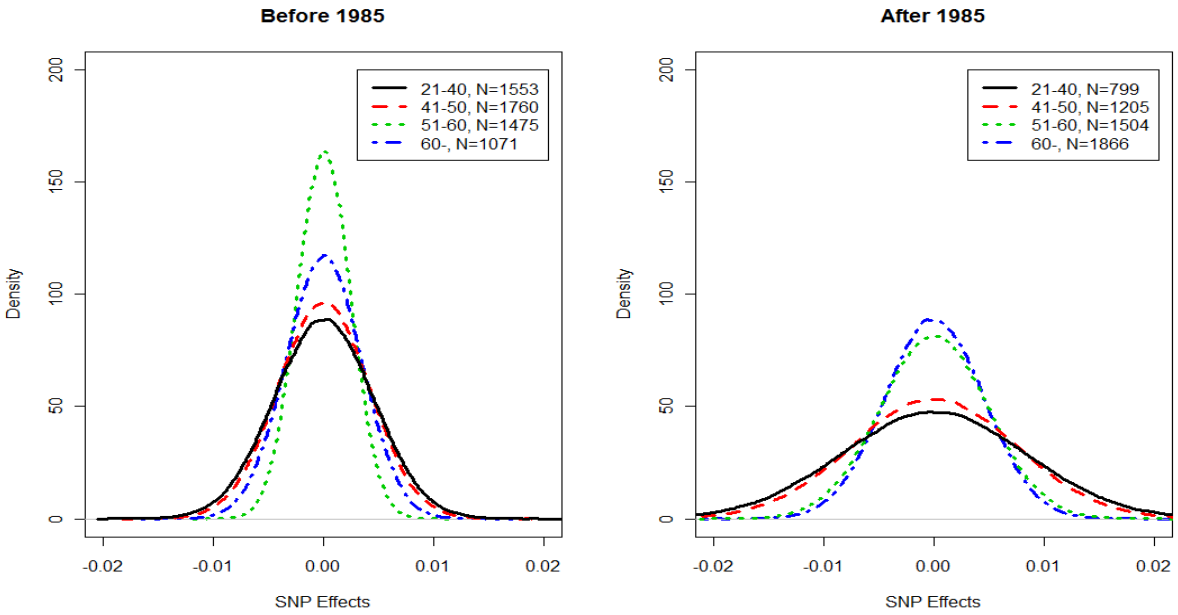


Figure 3. The two normal curves in each sub-figure represent two sets of estimated random effects, each random effect corresponding to one of a large number of SNPs. The GCTA analysis assumes that these random effects follow a normal distribution. The flatter the curve is, the large the random effects. A flatter curve indicates that more random effects are much larger than zero. Random effects of SNPs (Best Linear unbiased Predictors [PLUPs]) on BMI are substantially- larger after 1985 than before 1985 within each age group in the FHS (Panel 1); generally grow smaller with age within each historical period in the FHS (Panel 2); and substantially larger among those not engaged in heavy physical activity than those engaged in heavy physical activity in the age group of 21-50 (Panel 3). See the test results in Table 1.

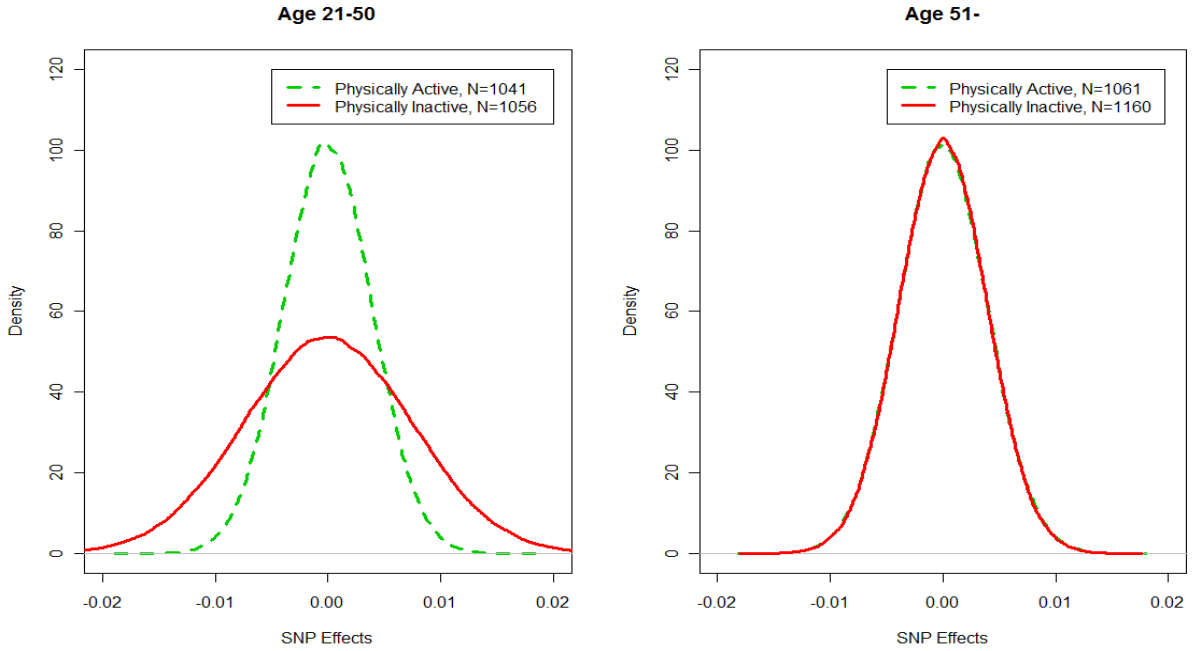
Panel 1



Panel 2


Before 1985
21-40, N=1553
41-50, N=1760
51-60, N=1475
60-, N=1071
Density
SNP Effects
After 1985
21-40, N=799
41-50, N=1205
51-60, N=1504
60-, N=1866
Density
SNP Effects

Panel 3


Age 21-50
Physically Active, N=1041
Physically Inactive, N=1056
Density
SNP Effects
Age 51-
Physically Active, N=1061
Physically Inactive, N=1160
Density
SNP Effects

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