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Genetic architecture of human obesity traits in the rhesus macaque

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Abstract

Objective: While the metabolic consequences of obesity have been studied extensively in the rhesus macaque, corollary genetic studies of obesity are non-existent. Here, we assess genetic contributions to spontaneous adiposity in this species.

Methods: We assessed phenotypic variation by age-class and sex for body mass index, waist-to-height ratio, waist-to-thigh ratio, and waist circumference in 583 macaques. We estimated total and sex-specific heritability for all traits, including waist-to-thigh ratio adjusted for BMI, as well as genotypic and phenotypic correlations. We also assessed functional genetic variation at *BDNF*, *FTO*, *LEP*, *LEPR*, *MC4R*, *PCSK1*, *POMC*, and *SIM1* in 4 animals with extreme spontaneous adiposity.

Results: Trait heritability in the combined sample was low-to-moderate (0.14–0.32), while sex-specific heritability was more substantial (0.20–0.67). Heritability was greater in females for all traits except BMI. All traits were robustly correlated, with genetic correlations of 0.63–0.93 indicating substantial pleiotropy. We discovered likely functional variants in the 4 macaques at all 8 human obesity genes, including 6 missense mutations in *BDNF*, *FTO*, *LEP*, *LEPR*, and *PCSK1*, and notably, 1 nonsense mutation in *LEPR*.

Conclusions: We find a moderate polygenic contribution to adiposity in rhesus macaques, and mutations with potentially larger effects in multiple genes that influence obesity in humans.

Keywords

Adiposity; obesity; animal models; genotype; obesity phenotypes

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INTRODUCTION:

Obesity affects ~36% of adults in the US[1], and reduces quality of life more severely than smoking, alcohol abuse, or 20 years of normal aging[2]. While poor diet and reduced physical activity play a large role in the current epidemic, obesity has long been known to have a strong genetic component. This is borne out by numerous reports of moderate to substantial heritability for body mass index (BMI), waist circumference (WC), and waist-to-hip ratio[3–7] (WHR), and by the well-described genetic forms of obesity, including monogenic, oligogenic, and polygenic forms[8]. Of these, polygenic obesity is considered to pose the greatest burden to public health. Numerous genetic variants have been associated with polygenic obesity, but almost all have effect sizes that account for only a fraction of reported heritability, even when multiple variants are considered in aggregate[3–5, 9–11]. Genetic determinants of obesity may also have effects that differ by sex; several recent studies have reported larger genetic effect sizes in women compared to men at loci influencing WHR and other indicators of central versus peripheral fat distribution[4–6,12]. These reports indicate that genotype-by-sex effects may also contribute to obesity.

Given this genetic complexity, studies in non-human primates (NHPs) that model closely both human physiology and segregating genetic variation would be a valuable complement to human studies. However, little is known about the genetic architecture of obesity in the Indian-origin rhesus macaque (*Macaca mulatta*), the NHP in which most academic biomedical research is conducted (https://orip.nih.gov/about-orip/research-highlights/nonhuman-primate-evaluation-and-analysis-part-1-analysis-future). Rhesus macaques develop spontaneous central obesity with age that is similar to that in humans, and with western-style diet or activity restriction is correlated with vascular dysfunction, dyslipidemia, insulin resistance, and overt type 2 diabetes[13–14]. More recently, Japanese macaques (*Macaca fuscata*), like rhesus macaques, were found to vary in their response to an energy dense diet, and several SNPs were associated with this resistance to obesity[15]. Genetic analysis of spontaneous adiposity in the rhesus macaque would thus be a natural extension of these previous studies, and is greatly facilitated by the availability of multi-generation pedigrees maintained by most national primate research centers.

In this study, we aimed to characterize genetic contributions to spontaneous adiposity in the rhesus macaque by assessing total and sex-specific heritability, as well as shared genetic effects, for BMI, waist-to-height ratio (WHtR), waist-to-thigh ratio (WTR), waist circumference (WC), and waist-to-thigh ratio adjusted for BMI (WTRadjBMI, a measure of fat distribution). We describe natural phenotypic variation by age and sex for these traits in 583 rhesus macaques belonging to a large, 6-generation pedigree, and maintained on chow diet. Further, to maximize the translational potential of results, we describe predicted functional sequence variation segregating at 8 genes that have been associated most repeatedly with monogenic and polygenic human obesity[8,16], i.e., *BDNF*, *FTO*, *LEP*, *LEPR*, *MC4R*, *PCSK1*, *POMC*, and *SIM1*, in 4 macaques with extreme values of BMI, WHtR, or WTR.

METHODS:

Animals and phenotyping:

Animals in this study were part of a single, 6-generation pedigree of 1,289 Indian-origin rhesus macaques housed at the Oregon National Primate Research Center (ONPRC). Macaques were included in this pedigree were ascertained at random with respect to adiposity. Under an IACUC protocol approved continuously from 2010–2015 (# 0875), animals were sedated for 15–20 minutes by intramuscular injection of 10–20 mgs/kg of ketamine, followed by the collection of adiposity measures and 20 mLs whole blood via femoral venipuncture into $EDTA^{2+}$. Measurements were taken using a pediatric stadiometer and measuring tape. Duplicate measures were collected for crown-rump length, abdominal circumference, and circumference of each thigh, and replicates were averaged to produce final data. Traits were derived as follows: BMI was calculated as weight in kilograms, divided by crown-rump length in meters-squared; WC was calculated as abdominal circumference in centimeters; WHtR was calculated as abdominal circumference in centimeters, divided by crown-rump length in centimeters; and WTR was calculated as abdominal circumference in centimeters divided by average thigh circumference in centimeters. WTRadjBMI was assessed as WTR adjusted for covariate effects of BMI during statistical analysis (phenotypic values for this trait are not shown). Macaques were removed from analyses if pregnant at the time of measurement, or if the animal had been in clinical care for at least 7 days within 30 days of measurement. Our combined analysis included 579–583 Indian-origin rhesus macaques (358–360 females and 221–223 males), spanning an age range of 1.3–24.7 years, which corresponds to a human age range of ~4–74 years. Macaques were assigned to age-classes as follows: “juveniles” aged 1 to <3 years, corresponding to 3–9 years in humans (pre-reproductive age in macaques); “young adults” aged 3 to <12 years (prime reproductive age in macaques), corresponding to 9–36 years in humans; “middle-age” adults aged ≥12 to <18 years, corresponding to 36–54 years in humans; and “geriatric” adults aged ≥18 years, corresponding to 55 to 74 years in humans (peri-menopausal and post-reproductive in macaques). Macaques <1 year of age were excluded from this study. To distinguish variation in adult adiposity from variation due to growth in macaques who had not yet achieved adult body size, we also assessed the distribution of all traits separately in males ≥8 years of age, and in females ≥7 years of age.

Diet and housing:

575 of the 583 animals were maintained on a commercial low-fat monkey chow diet (LabDiet® Lab Fiber-Balanced Monkey Diet, 14.7% fat, 27 ppm cholesterol, or LabDiet® High Protein Monkey Diet, 13.2% fat, 70 ppm cholesterol, PMI Nutrition International, Brentwood, MO) to which they had access *ad libitum*. Animals were offered food in excess to ensure that all animals had sufficient access to food irrespective of social dominance rank, which may confound heritability analysis of adiposity when food is limiting. Nine animals were assigned to the ONPRC Obese Resource at the time of measurement, having been identified previously as susceptible to weight gain, and thus were maintained on a high-fat diet. Housing type was recorded as either social group or paired-animal indoor housing, which reflects unlimited or limited physical activity, respectively.

Quantitative genetic analyses:

Heritabilities and trait correlations were estimated using a maximum likelihood-based variance components approach, which partitions phenotypic covariance among relatives into additive genetic and residual variance components[17] (details in SUPPLEMENTAL METHODS). Heritability is calculated as the proportion of phenotypic variance unexplained by covariates that can be attributed to additive genetic effects (i.e., $h^2 = [\sigma^2_G/\sigma^2_P]$. All traits were screened for covariate effects, which included age at the time of measurement, sex, age × sex, age^2, age^2 × sex, and housing type, and heritability was estimated from inverse-normalized residuals. Statistical significance of parameter estimates was assessed using likelihood ratio tests (LRTs)[18]. To estimate sex-specific heritability, raw data were stratified by sex, and covariate screening and estimation of heritability was conducted as described for the complete sample. Confidence intervals for heritability estimates were calculated using standard methods. All quantitative genetic analyses were conducted using SOLAR-Eclipse v.7.6.4 software[17] (© 1999–2015).

Sequencing:

Whole-exome sequence data from 4 macaques selected from the 85th–97th percentiles of BMI, WHtR, and WTR were used to discover functional genetic variants. Sequence data was collected as part of a larger study of macaque half-sibs matched for age-class and sex with large differences in HDL cholesterol levels. All sequencing data were generated on an Illumina HiSeq 2500 and processed to FASTQ format utilizing onboard Illumina software. Quality control, alignment to the MacaM version of the rhesus macaque genome[19], post-alignment processing, and subsequent variant identification was completed within the bioinformatics core at the ONPRC using methods as recommended in GATK Best Practices[20–22] (details in SUPPL. METHODS). The program SnpEff[23] (v3.6c) was used to annotate predicted functional variation in *BDNF*, *FTO*, *LEP*, *LEPR*, *MC4R*, *PCSK1*, *POMC*, and *SIM1*[8,16].

RESULTS

Effects of age, sex, and housing on phenotypic variation in adiposity:

As in humans, age, sex, and housing type as a proxy for physical activity all significantly influenced adiposity in macaques. All measures of adiposity increased with age for both males and females, but males consistently displayed larger values than females (FIGURE 1; FIGURE 2; TABLE S1). While age had a significant effect on all adiposity traits, significant differences by sex were also found in both the combined and adults-only samples for BMI, in the combined sample only for WTR, and in the adults-only sample for WC and WHtR (TABLE 1). Indoor-housed macaques had higher median values for all adiposity traits than did group-housed macaques (TABLE 2), and these differences were statistically significant for all traits except BMI. Fasting insulin levels increased with all measures of adiposity, and were also significantly greater in indoor-housed macaques than in group-housed macaques (one extreme outlier in fasting insulin levels was removed for this analysis) (FIGURE 3; TABLE 2).

In our variance components analyses, while age and its interactions with sex were a significant predictor of variation in most traits, sex had a significant effect on variation in BMI, WTR, WTRadjBMI, and WC, but not on WHtR (TABLE 3). In the same analyses, housing type had a significant influence on BMI, WHtR, WTR, and WTRadjBMI, but, unexpectedly, not on WC. Effects of sex, age, and housing type accounted for up to approximately one-half of total phenotypic variance in adiposity.

Heritability of adiposity:

Heritability estimated in the combined sample of males and females supports a low-to-moderate genetic contribution to adiposity in macaques (TABLE 3). However, for all traits, stratification by sex apportioned heritability differently between males and females, with sex-specific heritability consistently exceeding heritability in the combined sample. In most cases, heritability was increased in females and reduced or non-significant in males, with the exception of BMI, for which heritability was considerably greater in males. Notably, 95% confidence intervals for the sex-specific heritability estimates for BMI, WHtR, WTR, and WC do not overlap. In analyses of WTRadjBMI in macaques, BMI was a highly significant contributor to phenotypic variation in WTR ($P=1.2 \times 10^{-29}$), and together with effects of age and sex accounted for a substantial proportion of its phenotypic variance. Heritability for WTRadjBMI in the combined sample of males and females was low but statistically significant; as with other adiposity traits, stratification by sex revealed that heritability of this trait was increased in females and not statistically significant in males, although confidence intervals for this trait overlapped between males and females.

Phenotypic and genetic correlation among adiposity traits:

Phenotypic correlations between BMI, WHtR, WTR, and WC confirmed strong relationships among these 4 traits, with the weakest occurring between BMI and WTR (TABLE 4). Genetic correlations indicate substantial shared genetic effects (i.e., pleiotropy) among all 4 traits, accounting for ~40–86% of covariation between traits, but also confirmed the presence of independent genetic effects on each trait. However, we note that the same measures are used in multiple traits, and thus a portion of the correlation observed between these traits will be due to self-correlation.

Predicted functional genetic variation in macaques at established human obesity genes:

Functional variants were found in all 8 human obesity genes among the 4 animals selected from the extremes of spontaneous adiposity (TABLE S2; TABLE S3). A total of 840 functional variants were discovered, including several mutations with expected moderate or high impact. Most notably, these include a premature stop mutation in *LEPR*, and 6 additional missense (i.e., amino-acid changing) mutations in *BDNF*, *FTO*, *LEP*, *LEPR*, and *PCSK1*. An additional 833 mutations with expected lower-impact effects on protein function were also found.

DISCUSSION:

Phenotypic variation and effects of age and sex:

BMI, WHtR, WTR, and WC are all traits used in human populations as indirect measures of adiposity, due to their robust correlation with percentage body fat. Like humans, NHPs display considerable inter-individual variation in adiposity that is influenced by age, sex, physical activity, and genetics. While the effects of sex varied by trait, all trait values increased consistently with age and with indoor-housing, underscoring the impact of aging and reduced physical activity on adiposity. In addition to the effects of aging, sex, and physical activity on adiposity, all adiposity traits were also heritable. These contributions of age, sex, physical activity, and genetic variation illustrate the interaction of genetic and environmental components that regulate adiposity in NHPs and are consistent with a trait architecture in NHPs similar to that in humans.

BMI is used routinely in adults >20 years of age as an indirect measure of overall adiposity. BMI increases dramatically from young adulthood to middle-age, is greatest in middle-age, and decreases in old age[24,25]. Except for the youngest and oldest age-classes, men have higher BMI than women, but the decline in BMI at old age is greater for women[24]. In humans, BMI has defined clinical thresholds that correspond to adiposity: underweight (<18.5), normal weight (18.5–24.9), overweight (25–29.9), or obese (>30). While similar thresholds are not defined for macaques, an unpublished analysis of 11 female macaques fed a western-style diet for 1 year found that BMI was strongly correlated with total fat mass, percent body fat, and percent visceral fat as measured by dual-energy X-ray absorptiometry, i.e., DXA (r = 0.84, 0.75, and 0.78, respectively, P=0.0013–0.0084; C. Bethea, pers. comm.). These correlations are strikingly similar to those reported in Flegal et al.[26] between BMI and percentage body fat measured by DXA in a representative population sample of 12,901 men and women of all ages in the National Health and Nutrition Examination Survey (NHANES, 1999–2004). Moreover, in this study all 4 macaques selected from the upper percentiles of adiposity had BMI measures >30. Thus, the distribution of BMI in macaques reported here supports a similar degree of adiposity to those implied by clinical thresholds in humans.

In macaques, BMI increases consistently with age for both males and females, and is greatest in the oldest macaques. Additionally, males have greater BMI than females in every age-class, and these differences are the greatest in the oldest age-class. While BMI in macaques of both sexes is greatest in those defined as “geriatric” in this study (≥18 years), this age range in macaques is equivalent to 54–74 years in humans, an age range that is more appropriately described as spanning middle-to-early old age in humans, and during which BMI values in humans are also greatest[24,25]. Thus, the label of “geriatric” in this study should not be interpreted as equivalent to old age in humans, a life stage during which BMI declines.

WC measures central rather than overall adiposity, and for this reason is more closely associated with visceral fat and health risks associated with obesity[27,28]. When standardized by height, waist circumference (i.e., WHtR) is more closely correlated with percentage body fat than WC alone in both men and women of all ages and is a highly effective indicator of cardiovascular risk[26,29–30]. In humans, WC and WHtR increase across all age groups and

are consistently greater in men than in women[31]. In macaques, WC and WHtR also increase with age in both males and females, and achieve their largest values in the oldest macaques, reinforcing the idea that the greatest amount of adiposity occurs in middle-to-early old age in macaques. While the greatest differences in both traits between males and females occurs again in the oldest age-class, sex was a more consistent predictor of variation in WC than in WHtR. This findings suggests that WHtR may not capture aspects of adiposity in macaques that are most impacted by sex.

WTR summarizes the distribution of body fat between central and peripheral depots and may offer a more clinically important description of total adiposity than does waist circumference alone[32]. In human cohorts, WTR increases with age in both men and women, but there are striking differences by sex[32,33]. These differences by sex are highly age-specific, in that the largest gains in WTR occur between young adulthood and middle age for men, but between middle- and old age for women. Further, the greatest differences between men and women in WTR occur in early middle-age. In macaques, the largest gains in WTR occur between “middle-age” and “geriatric” animals for both males and females, and the largest differences between males and females are again found in the “geriatric” age-class. Interestingly, sex-specific differences in age-related gains are observed for WC rather than WTR in macaques, with the largest gains in WC occurring between “juvenile” and “young adult” age-classes for females, but between “young adult” and “middle-age” age-classes for males. Consistent with these differences between WTR and WC, differences by sex appear greater for WC than for WTR, and the contribution to variation in WC by age, sex, and their interaction is the largest among all the traits. We conclude that effects of age and sex on adiposity are similar between macaques and humans for some (BMI, WHtR, WC), but not all (WTR) traits.

Heritability and shared genetic effects on adiposity:

In macaques, the combined-sex heritability estimates for BMI, WHtR, WTR, and WC were moderate, but somewhat lower than that reported in some human studies[3–4,6,34–35,37]. However, our estimates of overall heritability for adiposity in macaques correspond reasonably well with heritability reported for total fat mass (0.41) and adipocyte cell volume (0.37) in baboons[36]. One potential limitation to our study is the possibility of mis-specified relationships in our pedigree that have yet to be revealed by whole-genome sequencing of all pedigree members, a project that is currently underway. Any such pedigree errors are likely to negatively impact the estimation of heritability. However, the estimated heritability of adiposity also depends greatly on experimental design, and may be inflated particularly in classical twin studies[38]. Indeed, family-based studies based on relative pairs other than twins typically produce lower and potentially more accurate estimates of heritability (see Robinson et al.[3] for a discussion of why ~0.4 is likely to be the true heritability for BMI).

We found that heritability increased considerably when analyses were stratified by sex. For example, heritability of BMI in male macaques was substantial, more than double the heritability in the combined sample. Greater heritability of BMI in men than in women has been reported previously in a twin study[39], although other studies have yielded conflicting results[6,12]. Our heritability estimates for BMI are significantly different between males and

females, as indicated by non-overlapping confidence intervals. However, differences in heritability between the sexes may indicate either differences in additive genetic variance between males and females, or differences in other contributions to total phenotypic variation, e.g., epistasis, gene-by-environment interactions, and/or environmental variance. In human studies, genotype-by-sex effects on BMI are controversial, with evidence reported that both supports[35,39] and rejects[40] this hypothesis.

Our sex-specific estimates of heritability for WHtR, WTR, and WC in macaques are also similar to those in several human studies, both in magnitude and in the direction of differences by sex[6,35]. Increased heritability in females may result if additive genetic variance between males and females is equivalent, but other sources of variation are reduced in females relative to males. Indeed, despite a substantially larger sample size, females displayed significantly lower phenotypic variation than males for all adiposity measures (data not shown). However, heritability was not uniformly greater in females for all traits, i.e., for BMI, heritability was greater in males despite their greater phenotypic variation. As this was a pilot study, we did not have sufficient power to test explicitly for genotype-by-sex effects on any trait, but our findings indicate this would be a valuable direction for future research.

In recent years, SNPs associated with WHR adjusted for BMI ("WHRadjBMI") have been reported in several large population-based studies[4,11]. In particular, WHRadjBMI has been used to explore sex-specific genetic effects on adiposity, with several studies finding locus-specific effects in women that were reduced or absent in men[4,5,12]. While sex was a significant predictor of phenotypic variation in WTRadjBMI, our analogous measure of fat distribution in macaques, both combined and sex-specific heritability estimates for WTRadjBMI were low, and did not differ significantly between males and females. This was an unexpected result, given the magnitude of sex-specific heritability in females for WTR, and the genetic correlation between BMI and WTR, which was the lowest among all trait combinations. Our results suggest that WTRadjBMI may not capture the same sex-specific genetic effects on fat distribution in macaques that WHRadjBMI does in humans. Despite these caveats, our finding of greater heritability for WTRadjBMI in female macaques compared to males is consistent with similar reports in human studies of the enrichment of genetic effects on WHRadjBMI in women.

Both phenotypic and additive genetic correlations among BMI, WHtR, WTR, and WC are similar to those reported in human studies[26,32,35]. In particular, the robust genetic correlations indicate the substantial degree to which additive genetic effects are shared among these traits. We note that the lowest phenotypic and genetic correlations were those found between WTR and BMI. This is consistent with reports in human studies of distinct loci influencing WHRadjBMI and BMI, and with our finding of heritability for WTRadjBMI, which taken together suggest underlying differences in the genetic architecture of fat distribution and overall adiposity[5].

Functional genetic mutation in 4 macaques with extreme spontaneous adiposity:

All 4 macaques carried from 4–8 missense mutations in *BDNF*, *FTO*, *LEP*, *LEPR*, and *PCSK1* (TABLE S3). An analysis of circulating leptin in all 4 macaques with extreme

adiposity, as compared with 4 unrelated, chow-fed, age- and sex-matched controls selected from the 40th-60th percentiles of BMI, WHtR, and WTR, found that the 4 macaque "cases" had substantially greater median circulating leptin (9.18 versus 5.24 ng/mL, data not shown) than did the healthy controls. Of particular interest, the only macaque carrying missense mutations in 4 out of 5 genes was also heterozygous for a high-impact premature stop mutation in *LEPR*. In addition to this nonsense mutation, this female was homozygous for 2 other missense variants in *LEPR*, and heterozygous for the missense variants described in *BDNF*, *FTO*, and *LEP* (TABLE S3). Colony records indicated that this female had been housed permanently in a large, natural social group enclosure since birth, and maintained on chow diet. Despite these considerable advantages of diet and physical activity, this female ranked above the 95th sex-specific percentile for BMI, above the 85th percentile for WHtR and WC, and above the 80th percentile for insulin levels. Her body condition score beginning at age 5 (corresponding to ~15 years of age in humans) was "4-Heavy", characterized as a loss of definition in bony contours, increasing subcutaneous fat, and the accumulation of fat deposits in the inguinal, axillary, or abdominal regions[41]. A review of her clinical history indicated a marked propensity for gaining weight, including an unusual weight gain of 0.5 kg while nursing an infant (K. Prongay, pers. comm.). These findings suggest that macaques segregate genetic mutations with effects on spontaneous adiposity via the leptin signaling pathway.

We conclude there is a close correspondence between macaques and humans for: 1) phenotypic variation in traits that are robust indicators of percentage body fat and associated health risks; and 2) moderate polygenic effects on adiposity, as well as functional genetic variation likely to have larger (i.e., oligogenic) effects on adiposity, all of which will influence response to obesogenic challenge. In the future, analyses of whole-genome sequence data in macaques will permit the identification of genetic variants, including rare or regulatory variants, that influence diet-induced obesity and associated sub-clinical precursors of disease, i.e., dyslipidemias, vascular inflammation, and insulin resistance, all of which have been demonstrated previously in this species by others.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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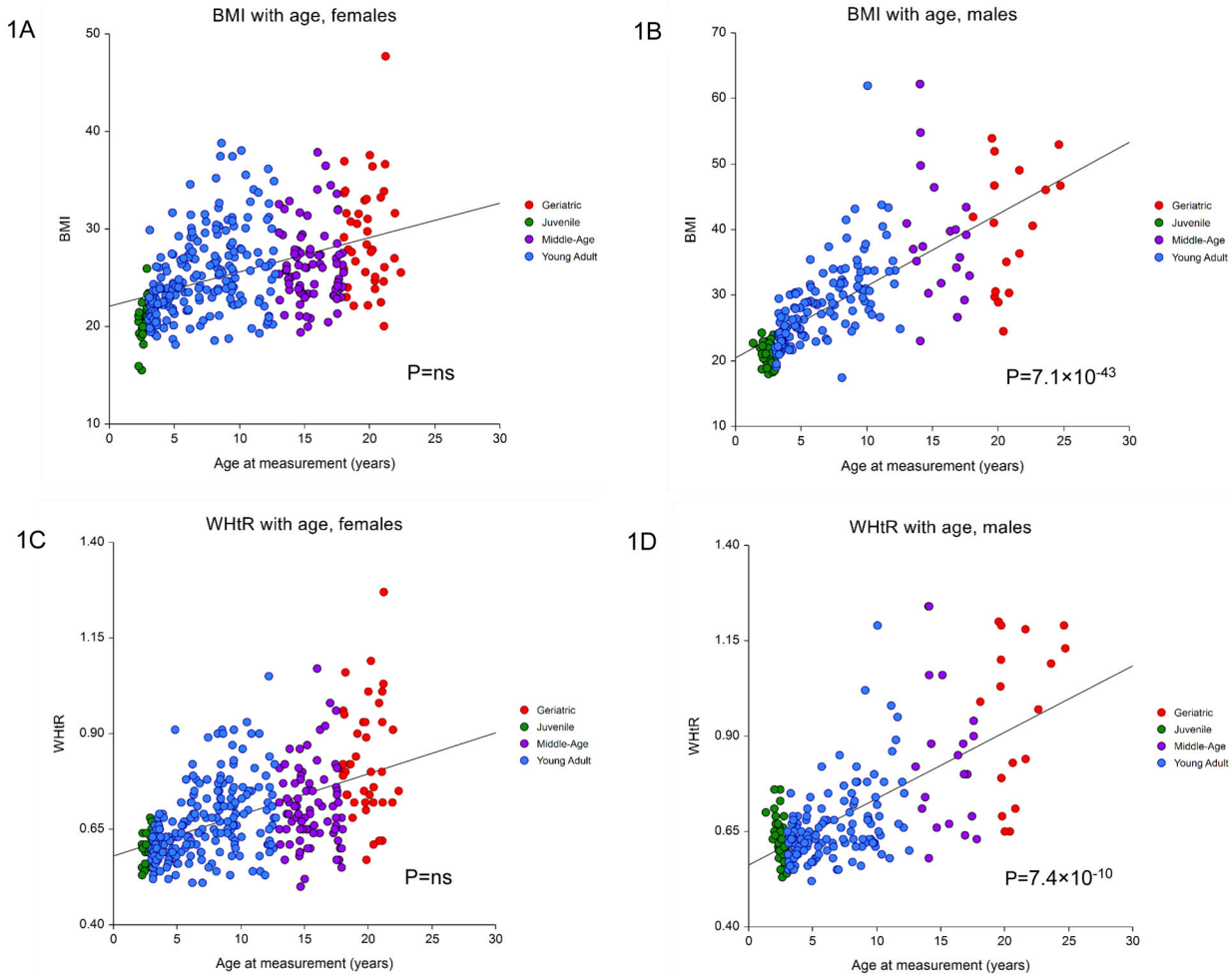
STUDY IMPORTANCE:

1. What is already known about this subject?

- The rhesus macaque is a well-established model for metabolic dysregulation that accompanies obesity;
- However, studies of genetic influences on adiposity or obesity in non-human primates are rare, and none have been conducted in this species

2. What does this study add?

- This study tests hypotheses of total and sex-specific heritability in the rhesus macaque for 5 traits associated with human obesity, and describes functional genetic variation at 8 obesity genes in 4 animals with spontaneous extreme adiposity



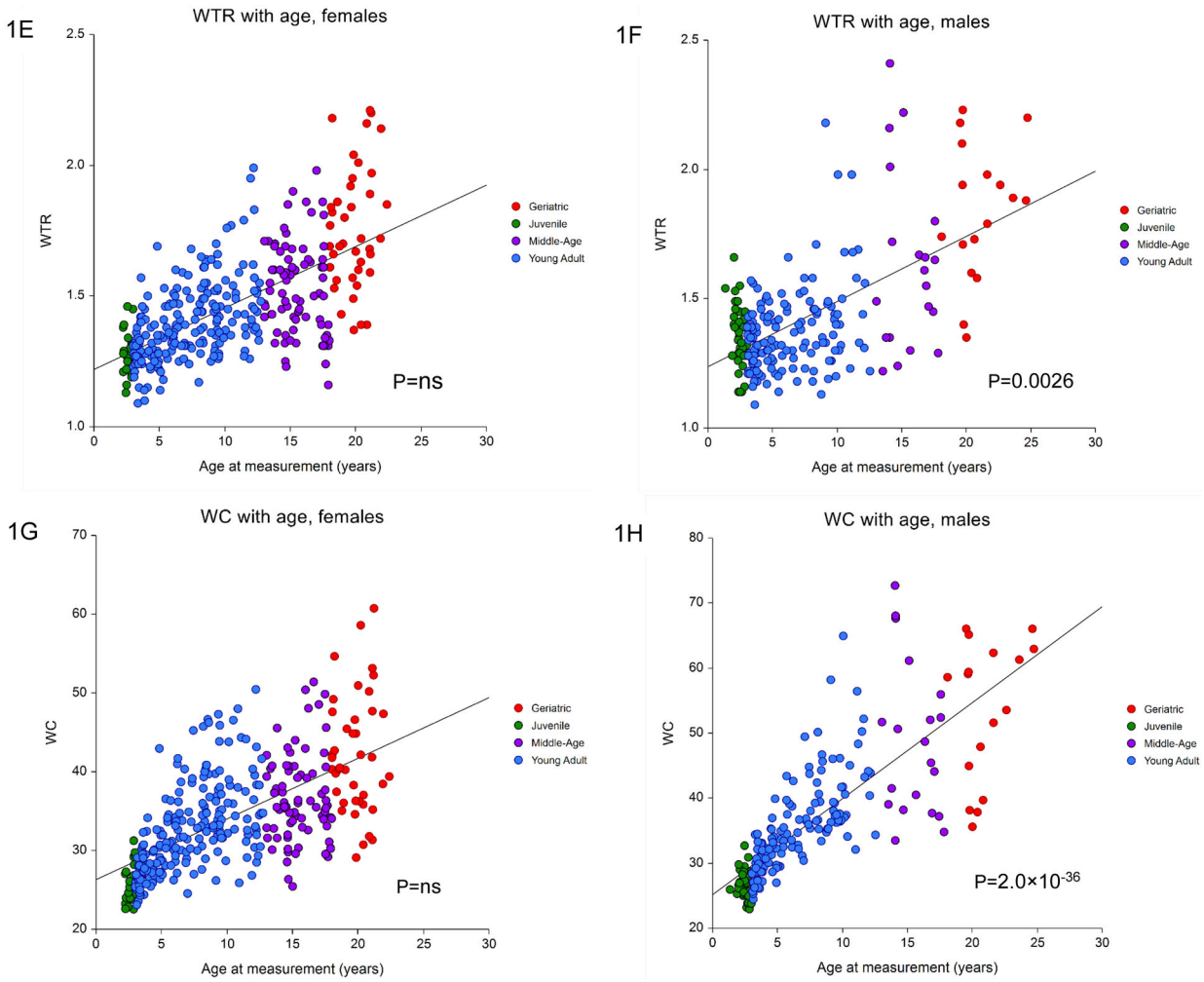


FIGURE 1, A–H.

Adiposity by age-class, plotted separately for males and females with regression lines. P-values are given for the effect of age in sex-stratified variance component analyses. Plots generated using NCSS 12 Statistical Software (2018). NCSS, LLC. Kaysville, Utah, USA, ncss.com/software/ncss.

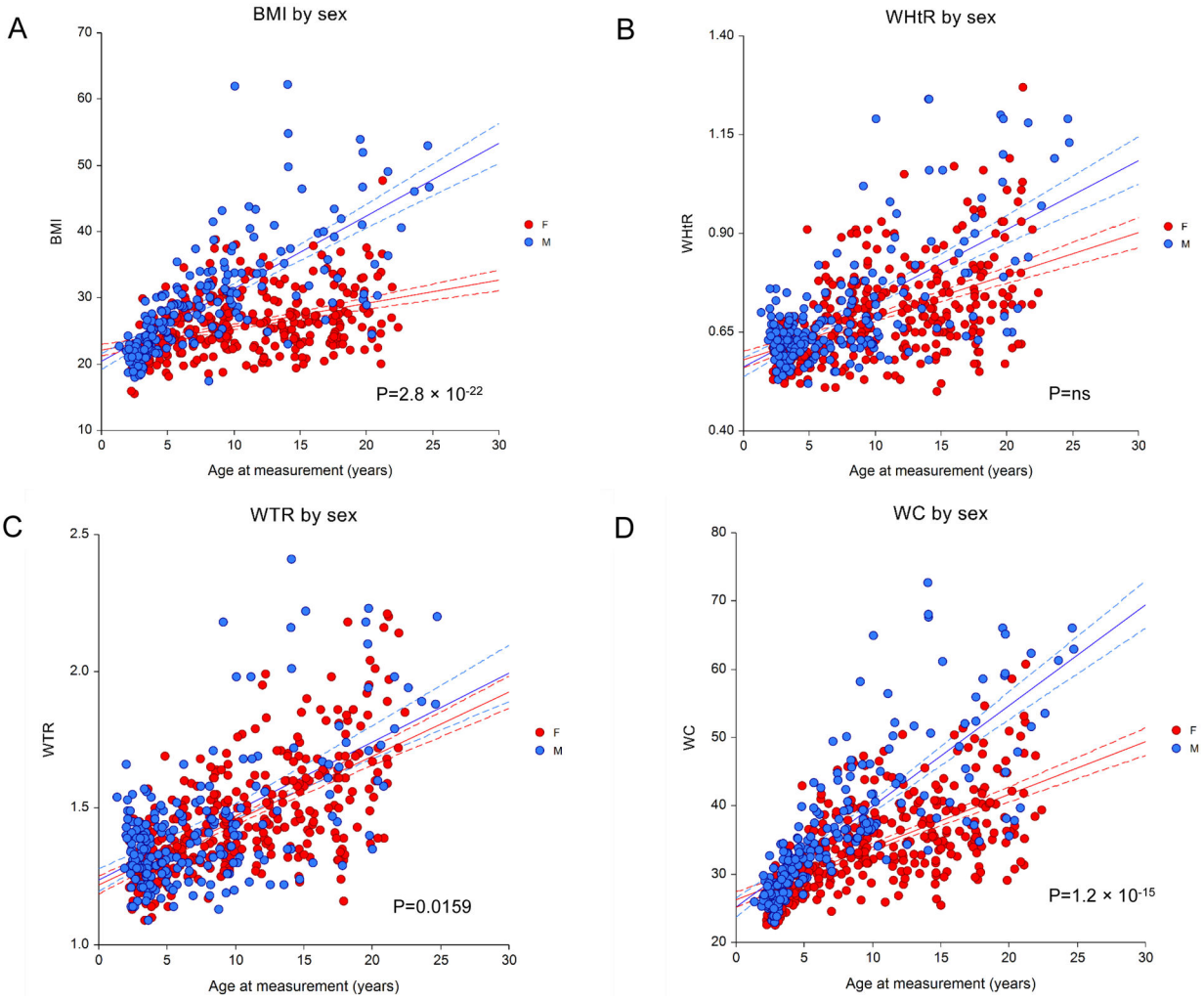


FIGURE 2, A–D.

Adiposity for males and females with age, with sex-specific regression lines. Dashed lines indicate 95% confidence intervals for the line. P-values are given for the effect of sex in variance component analyses of the combined sample. Plots generated using NCSS 12 Statistical Software (2018). NCSS, LLC. Kaysville, Utah, USA, ncss.com/software/ncss.

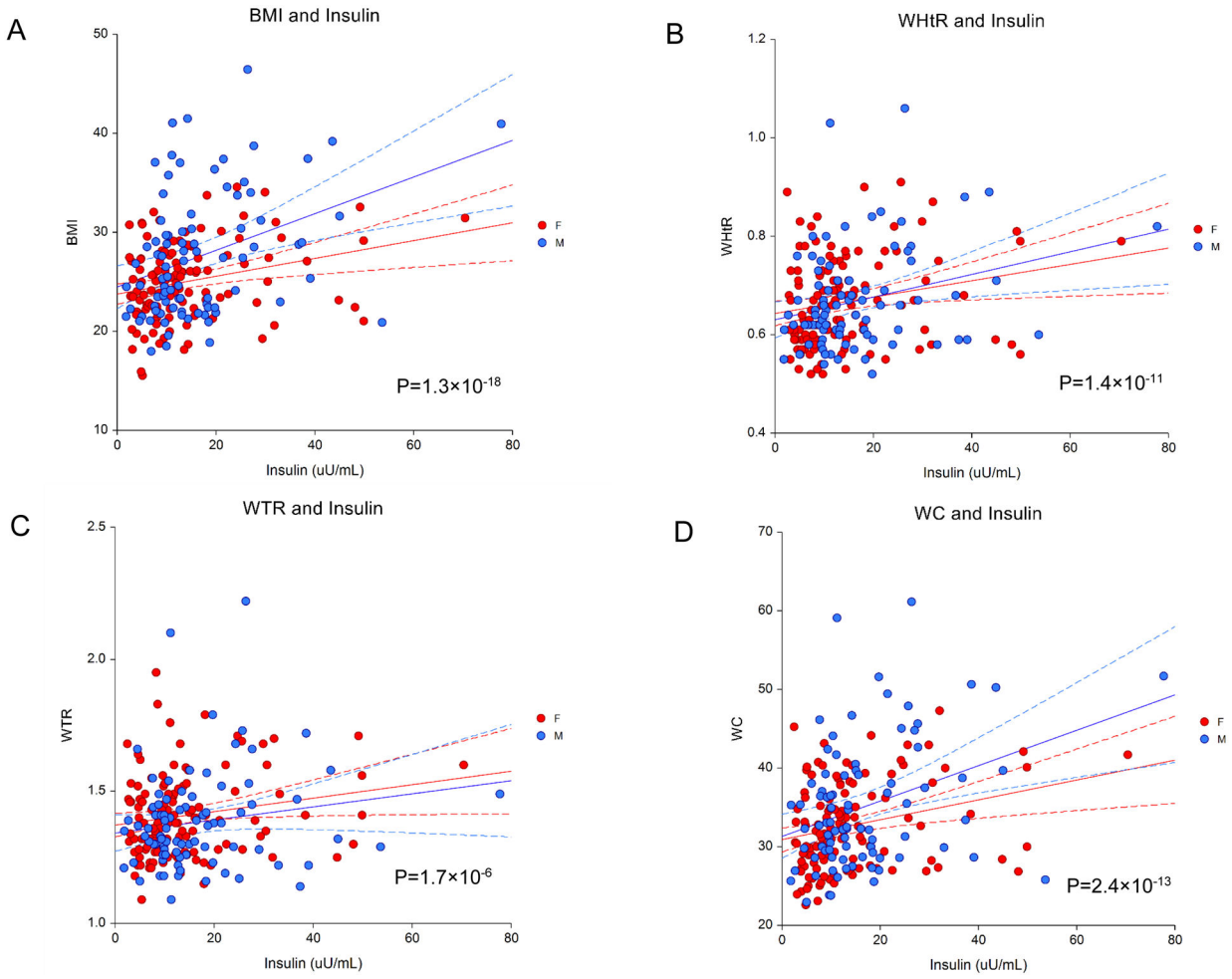


FIGURE 3, A–D.

Fasting insulin levels with adiposity for males and females, with sex-specific regression lines. Dashed lines indicate 95% confidence intervals for the regression line. P-values are given for the effect of insulin in variance component analyses in the combined sample. Plots generated using NCSS 12 Statistical Software (2018). NCSS, LLC. Kaysville, Utah, USA, ncss.com/software/ncss.

TABLE 1.

Distribution of spontaneous adiposity (N=579−583) in chow-fed rhesus macaques. All values are unadjusted. Results are shown both for the combined sample (i.e., “ALL”), and for only males and females that achieved full adult height and weight (i.e., “ADULT”). Body mass index (BMI); waist-to-height ratio (WHtR); waist-to-thigh ratio (WTR); waist circumference (WC). P-values for differences by sex determined using the Kolmogorov-Smirnov test (NCSS 12 Statistical Software (2018), NCSS, LLC. Kaysville, Utah, USA). P-values <0.05 are in bold.

	MALES	MIN, MAX	1ST QUARTILE	MEDIAN	3RD QUARTILE	FEMALES	MIN, MAX	1ST QUARTILE	MEDIAN	3RD QUARTILE	DIFFERENCES BY SEX
BMI (ALL)	223	17.44–62.21	22.52	26.53	31.84	360	15.53–47.71	22.35	24.83	27.90	**1.4×10^{-5}**
WHtR (ALL)	221	0.52–1.24	0.61	0.65	0.72	358	0.50–1.27	0.60	0.66	0.75	0.064
WTR (ALL)	221	1.09–2.41	1.28	1.38	1.49	358	1.09–2.21	1.31	1.41	1.58	**0.027**
WC (ALL)	221	22.95–72.7	28.65	33.50	39.80	359	22.55–60.75	28.90	33.10	38.25	0.099
BMI (ADULT)	82	17.44–62.21	30.25	33.84	40.68	222	18.56–47.71	23.86	26.40	30.29	**1.0×10^{-7}**
WHtR (ADULT)	82	0.56–1.24	0.65	0.74	0.89	222	0.50–1.27	0.65	0.71	0.80	**0.048**
WTR (ADULT)	82	1.13–2.41	1.33	1.47	1.72	223	1.16–2.21	1.39	1.51	1.65	0.184
WC (ADULT)	82	32.10–72.70	37.36	42.83	52.09	223	25.45–60.75	32.80	36.05	40.50	**$<1.0 \times 10^{-7}$**

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TABLE 2.

Distribution of spontaneous adiposity (N=579−583) and fasting insulin levels in chow-fed rhesus macaques housed in either social groups or indoors. All values are unadjusted. Body mass index (BMI); insulin (N=216; insulin levels in μU/mL); waist-to-height ratio (WHtR); waist-to-thigh ratio (WTR); waist circumference (WC). P-values for differences in adiposity by housing type determined using the Kolmogorov-Smirnov test (NCSS 12 Statistical Software (2018), NCSS, LLC. Kaysville, Utah, USA). P-values <0.05 are in bold.

Trait	Group-housed, Median (Range)	N	Indoor-housed, Median (Range)	N	P-value
BMI	24.57 (17.99–43.43)	323	26.06 (15.53–62.21)	260	0.111
WHtR	0.64 (0.52–1.05)	319	0.68 (0.5–1.27)	260	**2.8×10^{-3}**
WTR	1.34 (1.09–1.99)	319	1.47 (1.1–2.41)	260	**$<1.0\times10^{-7}$**
WC	31.83 (22.55–55.95)	320	35.20 (22.60–72.70)	260	**8.0×10^{-6}**
INSULIN	9.95 (1.79–70.4)	135	13.56 (2.67–516.9)	81	**0.003**

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TABLE 3.

Variance components analysis of spontaneous adiposity in the rhesus macaque. Heritability estimates (P-values) are shown for the combined sample, and when stratified by sex; heritability estimates with P-values <0.05, and non-overlapping confidence intervals for sex-stratified heritability are indicated in bold. c^2: proportion of total phenotypic variance explained by significant covariates in the combined sample. Weight-to-height ratio (WHtR); body mass index (BMI); waist-to-thigh ratio (WTR); waist-to-thigh ratio adjusted for BMI (WTRadjBMI); waist circumference (WC). All genetic analyses were conducted using SOLAR Eclipse v.7.6.4 software (© 1999–2015).

TRAIT	N	h^2, All (P-value)	Significant covariates	c^2	N, Females	h^2, Females (P-value)	95% CI, h^2 in females	N, Males	h^2, Males (P-value)	95% CI, h^2 in males
BMI	583	**0.31 (4.0×10^{-7})**	Age, Sex, Age × Sex, Age^2, Age^2 × Sex, Housing	0.49	360	**0.38 (1.5×10^{-4})**	**0.280, 0.484**	223	**0.67 (5.1×10^{-5})**	**0.537, 0.797**
WHtR	579	**0.32 (7.2×10^{-5})**	Age, Housing	0.29	358	**0.43 (1.8×10^{-4})**	**0.331, 0.535**	221	0.10 (0.228)	**−0.024, 0.233**
WTR	579	**0.23 (0.004)**	Age, Sex, Age × Sex, Age^2, Age^2 × Sex, Housing	0.38	358	**0.53 (1.8×10^{-6})**	**0.431, 0.635**	221	0.18 (0.063)	**0.056, 0.314**
WTRadjBMI	576	**0.14 (0.023)**	Age, Sex, Age × Sex, Age^2, Age^2 × Sex, Housing, BMI	0.51	357	**0.20 (0.027)**	0.098, 0.302	221	0.15 (0.090)	0.016, 0.274
WC	580	**0.32 (2.0×10^{-5})**	Age, Sex, Age × Sex, Age^2	0.56	359	**0.56 (4.0×10^{-7})**	**0.456, 0.659**	221	**0.29 (0.017)**	**0.164, 0.422**

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TABLE 4.

Phenotypic correlations (shaded, above the diagonal) and genetic correlations (unshaded, below the diagonal) between all adiposity measures in rhesus macaques (N=583). Body mass index (BMI); waist-to-height ratio (WHtR); waist-to-thigh ratio (WTR); waist circumference (WC). P-values are listed in parentheses; those <0.05 are indicated in bold. For genetic correlations, P-values indicate significant differences from 0 and from 1, respectively. All genetic analyses conducted using SOLAR Eclipse v7.6.4. (© 1999–2015) software.

	BMI	WHtR	WTR	WC
BMI	--------	**0.72 (1.4×10^{-76})**	**0.39 (1.8×10^{-20})**	**0.76 (3.8×10^{-92})**
WHtR	**0.89 (3.5×10^{-5}, 0.026)**	--------	**0.74 (1.3×10^{-81})**	**0.88 (2.8×10^{-159})**
WTR	**0.63 (0.008, 0.011)**	**0.82 (0.007, 0.011)**	--------	**0.69 (2.6×10^{-68})**
WC	**0.88 (2.0×10^{-5}, 0.020)**	**0.93 (1.8×10^{-4}, 0.005)**	**0.71 (0.014, 0.005)**	--------