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The sex-specific genetic architecture of quantitative traits in humans

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Mapping genetically complex traits remains one of the greatest challenges in human genetics today. In particular, gene-environment and gene-gene interactions, genetic heterogeneity and incomplete penetrance make thorough genetic dissection of complex traits difficult, if not impossible. Sex could be considered an environmental factor that can modify both penetrance and expressivity of a wide variety of traits. Sex is easily determined and has measurable effects on recognizable morphology; neurobiological circuits; susceptibility to autoimmune disease, diabetes, asthma, cardiovascular and psychiatric disease; and quantitative traits like blood pressure, obesity and lipid levels, among others. In this study, we evaluated sex-specific heritability and genome-wide linkages for 17 quantitative traits in the Hutterites. The results of this study could have important implications for mapping complex trait genes.

Recently, it was found that genes on the X chromosome show astounding variation in expression and action between males and females[1]; the same is likely to be true for autosomal genes. The cellular environment in men and women can be considered very different, given known differences in hormonal milieu and gene expression[2]. Therefore, it is not unreasonable to suggest that gene-environment interactions lead to different effects of the same variation in men and women, impacting gene mapping for complex traits.

In model organisms such as *Drosophila melanogaster*, *Mus musculus* and *Rattus norvegicus*, many quantitative traits show sex-specific architecture, from life span to bristle number to cholesterol level[3–5]. In humans, traits ranging from easily recognizable morphology to brain development to immune function are known to be sexually dimorphic. In fact, sex could be considered an easily measured environmental factor that can modify both penetrance and expressivity of a wide variety of traits. Many complex diseases with a large impact on public health are sexually dimorphic or have different disease courses in the sexes. For example, type 2 diabetes is more prevalent in males[6], as is depression in females[7]. Type 1 diabetes[8], asthma[9] and cardiovascular disease[10] show age-sex interactions in which the sex ratio varies with age or in which age-of-onset is affected by sex. The importance of sex differences in disease course and prevalence and in response to drugs has recently been highlighted (for examples, see the 10 June 2005 issue of *Science*), but little is known about the underlying genetic architecture of these differences. Failing to model for sex-specific architecture may significantly hamper detection of susceptibility loci in genome-wide screens for complex traits[11]. In humans, although candidate genes are sometimes tested for sex-specific effects, few traits have been tested for sex-specific susceptibility loci in genome-wide screens. Psychiatric traits such as autism[12], neuroticism[13] and mood disorders[14], as well as immune-mediated disorders such as inflammatory bowel disease[15] and osteoarthritis[16] have shown sex-specific linkages. Serotonin and serum cortisol levels in the Hutterites, a founder population, have also shown marked sex-specific architecture[17,18].

In order to determine whether sex-specific genetics is limited to certain traits or is a more general phenomenon, in this study, we evaluated sex-specific heritability and genome-wide linkages for 17 quantitative traits in the Hutterites: low-density lipoprotein cholesterol (LDL-c), high-density lipoprotein cholesterol (HDL-c), triglycerides, lipoprotein (a) (Lp(a)), diastolic blood pressure, systolic blood pressure, body mass index, fasting insulin, adult height, percent predicted forced expiratory volume at 1 s (percent predicted FEV_1), ratio of FEV_1 over forced vital capacity (FEV_1/FVC), eosinophilia, total serum immunoglobulin E (IgE), lymphocyte count, percentage of lymphocytes per white blood cell count, morning serum cortisol and whole blood serotonin. Here we assess the sex-specific genetic architecture of these 17 quantitative traits in a population-based sample.

Of the 17 quantitative traits assessed, the distribution of 11 differed between the sexes at $P < 0.05$ (**Table 1**). Two additional traits showed modest evidence for sex differences ($0.05 < P < 0.1$). Of the lipids, LDL-c was not sexually dimorphic, and Lp(a) measurements showed modest evidence of dimorphism, although HDL-c and triglycerides were strongly sexually dimorphic. All anthropometric measurements differed by sex, as did most immune measures (with the exception of lymphocyte count) and serotonin levels. Percent predicted FEV_1 was sexually dimorphic, but FEV_1/FVC was not, probably because the ratio cancels out the sex effects in both measures. Of the two

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Table 1 Quantitative traits examined in this study

Trait	*P*-value
LDL-c	0.27
HDL-c	3.6×10^{-15}
Triglycerides	7.2×10^{-5}
Lp(a)	0.086
DBP	1.9×10^{-12}
SBP	9.8×10^{-13}
BMI	0.0015
Insulin	0.51
Height	2.7×10^{-94}
FEV_1/FVC	0.24
FEV_1	9.0×10^{-5}
Eos	0.018
IgE	6.9×10^{-12}
Lymph	0.47
% Lymph	0.0073
Cortisol	0.051
Serotonin	2.0×10^{-9}

The *P*-values reported correspond to the effects of sex as a covariate in a linear regression model (Methods). In these analyses, we used FEV_1 measures and included height as a covariate in order to assess the significance of sex for this trait, and not the percent predicted FEV_1 (which is corrected for sex and height) that is used in the mapping studies.

hormones, insulin was not sexually dimorphic, and cortisol showed only modest evidence of a sex difference.

Five traits showed statistically significant sex interactions in the heritability models (**Figure 1**). For LDL-c, although the estimates of broad heritability (H^2) for males and females were similar and high, the estimates for narrow (or additive) heritability (h^2) were very different ($h^2_{male} = 0.52$, $h^2_{female} = 0.29$, $P_{interaction} = 0.0012$). Similarly, for systolic blood pressure, the narrow heritability was estimated to be 0 for each sample, but the broad heritabilities were significantly different ($H^2_{male} = 1.0$, $H^2_{female} = 0.20$, $P_{interaction} = 0.00047$). For HDL-c, the best model in males included an inbreeding component, whereas the best model in females included only additive components ($h^2_{male} = 0.54$, $H^2_{male} = 0.78$, $h^2_{female} = 0.70$, $P_{interaction} = 0.0024$). For height and insulin, the estimates for males and females were not significantly different, but there were significant sex interactions ($P_{interaction} = 0.021$ and 0.022, respectively). In addition to these five traits, for lymphocyte count, the male model included only additive variance, whereas the female model included a dominance component ($h^2_{male} = 0.57$, $h^2_{female} = 0.15$, $H^2_{female} = 0.99$, $P_{interaction} > 0.05$). For all other traits, there were neither significant differences between the heritability models in the sexes nor significant sex interaction, although in many cases the point estimates were different, but standard error was also large (for example, see Lp(a), body mass index and FEV_1/FVC in **Fig. 1**). In some cases, such as percent predicted FEV_1, IgE and serotonin, the best model was different in the combined sample than in both sex-specific samples. The difference in serotonin levels, for example, could be explained by sample size, in that the larger combined sample has more power to accept a model with additional parameters. In other cases, the omission of correlations between relatives of opposite sexes may influence the model in the sex-specific samples.

Of the 17 quantitative traits analyzed, only LDL-c did not show evidence for suggestive linkage in genome scans in any of the three samples. Of the remaining 16 traits, nine showed evidence for sex-specific linkage, defined here as linkage with differences between sexes meeting criteria for suggestive significance (**Table 2**); three genome-wide significant linkages were detected in the male-only sample ($P < 5.9 \times 10^{-5}$) (**Figure 2**). However, the presence of different linkage signals in males and females does not necessarily imply a genotype × sex interaction. To test explicitly for sex interactions at genome-wide significant peaks, we conducted additional analyses that tested for an HBD × sex interaction. All three genome-wide significant linkages had significant sex interactions ($P_{interaction} < 0.05$; **Figure 2**), as did the nine other sex-specific linkages denoted by an asterisk in **Table 2**.

Consistent with this evidence for significant HBD × sex interaction, none of the 36 signals detected in a sex-split sample reached suggestive significance in the other sex, and only two of the sex-specific signals were detected at the suggestive level in the combined sample (triglycerides on 21q and percentage of lymphocytes per white blood cell count on 19p). Of the remaining 22 suggestive linkage signals present in the combined sample, only eight were detected in even one of the sex-split samples.

In this study, we examined quantitative traits that are associated with common human diseases, such as heart disease, hypertension, diabetes, asthma and autoimmune disease. Of the 17 traits we

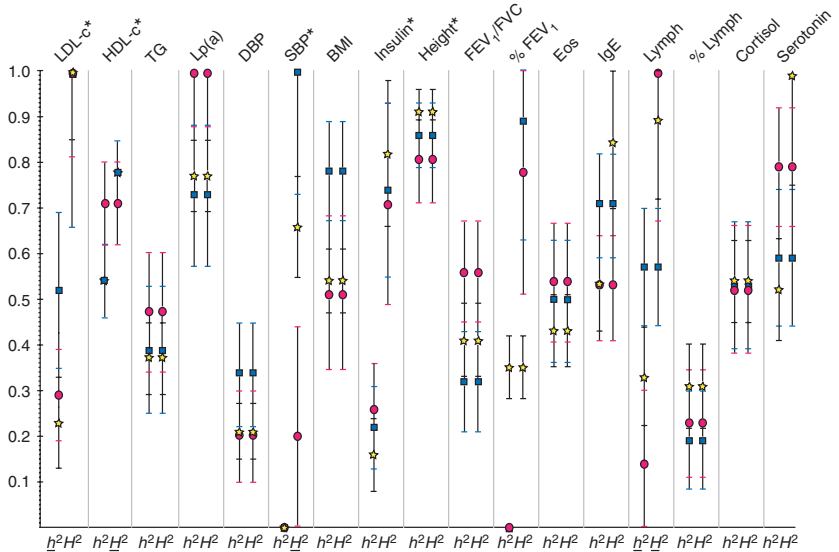

Figure 1 Heritability estimates for 17 quantitative traits. Narrow (h^2) and broad (H^2) heritability estimates are shown. Yellow stars represent the estimate in the combined sample, pink circles in females and blue squares in males. Standard errors around the estimates are represented by black (combined), pink (females) or blue (males) horizontal lines. Heritabilities for which the estimates differ significantly between the sexes are underlined. Those with a significant sex interaction ($P < 0.05$) are denoted by an asterisk. The broad heritability for HDL-c in males includes an inbreeding depression component. % Lymph, percentage of lymphocytes per white blood cell count; Lymph, lymphocyte count; TG, triglycerides; Lp(a), lipoprotein (a); DBP, diastolic blood pressure; SBP, systolic blood pressure; BMI, body mass index; Eos, eosinophilia; % FEV_1, percent predicted forced expiratory volume at 1 s; FEV_1/FVC, ratio of FEV_1 over forced vital capacity.

Table 2 Genome-wide linkage results

	All		Male		Female		HBD × sex interaction	
Trait	Chromosome	cM	Chromosome	cM	Chromosome	cM	Chromosome	*P*-value
LDL-c								
HDL-c			9q*	117			9q	0.0084
			19q*	95			19q	0.025
Triglycerides	21q	31	21q*	30	12q	73	21q	0.048
			9p	42				
Lp(a)	16p	44	14q	45				
	16q	59						
DBP	2q	140						
SBP	2p	3	2q	144	6p	47		
	2q	142	**8q***	**109**			8q	0.0007
	8p	50						
	9q	141						
BMI	2p	9	12p	9	2p	9		
Insulin	1q	158	16p	4				
	9q	143						
	16p	2						
Height	5p	11	5p	0				
FEV_1/FVC			1p	122				
			5q*	154			5q	0.022
			7p*	55			7p	0.0025
% FEV_1			3p	86	2q	135		
Eos	14q	106			2q*	256	2q	0.027
					10q*	77	10q	0.0041
IgE	2p	71	7p	18	2p	71		
	3q	198						
	7p	20						
	9p	2						
	17p	47						
Lymph			**16q***	**129**	3q	165	16q	0.029
			19p	43				
% Lymph	1q	225	1q	227				
	3q	138	11p	61				
	19p	46	15q	105				
			19p**	**43**			19p	0.027
Cortisol			4p	7	5q	168		
					10q*	97	10q	0.024
Serotonin	12q	153	12q	150	6q	184		
	16p	39	17p	41				
			17q*	70			17q	0.0049

Linkage results meeting criteria for suggestive significance are shown for each trait and sample. Three results meeting criteria for genome-wide significance are shown in bold font; results showing a suggestive significant difference between the sexes are denoted by one asterisk; one result showing a difference between the sexes meeting criteria for genome-wide significance is denoted by two asterisks. The test for sex interaction was performed only on linkages denoted by one or more asterisks.

surveyed, 11 were sexually dimorphic, and 12 showed evidence of differences between the sexes in heritability or linkage. Notably, the sexually dimorphic traits were not necessarily those with sex differences in heritability or linkage. For example, diastolic blood pressure was strongly sexually dimorphic but did not show significant sex-specific heritability or linkage differences, whereas LDL-c had a significant sex interaction in heritability as well as differences in narrow heritability (and high broad heritability in both sexes) but no sex-specific linkage or sexual dimorphism in trait values. These results suggest that many genes, or the genetic variation within them, may act differently in the sexes, even when the trait distributions do not differ by sex. Some of the sex-specific autosomal effects we observed could be due to interaction with sex-linked genes, but many may be the result of hormonal influences on gene expression and regulation or other nongenetic factors that are correlated with sex.

All three genome-wide significant linkage signals we detected were sex-specific, and only one of them was detected in the combined sample at all. In fact, only two of the 12 total sex-specific linkage signals were detected in the combined analysis, suggesting that autosomal genes that have sex-specific action or interaction may be hard to detect without separating the sexes or modeling for sex-specific differences. Moreover, those sex-specific signals that are picked up in combined-sex samples may be less likely to replicate across samples with different male-female ratios or sample sizes, similar to what occurs when replication samples are not well matched for other critical environmental factors.

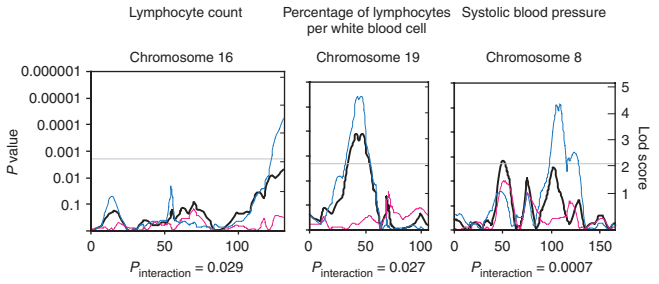

Figure 2 Genome-wide significant linkages. The black trace represents linkage in the combined sample, the pink trace represents the female-only sample and the blue trace represents the male-only sample. The gray line shows the threshold for suggestive significance. lod scores are shown on the right *y*-axis and *P* values on the left *y*-axis. *x*-axis shows distance (in cM) from the short arm telomeric end. HBD × sex interaction *P* values are shown for each linkage peak.

Our data indicate that the sex-specific genetic effects observed in model organisms and the few quantitative traits assessed in humans may be generalizable to a wide variety of human quantitative and disease-related traits. Thus, these results have broader implications for mapping complex trait genes. Failing to model for sex-specific architecture may substantially hamper detection of susceptibility loci in genome-wide screens, and using modified approaches may increase our power to identify genes underlying complex traits.

METHODS

Subjects. The Hutterites are a young founder population who practice a communal farming lifestyle. Details of the population, sampling strategy and the utility of this population for mapping complex traits have been described previously[19–21]. The 806 Hutterites in our studies are related to each other through multiple lines of descent in a known pedigree. The mean inbreeding coefficient of the individuals in this sample is 0.034 (s.d. 0.015), slightly greater than that of $1\frac{1}{2}$ cousins. A complete genealogy of 806 individuals was constructed from a >12,000-member Hutterite pedigree. This yielded a 1,623-person pedigree that included all known ancestors of the 806 individuals[22]. Each quantitative trait was measured in a subset of the Hutterites after obtaining informed consent, as previously described[19,21]. Quantitative trait measurements were transformed to establish a normal distribution[19]. Informed consent was obtained from subjects (or parents of minors), and this study was approved by the Institutional Review Boards of the University of Chicago and the University of South Dakota.

Genotyping. A genome screen using 658 autosomal microsatellite markers (Marshfield screening sets 9 and 51) was completed by the Mammalian Genotyping Service of the National Heart, Lung, and Blood Institute, yielding a ~5-cM map (Marshfield). In addition, 226 microsatellite markers and 239 intragenic SNPs or insertions/deletions related to asthma and cardiovascular diseases (see refs. 23–25) were genotyped in this sample. Distances for framework markers are based on the Marshfield map; all other markers were placed using the NCBI physical map (Build 35) and estimations of recombination within the Hutterite pedigree by CRI-MAP. The final map had an average intermarker distance of 3.2 cM.

Phenotype measurement. Phenotype measurements were performed as described previously[19], except for a modified formula for determination of LDL-c described previously[26]. Lymphocyte count and percentage of lymphocyte per white blood cell count were measured in whole blood on the basis of a differential blood count.

Analysis of sexual dimorphism in measured traits. The effect of sex on the trait distribution was measured using regression analysis. This analysis was not corrected for relatedness, as average relatedness is not expected to be correlated with sex. The significance of sex as a predictor in the regression model including other known covariates is reported. For one trait, percentage predicted FEV_1, the effect of sex was estimated using the raw FEV_1 measures and including height as an additional covariate, as the predicted FEV_1 is calculated based on sex and height.

Estimation of heritability. The methods used to estimate narrow (h^2) and broad (H^2) heritability have been described in detail elsewhere[27] but are briefly reviewed here. We analyzed each trait using a variance-component maximum-likelihood method that estimates additive, dominance and environmental variance using information about the kinship coefficient and the probability of a given pair of individuals to share two alleles identical by descent (IBD) without either being autozygous[22]. Accurate estimation of the dominance variance, as opposed to just a sibship correlation, is possible because essentially every pair of Hutterites has a nonzero probability of sharing two alleles IBD. We considered models that had, in addition to an environmental variance component, additive variance, dominance variance, both additive and dominance variance components and components reflecting inbreeding depression. To assess the best-fitting model, we compared the Bayesian information criterion (BIC)[28] for each model and used the likelihood ratio χ^2 test to determine which components were significant. In order to estimate the significance of sex interaction effects, we evaluated a model with different polygenic effects in males and females with a correlation between these two effects. Using either the likelihood ratio test or BIC we compared the model with separate sex effects with the model in which the effects are constrained to be equal in both sexes.

Mapping. Genome scans were performed using a multipoint homozygosity by descent (HBD) linkage method, and significance was assessed using a permutation test. The linkage method tests for correlations between a region's inherited HBD and trait value. We assess empirical locus-specific and genome-wide significance using a Monte Carlo permutation test. This test keeps the genotypes fixed while permuting trait values and has the advantage of assessing significance conditional on characteristics of the genotype data (such as informativeness, heterozygosity and linkage disequilibrium) while preserving the covariance structure in the phenotype data. The methods used in this study are identical to those described previously[29], except that we have now extended the original HBD computations to include genotype information from related individuals[17]. Because of these changes, as well as the inclusion of additional markers, the results of the genome-wide screens may vary from our previous reports[21,29,30].

All analyses were first run on the entire sample and then separately by sex. In the sex-specific analyses, the phenotype values for individuals of the opposite sex were entered as missing data. Genome-wide suggestive significance was met if the *P* value at a locus was less than the expected minimum *P* value under the null hypothesis, which was estimated by finding the minimum locus-specific *P* value across the genome for each of 1,000 permutations and averaging these values. Although likelihood ratios were not calculated, we can assign an equivalent one-degree-of-freedom lod score to each of our *P* values using the formula $\text{lod} = 0.217 \times F^{-1}(1 - p)$, where F^{-1} is the inverse cumulative distribution function of a χ^2 random variable with one degree of freedom.

Significance of the sex differences was also assessed by permutation testing. For each trait, 1,000 genome scans were run for two randomly selected subsets of the same size as the male and female subsets. Genome-wide suggestive significance for sex specificity was met if the difference in male and female *P* values at a locus was greater than the expected maximum difference in *P* values for random subsets, which was estimated by averaging the maximum *P* value difference across the genome for each of the 1,000 permutations and averaging these values. For those peaks that showed sex differences, we did an additional analysis to better understand whether the sex-specific peaks were indicative of a sex interaction. In this analysis we use the entire sample and included parameters for an overall effect on the phenotype due to sex and for an explicit HBD × sex interaction. We then tested for the presence of the sex interaction using a standard *F*-test.

URLs. University of California, Santa Cruz Genome Browser, http://www.genome.ucsc.edu; CRI-MAP, http://compgen.rutgers.edu/multimap/crimap/.

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COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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