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Evaluating human autosomal loci for sexually antagonistic viability selection in two large biobanks

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Abstract

Sex and sexual differentiation are pervasive across the tree of life. Because females and males often have substantially different functional requirements, we expect selection to differ between the sexes. Recent studies in diverse species, including humans, suggest that sexually antagonistic viability selection creates allele frequency differences between the sexes at many different loci. However, theory and population-level simulations indicate that sex-specific differences in viability would need to be very large to produce and maintain reported levels of between-sex allelic differentiation. We address this contradiction between theoretical predictions and empirical observations by evaluating evidence for sexually antagonistic viability selection on autosomal loci in humans using the largest cohort to date (UK Biobank, n = 487,999) along with a second large, independent cohort (BioVU, n = 93,864). We performed association tests between genetically ascertained sex and autosomal loci. Although we found dozens of genome-wide significant associations, none replicated across cohorts. Moreover, closer inspection revealed that all associations are likely due to cross-hybridization with sex chromosome regions during genotyping. We report loci with potential for mis-hybridization found on commonly used genotyping platforms that should be carefully considered in future genetic studies of sex-specific differences. Despite being well powered to detect allele frequency differences of up to 0.8% between the sexes, we do not detect clear evidence for this signature of sexually antagonistic viability selection on autosomal variation. These findings suggest a lack of strong ongoing sexually antagonistic viability selection acting on single locus autosomal variation in humans.

Keywords: sexually antagonistic selection; male–female FST; biobank; association study; probe mapping

Introduction

Understanding the relationship between genotype and sexually dimorphic phenotypes, and how selection shapes this relationship, is fundamental to understanding sex-specific responses in aging (Archer *et al.* 2018), fertility (Farquhar *et al.* 2019), disease susceptibility (Morrow 2015; Ferretti *et al.* 2018; Dumitrescu *et al.* 2019), and treatment (Khramtsova *et al.* 2019). For example, in humans, causes of mortality can differ between the sexes (Jurado-Coronel *et al.* 2018; Gold *et al.* 2019), and sex-specific response to treatments can occur (Sramek *et al.* 2016; Raparelli *et al.* 2017). Sexual dimorphism is also common across a range of plant and animal taxa (Rowe *et al.* 2018; Deegan and Engel 2019). Differences in optimal trait values between the sexes may result in sexually antagonistic selection (Arnqvist and Rowe 2005)—*i.e.* selection on variants that affect fitness in different directions for each sex. Surveys of natural selection suggest that the repeated evolution of sexual dimorphism is often associated with sexually antagonistic selection (Cox and Calsbeek 2009). Yet, we still lack an understanding of how this process shapes genomic variation within and between species. A major obstacle in assessing the genomic consequences of sexually antagonistic selection is that most of the hypothesized genomic signatures are not unique to this mode of selection. However, when the alleles at a single locus have opposite effects on viability between the sexes, *i.e.* intralocus sexual conflict (Rice and Chippindale 2008; Bonduriansky and Chenoweth 2009), the resulting association between genotype and sex may be detectable. Different alleles can also be favored in the sexes through non-divergent sex-specific selection that differs in magnitude between the sexes (Connallon and Clark 2014; Rowe *et al.* 2018). These processes are predicted to generate allele frequency differences between the sexes among adults, with sexually antagonistic selection predicted to give a stronger genomic signature (Kasimatis *et al.* 2017; Mank 2017).

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Recent research has sought this signature of selection by identifying alleles with high male–female F_{ST} (Cheng and Kirkpatrick 2016; Kasimatis *et al.* 2017, 2019), a normalized measure of allele frequency difference. Studies across a range of taxa have suggested that hundreds of autosomal loci are potentially subject to ongoing sexually antagonistic selection with many differentiated loci having male–female divergence values of at least 10% (Lucotte *et al.* 2016; Flanagan and Jones 2017; Wright *et al.* 2018; Dutoit *et al.* 2018; Bissegger *et al.* 2019), and some reaching even as high as 45% (Vaux *et al.* 2019). These results are surprising because the production and maintenance of such large male–female differences on autosomes requires strong, ongoing selection to overcome the homogenization of genotypes during meiotic segregation each generation (Cheng and Kirkpatrick 2016; Kasimatis *et al.* 2019). Theory suggests that a male–female F_{ST} value of 1% requires at least a 33% viability cost per sex per generation (Kasimatis *et al.* 2019). Given the high sex-specific viability cost, factors such as population structure, sampling variance due to small sample sizes, or bioinformatic artifacts may contribute to the high divergence values observed (Kasimatis *et al.* 2019). Of particular concern are the small sample sizes (15–100 individuals) used by many previous studies. Detecting the level of allelic differentiation expected at sexually antagonistic loci with moderate sex-specific mortality (≤10% per sex) requires substantially larger sample sizes as well as a careful consideration of confounding effects, such as population structure (Kasimatis *et al.* 2019). Indeed, a meta-analysis of 51 studies that included more than 100,000 European-ancestry individuals did not find any common variants associated with sex ratio (Boraska *et al.* 2012)—a phenotype representative of sexually antagonistic processes such as meiotic drive and segregation distortion (Immler and Otto 2018).

Here, we aim to reconcile empirical observations with theoretical predictions using a robust statistical framework to identify intralocus sexually antagonistic viability selection in the largest human cohort to date. We use two large-scale biobanks, the UK Biobank and the Vanderbilt Biobank (BioVU) to analyze >500,000 human genomes for signals of male–female divergence from which we can infer sexually antagonistic selection. Compared to previous studies examining sexual antagonism, these datasets significantly improve our statistical power to detect allele frequency differences among females and males by providing the largest available sample sizes to date—several orders greater than previous studies in humans (Lucotte *et al.* 2016; Cheng and Kirkpatrick 2016) and non-model taxa (Flanagan and Jones 2017; Wright *et al.* 2018; Dutoit *et al.* 2018; Bissegger *et al.* 2019; Vaux *et al.* 2019). Our association framework differs from traditional association studies as genetic sex is the phenotype of interest and the mechanism generating a true effect would be sex-specific viability. After controlling for multiple confounders, we are unable to detect conclusive evidence for ongoing sexually antagonistic viability selection at individual autosomal loci.

Materials and methods

Genotyping and quality control in BioVU

The DNA biobank at Vanderbilt University consists of DNA extracted from blood collected during routine clinical testing. For 93,864 individuals, genome-wide association study (GWAS)-level genotyping was performed using the Illumina MEGA-Ex chip, which includes >2 million common and rare variants before imputation. We obtained genotyped data in PLINK format from the Vanderbilt sequencing core after the following quality control steps: excluding either samples or variants with ≥5% missingness, and mismatched identifiers as detected by identity by descent checks. We also removed non-concordance between reported gender and genetically determined sex ($n = 791$ individuals). Overlapping variants with 1000 Genomes demonstrated ≥99.98% variant call concordance using HapMap sample aliquots. Using PLINKv1.90b3s (Chang *et al.* 2015), we additionally performed the following quality control steps. We first confirm that duplicate samples and those with high missing rate (≥5%) are not present and exclude samples with high heterozygosity on autosomes (>3 SD from observed data), or high relatedness (%IBD ≥0.2). Next, we removed duplicated variants and variants with high missing rate (≥5%) or significantly different missing rate between cases (females) and controls (males; $P < 0.00001$, Fisher's exact test). We then included only samples with a self or third party reported race as "white" and variants with minor allele frequency >0.01. This additional quality control resulted in a final European-ancestry dataset of 61,760 samples (34,269 females and 27,491 males) and 1,763,607 variants. We calculated the top 20 principal components on this cohort. We imputed variants that reached nominal or genome-wide statistical significance ($P < 5 \times 10^{-8}$) in the UK Biobank data but were not genotyped in the BioVU cohort. These variants were imputed using the Michigan Imputation Server (v1.2.4) (Das *et al.* 2016) using the HRC (Version r1.1 2016) reference panel and retaining variants with $R^2 > 0.3$. Imputed allele dosages were converted to hard calls using PLINK/2.00-alpha2 (Chang *et al.* 2015) and filtered to exclude variants with minor allele frequency <1% and genotyping rate <95%. During our quality control steps, we did not remove variants based on deviations from Hardy–Weinberg equilibrium (HWE) since theory indicates that sex-specific selection can violate the assumptions of HWE (Kasimatis *et al.* 2019). All PLINK code is available on the GitHub repository https://github.com/abraham-abin13/sexually_antagonistic_sel.git.

Genotyping and quality control in the UK Biobank

The UK Biobank is an international health resource with data from approximately 500,000 participants. Genotyping and quality control procedures have previously been described in detail by Bycroft *et al.* (2018). Briefly, two arrays—the UK Biobank Axiom Array ($n = 438{,}427$ participants) and the UK BiLEVE Axiom Array ($n = 49{,}950$ participants)—were used to genotype participants. Quality control procedures carried out before the data were released included: removal of participants with excess heterozygosity or missingness, removal of markers with batch, plate, array, or sex effects, and removal of markers with discordance across control replications (Bycroft *et al.* 2018). The removal of sex effects, via a Fisher's exact test on the 2 × 3 table of genotype counts between females and males at a given marker, does not preclude our analysis as the conservative threshold ($P < 10^{-12}$) removed only eight markers and the sex differences at these markers were due to technical artifacts, such as the probe sequence mapping to the Y chromosome (C. Bycroft, pers. comm.). We verified that these SNPs do not show consistent signals of true sexually antagonistic selection (Supplementary File S1). The released genotype data contain 805,462 markers from 488,377 participants (Field IDs 22100–22124). In addition, the genetic sex (Field ID 22001), year of birth (Field ID 34), date of assessment (Field ID 53), and assessment center (Field ID 54) were requested for each participant. The top 40 genetic principal components (Field ID 22009) were previously calculated using fastPCA (Bycroft *et al.* 2018).

Using PLINKv1.90b3s (Chang et al. 2015), we additionally performed the following quality control steps. We excluded samples with non-concordance between reported gender and genetically determined sex ($n = 292$), high missing rate (≥5%), and high heterozygosity on autosomes (>3 SD from observed data). Next, we pruned markers in linkage disequilibrium (window size = 50 kb, step rate = 5, r^2 threshold = 0.2). Finally, we removed variants with significantly different missing rates between females and males ($P < 0.00001$, Fisher's exact test). We included only variants with minor allele frequency >0.01 to exclude inaccurate calls made for low frequency alleles {Wright:2019hl, Weedon:2019bh}. This additional quality control resulted in a final dataset of 487,999 samples (264,578 females and 223,335 males) and 653,632 variants. We additionally subset the data to include only those individuals of genetic European-ancestry (Field ID 188052), which results in 409,406 samples (221,268 females and 188,052 males).

As in the BioVU quality control, we did not remove variants based on deviations from HWE. However, we tested for a lack of minor allele homozygotes relative to that expected under HWE using a binomial test. This was motivated by the observation that most candidate SNPs lacked minor allele homozygotes (see "Results" section). Overdominance—as can be generated by sexually antagonistic selection—can lead to an excess of heterozygotes, but theory does not predict a complete lack of homozygous genotypes (Kidwell et al. 1977). This test is conservative in that we expect a general excess of homozygotes due to population structure. All PLINK code is available on the GitHub repository https://github.com/abraham-abin13/sexually_antagonistic_sel.git.

Imputed genotype and phased haplotype values were used to compare significant loci in the BioVU cohort, which were not directly genotyped in the UK BIOBANK arrays. Imputation to a European-ancestry panel was completed prior to the data release using the Haplotype Reference Consortium and UK10K haplotype resource. The imputation methods are described in detail in Bycroft et al. (2018). Imputed allele dosages were converted to hard calls using PLINK/2.00-alpha2 (Chang et al. 2015).

Genome-wide association for an individual's sex

We performed a GWAS in UK Biobank and BioVU separately using logistic regression to test the association between an individual's sex (binary variable, encoding genetic sex) and the effect allele, defined as the minor allele by PLINKv1.90b3s (Chang et al. 2015), using an additive model. For the BioVU analysis, we controlled for genetic ancestry using 12 genetic principal components and included year of birth as a covariate. For the UK Biobank analysis, we again controlled for genetic ancestry using 12 genetic principal components, along with age at assessment and UK Biobank sampling center as covariates. All genome-wide association tests were performed using PLINKv1.90b3s (Chang et al. 2015). Our results remained robust even when considering 20 genetic principal components or when including only "genetic European ancestry" in the UK Biobank cohort (Supplementary File S2). We focused our analyses on the autosomes, where genomic divergence between the sexes is not confounded by sex chromosome processes.

Resampling of sex and generating a null distribution

We also applied our GWAS procedure to genotypes with randomly permuted genetic sexes to generate an empirical null distribution and verify if *P*-values were well calibrated (*i.e.* uniformly distributed on [0,1]). We resampled genetic sex 100 times to generate a set of random associations between genotype and genetic sex in the UK Biobank and BioVU cohorts. Within the UK Biobank cohort, we included only those variants that had a *P*-value of <0.01 in the original association analysis ($n = 8868$ SNPs) to specifically ascertain if the GWAS procedure is well calibrated for SNPs with potential associations (but note the distribution of *P*-values under permutation is still expected to be uniform). We then reran the logistic regression, again including 12 genetic principal components, age, and sampling center as covariates. These analyses generated a distribution of 100 permuted *P*-values at each variant. Permuted *P*-values are uniformly distributed (Supplementary Figure S1), even when the values were small, indicating that the permuted *P*-values for these association analyses are well calibrated, and therefore, a genome-wide Bonferroni significance threshold of $P < 5 \times 10^{-8}$ is appropriate. All R and PLINK codes are available on the GitHub repository https://github.com/abraham-abin13/sexually_antagonistic_sel.git.

Identifying SNPs with sequence similarity to sex chromosomes

High sequence similarity between a probe sequence for an autosomal variant and a sequence on a sex chromosome can produce mis-hybridization that results in statistically significant GWAS hits for sex due to the different effects on allele counts between females and males. We used BLAT (Kent 2002) with default parameters (stepSize = 5, repMatch = 2253, minScore = 20, minIdentity = 0) to identify sequence similarity between the probe sequences used on the genotyping arrays and sex chromosome regions. The MEGA-Ex array probe sequences used to genotyped the BioVU cohort were obtained directly from Illumina. Probe sequences for the UK Axiom Biobank array (Resource 149601) and UK BiLEVE array (Resource 149600) were download from https://biobank.ctsu.ox.ac.uk/crystal/label.cgi?id=263. MEGA-Ex probes are 50 base pair sequences adjacent to the variant being tested; MEGA-Ex uses single base extension to detect the variant allele. UK Biobank array probes are 71 base pairs long with the variant being genotyped located in the middle. BLAT hits to the X or Y chromosome were further filtered to identify regions likely to cross-hybridize by requiring at least 40 basepair overlap, sequence similarity ≥90%, and that the matching sequence overlaps (UK Biobank arrays) or flanks (MEGA-Ex array) the variant being tested. Predicting hybridization from sequence is a challenging problem that is influenced by sequence attributes (*e.g.* GC content) and array design (*e.g.* variant location in probe) (Zadeh et al. 2011; Beliveau et al. 2018). As a result, we used these sequence similarity-based criteria to identify sequences with sex-specific mis-hybridization potential. Similar criteria were used in a previous a study that reported cross-hybridization on the Illumina Infinium HumanMethylation27K microarray platform (Chen et al. 2012). Next, we identified the best BLAT hit to a sex chromosome for each probe sequence by selecting the hit with the highest BLAT score, which accounts for match length and sequence similarity. For this step, we considered the UK Axiom and UK BiLEVE array together thus selecting the probe sequence with the highest BLAT score from one of the two arrays per variant tested in the GWAS. In the BioVU (MEGA-Ex array) and UK Biobank arrays, 83,083 out of 798,051 and 128,090 out of 620,040 autosomal probes had at least one BLAT match (BLAT score ≥20) to a sex chromosome region.

Power analysis

We conducted a power analysis to determine the minimum allelic divergence between the sexes that could be detected within

the BioVU and UK Biobank cohorts (Supplementary File S3). Specifically, we determined the probability that we would reject the null hypothesis that the population frequency of each allele is equal at a P-value threshold of $P = 1 \times 10^{-8}$. Suppose we have N males and M females, and the allele frequencies in the two groups are P and Q. Since the cohort sample sizes are large, if the population frequencies are p and q, then $P \sim \text{Normal}(p, p(1-p)/2N)$ and $Q \sim \text{Normal}(q, q(1-q)/2M)$. The difference in population allele frequencies is then given by $P - Q \sim \text{Normal}(p-q, p(1-p)/2N + q(1-q)/2M)$. The variance is maximized when $p = q = 1/2$, so is at most: $V = (1/N + 1/M)/8$. The two-sided P-value for $P - Q$ being nonzero will be below 1×10^{-8} if $|P - Q|$ is larger than $z(0.5 \times 10^{-8}) \times \text{sqrt}(V)$, where $z(p)$ is the pth quantile for the standard Normal distribution. However, since $|P - Q|$ is random, a locus with a frequency difference of $|p - q| = z(0.5 \times 10^{-8} \times \text{sqrt}(V)$ will have a two-sided P-value below 1×10^{-8} only half the time. That is, at a P-value threshold of 1×10^{-8}, we would detect about half of the loci with frequency differences around $|p - q| = z(0.5 \times 10^{-8}) \times \text{sqrt}(V)$. To have higher power, the frequencies would have to be farther apart. For instance, we would have 95% power to detect any SNP with true $|p - q| > (z(0.5 \times 10^{-8}) + z(0.025)) \times \text{sqrt}(V)$. Power to detect small allele frequency differences will, therefore, be dataset dependent.

This analysis does not assume HWE in determining the population allele frequencies, p and q. In fact, departures from HWE will generate variance in allelic divergence. In the most extreme case, if all individuals were homozygous, then the standard deviation would be 40% larger. While our analysis ignores variance due to diploidy, it provides estimates of the limits of the procedure and is generalizable to other datasets.

Data availability

All the data generated from this study (Supplementary Files S1–S12) were deposited in the figshare repository https://figshare.com/s/e863ea11cc9dab30c1b9. All the codes generated for this study were deposited in a GitHub repository (https://github.com/abraham-abin13/sexually_antagonistic_sel.git).

Supplementary material is available at figshare DOI: https://doi.org/10.25386/genetics.13250417.

Results

Throughout this paper when we refer to an individual's sex, we are referencing that individual's sex chromosome composition as estimated in each biobank dataset and binarized (*i.e.* metadata reports each individual as XY or XX, although the datasets almost certainly include individuals not falling into these two categories; Lanfranco *et al.* 2004). We make no statements in relation to gender, which is determined by many factors beyond genetics.

Seventy-seven variants show genome-wide significance as candidates for sexually antagonistic selection

To identify autosomal variants that could be under sexually antagonistic selection, we performed a GWAS between genetically ascertained females and males in two large, independent cohorts (BioVU: 34,269 females and 27,491 males; UK Biobank: 264,813 females and 223,478 males). We first applied standard quality control steps to remove samples with high relatedness, discordant sex, or high heterozygosity and excluded genotyped variants with high overall missing rate ("Materials and methods" section). We account for potential confounders by including age and 12 principal components for population stratification as covariates. The resulting P-values are well calibrated, as verified by permuting the sex labels in the UK Biobank cohort (mean lambda_gc = 1.01; Supplementary Figure S1A) and BioVU cohort (mean lambda_gc = 0.99; Supplementary Figure S1B), and so the standard genome-wide significance threshold of $P < 5 \times 10^{-8}$ is appropriate for the association analysis ("Materials and methods" section). Applying this threshold resulted in 5 and 72 genome-wide significant variants in BioVU and UK Biobank, respectively.

Different amounts of missing data between females and males for a variant can lead to spurious associations (Moskvina *et al.* 2006). Indeed, we found a statistically significant difference in the missing rate between females and males ("Materials and methods" section) in 64 (of 72) genome-wide significant variants in the UK Biobank and in none in the BioVU genome-wide significant variants (Supplementary Figure S2 and Supplementary File S4). We evaluate these 64 variants in further detail below.

Following these quality control steps, eight and five variants remained significant in the UK Biobank and BioVU, respectively (Figure 1 and Table 1). One intriguing genome-wide significant variant in the UK Biobank cohort [rs11032483; odds ratio (OR) = 1.25, $P < 1.3 \times 10^{-53}$] lies in a known regulatory region on chromosome 11 and has evidence from association studies for increasing risk in males and being protective in females for a number of sex-specific reproductive pathologies (Cortes *et al.* 2018). Our results remained robust across BioVU and UK Biobank cohorts even after we increased the number of principal components and subset the UK Biobank cohort to only "genetic European ancestry" individuals (Supplementary File S5).

No candidate loci replicate across BioVU and the UK Biobank

Comparing the five autosomal significant hits from BioVU to the eight from the UK Biobank, none of the associations are genome-wide significant in both cohorts (Table 1). Furthermore, none of the significant hits in one cohort even meet a nominal significance threshold ($P < 0.05$) in the other cohort. For example, the variant with the strongest association in the UK Biobank cohort (rs11032483) had no evidence for association with sex in the BioVU cohort ($P = 0.99$).

The regions surrounding each of the significant variants do not exhibit the expected association signal clusters arising from variants in strong linkage disequilibrium (LD) with the causal variant. For example, the most strongly associated variant overall (rs9870157) has 33 variants with LD, as quantified by R^2, of at least 0.8 in the 1000 Genomes Phase 3 European-ancestry (EUR) populations. However, none of these variants have a strong association. The lack of replication across the two cohorts and the missing association peaks among variants in strong LD suggest that these signals could be false positives driven by technical or biological artifacts.

Significant associations are likely due to mis-hybridization with sex chromosome regions

Genotyping error can occur due to probe cross-reactivity between different regions of the genome. Sex-biased error has been observed in array-based studies of DNA methylation (Chen *et al.* 2013) and has been reported in the canid genome (Tsai *et al.* 2019), the stickleback genome (Bissegger *et al.* 2019), and on the Y chromosome in humans (Boraska *et al.* 2012). For instance, if an autosomal variant is assayed with a probe sequence that has sufficient sequence similarity to a Y chromosome region carrying the reference allele, then males homozygous for the alternate allele at the autosomal locus may instead be genotyped as heterozygous for the alternate allele. Females would not be subject to this bias, and thus there could appear to be an allele

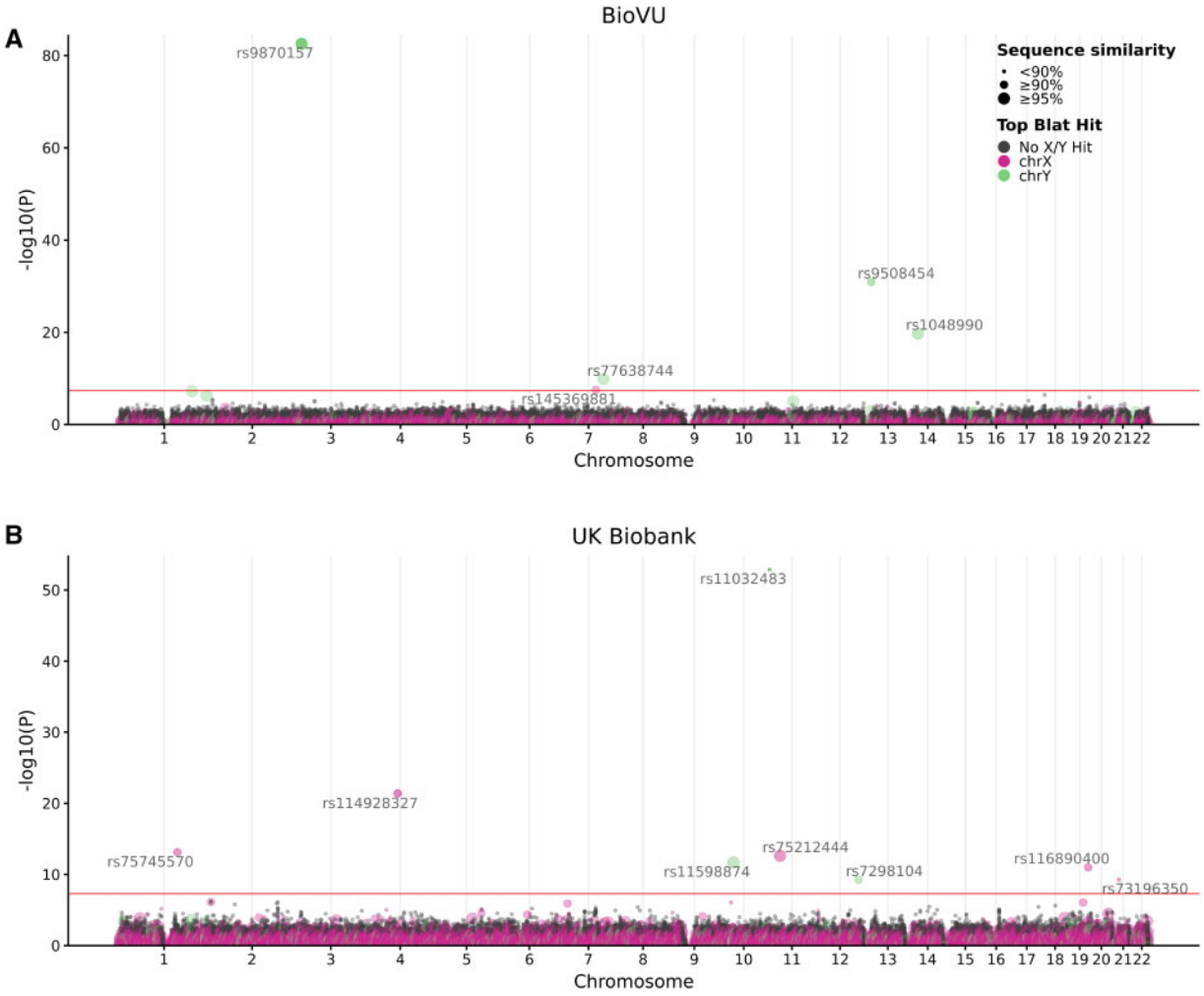

Figure 1 Genome-wide association tests for genetic sex reveal candidate variants for sexually antagonistic selection. To identify candidate variants for sexually antagonistic selection, we performed genome-wide association tests between females (cases) and males (controls) in two large biobank cohorts: (A) BioVU (females = 34,269, males = 27,491) and (B) UK Biobank (females = 264,813, males = 223,478). After standard quality control and sex-specific missingness filters ("Materials and methods" section), we identified five variants with genome-wide statistically significant associations ($P < 5^{-8}$, solid red line) in BioVU and eight in the UK Biobank. None of the significant variants in BioVU and UK Biobank replicated at genome-wide or nominal significance ($P < 0.05$) across the two cohorts (Table 1). The probe sequence for each associated variant (except rs11032483) had >90% sequence identity to at least one sequence on a sex chromosome (Table 2). Each point represents one variant. Each variant is colored by whether the best match of its probe sequence to a sex chromosome (according to BLAT score) is on X (pink) or Y (green). If it has no strong match to either sex chromosome, it is colored black. The size of each point indicates the degree of sequence similarity.

frequency difference between the sexes. Similarly, an autosomal variant with a probe sequence with high similarity to the X chromosome could result in a lack of homozygotes for the allele not on the X chromosome in both sexes, but the strength of this effect would differ between females and males. Furthermore, such cross-reactivity can lead the normalized intensities produced by genotyping arrays to lie outside of the regions corresponding to each genotype, and thus a missing genotype (Zhao *et al.* 2018). Cross-reactivity to a sex chromosome could therefore cause a differential missingness rate between the sexes. Indeed, we observe an almost complete lack of minor allele homozygotes in males across all 13 genome-wide significant SNPs, as well as for females in all but 4 genome-wide significant SNPs (Supplementary Files S4 and S6).

The same explanation is likely behind the aforementioned 64 genome-wide significant SNPs discarded for association between missingness and sex. Thirty-eight out of 64 SNPs with different missing rates had further evidence for technical artifacts as they had high potential for sex-specific mis-hybridization due to sequence similarity (see below, Supplementary File S6). In addition, all of the 64 SNPs have extremely low homozygous genotype counts in females or males based on a Bonferroni cutoff ($P < 6 \times 10^{-4}$), which is inconsistent with signatures of sexually antagonistic selection.

To quantify the potential for mis-hybridization of sex chromosome regions to autosomal probes, we used BLAT (Kent 2002) to find regions of the sex chromosomes with high sequence similarity to autosomal probe sequences on the MEGA-Ex (BioVU) and UK Axiom/BilEVE (UK Biobank) genotyping arrays ("Materials and methods" section). We assign each probe sequence to the sex chromosome region with the highest BLAT score.

The probes for each significantly associated variant have high sequence similarity to a sex chromosome region (Figure 2, Table 2, and Supplementary Files S8–S12). In contrast, the majority of probes (79% in UK Biobank, 89% in BioVU) do not have any detectable similarity (BLAT score <20) to a sex chromosome sequence. Compared to the distribution of BLAT scores for probes with a match to a sex chromosome region, all genome-wide significant variants had BLAT scores greater than the 99th and 95th percentiles for BioVU and UK Biobank, respectively (inset

Table 1 Genome-wide significant variants in BioVU and UK Biobank cohorts

Location (chr: position)	SNP ID	Allele	Max. OR	Individuals	BioVU, *P*-value	UK Biobank, *P*-value
BioVU significant SNPs						
3:16652240	rs9870157	T	1.31	61,709	2.82E−83	0.42
7:100351596	rs145369881	T	0.78	60,499	3.25E−08	0.06
7:121147858	rs77638744	A	1.12	61,361	1.52E−10	0.13
13:20119336	rs9508454	C	1.19	61,694	1.26E−31	0.87
14:35761675	rs1048990	G	1.16	61,712	1.94E−20	0.75
UK Biobank significant SNPs						
1:162075684	rs75745570	T	0.92	471,060	0.66	7.60E−14
4:88457099	rs114928327	T	0.89	413,257	0.30	4.09E−22
10:39006198	rs11598874	T	1.07	478,329	0.44	1.94E−12
11:4515024	rs11032483	T	1.25	482,581	0.99	1.33E−53
11:34104213	rs75212444	T	0.88	482,788	0.20	2.35E−13
12:118926685	rs7298104	T	0.91	475,771	0.93	5.97E−10
19:53535248	rs116890400	A	0.88	485,047	0.51	1.02E−11
21:18068575	rs73196350	A	0.94	479,137	0.76	5.19E−10

Variants passing genome-wide significance ($P < 5 \times 10^{-8}$) in the BioVU or UK Biobank cohorts are reported. Genome-wide significant variants did not replicate across the cohorts. Location is reported in GRch37/hg19 coordinates. Allele refers to the effect allele with which odds ratio (OR) is calculated. Individuals refer to the total number of individuals tested for the variant.

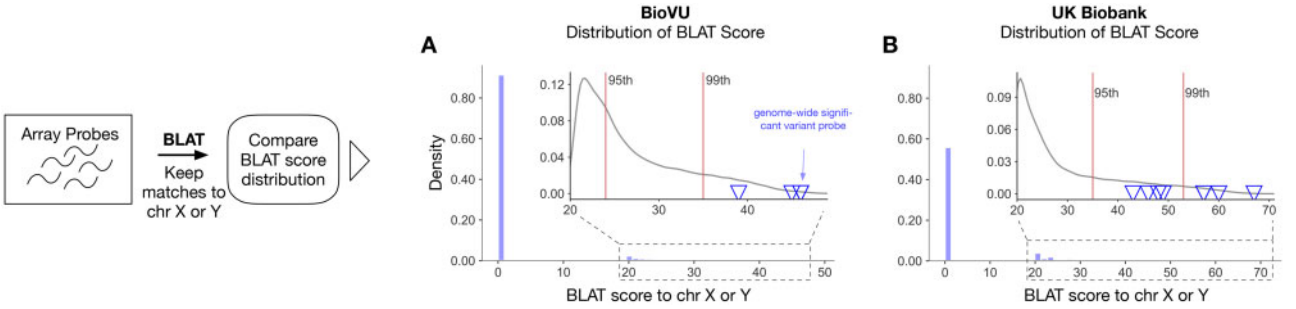

Figure 2 Probes for autosomal variants associated with genetic sex show high sequence similarity to sex chromosomes. We searched probe sequences used to genotype autosomal variants in the BioVU (798,051 autosomal probes) and UK Biobank (620,040 autosomal probes) cohorts for high sequence similarity to sex chromosome regions using BLAT ("Materials and methods" section). (A) More than 80% of BioVU autosomal probes do not have any sequence similarity (BLAT score ≤20) to a sex chromosome region; these are plotted at 0. Among the 83,083 BioVU probes with similarity to a sex chromosome sequence (inset), the probes for the variants with genome-wide significant associations with sex (blue triangles) are all in the tail of the distribution beyond the 99th percentile of the BLAT match score. (B) Patterns are similar for the UK Biobank probes; however, a higher fraction (20%, 128,090) has detectable similarity to a sex chromosome, likely due to their greater length than the BioVU probes.

Figure 2, A and B). Using a stricter criterion to define potential sex chromosome sequence similarity ("Materials and methods" section), we find that all genome-wide significant variants in BioVU (Supplementary Figure S3A) and six out of eight genome-wide significant variants in UK Biobank (Supplementary Figure S3B) still have strong sequence similarity to a sex chromosome region (Table 2). Only 0.57% (4587 probes) and 3.3% (20,528) of all probes in BioVU and UK Biobank, respectively, have such a sex chromosome match (Supplementary Figure S3). The difference in percentage is likely due to the UK Biobank arrays having longer probe sequences. Probes of genome-wide significant variants have similar BLAT matching properties as non-significant probes (Supplementary Figure S4) in UK Biobank and BioVU. Overall, the lack of homozygotes and the high sequence similarity between significant probes and sex chromosomes suggests that sex-specific genotyping error is the source of the significant associations rather than sexually antagonistic selection.

The lack of sex-specific allele frequency differences is not due to being statistically underpowered

To determine if the lack of significant associations might be a result of being underpowered to detect plausible effect sizes, we conducted a power analysis ("Materials and methods" section). Based on the large cohort sizes, we have 95% power to detect a variant with a true allele frequency difference greater than 2% between the sexes in the BioVU cohort and greater than 0.8% in the UK Biobank (Figure 3A). A frequency difference of f caused by sex-specific antagonistic selection at a locus requires a mortality of roughly $f/2$ (Supplementary File S3), so we should be able to detect segregating variants with sex-specific mortality effects of at least 0.4%. For comparison, a cohort of 100 individuals, as used in a previous HapMap study (Lucotte *et al.* 2016), only has 95% power to detect allele frequency differences between the sexes of 38% or greater (Figure 3B). The difference in allele frequencies between the sexes for the UK Biobank ranged from 0.12% to 1.5% for significant SNPs (Supplementary File S7), suggesting that the sample size is appropriate for identifying variants of small effect.

Discussion

Understanding how sex-specific effects are transmitted by autosomal variation is critical for understanding how sexual dimorphisms arise and become fixed in populations. Sexually antagonistic selection maintains sexual dimorphisms and is predicted to be a pervasive driver of genome evolution (Rowe *et al.* 2018). Yet empirically, the genomic signature of this process is not well characterized. In this study, we sought to identify the extent of one genomic signature of sexually antagonistic viability selection acting on autosomal variation in human populations.

Table 2 Best sex chromosome sequence match for genome-wide significant variant probes

Data	SNP ID	Location (chr: position)	*P*-value	Matched sex chromosome	BLAT score	Sequence similarity (%)	Match length (bp)
BioVU	rs9870157	3:16652240	2.82E−83	Y: 26964471–26964521	46	96.0	50
BioVU	rs145369881	7:100351596	3.25E−8	X: 26864979–2685116	39	90.0	50
BioVU	rs77638744	7:121147858	1.52E−10	Y: 23315613–23316169	45	98.0	50
BioVU	rs9508454	13:20119336	1.26E−31	Y: 28612640–28612689	46	91.9	50
BioVU	rs1048990	14:35761675	1.94E−20	Y: 15398460–15398510	46	96.0	50
UKBB	rs75745570	1:162075684	7.60E−14	X: 121952043–121952114	57	90.2	71
UKBB	rs114928327	4:88457099	4.09E−22	X: 79084149–79084223	60	93.0	71
UKBB	rs11598874	10:39006198	1.94E−12	Y: 13568059–13568130	67	97.2	71
UKBB	rs11032483	11:4515024	1.33E−53	Y: 19070733–19070803	48	84.3	70
UKBB	rs75212444	11:34104213	2.35E−13	X: 36967486–36967536	46	96.0	50
UKBB	rs7298104	12:118926685	5.97E−10	Y: 1513524–1513899	43	93.9	59
UKBB	rs116890400	19:53535248	1.02E−11	X: 38605892–38605963	57	90.2	71
UKBB	rs73196350	21:18068575	5.19E−10	X: 80780038–80780101	49	88.9	63

Variants with genome-wide significant associations with genetic sex are reported with GWAS P-value (P-value) and the matched sex chromosome region (matched sex chromosome) with the highest BLAT score (BLAT score). The sequence similarity and length of the matching region (match length) are also reported.

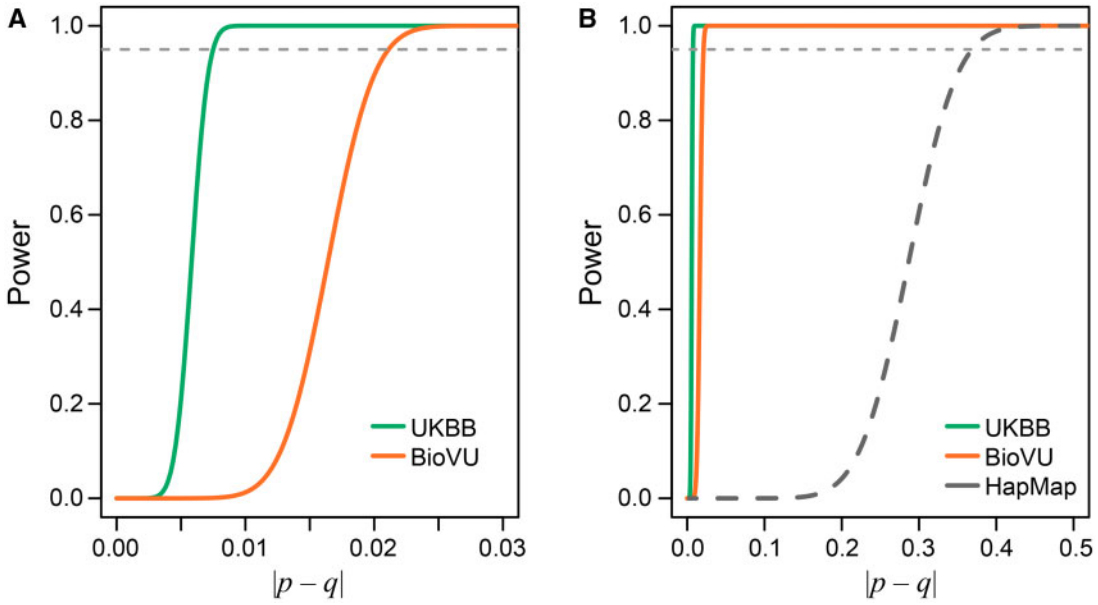

Figure 3 Statistical power was sufficient to detect small allelic divergence between the sexes. (A) The power to detect different levels of allelic divergence between the sexes was calculated for the BioVU (orange) and UK Biobank (green) cohorts. The dashed line shows the 95% power threshold. (B) Statistical power for the analyzed cohorts (same as in A) compared to previous analysis of human sequences (Lucotte *et al.* 2016) based on approximately 100 individuals per HapMap population (gray).

Capitalizing on two of the largest available biobanks, we performed genome-wide association tests for genetic sex that failed to replicate any genome-wide significant variants. On closer inspection, a number of promising genome-wide significant variants were likely driven by technical artifacts, with mis-hybridization due to high sequence similarity to a sex chromosome the most common.

For instance, the SNP in the UK Biobank with the strongest signal, rs11032483, at first seems to be a promising candidate, as it has been found to be strongly protective of a number of female-specific conditions (*e.g.* "excessive, frequent, and irregular menstruation") and a high risk factor of male-specific conditions (*e.g.* "diseases of male genital organs") (Cortes *et al.* 2018). However, given the moderately high sequence similarity of its probe to a Y chromosome sequence (Table 2), the lack of association among variants in high LD, and the complete lack of homozygous genotypes, it is much more likely that this variant is not a true positive. After all, what genetic marker is more protective against excessive menstruation than a Y chromosome? Thus, we conclude that there is no conclusive signal in these data of sexually antagonistic viability selection on genetic variants at individual loci based on male–female allelic divergence; however, the evidence is not definitive, and so a few loci might benefit from targeted genotyping and further analysis.

These results stand in contrast to recent male–female F_{ST} studies that have reported tens to hundreds of significantly differentiated variants (Lucotte *et al.* 2016; Flanagan and Jones 2017; Wright *et al.* 2018; Dutoit *et al.* 2018; Bissegger *et al.* 2019; Vaux *et al.* 2019). These studies suggest strong, pervasive sexually antagonistic viability selection acting across the genomes of various species, which would be puzzling in light of theoretical observations and simulations indicating that strong allelic divergence between the sexes requires high sex-specific mortality rates to overcome the homogenizing effect of meiotic segregation occurring every generation (Kasimatis *et al.* 2019). In contrast to these studies, the sample size of our study provided statistical power to

distinguish the selection of plausible magnitude from stochastic noise. In addition, our use of larger sample sizes provided power to detect smaller allelic divergence between the sexes—within the range predicted to be generated by weak sexually antagonistic selection. Our results are in line with a previous meta-analysis of sex-specific common variant differences in humans (Boraska *et al.* 2012), though our direct approach with larger sample sizes and a replication cohort mitigates potential confounders across different studies.

We found strict quality control measures for population structure and for an appropriate genome-wide significance threshold essential. In particular, rigorous testing for sequence similarity to the sex chromosomes showed that all significant SNPs had strong sequence matches. The potential for high sequence similarity between autosomes and sex chromosomes to generate sex-biased genotyping errors has been reported previously (Chen *et al.* 2012, 2013). However, the potential for these sex chromosome artifacts to affect population genetic statistics has not been fully appreciated until recently (Bissegger *et al.* 2019; Tsai *et al.* 2019) or has only been examined for the Y chromosome (Lucotte *et al.* 2016). In particular, probe sequences with high sequence similarity to one of the sex chromosomes can lead to skewed allele frequency estimates in a sex-specific manner due to sequence mis-hybridization and the different sex chromosome content between females and males. This problem extends beyond SNP-based genotyping to read-based sequencing data, where inaccurate mapping of short reads to an autosome instead of the sex chromosome could generate a similar skew in allele frequencies. Inaccurately mapping reads will be especially problematic if an autosomal gene duplicate has translocated to a sex chromosome, as is predicted by theory on the resolution of sexual conflict (Connallon and Clark 2011). This sex chromosome effect is potentially very common and, therefore, must be explicitly considered in any sex-specific or sex-stratified analyses to prevent technical and bioinformatic artifacts from generating false signals. Participation bias rather than differential mortality might also generate a signal of male–female divergence (Pirastu *et al.* 2020), though this source of error is not relevant in this study since we did not find candidate SNPs for sexually antagonistic selection that passed our quality controls. Such artifacts will be especially problematic in species with new sex chromosomes, poorly assembled genomes, or rapidly evolving sex chromosome systems. In our case, filtering out SNPs with large differences in missingness between sexes and/or lack of homozygotes was sufficient to remove potentially problematic SNPs.

Comparison of sequence similarity and match length for all probes indicates that thousands of other probes have similarly strong sex chromosome matches as the candidate variants analyzed here (Supplementary Figure S2). While previous studies have detected similar hybridization effects (Chen *et al.* 2012, 2013), the extent to which they can skew association results has not yet been reported on the UK Biobank and BioVU arrays. This high sequence similarity could suggest that more variants should show false positive signatures of sex-specific allele frequency differences. However, multiple factors contribute to the potential for mis-hybridization and inaccurate genotyping. For example, hybridization strength and kinetics are determined by sequence attributes beyond simple sequence similarity, including local GC content and the potential for DNA secondary structures to form (Zhang *et al.* 2018). Furthermore, the sequence region matched on the sex chromosome (*i.e.* pseudo-autosomal *vs* non-recombining) also matters. It is also likely that different quality control strategies used on different genotyping array platforms filter different problematic sites.

Although sexually antagonistic selection is certainly an important selective pressure, we see no evidence of it generating substantial autosomal allelic divergence between the sexes in the predominantly white populations we studied. This strong negative result is unusual, as genome-wide association studies for most traits on a biobank-scale find significantly associated SNPs, even in cases where heritability is low. We know that humans have the opportunity for sexually antagonistic effects, as seen through sex-specific mortality and disease susceptibility (Morrow 2015; Khramtsova *et al.* 2019). However, randomization of alleles every generation by meiotic segregation means that a large selective pressure is required to create a large difference in allele frequencies and, thus, this genetic process makes it harder to detect the results of sexually antagonistic selection. Furthermore, some sexually antagonistic variants are not stably polymorphic; we would not detect these because they move rapidly to fixation (Rowe *et al.* 2018; Kasimatis *et al.* 2019).

Given the confounding factors, technical artifacts, and high sampling variance, identifying variants with small sex-specific effect sizes is a formidable challenge. We strongly recommend that future studies avoid simple metrics, like the male–female F_{ST}, and instead incorporate strict quality filters and control for known confounders into their association tests. Sexually antagonistic viability selection is not the only action of sex-specific selection nor is male–female allelic divergence at a single locus the only possible signature of sexual antagonism. Given the extent of sexual dimorphisms in nature, there are almost surely autosomal loci subject to sexually antagonistic selection, which may be detectable through other genomic signatures and in other human sub-populations. However, our work illustrates that the field must reconsider our assumptions and develop new metrics for identifying the signatures of sexual antagonism in the light of theoretical expectations to understand how this process affects the genome. Such studies will help us understand the translation of sex across the genotype–phenotype map and apply this to human health.

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Conflicts of interest

None declared.

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