

# eXclusionary: 10 years later, where are the sex chromosomes in GWASs?

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## Summary

10 years ago, a detailed analysis showed that only 33% of genome-wide association study (GWAS) results included the X chromosome. Multiple recommendations were made to combat such exclusion. Here, we re-surveyed the research landscape to determine whether these earlier recommendations had been translated. Unfortunately, among the genome-wide summary statistics reported in 2021 in the NHGRI-EBI GWAS Catalog, only 25% provided results for the X chromosome and 3% for the Y chromosome, suggesting that the exclusion phenomenon not only persists but has also expanded into an exclusionary problem. Normalizing by physical length of the chromosome, the average number of studies published through November 2022 with genome-wide-significant findings on the X chromosome is ~1 study/Mb. By contrast, it ranges from ~6 to ~16 studies/Mb for chromosomes 4 and 19, respectively. Compared with the autosomal growth rate of ~0.086 studies/Mb/year over the last decade, studies of the X chromosome grew at less than one-seventh that rate, only ~0.012 studies/Mb/year. Among the studies that reported significant associations on the X chromosome, we noted extreme heterogeneities in data analysis and reporting of results, suggesting the need for clear guidelines. Unsurprisingly, among the 430 scores sampled from the PolyGenic Score Catalog, 0% contained weights for sex chromosomal SNPs. To overcome the dearth of sex chromosome analyses, we provide five sets of recommendations and future directions. Finally, until the sex chromosomes are included in a whole-genome study, instead of GWASs, we propose such studies would more properly be referred to as “AWASs,” meaning “autosome-wide scans.”

## Introduction

In the 10 years since Wise et al. (2013)<sup>1</sup> brought the exclusion of the X chromosome from genome-wide association studies (GWASs) to the attention of the community, little has improved regarding the analysis and reporting of the sex chromosomal variants in GWASs.<sup>2–4</sup> The X chromosome accounts for ~5% of the haploid genome and carries ~800 protein-coding genes. However, to date (November 2022), even after the call for including the X chromosome in GWASs by Wise et al.,<sup>1</sup> approximately only 0.5% of associated SNPs in the NHGRI-EBI GWAS Catalog<sup>5,6</sup> are on the X chromosome, a 10-fold paucity compared to the autosomes.

The paucity of research on the sex chromosomes includes both the X and Y chromosomes. For the Y chromosome,<sup>7</sup> as of November 29, 2022, only nine out of 447,939 associations reported in NHGRI-EBI GWAS Catalog<sup>5,6</sup> belong to the Y chromosome. Coverage is scarce on GWAS arrays for the male-only Y chromosome, in part because of repetitive sequences that make variant calling difficult. If Y chromosomal variants are available in the non-pseudo-autosomal region (NPR), they can be analyzed via existing methods. However, there appears to be “a lack of will” to do so.<sup>8</sup>

The X chromosome presents multiple analytical challenges,<sup>9–14</sup> including (1) a male has one copy of the X chromosome while a female has two, in contrast to the

autosomes; (2) the X chromosome in male germ cells only recombines with the Y chromosome in the pseudo-autosomal regions (PARs) but not in the NPR; (3) in contrast to males, the two copies in female germ cells recombine across the entire X chromosome; (4) the two female copies are also subject to X inactivation (i.e., X chromosome dosage compensation); (5) the X-inactivation status at the population level can be random, skewed, or absent (i.e., X-inactivation escape); and (6) the true X-inactivation status at the individual level cannot be derived from GWAS data alone.

Thus, the existing bioinformatic, statistical, and machine learning methods developed specifically for the autosomes are not suitable for the sex chromosomes. For example, most bioinformatic tools are autosome-centric, meaning that even if the sex chromosomes were included in the pipelines, tool developments were not tailored for the sex chromosomes.<sup>11</sup> These include variant calling,<sup>15,16</sup> data quality control (QC) prior to imputation<sup>17,18</sup> (e.g., cryptic relatedness,<sup>19,20</sup> Hardy-Weinberg equilibrium [HWE]<sup>21,22</sup>), and imputation.<sup>15,23–30</sup> Similarly, most association methods are not tailored for the sex chromosomes, including population stratification via principal-component analysis (PCA),<sup>31</sup> and the association methodology itself.<sup>32–36</sup> Finally, the recent polygenic risk score (PRS)-based disease risk prediction methods<sup>37–40</sup> rarely include the sex chromosomes.

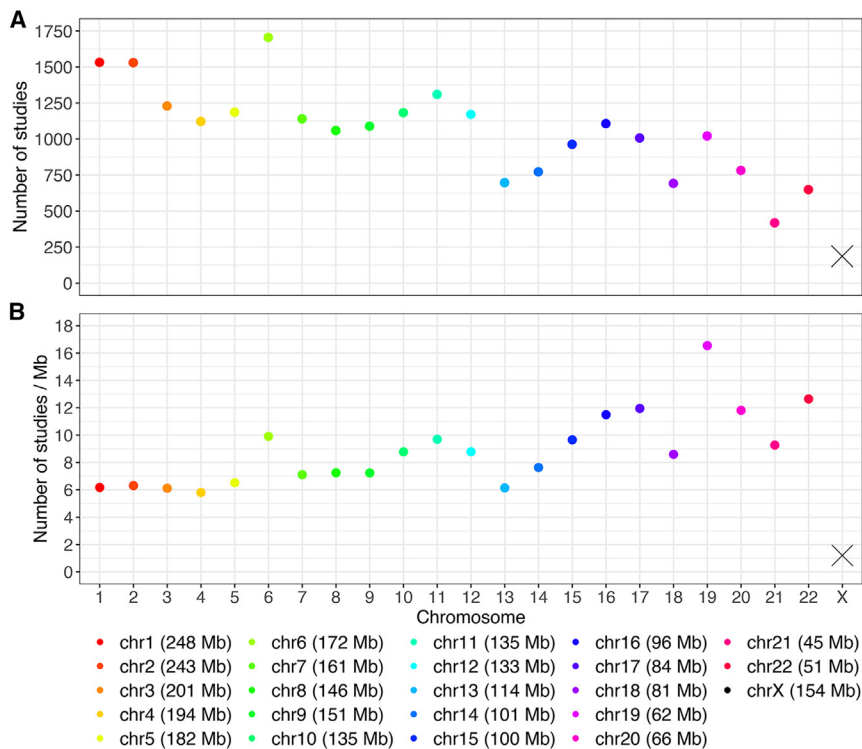
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<https://doi.org/10.1016/j.ajhg.2023.04.009>

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**Figure 1.** Total number of studies and average number of studies per Mb reporting at least one genome-wide significant finding ( $p$  value  $< 5 \times 10^{-8}$ ) stratified by chromosome, from the NHGRI-EBI GWAS Catalog

(A and B) Total number of studies (A) and average number of studies (B). Genetic associations were indexed by unique PubMed IDs up to November 29, 2022. Studies reporting associations with multiple traits were only counted once.

ported at least one genome-wide-significant association ( $p$  value  $< 5 \times 10^{-8}$ ).<sup>43</sup> However, only 186 studies (4.4%) had signals on the X chromosome (Figure 1A). In contrast, chromosome 21 had twice the number of signals (418 studies; 9.9%), despite being less than one-third the length (Figure 1A).

Before investigating how often the X chromosome was analyzed to begin with (in the next section), we first normalized each chromosome by its

physical length (Figure 1B). It is clear that signal densities vary across the autosomes. However, the most striking feature is the continued paucity of signals on the X chromosome since 2010–2011.<sup>1</sup>

To investigate whether the 2013 recommendation to include the X chromosome in GWASs had an impact on the practice of our field, we examined temporal changes. Figure 2 shows the average number of studies per Mb with at least one genome-wide-significant finding, separately for the autosomes and the X chromosome, from prior to 2008 to November 29, 2022. Unfortunately, the gap between the autosomes and the X chromosome appears to be widening in recent years. Between 2009 and 2021, the average number of studies with genome-wide-significant findings on the X chromosome grew at approximately 0.012 studies/Mb/year, remaining below 0.3/Mb every year (Figures 2 and S2). In contrast, the numbers increased consistently for the autosomes by approximately 0.086 studies/Mb/year. For comparison, Figure S1 shows the total number of studies reporting one or more signals per chromosome over time.

Examining GWAS array and sequencing studies (GWAS-by-WES [whole-exome sequencing], GWAS-by-WGS [whole-genome sequencing]) separately, using the “genotyping technology” variable, revealed that 4.6% of GWAS loci came from studies that included sequencing and only 0.76% of those loci (12 out of 1,576) were on the X chromosome; six of those 12 loci came from a single study.<sup>44</sup> Additionally, many of the studies that employed sequencing also used data from genotyping arrays.

Ironically, one of the most comprehensive X chromosome-wide studies (XWAS<sup>45</sup>) is not included in the

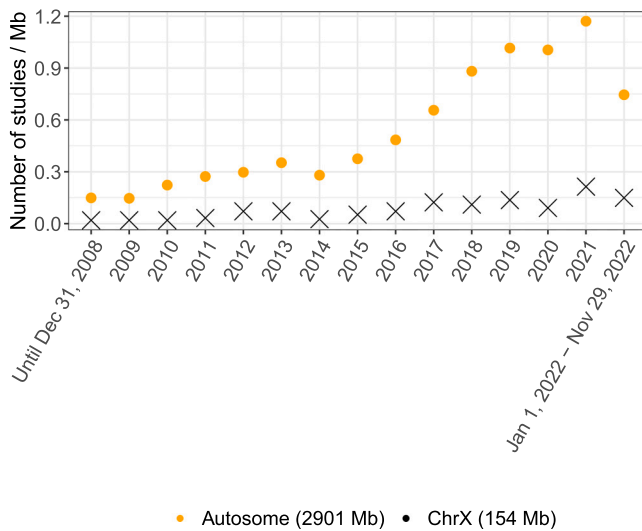
Back in 2013, after examining 743 GWAS papers published between January 2010 and December 2011 and in the NHGRI GWAS Catalog,<sup>41</sup> Wise and colleagues noted that only ~33% GWASs included the X chromosome<sup>1</sup>; the Y chromosome was not explicitly examined, though it is implicitly involved in the X chromosome through the PARs. Additionally, the authors commented on QC and power concerns, including poorer coverage of the X chromosome in early GWAS arrays and lower genotyping and imputation accuracy as compared to autosomes, as well as X-inactivation-related analytical complexities that may reduce power of an association study. Finally, the authors concluded that “many interesting biological insights could be revealed if we end the exclusion of the X chromosome in future GWAS.”

Thus, 10 years later, we first re-surveyed the research landscape to determine whether the earlier recommendations of including the X (and Y) chromosome(s) in GWASs had been translated into changes in practice. Second, as genotyping and sequencing technologies have also evolved, including imputation panels based on next-generation sequencing data,<sup>15,42</sup> we then scanned the literature for emerging issues and insights. Finally, we make new recommendations.

## Sex chromosome results in the NHGRI-EBI GWAS and PolyGenic Score (PGS) Catalogs

### Lack of X and Y SNP-trait associations in the NHGRI-EBI GWAS Catalog

As of Nov 29, 2022, the NHGRI-EBI GWAS Catalog<sup>5,6</sup> contained 6,130 published studies, of which 4,208 re-



**Figure 2. Average number of studies per Mb reporting at least one genome-wide significant finding ( $p$  value  $< 5 \times 10^{-8}$ ) over time, separately for the autosomes and X chromosome, from the NHGRI-EBI GWAS Catalog**

Genetic associations were indexed by unique PubMed IDs up to November 29, 2022. Studies reporting associations with multiple traits were only counted once.

NHGRI-EBI GWAS Catalog, presumably because it only reported the X chromosome association results, which does not meet the catalog inclusion criteria requiring genome-wide results. Although they showed that the contribution of X chromosome loci to trait variability may be smaller than similar-sized autosomes, for height in males, the X chromosome  $h^2$  estimate is similar to that for many shorter autosomes, including chromosomes 13 and 18.<sup>45</sup> These authors also observed interesting sex differences in X chromosome heritability across 20 quantitative traits in the UK Biobank on the basis of the central imputation from the Affymetrix arrays. Specifically, NPR X chromosome  $h^2$  estimates were on average twice as high for males as for females (0.63% vs. 0.30%), with the noticeable exception of educational attainment. When the XWASs were performed, hundreds of X chromosomal loci were identified across these 20 quantitative traits, with twice as many signals detected in males than females, and some loci had remarkable male-specific effects across numerous traits.

Genetic associations on the Y chromosome were even more rarely documented. Out of all 447,939 associations ( $p$  value  $< 1 \times 10^{-5}$ ), only nine, arising from two studies, were on the Y chromosome; among the 293,170 genome-wide-significant findings, only one was from the Y chromosome.

#### Lack of X and Y chromosome results in genome-wide summary statistics in the NHGRI-EBI GWAS Catalog

To address whether lack of sex chromosomal GWAS results were due to lack of appropriate (or any) analysis of the sex

chromosomes, we calculated the proportion of genome-wide summary statistics that included sex chromosome results, regardless of if there were significant findings.

There were 19,935 genome-wide summary statistics published in 2021 and posted at the NHGRI-EBI GWAS Catalog<sup>5,6</sup> (web resources). These GWAS submissions came from 136 publications, of which most provided one to two sets of summary statistics, but four provided  $>1,000$  sets (Data S1). To avoid analyzing multiple submissions from the same publication, we randomly selected one submission from each of the 136 publications (Data S1).

Out of the 136 GWAS summary statistics, only 34 (25%) contained X chromosome results (of the 34, only four also included Y chromosome results), which is less than the 33% based on the survey of GWASs conducted in 2010 and 2011.<sup>1</sup> Thus, exclusion has become more rather than less prevalent, contrary to the intent of the initial commentary! Further, exclusion appears to be an exclusionary problem, where both sex chromosomes have been routinely neglected in whole-genome studies.

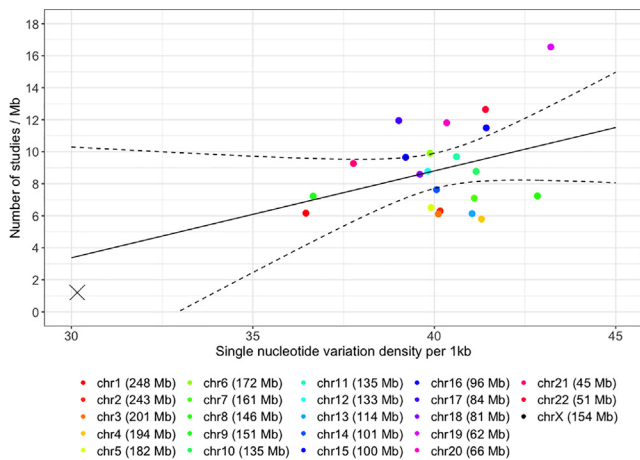
If we assume that the 136 studies with summary statistics in the NHGRI-EBI GWAS Catalog are a random sample of all GWASs in 2021 and recall from the previous section that there is a 6-fold difference in the average findings between chromosome 1 and the X chromosome (Figure 1B), it is then reasonable to hypothesize that much of the paucity would be resolved if the X chromosome were actually analyzed across all GWASs.

Some well-known contributing factors include the smaller effective population size ( $N_e$ ) and X chromosome inactivation in females, which reduce power to detect associations compared to autosomes.<sup>45,46</sup> Variation in single-nucleotide diversity<sup>47,48</sup> can be another contributing factor. For example, chromosome 19 has the highest density of single-nucleotide variations of 43.21/kb (based on the 1000 Genomes Project) among all chromosomes, while chromosome 1 has the lowest of all autosomes at 36.46/kb. However, cumulatively as of December 2022, there is no statistically significant linear relationship (slope = 0.54;  $p$  value = 0.13; Figure 3) between nucleotide diversity and the average number of genome-wide-significant findings among the autosomes. Even if we were willing to extrapolate the linearly fitted line to 30.16/kb, the nucleotide diversity of the X chromosome, the expected research yield on the X chromosome is 3.47/Mb, almost thrice the actual output of 1.21/Mb.

Based on high-coverage whole-genome sequencing of TOPMed<sup>15</sup> cohorts, the X chromosome has lower density of variants in coding sequences compared to the autosomes.<sup>49</sup> This can be an additional contributing factor to the paucity of signals on the X chromosome.

#### Lack of X and Y chromosome results in the PolyGenic Score (PGS) Catalog

Based on the above results from the GWAS Catalog, there is also a lack of sex chromosome results in the PGS Catalog, as expected. We downloaded PGS scoring files from the PGS Catalog<sup>50</sup> (web resources), focusing on the 430



**Figure 3. Average number of studies per Mb reporting at least one genome-wide significant finding ( $p$  value  $< 5 \times 10^{-8}$ ) per chromosome, from the NHGRI-EBI GWAS Catalog cumulatively, compared to chromosome-specific nucleotide diversity<sup>48</sup>**

Genetic associations were indexed by unique PubMed IDs up to November 29, 2022. Studies reporting associations with multiple traits were only counted once. The solid slope was fitted using the autosomal data only, and the dashed curves are the 95% confidence bands.

files (PGS001802 to PGS002231) all uploaded on January 10, 2022. Unsurprisingly, none of the 430 files contained any results from the sex chromosomes, confirming the current exclusionary practice in PGS research as well.

### Other emerging exclusionary issues: Quality control, association analysis and reporting, results interpretation, the Y chromosome, and clinical implications

#### Quality control

In addition to the QC discussed by Wise et al.,<sup>1</sup> many data quality pipelines and imputation tools<sup>15–18,23–30</sup> have been developed for GWASs. However, most are autosome-centric, ignoring the sex chromosomes either explicitly or implicitly. In 2014, König et al.<sup>11</sup> highlighted “the steps in which the X chromosome requires specific attention, and [gave] tentative advice for each of these,” including sex-stratified minor allele frequencies (MAFs) and missing rates, as well as testing for differential missingness. However, these recommendations have not been followed in practice. For example, sex-specific variant call rates are rarely reported.

There has been little work on chromosome-specific imputation quality. However, a recent study that compared the X chromosome with the autosomes<sup>51</sup> examined imputation from the Affymetrix 500k array in an admixed population with the Illumina MEGA array as the gold standard. They showed that, using the Michigan Imputation Server with the 1000 Genomes Project phase 3 data, the X chromosome had 70% imputation accuracy compared to 84% on the autosomes. Further, they showed

that imputation quality scores were also lower on the X chromosome across all imputation approaches. It would be interesting to study sex-specific imputation quality on the X chromosome.

#### Sex difference in minor allele frequency as QC revisited

Checking for sex difference in minor allele frequency (sdMAF) is rarely formed as part of GWAS QC. However, it was already noted a decade ago that “MAF checks might need to be conducted separately for the X chromosome because the expected frequencies are sex dependent,” based on an informal poll of leading statistical geneticists working in GWASs in 2013.<sup>1</sup> Others also suggested to include an sdMAF test as part of the QC for the X chromosome.<sup>11</sup> However, a recent work has shown that there are possible causes of sdMAF: genotyping errors and biology.<sup>52</sup> Delineating the two causes for each X chromosomal SNP is not straightforward, creating challenges in QC pipelines.

The recent study analyzed the high-coverage whole-genome sequencing data of the 1000 Genomes Project<sup>48</sup> and gnomAD v3.1.2<sup>53</sup> and identified many SNPs with genome-wide-significant sdMAF across the X chromosome, particularly at the boundaries between PAR and NPR.<sup>52</sup> Further, the study concluded that region-specific sdMAF at the PAR-NPR boundaries is most likely a biological phenomenon, possibly due to sex-specific linkage.<sup>54–56</sup> This illustrates the challenges of including sdMAF as a QC measure.

As sdMAF is statistically equivalent to GWAS of sex, both evaluating whether there is allele frequency difference between sexes, there is also a connection between the sdMAF study<sup>52</sup> and a recent GWAS of sex.<sup>57</sup> This GWAS of sex used data from 2.46 million customers of 23andMe but did not examine the X chromosome.<sup>57</sup> Although their main conclusion was that sdMAF is a result of participation bias, they also noted that 55% of their significant findings on the autosomes are most likely results of genotyping errors, further illustrating the importance and challenges of separating genotyping errors from biology (and other causes) that could lead to sdMAF.

#### Hardy-Weinberg equilibrium (HWE) test as QC revisited

Departure from HWE is routinely used as part of GWAS QC for autosomes,<sup>17</sup> as SNPs with severe Hardy-Weinberg disequilibrium (HWD) are typically believed to have genotyping errors.<sup>58</sup> However, how to evaluate HWE for the X chromosome is unclear and it remains debatable whether testing for HWD should be used at all as part of data QC, both of which we discuss next.

The standard HWE test is Pearson’s  $\chi^2$  test, testing for the difference between the observed and expected genotype counts based on HWE.<sup>59,60</sup> This test is typically applied to sex-combined genotype counts, which is reasonable for an autosomal SNP. But applying such an HWE test to an X chromosomal PAR or NPR SNP requires additional considerations.<sup>61</sup> For example, König et al.<sup>11</sup> recommended performing the HWE test with only females.

Alternatively, Graffelman and Weir<sup>21,62</sup> suggested using both females and males, and they proposed a new HWE test for an NPR SNP that includes the deviation of male genotype counts from the expected, based on sex-pooled allele frequency estimate. However, this alternative test has been shown to be simultaneously testing for HWD in females and sdMAF between males and females.<sup>61</sup> Therefore, if sdMAF were present, this sex-combined HWE test can be misleading. Instead of the Pearson's  $\chi^2_1$  test, testing for model fit has also been proposed.<sup>63</sup>

Regardless of the specific HWE test used, screening out variants with HWD is questionable for the X chromosome for two other reasons. First, it has been long (but not well) known that it takes several generations to achieve HWE on the X chromosome in contrast to a single generation for the autosomes under the same set of assumptions such as random mating.<sup>22</sup> Second, for the autosomes, recent works<sup>64–66</sup> have shown that association power can be improved by leveraging the *difference* in HWD between cases and controls while remaining robust to HWD caused by genotyping errors, but this has yet to be explored for the X chromosome.

### X-inactivation uncertainty and association results interpretation

Until very recently, the statistical genetics community believed that X inactivation was the main analytical challenge to achieving X chromosome-inclusive GWASs.<sup>1,11</sup> Therefore, most of the association methods developed so far have focused on X inactivation.<sup>67–72</sup> As the true model can be escaping, random, or skewed X inactivation, existing analytic methods include using minimum p value,<sup>67</sup> model selection,<sup>69</sup> or Bayesian model averaging.<sup>72</sup>

We note, however, that these statistical approaches rarely address the practical limitation that X inactivation can vary by cell and tissue, and until an association is identified, the relevant cells and tissues cannot even be guessed. Additionally, skewed X inactivation is confounded with non-additive genetic effect, statistically, based on GWAS data alone.<sup>9</sup> While these observations helped to develop a new association test that is robust to X-inactivation uncertainty,<sup>9</sup> both SNP and sex-effect estimates are biased if the model assumptions were incorrect.<sup>73</sup> As SNP-effect estimates are the bases for constructing PGS or PRSs, future research should consider how to correct for the biases when X chromosomal variants are included in PRS.

### Heterogeneous reporting of summary statistics and the X chromosome results

A workshop has resulted in recommendations for improving the standardization of genome-wide summary statistics,<sup>74,75</sup> having acknowledged that there has been large variation in reporting practices.<sup>76</sup> In one specific analysis, 127 unique formats were present among 327 summary statistics files analyzed. The authors then developed MungeSumstats, a Bioconductor package to stan-

dardize and perform QC of GWAS summary statistics. Interestingly, #27 among the total of 31 checks “for SNPs on chromosome X, Y and mitochondrial SNPs, [and] if any are found these are removed,” even though an option of retaining them was provided.

We further examined the reporting standard in the original publications of the X chromosomal signals documented in the NHGRI-EBI GWAS Catalog<sup>5,6</sup> (downloaded on 2020-03-08) with the genome-wide significance level of p value  $< 5 \times 10^{-8}$ . Out of the 3,869 studies available at that time (male-only studies excluded), 195 reported a total of 253 genome-wide-significant loci on the X chromosome. To streamline the analysis, we selected only one SNP from each associated region by retaining the SNP with the smallest association p value (Data S2).

We then extracted information on the analyses performed from the original publications; in total, there are 36 columns in Data S2. These details are crucial to the analysis and reporting of the X chromosome but are largely irrelevant to the autosomes. They include, for example, whether (1) the analysis was sex stratified (70% did sex-combined analysis); (2) for sex-combined analysis, sex was included as a covariate (57% did not); and (3) the genotype coding was documented (75% did not, presumably used the default X-inactivation assumption), because if X inactivation was assumed, males are typically coded 0 and 2 for the two hemizygous genotypes. These considerations were not included in the guidelines recommended by Little et al.<sup>77</sup> Not surprisingly, there was much heterogeneity in both the analysis and reporting among the 195 studies we examined. Such heterogeneity creates challenges for meta-analyses since the lack of necessary details may impact power if assumptions about how the analysis was performed are incorrect. We suggest sex-chromosome-aware research guidelines to be developed by the community.

### The Y chromosome

Non-recombining Y chromosome haplotypes (haplogroups) have a long history in population genetics and genealogy,<sup>78</sup> since these haplotypes can be determined without ambiguity, making them the patrilineal equivalent to mitochondrial haplogroups.<sup>79,80</sup> However, the Y chromosome has long been a thorn in the side of human geneticists<sup>81</sup>: more than half of the Y chromosome is absent from GRCh38.<sup>82</sup> Two recent papers used combinations of multiple long-read next-generation-sequencing technologies to generate much more complete sequence of the Y chromosome, and they also described a high degree of heterogeneity in chromosome length and content between individuals.<sup>82,83</sup>

The Telomere-to-Telomere Consortium has reported the sequence of an approximately 62 Mb long human Y chromosome,<sup>82</sup> which includes >30 Mb that were missing from the reference sequence. Human geneticists can often be criticized for exaggeration, claiming that their phenotype or gene of interest has extensive complexity, but the recent

analysis of 43 diverse Y chromosomes takes the crown.<sup>83</sup> For example, some Y chromosomes are only 45 Mb, while others are as long as 85 Mb, in part as a result of large duplications and inversions.

It has been shown that standard sequencing alignment methods may be problematic for females, without masking the Y chromosome from the reference genome.<sup>84</sup> For example, more variants were called after masking the Y chromosome in females, particularly in PARs. Similarly, for variant calling in PARs in males, it was recommended to provide only one PAR reference sequence from the two sex chromosomes (i.e., either the X or Y chromosome). Prior to variant calling, the authors recommended using read depths for the X and Y chromosomes, relative to the autosomes, to determine the sex chromosome composition of a sample, similar to that proposed for GWAS arrays.<sup>16</sup>

Additionally, in the past few years, age-dependent clonal loss of the Y chromosome has been reported in leukocytes.<sup>85,86</sup> This phenomenon may further affect data quality and analysis of PAR and Y chromosomal variants.

### Clinical implications

The exclusion of sex chromosomes from analysis and reporting also has significant clinical implications. Chief among these is failure to identify disease-associated SNPs or regions important in pathophysiology, prevention, diagnosis, or treatment. While common GWAS-identified SNPs tend to have small estimated effect sizes, this is not necessarily true for SNPs affecting drug responses, which have not generally been subjected to strong selective pressures.

Current pharmacogenetic guidelines such as those of the Clinical Pharmacogenetics Implementation Consortium<sup>87</sup> do not include genes on the sex chromosomes (quite possibly because of exclusion of these genes from analyses); were such variants to be identified, guidelines for screening or drug dosing might need to be modified on the basis of a patient's biologic sex. Similarly, adequate identification and inclusion of sex chromosome variants in PRSs might mandate stratification of these predictions by sex. It will be difficult, if not impossible, to assess these sex-stratified risks accurately until the dearth of analyses of sex chromosomes in clinically important traits is rectified.

### Recommendations and future directions

After 15 years, several authors have observed that GWASs are “realizing the promise”<sup>88</sup> with “no signs of slowing down.”<sup>89</sup> Interestingly, sex chromosomes were not discussed in the 5-year,<sup>90</sup> 10-year,<sup>4</sup> and 15-year<sup>88,89</sup> reviews of GWASs. Here, we revealed that, for example, sex chromosomes are omitted from ~75% of the GWASs in 2021, which is likely the major cause of the paucity of signals on the sex chromosomes. Given these observations, to achieve sex-chromosome-inclusive research, we make several recommendations and discuss related future research directions.

First, the existing bioinformatic and sequencing pipelines need to be revised for the sex chromosomes, from variant calling<sup>84</sup> to imputation, so that the downstream analyses improve the integrity and robustness of sex chromosome analyses and provide greater confidence in conclusions drawn from them.

Second, QC procedures need to follow previously recommended sex-stratified approaches.<sup>1,11</sup> Additionally, sex difference in MAF<sup>52,91,92</sup> needs to be examined, but whether attributing significant sdMAF solely to genotyping errors (then screening out such variants) is appropriate warrants future research. This is because sdMAF could also be a result of sex-specific linkage, particularly at the PAR-NPR boundaries.<sup>55</sup>

Third, the distinction between association testing and effect size estimation is particularly important for the X chromosome<sup>9,73</sup> Because of X-inactivation uncertainty, genetic effects may be more reliably estimated in a sex-stratified fashion to construct sex-specific PRSs,<sup>93</sup> conceptually analogous to population-specific PRSs.<sup>94</sup>

Fourth, obtaining and then incorporating SNP/gene/tissue/individual-specific X inactivation could improve association methods. To this end, recent advances in long-read next-generation-sequencing technology, enabling phased allele-specific methylation, could be useful.<sup>95</sup> Additionally, gene expression data such as the GTEx resource can be utilized.<sup>10,96</sup>

Fifth, many other existing statistical genetics analyses require sex-chromosome-aware development and implementation. These include, for example, rare variants,<sup>97,98</sup> meta-analysis,<sup>99</sup> LD score regression,<sup>100</sup> pleiotropy,<sup>101</sup> and causal inference via Mendelian randomization.<sup>102</sup> More work is also needed to better understand trait heritability<sup>103</sup> attributed to the sex chromosomes,<sup>45,46</sup> including the effect of imputation quality.

In summary, 10 years after the seminal work by Wise et al.,<sup>1</sup> the exclusion of the X and Y chromosomes from whole-genome analysis persists. Until the sex chromosomes are indeed included in a whole-genome study, instead of GWASs, we propose they be more properly referred to as “AWASs” for “autosome-wide scans.”

### Data and code availability

All data used are publicly available. The specific downloads of the time-stamped datasets and codes used for the different analyses are all available at <https://github.com/Paterson-Sun-Lab/eXclusionaryY/>.

### Supplemental information

Supplemental information can be found online at <https://doi.org/10.1016/j.ajhg.2023.04.009>.

### Acknowledgments

The authors would like to thank Karl Broman, Sara Good, Anthony Herzig, Inke König, Michael Schatz, Bhooma Thiruvahindrapuram,

Melissa Wilson, Stacey Winham, and Andreas Ziegler for valuable discussions. This research was funded by the Canadian Institutes of Health Research (CIHR, PJT-180460) and a University of Toronto Data Sciences Institute (DSI) Catalyst Grant.

### Author contributions

L.S., A.D.P., and T.A.M. conceptualized the study. L.S. and A.D.P. supervised the study and drafted the manuscript. Z.W. and T.L. performed the analyses and summarized the results. Z.W., T.L., and T.A.M. reviewed and edited the manuscript.

### Declaration of interests

T.L. is an employee and shareholder of 5 Prime Sciences Inc.

### Web resources

Genome-wide summary statistics reported in the NHGRI-EBI GWAS Catalog, <https://www.ebi.ac.uk/gwas/downloads/summary-statistics>

Significant SNPs reported in the NHGRI-EBI GWAS Catalog, <https://www.ebi.ac.uk/gwas/docs/file-downloads>

The PolyGenic Score (PGS) Catalog, <https://www.pgscatalog.org/downloads/>

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