

Summary of the *NIH Office of Research on Women's Health* *GWAS, Sex, and Chromosomes Think Tank* on February 27, 2019

Background

The consideration of sex as a fundamental biological variable is critical to enhancing scientific reproducibility through rigor and transparency, which is a great concern of the National Institutes of Health (NIH).¹ Sex differences are pervasive and often profound across body systems and disease categories.²⁻⁵ Nonetheless, there has been an overreliance on male animals and cells in preclinical research.⁶⁻⁸ Moreover, investigators have often neglected to perform sex-based analyses or report sex-disaggregated data in preclinical, as well as clinical, research.⁸⁻¹⁰ As a result, NIH released the Policy on Consideration of Sex as a Biological Variable (SABV) in NIH-funded Research¹¹ on June 9, 2015. The policy requires researchers to consider the possible role of sex at all stages of research in humans and other vertebrate animals, including study design, analysis, and reporting of study results.¹² This policy went into effect for grant applications submitted on or after January 25, 2016.

Genome-wide association studies (GWAS)—which are subject to the SABV Policy when conducted on humans, rodents, or other vertebrates—have transformed the search for genetic influences on human diseases and other complex traits.¹³ This approach involves scanning a large set of single nucleotide polymorphisms (SNPs) in a sample of individuals to identify SNPs that are statistically associated with one or more traits of interest. GWAS results could enhance personalized treatment paradigms, both by enabling population-level stratification of disease risk and by improving individual-level risk prediction.¹⁴ Moreover, GWAS data promise to help reveal disease mechanisms, improve understanding of the pathogenetic relatedness of different diseases, guide the repurposing of existing drugs, and inform the development of novel treatments.¹⁵ New drugs with mechanisms of action that are supported by GWAS data are nearly twice as likely to proceed successfully from phase I clinical trials through regulatory approval as drugs lacking GWAS support.¹⁶

Since the emergence of GWAS around the time that the Human Genome Project was completed, the number of SNPs found to be associated with disease phenotypes has grown dramatically.¹⁷ So have the sample sizes of GWAS and the total number of phenotypes investigated.¹⁷ Notably, the densities of SNP-trait associations discovered on the sex chromosomes (X and Y) have always remained far lower than the densities of such associations on the autosomes.^{13,18-20} This has been the case even though the X chromosome (ChrX) is thought to have a substantial effect on health and disease, especially on certain sexually divergent aspects of health and disease.²¹⁻²⁵

A survey of all GWAS papers published in 2010 and 2011 found that only one-third of them reported including ChrX in their analyses.¹⁹ Prior to 2017, less than 1 in 100 GWAS papers reported anything related to the sex chromosomes in their titles, abstracts, or keywords.²⁶ This omission of the sex chromosomes from GWAS has been caused by the following factors: low SNP coverage of the sex chromosomes on early genotyping arrays; low gene density on the Y chromosome (ChrY); low power to detect associations on the sex chromosomes; and the historical lack of statistical approaches for analyzing the haploid ChrY and for accounting for dosage compensation and X-inactivation in females.^{19,20,26,27} Low marker coverage for the sex chromosomes is no longer an issue, and some

statistical tools have been developed recently to facilitate the analysis of ChrX.^{22,28-31} Despite these developments, the rate at which significant SNP-trait associations are being added to the NHGRI-EBI Catalog of published GWAS associations (<https://www.ebi.ac.uk/gwas/home>) consistently has remained far lower for ChrX (and, indeed, for ChrY) than for any of the autosomes (see **Figure**), especially when one takes chromosome size or gene content into account.

Beyond the direct contributions of loci on the sex chromosomes to the genetic architectures of human diseases and other complex phenotypes, sex also modifies the autosomal genetic basis of such traits.³²⁻³⁹ The extent and importance of gene-by-sex interactions across diseases and conditions remain poorly known, because investigating such interactions has been widely neglected in the GWAS literature. Sex has often been treated as a confounding variable in human genetic studies, with sex being included as a cofactor in statistical models simply to regress its effect out of the results.^{26,40} Investigators have often opted against sex-stratified analyses because of concerns about loss of power in single-sex strata, as well as in comparisons of genetic effects between the sexes.^{20,26,41,42} This has been true especially in the smaller cohorts typical of earlier GWAS and in the cases of diseases with strong sex biases in incidence. Fortunately, combined datasets for meta-analysis and even individual cohorts have started to reach the requisite sample sizes for well-powered sex-stratified analyses and for tests of SNP-by-sex interactions.^{20,33} Methodological frameworks that are reasonable for these purposes are being developed.^{43,44} Yet in an analysis published as recently as mid-2017, Matthew S. Powers, Ph.D., and colleagues estimated that only about 1% of past genetic association studies made any mention (in their titles, abstracts, or keywords) of “sex differences” or analyses done “by sex” or in a “sex-specific” manner.²⁶

Clearly, building a better foundation of knowledge to fully realize the promise of precision medicine will require conducting and reporting human genetics research in a more “sex-aware” manner.²⁰ In a similar vein, a member of the NIH Advisory Committee on Research on Women’s Health—Louise McCullough, M.D., Ph.D., Professor at the University of Texas Health Science Center at Houston—commented to the NIH Office of Research on Women’s Health (ORWH) that human genetics is a field with notable gaps in terms of the application of SABV to science. Given the evident shortfalls in this area, ORWH planned a think tank discussion, involving several subject matter experts, to better understand gaps and opportunities related to genetic association analyses of the sex chromosomes and the consideration of SABV in GWAS. These goals align well with the mission of ORWH, which is the advancement of research to improve the health of women. The GWAS, Sex, and Chromosomes Think Tank was developed as an activity of the NIH Coordinating Committee on Research on Women’s Health. This trans-NIH committee exhibited a great deal of interest and engagement in the Think Tank, given the breadth of genetics research across NIH Institutes, Centers, and Offices.

Several external and internal subject matter experts participated in the Think Tank, which was held on February 27, 2019, with all external participants doing so virtually. The discussion was initiated by Janine A. Clayton, M.D., NIH Associate Director for Research on Women’s Health and Director of ORWH, and it was co-moderated by Dr. McCullough and Chris Cotsapas, Ph.D., Associate Professor at the Yale School of Medicine. Nine other individuals external to NIH participated, including scientific leaders in the field from the following institutions: Arizona State University; Broad Institute; Massachusetts General Hospital; University of California, Los Angeles; University of Chicago; University of Texas Health Science Center at Houston; University of Washington; and Vanderbilt University. Outside the U.S., two other leaders in the field, one from the University of Helsinki and one from the University of Oxford, also

joined the discussion. Inside NIH, the Think Tank was attended by 19 scientists with relevant expertise from 13 of the 27 Institutes and Centers, as well as a number of additional scientists representing three Offices within the NIH Office of the Director. The remainder of this document summarizes the key points made and major perspectives shared in the Think Tank discussion.

GWAS, Sex, and Chromosomes Think Tank: Expert Perspectives

Five topic areas were discussed at the Think Tank:

- (1) Specific technical and methodological hurdles affecting a more thorough consideration of the sex chromosomes and SABV in GWAS.
- (2) Broader topics and conceptual issues affecting a more thorough consideration of SABV in GWAS.
- (3) Limitations and advantages of analyzing older GWAS datasets for sex effects and associations on the sex chromosomes.
- (4) Long-term aspirations for understanding how sex and the sex chromosomes influence the genetic architectures and genetic risks of human disease.
- (5) Near-term opportunities (i.e., potential next steps) to better understand how sex and the sex chromosomes influence the genetic architectures and genetic risks of diseases.

References are omitted from the following sections, because the material contained hereunder is solely intended to be an informal synopsis of opinions and perspectives shared by the participating experts, for whom attributions are also omitted in this summary document.

Specific Technical and Methodological Hurdles

Reasonably sound methodological options and frameworks are now available for performing sex-stratified GWAS, conducting GWAS controlling for sex, analyzing sex-stratified heritability, and testing genetic correlations between sexes. Yet there is always room to advance these methods and refine the associated models (and model parameterizations) for considering sex effects on the genetic architectures of diseases and other complex traits. A research aim that is especially lacking in rigorous statistical methods is the goal of contrasting estimates of genetic correlations for pairs of traits, analyzed separately in males and females, and then determining whether the patterns of genetic correlations differ statistically between the sexes. This is not yet a solved problem, though addressing it would help reveal the distributions of genetic effects, genome-wide, in males and females. In turn, this would contribute to a more comprehensive understanding of the extent to which those effects are shared between the sexes for a certain disease—in other words, a better understanding of the causal genetic overlap of a given disease between the sexes.

One expert shared that generating good genotype data across ChrY is fraught with difficulties, even in most gametologous regions (i.e., regions of homology between ChrX and ChrY arising from the lack of recombination and subsequent differentiation of the sex chromosomes). These difficulties cannot be overcome by available methods for DNA sequence alignment and variant calling. It is well known that ChrY is characterized by regions with high copy number variation (i.e., genomic segments of 50 base pairs or more that differ in copy number among individuals in a population) and regions with long, identical repeats. This high repeat content makes the analysis of ChrY particularly difficult. Technically speaking, the copy number–variable regions on ChrY are similar to copy number–variable regions on other chromosomes in terms of alignment difficulties. According to the expert, the copy number–

variable regions on ChrY have received almost no attention in terms of the development of alignment methods and strategies, including those that consider multiple mapping regions, whereas copy number-variable regions on other chromosomes have received at least a little bit of attention in this regard.

One Think Tank participant stated that the ChrY datasets she had seen had used standard Genome Analysis Toolkit (GATK [Broad Institute]) pipelines for calling variants. She maintained that she does not trust any of the variants that have been called in these highly ampliconic regions of ChrY. (The ampliconic regions of ChrY are highly repetitive regions of the non-recombining portion of that chromosome, which contain large palindromes—up to 1.5 megabases in length—and multicopy genes predominantly expressed in testes.)⁴⁵ The field of genetics currently lacks a solid framework and methodology for aligning sequences and calling variants in those ChrY regions. Potentially, such regions should even be totally excluded from research until a lot more time has been spent developing robust methods for sequence alignment and variant calling in these ChrY regions, according to the expert.

General bioinformaticians at many institutions are likely to argue that reads for XX individuals should be aligned to a reference genome that lacks ChrY (in other words, to a female reference). One observes a reduction in the number of reads mapping to ChrX when data for XX individuals are aligned to an XY reference genome, owing to the spurious mapping of reads from females onto ChrY. The alignment of sequence data for XY individuals is especially problematic considering the mismapping that is often observed between ChrX and ChrY. One approach to solving this alignment problem might be to use statistical inference to consider estimates of minor allele frequencies on ChrY and ChrX and then to use this information to identify the specific reads that are mismapping between ChrY and ChrX. Such a solution to the sequence alignment problem for the sex chromosomes is probably a few years off, owing to the technical challenges inherent in this problem.

Methods for phasing sequence data (i.e., assigning alleles to paternal and maternal chromosomes) are clearly relevant to X-inactivation and the determination of which alleles are active in a female tissue sample under investigation. It might be possible to use RNA data in addition to DNA data, as well as information from the 1000 Genomes Project, for example, to phase ChrX and ChrY in males and to determine which alleles are on the active and inactive X chromosomes in females. However, an added layer of complexity is the heterogeneity of X-inactivation within bulk samples of cells in the real world. How can it be determined what proportions of maternal and paternal alleles are expressed in these situations? Moreover, how would this information be incorporated into any given analysis? Addressing these questions could be a whole area of research on its own.

Similarly, there are a few different ways to model the skewness of X-inactivation, but the field still does not have a good enough understanding of how X-inactivation varies across populations, tissues, and even cells within tissues. Knowing which of the available methods and models to use to address this problem is challenging. Some researchers end up using all available methods and reporting all the results, which frequently do not vary that much from method to method. Nevertheless, simply using and reporting all available methods is not a very satisfactory or elegant solution to a critically important problem in the field.

Overall, there seems to be a need for methods development at the basic technical levels of aligning reads, calling variants, determining which ChrX alleles are active, and performing quality control steps, even before the genotype data can be used for GWAS of the sex chromosomes. Overcoming these

hurdles would greatly advance sex chromosome–inclusive GWAS, because these challenges are among the main reasons that the sex chromosomes were omitted outright from so many previous studies.

Broader Topics and Conceptual Issues

One of the foremost conceptual issues discussed at the Think Tank is the effect of power differences among strata on the outcome and interpretation of stratified GWAS. When investigators find differences in genetic architecture between sexes for a given trait, this could be caused by true underlying sex differences in the genetics of that trait, or it merely could be a consequence of differences in statistical power between strata. For example, the latter possibility is probably a widespread problem for genetic studies of post-pubertal autoimmune diseases and psychiatric diseases, the incidences of which are strongly skewed between the sexes. Similarly, one of the Think Tank participants wondered whether there is a statistical approach (e.g., a bootstrap or permutation test) for distinguishing statistical support for associations detected in single-sex strata from the effects of subsampling. She also wondered whether taking an expression quantitative trait locus (eQTL) approach for every SNP-trait association showing an interaction with sex might help resolve this.

In response to these comments, another Think Tank participant noted that not much work has been done in the way of methods development for these issues. One thing her lab tried for GWAS of diseases showing strong skew in prevalence between the sexes was to calculate the effect sizes separately for males and females. Next, her group looked at the entire distribution of effect size differences. The group tried a Z-score approach to identify the tails of that distribution, which contained the most phenotypically differentiated genetic variants. It then examined the tails to determine whether they showed enrichment for certain functions. Regarding the eQTL approach, her lab has tried many ways of permuting the data. A major issue for the complex disease traits examined by her lab is that many essential covariates need to “travel” with the samples during the permutations, which strongly obscures interpretation of the null hypotheses for those tests. Her lab was unable to determine which of the permutation approaches was best or how to correct for the “multiple test burden” without being overly conservative and failing to detect any interactions at all. Despite the problem of losing too much statistical power in tests of this kind, given current sample sizes, another expert said, “I think we can still do some analyses that don’t rely on individual risk variant identification but instead do this bulk comparison of risk variants between the sexes.”

One possible solution to the intrinsic power differences between the sexes for sex-stratified GWAS of sex-biased traits would be to randomly subsample an equal number of cases for each stratum. In many instances, however, this approach could result in the exclusion of most of the dataset. One of the experts said, “Some of this comes down to needing to do some really targeted recruitment of individuals in the underrepresented sex.” Other participants in the discussion strongly agreed with this view.

During this line of discussion on power differences between strata in sex-stratified GWAS, another expert shared a question that he has been pondering for some time. He asked, “Are there specific biological differences that we can measure quantitatively and then leverage genetic influences to determine whether some of those biological differences that we observe are driving some of the [sex] differences in penetrance?” He continued, “Are certain sex hormones influencing risk for certain outcomes? And, if we can get a handle on the quantitative contribution to those sex hormones from

autosomal variation, can we use that to triangulate some of the source of the sex difference in the phenotype?” Currently, the data needed to answer these questions are lacking, according to the expert.

Switching subjects, one of the Think Tank participants pointed out that complex trait measurements can differ in fundamental ways, *in a phenotypic sense*, between males and females. One example of a phenotypic measurement that varies greatly between sexes is body fat distribution, as measured by waist-to-hip ratio and several other anthropometric measures. Another example among many is relapsing-remitting multiple sclerosis (RRMS), which presents differently in women than it does in men. This form of MS, which is more common than primary progressive MS (PPMS) overall, generally appears to be more severe in men, even though RRMS affects more women than men. How should we think about such basic phenotypic differences with these kinds of traits? When a given measure does not necessarily capture a biological variable in the same way for females and males, fundamental differences in the meaning of the phenotypic measurement between the sexes will often contribute to estimated sex differences in heritability and in genetic architecture. The expert posited that it might be prudent to think about the genetics of such traits differently with respect to men and women, though he stated that this remains an open question at present. “As we start dissecting these phenotypes out in men and women, it starts getting a little more complicated than just doing a sex-stratified analysis. There needs to be a *sex-aware* analysis,” he said.

Another Think Tank participant emphasized the need to carefully consider the models that one uses to adjust phenotypes according to important covariates prior to sex-stratified genetic analyses or genetic comparisons between sexes. She maintained that understanding each phenotype is important both in focused GWAS and genetic scans across large numbers of phenotypes. A single model for optimally adjusting a given phenotype has limited utility across a broad range of phenotypes. For each phenotype, it is important to understand the approach taken by the experts who focus on that trait, including the physicians who understand how certain traits are expressed, or presented, in a clinical setting.

Many variables that would help tremendously in the functional interpretation of genetic results—circulating sex hormone levels, in particular—were not measured for most previously collected human genomic datasets. This is something that the scientific community should encourage the large biobanks to start doing. According to a Think Tank participant, the “genetics of hormone levels” is an interesting topic of investigation in its own right. In addition to sex hormones, pubertal timing and a host of other sexually dimorphic or sexually divergent traits may modify how diseases manifest in the different sexes. Similarly, key variables for women include age of menarche, parity, health conditions during pregnancy (e.g., preeclampsia and gestational diabetes), age of menopause, and so forth. The scientific community should also develop phenotyping strategies or encourage common ways of collecting data across electronic health records (EHRs), not only to facilitate the sharing and merging of data among different EHR sites but also to reduce the biases that could characterize some biobank data.

Many genetic associations for disease traits map to non-coding regions of the genome, such as enhancers. One of the participants said that knowledge of circulating hormone levels would allow investigators to ask, “Are those enhancers also bound by sex steroid hormone receptors that act as transcription factors?” Answers to this and other molecular questions are needed to better understand the functional significance of GWAS results. To propose functional hypotheses, geneticists often layer other data on top of their GWAS results, such as data from the Encyclopedia of DNA Elements (ENCODE)—a research consortium funded by the National Human Genome Research Institute, which has sought

to identify and catalog all functional elements in the human genome. Thinking about how sex steroid hormones act at this molecular level is something geneticists seldom do in the context of their GWAS results, yet sex hormones are a key link between sex differences in genetic architectures and variation in disease traits between men and women.

One of the Think Tank participants offered that some anthropologists are investigating the effects of gonadal hormones on immune system function in non-industrialized and industrialized populations. Some of these investigators are collecting data-rich time series of androgen, estrogen, and cortisol levels and relating them to immunological endpoints, but they are not doing any genetics. Investigators such as these anthropologists may already have access to incredibly valuable datasets for investigating the genetic and molecular basis of sex differences in chronic diseases and other complex traits. Another participant agreed that human geneticists should think about the huge opportunities that may be afforded by those kinds of population cohorts.

Another expert maintained that a lot of information on previous pregnancies is available for many of the large-scale population-risk cohorts with genetic data, though as always, there are inevitably noise and error in the data. She said, "Pregnancy occurrences are relatively well documented, in my experience, in the biobanks that I'm working with, but hormone status and various hormone profiles are missing." According to the expert, a serious investigation of sex differences arising from the autosomes would require a large-scale study done in the proper setting, including full hormone profiling. In fact, truly understanding the influence of sex on genetic architecture and the difference in disease prevalence between the sexes would require a "dream study," meaning a longitudinal study of a large cohort. Several experts agreed that one or more large longitudinal studies would eventually be needed to make real progress in this area.

In some biobanks, sex hormones have already been measured on thousands of people, according to a Think Tank participant. These samples are already becoming a valuable resource for the passive collection of genetic and disease trait data accompanied by hormone profiles. However, the hormone data can have a lot of gaps and be quite messy, and there is certainly room to improve how EHRs are attached to biobank repositories in general. Reflecting on these comments, another expert said, "I agree with you, but my one concern is that the people that have sex hormones measured will not be an adequate representation of the population." The expert elaborated that cases with hormone data in the biobank would be enriched for patients with a prerequisite condition warranting the ordering of extra phlebotomy and hormone tests. In the U.S., individuals with hormone data in the biobank may also be biased toward patients with insurance, as opposed to those lacking insurance. In other words, there may be substantial ascertainment bias in data that are passively collected this way. The expert acknowledged that biobanks are a great starting point to begin looking at the influences of hormones on sex-stratified GWAS results, but this does not preclude the need for widespread hormone sampling for at least one of the large biobanks. "Ideally, we would want it to be done longitudinally, as well," she said.

One of the participants asked, "Is there an effort right now to look at whether the sex differences we observe in human diseases are replicated in animal models?" The participant reflected that the incidence of rheumatoid arthritis (RA) is something like three to five times higher in women than men, whereas mouse models of RA do not exhibit this kind of sex difference. For some mouse models of disease, the mice seem to recapitulate the sex differences seen in humans reasonably well. The expert said that for other models, the mice and humans may show sex differences in the opposite direction. In

many other cases, the phenotypes are so different between species that the mice are not good models of the human diseases at all. According to the expert, more effort needs to be spent identifying and cataloging mouse models of diseases exhibiting notable sex differences in humans—in other words, mouse models with sex differences that align well with those seen in humans—because many of the needed functional studies cannot be done in human volunteers. Two other participants voiced their enthusiasm for such an effort, particularly considering the historical lack of focus on SABV in preclinical research (see **Background**).

Another expert noted the importance of considering the sociobiology of any species used to model human diseases showing strong sex differences. He said that some hormonal influences on traits related to the brain and behavior, as well as other kinds of traits, may be evolutionarily labile and depend on an animal's social behavior, mating system, and so forth. The same participant acknowledged that hormonal influences on many other traits, including many neural and behavioral traits, may be much less evolutionarily labile and much more robust for comparisons between species. The speaker was not arguing against trying to model human diseases and disorders in non-model species, because doing so is obviously important to driving progress in biomedical research. Rather, the expert was arguing for the importance of being mindful that the ultimate (ecological and evolutionary) influences driving certain sex differences could vary substantially from species to species.

Limitations and Advantages of Analyzing Older GWAS Datasets

Though the majority of available GWAS datasets have sex chromosome data that could be analyzed, there are limitations and difficulties associated with the reanalysis of historical GWAS data for sex effects and/or genetic associations on the sex chromosomes. Consortia have traditionally analyzed data by getting summary statistics from individual cohorts and merging those into meta-analyses. Such an approach does not make it easy, or even feasible, to go back and obtain the individual-level data for a *de novo* study that considers the sex chromosomes and/or sex effects on genetic architecture.

Consortia have spent a tremendous amount of time and effort aggregating, summarizing, and analyzing data for genetic studies. Accordingly, to obtain the data needed to conduct sex-stratified GWAS or GWAS of the sex chromosomes, it makes more sense to look to the large biobanks than to reconsider and reanalyze several smaller cohorts. For instance, pulling together a GWAS of ChrX from older cohort studies would entail revisiting each individual sub-study, reanalyzing ChrX for each of those sub-study datasets, collating the results from all the individual analyses, and finally, performing the meta-analysis. This would not be a trivial task.

Harmonizing the phenotype data could be another major challenge when it comes to revisiting multiple older datasets or combining those data with newly collected cases. Oftentimes, the current researchers would need to go back and communicate directly with the original authors to determine exactly what measures were used, especially in the cases of complex or quantitative traits. Unless the original authors shared their raw data when they published their findings or unless they fastidiously saved their data and are now willing to share them, data harmonization might be difficult to impossible. In turn, if the data were not to be harmonized properly, the combined dataset might even produce genetic findings lacking loci that were previously detected and reported for the trait under investigation.

The UK Biobank, FinnGen biobank, Partners HealthCare Biobank, and other large biobanks already have ChrX data available for incorporation into new studies. Among all technical issues potentially affecting

GWAS, the availability of genotype data for ChrX is not one of the barriers preventing use of the large biobanks for sex chromosome–inclusive GWAS.

The rate of GWAS data generation today is expanding extraordinarily rapidly (see [Figure](#)). One of the Think Tank participants estimated that the amount of available genomic data is doubling approximately every 18–20 months. He also said that ChrX data are available for almost all GWAS data being generated today. The expert added, “Given this greatly expanding wave of new data, it makes little sense to go back to historical datasets to try to analyze ChrX. Instead, the community should ramp up its efforts to make ChrX data much more accessible and analyzable going forward.” In light of the deluge of new data, and considering the increasingly large sample sizes for many traits, there was broad consensus among Think Tank participants that the future looks bright for opportunities to perform GWAS on the sex chromosomes and conduct sex-stratified analyses.

Going back to published GWAS in the repository would be incredibly challenging. At the same time, there also seemed to be agreement among Think Tank participants that using historical data certainly could be worthwhile if doing so were to increase case aggregation in a way that is not possible with a biobank. With diseases that are not that common—such as MS, which affects roughly 1 in 1,000 people—biobanks may not offer substantial numbers of cases until they really get going. There are already consortia, such as the International MS Genetics Consortium, that have investigated about 50,000 cases of this disease. In situations like this, revisiting historical data might be well justified, but it would probably only be worth it to go back to older data when not enough power could be leveraged from a biobank for a disease of interest.

Long-term Aspirations

All Think Tank participants were asked to share their biggest aspirational goals (one per participant) for advancing a better understanding of how sex and the sex chromosomes influence the genetic architectures and genetic risks of human diseases. For the list of aspirational goals that follows, no attempt was made to prioritize the goals based on the amount of support or consensus they received from the Think Tank participants. Prioritizing goals was beyond the scope of the February 27, 2019, discussion.

- Deepen the examination of documented biological differences between the sexes.
- Attain complete hormone profiling in a longitudinal study of a very large population cohort.
- Perform a thorough population-based assessment of sex differences in biology across the lifespan using a wide range of biomarkers (from imaging, blood tests, etc.) and/or other endpoints (e.g., growth curves).
- Achieve a fundamental understanding of what “sex” and “gender” are, as well as a better understanding of the distinction between “dimorphism” and “difference” in the context of variation by sex or gender.
- Synthesize all the layers of “omics” data to better understand sexual dimorphisms and sex differences in diseases and other complex traits (e.g., to better understand the “multi-omic” associations between sex hormones and genes).
- Expand the separate estimation of genetic effect sizes in women and men to advance the use of polygenic risk scores for risk prediction and precision medicine.

- Develop a methodological framework to distinguish genuine sex differences (e.g., those caused by genetics and/or sex hormones) from the consequences of simply subdividing a population.
- Advance phenotyping and bioinformatic approaches to extract key covariates from clinical populations and EHR data, such as pubertal age, age of menopause, important hormone data, and other variables of relevance to sex differences in health and disease.

Near-term Opportunities (Potential Next Steps)

Think Tank participants also identified their highest-priority near-term opportunities, or “next steps” (one per participant), for advancing a better understanding of how sex and the sex chromosomes influence the genetic architectures and genetic risks of human diseases. As before, the following list of potential next steps has not been ordered or prioritized in any way.

- Develop centralized resources for the “garden-variety” bioinformatician (e.g., one or more publications, an online portal, GitHub repository, etc.) with easy-to-follow methods, guidelines, best practices, analysis tools, and other resources for working with sex chromosome data on a technical level (e.g., alignment and variant calling) and also for incorporating the sex chromosomes and SABV into GWAS (e.g., robust methods for sex-stratified analyses).
- In addition to developing centralized resources for working at the genetic level, develop similar best practices and resources for working on the phenotypic side, at the levels of gonadal sex and gender.
- Identify and address the barriers that inhibit many researchers from more widely including the sex chromosomes and considering SABV in GWAS and from adopting existing methods and tools for these purposes. In other words, take steps to increase the uptake of available methods.
- Encourage the growth of a more sex-aware approach to medical genetics through some kind of media or public relations campaign.
- Conduct a public relations campaign to educate the scientific community about the distinction between “sex” and “gender,” as well as the correct use of those terms.
- Geneticists who are already analyzing the sex chromosomes and performing sex-stratified GWAS (e.g., for noncommunicable diseases) should be encouraged to continue doing so.
- Make gonadal hormone testing nearly as routine in medical evaluations as cholesterol testing. Similarly, make the consideration of risk factors such as preeclampsia, pregnancy history, and adverse life events as common as the consideration of hypertension when it comes to thinking about cardiovascular health.
- Expand the number of traits for which sex-stratified GWAS analyses have been performed, and make this approach much more routine going forward.
- Harness existing EHRs attached to large biobanks to better understand how genetic risk for complex disease traits manifests differently in males and females as patients present with those diseases in real-world clinical settings.
- Translate polygenic risk scores into biobank settings and investigate how sex modifies the effects of those risk scores in order to gain insights into how sex may act as both a biological variable and an environmental variable.
- Encourage systems such as ENCODE to have a better representation of male and female tissues—for example, in terms of the kinds of epigenetic changes that are entered in such

systems. This would facilitate a better understanding of sex differences in gene expression in different tissues.

- Systematically investigate and evaluate the nature of the genetic correlation between men and women.
- Develop methods for factoring biological priors from X-inactivation and escape from X-inactivation into analyses of ChrX.
- For human diseases exhibiting sex differences, identify biologically sound animal models with which to advance our understanding of the functional basis of polygenic risk. In other words, figure out what the right genetic models are for preclinical work that can be translated to human diseases with salient sex differences.
- Identify genetic variants in humans and other species associated with evolutionarily conserved sex differences.

Closing

At the conclusion of the 2 ½-hour discussion, Dr. Cotsapas and Dr. McCullough thanked all the experts both outside and inside NIH for participating in the ORWH GWAS, Sex, and Chromosomes Think Tank. The two moderators encouraged continued momentum on addressing the inadequate consideration of the sex chromosomes and SABV in GWAS, which is clearly a big problem in human genetics. Dr. McCullough acknowledged that the Think Tank was only a small start to a very large and important set of issues—and opportunities. The ability to work through issues and come to a consensus by telephone or via the internet is extremely limited. As a result, Dr. McCullough encouraged attendees from the scientific community outside NIH to try to figure out a way to keep this important conversation going and possibly even develop a face-to-face meeting involving many more partners.

Dr. Clayton thanked the two moderators and everyone else for participating in the discussion and for shedding light on some very significant issues affecting research on the health of women (and men) across disease categories. Dr. Clayton then concluded the Think Tank gathering.

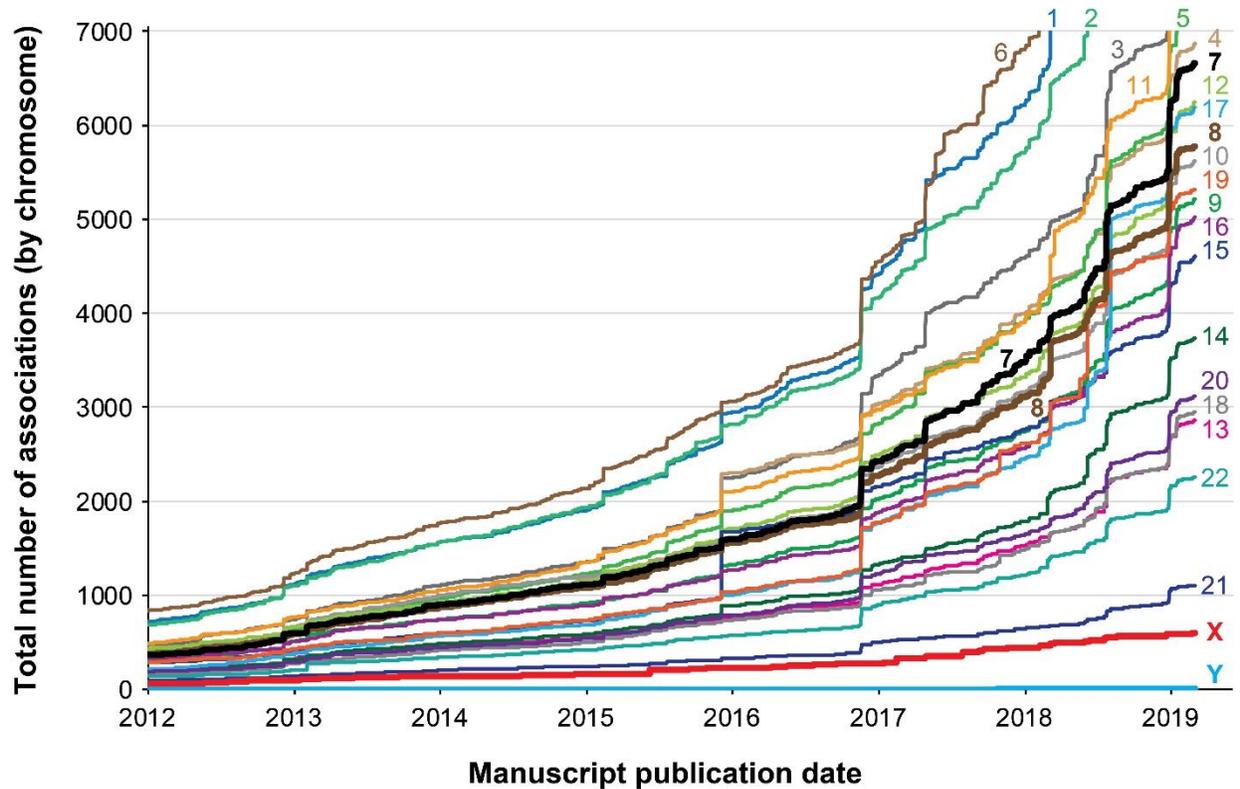
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Figure



Caption: Accumulation of significant SNP-trait associations in the NHGRI-EBI GWAS Catalog, shown separately for each unique chromosome of the human genome (chromosome designations given at the right for the 22 pairs of autosomes [1–22] and one pair of sex chromosomes [X and Y]). Thicker lines are used for Chr7 (black), Chr8 (dark brown), and ChrX (red), to draw attention to their similar sizes in terms of base pairs and gene content: Chr8 = 146 Mb, *ca.* 700 protein coding genes; ChrX = 155 Mb, 800–900 genes, Chr7 = 159 Mb, 900–1,000 genes (NLM Genetics Home Reference, 2019; <https://ghr.nlm.nih.gov>). As such, Chr7 and Chr8 serve as good autosomal references for ChrX in terms of their target sizes for possible GWAS associations. Data in the figure were taken from ver. 1.0 of the GWAS Catalog (06-April-2019 release date; Ensembl release ver. 96; downloaded from <https://www.ebi.ac.uk/gwas/docs/file-downloads>). Data were not filtered on the basis of genome-wide significance level. Accordingly, the figure represents all accessions in the GWAS Catalog except for the small number of associations that have not been mapped to chromosomes. Large, synchronous steps in cumulative sum profiles across many autosomes resulted from the same large publications uploading huge numbers of GWAS associations to the catalog.