Sex Matters in Health and Disease: a review of biological sex differences with an emphasis on glioma

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Abstract: Humans are sexually dimorphic, with sex being the most persistent difference among humans over the course of our evolutionary history. Beyond the visible sex differences that can be considered true dimorphisms, there are also sex differences at the molecular and cellular scales. The role of these biological sex differences for human health, while being increasingly recognized, have long been underappreciated and underexplored. Frequently, these differences are only recognized in sex–specific diseases, such as genitourinary diseases and cancers. However, given the evidence for sex differences in the most basic aspects of human biology, including metabolism, cellular composition, and immune activity, these differences could have consequences for the etiology and pathophysiology of a majority of diseases. It is thus essential to consider the extent to which these differences may influence the various mechanisms underlying disease processes, response to treatment, and the maintenance of health in order to better improve patient outcomes. Here we review the evidence for a broad array of biological sex differences in humans and discuss how they may relate to observed sex differences in various diseases, with an emphasis on cancer, specifically glioblastoma. We further propose that mathematical approaches can be useful for exploring the extent to which sex differences affect disease outcomes and accounting for those in the development of therapeutic strategies.

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INTRODUCTION

From a very young age, we are introduced to the dichotomies of sex and gender. Based on external anatomical sexual dimorphism, children are typically assigned a gender at birth and brought up according to distinct gender norms. However, the resulting impacts of this genderedness as a social construct and efforts to avoid gender–based discrimination have at times led to an avoidance of robustly studying and understanding biological sex differences and their role in human medicine. These biological sex differences are related to human sexual dimorphism, but go beyond external anatomical differences and even the more widely understood hormonal differences that we typically associate with gender. At the base of biological sex in humans lies the 23rd chromosome pair—typically XX in genetic females and XY in genetic males, though other combinations such as XXY and X0 are both viable and not rare.[1,2] The sex determining region Y (*SRY*) gene on the Y chromosome is initially responsible for gonadal differentiation and contributes to regulating differences in expression of testosterone, but this is not the only difference seen at the chromosomal level between the sexes.[3,4] As such, treatment with cross–sex hormones in transgender individuals[5] and people with atypical sex chromosome karyotypes does not completely change all of the underlying biological factors associated with chromosomal sex. The impacts of sex can be observed throughout the lifespan—from metabolic differences following conception, to differences in lifespan length and response to infectious disease.[6,7]

In this review, we use “sex” to denote biological sex as determined by chromosomes, following NIH guidelines,[8] focusing on XX females and XY males. This is distinct from gender, which is a social construct. The societal impacts of gender also have implications for human health, including the conditions referred to in this review, but that is not the focus of this article. We begin by highlighting known biological sex differences in healthy individuals, including the immune system and metabolism. Next, we discuss sex differences observed in nonneoplastic disease and how these relate to underlying biological sex differences. Finally, we review sex differences in neoplastic disease broadly, and focus on one particular cancer for which understanding sex differences may be impactful: glioblastoma. We conclude with recommendations for using computational approaches to facilitate studies investigating the complex impacts of sex in human health and medicine.

SEX DIFFERENCES IN HEALTHY INDIVIDUALS

Sex differences in the immune system.

The human X chromosome contains many genes related to immune function.[9] Because the human Y chromosome does not contain alleles for these genes, dosage compensation evolved so that only one complement is needed, thereby necessitating X-inactivation in females (XX) to avoid the consequences of over-expression of these same genes.[10] This inactivation is achieved through a variety of epigenetic mechanisms;[11] however, a number of studies have shown that X-inactivation is not complete, with as many as 30% of genes on the inactivated X (Xi) escaping inactivation.[12,13] Furthermore, recent work has shown that the Xi can be partially reactivated in lymphocytes, leading to the overexpression of X-linked immune genes.[14]

Immune differences between the sexes are also reflected in different relative abundances of various immune cells. In their review of sex differences in immunology across a variety of species, including humans, Klein and Flanagan note that human females have higher T-cell numbers and increased antibody response.[15] On average, females have higher numbers of CD4+ T-cells than males, as well as a higher ratio of CD4+ to CD8+ cells, and this difference is maintained across all adult ages, even as this ratio increases with age in both males and females.[16–19] Other studies have found a higher count in total lymphocytes among males, but a higher abundance of

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granulocytes in blood samples from females.[20–22] These trends have been observed across different ancestries and various geographic regions, suggesting that these differences are maintained in the presence of various genetic and environmental influences.

In addition to differences attributable to genetics, there are also influences due to sex hormones, which are more notable following puberty. Because technology to detect hormones and to produce synthetic hormones has existed much longer than genetic sequencing technology and other sophisticated microbiological approaches, many studies have focused on immune differences attributable to sex steroids.[23] Physiologic levels of estradiol have been shown to be immunostimulatory.[24] One study showed that estradiol may promote the production of corticotropin stimulating hormone by enhancing *CRH* gene transcription in the hypothalamus, suggesting one mechanism for this effect.[25] Furthermore, hormonal fluctuations during the female menstrual cycle are associated with alterations in T cell numbers. The overall number of regulatory T-cells (T_{reg}) increase during follicular phase and decrease during the luteal phase.[26] Additionally, the relative abundance of type 1 versus type 2 helper T cell (T_h1 and T_h2, respectively) responses vary with estrogen levels, with T_h2 responses being predominant during the follicular phase (when estrogen levels are high) and T_h1 responses predominating during the luteal phase.[27,28] Studies have also shown that estrogens play a dynamic role in wound healing. Estrogens stimulate various growth factor pathways to improve re-innervation and -epithelialization, as well as enhance the formation of granulation tissue.[29] There is an age-related decline in wound healing in healthy females, which is counteracted by hormone replacement therapy with progesterone and either conjugated estrogen or estradiol.[30]

Immune Sex Differences seen in Animal Studies. Studies among murine models have revealed further immunological sex differences, suggesting the existence of additional immunological differences between healthy human males and females. For example, resident leukocyte populations in murine females are more numerous than in males, and they have a greater density of pathogen/injury-sensing toll-like receptors,[31] and dendritic cells express estrogen receptor alpha.[32] Experiments have also shown a role for the X chromosome in autoimmune disease susceptibility in females.[33] In the brain, there are also sex differences in microglia, suggesting implications for neuroimmune differences between the sexes. In particular, the abundance and morphology of microglia in various brain regions differs between the sexes,[34,35] as do their phenotype and transcriptome.[36,37] Due to the role of microglia in modulating synaptic connectivity, neuroimmune sex differences have implications for neurological development, as reviewed elsewhere.[38,39] These immunological sex differences may even have implications for pain perception and morphine response, with microglia required for sensing pain in male rodents, but not in females,[40] and sex differences in microglia may drive the observation of reduced sensitivity to morphine in females.[41] Neurons and astrocytes can produce estrogen and microglia and oligodendrocytes express estrogen receptor, particularly ERb.[42] In vivo studies in mice with experimental autoimmune encephalomyelitis (a model of multiple sclerosis) have demonstrated that Δ^5-androstandediol bound to ERb inhibits inflammation, while E2 bound to ERb prevents this in brain vascular SMCs.[43] Additionally, there has been increased recognition of the role that the microbiome plays in immune response. In one study using the nonobese diabetic mouse model of type 1 diabetes, gut microbiota were transferred from adult male subjects to immature females, which resulted in elevated testosterone production and reduced islet inflammation, protecting against development of diabetes. This suggests that microbiota may participate in signaling loops that can influence sex hormone levels and thereby affect immune response and metabolism.[44]

Sex differences in metabolism.

Sex differences in human metabolism have been noted during periods of exercise and fasting, as well as in hypoglycemia, with females having increased lipolysis relative to males and males

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having increased carbohydrate oxidation relative to females.[45,46] There are also differences in protein use and muscle turnover, with slightly less amino acid metabolism in females (particularly reduced leucine oxidation).[46] While these differences are small between young males and females, they may be more pronounced in response to resistance exercise and feeding among older adults.[47,48] After adjusting for body composition, there does not appear to be a sex difference in overall basal metabolic rate (BMR).[49,50] However, in one study, the sample size was quite small after restricting subjects to postmenopausal ages (>49 years) for females and age-matched males in order to exclude the effect of circulating sex steroid hormones.[50] Another study noted that females had higher levels of circulating leptin which did not impact residual BMR, although there was an association between residual BMR and the thyroid hormone thyroxine that remained significant for males but not females when the sex cohorts were analyzed separately.[49] A review of metabolic sex differences by Mauvais-Jarvis goes into further depth on these and also includes animal studies that may help distinguish the hormonal versus chromosomal impacts of sex on metabolism.[51]

Studies have also revealed metabolic sex differences in the brain, specifically in cerebral glucose metabolism. Although these have sometimes been framed to discuss potential differences in cognitive abilities and emotional processing, this is not our focus here; rather, we include them to highlight possible (sub)cellular biological differences that could be related to disease pathogenesis and outcome. Most studies in cerebral glucose metabolism largely rely on 2-deoxy-2-(^{18}F)fluoro-D-glucose (^{18}F-FDG) positron emission tomography (PET) to study global and regional differences in the brain. Two studies have showed increased whole brain glucose metabolism in females.[52,53] Another study found that resting glucose metabolism in the temporal limbic region and the cerebellum was higher in males than females, while the opposite was true in the cingulate region.[54] Still another study found that cerebral glucose metabolism was higher in the orbital frontal region in females than in males, in addition to a global increase, but no difference was found in the left anterolateral prefrontal cortex.[53] The significance of these differences is not well–understood, but they point to potential differences in underlying biology at the cellular level.

Some have focused on the role of the menstrual cycle, hypothesizing that metabolic sex differences in the brain may be driven by hormonal differences. One study showed globally elevated (19% higher) glucose metabolism on ^{18}F-FDG PET in the whole brain of females in the follicular phase of the menstrual cycle as compared to males, with no particular neuroanatomical structures or regions outstanding.[52] Another compared cerebral glucose metabolism during the follicular and luteal phases in menstruating females.[55] This study found no difference in whole brain glucose metabolism between the menstrual phases, but did find regional differences, with higher glucose metabolism in the thalamic, prefrontal, temporoparietal, and inferior temporal regions during the mid-follicular phase, and in the superior temporal, anterior temporal, occipital, cerebellar, cingulate, and anterior insular regions during the mid-luteal phase. These regional differences may or may not be associated with regional differences in hormone receptor expression, and the finding of no differences in whole brain glucose metabolism during different phases of the menstrual cycle suggests that sex hormones may not be the primary cause of sex differences in whole brain glucose metabolism.

There are also important sex differences in drug metabolism, with some drugs being metabolized slower in females than males and other drugs metabolized faster in females than males.[56] For example, drugs catalyzed by the cytochrome P450 CYP3A have faster rates of clearance in females, who demonstrate twice the level of CYP3A4 expression in their livers.[57] Differences in expression of various cytochrome P450s (CYPs) may be related to their role in steroid hormone synthesis and metabolism.[58] Other drugs have slower clearance in females than males, and may thus have higher toxicity.[56] This difference has also been observed among children treated with

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6-mercaptopurine for leukemia, with males requiring higher levels of the drug to attain similar efficacy.[59,60] Sex differences in growth hormone secretion patterns may be just one factor contributing to observed sex differences in CYP expression, through effects on expression of STAT5b, which has regulatory effects on a number of CYP genes.[61,62]

Sex differences in development.

Sex differences in metabolism start as early as conception, prior to the development of gonads or existence of gonadal hormones, and are linked to developmental differences between the sexes. One large retrospective study found that low gestational weight gain results in more male fetal losses than female,[63] a finding that was also observed in a study of births occurring during the 1959-1961 Chinese Great Leap Forward famine.[64] Another study demonstrated differences in cell count and uptake of resources during the early stages of human embryonic development.[6] Sex plays a significant role in the physical development of humans, particularly in sexual differentiation through the development of gonads and secondary sexual characteristics that lead to physical sexual dimorphism. Further, female life expectancy is longer than that for males, a finding that persists in survival data across countries throughout the lifespan, including in very early life (birth to age 5) and in later life (ages 50+), indicating that these differences are not solely attributable to sex-specific societal exposures (e.g., war and violence, or different pressures toward risk-taking behaviors).[7]

Beyond the more obvious sexually dimorphic traits, such as physical size and gonads, other morphological differences between males and females occur throughout the body. Females have stiffer arterial walls (as measured by pulse pressure) in prepubescent childhood and post menopause as compared to menstruating females, while males' arterial stiffness increases linearly over the lifespan.[65] Further, males have larger brain volumes than females,[66] with a higher percentage of that volume consisting of white matter.[67,68] Regional differences in gray matter volume between males and females has been shown to be independent of overall brain size in studies where male and female subjects were matched on the basis of total brain volume.[69] Further, there appear to be sex differences in the timing of volumetric growth and maturation of various brain regions during development.[70,71] Still other studies have examined inter- and intra-hemispheric brain connectivity and found sex differences.[72,73] However, it is worth noting that these studies in humans have been conducted on subjects of age 8 years and older, and therefore we cannot rule out the possible contributions of socialization and gender roles on these observed brain differences. This is particularly important to be aware of, since some studies on sex differences in the brain have erroneously been interpreted to reinforce stereotypes about differential cognitive capabilities between the sexes, as discussed comprehensively elsewhere.[74,75]

Developmental Sex Differences seen in Animal Studies. Because of the difficulty in teasing apart the contributions of socialization and innate biology on neurocognitive development in humans, animal studies can be particularly useful. In a series of murine experiments, alterations in prostaglandin-E2 (PGE2) expression during development were shown to affect neurogenesis in the rat preoptic area (POA). Specifically, increased PGE2 was associated with increased dendritic spine density (and vice versa), as well as masculine sexual behavior.[76,77] Studies using the four core genotypes (FCG) mouse model allow for the separation of gonadal vs chromosomal contributions to biological sex differences by moving the SRY gene to an autosome to create XX and XY individuals with ovaries as well as XX and XY mice with testes.[78] In one study using FCG mice, both chromosomes and estrogen were shown to contribute to differences in growth hormone (GH) regulation in some regions of the brain.[79] Specifically, estradiol increased GH in the hippocampus and cerebellum, while XX mice had more GH in the arcuate nucleus of the hypothalamus than XY mice. Various other sex hormones, including androgens and progestins,

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have been shown to affect adult hippocampal neurogenesis as well.[80] Early life adverse events also have sex- and age-specific impacts on hippocampal neurogenesis in developing rodents.[81]

SEX DIFFERENCES IN PATHOLOGICAL CONDITIONS

Sex differences in nonneoplastic disease.

Sex differences not only impact healthy day-to-day functioning, there are also many differences that influence disease risk/incidence, pathophysiology, and outcome. This has been particularly noted for autoimmune diseases, which generally affect females more frequently than males.[82] Given the normal differences between the sexes in immune function, with females generally having higher levels of immune activation (as discussed above), this difference is perhaps unsurprising, although it must be noted that the relationship between sex and disease incidence is not necessarily clear. Some diseases, such as Sjogren's syndrome, Hashimoto's thyroiditis, and Graves' disease, affect women far more frequently than men (more than 4:1), while other diseases such as sarcoidosis and ulcerative colitis are only slightly more common in females or exhibit no sex differences.[82,83] A few autoimmune diseases may even affect males more frequently—in particular, studies have found a male bias in incidence of type 1 diabetes among patients diagnosed following puberty,[84] which may also be connected to sex differences in insulin sensitivity.[51] Sex differences in neuroimmunology, in combination with sex differences in dopamine and glutamate signaling, may contribute to observed sex differences in incidence and/or clinical outcomes of various neurological and psychiatric illness, including multiple sclerosis, Alzheimer's and Parkinson's diseases, autism and schizophrenia.[85–87] One systematic review found stroke to be more common in males,[88] while another review found it to be more common among females;[89] however, both found worse outcomes for females. Sex differences in arterial wall stiffness and inflammatory pathways may explain some of the differences observed in hypertension and cardiovascular disease between males and females.[65,90,91] There also appear to be contributions from sex hormones, but results have been contradictory.[92] A study of cardiovascular disease in transgender patients found that male to female transgender individuals taking cross hormones in the form of oral estrogen had worse cardiovascular outcomes, and thus recommend other routes of administration.[93] Beyond diseases themselves, immunological sex differences also contribute to the disparate outcomes in wound healing and susceptibility to infectious disease following injury.[94] One retrospective study of patients treated for injuries demonstrated that males had a greater prevalence of major infections following moderate injury than female patients.[95]

Sex differences in neoplastic disease overall.

These sex differences also impact incidence and outcome in neoplastic disease. Nonreproductive cancers affect males more frequently than females, and carry poorer prognoses in males.[96–98] While this is sometimes attributed to different sociological factors, such as a greater propensity to have been a smoker, a number of studies that controlled for these (such as looking at only those who were previously smokers) suggest that such sociological differences are not wholly responsible for the observed differences in incidence and outcome.[98] This is further supported by studies among childhood cancers, where males make up a greater proportion of affected individuals overall and among most cancer types.[99] The preponderance of males among children affected by cancers also suggests that hormonal differences may not necessarily be primarily responsible for the observed sex difference at other ages. In one recent study, it was found that sex differences relating to metabolism may enable prognostic stratification of females with clear cell renal cell carcinoma.[100] Specifically, high relative visceral fat area (compared to subcutaneous fat area) on computed tomography was associated with poorer survival outcomes in females but not males. Conversely, females with low relative visceral fat area and low tumor glycolysis rates had remarkably good survival outcomes, which was not seen to be as strong in males. Additionally, an analysis of gene regulatory networks in colon cancer identified sex differences in

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expressed and targeted genes.[101] Interestingly, while all of the 20 most sex differentially expressed genes in this study were linked to sex chromosomes, 19 of the 20 most sex differentially targeted genes were of autosomal origin, and many of those more highly targeted among females were genes involved in drug metabolism. Of course, sex hormones interact with immune and metabolic functions, and thus likely play some further role in sex differences among cancers. Estrogens and androgens can modulate immune responses,[102,103] as well as gene expression *in vitro* and *in vivo*, with effects on tissues being further mediated by intracellular sex hormone receptors.[104–106] A lower risk of hepatocellular carcinoma in females has been attributed to prolactin,[107] and estrogen has been associated with colorectal cancer risk reduction in premenopausal females.[108–110] Further, women were found to be more susceptible to oral cancers following menopause.[111] While research on sex differences in cancer has historically focused more on the contributions of sex hormones, this is only one facet of the biological sex differences that may impact disparate incidence and outcome in neoplastic disease.

SEX DIFFERENCES IN GLIOMA

Thus far, we have reviewed the significant sex differences observed in healthy bodies, pathologic conditions, and non-brain cancers. The presence of consistent sex differences throughout the body and in healthy and pathologic conditions have led researchers to hypothesize that sex differences play a role in both primary and secondary brain cancers.[112,113] There are known hormonally driven sex differences seen particularly in meningioma and pituitary adenoma.[113,114] In this section, we will focus on the most common primary malignant brain cancer, glioma and glioblastoma (GBM, grade IV glioma), in which sex differences has been relatively understudied. The strongest and most consistent evidence for sex differences in GBM is related to incidence, with GBM being more common in males, resulting in a M:F ratio of GBM patients of about 1.4-1.6:1.[115,116] The M:F ratio of the average annual age-adjusted incidence rate from the most recent CBTRUS report is also about 1.6:1.[116] Additionally, female GBM patients have been observed to live longer than their male counterparts when given the same standard-of-care treatment.[117] These two differences allude to the existence of underlying biological sex differences that enhance male risk for GBM and extend female life during treatment.

Sex differences in glioma metabolism

Aerobic glycolysis, or the Warburg effect, refers to the metabolism of glucose to lactate in proliferating cancer cells despite the presence of oxygen that would otherwise support the complete oxidation of glucose in mitochondria.[118] Cancer cells, including glioma cells, use this pathway to rapidly produce ATP and other metabolic precursors that are needed to combat oxidative stress and enable rapid proliferation.[119–121] Considering the observed metabolic sex differences in glucose uptake in proliferating embryos, as well as those in healthy adults during exercise and conditions of oxidative stress, one might hypothesize that nutrient uptake and metabolism in cancer cells may also display sex differences. One study found that the level of expression of glycolytic genes significantly stratified survival among males with lower grade gliomas, even when the males were split up by grade, histology, and select mutations. For females, glycolytic gene expression level only stratified survival among IDH1 wild-type patients. Additionally, glycolytic metabolite levels (pyruvate and the lactate/pyruvate ratio) stratified male survival, and not female, among grade II glioma patients.[119] A different study used advanced imaging and found differences in magnetic resonance imaging (MRI) metrics and relative metabolite levels between male and female high-grade glioma rat models. They concluded that the male tumors were more aggressive than female tumors, warranting further investigation in human subjects.[122] With the increased utilization of FDG-PET and other advanced imaging on brain tumor patients and the potential for this information to be used to predict tumor grade and patient prognosis,[123] it will be increasingly important that we understand how sex impacts tumor metabolism and patient outcomes.

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Sex differences in glioma and immune system

Once thought to be immune-privileged with limited intervention against antigens, the immune system in the CNS is now known to have both adaptive and innate components, with antigens triggering both T cell and macrophage responses. Ideally, the immune system combats cancerous growth by detecting tumor-associated antigens on malignant cells. While glioblastoma is usually accompanied by inflammation and an immune response consisting of T cells, macrophages, and microglia, this is not necessarily a sign of tumor rejection, since these cancer cells are known to secrete immunosuppressive cytokines and manipulate immune activity.[124,125] There is very little information on sex differences in the neuroimmune system based on the analysis of human subjects, but microglia are known to play an important role in human brain development, and rat models have shown sex differences in the abundance of microglia and effect of T-cells on the development of rat brains.[38] Considering the previously described role of X inactivation in immune activity and the observed immune sex differences in the rest of the body, one would hypothesize that the interactions between GBM cells and the immune system might also be impacted by sex. Two studies using case-control methods found an inverse relationship between pre-diagnostic IgE levels and risk for high-grade glioma among females only.[126,127] Contrarily, another study found an inverse relationship between pre-diagnostic IgE levels and glioma risk among all patients and did not find that this relationship was more significant among females.[128] At baseline, males were found to have higher levels of total IgE compared to females among both glioma cases (tested after diagnosis) and healthy controls.[124] Considering the vast potential impact of sex differences on immune-glioma interactions and the necessity of understanding sex's role in these interactions when deploying immune-dependent treatments (e.g., chimeric antigen receptor (CAR) T cell therapy), there is a startling shortage of research on this subject.

Sex differences in glioma related to hormones

The sex differences in glioblastoma incidence have been observed across age groups,[129,130] indicating that sex hormones alone do not cause this disparity. However, it is reasonable to hypothesize that sex hormones influence glioma growth and/or treatment response. Literature on this subject has been primarily focused on the role of sex hormones in glioma risk and the results have been largely inconsistent. A prospective study of over 200,000 women (European Prospective Investigation into Cancer and Nutrition) found no significant association between glioma risk and reproductive factors like age at menarche, parity, age at first birth, menopausal status, and age at menopause.[131] A meta-analysis of multiple case-control studies found that higher age at menarche was associated with increased risk for glioma, but did not find any risk associated with other reproductive factors.[132] Oral contraceptives (OC) have also been investigated for their impact on glioma risk. The meta-analysis found that OC use was associated with lower risk for glioma,[132] while the prospective study found no association between glioma risk and OC use.[131] Among postmenopausal women, the meta-analysis found that hormone replacement therapy (HRT) users had a lower risk for glioma,[132] and the prospective study found no association between HRT usage and glioma risk.[131] However, neither of these studies examined the role of the dosage strength or type of hormones used. A different prospective study of over one million postmenopausal women found that estrogen-only HRT users had an increased risk for glioma, while estrogen-progesterone users did not have an increased risk compared to never users.[133] There is minimal research on the impact of sex hormones on glioma growth or treatment response and the role of sex hormones in the observed sex differences in prognosis and outcome has yet to be elucidated.

Other observed sex differences in glioma

Genetic differences, either in coding or expression, are thought to play a role in the sex differences observed in GBM. A comprehensive study of both the mutation and expression profiles of multiple kinds of cancer found that low grade gliomas and GBMs both fit into the "weak sex-effect" group,

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indicating that there were less sex-biased patterns in gene coding and expression in glioma compared to cancers like bladder urothelial carcinoma and thyroid carcinoma.[134] Despite being a "weak sex-effect" cancer, multiple studies have used genetic coding and expression data to reveal sex differences in GBM. By applying a framework for assessing mutational clonality to the genetic coding data of glioma patients, one study found that females had higher overall and subclonal mutation burden than males among both LGG and GBM groups. While the X chromosome contributed to the higher overall mutation burden in females, other chromosomes were implicated in this finding as well.[135] Their results suggest that sex-biased mutagenesis may play a role in glioma development and that sex chromosomes may play an important role in cancer evolution. Focusing on genetic expression, a recent study on GBM patients used a joint and individual variance explained (JIVE) analysis to identify sex-specific patterns of gene expression. After clustering patients into five male and five female groups based on patterns of gene expression, they found that the longest surviving male group had unique expression of genes related to cell cycle regulation and the longest-surviving female group had unique expression of genes related to regulation of integrin signaling.[136] Additionally, IDH1 mutant female patients mostly clustered into a single group that had improved survival over the other female groups, while IDH1 mutant male patients did not cluster in the same way. These results suggest that while males and females may have similar patterns of genetic expression at a population level, these expression patterns may have sex-specific implications for outcome. The same study also used segmented, serial MR imaging of GBM to find that females had a stronger volumetric response to adjuvant temozolomide therapy compared to males. Finally, using a larger cohort of GBM patients with segmented pre-surgical images, this study found that patient-specific, estimated parameters of tumor growth kinetics, specifically estimated tumor cell diffuse invasion rate, was predictive of overall survival among females and not males.[136] Two studies have used segmented MR imaging to find sex differences in tumor volume with mixed results,[137,138] while another study found that these volumes have a sex-specific impact on overall survival.[139] Taken together, these studies emphasize the need to consider sex differences in studies of glioma genetics and neuroimaging, particularly in the growing field of radiomics.

COMPLEX ADAPTIVE SYSTEMS AND MODELING

During a meeting on sex differences in the brain and brain tumors sponsored by the James S. McDonnell Foundation in March 2018, we concluded that understanding the contributions of sex to health and disease is imperative for advancing precision medicine. The myriad differences between the sexes and their impact on normal biology and pathology are highly interconnected and complex, necessitating mathematical and computational approaches for investigation. Mechanistic mathematical models, including differential equation models, can allow us to bridge spatiotemporal scales in testing hypotheses about the impacts of biological sex differences on health and disease outcomes. Agent–based models, in particular, can be useful for discovering emergent phenomena in complex adaptive systems, integrating various processes that have been well-described in isolation to better understand their interacting effects. Computational machine learning models can be useful for identifying patterns in data that provide further understanding of the extent to which various biological sex differences affect health outcomes. Machine learning models can also be combined with mechanistic models wherein we constrain algorithms with well-understood phenomena in order to better elucidate that which is less well understood. In order to fully make use of these quantitative methods, it is vital to collect data related to sex as part of clinical studies. In particular, clinical trial coordinators should pre–consider which data might be needed to examine sex differences prior to initiating a clinical study (for example, data on subjects' menstrual status and noting any type of hormone therapy a subject is taking, or explicitly measuring subjects' hormone levels, including sex steroids other than estrogen/testosterone). Additionally, clinical trial coordinators should be informed about the social situations surrounding sex and gender disparities, including gender identity, and be sensitive to potential patient

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concerns to improve data collection.[135] To reduce the impact of gendered social norms for self–reported symptoms, emphasis in clinical studies should be placed on quantitative assessment of symptoms whenever possible. With these approaches, we can build the individualized patient-specific medicine of the future, wherein all aspects of a patient's biology are fully considered—including their sex.

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