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Sex Differences in Health and Disease: a review of biological sex differences relevant to cancer with a spotlight on glioma

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Abstract: The influence of biological sex differences on human health and disease, while being increasingly recognized, has long been underappreciated and underexplored. While humans of all sexes are more alike than different, there is evidence for sex differences in the most basic aspects of human biology and these differences have consequences for the etiology and pathophysiology of many diseases. In a disease like cancer, these consequences manifest in the sex biases in incidence and outcome of many cancer types. The ability to deliver precise, targeted therapies to complex cancer cases is limited by our current understanding of the underlying sex differences. Gaining a better understanding of the implications and interplay of sex differences in diseases like cancer will thus be informative for clinical practice and biological research. Here we review the evidence for a broad array of biological sex differences in humans and discuss how these differences may relate to observed sex differences in various diseases, including many cancers and specifically glioblastoma. We focus on areas of human biology that play vital roles in healthy and disease states, including metabolism, development, hormones, and the immune system, and emphasize that the intersection of sex differences in these areas should not go overlooked. 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.

Keywords: sex differences, sex factors, precision medicine, glioma, patient-specific computational modeling

Abbreviations: ^{18}F-FDG PET 2-deoxy-2-(^{18}F)fluoro-D-glucose positron emission tomography, CYP cytochrome p450, GBM glioblastoma, IgE immunoglobulin E, IDH1 isocitrate dehydrogenase 1, SRY sex–determining region Y

1. INTRODUCTION

The impacts of biological sex on human health can be observed throughout the lifespan—from metabolic differences following conception, to differences in lifespan length and response to infectious disease, as well as in cancer incidence rates and outcomes.[1,2] 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.[3,4] 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 only one of many differences seen at the chromosomal level between the sexes.[5,6] As such, treatment with cross–sex hormones in transgender individuals[7] and people with atypical sex chromosome karyotypes does not completely change all of the underlying biological factors associated with chromosomal sex. Thus, 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. Based on external anatomical sexual dimorphism, children are typically assigned a gender at birth and brought up according to distinct gender norms. 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. We have also highlighted some compelling studies in animals that suggest similar but as yet unconfirmed sex differences in humans. Next, we discuss sex differences observed in nonneoplastic disease that have been reported in the literature and how these may relate to underlying biological sex differences. This is not exhaustive, but highlights the breadth of potential interactions between disease mechanisms and normally occurring 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.

2. SEX DIFFERENCES IN HEALTHY INDIVIDUALS

2.1 Sex differences in the normal 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 one review of sex differences in immunology across a variety of species, human females are noted to 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 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] affecting corticotropin stimulating hormone production.[25] Furthermore, hormonal fluctuations during the female menstrual cycle are associated with alterations in T cell numbers between the follicular and luteal phases.[26–28] Studies have also shown that estrogens play a dynamic role in wound healing. Estrogens stimulate various growth factor pathways to improve re-innervation and re-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 suggest the existence of additional immunological sex differences between healthy human males and females. For example, resident leukocyte populations in murine females are more numerous than in males and 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, 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, such neuroimmune sex differences have implications for neurological development.[38,39] These differences may even have implications for sex differential 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, while microglia and oligodendrocytes express estrogen receptor (ER), particularly ERb,[42] and various estrogens in murine experimental autoimmune encephalomyelitis (a model of multiple sclerosis) have shown differential inhibitory effects on neuroinflammation.[43] Additionally, there has been increased recognition of the role that the microbiome plays in immune response, which may also interact with sex differences. In one study using the nonobese diabetic mouse model of type 1 diabetes, gut microbiota transferred from adult male subjects to immature females 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] All of these studies highlight the complexity of the interactions involved in biological sex differences in immune response which may be found to similarly impact humans.

2.2 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 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] 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 some authors have framed these to discuss potential differences in cognitive abilities and emotional processing, we are not validating those claims; rather, we include these studies 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 showed increased whole brain glucose metabolism in females,[52,53] and some regional differences in which either males or females have higher resting metabolism than the other have also been demonstrated.[53,54] The significance of these differences is not well–understood, but they point to potential differences in underlying biology at the cellular level. Some investigation has been done into the role of hormonal differences on these brain metabolic sex differences by examining them in relation to the menstrual cycle. 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 comparing cerebral glucose metabolism during the follicular and luteal phases in menstruating females found no difference in whole brain glucose metabolism between the menstrual phases, but did find regional differences.[55] Thus, it may be that regional differences in glucose metabolism between the sexes are associated with regional differences in hormone receptor expression, while the finding of no differences in whole brain glucose metabolism during different phases of the menstrual cycle suggests that sex hormones are not the primary cause of these sex differences overall.

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 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]

2.3 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.[1] 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).[2]

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, on the whole, 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 the results of some studies on sex differences in the brain have been extrapolated beyond the evidence 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. 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 and 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 brain regions.[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, 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]

3. SEX DIFFERENCES IN PATHOLOGICAL CONDITIONS

3.1 Sex differences in nonneoplastic disease.

Given the wide array of sex differences that impact healthy day-to-day functioning, it is not surprising that sex differences arise in risk, incidence, etiology, pathophysiology, and outcomes across many diseases. Many autoimmune diseases affect females more frequently than males,[82] and some (such as Hashimoto's thyroiditis and Grave's disease) pronouncedly so,[82,83] consistent with the immune sex differences noted earlier. However, sex differences can interact in complex ways in disease. For example, male bias in the incidence of type 1 diabetes among patients diagnosed following puberty[84] may also be connected to sex differences in insulin sensitivity,[51] suggesting that increased immune activation is not the only factor driving the disease. Similarly, 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] Sex differences in both arterial wall stiffness and inflammatory pathways may explain some of the differences observed in hypertension and cardiovascular disease symptoms between males and females.[65,88,89] There also appear to be contributions from sex hormones, but results have been contradictory.[90] 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.[91] These also interact with immunological differences—in stroke, while studies conflict on sex differences in incidence, many are consistent in finding that outcomes are worse in female patients.[92,93] Female bias toward higher immune activation contributes to the sex 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] These examples are helpful for thinking about the complex interactions of biological sex differences in the context of cancers, which are themselves inherently complex and whose hallmarks frequently involve many of these systems.

3.2 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 (Table I), and carry poorer prognoses in males.[96–99] While this is sometimes attributed to different sociological factors, a number of studies that controlled for these 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.[100] 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.[101] 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 expressed and targeted genes.[102] 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,[103,104] as well as gene expression *in vitro* and *in vivo*, with effects on tissues being further mediated by intracellular sex hormone receptors.[105–107] A lower risk of hepatocellular carcinoma in females has been attributed to prolactin,[108] and estrogen has been associated with colorectal cancer risk reduction in premenopausal females.[109–111] Further, women were found to be more susceptible to oral cancers following menopause.[112] 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.

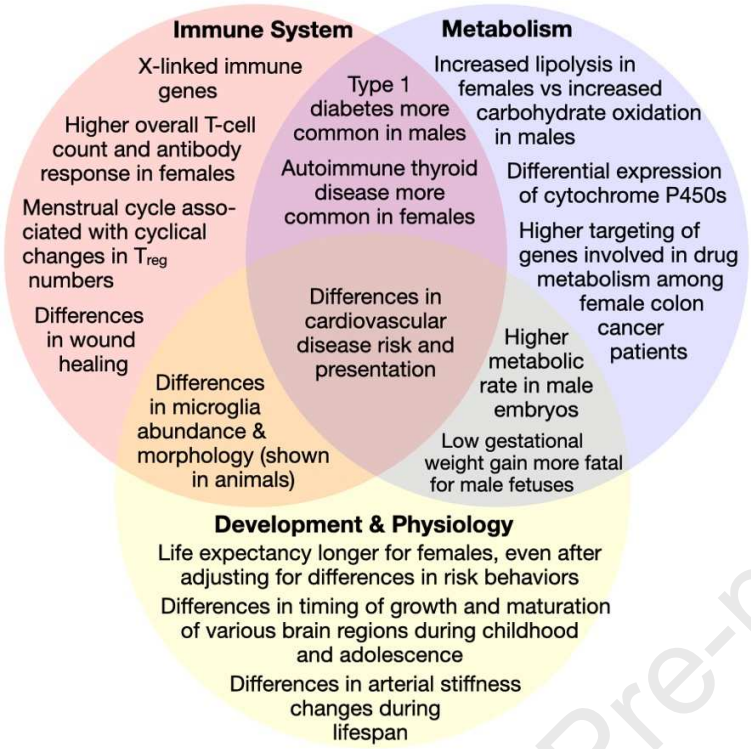

Figure 1. Biological sex differences in humans cut across domains. Biological sex differences do not all fit neatly into typical domains of medical study, and frequently occur in the intersection of these, as shown in the intersecting regions of the Venn diagram. This highlights both the need for collaborative approaches in the study of sex differences, as well as their potential for broad implications for human health.

Table I. U.S. cancer incidence rates from 1975–2004 by sex.

Cancer site	Incidence per 100,000			M:F IRR
	Total	Male	Female	
Lung	138.34	93.16	45.18	5.17
Breast	127.48	1.10	126.38	0.01
Colorectal	121.49	70.33	51.16	1.37
Blood	80.60	49.03	31.57	1.55
Hodgkin's lymphoma	5.91	3.40	2.51	1.35
Non-Hodgkin's lymphoma	35.75	21.35	14.40	1.48
Myeloma	11.62	6.98	4.64	1.51
Lymphocytic leukemia	13.11	8.56	4.55	1.88

Myeloid & monocytic leukemia	12.05	7.38	4.67	1.58
Other leukemia	2.16	1.36	0.80	1.69
Bladder	47.11	37.54	9.57	3.92
Skin	33.51	19.70	13.81	1.43
Pancreas	23.85	13.65	10.20	1.34
Kidney	22.01	14.84	7.17	2.07
Stomach	20.53	14.10	6.43	2.19
Brain	12.70	7.55	5.15	1.47
Thyroid	12.43	3.50	8.93	0.39
Esophagus	9.93	7.72	2.21	3.49
Liver	9.06	6.60	2.46	2.69

SEER data 1975 to 2004, reported in Cook, et al. 2009 [99]; M:F IRR = Male to Female Incidence Rate Ratio.

4. SEX DIFFERENCES IN GLIOMA

Thus far, we have reviewed the significant sex differences observed in healthy bodies, pathologic conditions, and non-brain cancers (Figure 1). 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.[113,114] There are known hormonally–driven sex differences seen particularly in meningioma and pituitary adenoma.[114,115] In this section, we will focus on the most common primary malignant brain cancer, glioblastoma (GBM, grade IV glioma), in which sex differences have 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.[116,117] Additionally, female GBM patients have been observed to live longer than their male counterparts when given the same standard-of-care treatment.[118] These two differences allude to the existence of underlying biological sex differences that enhance male risk for GBM and extend female life during treatment.

4.1 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.[119] 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.[120–122] 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, independent of grade, histology, and select mutations, including isocitrate dehydrogenase 1 (*IDH1*) mutation, with the lower glycolytic group surviving longest. Among *IDH1* wild-type patients, however, glycolytic genes expression level stratified survival only among females, with the high glycolytic group surviving longest. Additionally, glycolytic metabolite levels (pyruvate and the lactate/pyruvate ratio) stratified male survival, and not female, among grade II glioma patients.[120] Another study used advanced imaging and found differences in perfusion metrics and relative metabolite levels (taurine and *myo*-inositol) between male and female high-grade glioma rat models (C6 cell line). These suggested more aggressive features for male tumors and warrants further investigation in human subjects.[123] With the increased utilization of ^{18}F-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,[124] it will be increasingly important that we understand how sex impacts tumor metabolism and patient outcomes.

4.2 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 GBM 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.[125,126] 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 immunoglobulin E (IgE) levels and risk for high-grade glioma among females only.[127,128] 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.[129] 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.[125] Contributing to an area of growing research, one study found evidence of fetal microchimerism in 80% of their glioma cases from women with a history of male pregnancy.[130] While the relationship between fetal microchimerism, cancer, and the immune system has not been fully elucidated, the results of this study demonstrate the need to further study this phenomenon and its immunological implications in the context of glioma.

Myeloid-derived suppressor cells (MDSCs) also contribute to the tumor-immune landscape of glioma, wherein they function to inhibit anti-tumor immune response. One recent study found that among mouse models of GBM, male tumors were enriched in monocytic MDSCs, while

female blood had elevated granulocytic MDSCs, each involving distinct biological pathways and therapeutic targets.[131] The authors also analyzed patient samples and found that proliferating monocytic MDSCs predominated male tumors, whereas a high granulocytic MDSC/IL-1b gene signature was associated with poor prognosis in females, affirming the potential for effective sex-specific immunotherapeutic intervention in the clinic. While existing literature has described sex differences related to the use of immunotherapy agents like check-point inhibitors in non-glioma cancers[132] and one study found sex differences in the outcomes of a bevacizumab trial for non-small-cell-lung cancer,[133] our search was unable to find any sex-specific analysis of the efficacy of immunotherapies on glioma patients. Considering the vast potential impact of sex differences on immune-glioblastoma interactions and the necessity of understanding sex's role in these interactions when deploying immune-dependent treatments (e.g., chimeric antigen receptor T-cell therapy), there is a startling shortage of research on this subject.

4.3 Sex differences in glioma related to hormones

The sex differences in glioblastoma incidence have been observed across age groups,[134,135] 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, EPIC) 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.[136] 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.[137] The meta-analysis also found that oral contraceptive (OC) use was associated with lower risk for glioma, as was hormone replacement therapy (HRT) among post-menopausal women,[137] while the EPIC study found no

association between glioma risk and OC or HRT use.[136] However, neither of these studies examined the dosage or particular 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.[138] There is minimal research on the impact of sex hormones on mechanisms of glioma growth or treatment response, and any role of sex hormones in the observed sex differences in prognosis and outcome has yet to be elucidated.

4.4 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, 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.[139] 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 low-grade glioma 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.[140] 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. While most existing glioma GWAS studies have not stratified for sex, two that did found sex-specific associations between genes and glioma risk, suggesting that sex may play a role in genetic risk for glioma.[141,142]

Focusing on genetic expression, a murine cell line study found that female GBM astrocytes undergo p16- and p21-dependent cell cycle arrest in conditions of serum deprivation and induced DNA damage, while the male cells continued to proliferate and accumulate mutations.[143] These results allude to potential sex differences in cellular response to treatment that need further investigation. Using patient-derived samples, 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.[144] 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. Similar to the previously mentioned study on glycolytic gene expression, 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 analyzed segmented, serial magnetic resonance imaging (MRI) of GBM and found 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.[144] Two other studies have used segmented MRI to investigate sex differences in tumor volume with mixed results,[145,146] while a third study found that these volumes have a sex-specific impact on overall survival.[147] 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, as well as in connection with other observed sex differences in glioma to understand the extent to which these may be genetically driven (Figure 2).

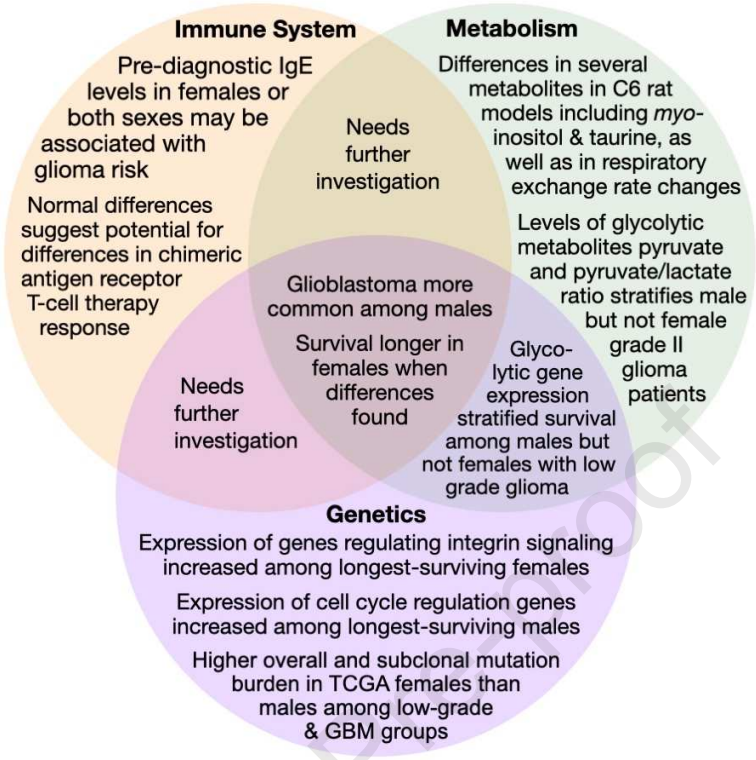

Figure 2. Summary of known biological sex differences in glioma and glioblastoma. The sex differences observed in glioma in various domains of study interconnect and may together contribute to sex differences in glioblastoma incidence and overall survival. However, many areas remain understudied, particularly in the intersecting areas shown in the Venn diagram. (GBM = glioblastoma, IgE = immunoglobulin E, TCGA = the Cancer Genome Atlas)

5. RECOMMENDATIONS FOR FUTURE RESEARCH

5.1 Complex adaptive systems 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, since they explicitly allow for the integration of known biological processes and importantly can include realistic interactions across different biological scales that together drive observed behaviors. Computational machine learning models can be useful for identifying patterns in noisy 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 the latter constrain the former such that known biological processes can define the inputs, shape the temporal dynamics or constrain the outputs, allowing us to deliver better predictions. It is worth noting that sex differences have essentially been ignored by the mathematical and computational modeling community, further emphasizing the need for a change in how we perceive the specific biological systems we model to explicitly consider sex when relevant.

5.2 Considerations for preclinical and clinical study design

In order to make full use of the aforementioned quantitative methods, it is vital to collect and report data related to sex as part of basic science and preclinical research, as well as clinical studies.

Experimental work is a critical component of understanding complex cancer mechanisms, facilitating this computational work, and it ought to be carried out in a sex–conscious fashion. In particular, the sex of tumor cell lines and model animals should be considered, with both sexes used in studies to the fullest extent possible. To more deeply investigate sex–associated mechanisms, the use of the four core genotype model may be appropriate, differentiating hormonal versus genetic contributions to sex differences in cancer. This model may also be especially useful for understanding how hormones versus genetics impact the metabolism of

therapies, providing insight for clinical studies and the best use of these treatments among transgender patients taking cross sex hormones.

At a minimum, all clinical trial analyses should report response sub-divided by sex. Small sample size in studies is a frequent challenge to observing whether there are in fact sex differences in response, as it may not be possible to assess the significance of either any differences or an apparent lack thereof with the even smaller subcohorts. However, it may be possible for subsequent investigators to combine results from multiple studies to power such an analysis, and it is a good habit to practice, as in large drug trials reporting response in sex–specific subcohorts is essential. Additionally, 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). Coordinators should also be informed about the social situations surrounding sex and gender disparities, including gender identity, and be sensitive to potential patient concerns to improve data collection.[148] 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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ARTICLE HIGHLIGHTS

- Biological sex differences appear in many aspects of normal human biology
- Sex differences may influence the etiology of many diseases—more work is needed
- Immune, metabolic, & genetic sex differences affect glioblastoma treatment outcomes
- Patients may benefit from sex–specific therapeutic strategies
- Computational models can elucidate molecular sex–difference impacts on disease

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