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White Matter Microstructure Among Straight and Gay Cisgender Men, *Sao Praphet Song*, and Straight Cisgender Women in Thailand

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ABSTRACT

White matter (WM) microstructure is differentiated in relation to sex/gender, psychosexuality, and, among transgender people, gender-affirming hormone (GAH) use. Prior research focused on Western samples, which limits generalizability to other populations. Here, diffusion tensor imaging (DTI) was used to assess WM microstructure in a Thai sample ($N = 128$) of straight cisgender men, straight cisgender women, gay cisgender men, and *sao praphet song* (i.e., transfeminine individuals assigned male at birth and sexually attracted to cisgender men). *Sao praphet song* were further grouped by GAH use. Groups were compared on fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD), and radial diffusivity (RD) using whole-brain tract-based spatial statistics (TBSS). FA, AD, and RD were further examined via multivariate analysis to assess covariance across WM microstructural indices and participant groups. A significant multivariate pattern differentiated the feminine- from masculine-identifying groups irrespective of sex assigned at birth and suggested WM tissue organization was greater among the latter in the bilateral cingulum, anterior corona radiata, left corpus callosum, and right superior longitudinal fasciculus, forceps minor, and corticospinal tracts. TBSS analyses reinforced that WM differed by gender identity in various regions. Among *sao praphet song*, GAH use was associated with lower regional FA, suggesting less WM organization bilaterally in the corpus callosum, cingulum, and anterior corona radiata. The findings aligned with prior studies in Western samples, indicating cross-population generalizability of WM microstructural differentiation in relation to sex/gender, psychosexuality, and GAH use.

Suwat Chariyalertsak and Suwit Saekho are co-site primary investigators.

Summary

- White matter microstructure is differentiated in relation to sex/gender, psychosexuality, and, among transgender people, gender-affirming hormone use.
- A significant multivariate pattern differentiated feminine- from masculine-identifying adults irrespective of sex assigned at birth and suggested white matter tissue organization was greater among the masculine-identifying groups.
- Among the transfeminine *sao praphet song*, gender-affirming hormone use was associated with lower regional white matter tract organization.

1 | Introduction

Sex steroid hormone exposure in the brain has been posited by neurohormonal theory as one key influence on psychosexual and behavioral traits, including gender identity and sexual orientation (Hines 2020). Current neuroimaging literature on the associations between gender and sexual diversity and brain characteristics is limited almost entirely to Western populations (Smith et al. 2015; Guillamon et al. 2016; Frigerio et al. 2021), making it challenging to generalize our understanding of neural development to non-Western populations. If neurohormonal theory explains psychosexual differentiation in the brain irrespective of socio-cultural contexts, then similar neurohormonal influences are expected in sexually and gender-diverse people in non-Western populations. Thus, possible neurohormonal influences toward sexual and gender differentiation need to be tested in non-Western populations, including with respect to broader definitions of sexual and gender diversity seen in many non-Western cultures.

In Thailand, the concept of *pheet thii saam* (translated as "third sex/gender") includes identities beyond a woman-man binary (Jackson 1999; Totman 2003; Sinnott 2004). Gender and sexual diversity are often socially visible and reputedly accepted in Thai culture (Winter 2006a; Gooren et al. 2015), making it particularly informative to study psychosexual variability in this population. If the neural patterns associated with sexual and gender diversity in prior Western research generalize, parallels are expected in the neural phenotypes that differentiate participants with respect to sex/gender, gender identity, and sexual orientation among Thais. Here, the intentional use of the term *sex/gender* reminds us of the closely intertwined aspects of biological sex (e.g., the sex label assigned at birth that indicates the joint effects of multiple sex-related biological factors) and the social concept of gender. These aspects cannot be easily disentangled and, therefore, are studied in tandem in human psychology (Kaiser 2012).

Sao praphet song (translated as "a second kind of woman") is one example of a gender-nonbinary group that can help elucidate neural differentiation across axes of gender and sexual diversity. *Sao praphet song* are Thai transfeminine individuals assigned male at birth (AMAB) who are typically attracted to cisgender men (Winter 2006a, 2006b; Coome et al. 2020) and are characterized as markedly feminine relative to gay cisgender men (Totman 2003; Coome et al. 2020). A large majority (i.e., 75%–90%) of *sao praphet song* use exogenous gender-affirming hormones (GAH), which may include estrogenic, progesteronic, and/or anti-androgenic doses (Guadamuz et al. 2011; Gooren et al. 2015; Humphries-Waa 2014; Salakphet et al. 2022; Skorska et al. 2023). GAH use onset typically occurs during adolescence and emerging adulthood for *sao praphet song* (Skorska et al. 2023). Unlike in the West, where GAH use is typically physician-prescribed (Rowniak et al. 2019), *sao praphet song* most often obtain exogenous hormones from pharmacies or via non-medically affiliated means (e.g., friends), and administration and dosing are often self-regulated (e.g., Gooren et al. 2015; Skorska et al. 2023). Also, recent studies show that once GAH use has been initiated, frequency of use is daily (Ittiphisit et al. 2022), and *sao praphet song* often do not go more than six months without using GAH (Skorska et al. 2023).

Prior research with *sao praphet song* presented anthropometric measures that reflect, to some extent, sex steroid hormone action during development (Skorska et al. 2021). Compared with straight cisgender men, *sao praphet song* exhibited less "male-typical" height, long-bone growth, and second-to-fourth digit length ratios, whereas gay cisgender men showed height and long-bone growth intermediate between *sao praphet song* and straight cisgender men, but digit ratios that differed from straight cisgender men's and were similar to those of *sao praphet song*. These align with some Western findings (for review, see VanderLaan et al. 2023), but whether these Thai groups show parallel patterns in brain characteristics has yet to be investigated.

In addition, GAH use in *sao praphet song* might be associated with brain characteristics. Exogenous sex steroid hormones have been associated with neural development in nonhuman animal models (McCarthy 2008; Abi Ghanem et al. 2017) and as brief as one month of GAH exposure was associated with white matter (WM) changes in Western transgender people (for review, see Kranz et al. 2020). In *sao praphet song*, GAH use has been associated with altered visuospatial cognitive performance (Thurston et al. 2021). Thus, sex steroid hormones influence neural changes, and extending the investigation of this influence to *sao praphet song* is warranted.

WM microstructure, defined as the organization of axons and distribution of myelin (O'Donnell and Westin 2011), is an important neural index to explore in relation to sex/gender, psychosexuality, and hormonal mechanisms. The investigation of WM microstructure informs our understanding of neural connectivity and signal propagation between gray matter regions. Both testosterone and estradiol influence WM organization and myelination (human: Herting and Sowell 2017; rodent: Prayer et al. 1997; Marin-Husstege et al. 2003; Juraska et al. 2013; Abi Ghanem et al. 2017) but have differing effects. Testosterone increases WM tissue density, and estradiol promotes myelination (Perrin et al. 2008; Pesaresi et al. 2015; Pangelinan et al. 2016). Further, WM microstructure development may be influenced by hormone exposure during dynamic developmental periods (i.e., prenatal, pubertal; Lebel and Deoni 2018), potentially leading to sex/gender and psychosexual differences in neural structure in adulthood.

Diffusion tensor imaging (DTI), a magnetic resonance technique that can quantify water diffusion within neural tissue (O'Donnell

and Westin 2011), has demonstrated sex/gender differentiation in WM microstructure between cisgender and transgender adults naïve to exogenous hormone therapies, although somewhat inconsistently. Tissue organization was often measured by fractional anisotropy (FA), reflecting the directionality of water diffusion, and mean diffusivity (MD), reflecting the average water movement in all directions (O'Donnell and Westin 2011). In transgender adults, these metrics were reported to align with those of cisgender adults of the same gender (Rametti et al. 2011b), the same sex assigned at birth (Burke et al. 2017), or to be intermediate between cisgender men and women (Rametti et al. 2011a; Kranz et al. 2014). Across these studies, group differences were reported with some consistency in the right superior longitudinal fasciculus (SLF), corticospinal tract, inferior fronto-occipital fasciculus (IFOF), and forceps minor—although Kranz et al. (2014) reported more widespread WM tract associations. Yet, the studies do not agree on the direction of the relationship, making it challenging to interpret specific underlying mechanisms associated with the WM patterns across groups.

Studies of GAH use have helped elucidate the effects of sex steroid hormones on the brain, but few studies assessed the effects of GAH use on WM microstructure in transfeminine populations (for review, see Kranz et al. 2020). In transgender women, one study longitudinally examined WM microstructure before and after four months of so-called feminizing-GAH therapy (e.g., hormonal therapy containing hormones historically associated with "feminized" physiology; Kranz et al. 2017). The study reported increased MD in the right splenium of the corpus callosum and right temporal lobe, decreased MD in the right splenium of the corpus callosum, and decreased FA in the right postcentral blade—although these effects were not correlated with the concomitant changes in serum estradiol or testosterone concentrations (Kranz et al. 2017). The observed decrease in FA and increase in MD could indicate a change in WM tissue towards a more female-typical WM organization in these regions (Schwartz et al. 2005). Notably, the above Western neuroimaging findings may be limited in their generalizability to the Thai context due to genetic, ethnic, and cultural differences, as well as differences in access to GAH or the age of GAH onset (Skorska et al. 2023). Therefore, investigating GAH use in *sao praphet song* offers an opportunity to assess how alternative access to, and use of, exogenous hormones may impact the brain beyond what is known from Western research.

With respect to sexual orientation, there is some evidence that it relates to aspects of brain structure and function shown to be sex/gender-differentiated (for review, see Frigerio et al. 2021), but little WM microstructure research has investigated both sexual orientation and gender identity concurrently. Burke et al. (2017) reported an effect of sex, but not sexual orientation, on FA in cisgender adults in a comparison of straight and gay cisgender men and women. In GAH-naïve transgender adults, sexual orientation was treated as a covariate due to insufficient numbers of participants to form separate groups and was not associated with FA (Burke et al. 2017). Similarly, Kranz et al. (2014) reported that controlling for sexual orientation did not affect the outcome when comparing cisgender and transgender participants' WM microstructure. Other studies included exclusively straight transgender adults (i.e., attraction to the opposite gender; Rametti et al. 2011a, 2011b), which made sexual orientation a confound in comparisons of transgender and cisgender groups. The relationship between sexual orientation and WM microstructure requires further study, including disentangling it from gender identity.

This literature may also benefit from investigating multiple WM microstructure metrics. Prior DTI studies investigating WM microstructure in transgender individuals most often focused on FA and MD (e.g., Rametti et al. 2011a, 2011b, 2012; Kranz et al. 2014, 2017; Burke et al. 2017). Diffusion can also be measured by the more specific indices of axial (AD) and radial diffusivity (RD; Tamnes et al. 2018). These indices offer potential proxy measurements of axonal tortuosity (Takahashi et al. 2000; Schwartz et al. 2005) and degree of myelination (Song et al. 2002, 2005), respectively. Concurrent consideration of FA, AD, RD, and MD can be beneficial for accurately characterizing WM tissue composition (Tamnes et al. 2018).

The current study used these four DTI metrics to investigate WM microstructure. As past literature has yet to pinpoint specific WM regions related to psychosexual differentiation and GAH use, we employed a whole-brain approach. In doing so, we present both mass-univariate analyses examining one DTI metric at a time (as in most prior DTI studies of psychosexuality and GAH), and a multivariate analysis that better captures how the DTI metrics combine to distinguish groups and inform differences in the underlying WM tissue characteristics. Our analyses focus on straight cisgender men, gay cisgender men, straight cisgender women, and two groups of *sao praphet song*: those who reported GAH use for at least 6 months (GAH+) and those who reported no lifetime use of GAH (GAH−). These five groups allowed us to assess several parameters in relation to WM differentiation, including sex/gender, gender identity, sexual orientation, and GAH use. As such, we provide a test of the neurohormonal theory on gender and sexual diversity as well as GAH (Hines 2020; Kranz et al. 2020), and we clarify the generalizability of findings from previous Western studies to a non-Western population.

2 | Methods

2.1 | Ethics Statement

This research was approved by the research ethics boards at Chiang Mai University (Protocol #22/59) and the University of Toronto (Protocol #34425).

2.2 | Participants

Participants ($N = 140$) were recruited via sexual health clinics affiliated with the Research Institute for Health Sciences at Chiang Mai University in Chiang Mai, Thailand, for a neuroimaging study. All participants were ethnically Thai. Data from 12 participants were excluded due to errors during scanning (i.e., incorrect scanning protocol; $n = 11$) and errors during preprocessing ($n = 1$). Participants were grouped by self-report and interviewed identity (i.e., gender identity and sexual attractions) into five groups: straight cisgender men ($n = 28$), gay cisgender men ($n = 28$), *sao praphet song* who had never used GAH (GAH−; $n = 20$), *sao praphet song* who had used GAH (GAH+; $n = 21$), and straight cisgender women ($n = 31$). *Sao praphet song*

TABLE 1 | Age, education, and income distribution by group.

	Straight cisgender men	Gay cisgender men	GAH− *sao praphet song*	GAH+ *sao praphet song*	Straight cisgender women	*F* (df)	*p*
n	28	28	20	21	31		
Age (years) Mean (SD)	26.07 (6.10)	25.18 (5.14)	21.75 (3.84)	24.33 (4.04)	26.1 (6.13)	2.58 (4, 123)	0.04
						χ^2 (df, *N*)	*p*
Completed college or university (*n* [%])	12 (42.9)	15 (53.6)	7 (35.0)	11 (52.4)	16 (51.6)	2.32 (4, 128)	0.68
Monthly income > 9999 Baht (*n* [%])	14 (50.0)	13 (46.4)	5 (25.0)	12 (57.1)	17 (54.8)	5.59 (4, 128)	0.23

Abbreviations: GAH− = haven't used gender affirming hormones; GAH+ = has used gender affirming hormones; SD = standard deviation.

were included in the GAH+ group if they reported continued use of GAH for 6 months or longer. Further, participants in this group included only individuals who reported that they had never ceased GAH for a period of more than 6 months since they started GAH. All participants completed a Childhood Gender Identity Scale (e.g., VanderLaan et al. 2017; see SI for details) as an additional measure to confirm the groups differed as expected in their gender expression histories. Notably, the two *sao praphet song* groups did not differ from each other on this measure, suggesting that any WM differences observed between these groups were not confounded by gender expression history.

Additional inclusion criteria were that all participants were HIV-negative (confirmed via blood serum sample); straight cisgender men were exclusively attracted to cisgender women; and individuals in all other groups were exclusively attracted to cisgender men. All participants were screened for contraindications to MRI. Participants received 1000 Thai Baht as an honorarium for completing the study.

2.3 | Measures

Participants completed a psychological and behavioral assessment as part of a larger study. The measures in the questionnaire were translated and back-translated between English and Thai to ensure the accuracy of translation.

2.3.1 | Age

Participants were aged 18 to 44 years (mean = 24.92 years; standard deviation [SD] = 5.43). A one-way analysis of variance (ANOVA) found a significant age difference across the five groups ($F(4,123) = 2.58$, $p = 0.04$, $\eta^2 = 0.08$; Table 1). Post hoc LSD tests revealed that GAH− *sao praphet song* were significantly younger than straight cisgender men, gay cisgender men, and straight cisgender women (all pairwise $p < 0.05$). The two *sao praphet song* groups did not significantly differ in age ($p = 0.12$).

2.3.2 | Education and Income

Education and income were used as proxies of socioeconomic status. Participants were asked to provide their highest level of education achieved at the time of participation and to report their monthly income in Thai Baht. Education and income responses were dichotomized as in prior research (Thurston et al. 2021). Education was categorized into low (completed some college or university or less) and high (completed college or university). Monthly income was categorized into low (less than 9999 Baht) and high (more than 10,000 Baht). The groups did not significantly differ in level of education (χ^2 (4, 128) = 2.32, $p = 0.68$, $\varphi = 0.14$) or monthly average income (χ^2 (4, 128) = 5.59, $p = 0.23$, $\varphi = 0.21$; Table 1).

2.3.3 | Psychosexual Identity

All participants answered a question about the sex they were assigned at birth with options of male, female, ambiguous/other, and prefer not to answer. In addition, each participant reported their sexual/gender identity during an individual interview with a research assistant and in response to a questionnaire item with options of male/man, female/woman, bi-woman, bi-man, *tom* (a transmasculine individual assigned female at birth [AFAB]; similar connotation to the Western term "tomboy"), *dee* (a feminine individual AFAB who is attracted to *toms*; derived from the latter syllable of the English word "lady"), gay, lesbian, *sao praphet song*, other, and prefer not to answer. For each question, if participants selected ambiguous/other, they were given the option to specify.

At screening, inclusion in the present study required straight cisgender men to report sexual attraction exclusive to cisgender women. If a prospective straight cisgender man indicated attraction to other identity groups, the participant was excluded. Similarly, prospective straight cisgender women, gay cisgender men, and *sao praphet song* participants had to indicate sexual attraction exclusive to cisgender men to be included in the present analysis.

2.3.4 | Exogenous Hormone Use

All participants completed a hormone use questionnaire beginning with the question "have you ever used hormones such as estrogen or testosterone?" Participants who answered "no" ended the questionnaire and those who responded "yes" answered further questions. The questionnaire asked about the

age of first hormone use and the type(s) of hormones used (lifetime and past 6 months, respectively). Participants indicated which types of hormones they had used from a list of brands of exogenous estrogens, progestogens, combination pills of estrogens and progestogens, and anti-androgens, or to specify if a brand they had used was not listed. *Sao praphet song* were the only participants categorized by their use of GAH in the current study.

Twenty-one *sao praphet song* reported having ever used GAH and had used GAH in the past six months at the time of participation in the study (i.e., GAH+ *sao praphet song*). The mean (SD) age for GAH onset in this group was 17.48 (2.9) years (range: 12–20 years) and the duration of GAH use averaged 6.86 (5.12) years (range: 0.5–19 years). A variety of GAH brands were reported across estrogenic, progesteronic, anti-androgenic, and combination hormones. The number of *sao praphet song* who used each GAH brand (lifetime and past 6 months) can be viewed in Table S2. Straight cisgender women were the only other participants who reported exogenous hormone use; 23 straight cisgender women reported ever using hormones and nine reported use in the past 6 months. Their exogenous hormone use histories are reported in Table S3.

2.4 | Neuroimaging

2.4.1 | Acquisition

Diffusion-weighted data were acquired using a 1.5 T Philips Ingenia magnetic resonance scanner with a 16-channel head coil at Chiang Mai University, Thailand. Images were acquired with a 2D spin echo pulse imaging sequence with oblique-axial slices; 2.5 mm isotropic resolution; $b = 1000\,s/mm^2$; 32 diffusion gradient directions and 1 non-diffusion-weighted image were acquired in reversed phase-encoding directions (i.e., anterior to posterior, posterior to anterior).

2.4.2 | Image Processing

Diffusion-weighted MRI data were processed using FSL (v 6.0; Smith et al. 2004). The diffusion-weighted images were corrected for susceptibility (Andersson et al. 2003) and eddy current-induced distortions (Andersson and Sotiropoulos 2016). Reversed phase-encode blips were used to estimate the susceptibility-induced off-resonance field (Smith et al. 2004) and FSL's eddy current correction method was applied. Next, the distortion-corrected data were processed using FSL's DTIFIT, whereby each voxel was fit with a diffusion tensor model.

The tract-based spatial statistics (TBSS) pipeline (Smith et al. 2006) was used to register individual FA maps to MNI standard space. Specifically, and as recommended for non-Western samples, a study-specific registration process was used by identifying the most representative FA map for the full sample to be used to non-linearly transform the individual FA maps to the MNI space (i.e., using the *–n* flag in tbss_2_reg). A threshold of 0.2 was applied to the mean FA skeleton to reduce partial volume effects. A WM skeleton was created from the mean FA image of the sample. The non-FA brain maps (AD, RD, and MD) were then transformed to MNI standard space using the transformations determined by the TBSS pipeline. Before multivariate analysis, the AD and RD maps were scaled by 10^2 to make the range of values comparable to those of the FA maps.

2.5 | Statistical Analyses

2.5.1 | Demographics

Demographic analyses were conducted using SPSS version 28 (IBM Corp. 2021). Group differences in age were assessed by one-way ANOVA. Post hoc pairwise comparisons were performed using Fisher's LSD. Group differences in education and income were assessed by chi-square analysis with φ coefficient. A conventional alpha level of $p < 0.05$ was used to indicate statistical significance in all analyses.

2.5.2 | TBSS

An initial mass-univariate analysis was conducted on each of the four diffusion indices (i.e., FA, MD, AD, and RD) using TBSS. One-way ANOVAs were run using FSL's randomise (Winkler et al. 2014) on each diffusion index independently, with post hoc two-group comparisons. Randomise applied whole-brain, voxel-wise statistics with 5000 permutations and threshold-free cluster enhancement to identify group differences for each diffusion index. Due to the significant group differences in age, additional analyses of covariance (ANCOVA) were run, controlling for age (see details in the Data S1). The ANCOVA was significant for AD only, indicating a negative correlation between AD and age (5 clusters $k \geq 100$, all $p < 0.014$, k range: 649–2496 voxels; see Figure S1 and Table S4); however, age did not change the pattern of associations between AD and group, and we, therefore, reported the findings without statistically controlling for age here. Clusters were considered significant at $p_{FWE} < 0.05$ (Family-Wise Error corrected) and a minimal cluster size of $k \geq 100$ voxels ($1\,mm^3$ voxel size) following methods reported by Burke et al. (2017).

2.5.3 | Partial Least Squares (PLS)

A multivariate PLS analysis was performed on FA, AD, and RD to aid tissue characterization (Bennett et al. 2010; Tamnes et al. 2018). MD was not included because it is a linear combination of AD and RD (Bennett et al. 2010). PLS was run using methods outlined by McIntosh and Lobaugh (2004). PLS analyses were conducted with PLS software (v. 6.1311050) using MATLAB (2021a, version 8.3.0.532).

PLS was used to identify similarities and differences among groups (i.e., straight cisgender men, gay cisgender men, GAH– *sao praphet song*, GAH+ *sao praphet song*, straight cisgender women) and WM microstructure metrics, FA, AD, and RD. In PLS, singular value decomposition is used on mean-centered data (for each metric separately) to create latent variables (LVs) with three components: contrasts of group-metric differences, brain saliences or a measure of each voxel's contribution to the observed contrast, and a measure of relationship strength

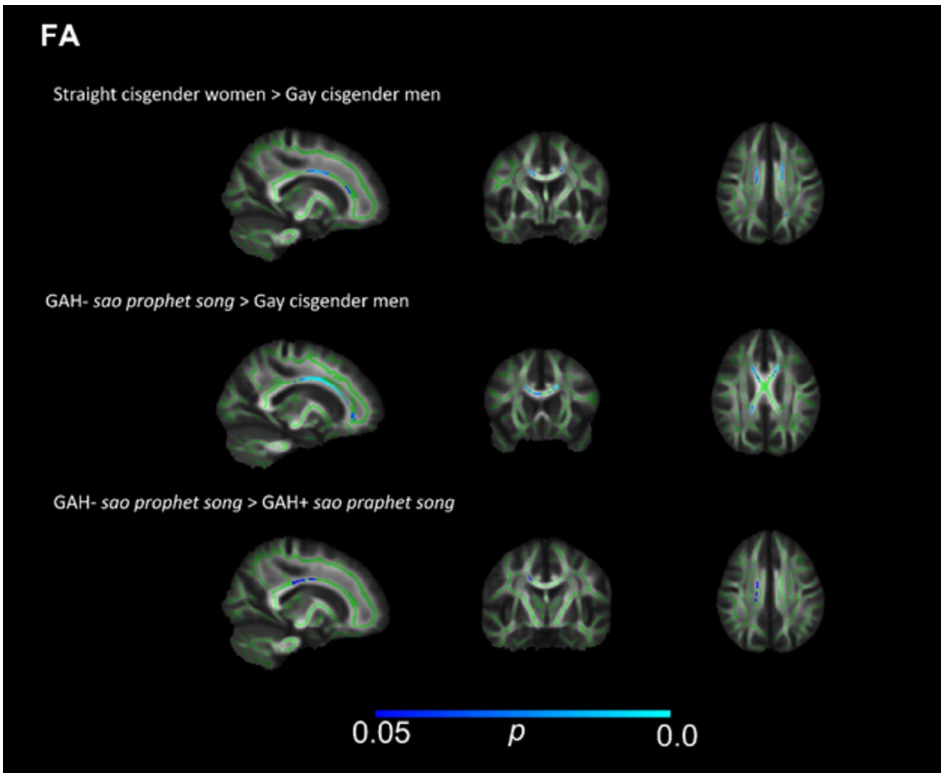

FIGURE 1 | Significant TBSS clusters indicating group differences in FA. Significant clusters ($p_{FWE} < 0.05$; $k > 100$ voxels) identified in FA (blue-light blue) by TBSS analysis. Sample mean FA skeleton mask (green) overlaid on the sample mean FA map. Anatomical left is right.

(McIntosh and Lobaugh 2004). The probability of the model is assessed with permutation testing, which estimates the number of times the strength of the permuted LV exceeds the observed strength. An estimate of the standard error of the brain saliences was assessed with bootstrap resampling. Using the ratio of the brain salience to its standard error approximates a z-score and, therefore, it was used to assess the reliability and stability of the brain salience. Here, the analysis used 5000 permutations and bootstrap samples, and a threshold of ±2.5 was applied to all analyses to focus on stable saliences only. The regional labels for stable voxels were identified using the John Hopkins University (JHU) DTI-based white-matter atlases (Mori et al. 2005).

3 | Results

3.1 | TBSS Analyses

The one-way ANOVAs for FA (*F*-test, $p_{FWE} = 0.01$), AD (*F*-test, $p_{FWE} < 0.001$), and RD (*F*-test, $p_{FWE} = 0.037$) were significant, whereas the one for MD was not (*F*-test, $p_{FWE} = 0.21$). Post hoc pairwise comparisons revealed significantly greater FA in straight cisgender women compared with gay cisgender men ($p_{FWE} = 0.003$) bilaterally in the body of the corpus callosum and the left forceps minor. GAH− *sao praphet song* had significantly greater FA than gay cisgender men ($p_{FWE} < 0.001$) bilaterally in the corpus callosum and greater FA than GAH+ *sao praphet song* ($p_{FWE} = 0.03$) in the right body of the corpus callosum. Significant group comparisons for FA using TBSS are included in Figure 1 and Table 2. No other pairwise comparisons were significant.

Post hoc *t*-tests revealed higher AD in straight cisgender women compared with straight cisgender men ($p_{FWE} = 0.001$) and gay cisgender men ($p_{FWE} < 0.001$; Figure 2). Statistically significant clusters were found in the left corticospinal tract and right superior corona radiata, and bilateral corpus callosum, respectively (Table 3). In addition, both *sao praphet song* groups had higher AD compared with gay cisgender men. GAH− *sao praphet song* had higher AD ($p_{FWE} < 0.001$) bilaterally in the corpus callosum and GAH+ *sao praphet song* had higher AD ($p_{FWE} = 0.02$) in the left forceps minor. No other pairwise comparisons were statistically significant.

Gay cisgender men were found to have higher RD compared with GAH− *sao praphet song* ($p_{FWE} < 0.001$) in the right corpus callosum and left superior corona radiata (Figure 3 and Table 4). There were no other significant pairwise comparisons identified for RD.

3.2 | PLS Analysis

The PLS analysis identified a distinct brain pattern for FA, AD, and RD across groups. One significant LV ($p = 0.034$) explained 16.97% of the model covariance (Figure 4). The patterns identified between brain score and group in this LV were generally negative, indicated by the blue-light blue voxels (Figure 4B) and, thus, the associations depicted in Figure 4A are inversely interpreted. The LV showed a shared pattern in AD and RD between straight cisgender women and both *sao praphet song* groups that differed from straight and gay cisgender men (Figure 4A). AD and RD were higher in the feminine-identifying groups (i.e.,

TABLE 2 | Size and location of significant clusters identified by pairwise comparison of FA.

Region	Cluster Size, *k*	p_{FWE}	MNI coordinates		
			X	Y	Z
Straight cisgender women > Gay cisgender men					
Left genu of corpus callosum; forceps minor	1167	0.013	99	153	84
Right body of corpus callosum	285	0.014	74	125	106
GAH− *sao praphet song* > Gay cisgender men					
Bilateral corona radiata; corpus callosum	2674	0.001	106	144	100
GAH− *sao praphet song* > GAH+ *sao praphet song*					
Right body of corpus callosum	132	0.046	74	118	106

Abbreviations: *k* = number of voxels in a cluster; FWE = family-wise error corrected.

straight cisgender women, GAH− *sao praphet song*, and GAH+ *sao praphet song*) and lower in the two groups of cisgender men. The LV also showed a shared brain pattern of higher FA in cisgender men. FA was lower in GAH+ *sao praphet song* and did not contribute to the brain pattern of GAH− *sao praphet song* or that of straight cisgender women. This brain pattern was found in stable clusters bilaterally in the cingulum and anterior corona radiata, left corpus callosum (genu), and right SLF, forceps minor, and corticospinal tract (Figure 4B).

4 | Discussion

The present study assessed WM microstructure in five sexually and gender-diverse groups of Thai adults using whole-brain mass-univariate as well as multivariate analyses. The inclusion of the multivariate analysis expands upon the traditional analysis techniques of DTI research by assessing covariance across multiple diffusion indices, which helps to better characterize tissue (Tamnes et al. 2018). The findings demonstrated WM microstructural differences in relation to sex/gender, including masculine versus feminine gender identity irrespective of sex assigned at birth and GAH use by transfeminine *sao praphet song*. There was no clear sexual attraction pattern differentiating straight cisgender men from the four groups exclusively attracted to cisgender men. Here, we summarize the findings and discuss the ways in which they do and do not align with a neurohormonal perspective on sexual and gender diversity, keeping in mind the absence of measuring organizational effects directly. In addition, we note parallels between the present findings and those of prior Western studies, indicating ways that WM tissue differentiation generalizes beyond the Western populations studied previously.

Our findings are consistent with the hypothesized organizational effects of sex steroid hormones on the brain that are linked to gender identity. The multivariate PLS analysis identified a significant LV reflecting a WM microstructure pattern that differentiated masculine- from feminine-identifying groups in the bilateral cingulum and anterior corona radiata, left corpus callosum (genu), and right SLF, forceps minor, and corticospinal tracts. In these regions, compared with the masculine-identifying straight and gay cisgender men, the feminine-identifying adults (i.e., straight cisgender women and both *sao praphet song* groups) evidenced higher AD and RD. In addition, FA was lower in GAH+ *sao praphet song* and higher among both straight and gay cisgender men, but FA did not significantly contribute to the latent variable for GAH− *sao praphet song* and straight cisgender women. The combination of higher FA and lower AD and RD in these regions among cisgender men could indicate relatively greater WM tissue organization, density, and myelination, whereas the inverse pattern in GAH+ *sao praphet song* and the higher AD and RD in all feminine-identifying groups could indicate less WM tissue density (Schwartz et al. 2005) and myelination (Song et al. 2005) relative to cisgender men. These findings align with some previously reported sex/gender differences in FA and RD between cisgender men and women in some of the same regions, such as the corpus callosum and cingulum (Menzler et al. 2011; van Hemmen et al. 2017). Here, the multivariate pattern observed in this more diverse set of groups expands our understanding of WM microstructural differentiation in these regions. It demonstrates a difference between cisgender men irrespective of sexual orientation on the one hand and feminine-identifying adults attracted to cisgender men irrespective of sex assigned at birth on the other.

The findings from the TBSS analyses reinforce that WM microstructure is differentiated between cisgender men and the feminine-identifying groups. Of note, the TBSS findings pertained to different WM voxel clusters than those highlighted in the PLS and, in contrast to the multivariate PLS analysis, the mass-univariate TBSS analyses revealed differences suggestive of less WM organization regionally among cisgender men. The significant group differences were mainly observed between gay cisgender men and the feminine-identifying groups, particularly GAH− *sao praphet song*. Gay cisgender men had lower FA than straight cisgender women and GAH− *sao praphet song* in similar regions bilaterally in the forceps minor and corpus callosum. In the right corpus callosum and left superior corona radiata, gay cisgender men had higher RD compared with GAH− *sao praphet song*. Also, AD in gay cisgender men was lower compared with straight cisgender women in the left corticospinal tract, right superior corona radiata, and bilateral corpus callosum; GAH− *sao praphet song* bilaterally in the corpus callosum; and GAH+ *sao praphet song* in the left forceps minor. Lower FA and AD, and higher RD in gay cisgender men in these regions could indicate higher axonal tortuosity and crossing fibers and, therefore, less organization in the WM microstructure, as well

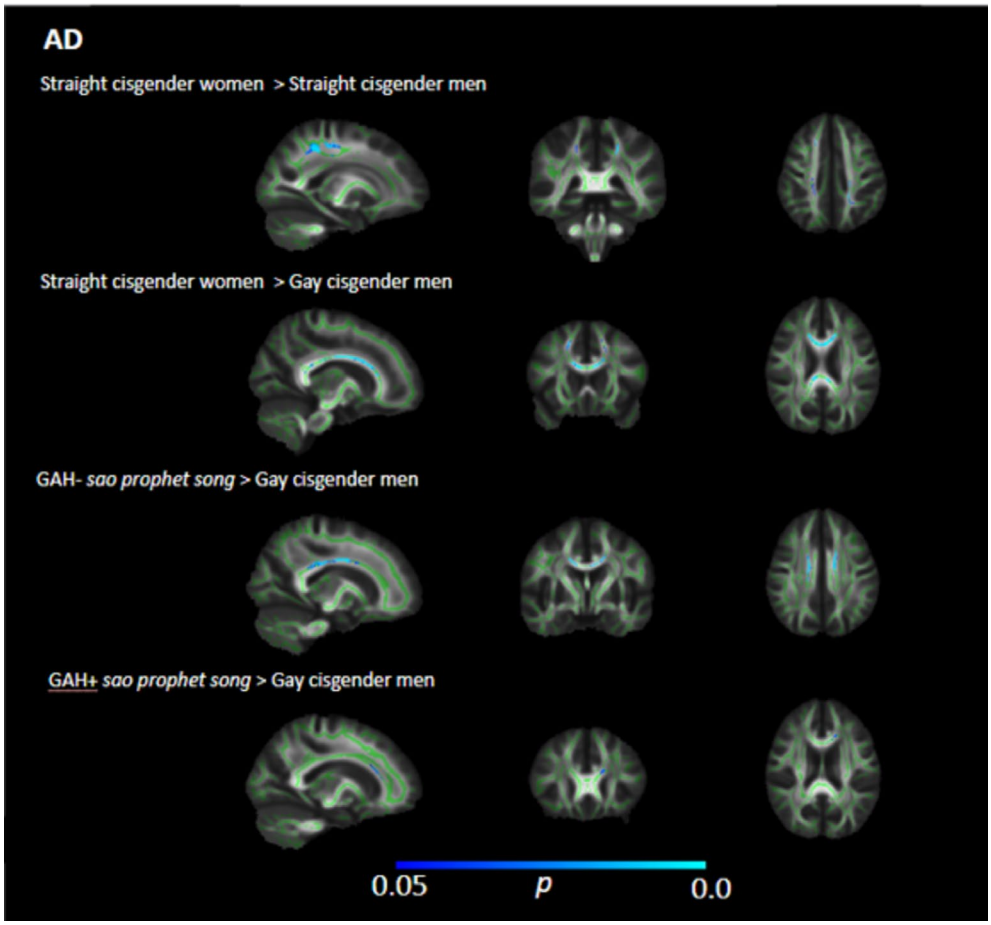

FIGURE 2 | Significant TBSS clusters indicating group differences in AD. Significant clusters ($p_{FWE} < 0.05$; $k > 100$ voxels) identified in AD (blue-light blue). Sample mean FA skeleton mask (green) overlaid on FA map. Anatomical left is right.

as less myelination compared with the feminine-identifying groups, particularly GAH− *sao praphet song*. AD was also lower among straight cisgender men than among women in the left corticospinal tract, right superior corona radiata, and bilateral corpus callosum, potentially suggesting greater axonal tortuosity and less WM tissue organization in these regions among straight men compared with women.

The group differences highlighted by the TBSS analyses correspond, to some extent, with prior WM microstructural research on sex/gender and sexual orientation. WM organization is often greater in cisgender men than women in most brain regions, but regionally the reverse (as seen in our TBSS analyses) has been reported (e.g., Buyanova and Arsalidou 2021; Lawrence et al. 2021; Lebel and Deoni 2018). Regarding sexual orientation, a neurohormonal perspective suggests that gay cisgender men would bear similarities to straight cisgender women and *sao praphet song*, as was found in an earlier Thai study examining androgen-mediated anthropometrics (Skorska et al. 2021). Yet, the present findings correspond with prior Western research reporting that the FA of cisgender gay and straight men was similar and varied from that of cisgender and transgender women (Burke et al. 2017). That said, Burke et al. (2017) found greater FA, suggesting greater WM organization, among cisgender men (as shown in our multivariate PLS analysis for the identified significant LV) and the effects were most pronounced for straight cisgender men, whereas our TBSS findings suggested less WM organization in cisgender men, particularly gay cisgender men. These somewhat mixed WM findings are reminiscent of cortical structure neuroimaging research on cisgender men's sexual orientation in which the directions of effects and regions in which they are found, if at all, often vary across studies (e.g., Abé et al. 2014, 2021; Manzouri and Savic 2018; Votinov et al. 2021; Wang et al. 2020). In any case, the lack of difference in WM microstructure between gay and straight cisgender men suggests that neurohormonal influences related to sexual orientation may be more evident in other brain characteristics—for example, as indicated by prior studies on cortical structure (e.g., Abé et al. 2014, 2021; Manzouri and Savic 2018; Wang et al. 2020) and functional connectivity (Manzouri and Savic 2018).

Notably, and for the first time, our findings indicate that WM microstructure is differentially organized in cisgender versus transfeminine individuals assigned male at birth with sexual attractions to cisgender men. WM microstructural differences were apparent between the two groups of *sao praphet song* and gay cisgender men in the PLS analysis. Likewise, in the TBSS analyses, gay cisgender men differed on AD from both *sao praphet song* groups and on FA and RD from GAH− *sao praphet song*. Further, the WM microstructure of *sao praphet song* bore more similarity to that of straight cisgender women, while no significant WM microstructure differences were observed between straight and gay cisgender men. Thus, the present findings are consistent with the hypothesis that transfeminine individuals

TABLE 3 | Size and location of significant clusters identified by pairwise comparison of AD.

Region	Cluster Size, *k*	p_{FWE}	MNI coordinates		
			X	Y	Z
Straight cisgender women > Straight cisgender men					
Left corticospinal tract	482	0.005	77	126	103
Right cerebral WM (IFOF)	211	0.011	71	153	109
Right superior corona radiata	164	0.039	71	107	108
Straight cisgender women > Gay cisgender men					
Bilateral corpus callosum	3571	0.001	77	126	103
Left cerebral WM (cingulum)	487	0.025	105	160	106
Right cerebral WM (anterior corona radiata)	194	0.014	72	145	115
GAH− *sao praphet song* > Gay cisgender men					
Right body of corpus callosum	732	0.006	74	123	106
Left genu of corpus callosum	655	0.008	104	146	97
GAH+ *sao praphet song* > Gay cisgender men					
Left anterior corona radiata; forceps minor	201	0.031	106	152	93

Abbreviations: *k* = number of voxels in a cluster; FWE = family-wise error corrected; WM = white matter; IFOF = inferior fronto occipital fasciculus.

attracted to cisgender men, compared with gay cisgender men, have a neural phenotype that is more similar to that of straight cisgender women—though it should be noted that such a pattern may only apply to particular neural regions (VanderLaan et al. 2023).

The present findings parallel other research that aligns with this hypothesis. For example, two prior Western studies reported that, in regions identified as different between cisgender men and women, the WM microstructural properties of transgender women naive to GAH were intermediate to these groups, and microstructural patterns were more similar to those of cisgender women (Kranz et al. 2014; Rametti et al. 2011a; but see Burke et al. 2017). One of these studies reported that the relation between WM microstructure and gender identity was not moderated by sexual orientation (Kranz et al. 2014). Another study demonstrated a gradient of neural feminization by showing that functional brain activation during a visuospatial task was more similar to that of straight cisgender women among gender-nonconforming but not gender-conforming gay cisgender men (Folkierska-Żukowska et al. 2020). Together, the findings presented here and in these prior studies challenge the notion that all individuals assigned male at birth with sexual attractions to cisgender men have a similar brain phenotype regardless of their gender identity and expression. Also, in the present study, there does not appear to be a clear delineation in brain phenotype between sexual attraction to cisgender men or women, given the absence of a WM difference distinguishing straight cisgender men from all of the other four groups. However, due to the relative paucity of research on this topic, additional studies concurrently examining sexual orientation, gender identity, and gender expression in relation to a variety of brain features are needed before firmer conclusions can be drawn.

Importantly, given our cross-sectional design and lack of direct assessment of hormonal influences during periods of brain organization, alternatives to a neurohormonal perspective should also be considered. For example, one can consider childhood gender role expression differences and their potential relations with social and brain development. In the present sample, the two *sao praphet song* groups reported more feminine and less masculine childhood gender role behavior than gay cisgender men (see Data S1)—a pattern that mirrored the WM microstructural brain pattern. Although these childhood gender role differences could reflect downstream effects of hormonal influences on brain organization (Hines 2020), another reasonable interpretation, in light of our cross-sectional design, is that the experience of being relatively masculine or feminine drives neural differentiation. Additional experiential factors that may differentiate development among feminine-identifying individuals compared with cisgender men may include stigma and gender minority stressors (Collet et al. 2023), which are reported to influence neural connectivity (e.g., Nicholson et al. 2021). Although *sao praphet song* are visible and reputedly accepted within Thai society, Thai gender-diverse people do nevertheless experience gender-based discrimination (Srikummoon et al. 2022). As such, developmental and social differences beyond hypothesized neurohormonal factors could contribute to neural structural differences on an axis of gender identity as observed here.

The relative lack of apparent differences in WM microstructure between *sao praphet song* and straight cisgender women also presents some challenges to a neurohormonal perspective and offers novel hypotheses on the relations of gender identities and their brain substrates (Frigerio et al. 2021). These groups evidenced largely similar WM microstructure despite heterogeneity in exposure to so-called feminizing hormones. The current findings suggest WM microstructure among feminine-identifying people can be similar regardless of whether exposure to so-called feminizing hormones during adolescence and adulthood was absent (as with GAH− *sao praphet song*), occurred exogenously (as with GAH+ *sao praphet song*), or endogenously (as with straight cisgender women). Further research is needed to explore why these similarities exist despite differences in

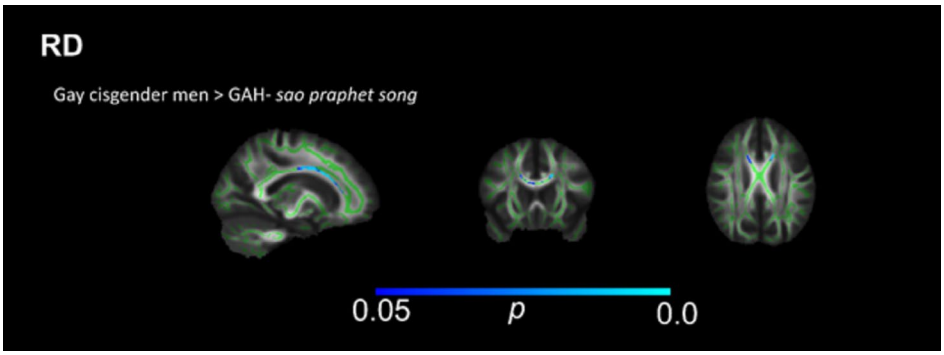

FIGURE 3 | Significant TBSS clusters indicating group differences in RD. Significant clusters ($p_{FWE} < 0.05$; $k > 100$ voxels) identified in RD (blue-light blue). Sample mean FA skeleton mask (green) overlaid on FA map. Anatomical left is right.

TABLE 4 | Size and location of significant clusters identified by pairwise comparison of RD.

Region	Cluster Size, *k*	p_{FWE}	MNI coordinates		
			X	Y	Z
Gay cisgender men > GAH− *sao praphet song*					
Left anterior corona radiata	631	0.009	106	144	100
Right body of corpus callosum	541	0.024	74	119	106

Abbreviations: *k* = number of voxels in a cluster; FWE = family-wise error corrected.

hormonal exposure, such as the roles of non-sex-steroid factors shared across feminine-identifying people (e.g., gendered social experiences shaping brain organization).

Still, certain patterns observed for FA among the GAH+ *sao praphet song* lend some limited support to the hypothesis that GAH influences WM microstructure. The TBSS analyses identified lower FA in GAH+ *sao praphet song* compared with GAH− *sao praphet song* in a relatively small cluster of voxels in the right corpus callosum. This lower FA in GAH+ *sao praphet song* might be reflective of why GAH− *sao praphet song* and straight cisgender women, but not GAH+ *sao praphet song*, showed greater FA than gay cisgender men in the pairwise TBSS analyses. Further, although this lower FA suggests a decrease in FA related to GAH, we cannot conclude that this decrease is associated with so-called feminizing hormone use given that the TBSS group comparisons for FA indicated straight cisgender women as having greater FA in the right corpus callosum. In the PLS analysis, significantly lower FA among the GAH+ *sao praphet song*, but not among the GAH− *sao praphet song* or straight cisgender women, contributed to the latent variable capturing significant group contrasts in WM microstructure bilaterally in the cingulum and anterior corona radiata and left corpus callosum. This pattern of lowered FA among GAH+ *sao praphet song* revealed by the PLS analysis is consistent with a possible effect of GAH whereby it alters WM tissue to be increasingly dissimilar in its organization from cisgender men's, given that the latter showed higher FA in these regions.

Thus, despite the mixed evidence bearing on whether GAH is associated with decreased WM microstructure organization, the FA patterns from both sets of analyses provide some evidence to suggest that GAH+ *sao praphet song* have less WM organization in these regions. DTI literature investigating GAH use in transfeminine individuals is unfortunately scant, even in comparison with GAH research in transmasculine individuals (Kranz et al. 2020). However, the one prior study reporting on WM microstructure changes following four months of GAH therapy in 15 transgender women also found evidence of regionally decreased FA—although there was no direct association with the observed change in plasma estradiol concentration (Kranz et al. 2017). Thus, while emerging empirical research exists and provides some limited support for an effect of GAH on brain WM FA in transfeminine individuals, more research investigating the effects of GAH on WM microstructure in transgender populations is needed.

The above findings offer insight into the relationships between identity and WM structure, but are limited in their extension to functional connectivity and behavioral relationships. Several of the WM regions identified in the significant LV are related to motor function (i.e., anterior corona radiata, corticospinal tract; Natali et al. 2023) and spatial awareness (i.e., SLF; Janelle et al. 2022), as well as emotion, cognitive, and pain functions (i.e., forceps minor, cingulum; Bubb et al. 2018). Additionally, the cingulum is related to pain appraisal and reinforcing pain reduction behaviors (Bubb et al. 2018). Yet, despite the role of WM tissue in signal conduction (Buyanova and Arsalidou 2021), the present analyses are unable to indicate whether there are functional or behavioral differences that correspond to the identified microstructural differences.

5 | Limitations

The current findings should be considered in light of some limitations. Although the current study uniquely used five groups to differentiate gender identity, sexual orientation, and GAH use, the findings can be limited by smaller sample sizes relative to the substantial sample sizes available in amalgamated neuroimaging data (e.g., Human Connectome Project [Lawrence

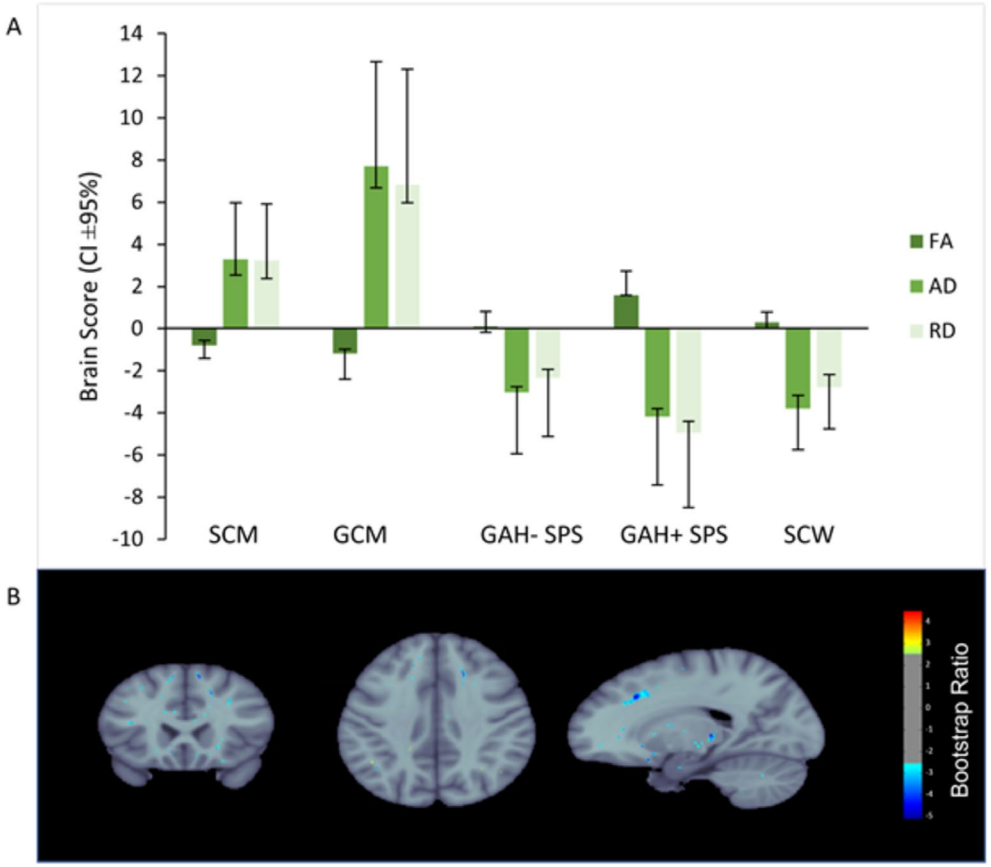

FIGURE 4 | One latent variable reveals a significant difference in WM microstructure across groups. (A) Brain pattern identified by the latent variable across groups. The direction of associations is interpreted in the inverse due to the blue clusters in panel B indicating a negative association with brain scores in panel A. Correlations with confidence intervals that do not overlap with zero indicate a stable contribution to the latent variable. AD = axial diffusivity; FA = fractional anisotropy; GAH+ SPS = *sao praphet song* using gender-affirming hormones; GAH− SPS = *sao praphet song* not using gender-affirming hormones; GCM = gay cisgender men; RD = radial diffusivity; SCM = straight cisgender men; SCW = straight cisgender women. (B) Stable regions of the latent variable. Bootstrap ratio is thresholded at ±2.5 as indicated by the gradient scale on the right. Anatomical left is left for coronal and axial view; the sagittal view shows the right hemisphere.

et al. 2021]; ENIGMA [Mueller et al. 2021]). That said, our group sample sizes were comparable to those reported in studies of similar research paradigms (e.g., Rametti et al. 2011a; Kranz et al. 2014; Burke et al. 2017) and the median sample size of neuroimaging studies in general (Szucs and Ioannidis 2020). Also, to disentangle sexual orientation from gender identity among individuals assigned male at birth, we sampled straight cisgender men, gay cisgender men, and transfeminine *sao praphet song* attracted to cisgender men; however, we did not sample transfeminine *sao praphet song* with other sexual attractions (e.g., attracted to cisgender women). Studies report a lower prevalence of gynephilia or attraction to women among transfeminine individuals (Lawrence 2010; Kuper et al. 2011; Reisner et al. 2023). As such, the present study indicates that the WM microstructure of transfeminine *sao praphet song* attracted to cisgender men aligns with that of straight cisgender women and differs from that of both straight and gay cisgender men; whether this pattern extends to transfeminine individuals with other sexual attractions cannot be discerned given the present study design.

Additionally, the measurement of GAH use may have posed some limitations to the study. Access to exogenous hormones in Thailand is not limited to those with a physician prescription or referral (Salakphet et al. 2022; Skorska et al. 2023), which may have contributed to the varied hormone use among participants. GAH is relatively accessible in Thailand in the form of hormonal contraception from pharmacies, and this likely contributes to there being little consistency in use patterns across individuals (Skorska et al. 2023). The type and consistency of hormone use in the present sample of GAH+ *sao praphet song* were similarly inconsistent across participants. Therefore, the current study was unable to assess differences related to the type of hormone or administration and did not have a direct measure of hormone concentration for comparison across individuals. Despite these limitations, the present study assesses the association between GAH use and neural structure among Thai *sao praphet song*, and thus, provides important information for this population and other transfeminine populations that may access GAH in similar ways (China: Liu et al. 2020; Japan: Baba et al. 2022; Russia: Makarova et al. 2022). Among *sao praphet song*, GAH use may include one or a combination of estradiol, progestogen, and anti-androgen agents (Skorska et al. 2023). Estrogens and progestogens have been inconsistently related to WM microstructure (Herting and Sowell 2017; van Hemmen et al. 2017; Kranz et al. 2020), while literature specific to the effects of anti-androgen is lacking altogether.

Our sample also included a small number ($n = 9$) of straight cisgender women who reported using hormonal contraception. There were too few of these participants to create their own hormone-use group and, because the study's aim of recording hormone use was to characterize the influence of GAH in *sao praphet song* specifically, the cisgender participants using contraception were not removed. The degree with which hormonal contraception influences the brain in cisgender women is still unclear (Concas et al. 2022). Despite these limitations, our findings contribute to the current literature on GAH influence toward WM microstructure and offer a novel exploration of sex/gender-related differences in WM tissue using DTI in a non-Western population.

6 | Conclusions

Here, we presented whole-brain mass-univariate and multivariate analyses of WM microstructure indices in five sexually and gender diverse groups of Thai adults. Regional differentiation of WM microstructure was found in relation to gender identity irrespective of sex assigned at birth, as well as between cisgender and transfeminine individuals assigned male at birth with sexual attractions to cisgender men. These findings are partially consistent with a neurohormonal perspective on brain organization and gender identity/role expression, although we note that alternative explanations can apply and should not be ruled out given our cross-sectional design. There was no clear sexual orientation-related pattern as no comparison showed straight cisgender men differing from the four groups attracted to cisgender men. In addition, regional differentiation of WM microstructure was found in relation to GAH use by *sao praphet song*. Importantly, our findings parallel some prior Western WM microstructure studies in relation to sex/gender, psychosexual variation, and the use of GAH by transfeminine people, thus supporting the cross-population generalizability of research findings in this area. To further advance the field, future research assessing a wide variety of brain metrics in relation to broad psychosexual variation, as well as GAH use, is needed.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are openly available in Borealis at https://doi.org/10.5683/SP3/MED7LN (Thurston and VanderLaan 2024).

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.