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TRANSGENDER HEALTH

The Neuroanatomy of Transgender Identity: Mega-Analytic Findings From the ENIGMA Transgender Persons Working Group

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ABSTRACT

Background: In contrast to cisgender persons, transgender persons identify with a different gender than the one assigned at birth. Although research on the underlying neurobiology of transgender persons has been accumulating over the years, neuroimaging studies in this relatively rare population are often based on very small samples resulting in discrepant findings.

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Aim: To examine the neurobiology of transgender persons in a large sample.

Methods: Using a mega-analytic approach, structural MRI data of 803 non-hormonally treated transgender men (TM, $n = 214$, female assigned at birth with male gender identity), transgender women (TW, $n = 172$, male assigned at birth with female gender identity), cisgender men (CM, $n = 221$, male assigned at birth with male gender identity) and cisgender women (CW, $n = 196$, female assigned at birth with female gender identity) were analyzed.

Outcomes: Structural brain measures, including grey matter volume, cortical surface area, and cortical thickness.

Results: Transgender persons differed significantly from cisgender persons with respect to (sub)cortical brain volumes and surface area, but not cortical thickness. Contrasting the 4 groups (TM, TW, CM, and CW), we observed a variety of patterns that not only depended on the direction of gender identity (towards male or towards female) but also on the brain measure as well as the brain region examined.

Clinical Translation: The outcomes of this large-scale study may provide a normative framework that may become useful in clinical studies.

Strengths and Limitations: While this is the largest study of MRI data in transgender persons to date, the analyses conducted were governed (and restricted) by the type of data collected across all participating sites.

Conclusion: Rather than being merely shifted towards either end of the male-female spectrum, transgender persons seem to present with their own unique brain phenotype. **Mueller SC, Guillamon A, Zubiaurre-Elorza L, et al. The Neuroanatomy of Transgender Identity: Mega-Analytic Findings From the ENIGMA Transgender Persons Working Group. J Sex Med 2021;18:1122−1129.**

Key Words: Transgender; MRI; Mega-analysis; ENIGMA; Anatomy; Sex differences

INTRODUCTION

Sex differences in human neuroanatomy are well documented.[1] However, the role of gender identity in contributing to this dimorphism is unknown. A small percentage of individuals in society - transgender persons - identify with a gender different from the sex they were assigned at birth.[2] Whether transgender persons resemble, neurobiologically, their sex assigned at birth or their gender identity is currently unclear. Research initiated more than two decades ago based on *post-mortem* data of 6 transgender women (male sex assigned at birth with a female gender identity) suggested a brain pattern that was concordant with their gender identity.[3] However, this effect was highly localized and restricted to the bed nucleus of the stria terminalis (while other nuclei did not follow that pattern). Moreover, these individuals were examined after they underwent hormone treatment. Over the last 10 years, a surge in magnetic resonance imaging (MRI) studies have aimed to clarify the neurobiology and contribution of gender identity to human neurobiology by examining transgender persons before hormone treatment.[4,5] Nevertheless, the outcomes of these studies have also been largely inconsistent possibly due to a large heterogeneity across (relatively small) cohorts as well as the diversity in analysis methods applied.[6−11] In addition, studies often aimed to test whether the neurobiology of transgender persons was similar to (or different from) the one of cisgender persons rather than acknowledging that transgenderism comes with its own unique profile. Moreover, these findings may be region-specific including the hypothalamus,[3] the primary motor and somatosensory cortices[12] and the frontal and/or limbic lobe[8] (just to name a few).

Indeed, the possibility that both distinct and unique brain patterns may exist has been fervently discussed. For example, it has recently been demonstrated that human brains may be mosaics consisting of male and female patterns.[13] Moreover, it has been theorized that the brains of transgender men and women may be different due to their underlying genetic sensitivities to sex steroid receptors or as a consequence of individual neurodevelopmental patterns.[14,15] However, studies in transgender people to date have lacked the necessary statistical power to examine this issue. Relatedly, more studies are needed to ensure diversity and inclusivity to understand issues and disparities related to sex and gender in brain health; for example, to provide a baseline for longitudinal effects of gender-affirming hormone treatment.

To further advance this understudied (and underpowered) field of research, the ENIGMA Transgender Persons Working Group was created to collaboratively evaluate available MRI data in transgender persons using a mega-analytic approach.[16] In contrast to more traditional meta-analyses (which merely pool statistical effect sizes across sites), a mega-analysis fits a statistical model to all individual level data across all sites while, at the same time, adjusting for site-dependent effects.

The current study addressed the central question of whether the neuroanatomy of transgender persons resembled that of their gender identity, their sex assigned at birth, a combination of both, or something entirely different. Of note, our mega-analytic study is conducted using data from previously published transgender MRI studies. Thus, we deliberately abstained from making predictions based on the prior data in order to avoid the risk of running a circular argument.

MATERIAL AND METHODS

Participants

The sample consisted of a total of 803 structural brain MRI scans including non-hormonally treated transgender men (TM, $n = 214$, female assigned at birth with male gender identity, mean age = 28.17 years, SD=10.69 years), transgender women (TW, $n = 172$, male assigned at birth with female gender identity, mean age = 27.92 years, SD = 9.66 years), cisgender men (CM, $n = 221$, male sex assigned at birth with male gender identity, mean age = 28.83 years, SD = 10.10 years) and cisgender women (CW, $n = 196$, female sex assigned at birth with female gender identity, mean age = 27.24 years, SD = 7.18 years). Brain images and accompanying individual information were contributed by eight sites. Criterion for inclusion in the present mega-analysis was availability of structural MRI data in transgender persons before gender affirming hormone treatment and that sites were willing to share either raw structural MRI data or pre-process their MRI data locally according to ENIGMA protocol. All invited sites except for one agreed to participate. For site-specific details, including individual study N and image acquisition parameters, as well as inclusion/exclusion criteria please refer to Supplementary Table S1. All sites (except 1, self report) used the most appropriate criteria at-the-time for diagnosis of gender dysphoria or gender identity disorder (DSM-IV or 5). Groups did not differ by age ($F(3,799) = 0.98$, $P = .401$) or level of education (minimum number of years of education received) ($F(3,572) = 01.23$, $P = .30$). Institutional Review Board approval was granted by the respective boards at each site and written informed consent was obtained from all participants prior to data collection following the Declaration of Helsinki (Supplementary Table S1). In addition, the secondary analysis was approved by the ethical committee of the Faculty of Psychology at the University of Ghent (2020/170).

MRI Data Acquisition and Preprocessing

Similar to other multi-site efforts,[17] all anatomical MRI data (T1-weighted scans) were preprocessed using FreeSurfer v. 5.3 (sites (Aachen, Amsterdam, Florence, Muenster, Los Angeles, Madrid, Mashhad) or FreeSurfer v.6 (site Vienna) either locally or on the high performance cluster (HPC, Freesurfer v5.3) at Ghent University using a fully-automated MRI processing pipeline.[17] The reason for whether data were processed locally or collected at Ghent University were site-specific ethical regulations regarding sharing of data. Screening for image artifacts and outliers was performed as per the semi-automatic ENIGMA quality and assurance (Q&A) standardized protocol (e.g.,[16,17] see also http://enigma.ini.usc.edu/protocols/imaging-protocols/). This Q&A procedure includes visual inspection and detection of outliers in a series of standard planes. All preprocessed data was anonymized and further analyzed at Ghent University.

Experimental Design and Statistical Analyses

Three main anatomical measures of interest were analyzed: (i) grey matter volume (cortical and subcortical, GMV), (ii) cortical surface area (CSA), and (iii) cortical thickness (CTh) using a region-of-interest (ROI) approach. To reduce the number of comparisons several *a priori* ROIs were selected based on prior findings[6–8,18,19] as well as based on specific research interests of specific sites. Specifically, these regions included for *cortical and subcortical volume* (GMV) as well as *cortical thickness* (CTh) and *cortical surface area* (CSA): (i) inferior parietal gyrus, (ii) precentral gyrus, (iii) postcentral gyrus, (iv) fusiform gyrus, (v) IFG pars orbitalis, (vi) IFG pars opercularis, (vii) IFG pars triangularis, (viii) superior frontal gyrus, (ix) frontal pole, (x) rostral middle frontal gyrus, (xi) caudal middle frontal gyrus, (xii) insula. In addition to these, GMV was also obtained from (xiii) cerebellum, (xiv) ventricles, (xv) thalamus, (xvi) putamen, and the (xvii) caudate. All statistical analyses were performed on the mean values for each ROI obtained from the FreeSurfer software in SPSS (V.24) using analysis of covariance (ANCOVA). As we were predominantly interested in relative structural differences, for all analyses, age and intracranial volume (ICV) were used as covariates of no interest. However, as findings may differ after correction for overall brain volume,[20] the uncorrected findings are presented in Supplementary Tables S4 and S5.

Importantly, in terms of correction for multiple comparisons, all main effects were controlled for the number of ROIs using false-discovery rate (FDR) correction[21] ($P < .05$, two tailed). A significant main effect within a given ROI was followed by individual group comparisons using an ANCOVA with only two groups at a time. Here, Bonferroni corrections taking into account the number of group comparisons were applied (N = 6, adjusted $P < .008$, $P < .05$ corrected, two-tailed). This was done separately for the independent variables of GMV, CSA, and CTh. Decision for the respective use of these two correction methods was based on the notion that FDR seemed more appropriate to control for the overall number of tests, while the Bonferroni method seemed best for the post hoc follow-up. In addition, *post-hoc*, the analyses of main effects were re-run to assess a potential impact of study site, scanner strength, or FreeSurfer version, but all reported findings remained significant ($P < .05$, corrected for both level corrections, two-tailed). Although some previous studies have tried to account for sexual orientation,[8] there were several issues. Predominantly, a) each centre either assessed sexual orientation very differently (e.g., using a 10 point or a 100 point scale, the 7 point Kinsey scale, or a simple categorical self-definition as homosexual, heterosexual, or bisexual) or did not collect information on sexual orientation at all. Given this difficulty to adequately synthesize sexual orientation across sites, analysis of this factor can be found in the supplementary materials (Supplementary Table S6), and should be considered exploratory and taken with great caution.

RESULTS

GMV: 5 main patterns of brain anatomy became apparent for GMV (Figure 1), with four patterns for CSA (Figure 2; both Supplementary Tables S2 and S3). GMV: In pattern 1 (CM>

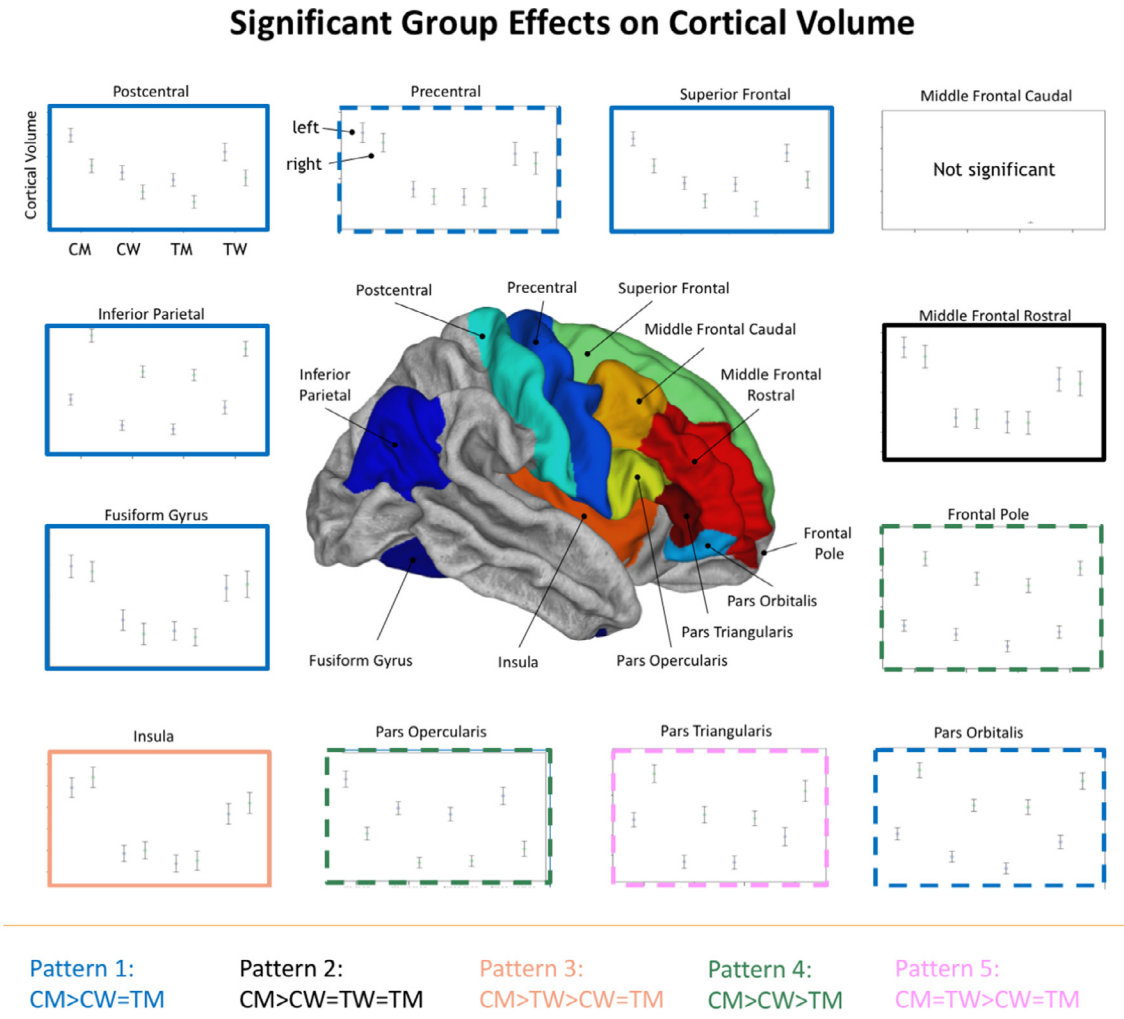

Figure 1. Significant group effects on cortical volume. Shown are error bar graphs, including means and 95% confidence intervals. 5 different patterns emerged (The > (larger than) indicates a statistically significant difference between groups; = indicates that there was no statistical difference between groups. Note that significance was established while removing the variance associated with age and ICV (for *P*-values refer to Supplementary Tables S2 and S3), but that the scatterplots indicate the raw values. The dashed border indicates a minor variation of the main pattern or lateralized effects. For patterns 1 and 4, TW were not included as they showed a pattern not easily expressed within the patterns (eg, TW>TM but TW = CM/CW). Please see Supplementary Tables S2 and S3 for details. Significant findings within the cerebellum, thalamus, caudate, and putamen have been omitted in this figure. Figure 1 is available in color online at www.jsm.jsexmed.org.

CW=TM) CM differed from both CW and TM, that is TM resembled their sex assigned at birth (CW) (for example, in the *pre-* and *postcentral gyri* as well as *superior frontal gyrus*, *inferior parietal gyrus,* and *fusiform gyrus*. In pattern 2 (CM>CW=TW=TM), CM had larger volumes or surface area than any other groups showing that TW were similar to their gender identity but TM were similar to their sex assigned at birth (*middle frontal rostral* gyrus and *thalamus*). Pattern 3 (CM>TW>CW=TM) is similar to pattern 1 but additionally shows statistically significant differences that place TW in-between CM and CW, whereas TM resemble their sex assigned at birth (*insula*). In pattern 4 (CM > CW> TM), CM had larger volumes and surface areas than CW, who in turn had larger GMV and CSA then TM. This patterns documents that TM had even lower brain anatomy measures than CW, thus showing a phenotype distinct from their gender identity or their sex assigned at birth (*frontal pole* and *pars opercularis*). Finally, pattern 5 showcased a clear sex-assigned at birth pattern, in which CM and TW differed from CW and TM (*cerebellum* and *pars triangularis*).

CSA: 4 patterns seen in GMV were also apparent in CSA but most regions varied in their pattern relative to the one found in GMV. Pattern 1 was seen in the *caudal middle frontal gyrus*, whereas pattern 2 was apparent in the *superior frontal*, *inferior parietal*, the *middle frontal rostral*, *pars opercularis, and fusiform gyrus*. Pattern 3 could be seen in the *precentral gyrus, pars triangularis, and the insula* and pattern 4 was apparent in the *frontal pole*, *pars orbitalis, and postcentral gyrus*. By contrast, pattern 5 was absent for CSA.

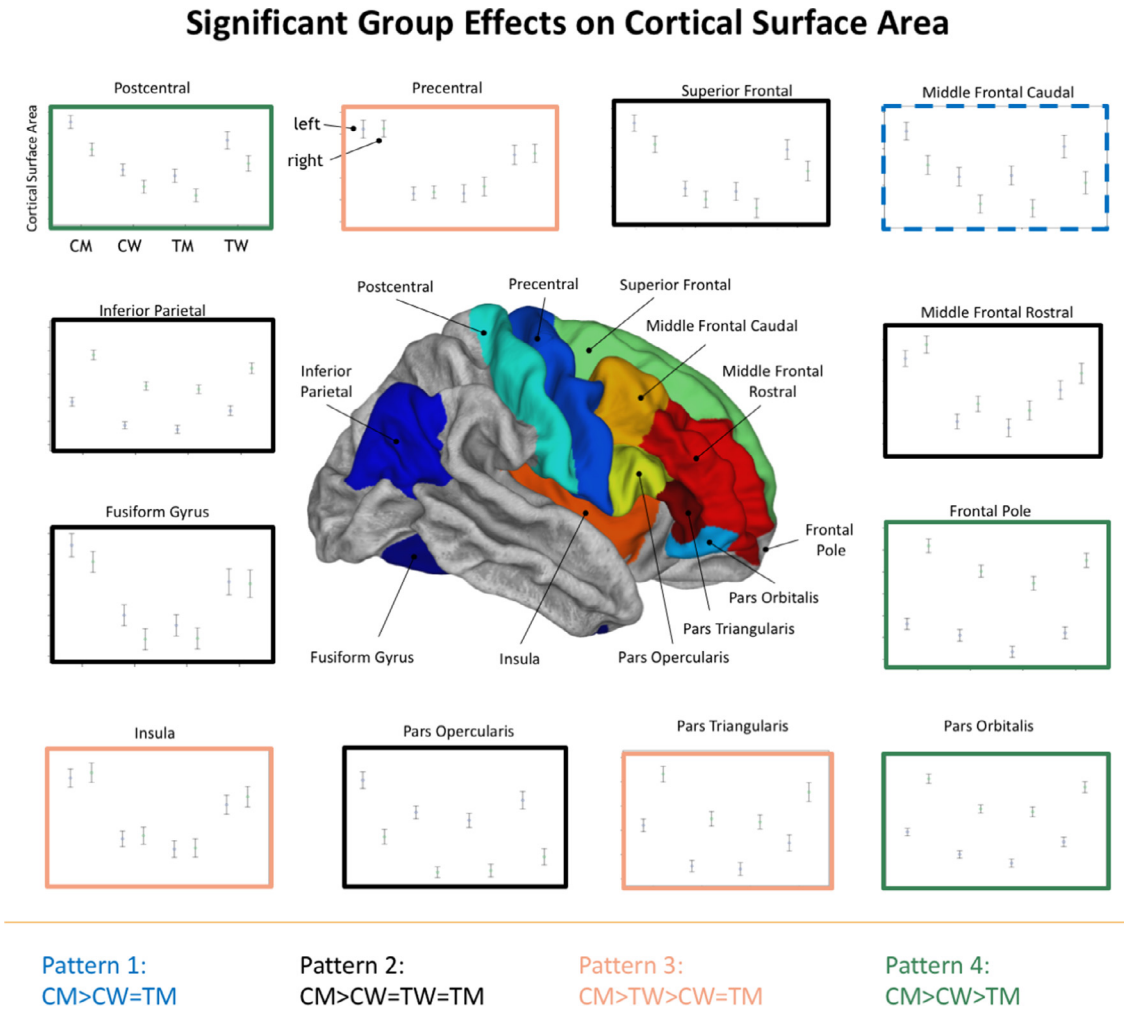

Figure 2. Significant group effects on cortical surface area. Error bars, symbols, and patterns as in Figure 1. Since pattern 5 did not occur in CSA, it was omitted from the legend. Figure 2 is available in color online at www.jsm.jsexmed.org.

CTh: No statistically significant effects emerged for CTh (Supplementary Tables S2 and S3).

DISCUSSION

Due to previously smaller sample sizes (e.g.,[7,12]) and disparate findings[3,6–8,22] published in the literature, the goal was, by virtue of mega-analysis, to examine structural brain patterns in transgender persons prior to gender affirming hormonal treatment. Applying such a mega-analytic approach to the largest available dataset to date in over 800 participants, this study uncovered that transgender men and women may have their own unique neurobiological phenotypes depending on the brain region assessed.

Previous studies have published a variety of neurobiological findings in transgender people. For example, particularly contentious is putamen volume. While Luders et al.[6] reported lager volume in TW relative to CM, Savic & Arver[8] documented smaller putamen volume in TM relative to CM, whereas in Mueller et al.,[7] putamen volume was larger in TW relative to CW. Using a variety of regions of interest, we were able to identify 5 distinct patterns in the current data that emerged depending on the brain area queried. Generally speaking, while CM and CW differed from one another with CM having consistently larger volume and/or surface area, TW either shifted (depending on the pattern) between resembling their sex assigned at birth or their gender identity, or they were in-between. By comparison, TM did not shift as much and, interestingly, shifted in one instance to be lower in volume than CW. Reduced volumes and surface areas of parts of the frontal lobe and the insula in transgender men relative to their sex assigned at birth have previously not been documented in untreated transgender persons. Moreover, a prior, independent study of treated transgender persons highlighted this pattern, showcasing reduced fusiform volume in TM relative to CW.[7] Given that a reduction in volume also appears to be present in non-hormonally treated transgender men relative to CW indicates that the findings by Mueller et al.[7] were probably not due to treatment effects (although the brain region differed). Importantly, the current data are inconsistent with earlier

thought on transgender people resembling, neurobiologically, either their sex assigned at birth[6,8,15,20] or their gender identity[3] and speak for a unique phenotype.

Indeed, regionally-specific variations are consistent with the view by Guillamon et al.,[14] who suggested that transgender people demonstrate different extents of phenotypically male and female brain patterns. These differences may be due to a variety of factors including molecular[23–25] and neurodevelopmental[26] factors, either alone or in interaction with one another. Such interactions may account for some of the variability in patterns and explain why the TW group might also display demasculinized patterns (although one might expect solely defeminized patterns). As a result, each group might present itself with its own unique brain phenotypes. Such a view is somewhat consistent with recent empirical data from CM and CW that documented a "mosaic" pattern of a "neurophenotype" along the maleness-femaleness spectrum.[13]

Another theory that has been proposed to account for brain differences between transgender and cisgender people are not anatomical differences inherent to sex assigned at birth *per se* but are rather linked to brain correlates of how one perceives one's body in relation to one's gender identity. This is noteworthy as all but one study in the present mega-analysis recruited participants based on DSM-IV/5 criteria of gender identity disorder or gender dysphoria, both of which entail a crucial component of persistent discomfort (or suffering) with the assigned gender. One possibility is that at least some part of this suffering may be associated with a mismatch between the brain correlates of the identified gender and the physical characteristics of the sex assigned at birth. Towards this point, Savic and colleagues[15,27,28] proposed that structural and functional alterations in brain morphometry may be linked to an incongruence between the gender assigned at birth and the gender identity in transgender people. However, anatomically, these effects of incongruence have predominantly been documented in CTh,[15] but not in GMV or for CSA (i.e., where the current study revealed pronounced effects). In fact, our findings indicate a lack of group differences with respect to CTh altogether, which is in contrast to many prior outcomes.[9,15,29] Future work will need to examine more closely the potential reasons for this discrepancy. One possible explanation is that brain volume and cortical surface area are more sensitive and robust measures of gender identity and sex than CTh.[30]

Despite these intriguing findings, some strengths and limitations of the present work require discussion. Specifically, one caveat of large-scale mega-analyses is that it is difficult to control what information is being collected, given that different research groups may embark on their studies with different aims and agendas. As a consequence, this may inevitably result in increased sample heterogeneity. For example, whereas some investigators might specifically recruit people according to their age of onset of gender dysphoria[9,31] or sexual orientation,[8,32,33] others might not.[7,29] In point of fact, in addition to some missing data, every centre essentially collected and defined these variables differently. Nonetheless, while groups did not differ with regards to number of years of education received, the point regarding sexual orientation was more difficult to address and cannot be resolved here satisfactorily. However, an exploratory analysis (supplementary material) revealed that trans women showed the same directionality of findings as cis men in GMV and as cis men and cis women in CSA with no effects in trans men for either measure. This is consistent with prior MRI work in transgender persons that has not found modulating effects of sexual orientation.[32,33] However, to fully and adequately assess effects of sexual orientation on neuroanatomy, we hope that standardized instruments across studies with larger sample sizes will be used in the future to facilitate such analyses. Similarly, although disorders of sexual development served as an exclusion criterion for all studies, information on sexual maturation in this cohort was not available, which could be an interesting point for future MRI work. In addition, we were not able to examine the influence of culture or ethnicity *per se* as all but one sample stemmed from participants with a white ethnic background. Despite these disadvantages, the mega-analytic approach also confers several strong advantages.

On the plus side, we were able to account for potential effects of age given that the brain continues to change over the course of the lifespan (e.g.,[34]). Critically, MRI work in clinical and/or endocrine populations is commonly accompanied by relatively small sample sizes. Increasing sample size by a power of ~8 in relation to prior work and stringently controlling for multiple comparisons provides the opportunity for some limited generalization that is not possible with individual studies. Moreover, were any individual finding be caused by an unknown confound, this should have averaged out in the mega-analysis. Demonstration of robust findings in the present work suggest some brain patterns that survived despite heterogeneity adding further strength to the conclusions. As noted earlier, all but one center recruited their transgender population via clinical criteria of the DSM-VI/5 / ICD-10 manuals meaning that gender dysphoria was likely present in all participants. Additionally, previously, little attention was being paid to non-binary genders. As a result, most studies have relied on a subsample of transgender persons identifying with the gender opposite to the one assigned at birth. Only recently the need for inclusion of gender non-binary people[35] has been advocated. Thus conclusions to the entire spectrum of transgender persons are limited. Finally, by reporting all three brain structural measures, we were able to show that these outcomes might be differentially sensitive to sex and gender and may not necessarily be consistent with one another (CMV/CSA vs CTh). Consequently, future work will need to more carefully report the influence of such extraneous variables on brain data.

With regards to clinical implications, the current data contribute to providing normative data and to promote inclusivity and ensure diversity to understand potential issues related to brain health. Such data are required to fully understand risk and resilience factors. In addition, these data may provide a baseline for future longitudinal studies that examine the influence of gender-affirming hormone treatment on the brain in transgender persons.

In sum, this is the first large-scale and well-powered study of structural neuroimaging data in transgender persons. The outcomes of our study highlight the underlying neurobiology of gender identity and pose novel questions to be followed up regarding neurodevelopmental, genetic, and hormonal factors. In addition, future research will need to address other unresolved aspects, such as whether cross-cultural comparisons are needed and examine the possible influence of sexual orientation.[5,36]

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STATEMENT OF AUTHORSHIP

Author contributions Aachen site: Ute Habel supervised the project, and Ute Habel and Birgit Derntl provided the resources and acquired the funding, Mikhail Votinov processed the data; Madrid site: Carme Junqué conceived the study, contributed with conceptual advice and design the MRI protocol and revised the current manuscript; Carme Uribe recruited the sample and collected MRI data; Esther Gómez-Gil recruited the sample, made gender dysphoria diagnosis and gathered clinical data; Antonio Guillamon conceived the study, conceptual advice, design the MRI protocol and gathered the MRI data and revised the current manuscript; Leire Zubiaurre-Elorza collected MRI data, revised quality of MRI data and gathered the MRI data at the and revised the current manuscript; Amsterdam site: Conception and design original study: Dick Veltman, Baudewijntje Kreukels, Peggy Cohen-Kettenis, Nils Lambalk; Acquisition, Analysis and Interpretation original study: Dick Veltman, Baudewijntje Kreukels, Peggy Cohen-Kettenis, Nils Lambalk; Preparation and analysis of data: Sarah Burke, Mathilde Kennis, Dick Veltman, Baudewijntje Kreukels; Revising the article for intellectual content: Sarah Burke, Dick Veltman, Baudewijntje Kreukels; Brazil site: Maikel Abel Schneider edited the manuscript. Florence site: Alessandra D. Fisher contributed to patients' enrollment and the final version of the manuscript; Jiska Ristori contributed to patients' enrollment and the final version of the manuscript; Giovanni Castellini perfomed the psychiatric assessment, psychometric assessment, data collection and editing; Mario Maggi: supervision of the project; Mario Mascalchi performed the neurological assessment of MRI scans and editing; Gioele Gavazzi & Stefano Orsolini = Data Acquisition and Analysis; Vienna site: Rupert Lanzenberger and Georg S. Kranz designed and performed the research, and Rene Seiger analysed the data. Ghent site: Sven Mueller planned the project, analysed the data, created the figures, wrote the paper, and coordinated local data processing, Guy T'Sjoen edited the manuscript; LA site: Eileen Luders wrote the manuscript.

REFERENCES

1. Ruigrok AN, Salimi-Khorshidi G, Lai MC, et al. A meta-analysis of sex differences in human brain structure. **Neurosci Biobehav Rev 2014;39:34–50.**
2. Arcelus J, Bouman WP, Van Den Noortgate W, et al. Systematic review and meta-analysis of prevalence studies in transsexualism. **Eur Psychiatry 2015;30:807–815.**
3. Zhou J-N, Hofman MA, Gooren LJ, et al. A sex difference in the human brain and its relation to transsexuality. **Nature 1995;378:68–70.**
4. Smith ES, Junger J, Derntl B, et al. The transsexual brain—A review of findings on the neural basis of transsexualism. **Neurosci Biobehav Rev 2015;59:251–266.**
5. Mueller SC, De Cuypere G, T'Sjoen G. Transgender research in the 21st century: A selective critical review from a neurocognitive perspective. **Am J Psychiatry 2017;174:1155–1162.**
6. Luders E, Sanchez FJ, Gaser C, et al. Regional gray matter variation in male-to-female transsexualism. **Neuroimage 2009;46:904–907.**

7. Mueller SC, Landre L, Wierckx K, et al. A structural magnetic resonance imaging study in transgender persons on cross-sex hormone therapy. **Neuroendocrinology 2017;105:123–130.**
8. Savic I, Arver S. Sex dimorphism of the brain in male-to-female transsexuals. **Cereb Cortex 2011;21:2525–2533.**
9. Zubiaurre-Elorza L, Junque C, Gomez-Gil E, et al. Cortical thickness in untreated transsexuals. **Cereb Cortex 2013;23:2855–2862.**
10. Kranz GS, Hahn A, Kaufmann U, et al. Effects of testosterone treatment on hypothalamic neuroplasticity in female-to-male transgender individuals. **Brain Struct Funct 2018;223:321–328.**
11. Seiger R, Hahn A, Hummer A, et al. Subcortical gray matter changes in transgender subjects after long-term cross-sex hormone administration. **Psychoneuroendocrinology 2016;74:371–379.**
12. Simon L, Kozak LR, Simon V, et al. Regional grey matter structure differences between transsexuals and healthy controls—a voxel based morphometry study. **PLoS One 2013;8:e83947.**
13. Joel D, Berman Z, Tavor I, et al. Sex beyond the genitalia: The human brain mosaic. **PNAS 2015;112:15468–15473.**
14. Guillamon A, Junque C, Gomez-Gil E. A review of the status of brain structure research in transsexualism. **Arch Sex Behav 2016;45:1615–1648.**
15. Manzouri A, Kosidou K, Savic I. Anatomical and functional findings in female-to-male transsexuals: Testing a new hypothesis. **Cereb Cortex 2017;27:998–1010.**
16. Thompson P, Jahanshad N, Ching CRK, et al. ENIGMA and global neuroscience: a decade of large-scale studies of the brain in health and disease across more than 40 countries. **Transl Psychiatry 2020;10:100.**
17. de Kovel CGF, Aftanas L, Aleman A, et al. No alterations of brain structural asymmetry in major depressive disorder: an ENIGMA consortium analysis. **Am J Psychiatry 2019;176:1039–1049.**
18. Mueller SC, Wierckx K, Jackson K, T'Sjoen G. Circulating androgens correlate with resting-state MRI in transgender men. **Psychoneuroendocrinology 2016;73:91–98.**
19. Nota NM, Burke SM, den Heijer M, et al. Brain sexual differentiation and effects of cross-sex hormone therapy in transpeople: A resting-state functional magnetic resonance study. **Neurophysiol Clin 2017;47:361–370.**
20. Khorashad BS, Khazai B, Talaei A, et al. Neuroanatomy of transgender persons in a Non-Western population and improving reliability in clinical neuroimaging. **J Neurosci Res 2020;98:2166–2177.**
21. Benjamini Y, Hochberg Y. Controlling the false discovery rate: A practical and powerful approach to multiple testing. **J Roy Statist Soc Ser A 1995;57:289–300.**
22. Hahn A, Kranz GS, Kublbock M, et al. Structural connectivity networks of transgender people. **Cereb Cortex 2015;25:3527–3534.**
23. Fernandez R, Guillamon A, Cortes-Cortes J, et al. Molecular basis of gender dysphoria: Androgen and estrogen receptor interaction. **Psychoneuroendocrinology 2018;98:161–167.**
24. Hare L, Bernard P, Sánchez FJ, et al. Androgen receptor repeat length polymorphism associated with male-to-female transsexualism. **Biol Psychiatry 2009;65:93–96.**
25. Henningsson S, Westberg L, Nilsson S, et al. Sex steroid-related genes and male-to-female transsexualism. **Psychoneuroendocrinology 2005;30:657–664.**
26. Mills KL, Goddings A-L, Herting MM, et al. Structural brain development between childhood and adulthood: convergence across four longitudinal samples. **Neuroimage 2016;141:273–281.**
27. Burke SM, Manzouri AH, Dhejne C, et al. Testosterone effects on the brain in transgender men. **Cere Cortex (New York, NY: 1991) 2018;28:1582–1596.**
28. Feusner JD, Lidström A, Moody TD, et al. Intrinsic network connectivity and own body perception in gender dysphoria. **Brain imaging Behav 2017;11:964–976.**
29. Luders E, Sanchez FJ, Tosun D, et al. Increased cortical thickness in male-to-female transsexualism. **J Behav Brain Sci 2012;2:357–362.**
30. Walhovd KB, Fjell AM, Giedd J, et al. Through thick and thin: a need to reconcile contradictory results on trajectories in human cortical development. **Cereb Cortex 2017;27:1472–1481.**
31. Uribe C, Junque C, Gómez-Gil E, et al. Brain network interactions in transgender individuals with gender incongruence. **Neuroimage 2020;211:116613.**
32. Kranz GS, Hahn A, Kaufmann U, et al. White matter microstructure in transsexuals and controls investigated by diffusion tensor imaging. **J Neurosci 2014;34:15466–15475.**
33. Junger J, Habel U, Brohr S, et al. More than just two sexes: The neural correlates of voice gender perception in gender dysphoria. **PLoS One 2014;9:e111672.**
34. Luo N, Sui J, Abrol A, et al. Age-related structural and functional variations in 5,967 individuals across the adult lifespan. **Hum Brain Mapp 2020;41:1725–1737.**
35. Richards C, Bouman WP, Seal L, et al. Non-binary or genderqueer genders. **Int Rev Psychiatry 2016;28:95–102.**
36. Kreukels BP, Haraldsen IR, De Cuypere G, et al. A European network for the investigation of gender incongruence: The ENIGI initiative. **Eur Psychiatry 2012;27:445–450.**

SUPPLEMENTARY MATERIALS

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