nature portfolio

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Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒ A description of all covariates tested
☐	☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒ For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐ Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Olink NPX software (https://olink.com/software/npx-software) was used to generate the Olink protein expression data values. FLEXMAP 3D Luminex instrument system (Luminex, Austin, TX, USA) was used to generate cytokine median fluorescence intensity values.
Data analysis	Data analysis was performed using R version 4.1.2. Statistical tests were performed using the nlme: Linear and Nonlinear Mixed Effects Models tool (https://www.rdocumentation.org/packages/nlme/versions/3.1-166/topics/nlme). Gene Ontology (GO) and Kyoto Encyclopaedia of Genes and Genomes (KEGG) enrichment analyses were performed using Homer (https://homer.ucsd.edu/homer/motif/). Additional R packages were used to transform data and generate figures, including dplyr (https://dplyr.tidyverse.org/) and ggplot2 (https://ggplot2.tidyverse.org/). The TissueEnrich online tool was used to map protein expression to tissues in the Human Protein Atlas data (https://tissueenrich.gdcb.iastate.edu/) Codes for generating figures in the manuscript are available on GitHub: https://github.com/BNovLab/Feminizing-Gender-Affirming-Hormone-Therapy-Proteomics

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

Original data generated and analyzed during this study for each participant is available on figShare: https://figshare.com/s/9b750f56ffe4914c5988

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	Sex and gender were carefully reported. The participants in this study are transgender individuals (identifying as transgender women and/or non-binary) receiving feminizing gender-affirming hormone therapy. When we compare our findings to protein associations in the UK Biobank we use the term sex to refer to sex-specific proteins, which were identified by comparing female to male groups. In the discussion we use the term transgender individuals on GAHT when we refer to our cohort, but female and male when we discuss sex-specific results in the literature.
Reporting on race, ethnicity, or other socially relevant groupings	Race and ethnicity are not reported. Our cohort primarily identifies as white Australian, which is representative of the transgender population seeking GAHT in Australia.
Population characteristics	For participants that Olink data was generated for, age at baseline ranged from 18 to 36 years, with a mean of 25.7 years at the baseline blood collection. Demographic information, including age and body mass index (BMI), is presented in Table 1 of the manuscript. For the 49 participants for which cytokine data was generated, the age range was 18 to 58 years, with a mean of 25.9 years at baseline blood collection, and BMI range was 17.7 to 41.5 with a mean of 26.8 at baseline.
Recruitment	Participants were recruited from a tertiary referral hospital in Melbourne, Australia between August 31, 2020, and March 15, 2022. Inclusion criteria included transgender people (transgender women and non-binary people assigned male at birth) aged 16 to 80 years who were about to commence treatment with feminizing GAHT. Participants were provided with a Participant Information Sheet/Consent Form and gave written informed consent for their blood samples to be used in future studies, including blood analysis and genetic analysis.
Ethics oversight	Austin Health Human Research Ethics Committee

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	The number of included subjects was maximized but not pre-specified by a priori power analysis.
Data exclusions	No samples were excluded from analysis.
Replication	Cross platform replication of several top proteins identified by Olink was performed using the Luminex platform, showing a strong correlation.
Randomization	All participants received feminizing GAHT. For data generation samples were randomized on 96-well plates for both Olink and Luminex, to minimise technical error.
Blinding	Analysis was not blinded, because we controlled for individual in the longitudinal analysis. Individuals that generated the data did not know which group the samples belonged to.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☐	☒ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	Universal Trial Number: U1111-1248-7232, ANZCTR registration ACTRN12620000339954
Study protocol	The manuscript reporting the clinical trial findings can be found at doi: 10.1210/clinem/dgae650. The Study protocol is not publicly available
Data collection	Clinical data was collected at visit to the Gender Clinic using a RedCap questionnaire.
Outcomes	For this study, only age and BMI information was used in the models.

Plants

Seed stocks	N/A
Novel plant genotypes	N/A
Authentication	N/A