Gene, the Sexual Brain, and Cognition: Machine-Predicted Brain Sex Score Explains Individual Differences in Cognitive Development and Genetic Influence.

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Abstract

Sex impacts the development of the brain and cognition differently across individuals. We investigated the biological underpinnings of the individual variability of sexual dimorphism in the brain and its impact on cognitive development. In prepubertal children (N=9,658, ages 9~10 years old; the Adolescent Brain Cognitive Development study), we tested whether the individual difference in brain sex development was related to that in cognitive development, known to be influenced by genetic factors. We estimated an individual's brain sex score from machine learning models trained on brain morphometry and diffusion white matter connectomes that accurately classified the biological sex with a test ROC-AUC of 93.32%. A greater brain sex score correlated significantly with greater intelligence ($P_{\text{fdr}} < 0.001$, $\eta_p^2 = 0.034 \sim 0.050$; adjusted for covariates) and higher cognitive genome-wide polygenic scores (GPSs) ($P_{\text{fdr}} < 0.001$, $\eta_p^2 < 0.005$). Structural equation models revealed that the GPS-intelligence association was modulated by the brain sex score, such that a brain with a higher maleness score (or a lower femaleness score) mediated a positive GPS effect on intelligence (indirect effects = 0.006~0.009; $P = 0.002 \sim 0.022$; sex-stratified analysis). The novel gene-brain-cognition relationship reported in this study presents a biological pathway to the individual and sex differences in the brain and cognitive development in preadolescence.

1 Introduction

2 Sex affects the development of the brain, cognition, and behaviors. Sex differences in
3 mental ability^{1,2}, psychological³ cognitive well-being^{4,5}, mental and cognitive health outcomes⁶
4 along the lifetime trajectory may originate from sex differences in brain structure and function and
5 from those in developmental and aging courses. Several studies attempted to test the sex-
6 dependent structural and functional variations in the brains of males and females⁷⁻⁹. From the
7 moment of birth, female and male brains embark on different developmental trajectories^{10,11}.
8 Human neuroimaging reports sex differences in brain gross anatomy (e.g., total brain volume),
9 cortical thickness, the functional and the white matter structural connectomes¹⁰⁻¹⁵. On the other
10 hand, some view that the human brains present the mosaics of female and male characteristics
11 with a great extent of individual variability¹⁶.

12 In humans, the brain sex differences do not necessarily map onto cognitive differences.
13 This may be related to the fact that the prior research primarily focused on the group differences
14 between sex and thus failed to account for individual variations of the impact of sex on the brain
15 and cognition. Since numerous brain, cognitive, and behavioral traits have extensively
16 overlapping distributions between sex¹⁶⁻¹⁸, sex may be a continuous rather than binary factor¹⁸.
17 Indeed, a recent elegant study shows that the continuum of sex derived from brain or body
18 measures accounts for the individual variability of sex hormone levels and behaviors in
19 adolescents¹⁸. Therefore, determining how sex affects one's brain, cognition, and individuality is
20 an important research question towards precision neuroscience.

21 A crucial but understudied factor in the sex differences in brain and cognition is the genetic
22 underpinning. The genetic basis of the brain and cognitive development, and the relationship with
23 sex may provide new mechanistic insights into the impact of sex on the brain and cognitive
24 development. Literature shows the heritability of general intelligence and genetic differences

across individuals account for between approximately 20% to 70% of the variance in general intelligence¹⁹. Large-scale genome-wide association studies (GWAS) have discovered the variants significantly related to cognitive ability in European populations^{20–22}. Concerning the genetic variants of small effects associated with the heritability of intelligence, a genome-wide polygenic score (GPS) could provide an overall estimate of the genetic influence on a trait at the individual level²³ and expand its utility to multiethnic populations^{24,25}. Indeed, the GPS approach shows the inherited genome-wide genetic effect accounts for up to 13% of the variance in educational attainment and 7~10% in cognitive performance²⁶. An important outstanding question regarding neurocognitive development is whether the genetic influence on cognitive development is related to the impact of sex on the brain and cognition. Testing this relationship may provide an insight into the understanding of the vital biological factors of neurocognitive development.

In this study, we investigate the individual differences in brain sex development in a rigorous, unbiased, data-driven manner leveraging large-scale multimodal brain imaging of grey matter morphometric and white matter connectomes, together accounting for the key brain developmental processes in prepubertal children. We then test whether the individual differences of the brain sex development map onto that of cognitive development. Finally, we investigate the relationship between sexual brain development and cognitive development concerning the genetic influence on cognitive development. The integrative analysis of brain imaging, cognition, and the GPS in the large pediatric samples provides new and mechanistic insights.

Materials and Methods

ABCD Participants

We obtained the study data from the Adolescent Brain Cognitive Development (ABCD) study release 2.0 (<http://abcdstudy.org>). The ABCD study is the largest longitudinal study of brain development and child health across the United States⁴⁹. The ABCD study aims to investigate psychological and neurobiological development trajectories for adolescent mental health⁵⁰. 11,875 multiethnic children with ages of 9 to 10 years were recruited from 21 research sites composed of 52.3% Caucasians, 20.3% Mexican-Americans, 14.7% African-Americans, and 12.5% Asian-Americans and others based on self-reported ethnicities. Informed consent and assent forms were collected from all the participants and parents, and/or legal guardians. 9,658 participants were used for the analysis after quality control (See “Brain Imaging-Quality Assessment and Control”). Missing values of the income variable (403 missing values in males, 362 missing values in females) were replaced with median values as part of the quality control process. All experimental protocols were approved by the Seoul National University’s institutional review board (IRB), and all methods were carried out following relevant guidelines and regulations.

Cognitive Intelligence

We used total intelligence, fluid intelligence, crystallized intelligence composite scores measured by the NIH Toolbox⁵¹. They are based on the following tests: dimensional change card sort test, flanker inhibitory control, and attention test, oral reading recognition test, pattern speed test, picture vocabulary test, and list sorting working memory test. The test scores were z-scored and not normalized to age and sex; instead, these were used as covariates in the statistical

models. The NIH Toolbox assessment scores of each participant were available from the neurocognitive data of the ABCD release 2.0.

Genome-wide Polygenic Scores (GPSs)

We created genome-wide polygenic scores (GPSs) of three representative cognitive traits: educational attainment, cognitive performance, and intelligence quotient (IQ). The saliva DNA samples were collected for 10,659 participants and genotyped at Rutgers University Cell and DNA Repository (RUCDR) with Affymetrix NIDA Smoke Screen Array. After removing the SNPs with genotype call rate <95%, sample call rate <95%, and minor allele frequency (MAF) <1%, we performed imputation using the Michigan Imputation Server using the 1000 Genome phase3 version5 panel and Eagle v2.4 phasing. Among the imputed variants with INFO score >0.3, we additionally removed the inferior SNPs with genotype call rate <95%, sample call rate <95%, rare variants with MAF<1%, and Hardy–Weinberg equilibrium (HWE) p-value<1x10⁻¹⁰ for an ethnically diverse population. We performed identity-by-descent analysis on the pruned data and excluded genetically related individuals closer than first cousin relatedness or grandparent-grandchild relatedness (ϕ hat>0.18) using PLINK software, which resulted in keeping only one subject from each biological family⁵². Then, principal component analysis (PCA) of the study samples with 1000 Genome phase3 reference samples was conducted, showing that all the study samples were clustered as admixed American population (superpopulation code AMR), falling inside of the mean pairwise Euclidean distance between admixed American samples (**Supplementary Figure 1**). Next, GPSs of cognitive phenotypes were computed as the sum of effect allele count (0,1,2) weighted by the effect based on the publicly available summary statistics from the large-scale GWASs on educational attainment, cognitive performance, and intelligence quotient (IQ)^{22,26}. The score estimation with linkage-disequilibrium clumping was performed using r^2 >0.2 thresholds over 500kb sliding windows over the reference panel (p-value<1) using PRSice2

software^{51,53}. Considering the multiethnicity of the study participants, the GPSs were adjusted for the first ten ancestrally-informative principal components of the genotype data of the participants and further corrected with self-reported ethnicity covariate. To evaluate the generalizability of our results to multiethnic populations, we performed sensitivity analyses with European-ancestry participants (44.04% of total ancestry) by selecting individuals with European ancestry using the *PLINKQC* R package^{54,55}.

Sex Hormones

We used saliva salimetrics mean scores (pg/mL) of testosterone and DHEA (Dehydroepiandrosterone) available from the curated ABCD dataset. Since estradiol was not collected in males, we excluded the estradiol in the present study. The saliva collection was implemented following Granger et al. (2012)⁵⁶. Participants did not eat or any drink before the saliva collection. There are variations in collection time and the cold chain for the sample protection depending on the ABCD study site⁵⁷.

Brain Imaging-Anatomical Imaging - T1/T2, Freesurfer 6

T1-weighted (T1w) and T2-weighted (T2w) 3D structural MRI were acquired in the ABCD study. The image was processed using the following protocol^{50,58}: To improve geometric accuracy and image intensity reproducibility, the gradient nonlinearity distortion correction method was performed in structural MRI⁵⁹. T2w images registered to T1w images using mutual information⁶⁰. Based on tissue segmentation and sparse spatial smoothing, intensity non-uniformity was corrected. Then, the data were resampled with 1 mm isotropic voxels into rigid alignment with an atlas brain.

Cortical surface reconstruction was applied using the following procedures: Structural MRI was processed using FreeSurfer v6.0 (<https://surfer.nmr.mgh.harvard.edu>) for cortical surface reconstruction⁶¹, which includes skull-stripping⁶², white matter segmentation and initial mesh creation⁶¹, correction of topological defects, surface optimization^{63,64}, and nonlinear registration to a spherical surface-based atlas⁶⁵.

Brain Imaging-Diffusion Spectrum Imaging

We used the diffusion spectrum images from the ABCD study preprocessed using the following protocol⁶⁶ by the ABCD Data Analysis and Informatics Center (DAIC). Eddy current distortion correction was used with a nonlinear estimation using diffusion gradient orientations and amplitudes to predict the pattern of distortions⁶⁷. Head motion was corrected by registering to images synthesized from tensor fit⁶⁸. Diffusion gradients were adjusted for head rotation^{68,69}. We then fitted the diffusion tensor model⁷⁰. B0 distortion was corrected with reversing gradient method⁷¹. Gradient nonlinearity distortion correction was applied⁵⁹. T2-weighted b0 images to T1w structural images using mutual information⁶⁰. Then, the data were resampled to a standard orientation with 1.77 mm isotropic resolution.

To estimate accurate brain imaging phenotypes, we used individual connectome data. First, we applied MRtrix3⁷² for whole-brain white matter tracts estimation and individualized connectome generation. For connectivity metrics, we used streamline counts associated with fiber connection strength^{73,74} associated with fiber integrity. We decreased the noise⁷⁵. Then, we performed bias correction with the Advanced Normalization Tools (ANTs) pipeline' N4 algorithm⁷⁶. To obtain a connectivity index with a white matter pathway⁷⁷, we performed probabilistic tractography by 2nd order integration over fiber orientation distributions⁷⁸ with random seeding across the brain and target streamline counts of 20 million. These initial tractograms were filtered

out preliminary tractograms with spherical-deconvolution informed filtering (2:1 ratio). With a final streamline count of 10 million, we generated an 84 x 84 whole-brain connectome matrix for each participant using the T1-based parcellation and segmentation from FreeSurfer. This pipeline thus ensures that individual participants' connectomes are restricted to their neuroanatomy. Computation was carried out on the supercomputers at Argonne Leadership Computing Facility Theta and Texas Advanced Computing Center Stampede2.

Brain Imaging-Quality Assessment and Control

Quality Assessment of the ABCD brain imaging data was performed by the ABCD Data Analysis and Informatics Center (DAIC). The ABCD MRI quality assessment consisted of protocol compliance checking, automated quality control metrics, and manual review of data quality⁶⁶. In the first part, the protocol compliance checking included the match between key imaging parameters and expected values for a given scanner, such as voxel size or repetition time. The protocol compliance checking was performed by on-site FIONA workstations for feedback to scan operators. The presence or absence of the B0 distortion field map series was also checked for diffusion MRI (dMRI). In the second part, automated quality metrics were used. For structural MRI (sMRI), the mean and standard deviation of brain values and spatial SNR was used. For diffusion MRI (dMRI), mean motion and the number of slices and frames affected by slice dropout due to head motion. Finally, with the combination of automated and manual methods, the DAIC reviewed data quality, such as incorrect acquisition parameters, imaging artifacts, or corrupted data files.

For FreeSurfer cortical surface reconstruction and diffusion MRI preprocessing, we used the quality control results from the ABCD DAIC⁶⁶. The DAIC reviewed FreeSurfer cortical surface construction and DTI reconstruction and generated a binary QC score. A sample is deemed unusable when the data has the most severe artifacts or irregularities, results still included in

shared tabulated data, and recommended exclusion from group analyses involving cortical, subcortical, and tract-based ROIs. Reviewers gauged the severity of five types of artifact for FreeSurfer QC: motion, intensity inhomogeneity, white matter underestimation, pial overestimation, and magnetic susceptibility artifact; and, for DTI, B0 wrapping, image quality based on motion, intensity inhomogeneity, and magnetic susceptibility artifact, full head coverage, registration with T1w image, and registration with FreeSurfer segmentation.

Sex Classification with Machine Learning

We conducted machine learning modeling to classify biological sex, using the brain morphometric and white matter structural connectomic estimates as the input. The features related to the whole brain volume were excluded because of the apparent volume difference between the male and female brains: e.g., total intracranial volume, total gray matter volume. Finally, we used 988 features for structural MRI and 3,486 features for white matter connectomic. We used an ensemble machine learning pipeline consisting of data loading, feature preprocessing, model and feature tuning for the optimal hyperparameters of light gradient boost machine⁷⁹, general linear model, and xgboost⁸⁰. We applied feature evolution to the best model in validation sets to determine the best algorithm and parameters. In this stage, we used feature engineering and trained the models on the large feature engineering space to find the best representation of the data⁸¹. Finally, we built a stacked ensemble model with the three best models in discovery sets. To test the reproducibility and generalizability, we evaluated the final model's performance in the replication set (20% split of the ABCD). To compare model performance between machine learning and deep learning, we performed deep neural network (DNN), TabNet, to predict the biological sex of the participants. TabNet is a high-performance and

interpretable deep learning architecture with a sequential attention mechanism for feature selection that enables feature interpretability and efficient learning⁸².

Machine Learning Interpretation

To make inferences, we applied a machine learning interpretation framework, 'K-Local Interpretable Model-agnostic Explanation (K-LIME)', a variant of LIME⁸³. The K-LIME technique allows both global (i.e., group-level) and local (i.e., subject-level) explanations of given machine learning models. K-LIME generates a single surrogate linear model on the entire training samples for global explanations and multiple local surrogate linear models on samples created from K-means clusters in the discovery data set for local explanations. The features of K-means were selected from the variable importance in a random forest surrogate model. To estimate the predictions in a final ensemble model, linear surrogate models were trained. The number of clusters for local explanations was selected based on a grid search where the r^2 of predictions was maximized between the final ensemble and all the local K-LIME models. K-LIME is available in R or Python through an open-source machine learning package, H2O (<https://www.rdocumentation.org/packages/h2o/versions/3.10.5.2/topics/h2o.klime>).

Cross-Validation

We split the ABCD data into a discovery (80%) and replication set (20%) stratified by biological sex. Distributions of the covariates (age, race/ethnicity, sites, parental education, marital status, income, height, weight, and BMI) were consistent between the split. We used leave-one-site-out-cross-validation (21 folds) to test the generalizability of model performance across different data collection sites. In addition, we performed random 5-fold cross-validation.

209

210 ***General Linear Model***

211 We tested the relationship between brain-based sex scores from the ML model and NIH
212 toolbox scales and the cognitive GPSs using a general linear model (GLM). The following
213 covariates were used: age, race/ethnicity, sites, parental education, marital status, income,
214 height, weight, BMI, additionally family ID as a random variable. All the models were fit to the
215 males and females separately.

216 ***Structural Equation Modeling***

217 To test the putative causal relationship among the cognitive GPSs, brain, and intelligence,
218 we performed the structural equation modeling (SEM) using the lavaan R package⁸⁴. We defined
219 three latent variables: cognitive GPSs, brain-based sex, total intelligence from different data
220 sources described above. The GPSs of three representative cognitive traits (educational
221 attainment, cognitive performance, and intelligence quotient) were used as observed variables
222 for cognitive GPSs. Fluid intelligence composite score (flanker inhibitory control and attention,
223 dimensional change card sort, pattern comparison processing speed, picture sequence memory,
224 list sorting working memory) and crystallized intelligence composite score (picture vocabulary,
225 oral reading recognition) from NIH Toolbox assessment data were observed for total intelligence.
226 Parameters were estimated using a bias-corrected bootstrap method with a 95% confidence
227 interval. We included the following covariates in the models: age, race/ethnicity, sites, parental
228 education, marital status, income, height, weight, and BMI (categorical variables were dummy
229 coded).

Results

Demographic Characteristics

The total brain volume showed a significant sexual difference among the demographic variables, with males having larger volumes ($P < 0.001$). However, all the others showed trivial sexual differences (Cohen's $D_s < 0.06$; $P_s < 0.037$) in the study subjects (**Figure 1, Supplementary Table 1**).

Brain Sex Classification

We classified sex using machine learning and deep neural networks with morphometric data (including surface area, mean curvature, thickness, and volume) and white matter structure connectome. Across the cross-validation methods, combining morphometric data and white matter structure connectome consistently outperformed the models with morphometric data and white matter structure connectome, respectively (**Figure 2.A & 2.B, Supplementary Table 2**). In addition, 5-fold cross-validation showed slightly better performance than leave-one-site-out-cross-validation. The brain-based sex score from the optimized ML model showed considerable individual differences in both males and females (**Figure 2.C**).

Machine learning interpretation showed specific brain features contributing to the classification of each sex (**Figure 2.D, Supplementary Table 3**). In predicting maleness, the following brain features contributed: right white matter surface area, left lateral occipital area, and the right cerebellar cortex volume, left precuneus thickness, and left cerebellar cortex volume; in predicting femaleness: the right white surface area, cerebrospinal fluid, left posterior thickness, left white surface area, and white matter connection between the right thalamus and right middle temporal gyrus.

Individual Differences in Brain-Based Sex Score Correlate with Cognitive Intelligence

We then tested whether the machine-predicted brain-based sex score is linked to cognitive development in each sex. As a result, a greater brain-based maleness score positively correlated with greater cognitive intelligence; reversely, a greater femaleness score negatively correlated with greater cognitive intelligence (**Figure 3**). Crystallized intelligence showed the most significant positive association with the brain-based maleness score (Male, $\beta=2.542$, $P_{\text{fdr}}<0.001$, $\eta_p^2=0.050$, Female, $\beta=2.125$, $P_{\text{fdr}}<0.001$, $\eta_p^2=0.034$), followed by total intelligence (Male, $\beta=2.128$, $P_{\text{fdr}}<0.001$, $\eta_p^2=0.005$, Female, $\beta=1.912$, $P_{\text{fdr}}<0.001$, $\eta_p^2=0.004$). Fluid intelligence showed the smallest, non-significant association with brain-based maleness score (Male, $\beta=1.074$, $P_{\text{fdr}}=0.094$, $\eta_p^2=0.001$, Female, $\beta=1.094$, $P_{\text{fdr}}=0.076$, $\eta_p^2=0.001$) (**Figure 3, Supplementary Table 4**).

Individual Differences in Brain-Based Sex Score Correlate with Cognitive GPSs but non-significant associations with sex hormones

What is the biological underpinning of the association between brain-based sex score and intelligence? We tested whether the brain sex-intelligence association is related to sex hormones and a well-established genetic influence on cognitive ability²⁶. As a result, sex hormones showed no significant associations with the score of brain sex (maleness) or cognitive abilities in prepubertal children (**Supplementary Table 5**).

On the other hand, the cognitive GPSs significantly correlated with brain-based sex (maleness) score and cognitive abilities. Of the three cognitive GPSs, the cognitive performance

GPS significantly correlated with the brain-based sex (maleness) score when adjusted for effects of biological sex ($\beta=0.252$, $P_{\text{fdr}}<0.001$, $\eta_p^2=0.004$). In the subsequent, sex-stratified analysis, we found the effect size of this correlation was greater in females than in males (Female - Educational Attainment: $\beta=0.287$, $P_{\text{fdr}}<0.001$, $\eta_p^2=0.001$; Cognitive Performance: $\beta=0.306$, $P_{\text{fdr}}<0.001$, $\eta_p^2=0.005$) (Male - Educational Attainment: $\beta=0.131$, $P_{\text{fdr}}=0.082$, $\eta_p^2=0.001$; Cognitive Performance: $\beta=0.179$, $P_{\text{fdr}}=0.077$, $\eta_p^2=0.002$, Intelligence Quotient: $\beta=0.148$, $P_{\text{fdr}}=0.083$, $\eta_p^2=0.001$, **Figure 4, Supplementary Table 6**). In European only, educational attainment and cognitive performance is significantly related in females ($\beta=0.424$, $P_{\text{fdr}}=0.001$, $\eta_p^2=0.014$; Cognitive Performance: $\beta=0.432$, $P_{\text{fdr}}=0.002$, $\eta_p^2=0.011$, **Supplementary Table 7**)

Brain-Based Sex Score Modulates the Relationship between Cognitive GPSs and Cognitive Intelligence

To test the potential causal relationship among the cognitive GPSs, brain, and intelligence, we performed SEM. In the admixed American participants, sex-stratified SEM showed acceptable model fits (Comparative Fit Index (CFI) - Male: 0.921, Female: 0.940, Root Mean Square Error of Approximation (RMSEA) - Male: 0.022, Female: 0.019) (**Figure 5**). As in the GLMs, the total effect of the cognitive GPSs on intelligence was confirmed significant and positive in both sex (Male - total effect= 0.253 , $P<0.001$, bias-corrected percentile method in bootstrap samples; Female - total effect= 0.326 , $P<0.001$). Of note, the brain maleness score partially modulated the cognitive GPS's effect on the total Intelligence in both sexes (Male - indirect effect= 0.006 , $P=0.022$; Female - indirect effect= 0.009 , $P=0.002$). Note that this means that while the cognitive GPS has a *positive* direct effect on intelligence in females, a greater brain maleness score (i.e., a lower brain femaleness score) strengthens it. However, when modeling in only European-ancestry samples, we found no significant indirect modulatory effects of brain sex score (P 's >0.204)

300 **(Supplementary Figure 5).** These results may suggest the modulatory effect of brain sex
301 development on the genetic pathway to cognitive development.

Discussion

We report the novel relationship between brain sex development, cognitive development, and shared genetic influence in an admixed American population of prepubertal children. As trained on the grey matter morphometric and white matter connectomes, our machine learning showed the accurate classification of sex with over 93.32% ROC-AUC in a replication set. Furthermore, the individual variability of the sexual brain development, represented by the brain-based sex score, showed significant correlations with general intelligence and the inherited genetic influence to general intelligence, the cognitive GPSs. Moreover, the SEM showed that the effect of the cognitive GPSs on cognitive development outcomes was modulated by the brain sex score significantly in females and with a trend in males. Thus, this study indicates the critical role of brain sex development in cognitive development in prepubertal children, influenced by genetic factors, providing a biological account for the individual variability of neurocognitive development.

Our study departs from the prior literature on sex differences in intelligence in children by showing the role of the continuum of brain sex development on cognitive development. Literature shows mixed findings of sex differences in intelligence²⁷. Some studies show that males have advantages^{28–30} in general intelligence over females, while others show females have advantages over males³¹. These studies allude to the great individual variability in intelligence within sex. A recent seminal study shows the biological underpinnings of the individual variability in behavioral phenotypes in adolescents¹⁸. This study presents an estimate of the continuum of sex based on the brain and body traits, which predicts within each sex the individual variability in sex hormones, personality traits, and internalizing-externalizing behaviors. In line with this, our study further demonstrates the utility of multimodal brain imaging combined with machine learning in estimating an individual status of brain sex development. Indeed, our method permitted the accurate estimation of an individual's developmental status of the brain sex and revealed that the brain sex development estimates varied across individuals even within the narrow age range. The discovery

of the correlation of the brain sex development variability with the genetic and cognitive variables further reflects that this novel estimate represents a critical neurobiological process rather than a trivial modeling error.

Our structural equation models show the potential causal relationships among the genes, brain sex, and cognition. The results indicate that a higher brain maleness score (a lower femaleness score) positively modulates the positive effect of the cognitive GPS on general intelligence significantly in both sexes. Considering that the modulatory effect remains significant after controlling for several potential confounding factors of the brain and cognitive development, this GPS-brain sex-intelligence pathway is likely causal. These results thus suggest the novel role of brain sex development in children linking the genetic influence to cognitive development.

Then, what is the biological account of the modulatory effects of the brain sex on the genetic influence on cognitive development? That is, positive towards maleness and negative towards femaleness? Literature shows that sex chromosomes play a crucial role in cognitive development^{32–34}. However, since we did not include the sex chromosomes when constructing the GPSs (following the common practice of the GWAS designs to boost statistical power), the mediation effects difference across the sexes might not be directly related to the effect of the sex chromosome. Instead, we speculate that the expression patterns of autosomal variants different across the sexes^{35–37} may account for the modulatory effects of sex. Also, in line with this speculation, recent literature highlights sex differences in brain transcriptomes related to schizophrenia and alcohol effects^{38,39}. It remains to be tested whether the modulatory effects last in the later development. Of note, regardless of the modulatory effects of sex, in both females and males, the influence of the cognitive GPSs on cognition was positive, similar to the literature in adults^{22,26}. Therefore, our integrative analysis reveals the subtle relationships among sex, genes, brains, and cognition, otherwise undetectable. We suggest this is a novel biological pathway to

individual differences in brain sex development. It may be interesting to test whether this pathway is related to epigenetic effects of environmental factors, such as early life stress.

This study confirms that biological sex can be classified accurately based on morphometric and white matter connectivity. Adeli et al. (2020) show that the biological sex was classified with 89.6% accuracy in the replication set using a deep neural network trained on ABCD T1-weighted structural MRI⁴⁰. Our study extends this prior work by showing the additive performance increase with the diffusion white matter connectomes. The performance increase in our study perhaps presents that the multimodal MRI effectively accounts for the heterogeneous developmental trajectories of grey and white matter⁴¹. It further shows the importance of the multimodal MRI approach in brain development research.

Our brain features do not include the total volumes of the brain, grey or white matter, of which the sex differences have been reported⁸. Though the whole brain volume difference between sexes may be a biological aspect, in this study, we reasoned that the measures of gross anatomy might confound the brain-cognition relationship. Therefore, beyond the sex difference in the gross anatomy, this study shows that the patterns of the grey matter and white matter fibers are associated with the continuum of brain sex.

In our study, we found no significant relationship among our key variables with salivary measures of sex hormones. Given the prepubertal stages of the participants, the negative statistical findings may reflect that the gene-brain sex-cognition relationship is not significantly related to the effects of sex hormones. Literature shows a complex relationship between the level of sex hormones and cognitive development^{42,43}. Though different sex hormonal levels across the sexes are observed from the prepubertal ages⁴⁴, the actual effect of the sex hormones on cognitive development (or its modulation) may not appear until puberty⁴⁵.

This study shows a novel relationship among genetic factors, brain sex development, and cognitive development. The link between genome-wide factors and cognitive ability has been shown in previous studies. Cognitive GPSs account for general cognitive ability up to 3.5% in pre-adolescence children⁴⁶, 11% of the variance in general intelligence, and 16% of the variance in educational achievement in adolescents⁴⁷. Extending this literature, our study shows that an individual's degree of brain sex development may modulate the impact of the genetic factor on cognitive development. Since this modulatory effect is positive towards brain maleness and negative towards brain femaleness, it adds another source of sex and individual variability in intelligence. This inference also presents the benefit of using the brain data as an endophenotype in assessing the genotype-phenotype association⁴⁸. Taken together, brain sex development is linked to the inherited genetic influence of cognition, accounting for a novel pathway to the individual difference in cognitive development in preadolescence.

In contrast to the multiethnic participants, the SEM in the European participants only showed non-significant indirect effects of brain sex score. The discrepant results may not be easily reconciled. It should be noted that the cross-ethnic transferability of our cognitive GPS based on the European-ancestry GWAS remains to be validated. However, our cognitive GPS was rigorously adjusted for the potential ethnic confounding. Our result of the significant modulatory effects in the admixed American participants needs to be interpreted with caution.

This study shows the novel relationships among brain sex, cognition, and cognitive GPSs. The brain sex score based on grey matter morphometric and white matter connectivity may represent a neurodevelopmental process in preadolescence related to the inherited genetic influence on cognitive development and unrelated to sex hormonal levels. This study thus provides a novel framework for future research in neurocognitive development and mental illnesses such as Autism Spectrum Disorders.

Author contributions

Conceived and designed the experiments: K.K., J.C.

Performed the experiments: K.K., G.A.

Analyzed the data: K.K.

Interpreted the data: K.K., Y.J., J.C.

Contributed materials/analysis tools: K.K., Y.J., H.W., S.M.

Wrote the paper: K.K., Y.J., J.C.

Critical revision of the manuscript for important intellectual content: K.K., Y.J., H.K., W.-Y.A., J.C.

Obtained funding: J.C.

Study supervision: J.C.

Data and Code Availability

Codes are available for reproducibility (<https://github.com/Transconnectome/cGPS-BrainSex-Intelligence>)

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Figures

A. Data Processing Procedures

B. Sex Differences in the Demographic Characteristics of the Study Participants

Figure 1. The descriptions of ABCD data A, Data processing procedure B, Sex differences in the Demographic Characteristics of the study participants.

A. Classification Performance of Machine Learning
(cross validation with leave one site out)

B. Classification Performance of Deep Tabular Network
(cross validation with leave one site out)

C. Histograms of Brain-Based Sex Score across Sex

D. Important Features Contributing to Sex Classification

Figure 2. Brain-Based Sex Score Shows Individual Sex Difference in Brain A, Classification Performance of Machine Learning (leave one site out cross-validation). B, Classification Performance of Deep Tabular Network. C, Histograms of Brain-Based Sex Score across Sex. The brain-based sex score was from the optimized ML model trained on morphometric and structural connectome data. D, Important Features Contributing to Sex Classification.

Figure 3. Brain-Based Sex Score Correlates with Cognitive Intelligence. Associations between Brain-Based Sex Score and Cognitive Intelligence. Effects adjusted for covariates. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$

Figure 4. Cognitive GPSs Explains Genetic Underpinnings of the Relations between Brain and Cognitive GPS. Effects adjusted for covariates. *** $P<0.001$, ** $P<0.01$, * $P<0.05$

A. In Males

B. In Females

Figure 5. Structural Equation Modeling of Tripartite Relationships: Cognitive GPSs-Brain Sex Score-Intelligence A, Structural Equation Modeling of tripartite relationships in males B, Structural Equation Modeling of tripartite relationships in females.

Supplementary Materials

Gene, the Sexual Brain, and Cognition: Machine-Predicted Brain Sex Explains Individual-Specific Cognitive Development and Its Genetic Influence in Prepuberty Children.

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Supplementary Table 1 - 7

Supplementary Figure 1 - 5

Supplementary Table 1. Demographic Characteristics

Demographic Data (n=9,658)	Mean (SD)		t / χ^2 (P)	Cohen's d / ϕ
	Male (n=4,980)	Female (n=4,678)		
Age (months)	119.36 (7.50)	118.91 (7.47)	2.943 **	0.06
Height (inches)	55.25 (3.11)	55.35 (3.26)	-1.59	-0.03
Weight (lbs)	81.49 (22.29)	82.91 (23.79)	-3.03 **	-0.06
Volume (cm ³)	1579.61 (140.45)	1450.47 (127.02)	47.44 ***	0.96
BMI (inches/lbs ²)	18.607 (4.01)	18.82 (4.20)	-2.57 *	-0.05
Maternal Education (highest grade)	16.71 (2.67)	16.69 (2.74)	0.52	0.01
Income	7.33 (2.36)	7.32 (2.36)	0.36	0.01
Ethnicity	White: 2,756, Black: 642, Hispanic: 992, Asian: 93, Other: 497	White: 2,469, Black: 681, Hispanic: 951, Asian: 103, Other: 474	9.34 *	0.03

Site	1: 152, 2: 275, 3: 283, 4: 292, 5: 159, 6: 254, 7: 156, 8: 117, 9: 176, 10: 275, 11: 194, 12: 257, 13: 239, 14: 294, 15: 174, 16: 530, 17: 245, 18: 137, 19: 195, 20: 307, 21: 269	1: 149, 2: 235, 3: 265, 4: 288, 5: 169, 6: 262, 7: 139, 8: 105, 9: 188, 10: 267, 11: 193, 12: 258, 13: 246, 14: 250, 15: 155, 16: 421, 17: 230, 18: 117, 19: 216, 20: 306, 21: 219	22.44	0.05
Married	Married: 3,488, Widowed: 38, Divorced: 460, Separated: 174, Never Married: 539, Living with partner: 281	Married: 3,210, Widowed: 40, Divorced: 401, Separated: 184, Never Married: 584, Living with partner: 259	9.18 *	0.03

*** P<0.001, ** P<0.01, * P<0.05

Supplementary Table 2. Classification Performance of Machine Learning and Deep Tabular Neural Networks

	Automated Machine Learning (cross-validation with leave one site out)		Deep Tabular Neural Networks (cross-validation with leave one site out)		Automated Machine Learning (5-cross validation)	
	Discovery ROC-AUC	Replication ROC-AUC	Discovery ROC-AUC	Replication ROC-AUC	Discovery ROC-AUC	Replication ROC-AUC
Morphometric only	91.02	90.10	94.86	93.19	90.88	90.79
Structural Connectome Only	85.05	85.01	86.30	85.33	86.15	85.46
Morphometric and Structural Connectome Both	92.59	90.99	93.91	93.32	92.64	91.23

Supplementary Table 3. Important Features Contributing Sex Classification in Morphometric and Structural Connectome Data

Rank	Features contributing to brain-femaleness		Features contributing to brain-maleness	
	Variable	Weight	Variable	Weight
1	R white surface area	0.052	R white surface area	0.087
2	Cerebrospinal Fluid (CSF)	0.031	L lateral occipital area	0.034
3	L postcentral thickness	0.031	R cerebellum cortex volume	0.029
4	L white surface area	0.030	L precuneus thickness	0.027
5	R thalamus - R middle temporal gyrus	0.028	L cerebellum cortex volume	0.026
6	R superior circular sulcus of insula mean curvature	0.027	L subcentral gyrus and sulcus area	0.026
7	R parahippocampal thickness	0.027	Fourth ventricle	0.025
8	L pericallosal sulcus mean curvature	0.024	L putamen volume	0.024
9	L superior temporal gyrus	0.023	L rostral middle frontal thickness	0.023
10	L WM transverse temporal	0.023	L amygdala volume	0.022

Supplementary Table 4. General Linear Model between Brain-Based Sex Score and Cognitive Intelligence Effects adjusted for covariates. *** P<0.001, ** P<0.01, * P<0.05

NIH toolbox Cognition Battery	Brain-Based Sex Score In Both Group				Brain-Based Sex Score In Male Group				Brain-Based Sex Score In Female Group			
	β	SE	P _{tdr}	η_p^2	β	SE	P _{tdr}	η_p^2	β	SE	P _{tdr}	η_p^2
Total Intelligence	1.999	0.348	0.000	0.004	2.128	0.502	0.000	0.005	1.912	0.484	0.000	0.004
Fluid Intelligence	1.076	0.444	0.015	0.001	1.074	0.641	0.094	0.001	1.094	0.616	0.076	0.001
Crystallized Intelligence	2.254	0.270	0.000	0.009	2.542	0.386	0.000	0.050	2.125	0.383	0.000	0.034

Supplementary Table 5. General Linear Model between Sex Hormones and Brain-Based Sex Score or Cognitive Intelligence

General Linear Model between Sex Hormones and Brain-Based Sex Score												
Brain-Based Sex Score	Brain-Based Sex Score In Both Group				Brain-Based Sex Score In Male Group				Brain-Based Sex Score In Female Group			
	β	SE	P _{tdr}	η_p^2	β	SE	P _{tdr}	η_p^2	β	SE	P _{tdr}	η_p^2
Testosterone	-0.579	1.313	0.659	0.000	-0.449	1.842	0.808	0.000	-0.677	1.912	0.724	0.000
DHEA	-4.994	3.822	0.191	0.001	-5.911	4.833	0.222	0.002	-4.862	5.977	0.417	0.002
General Linear Model between Testosterone and Cognitive Intelligence												
NIH toolbox Cognition Battery	Brain-Based Sex Score In Both Group				Brain-Based Sex Score In Male Group				Brain-Based Sex Score In Female Group			
	β	SE	P _{tdr}	η_p^2	β	SE	P _{tdr}	η_p^2	β	SE	P _{tdr}	η_p^2
Total Intelligence	-0.004	0.010	0.916	0.000	-0.001	0.014	0.999	0.000	-0.010	0.014	0.669	0.001

Fluid Intelligence	-0.001	0.012	0.916	0.000	-0.003	0.017	0.999	0.001	-0.005	0.017	0.768	0.001
Crystallized Intelligence	-0.004	0.008	0.916	0.000	0.000	0.011	0.999	0.000	-0.011	0.011	0.669	0.000
General Linear Model between DHEA and Cognitive Intelligence												
NIH toolbox Cognition Battery	Brain-Based Sex Score In Both Group				Brain-Based Sex Score In Male Group				Brain-Based Sex Score In Female Group			
	β	SE	P_{fdr}	η_p^2	β	SE	P_{fdr}	η_p^2	β	SE	P_{fdr}	η_p^2
Total Intelligence	0.004	0.003	0.296	0.001	0.007	0.005	0.245	0.001	0.001	0.004	0.954	0.001
Fluid Intelligence	0.004	0.004	0.296	0.001	0.006	0.007	0.373	0.000	0.002	0.005	0.954	0.001
Crystallized Intelligence	0.003	0.003	0.296	0.001	0.006	0.004	0.245	0.003	-0.000	0.003	0.954	0.000

Supplementary Table 6. General Linear Model between Brain-Based Sex Score and Cognitive GPS

Cognitive GPS	Brain-Based Sex Score In Both Group				Brain-Based Sex Score In Male Group				Brain-Based Sex Score In Female Group			
	β	SE	P _{fd}	η_p^2	β	SE	P _{fd}	η_p^2	β	SE	P _{fd}	η_p^2
Educational Attainment	0.213	0.054	0.000	0.003	0.131	0.075	0.082	0.001	0.287	0.077	0.000	0.001
Cognitive Performance	0.252	0.058	0.000	0.004	0.179	0.080	0.077	0.002	0.306	0.083	0.000	0.005
Intelligence Quotient	0.028	0.058	0.634	0.000	0.148	0.083	0.082	0.001	-0.863	0.082	0.292	0.000

Supplementary Table 7. General Linear Model between Brain-Based Sex Score and Cognitive GPS in European only

Cognitive GPS	Brain-Based Sex Score In Both Group				Brain-Based Sex Score In Male Group				Brain-Based Sex Score In Female Group			
	β	SE	P _{fd}	η_p^2	β	SE	P _{fd}	η_p^2	β	SE	P _{fd}	η_p^2
Educational Attainment	0.313	0.080	0.000	0.007	0.200	0.114	0.120	0.006	0.424	0.112	0.001	0.014
Cognitive Performance	0.349	0.094	0.000	0.005	0.269	0.133	0.120	0.007	0.432	0.133	0.002	0.011
Intelligence Quotient	-0.009	0.089	0.921	0.001	0.149	0.132	0.260	0.001	-0.143	0.122	0.243	0.001

Supplementary Figure 1. PCA biplots of the genotype data of the study samples with 1000 Genomes phase3 reference samples. All the study samples were clustered as an admixed American population (superpopulation code AMR), falling inside of the mean pairwise Euclidean distance between admixed American samples. The color blue indicates ABCD subjects. We marked European-ancestry subjects selected for the sensitivity analysis as circles. (AMR: Admixed American, AFR: African, EAS: East Asian, EUR: European; 1000 Genomes super population codes)

A. Cognitive Outcomes across Sex

NIH Toolbox Cognition Battery

B. Cognitive Outcomes across Brain-Based Sex

NIH Toolbox Cognition Battery

Supplementary Figure 2. Cognitive Intelligence across Sex and Brain-Based Sex Group A, Cognitive Intelligence across Sex B, Cognitive Intelligence across Brain-Based Sex (brain-based maleness score ≥ 0.5 , higher maleness; < 0.5 , lower maleness) Group difference of cognitive intelligence between two groups were larger across brain-based sex group than biological sex group (Cohen's $d < 0.21$)

A. Characteristics of Hormone across Biological Sex Group

B. Characteristics of Hormone across Brain-Based Sex Group

Supplementary Figure 3. Brain-Based Sex Score Do Not Correlates with Sex Hormones A, Characteristics of Hormone across Biological Sex Group B, Characteristics of Hormone across Brain-Based Sex Group

A. Cognitive GPS Characteristics across Biological Sex Group

B. Cognitive GPS Characteristics across Brain-Based Sex Group

Supplementary Figure 4. Cognitive GPS Characteristics across Sex and Brain-Based Sex Group A, Cognitive GPS Characteristics across Sex B, Cognitive GPS Characteristics across Brain-Based Sex

A. In Males

B. In Females

Supplementary Figure 5. Structural Equation Modeling of Tripartite Relationships: Cognitive GPSs-Brain Sex Score-Cognitive Intelligence in European only A, Structural Equation Modeling of tripartite relationship in males B, Structural Equation Modeling of tripartite relationship in females.