Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Prenatal exposure to maternal steroid hormones and child neurodevelopment: evidence for sex-specific effects in a longitudinal birth cohort

Estelle Renard-Dausset
estellerenarddausset@gmail.com

Barcelona Institute for Global Health
Emily P. Laveriano-Santos
Barcelona Institute for Global Health
Mariona Bustamante
Barcelona Institute for Global Health
Muriel Ferrer
Barcelona Institute for Global Health
Mireia Gascon
Barcelona Institute for Global Health
Mònica Guxens
Barcelona Institute for Global Health
Noemí Haro
Hospital del Mar Medical Research Institute
Jordi Julvez
Barcelona Institute for Global Health
Aurélie Portefaix
Hospices Civils de Lyon
Martine Vrijheid
Barcelona Institute for Global Health
Óscar J Pozo
Hospital del Mar Medical Research Institute
Léa Maitre
Barcelona Institute for Global Health

Research Article

Keywords: Maternal steroid metabolism, steroid hormones, pregnancy, child neurodevelopment, neurodevelopmental disorders, biomarkers.

Posted Date: April 23rd, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9198207/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Abstract

Background

Neurodevelopmental disorders affect millions of children worldwide, yet their prenatal biological origins remain unclear. Steroid hormones regulate key processes in fetal brain development, but the role of maternal steroid metabolism during pregnancy on children's neurodevelopment remains poorly understood.

Methods

We report the first comprehensive longitudinal analysis of maternal steroid metabolism in late pregnancy in relation to offspring neurodevelopment. We quantified 50 maternal urinary steroid metabolites and derived 40 indicators of steroid metabolism in third-trimester samples from two Spanish birth cohorts (INMA-Sabadell, n = 500; BiSC, n = 556). In INMA-Sabadell, child cognition, motor development, attention and behaviour were assessed repeatedly from 15 months to 15 years, and associations were estimated using linear mixed-effects models, including sex-stratified analyses. As a secondary analysis, we evaluated cross-cohort consistency by testing whether associations with early-life cognitive and motor outcomes identified in INMA-Sabadell were also observed in BiSC at 18 months using linear regression models.

Results

In INMA-Sabadell, higher maternal levels of the cortisol metabolite 20α-dihydrocortisol-glucuronide were associated with poorer early cognitive abilities (estimate β = -2.05, 95% confidence interval (CI): [-3.08, -1.02]), whereas indicators of enhanced cortisol inactivation were associated with better attention, as reflected by lower variability in reaction time (β = -12.99, 95% CI: [-20.22, -5.76]). The association with 20α-dihydrocortisol-glucuronide was consistently observed in the BiSC cohort at 18 months (β = -0.29, 95% CI: -0.51, -0.06). Sex-stratified analyses revealed marked sexual dimorphism. Notably, indicators of greater androgen bioavailability were associated with increased externalizing behaviour in females (β = 0.23, 95% CI: [0.11, 0.35]) but better fluid intelligence in males (β = 4.76, 95% CI: [2.02, 7.52]).

Conclusions

These findings establish maternal steroid metabolism as a determinant of child neurodevelopment, highlight the relevance of including metabolic transformation indicators, and identify both shared and sex-specific in utero biomarkers of neurodevelopmental trajectories.

BACKGROUND

Neurodevelopmental disorders are a major public health concern, affecting millions of children worldwide [1, 2] and placing a heavy burden on families and societies. Increasing evidence suggests that these conditions, such as autism spectrum disorder and behavioural disorders, may originate from early metabolic disturbances, with pregnancy representing a particularly sensitive window. During this period of profound maternal hormonal and metabolic changes, even subtle deviations in maternal physiology may have lasting consequences for the child. Yet, the specific early biological processes that contribute to neurodevelopmental vulnerability remain poorly understood, limiting opportunities for prevention.

Steroid hormones have emerged as central regulators of fetal brain development and potential in utero biomarkers of early behavioural problems [3, 4]. Neuroactive steroids are synthesized either in the brain, or peripherally and reach the brain through circulation. Some of these metabolites are highly produced by the placenta and the fetus during pregnancy, influencing critical processes of fetal brain development, and giving rise to the notion of placental-brain axis [5, 6]. For instance, allopregnanolone modulates GABA-A (gamma-aminobutyric acid-A) receptors, enhancing central nervous system inhibition and providing neuroprotection in late gestation [5]. Disruption of this pathway, through impaired synthesis or preterm birth, has been associated with lasting brain injury and behavioural deficits [7, 8], supporting the idea that neurosteroids act as endogenous neuroprotectants for the developing brain. Human case-control studies further implicate prenatal steroidogenic activity in shaping neurodevelopment. Elevated prenatal estrogens in amniotic fluid have been associated with increased likelihood of autism [3], while distinct steroidogenic signatures in maternal serum have been identified in pregnancies of children later diagnosed with autism [9]. Other studies highlight how steroid hormones contribute to sexual differentiation of the brain and influence later behavioural traits such as play preferences and social cognition [4, 10, 11].

Despite these advances, prior research has been limited by small sample sizes, lack of longitudinal assessment of child neurodevelopment, and a focus on individual hormones rather than the broader metabolic pathways that regulate steroid activity. This narrow focus is problematic because steroidogenesis involves tightly interconnected biosynthetic pathways, and studying steroid hormones in isolation risks overlooking the coordinated metabolic patterns and regulation dynamics that may influence fetal brain development. In particular, prenatal phase II conjugation (glucuronidation or sulfation), essential for steroid clearance and bioavailability, remains unexplored in relation to child neurodevelopment.

We hypothesized that maternal steroid hormones and their metabolism in late pregnancy influence offspring neurodevelopment, from infancy through adolescence.

To test this, we characterized maternal steroid metabolism in urine, from samples collected in the third trimester of pregnancy in two population-based birth cohorts, using targeted liquid chromatography-tandem mass spectrometry (LC-MS/MS). This approach allows to simultaneously quantify a wide range of steroid metabolites, including their conjugated forms, at very low concentrations [12–14]. Using longitudinal neurodevelopmental assessments from these cohorts, we then evaluated associations

between maternal steroid profiles and children's cognitive, motor and behavioural outcomes from infancy into adolescence.

Here, we present the first comprehensive longitudinal study of 90 maternal steroid metabolites and derived indicators of steroid metabolism in the third trimester of pregnancy, in relation to offspring neurodevelopmental outcomes in 1,056 mother-child pairs from two population-based birth cohorts. By integrating high-resolution steroid metabolomics with longitudinal neurodevelopmental assessments, our study moves beyond prior research focused on single hormones or small cross-sectional designs, offering a unique opportunity to identify early-life biomarkers and biological pathways contributing to neurodevelopmental vulnerability.

METHODS

Study design and populations

We analyzed data from two prospective population-based birth cohorts in Spain to investigate associations between maternal steroid hormone concentrations in the third trimester of pregnancy and neurodevelopmental outcomes in the offspring (Fig. 1).

The main discovery analysis was conducted in the INMA-Sabadell cohort, a regional cohort part of the INfancia y Medio Ambiente (INMA) project. Between July 2004 and July 2006, 657 pregnant women aged ≥ 16 years were recruited in their first-trimester ultrasound visit in the primary health centre of Sabadell in Catalunya, Spain. Detailed recruitment and data collection protocols have been described elsewhere [15]. Spot urine samples were collected during morning interviews during the third trimester routine visit (mean gestational age = 34.3 weeks, SD (Standard Deviation) 1.6) and stored at − 20°C until analysis.

As a secondary cross-cohort consistency analysis, we used data from the Barcelona Life Study Cohort (BiSC), an independent birth cohort that recruited 1,080 pregnant women during the first routine prenatal visit (11–15 weeks of gestation) at three hospitals in Barcelona in Catalunya, Spain, between October 2018 and April 2021. Recruitment, data collection, and follow-up procedures have been described previously [16]. Pregnant women collected morning and bedtime urine samples from days 2–7 following the baseline visit (mean gestational age 34.7 weeks, SD 1.5), storing them at home at approximately − 20°C. On day 8, fieldworkers transported samples to ISGlobal-Campus Clínic (-20°C). For analysis, pooled 0.5 mL aliquots representing at least ten voids per participant were prepared, following the sample collection and analytical protocol previously reported [17].

In both cohorts, all participating women provided informed consent, and ethics approval were obtained from the relevant committees at each participating institution. For INMA-Sabadell, the study was approved by the Ethical Committee of the Municipal Institute of Medical Investigation and by the Ethical Committee of the Hospital of Sabadell. For BiSC, approvals were granted by the Clinical Research Ethics Committee of Parc de Salut Mar (2018/8050/I), the Medical Research Committee of the Fundació de

Gestió Sanitària del Hospital de la Santa Creu i Sant Pau de Barcelona (EC/18/206/5272), and the Ethics Committee of the Fundació Sant Joan de Déu (PIC-27-18).

For the present study, we included mother-child pairs with available maternal urinary steroid hormone measurements at the third trimester of pregnancy and at least one available neurodevelopmental outcome: 500 from the INMA-Sabadell cohort and 556 from the BiSC cohort (Supplementary Table 1).

Maternal steroid hormone measurements and derived indicators

Urinary steroid hormone metabolites were quantified using validated targeted liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods [18]. Chromatographic separation was performed using an Acquity UPLC system with a CSH C18 column. Mass spectrometric analysis was conducted on a triple quadrupole (XEVO TQ-S) micro mass spectrometer using negative ionization mode with selective reaction monitoring for quantification. Full chromatographic and mass spectrometric parameters are described in a previous study [18]. Quality control included calibration curves and pooled quality control samples in each of four analytical batches. Limits of detection (LODs) ranged from approximately 1-200 ng/mL, with most androgens and estrogens < 10 ng/mL and higher LODs for certain progestogens and precursors. Values below the LOD were imputed using a left-censored approach after log2-transformation [19].

We examined 50 individual metabolites of corticosteroids, androgens, estrogens and progestogens, and derived three classes of metabolic indicators (detailed in Supplementary Tables 2 and 3). These indicators were selected to capture functional aspects of steroidogenesis and hormone metabolism beyond absolute metabolite concentrations:

(1) Hormone family or subfamily sums (p = 13) representing total output within specific biosynthetic pathways.

(2) Sulfate/Glucuronide (S/G) ratios (p = 9), reflecting the balance between conjugation pathways (sulfation and glucuronidation). Since sulfated metabolites can be enzymatically reactivated, these ratios provide insight into potential hormonal bioavailability [20], with a higher S/G ratio potentially indicating a metabolic profile favoring hormonal reactivation.

(3) Steroidogenic enzymatic activity markers (p = 18), calculated as product-to-precursor molar concentration ratios along specific steroid biosynthesis pathways, providing *in vivo* proxies for the activity of key phase I enzymes [21, 22].

Urinary hormone concentrations (μmol/L) were adjusted for dilution by regressing out specific gravity (SG), measured using a digital refractometer (ATAGO Co., Ltd); removing SG dependence separately for each hormone rather than applying a common denominator, while preserving biological variation. Concentrations and metabolic indicators were further adjusted for gestational age using the multiples of the median approach, based on quantile regression, to express each value relative to the gestational-age-specific median [23].

3. Neurodevelopmental outcome assessments in children

Table 1: Description of neurodevelopmental outcomes and sample sizes in the INMA-Sabadell and BiSC cohorts

Neurodevelopmental Outcomes	Assessement Tools	Age and Sample Size	Total Observations
INMA Sabadell (N = 500)			
Early cognitive and psychomotor development • Early cognitive skills • Early motor skills	• Bayley Scales of Infant and Toddler Developement (BSID-I) at 15 months • McCarthy Scales of Children's Abilities (MSCA) at 4 years	• 15 months (N = 500) • 4 years (N = 414)	T = 914
Fluid intelligence	• Raven's Coloured Progressive Matrices (CPM)	• 9 years (N = 378) • 15 years (N = 277)	T = 655
Attention • Hit reaction time standard error	• Conners Kiddie Continuous Performance Test (K-CPT) at 4-5 years • Attention Network Test (ANT) at 7 and 9 years • Flankers task at 11 years	• 4-5 years (N = 258) • 7 years (N = 412) • 9 years (N = 382) • 11 years (N = 378)	T = 1430
Behavioural problems • Internalizing scale • Externalizing scale • Total scale	• Child Behavior Checklist (CBCL)	• 9 years (N =383) • 15 years (N = 286)	T = 669
ADHD symptoms	• Conners Rating Scale	• 7 years (N = 413) • 9 years (N = 379) • 11 years (N = 378)	T = 1170
BiSC (N = 556)			
Early cognitive and psychomotor development • Cognitive score • Expressive communication • Receptive communication • Fine motor skills • Gross motor skills	• Bayley Scales of Infant and Toddler Developement, 3rd edition (BSID-III) at 18 months	• 18 months (N = 552-556)	T = 552-556

INMA-Sabadell cohort

Early cognitive and psychomotor development was evaluated with the Bayley Scales of Infant Development, first edition (BSID-I) at 15 months and the McCarthy Scales of Children's Abilities (MCSA) at 4–5 years of life. BSID yields two indices, the Mental Scale and the Psychomotor Scale. MCSA provides a global score for general cognition and scores for five subscales (verbal, perceptive-

performance, memory, quantitative and motor) [24]. For both tests, all raw scores were standardized for child's age in days at the time of test administration. The resulting standardized residuals were then scaled to have a mean of 100 and a standard deviation of 15. Early cognitive skills were derived by compiling the BSID-I Mental Scale and the MSCA general cognition score, and early motor skills by compiling the BSID-I Psychomotor Scale with the MSCA motor score.

Child's behaviour was evaluated using questionnaires filled by the parents, including the Child Behaviour Checklist (CBCL) at 9 and 15 years, and the Conner rating scales for ADHD (Attention Deficit Hyperactivity Disorder) symptoms at 7, 9 and 11 years [24, 25]. The 99-item CBCL/6–18 version for school children was used to obtain standardized parent reports of children's problem behaviours. The parents responded along a 3-point scale with the code of 0 if the item is not true of the child, 1 for sometimes true, and 2 for often true. The internalizing score includes the subscales of emotionally reactive and anxious/depressed symptoms, as well as somatic complaints and symptoms of being withdrawn. The externalizing score includes attention problems and aggressive behaviours. In addition, an ADHD index based on the short form of the Conners' rating scales of 27 items provided information on inattention and hyperactivity symptoms. The internal consistency (Cronbach's alpha) of each of the study scales was > 0.80. All outcomes were analyzed as raw count scores, which were square root-transformed and subsequently z-standardized.

Finally, trained fieldwork technicians measured two cognitive domains in children using a battery of computer-based tests: fluid intelligence at 9 and 15 years (Raven Coloured Progressive Matrices Test [CPM]) and attention function at 4, 7, 9 and 11 years (Attention Network Test [ANT]). The CPM comprised a total of 36 items and we used the percentiles of the total number of correct responses as the outcome. A higher CPM scoring indicates better fluid intelligence. Fluid intelligence is the ability to solve novel reasoning problems and depends only minimally on prior learning. For ANT, we used the outcome of hit reaction time standard error (HRT-SE), a measure of response speed consistency throughout the test. A high HRT-SE indicates highly variable reaction time during the attention task and is considered a measure of inattentiveness.

All examiners were previously trained following a standardized assessment protocol by the study expert psychologist. Complete outcome descriptions are provided in previous publications [24].

BiSC cohort

Early neurodevelopmental assessment was conducted at 18 months by a trained neuropsychologist using the Bayley Scales of Infant Development, third edition (BSID-III). The following direct subscale scores were extracted for analysis: cognition, expressive and receptive communication, and fine and gross motor skills. For comparability across domains, raw scores were standardized to a distribution with a mean of 10 and a standard deviation of 3.

All the time points and sample sizes for each neurodevelopmental outcome assessed in children of both cohorts are provided in Table 1.

4. Statistical analysis

INMA-Sabadell was selected as the primary cohort because of its longer follow-up period, repeated outcome measurements, and extensive neurodevelopmental characterization, allowing robust longitudinal analyses. The BiSC cohort served as a secondary independent cohort to conceptually replicate associations for early cognitive and psychomotor development, measured at 18 months in 556 children. Although assessed with different tools, the same developmental domains were captured, providing independent confirmation of findings while acknowledging differences in timing, and scoring metrics.

Discovery analysis in the INMA-Sabadell cohort.

First, in the INMA-Sabadell cohort, we used linear mixed-effects models to examine associations between each steroid hormone exposure and 8 neurodevelopmental outcomes measured repeatedly across childhood and up to adolescence. For a given neurodevelopmental outcome, all available measurements across timepoints were pooled and modeled jointly, with a random intercept for each child to account for within-subject correlation.

Exposures included 50 individual steroid metabolites and the three classes of derived metabolic indicators: (1) 13 hormone family and subfamily sums, (2) 9 S/G ratios, and (3) 18 steroidogenic enzymatic activity indicators; for a total of 720 models. All exposures were log2-transformed prior to analysis to improve normality.

All models were adjusted for potential confounders and precision variables: maternal age, maternal education, maternal origin (European or Latin American country of birth), pre-pregnancy body mass index (BMI), parity, active smoking and passive smoking during pregnancy, child sex, age of the child at outcome assessment, and nursery attendance. Missing covariate data were imputed using the *missForest* algorithm, a machine learning-based data imputation algorithm based on the random forest method [26]. A sensitivity analysis was conducted by additionally adjusting for breastfeeding duration (ranging from 0 weeks to more than 24 weeks). To explore potential sex-specific associations, all analyses were also stratified by child sex, and models with an exposure-by-sex interaction term were fitted to formally assess effect modification. Sex (male/female) was extracted from medical records; gender identity was not assessed. All stratified analyses refer to sex assigned at birth.

The number of observations varied across models depending on the specific neurodevelopmental outcome tested, ranging from 655 to 1,170. Exact numbers of participants and repeated outcome measurements used in each analysis are reported in Table 1.

To address multiple testing while accounting for correlation among exposures, we applied the Effective Number of Tests (ENT) correction [27, 28]. This approach accounts for the correlation structure among

exposures by estimating the effective number of independent tests, thereby providing a less conservative adjustment than Bonferroni. ENT values were estimated separately for each group of exposures (individual metabolites, hormone sums, S/G ratios and enzymatic activity ratios), and significance thresholds were calculated as: $P_{ENT} = 0.05/ (ENT\ exposures \times \ n\ outcomes)$, resulting in corrected thresholds of 0.0003 for hormone metabolites, 0.0012 for hormone sums, 0.0014 for S/G ratios and 0.0006 for enzymatic activity ratios.

Cross-cohort consistency analysis

As a secondary analysis, we evaluated whether associations between prenatal hormone levels and early-life cognitive and psychomotor outcomes that reached nominal statistical significance in INMA-Sabadell (p-value < 0.05) were also observed in the second, independent birth cohort, BiSC. Although outcomes were assessed using different tools (Bayley-I and MSCA in INMA-Sabadell vs. Bayley-III in BiSC), the core neurodevelopmental domains were comparable. Accordingly, we focused on identifying consistent patterns of association across cohorts, rather than attempting formal replication. We considered findings to be consistent across cohorts when effect directions matched within the same developmental domain (cognitive or motor) and both associations reached statistical significance (p-value < 0.05) in INMA-Sabadell and BiSC. Given differences in instruments, timing, and scoring metrics, effect estimates were not directly comparable in magnitude; therefore, we treated this as a conceptual (construct-level) replication. To enhance comparability, we prespecified the same exposure definitions and covariate set in both cohorts and mapped outcomes to comparable developmental domains (cognitive and motor).

In BiSC, we fitted linear regression models for each exposure-outcome pair, adjusting for maternal age, maternal education, maternal ethnicity, maternal BMI at 12 weeks of gestation, parity, active and passive smoking during pregnancy, child sex, age at Bayley test, and nursery attendance. Missing covariates were imputed using *missForest*. The sample size ranged from 552 to 556 mother-child pairs, depending on the domain assessed. The same ENT correction strategy was applied to account for multiple comparisons; however, in this case, the correlation structure between outcomes was additionally considered. The adjusted significance threshold was calculated as $P_{ENT} = 0.05/ (ENT\ exposures \times \ ENT\ outcomes)$, resulting in corrected thresholds of 0.0007 for hormone metabolites, 0.0026 for hormone sums, 0.0031 for S/G ratios and 0.0014 for enzymatic activity ratios. All analyses were conducted in R (v4.3.3) using the *lmerTest* and *stats* packages. All tests were two-tailed.

RESULTS

Mother and child characteristics

A total of 500 mother-child pairs from the INMA-Sabadell cohort and 556 from the BiSC cohort with urinary steroid hormone measurements and at least one available neurodevelopmental outcome were

included in the analyses (Fig. 1, Supplementary Table 1). INMA-Sabadell (recruited in 2004–2006) was used as the primary cohort due to longer follow-up period of the children, whereas BiSC (recruited in 2018–2021) served as the secondary one. Because these cohorts were established nearly two decades apart, some differences in participant characteristics likely reflect the distinct social and historical contexts in which recruitment took place.

Regarding maternal characteristics, the mean age at delivery was 30.0 years in INMA-Sabadell and 34.6 years in BiSC. Most participants in both cohorts were of European origin, although BiSC included a higher proportion of Latin American mothers (20% vs. 7.3%). Smoking during pregnancy was more common in the earlier cohort (14% in INMA-Sabadell vs. 7.1% in BiSC), while a university education was more prevalent among BiSC mothers (74% vs. 32%). For child characteristics, the sex distribution was similar across cohorts, with girls representing 48% in INMA-Sabadell and 53% in BiSC. Overall, despite some sociodemographic differences, the two cohorts were broadly comparable. The exact number of participants contributing to each analysis varies depending on the outcome assessed and is provided in Table 1.

Main effect of prenatal steroid hormones on neurodevelopment in the INMA-Sabadell cohort

Associations between 90 maternal steroid hormone metabolites and metabolic indicators and eight neurodevelopmental outcomes (behavioural, cognitive and motor) in the offspring from 15 months to 15 years were estimated using linear mixed-effects models. Models were adjusted for relevant maternal characteristics (age, education, country of origin, pre-pregnancy BMI, parity, and active and passive smoking during pregnancy) and child characteristics (sex, age at outcome assessment, and nursery attendance). Multiple testing was addressed using the Effective Number of Tests (ENT) method, which accounts for both the number of outcomes and the correlation among exposures.

As shown in Figs. 2 and 3, 42 out of 720 associations tested, reached nominal statistical significance (p-value < 0.05), with approximately half of these involving early-life cognitive and motor skills (assessed up to 4 years old). After applying the ENT correction for multiple testing, three associations remained statistically significant. Specifically, two cortisol-related variables, 20α-dihydrocortisol glucuronide (20αDHF-G) (estimate β = -2.05, 95% confidence interval (CI): -3.08, -1.02) and 20α-reductase activity (β = -2.16, 95% CI: -3.26, -1.05) were associated with lower early cognitive abilities. In addition, increased activity of the combination of 5β-reductase and 3α-hydroxysteroid dehydrogenase was associated with better attentional performance (repeatedly assessed from 4 to 11 years old), reflected by lower variability in reaction time (β = -12.99, 95% CI: -20.22, -5.76). Residual plots for ENT-significant associations (Fig. 4) illustrate the direction, strength, and linearity of these relationships after adjusting for confounders. Similar results were obtained when breastfeeding duration was added to the models.

Cross-cohort consistency of prenatal steroid hormones on early-life neurodevelopment in the BiSC cohort

Then, to assess the consistency of findings across cohorts, we examined whether exposures nominally associated with early-life cognitive or motor abilities in INMA-Sabadell showed similar patterns of associations in the BiSC cohort.

Of the 16 exposures nominally associated with early cognitive or motor skills in INMA-Sabadell, one replicated in BiSC with consistent direction and statistical significance: increased maternal cortisol metabolite 20αDHF-G was associated with lower expressive communication scores (β = -0.29, 95% CI: -0.51, -0.06) (Supplementary Table 4-G). This finding mirrors the association observed in INMA-Sabadell between elevated 20αDHF-G and poorer early cognitive abilities (Figs. 2 and 4), strengthening evidence for an inverse relationship between this cortisol metabolite and early cognitive functioning.

Sex-specific associations of prenatal steroid hormones on neurodevelopment

We next conducted sex-stratified analyses to explore potential sex-specific associations in the INMA-Sabadell cohort.

In female children, 93 associations reached nominal significance (p-value < 0.05), more than double the number observed in male children (only 37). The associated outcomes also differed substantially between sexes. In females, the vast majority of nominally significant associations (∼80%) involved behavioural outcomes assessed via CBCL and ADHD symptom scales, compared with only 15% in males (Fig. 3). Volcano plots (Fig. 5) further show that for behavioural outcomes in females, estimates were predominantly positive, indicating higher symptom scores and suggesting a consistent pattern towards increased behavioural problems. In contrast, associations in males tended to be closer to null or even negative. This divergence was particularly clear when examining mean estimates across behavioural domains. For example, mean estimates for the total problems scale were negative in males (-0.015) but positive in females (0.052), and a similar pattern was observed for internalizing (male mean = -0.031 vs. female mean = 0.050), externalizing (male mean = 0.011 vs. female mean = 0.038), and ADHD symptoms (male mean = -0.028 vs. female mean = 0.038). After ENT correction, one association remained significant in females: increased sulfate to glucuronide conjugation balance (S/G ratio) of androgens was associated with increased externalizing behaviour scores (β = 0.23, 95% CI: 0.11, 0.35).

In males, nominally significant associations were more evenly distributed across domains, with a larger proportion (46%) involving early cognitive and psychomotor development. Two associations survived ENT correction (Fig. 5). First, cortisol 20αDHF-G again showed an association with poorer early cognitive skills (β = -2.71, 95% CI: -4.09, -1.35), consistent with findings in the full INMA-Sabadell and BiSC cohorts. Second, increased S/G ratio of testosterone was associated with better fluid intelligence (β = 4.76, 95% CI: 2.02, 7.52).

Residual plots for ENT-significant associations (Fig. 6) depict exposure-residual relationships separately for males and females, with independent regression lines and correlation coefficients, highlighting sex-

dependent differences in association patterns.

To formally assess whether associations differed by sex, we tested exposure-by-sex interaction terms in the models. While stratified analyses showed significant associations in both males and females, interaction terms did not reach statistical significance after correction for multiple testing. Of the 720 interaction tests performed, 75 were nominally significant (p-value < 0.05), but none remained significant after ENT correction. Specifically, for the ENT-significant associations identified in the sex-stratified analyses, only the S/G ratio of androgens with externalizing behaviour showed a nominally significant interaction with sex (p-value = 0.023), while the interaction terms for cortisol 20αDHF-G with early cognitive skills (p-value = 0.217) and testosterone S/G ratio with fluid intelligence (p-value = 0.069) did not.

Full model outputs, including estimates, standard errors, test statistics, p-values, and 95% confidence intervals are provided in Supplementary Table 4.

Results from linear mixed-effects models are shown as signed −log10(p-value), where the sign reflects the direction of the association (positive associations to the right of zero, negative ones to the left). The black dashed line indicates the nominal significance threshold (p-value = 0.05), and the red dashed line the threshold corrected for multiple testing using the Effective Number of Tests (ENT) method. ADHD: Attention Deficit Hyperactivity Disorder; SE: Standard Error; DHEA: Dehydroepiandrosterone; SG ratio: Sulfate/Glucuronide ratio

(p-value < 0.05) in INMA-Sabadell

Pie charts display the percentage of nominally significant associations attributed to each neurodevelopmental outcome in the full population, and separately in sex-stratified analyses (females and males). ADHD: Attention Deficit Hyperactivity Disorder; SE: Standard Error.

For exposure-outcome pairs passing the ENT-corrected significance threshold, residual plots show the association between log2 transformed exposure levels and residuals from models adjusted for covariates only (maternal characteristics: age, education, country of origin, pre-pregnancy body mass index, parity, and active and passive smoking during pregnancy; child characteristics: sex, age at outcome assessment, and nursery attendance). A linear regression line is overlaid, with Pearson's correlation coefficient (R) and p-value shown in the subtitle. SE: Standard Error.

Volcano plots show the results of the sex-stratified linear mixed models for each outcome, with estimates plotted on the x-axis and -log10(p-values) on the y-axis. Red points represent associations in females, blue in males. Labeled exposures indicate associations that pass the ENT-adjusted significance threshold. ADHD: Attention Deficit Hyperactivity Disorder; SE: Standard Error; SG ratio: Sulfate/Glucuronide ratio.

For associations passing the ENT threshold in either males or females, residual plots display the relationship between log2-transformed exposure levels and residuals from models adjusted for covariates only (maternal characteristics: age, education, country of origin, pre-pregnancy body mass index, parity, and active and passive smoking during pregnancy; child characteristics: age at outcome assessment, and nursery attendance), computed separately by sex. Separate linear regression lines are shown for males and females, with corresponding Pearson's correlation coefficients (R) and p-values indicated for each group. F: Females; M: Males; SG ratio: Sulfate/Glucuronide ratio.

DISCUSSION

This prospective study integrated comprehensive prenatal steroid metabolomics with longitudinal assessment of neurodevelopment from infancy through adolescence. We found consistent links between maternal steroid metabolism during pregnancy and child neurodevelopment across two independent cohorts. Most notably, increased maternal levels of the cortisol metabolite 20αDHF-G during pregnancy were consistently associated with poorer early cognitive performance. Apparent sexual dimorphism emerged in sex-stratified analyses. In females, increased androgens sulfate-to-glucuronide ratio was associated with more externalizing behavioural problems. In contrast, in males, increased testosterone sulfation-to-glucuronide balance was linked to better fluid intelligence. These findings suggest that maternal steroid hormone metabolism influences offspring neurodevelopment through both shared and sex-specific biological mechanisms, potentially mediated by differences in placental steroidogenesis and fetal neuroendocrine sensitivity. The results also highlight the potential of maternal steroid metabolism during pregnancy as an early biomarker framework to identify children at risk for altered neurodevelopment and to inform preventive strategies.

Prenatal cortisol metabolism and cognition in the offspring

Cortisol during pregnancy plays a central role in fetal brain development, where it regulates neuronal differentiation, synaptogenesis, myelination, and overall maturation of the central nervous system [29, 30]. Evidence from human studies shows mixed effects of prenatal cortisol and indicates that its neurodevelopmental effects are dependent on the timing of exposure. Elevated maternal cortisol in late pregnancy has been associated with enhanced child brain maturation and cognitive functioning, whereas higher levels in early gestation have been linked to poorer language, inhibition and general cognitive performance [31, 32]. In contrast, other evidence suggests a distinct pattern, with increased maternal cortisol in the third trimester of pregnancy being associated with reduced cognitive skills in the offspring [33]. These findings underscore the timing sensitivity of neurodevelopmental processes to glucocorticoid exposure.

In this context, our study identified a consistent inverse association between the maternal cortisol metabolite 20αDHF-G and child cognition across two independent cohorts. In INMA-Sabadell, both increased 20αDHF-G and greater 20α-reductase activity were linked to poorer early-life cognitive abilities, while in BiSC, increased 20αDHF-G was associated with reduced expressive communication. This

concordance is biologically plausible since the 20α-reductase enzyme reduces cortisol into 20α-dihydrocortisol, which is subsequently conjugated into 20αDHF-G. Thus, higher 20α-reductase activity reflects increased production of 20αDHF-G. Although the biological activity of 20α-dihydrocortisol is not fully established, its stereoisomer 20β-dihydrocortisol has been shown to act as a weak glucocorticoid receptor agonist and a potent mineralocorticoid receptor agonist [34], raising the hypothesis that 20α-dihydrocortisol could also be biologically active and potentially detrimental for early life cognition.

We also found that increased maternal activity of the 5β-reductase and 3α-hydroxysteroid dehydrogenase (3α-HSD) complex was associated with better attentional performance in the offspring. This enzyme complex mediates the irreversible inactivation and clearance of cortisol and cortisone, reducing fetal exposure to active glucocorticoids [35, 36]. Although its role in pregnancy has been little studied, our finding is consistent with evidence that excess prenatal glucocorticoid exposure can impair neurodevelopment [32, 33], suggesting that effective maternal glucocorticoid clearance through the 5β-reductase and 3α-HSD complex may protect fetal brain development.

Together these findings underscore that prenatal cortisol metabolism - the specific pathways through which the hormone is processed and inactivated - rather than absolute cortisol levels alone, shapes neurodevelopmental outcomes. Maternal cortisol metabolism is influenced by multiple factors, including stress, genetic variation, body mass index and lifestyle or environmental exposures such as diet, and tobacco or alcohol use [17], suggesting that it could act as a key mediator of the maternal exposome's impact on child neurodevelopment.

Conjugation balance of androgens and sex-specific associations

The sex-stratified analyses revealed different patterns of associations between prenatal steroid metabolome and neurodevelopment, particularly in the balance between androgens sulfation and glucuronidation These S/G ratios are biologically meaningful and reflect steroid fate: sulfated hormones can act as a circulating reservoir that can be locally desulfated back to bioactive steroids by steroid sulfatase, while glucuronidation largely marks steroids for excretion [37]. Thus, a higher S/G ratio of androgens may indicate greater bioavailability of reactivable androgen precursors to the developing fetus during sensitive windows of brain development, and the associated neurodevelopmental implications appear to be sex-specific.

In females, we observed that increased S/G ratio of androgens was associated with more externalizing behavioural problems. In males, increased S/G ratio of testosterone was associated with better fluid intelligence. These sex-specific patterns align with previous studies indicating that prenatal androgen exposure affects males and females differently. Increased prenatal testosterone (inferred from 2D:4D ratios) has been associated with higher hyperactive-impulsive ADHD symptoms in girls but not boys [38]. Similarly, girls with congenital adrenal hyperplasia, who experience excess prenatal androgen exposure, exhibited higher aggression and activity levels [39]. Then, bioavailable testosterone from umbilical cord blood at birth has also been shown to predict better attention scores in boys but more withdrawn

behaviour in girls, though associations with total, internalizing, and externalizing behaviour scores were not observed [40]. Together, these findings support the notion that female neurodevelopment may be particularly sensitive to androgen exposure, consistent with our observation that increased androgens S/G ratios in girls were linked to more externalizing behaviours. In contrast, the positive association of testosterone S/G ratio with fluid intelligence in boys aligns with literature highlighting the importance of prenatal testosterone in male brain development [10, 41].

These divergent associations likely reflect not only sex-specific sensitivities to androgens but also the sexually dimorphic nature of placental steroid metabolism. Human studies indicate that enzyme expression and circulating steroid conjugates vary by fetal sex: for instance, pregnancies with female fetuses show lower maternal dehydroepiandrosterone-sulfate (DHEA-S) alongside higher placental expression of steroid sulfatase [42]. Such differences suggest that the balance between sulfation and desulfation, and therefore the potential reactivation of sulfated androgens, may be modulated by fetal sex, with indications of greater desulfation capacity in female pregnancies.

Overall, our findings suggest that the prenatal conjugation balance of androgens represents a biologically meaningful axis with sex-specific effects on neurodevelopment, influencing cognitive and behavioural outcomes in distinct ways. By regulating the availability of bioactive androgens during sensitive windows of brain development, this balance may play a critical role in shaping these neurodevelopmental trajectories.

Strengths, limitations and future directions

Strengths of this study include the use of two independent, well-characterized birth cohorts with relatively large sample sizes. Comprehensive prenatal hormone profiling using highly sensitive LC-MS/MS techniques allowed us to compute metabolic indicators and capture pathway-specific information beyond absolute hormone concentrations.

Repeated neurodevelopmental assessments in INMA-Sabadell until adolescence, rigorous adjustment for multiple confounders and cross-cohort consistency for key findings further strengthen the robustness of our findings. Moreover, while cortisol excretion follows a pronounced diurnal rhythm, peaking shortly after awakening and declining throughout the day, our findings were supported across cohorts despite differences in sampling design: INMA-Sabadell relied mainly on single morning spot urine samples, whereas BiSC collected repeated samples over a week, providing a more stable estimate of average cortisol metabolite excretion. The consistent validation of one cortisol metabolite across both cohorts reinforces confidence in the robustness of the association and supports its plausibility as a causal link.

However, some limitations should be noted. Early-life neurodevelopmental assessments differed between cohorts (different assessment tools of early cognitive and psychomotor development and different ages at testing), limiting direct comparability and formal replication. Analyses of sex-interactions were limited by the relatively small sample sizes and the reduced number of timepoints for

each outcome, which may have reduced statistical power to detect meaningful differences between males and females. Then, despite extensive covariate adjustment, unmeasured factors such as maternal stress, fat composition or nutrition could influence both hormone levels and child neurodevelopment. Finally, because hormone measurements were limited to the third trimester, earlier windows of vulnerability, such as those critical for brain sexual differentiation, may have been missed.

Future research should extend hormone profiling across different gestational windows and biological matrices, including maternal serum and placenta, to confirm the production and activity of 20α-dihydrocortisol and its impact on early-life cognition. As children in BiSC continue to grow, it will also be valuable to replicate and expand these analyses with more comprehensive neurodevelopmental assessments at later ages, providing further insight into the mechanisms linking maternal steroid metabolism to long-term neurodevelopmental outcomes.

CONCLUSIONS

Our findings show that prenatal maternal steroid metabolism associates with child cognitive and behavioural development in a sex-specific manner. By moving beyond single hormone concentrations and including indicators of metabolic pathways, we reveal mechanisms through which prenatal endocrine regulation may contribute to neurodevelopmental vulnerabilities, with potential relevance for early risk identification and preventive strategies. By linking maternal endocrine dynamics to long-term child neurodevelopment, this study bridges endocrinology, neuroscience, and developmental epidemiology, highlighting the importance of integrating hormonal pathways into life-course models of neurodevelopmental health.

Abbreviations

20αDHF-G
20α-dihydrocortisol glucuronide
2D
4D ratio:second-to-fourth digit ratio
3α-HSD
3α-hydroxysteroid dehydrogenase
ADHD
attention deficit hyperactivity disorder
ANT
attention network test
BiSC cohort
Barcelona Life Study Cohort
BSID-I
Bayley Scales of Infant Development, first edition
BSID-III

Bayley Scales of Infant Development, third edition
CBCL
Child Behaviour Checklist
CI
confidence interval
CPM
Raven Coloured Progressive Matrices Test
DHEA
dehydroepiandrosterone
DHEA-S
dehydroepiandrosterone sulfate
ENT
effective number of tests
GABA-A
gamma-aminobutyric acid A
HRT-SE
hit reaction time standard error
INMA cohort
INfancia y Medio Ambiente cohort
LC-MS/MS
liquid chromatography–tandem mass spectrometry
LOD
limit of detection
MCSA
McCarthy Scales of Children's Abilities
SD
standard deviation
SE
standard error
SG
specific gravity
S/G ratio
sulfate/glucuronide ratio

Declarations

Ethics approval and consent to participate

In both cohorts, all participating women provided informed consent, and ethics approval were obtained from the relevant committees at each participating institution. For INMA-Sabadell, the study was approved by the Ethical Committee of the Municipal Institute of Medical Investigation and by the Ethical

Committee of the Hospital of Sabadell. For BiSC, approvals were granted by the Clinical Research Ethics Committee of Parc de Salut Mar (2018/8050/I), the Medical Research Committee of the Fundació de Gestió Sanitària del Hospital de la Santa Creu i Sant Pau de Barcelona (EC/18/206/5272), and the Ethics Committee of the Fundació Sant Joan de Déu (PIC-27-18).

Consent for publication

Not applicable

Availability of data and materials

The participants data reported in this study cannot be deposited in a public repository due to participant confidentiality and privacy concerns. Therefore, data is available upon written request. According to standard controlled access procedure, applications to use the BiSC and INMA-Sabadell data will be reviewed by the Steering Committee, evaluation of the fit of the data for the proposed methodology, and verification that the proposed use meets the guidelines of the Ethic and Governance Framework and of the consent that was provided by the participants. To request BiSC data follow the instructions described in the website.

All original analytical code has been deposited in a GitHub repository and is publicly available at https://github.com/EstelleRD/prenatal-steroids-neurodevelopment

Competing interests

The authors declare that they have no competing interests

Funding

This work was supported by the FIS grant ISCIII-AES-2021/001814 (Instituto de Salud Carlos III), project code: FIS 2021 - IGRO PI21/01269.

The BiSC cohort has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (785994 – AirNB project), from the Health Effects Institute (4959-RFA17-1/18-1 – FRONTIER project), from the European Union's Horizon 2020 research and innovation programme-EU.3.1.2. (874583 - ATHLETE project) and H2020-EU.3.1.1. (GA964827 – AURORA project), from AXA Research Fund (MOOD-COVID project), from Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail (ANSES) (2019/01/039 - HyPAXE project), from the AGAUR-Agència de Gestió d'Ajuts Universitaris de Recerca (2021 SGR 01570 - Population Neuroscience group), from the Centro de Investigación Biomédica en Red de Epidemiología y Salud Pública (CIBERESP) (CB06/02/0041), from the Instituto de Salud Carlos III (ISCIII) and the European Regional Development Fund (ERDF) - Maternal and Child Health and Development Network (SAMID) (RD16/0022/0014 and RD16/0022/0015), and from the Instituto de Salud Carlos III (ISCIII) and the European Union Next Generation EU - Primary Care Interventions to Prevent Maternal and Child

Chronic Diseases of Perinatal and Developmental Origin Network (RICORS-SAMID) (RD21/0012/0001 and RD21/0012/0003).

INMA data collections were supported by grants from the Instituto de Salud Carlos III, CIBERESP, and the Generalitat de Catalunya-CIRIT.

Léa Maitre has received funding from the Ramon y Cajal Research Fellowship RYC2022-036475-I funded by MICIU/AEI/10.13039/501100011033. E.P.Laveriano-Santos is supported by the post-doctoral grant JDC2022-049842-I funded by MICIU/AEI/10.13039/501100011033 and by “European Union NextGeneration EU/PRTR”.

Authors' contributions

Conceptualization: ERD and LM; Data Curation: ERD and EPL-S; Formal Analysis: ERD; Funding Acquisition: MV and M.Guxens, LM Investigation: M.Gascon, JJ, MB, NH, OJP, ERD and LM; Methodology: ERD, LM and OJP; Project Administration: LM; Resources: LM; Software: ERD; Supervision: LM; Validation: ERD and LM; Visualization: ERD; Writing - Original draft Preparation: ERD; Writing - Review & Editing: All authors; All authors have read and approved the manuscript.

Acknowledgements

We acknowledge all participants and their families for their collaborations. We also acknowledge all BISC and INMA-Sabadell team members for their contributions to these cohorts.

Authors' information

The corresponding author is Estelle Renard-Dausset

ISGlobal, Barcelona, Spain

Estelle Renard-Dausset, Emily P. Laveriano-Santos, Mariona Bustamante, Muriel Ferrer, Mireia Gascon, Mònica Guxens, Jordi Julvez, Martine Vrijheid, Léa Maitre

Centro de Investigación Biomédica en Red de Epidemiología y Salud Pública (CIBERESP), Madrid, Spain

Estelle Renard-Dausset, Mariona Bustamante, Muriel Ferrer, Mireia Gascon, Mònica Guxens, Jordi Julvez, Martine Vrijheid, Léa Maitre

Centro de Investigación Biomédica en Red Fisiopatología de la Obesidad y la Nutrición (CIBEROBN), Institute of Health Carlos III, Madrid, Spain

Emily P. Laveriano-Santos

Universitat Pompeu Fabra, Barcelona, Spain

Mariona Bustamante, Muriel Ferrer, Mireia Gascon, Mònica Guxens, Martine Vrijheid, Léa Maitre

Applied Metabolomics Research Group, Hospital del Mar Research Institute, Barcelona, Spain

Noemí Haro, Óscar J Pozo

Clinical and Epidemiological Neuroscience (NeuroÈpia), Institut d'Investigació Sanitària Pere Virgili (IISPV), 43204, Reus, Spain

Jordi Julvez

Department of Child and Adolescent Psychiatry/Psychology, Erasmus MC, University Medical Centre, Rotterdam, The Netherlands

Mònica Guxens

ICREA, Barcelona, Spain

Mònica Guxens

Unitat de Suport a la Recerca de la Catalunya Central, Fundació Institut Universitari per a la Recerca a l'Atenció Primària de Salut Jordi Gol i Gurina (IDIAPJGol), Manresa, Spain

Mireia Gascon

Hospices Civils de Lyon Centre d'Investigation Clinique de Lyon, INSERM P1407, Hôpital

Louis Pradel, 28 avenue du Doyen Lépine, 69500 Bron, France

Aurélie Portefaix

Université Claude Bernard Lyon 1, Unité INSERM 1290 RESHAPE, France

Aurélie Portefaix

References

1. Gidziela A, Ahmadzadeh YI, Michelini G, Allegrini AG, Agnew-Blais J, Lau LY, et al. A meta-analysis of genetic effects associated with neurodevelopmental disorders and co-occurring conditions. Nat Hum Behav. 2023;7:642–56. https://doi.org/10.1038/s41562-023-01530-y.
2. Francés L, Quintero J, Fernández A, Ruiz A, Caules J, Fillon G, et al. Current state of knowledge on the prevalence of neurodevelopmental disorders in childhood according to the DSM-5: a systematic review in accordance with the PRISMA criteria. Child Adolesc Psychiatry Ment Health. 2022;16:27. https://doi.org/10.1186/s13034-022-00462-1.

3. Baron-Cohen S, Tsompanidis A, Auyeung B, Nørgaard-Pedersen B, Hougaard DM, Abdallah M, et al. Foetal oestrogens and autism. Mol Psychiatry. 2020;25:2970–8. https://doi.org/10.1038/s41380-019-0454-9.
4. Auyeung B, Lombardo MV, Baron-Cohen S. Prenatal and postnatal hormone effects on the human brain and cognition. Pflüg Arch - Eur J Physiol. 2013;465:557–71. https://doi.org/10.1007/s00424-013-1268-2.
5. Hirst JJ, Kelleher MA, Walker DW, Palliser HK. Neuroactive steroids in pregnancy: Key regulatory and protective roles in the foetal brain. J Steroid Biochem Mol Biol. 2014;139:144–53. https://doi.org/10.1016/j.jsbmb.2013.04.002.
6. Rosenfeld CS. The Placenta-Brain-Axis. J Neurosci Res. 2021;99:271–83. https://doi.org/10.1002/jnr.24603.
7. Hirst JJ, Cumberland AL, Shaw JC, Bennett GA, Kelleher MA, Walker DW, et al. Loss of neurosteroid-mediated protection following stress during fetal life. J Steroid Biochem Mol Biol. 2016;160:181–8. https://doi.org/10.1016/j.jsbmb.2015.09.012.
8. Shaw JC, Berry MJ, Dyson RM, Crombie GK, Hirst JJ, Palliser HK. Reduced Neurosteroid Exposure Following Preterm Birth and Its' Contribution to Neurological Impairment: A Novel Avenue for Preventative Therapies. Front Physiol. 2019;10:599. https://doi.org/10.3389/fphys.2019.00599.
9. Ritz B, Yan Q, Uppal K, Liew Z, Cui X, Ling C, et al. Untargeted Metabolomics Screen of Mid-pregnancy Maternal Serum and Autism in Offspring. Autism Res. 2020;13:1258–69. https://doi.org/10.1002/aur.2311.
10. Lombardo MV, Ashwin E, Auyeung B, Chakrabarti B, Taylor K, Hackett G, et al. Fetal Testosterone Influences Sexually Dimorphic Gray Matter in the Human Brain. J Neurosci. 2012;32:674–80. https://doi.org/10.1523/JNEUROSCI.4389-11.2012.
11. Hines M. Sex Steroids and Human Behavior: Prenatal Androgen Exposure and Sex-Typical Play Behavior in Children. Ann N Y Acad Sci. 2003;1007:272–82. https://doi.org/10.1196/annals.1286.026.
12. Marcos J, Pol M, Fabregat A, Ventura R, Renau N, Hanzu FA, et al. Urinary cysteinyl progestogens: Occurrence and origin. J Steroid Biochem Mol Biol. 2015;152:53–61. https://doi.org/10.1016/j.jsbmb.2015.04.015.
13. McLeod MD, Waller CC, Esquivel A, Balcells G, Ventura R, Segura J, et al. Constant Ion Loss Method for the Untargeted Detection of Bis-sulfate Metabolites. Anal Chem. 2017;89:1602–9. https://doi.org/10.1021/acs.analchem.6b03671.
14. Pozo OJ, Marcos J, Khymenets O, Pranata A, Fitzgerald CC, McLeod MD et al. SULFATION PATHWAYS: Alternate steroid sulfation pathways targeted by LC–MS/MS analysis of disulfates: application to prenatal diagnosis of steroid synthesis disorders. 2018. https://doi.org/10.1530/JME-17-0286
15. Guxens M, Ballester F, Espada M, Fernández MF, Grimalt JO, Ibarluzea J, et al. Cohort Profile: The INMA—INfancia y Medio Ambiente—(Environment and Childhood) Project. Int J Epidemiol.

2012;41:930–40. https://doi.org/10.1093/ije/dyr054.

16. Dadvand P, Gascon M, Bustamante M, Rivas I, Foraster M, Basagaña X, et al. Cohort Profile: Barcelona Life Study Cohort (BiSC). Int J Epidemiol. 2024;53:dyae063. https://doi.org/10.1093/ije/dyae063.
17. Laveriano-Santos EP, Renard-Dausset E, Bustamante M, Pelegri D, García-Ruiz Z, Ruiz-Rivera M, et al. Mapping of Determinants of Urinary Sex Steroid Metabolites During Late Pregnancy: Results from Two Spanish Cohorts. Int J Mol Sci. 2025;26:11598. https://doi.org/10.3390/ijms262311598.
18. Servin-Barthet C, Martínez-García M, Paternina-Die M, Marcos-Vidal L, Martín de Blas D, Soler A, et al. Pregnancy entails a U-shaped trajectory in human brain structure linked to hormones and maternal attachment. Nat Commun. 2025;16:730. https://doi.org/10.1038/s41467-025-55830-0.
19. Lubin JH, Colt JS, Camann D, Davis S, Cerhan JR, Severson RK, et al. Epidemiologic Evaluation of Measurement Data in the Presence of Detection Limits. Environ Health Perspect. 2004;112:1691–6. https://doi.org/10.1289/ehp.7199.
20. Pacifici GM. Sulfation of drugs and hormones in mid-gestation human fetus. Early Hum Dev. 2005;81:573–81. https://doi.org/10.1016/j.earlhumdev.2004.10.021.
21. Ondřejíková L, Pařízek A, Šimják P, Vejražková D, Velíková M, Anderlová K, et al. Altered Steroidome in Women with Gestational Diabetes Mellitus: Focus on Neuroactive and Immunomodulatory Steroids from the 24th Week of Pregnancy to Labor. Biomolecules. 2021;11:1746. https://doi.org/10.3390/biom11121746.
22. Magyar BP, Santi M, Sommer G, Nuoffer J-M, Leichtle A, Grössl M, et al. Short-Term Fasting Attenuates Overall Steroid Hormone Biosynthesis in Healthy Young Women. J Endocr Soc. 2022;6:bvac075. https://doi.org/10.1210/jendso/bvac075.
23. Lobmaier SM, Figueras F, Mercade I, Crovetto F, Peguero A, Parra-Saavedra M, et al. Levels of Maternal Serum Angiogenic Factors in Third-Trimester Normal Pregnancies: Reference Ranges, Influence of Maternal and Pregnancy Factors and Fetoplacental Doppler Indices. Fetal Diagn Ther. 2014;36:38–43. https://doi.org/10.1159/000360078.
24. Gascon M, Guxens M, Vrijheid M, Torrent M, Ibarluzea J, Fano E, et al. The INMA—INfancia y Medio Ambiente—(Environment and Childhood) project: More than 10 years contributing to environmental and neuropsychological research. Int J Hyg Environ Health. 2017;220:647–58. https://doi.org/10.1016/j.ijheh.2017.02.008.
25. Maitre L, Julvez J, López-Vicente M, Warembourg C, Tamayo-Uria I, Philippat C, et al. Early-life environmental exposure determinants of child behavior in Europe: A longitudinal, population-based study. Environ Int. 2021;153:106523. https://doi.org/10.1016/j.envint.2021.106523.
26. Stekhoven DJ, Bühlmann P. MissForest—non-parametric missing value imputation for mixed-type data. Bioinformatics. 2012;28:112–8. https://doi.org/10.1093/bioinformatics/btr597.
27. Li J, Ji L. Adjusting multiple testing in multilocus analyses using the eigenvalues of a correlation matrix. Heredity. 2005;95:221–7. https://doi.org/10.1038/sj.hdy.6800717.

28. Nyholt DR. A Simple Correction for Multiple Testing for Single-Nucleotide Polymorphisms in Linkage Disequilibrium with Each Other. Am J Hum Genet. 2004;74:765–9. https://doi.org/10.1086/383251.
29. Drake AJ, Tang JI, Nyirenda MJ. Mechanisms underlying the role of glucocorticoids in the early life programming of adult disease. Clin Sci. 2007;113:219–32. https://doi.org/10.1042/CS20070107.
30. Trejo JL, Cuchillo I, Machín C, Rúa C. Maternal adrenalectomy at the early onset of gestation impairs the postnatal development of the rat hippocampal formation: Effects on cell numbers and differentiation, connectivity and calbindin-D28k immunoreactivity. J Neurosci Res. 2000;62:644–67. https://doi.org/10.1002/1097-4547(20001201)62:5%3C644::AID-JNR4%3E3.0.CO;2-3.
31. Davis EP, Head K, Buss C, Sandman CA. Prenatal maternal cortisol concentrations predict neurodevelopment in middle childhood. Psychoneuroendocrinology. 2017;75:56–63. https://doi.org/10.1016/j.psyneuen.2016.10.005.
32. Womack SR, Murphy HR, Arnold MS, Duberstein ZT, Best M, Qiu X, et al. Timing sensitivity of prenatal cortisol exposure and neurocognitive development. Dev Psychopathol. 2025;37:1707–20. https://doi.org/10.1017/S0954579424001287.
33. LeWinn KZ, Stroud LR, Molnar BE, Ware JH, Koenen KC, Buka SL. Elevated maternal cortisol levels during pregnancy are associated with reduced childhood IQ. Int J Epidemiol. 2009;38:1700–10. https://doi.org/10.1093/ije/dyp200.
34. Morgan R, Beck K, Nixon M, Homer N, Melchers D, Houtman R, et al. 20[beta]-Dihydrocortisol; a weak endogenous agonist of the glucocorticoid receptor but a potent agonist of the mineralocorticoid receptor. Endocr Abstr. 2017. https://doi.org/10.1530/endoabs.50.P005.
35. Yang C, Nixon M, Kenyon C, Livingstone D, Duffin R, Rossi A, et al. 5α-Reduced glucocorticoids exhibit dissociated anti-inflammatory and metabolic effects. Br J Pharmacol. 2011;164:1661–71. https://doi.org/10.1111/j.1476-5381.2011.01465.x.
36. Penning TM, Jin Y, Steckelbroeck S, Lanišnik Rižner T, Lewis M. Structure–function of human 3α-hydroxysteroid dehydrogenases: genes and proteins. Mol Cell Endocrinol. 2004;215:63–72. https://doi.org/10.1016/j.mce.2003.11.006.
37. Mueller JW, Gilligan LC, Idkowiak J, Arlt W, Foster PA. The Regulation of Steroid Action by Sulfation and Desulfation. Endocr Rev. 2015;36:526–63. https://doi.org/10.1210/er.2015-1036.
38. Roberts BA, Martel MM. Prenatal Testosterone and Preschool Disruptive Behavior Disorders. Personal Individ Differ. 2013;55:962–6. https://doi.org/10.1016/j.paid.2013.08.002.
39. Pasterski V, Hindmarsh P, Geffner M, Brook C, Brain C, Hines M. Increased aggression and activity level in 3- to 11-year-old girls with congenital adrenal hyperplasia. Horm Behav. 2007;52:368–74. https://doi.org/10.1016/j.yhbeh.2007.05.015.
40. Robinson M, Whitehouse AJO, Jacoby P, Mattes E, Sawyer MG, Keelan JA, et al. Umbilical Cord Blood Testosterone and Childhood Internalizing and Externalizing Behavior: A Prospective Study. PLoS ONE. 2013;8:e59991. https://doi.org/10.1371/journal.pone.0059991.
41. Karaismailoğlu S, Erdem A. The effects of prenatal sex steroid hormones on sexual differentiation of the brain. J Turk Ger Gynecol Assoc. 2013;14:163–7. https://doi.org/10.5152/jtgga.2013.86836.

42. Gong S, Sovio U, Aye ILMH, Gaccioli F, Dopierala J, Johnson MD, et al. Placental polyamine metabolism differs by fetal sex, fetal growth restriction, and preeclampsia. JCI Insight. 2018;3. https://doi.org/10.1172/jci.insight.120723.

Figures

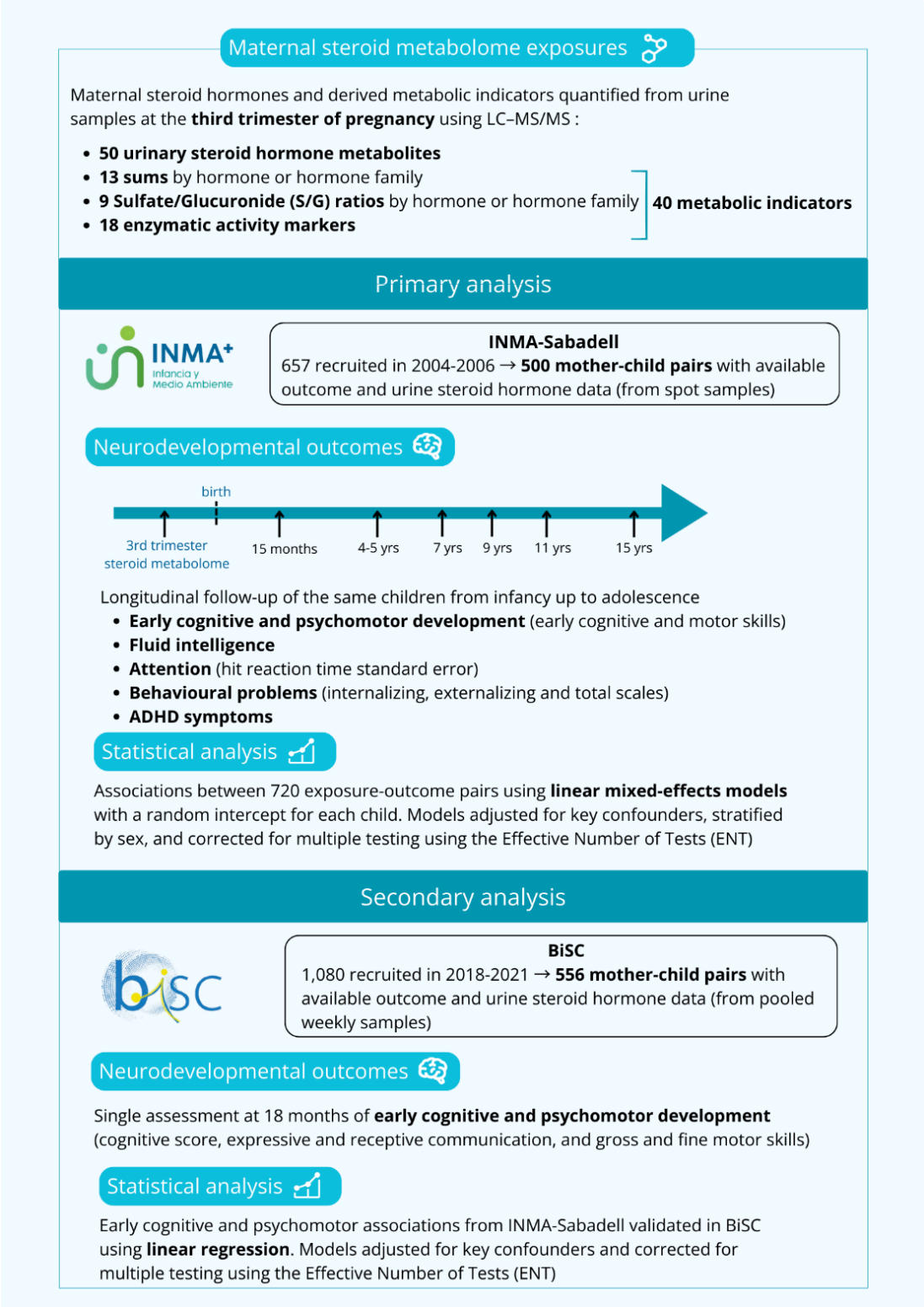


Figure 1

Study Workflow

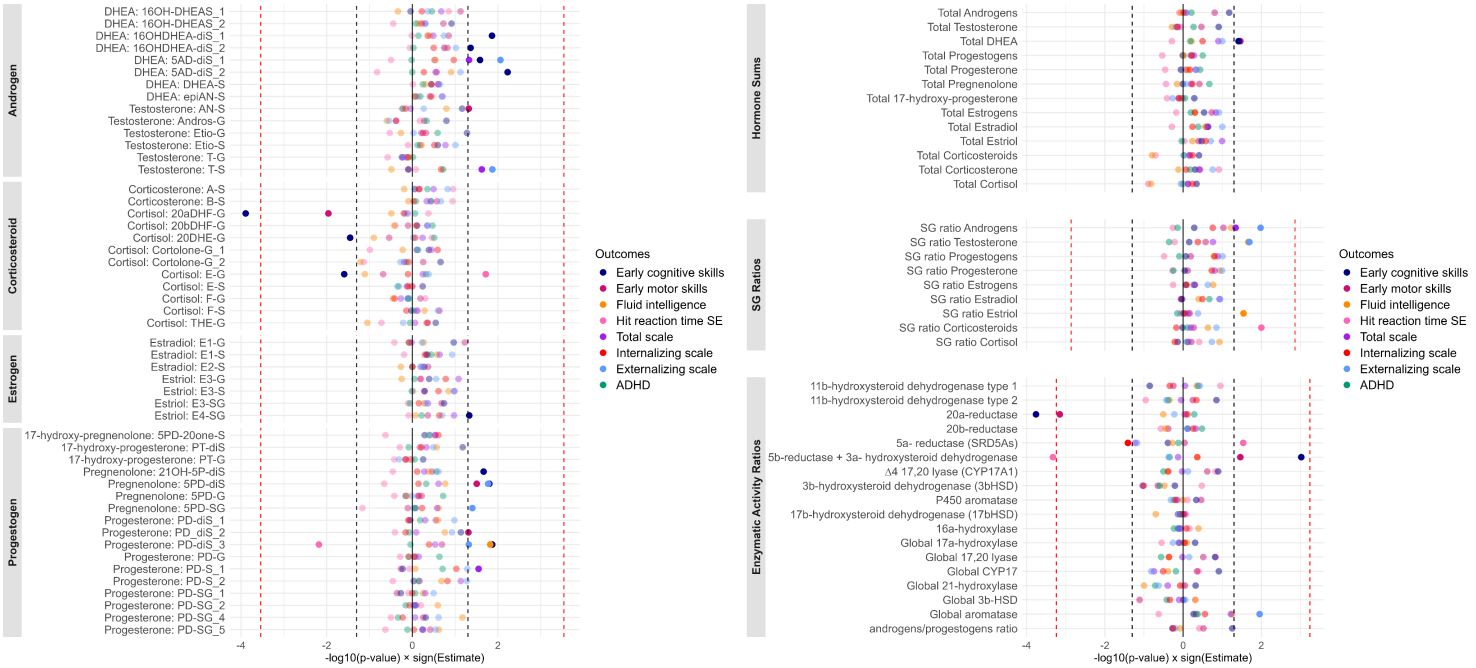


Figure 2

Associations between prenatal steroid hormone exposures and neurodevelopment in INMA-Sabadell

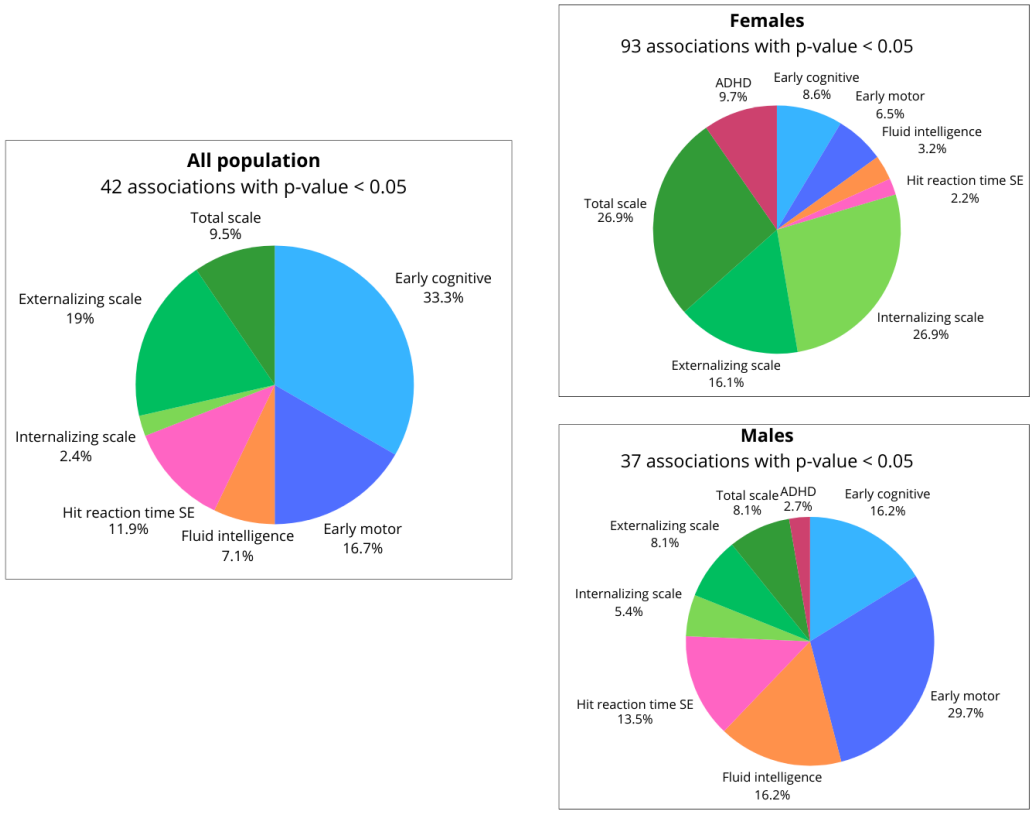


Figure 3

Distribution of outcomes among nominally significant associations (p-value < 0.05) in INMA-Sabadell

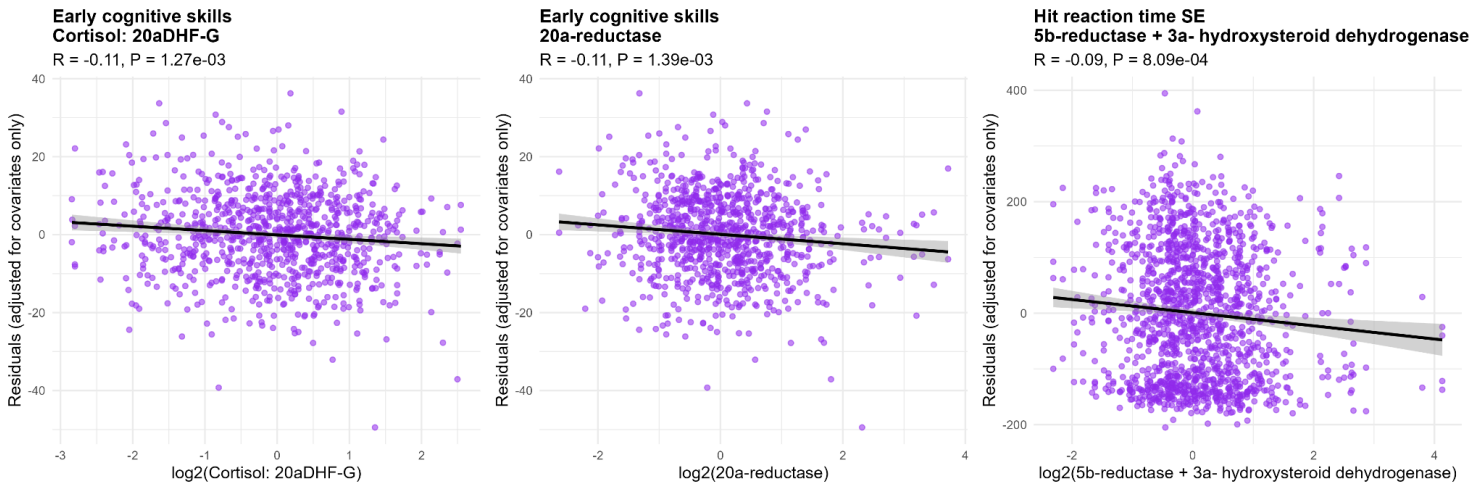


Figure 4

Exposure-outcome residual associations for significant models in INMA-Sabadell

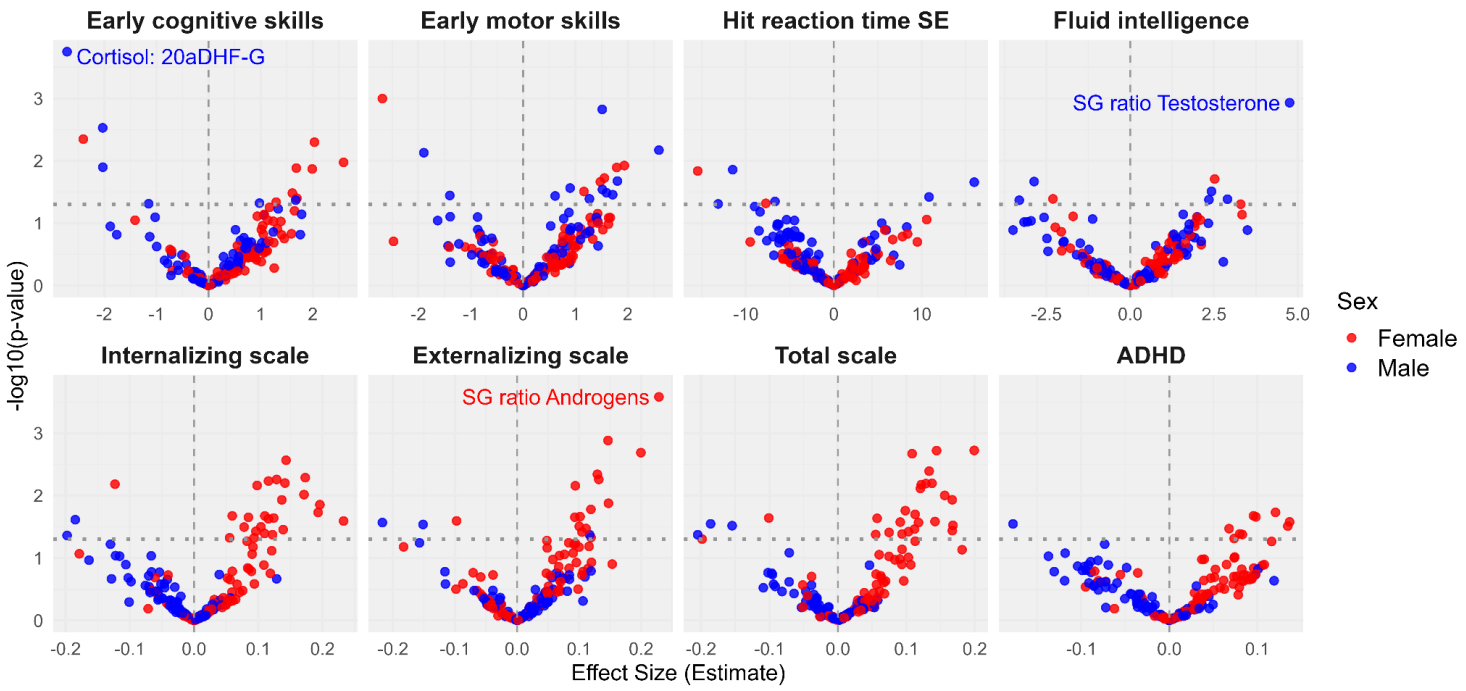


Figure 5

Sex-stratified associations with neurodevelopmental outcomes in INMA-Sabadell

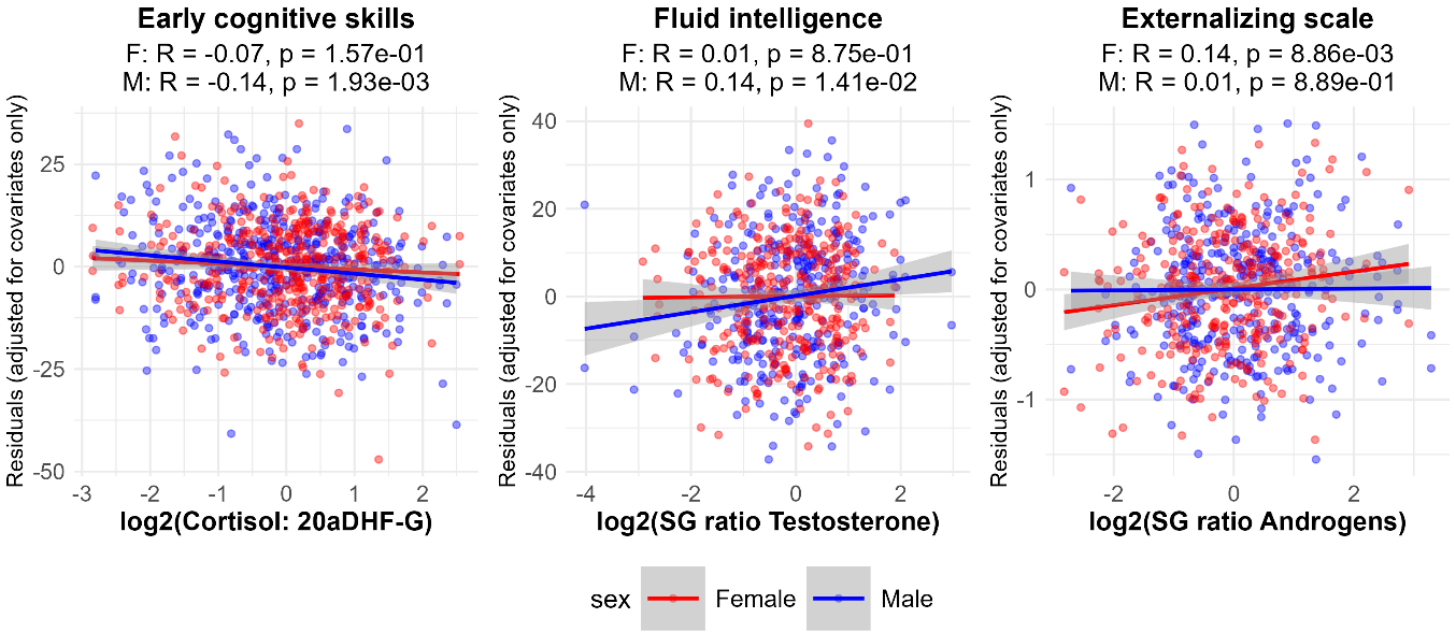


Figure 6

Residual plots for sex-stratified exposure–outcome pairs passing the ENT-corrected significance threshold in INMA-Sabadell

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTables.xlsx