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Deep Screening for X Chromosome Parent-of-Origin Effects on Neurobehavioral and Neuroanatomical Phenotypes in 47,XXY Klinefelter Syndrome

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ABSTRACT

BACKGROUND: X chromosome parent of origin (POX) has been proposed as a source of phenotypic variation within sex chromosome aneuploidies such as Klinefelter syndrome (XXY/KS) and between XX and XY individuals. However, previous studies have yielded conflicting results regarding the presence and nature of POX effects, which we sought to clarify in an expanded sample with deeper neurobehavioral phenotyping.

METHODS: A cohort of 58 individuals with XXY/KS underwent duo or trio genome sequencing with parents (n = 151), measurement of 66 neurobehavioral phenotypes by standardized research assessments, and measurement of over 1000 anatomical phenotypes by structural magnetic resonance imaging. We developed a novel algorithm, the uniparental disomy visualization for variant call format files, to determine proband POX and then systematically tested for POX associations with all neurobehavioral and neuroanatomical outcomes.

RESULTS: The uniparental disomy visualization for variant call format files algorithm showed maternal POX in 35 of 58 cases (60.3%). There were no statistically significant POX effects on any of the 66 subscale measures of cognition, psychopathology, or behavior. Neuroimaging analysis identified 2 regions in the right hemisphere with significantly higher surface area (mean effect size = 1.20) among individuals with paternal versus maternal POX (q = .021).

CONCLUSIONS: Using deeper phenotyping in an expanded sample, we did not find evidence for substantial POX effects on neurobehavioral variability, except for localized unilateral modulations of surface area in the absence of co-occurring behavioral associations. These findings help to clarify previous inconsistencies in POX research and direct attention toward other sources of clinical variability in sex chromosome aneuploidies.

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X chromosome parent of origin (POX) has been proposed as a source of phenotypic variability in sex chromosome aneuploidies (SCAs) as well as a potential driver of normative sex differences given the POX difference between XX and XY individuals. The molecular basis for POX effects is that some X-linked genes show differential epigenetic regulation (or imprinting) in the maternal versus paternal germline that can impact somatic gene expression during offspring development with lasting phenotypic effect (1,2).

SCAs provide a powerful naturally occurring model to test for POX effects because in SCAs (unlike euploidy), these effects can vary independently of gonadal status (3,4). In 47,XXY or Klinefelter syndrome (XXY/KS), POX refers to the parental origin of the supernumerary X chromosome (maternal or paternal) because one X chromosome is always expected to be maternally inherited. XXY/KS is particularly suited to this area of study because paternal nondisjunction is involved in approximately half of the cases (5,6), whereas the rate of paternal nondisjunction is typically higher in Turner syndrome (45X/TS) (7). Evidence for POX effects in SCAs would not only be relevant for understanding broader sex differences but would also represent a potential source of the prominent variability in outcomes within individual SCA groups. Specifically, XXY/KS is characterized by a highly variable neuropsychological phenotype with enrichment for developmental and cognitive difficulties, autism spectrum disorder, and psychiatric diagnoses such as attention-deficit/hyperactivity disorder, anxiety, and depression (8,9). The presentation and severity of XXY/KS are extremely variable, ranging from minimal discernable symptoms to significant impairment (10).

Given the above considerations, there has been extensive previous research into POX effects on both behavioral and brain features in SCAs, but results to date have been strikingly mixed. Previous studies of XXY/KS have yielded inconsistent

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results regarding POX influences on cognition and psychopathology. For example, one study showed that paternal POX (POXp) was associated with greater challenges in both speech and motor development, while another suggested that maternal POX (POXm) was associated with a greater burden of schizotypal traits (5,11). It is also notable that the reported patterning of POX effects conflicts with well-established correlations between human cognition and behavior. For example, POXm status has been associated with both increased schizotypal traits and decreased developmental delays, but risk for schizophrenia is strongly positively associated with the presence of developmental delay in the general population (12,13). In addition to these mixed positive POX findings in XXY/KS, numerous studies have failed to find any statistically significant associations between cognition and behavior with POX (14–17). There are also inconsistencies regarding the direction of the POX effect reported in studies of 45X/TS and compared with those in XXY/KS (18–20).

There have been fewer studies of POX effects on brain organization than behavior, but available neuroimaging findings among individuals with 45X/TS are also mixed. Specifically, there are reports that POXm is associated with increased surface area (SA) in the left temporal lobe (21) and increased gray and white matter volume in the superior temporal gyrus (22), while another study showed the opposite effect, with POXm being associated with significantly decreased white and gray matter volumes bilaterally compared with POXp (23). Other reports showed no significant differences in brain morphology based on POX in 45X/TS (24,25).

Given the profound heterogeneity in past studies of POX effects, we sought to revisit the question of POX impact by harnessing new approaches for calling POX in a deeply phenotyped sample of individuals with XXY/KS and applying expanded outcome measures including both cognitive and psychological domains. Utilizing a novel method for determining POX from duo and trio genome sequencing data, we tested for potential POX effects in the largest sample of XXY/KS with combined behavioral and imaging data reported to date (5,11,15). We sought to determine whether POX in XXY/KS was associated with differences across 66 measures of neuropsychiatric phenotypes (Table 1 and Table S1 in Supplement 1), as well as 1084 measures of brain morphology including cortical volume (CV), SA, and thickness.

METHODS AND MATERIALS

Participants

Our study included 58 individuals with a cytogenetically confirmed nonmosaic XXY/KS karyotype (based on metaphase spreads) who were drawn from a previously published, larger XXY/KS cohort of 102 (26) based on the availability of parental genotypic data enabling POX analysis (Table 2). All individuals were included in analyses of both behavioral and neuroimaging data with 2 exceptions: 1) one individual was excluded from behavioral analyses based on detection of a de novo likely pathogenic variant in the *RAI1* gene (NM_030665.4:c.4891G>T, p.Ala1631Ser) associated with Smith-Magenis syndrome, and 2) one was excluded from neuroimaging analysis due to a co-occurring seizure disorder. Participants provided written informed consent prior to completing study procedures. This research was approved by the National Institutes of Health Institutional Review Board (NCT03206099 and NCT00001246).

Classification of POX Status

Participants underwent clinical-grade genome sequencing and chromosomal microarray in 2021 and 2022 with return of novel primary and secondary findings. Sequencing was also offered to parents when available for trio or duo analysis and determination of POX. Deidentified genome sequence data for participants is shared in dbGaP (Accession No. phs001899.v3.p1).

We developed an algorithm called uniparental disomy (UPD) visualization for variant call format files, adapted from the trio parentage/UPD studies algorithm (27). Our algorithm was designed to 1) use variant call format files from genome

Table 1. Instrument Names, Abbreviations, Descriptive Domain, and Reference for Measures of Psychopathology, Behavior, and Cognition

Instrument (Abbreviation)	Domain of Psychopathology, Behavior, and Cognition	Reference
Child Behavior Checklist/Adult Behavior Checklist (CBCL)[a]	Multidimensional	(32)
Strengths and Difficulties Questionnaire (SDQ)	Multidimensional	(33)
Social Responsiveness Scale (SRS)	Features of autism spectrum disorder	(34)
Social Communication Questionnaire (SCQ)	Features of autism spectrum disorder	(35)
Obsessive-Compulsive Inventory-Revised (OCI-R)	Features of obsessive-compulsive disorder	(36)
Developmental Coordination Disorders Questionnaire (DCDQ)	Features of motor coordination disorder	(37)
Conners 3 (CON)	Features of attention-deficit/hyperactivity disorder and impulsivity	(38)
Barratt Impulsiveness Scale (BIS)	Features of attention-deficit/hyperactivity disorder and impulsivity	(39)
Depression, Anxiety and Stress Scales-21 (DASS-21)	Features of mood disorders—depression, anxiety, and stress	(40)
Affective Reactivity Index (ARI)	Irritability	(41)
Antisocial Process Screening Device (APSD)	Features of conduct/dissocial disorders	(42)
Children's/Adult Scale of Hostility and Aggression: Reactive/Proactive (SHRP)	Features of conduct/dissocial disorders	(43)
Wechsler Intelligence Scale for Children, Fifth Edition (WISC-V)	General cognitive ability	(44)
Wechsler Adult Intelligence Scale, Fourth Edition (WAIS-IV)	General cognitive ability	(45)
Wechsler Abbreviated Scale of Intelligence, Second Edition (WASI-II)	General cognitive ability	(46)

[a]Referred to as CBCL in the Results section.

Table 2. Participant Characteristics

Characteristic	X Chromosome Parent of Origin		Test for Group Difference	
	Maternal, n = 35	Paternal, n = 23	Statistic (df)	P
Age, Years	16.9 (4.5) [8.1–25.8]	13.9 (5.4) [6.7–25.0]	t = 2.18 (41)	.035[a]
Race				
American Indian/Alaska Native	1 (3%)	0 (0%)	χ^2 = 5.96 (4)	.202
Asian	0 (0%)	1 (4%)		
Black	1 (3%)	0 (0%)		
More Than 1 Race	0 (0%)	2 (9%)		
White	33 (94%)	20 (87%)		
Ethnicity				
Hispanic	2 (6%)	2 (9%)	χ^2 = 0.00 (1)	1
Non-Hispanic/unknown	33 (94%)	21 (91%)		
Socioeconomic Status	47.5 (15.1) [20–82]	47.9 (21.4) [20–120]	t = −0.07 (36)	.941
Gestation, Weeks	39.1 (1.5) [36–42]	38.6 (1.8) [33–41]	t = 1.08 (40)	.286
Birth Weight, kg	3.3 (0.4) [2.3–4.1]	3.3 (0.4) [2.7–4.0]	t = 0.08 (51)	.939
Maternal Age at Birth, Years	36.5 (5.3) [25.5–47]	33.0 (5.4) [23–42]	t = 2.36 (46)	.022[a]
Time of XXY/KS Diagnosis				
Prenatal	16 (46%)	7 (30%)	χ^2 = 0.79 (1)	.374
Postnatal	19 (54%)	16 (70%)		

Values are presented as n (%) or mean (SD) [range]. Characteristics of the 58 individuals included in this study, separately by parent of origin of the supernumerary X chromosome, with χ^2 and t test results for comparison are presented in the table.

[a]Indicates statistical significance ($p < .05$).

sequencing as input rather than microarray data and 2) identify UPD in probands based on duos (father + child or mother + child) in addition to trios. This algorithm takes variants called by GATK (28), sex assignments called by GATK-SV (29), and sample IDs for the proband, mother, and/or father. The variants were filtered by bcftools (30) to remove variants that meet any of the following criteria: overlapping low complexity regions (31) or pseudoautosomal regions; non-PASS (i.e., GATK Variant Quality Score Recalibration < 99.9% truth sensitivity threshold); indel; multiallelic; "*" alternate allele; genotype quality <60; allelic depth <10; variant allele fraction from 10% to 30%, 60% to 90%, or missing genotype call for any individual in the trio/duo; and genotype quality <90 for autosomal variants in the proband. These threshold criteria were determined based on the distribution of each metric among the highest quality ~1 to 3 million variants in each trio or duo.

For trio analysis, each proband allele was assigned an inheritance pattern, and UPD status was called using discriminative thresholds for father-only or mother-only inherited variants. For duo analysis, each proband variant was categorized based on the number of alleles that matched parent alleles (i.e., 0, 1, or 2) and/or variant allele fraction (i.e., 0%, 50%, or 100%), and UPD status was called using discriminative thresholds. Validity of the duo pipeline was confirmed by repeating the trio cases as duos, with 100% concordance observed.

Phenotypic Measures

Questionnaire-Based Measures of Psychopathology, Behavior, and Cognition. All XXY/KS participants were evaluated with a structured medical history and physical examination. The age of diagnosis of XXY/KS was determined from caregiver reports and reviews of prior medical documents.

Twelve self- and parent-report instruments were used to gather deep phenotypic information related to psychopathology and behavior from all probands (32–43), which is detailed in Table 1. These 12 instruments yielded a total of 66 scales (Table S1 in Supplement 1). Additionally, general cognitive ability was measured using an age-appropriate Wechsler scale (44–46). If a participant was tested with one of the Wechsler scales within 1 year (n = 1), the Wechsler Abbreviated Scale of Intelligence, Second Edition (46) was used. Full Scale IQ (FSIQ) was derived from the Wechsler scale for each participant.

Standardization. To standardize scores and allow for direct comparison across the parent of origin groups, all psychopathology and behavior scale scores were standardized against a sample of typically developing XY individuals (n = 74) that was age matched to the XXY/KS sample (XY mean age = 17.1, XXY mean age = 15.7; p = .131). These z scores were used because not all measures provided published normative population scores on which the sample could be adjusted. For all 66 scales of psychopathology and behavior, only our standardized scores were used in analysis. The standardization method is described in Raznahan *et al.* (47) and in Schaffer *et al.* (48). Briefly, the scores for each instrument were standardized against the XY control group using a general linear model with age as a predictor (when there were significant age × group interactions) or standardized as z scores from the distribution of scores in the XY control group (when there were no significant age × group interactions). As a sensitivity analysis, we used a complementary analytic approach to compare the scores between groups. We used scaled scores with age as a covariate, compared the resulting standardized betas to those derived from the z-scored scales, and observed a Pearson correlation of r = 0.8 across effect sizes. We provide the full model output in Table S5 in Supplement 2.

Neuroimaging. Forty-two participants from the total sample were included in neuroimaging analyses (Table S2 in Supplement 1). These participants had a 3-dimensional T1-weighted magnetization-prepared rapid acquisition gradient-echo structural brain magnetic resonance imaging scan gathered on the same 3T Discovery MR750 (General Electric) scanner (parameters: 172 sagittal slices, field of view = 256 mm, 256 × 256 in-plane acquisition matrix, 1-mm isotropic voxels, flip angle = 7°, inversion time = 1100 ms, echo time = 3.5 ms, repetition time = 7.948 ms) and passed quality control based on visual inspection.

As previously described (26,49–51), each participant's structural magnetic resonance imaging scan was run through the FreeSurfer version 7.1.0 (52) recon-all pipeline to derive measures of CV, SA, and cortical thickness (CT) for each of 360 cortical regions from the Glasser parcellation (53). Briefly, the recon-all pipeline involves image intensity normalization, skull stripping, atlas registration, tissue classification and image segmentation, creation of tessellated pial and gray/white surfaces, surface-based intersubject registration, and parcellation (54–62).

Statistical Analysis

Proportions and means were used as descriptive statistics for categorical and continuous variables, respectively. χ^2 and *t* tests were used for comparison of demographic variables (for categorical and continuous variables, respectively) between POX groups. A power analysis using the R package *pwr* (63) was conducted to estimate the effect sizes that could be reliably detected (>80% power) with our sample sizes. All statistical analyses and data visualizations were completed using the R language for statistical computing. Behavioral data preprocessing methods are the same as those reported in Raznahan *et al.* (47) and Schaffer *et al.* (48).

Analysis of POX Differences in the Behavioral Phenotype. All continuous behavioral phenotypes were compared between the POXm and POXp groups using linear regression. We provide raincloud plot visualizations using the R package *raincloudplots* (64) for 3 commonly studied broad-scale scores of cognition and behavior provided by our battery: general cognitive ability (FSIQ), total psychopathology (total Child Behavior Checklist [CBCL] *z* score), and total level of autism spectrum disorder–related traits (total Social Responsiveness Scale [SRS] *z* score). These broad-scale scores were tested for significant differences between POX groups at a nominal $p < .05$ threshold. We present POX associations with all 66 fine-grained measures of behavior as *z* score effect sizes and confidence intervals [effect sizes are standard model coefficients given that all scales were *z*-scored prior to analysis as per Raznahan *et al.* (47) and Schaffer *et al.* (48)]. FSIQ scores are population normed, and the corresponding POX model coefficient is therefore divided by the instrument's standard deviation (SD = 15) to be interpreted as an effect size. These 66 scale scores were tested for statistically significant POX differences using a Bonferroni-adjusted *p* value (adjusted *p* value = .05/66 = .0008) as well as a nominal threshold ($p < .05$).

Analysis of POX Differences in Neuroanatomy. We used linear models to systematically test for a main effect of POX on measures of brain anatomy from surface-based estimation of CV, SA, and CT in the XXY/KS group. We first tested for POX differences in 3 commonly studied global indices of neuroanatomy, covarying for age and using a nominal $p < .05$ threshold: total tissue volume (TTV), total CV, total SA, and mean CT. We then tested for POX differences in CV, SA, and CT at each of 360 Glasser Human Connectome Project regions, covarying for age and TTV and correcting for multiple comparisons using false discovery rate (FDR) ($q < .05$). We report all effect sizes as standardized model coefficients (β) based on scaling each anatomical variable in XXY/KS to a mean of 0 and a standard deviation of 1. All imaging analyses and brain atlas visualizations were conducted using the R package *ggsegGlasser* (65).

We complemented our linear regression analyses by testing for POX effects on regional cortical anatomy using nonparametric permutation-based tests. We randomized POX across participants and randomly divided participants into 2 groups of the same size as the original POX groups from the imaging subsample. After each round of permutation, we estimated group differences in CV, SA, and CT across regions between POX groups using linear regression with age and TTV as additional predictors, storing the resulting effect size (standardized β) estimate for POX. This procedure was iterated 10,000 times to construct a null distribution of POX effect size estimates for each regional measure of CV, SA, and CT for all 360 regions. A *p* value with a significance threshold of .05 was derived from the null distribution by determining the ratio of times that the absolute value of the observed effect size was smaller than the absolute value of the permuted effect size under the null distribution and dividing by the number of iterations (10,000). This empirical *p* value was FDR adjusted for 360 comparisons ($q < .05$).

RESULTS

Participant Characteristics

The total study sample included 58 individuals diagnosed with XXY/KS and trio ($n = 35$) or duo ($n = 23$) genome data. Demographic characteristics are described in Table 2. Output of the UPD visualization for variant call formats demonstrated that the supernumerary X chromosome was inherited from the mother in 35 cases (POXm 60.3%) and from the father in 23 cases (POXp 39.7%). Of the samples with maternal inheritance, 4 (11.4%) were isodisomic for the supernumerary X (identical due to nonsegregation in meiosis II), and 31 (88.6%) were heterodisomic (not identical due to nonsegregation in meiosis I). The POX breakdown of the imaging analysis subsample was 59.5% POXm ($n = 25$) and 40.5% POXp ($n = 17$) (Table S2 in Supplement 1). Participant characteristics of the subsample included in imaging analysis ($n = 42$) did not differ from characteristics of the total sample (Table S2 in Supplement 1).

As expected (66,67), those with POXm had significantly greater maternal age at birth on average than those with POXp (36.5 years and 33.0 years, respectively; $p = .022$) (Table 2). Unexpectedly, individuals in our sample with a supernumerary

X chromosome with POXm were significantly older at the time of the study visit on average than those in the POXp group (16.9 years and 13.9 years, respectively; p = .035) (Table 2). Because the z scores used in the analysis of behavioral variables accounted for age when scaling the scores relative to the XY control group, we did not further adjust for age when testing for POX effects on behavioral traits. However, age was included as a covariate in analyses of POX influences on brain anatomy. The 2 POX groups did not differ on race, ethnicity, socioeconomic status, gestational age, birth weight, or time of XXY/KS diagnosis (prenatal vs. postnatal).

POX Influences on Cognitive and Behavioral Traits

We did not observe statistically significant associations between POX and any measured cognitive or behavioral traits. Our power analysis indicated that, for the cognitive and behavioral phenotype analysis, our sample size (n = 58) was sufficient to reliably (power = 0.80) detect a small effect size (0.14) at an alpha level of 0.05. Distributions of FSIQ, total CBCL score, and total SRS score in the POX groups are shown in Figure 1A. POX effects on these 3 variables failed to reach statistical significance and were of small to moderate effect size (FSIQ: $\beta = -0.24$ [−3.64/15], p = .27; CBCL: $\beta = -0.99$, p = .23; SRS: $\beta = -0.63$, p = .280) (Table S3 in Supplement 1). Relationships between POX and all 66 measured scales of psychopathology are shown in Figure 1B. None of these associations reached statistical significance at a Bonferroni-corrected p value of .0008. We replicated this analysis using FDR correction for multiple comparisons and again found that no scale reached statistical significance ($q < .05$).

Observed effect sizes spanned a wide effect size range ($-2.03 \leq \beta \leq 1.34$) (see Table S5 in Supplement 2 for the full regression results) and often showed opposing directions for scales known to be highly concordant across individuals. For example, as shown in Figure 1B, affective symptom domains measured by the CBCL, anxious/depressed (ax.dep_CBCL) and withdrawn/depressed (wt.dep_CBCL), were split across the range of effect sizes and had opposite directionality (ax.dep_CBCL: $\beta = 0.56$ vs. wt.dep_CBCL: $\beta = -1.03$). Additionally, a measure of reciprocal social interaction on the Social Communication Questionnaire (soc_SCQ) and other measures of social behavior such as autism spectrum disorder social communication/interaction (asd_SRS) and peer relations (peer_CON) that one would expect to exhibit similar POX effects had opposing effect sizes (soc_SCQ: $\beta = 0.35$ vs. asd_SRS: $\beta = -0.65$ and peer_CON: $\beta = -1.23$).

POX Influences on Neuroanatomical Traits

Our sample size for the neuroimaging analysis (n = 42) is sufficient to reliably (power = 0.80) detect a medium effect size (0.23) at an alpha level of 0.05. Distributions of TTV, CV, SA, and CT for the 2 POX groups are shown in Figure 2. None of these global measures of brain anatomy showed a statistically significant association with POX, although we observed larger values of all 4 measures in POXp than in POXm individuals, with borderline statistical significance for TTV and the effect size for SA reaching the nominal $p < .05$ threshold (TTV: $\beta = 0.62$, p = .06; CV: $\beta = 0.43$, p = .12; SA: $\beta = 0.65$, p = .046; CT: $\beta = -0.09$, p = .734) (Table S4 in Supplement 1).

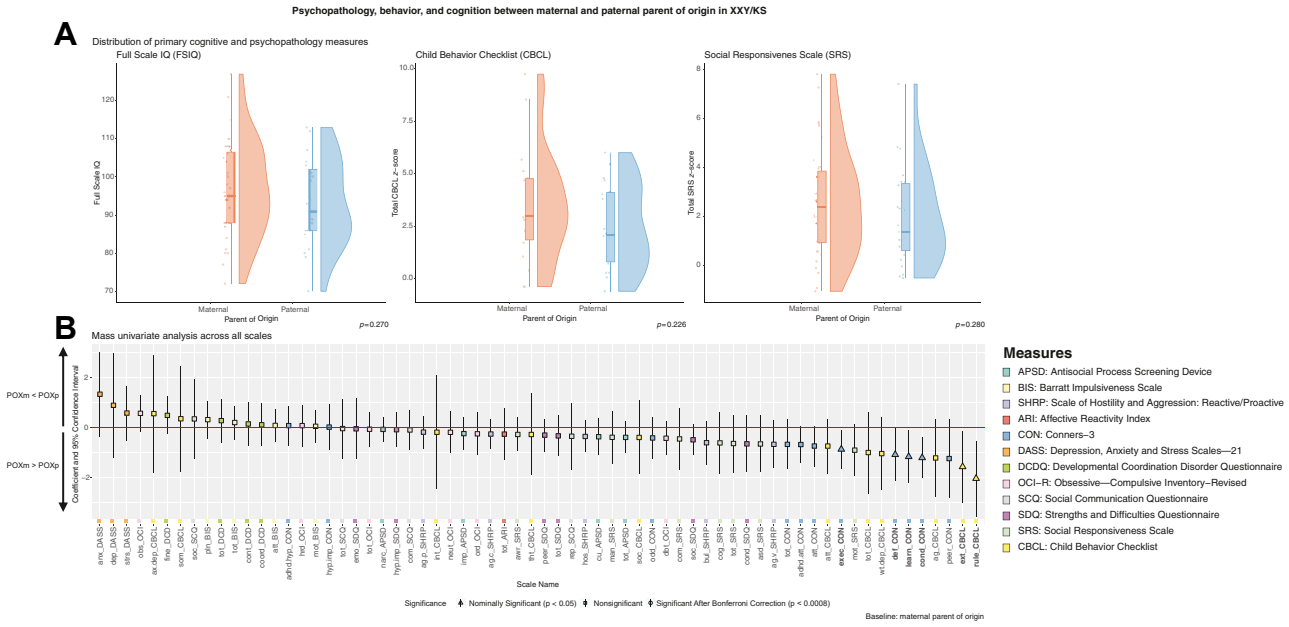

Figure 1. Mass univariate analysis of the 66 scales of psychopathology, behavior, and cognition from 12 measures between POX groups. **(A)** Comparing the scores on 3 primary measures (Full Scale IQ scores, total Child Behavior Checklist z scores, and total Social Responsiveness Scale z scores) in the POXm and POXp groups. **(B)** Model coefficients and 95% CIs for all individual scales, with POXm as the baseline. All point estimates above the red line indicate that scores were higher among the POXp group. The shape of the point estimate indicates statistical significance (nominally significant, nonsignificant, and significant after correction). No scales were significant after Bonferroni correction. POX, parent of origin; POXm, maternal POX; POXp, paternal POX; XXY/KS, Klinefelter syndrome.

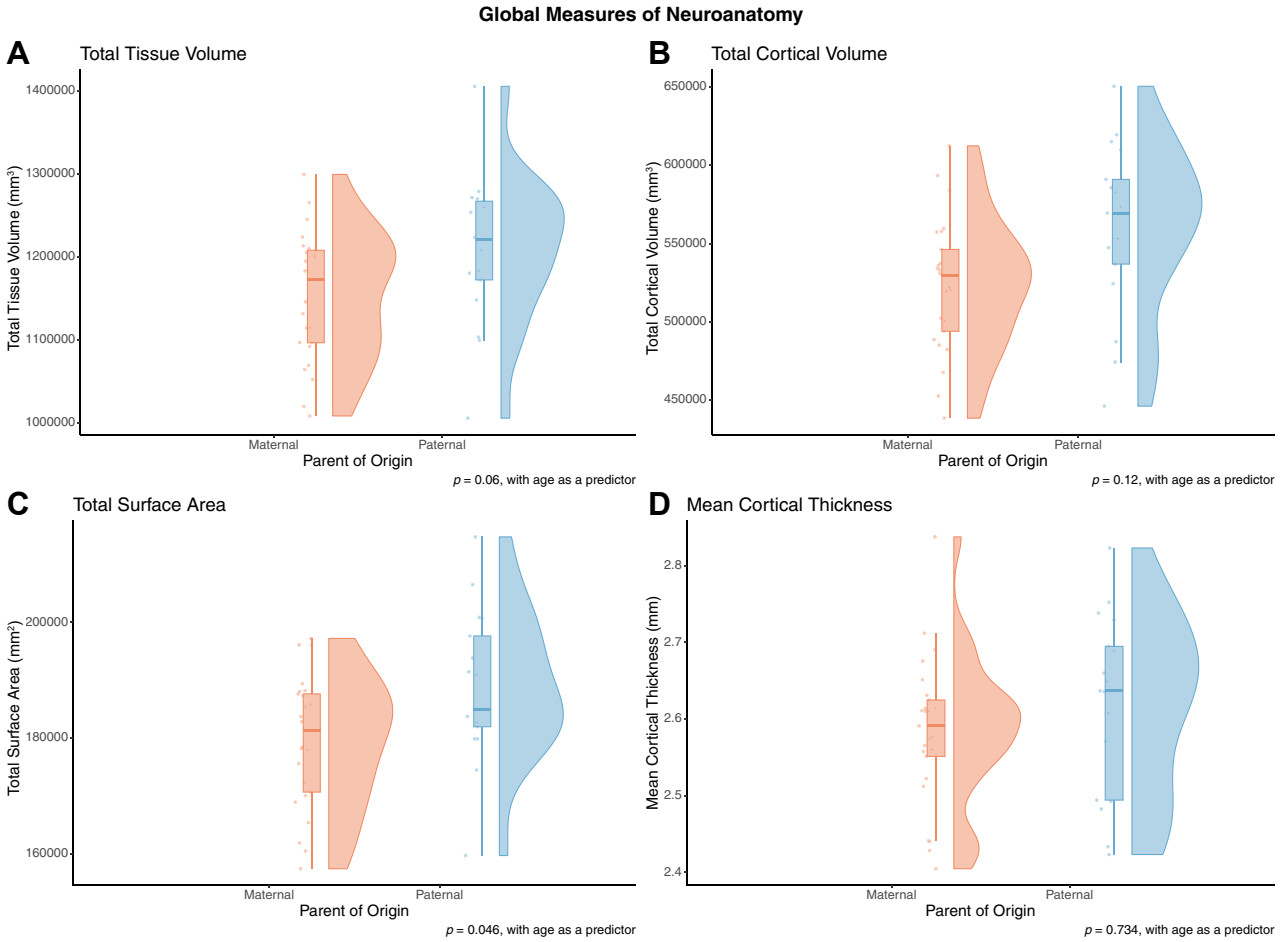

Figure 2. Distribution of 4 global indices of neuroanatomy between parent of origin. Statistical significance is derived from a linear model with age as a predictor for each metric. **(A)** Total tissue volume, **(B)** total cortical volume, **(C)** total surface area, and **(D)** mean cortical thickness.

Estimating POX effects on regional measures of CV, SA, and CT by linear regressions identified 2 right hemisphere regions with statistically significant differences in SA between POX groups that survived FDR correction for multiple comparisons (Figure 3A; Table S6 in Supplement 2). Regional SA was greater in POXp than POXm groups in area 55b ($\beta = 1.15$, $q = .021$) and the frontal eye field (FEF) ($\beta = 1.24$, $q = .021$). The SA distributions in POX groups for these 2 regions are shown in Figure 3B.

Permutation-based analysis with 10,000 iterations confirmed the results from linear regression analyses and also detected statistically significant differences in SA between POX groups in the right hemisphere regions of area 55b and the FEF (see Figure S2 in Supplement 1). After permutation, 1 additional left hemisphere region and 1 right hemisphere region emerged with a statistically significant difference in SA, and 1 additional right hemisphere region emerged with a statistically significant difference in CV between POX groups that survived FDR correction. Regional SA was smaller in the POXp group than in the POXm group in the left hemisphere area, frontal operculum 3 (observed $\beta = -0.98$, $q = .018$). Regional SA and CV were greater in the POXp group than the POXm group in the right hemisphere region 6a (SA: observed $\beta = 1.09$, $q = .018$; CV: observed $\beta = 1.11$, $q = .036$) (Figure S2 in Supplement 1).

DISCUSSION

Our study design substantially expands the available body of empirical information regarding potential POX effects on human brain and behavior by combining the strengths of a new pipeline for calling POX from genome sequencing data with access to unprecedentedly deep phenotypic data in a cohort of individuals with XXY/KS.

The novel method used herein for determining parent of origin using genome sequencing data with duos or trios could be employed in other research areas. Because this algorithm does not rely on short tandem repeats unique to the X chromosome, it can be adapted to examine parent of origin impacts of other chromosomes (27). Moreover, by screening for POX effects across a deep phenotypic battery, we limit the risk of false inference that can result when considering isolated measures of behavior or brain organization. Deep phenotypic data provides better coverage of the diverse aspects of brain

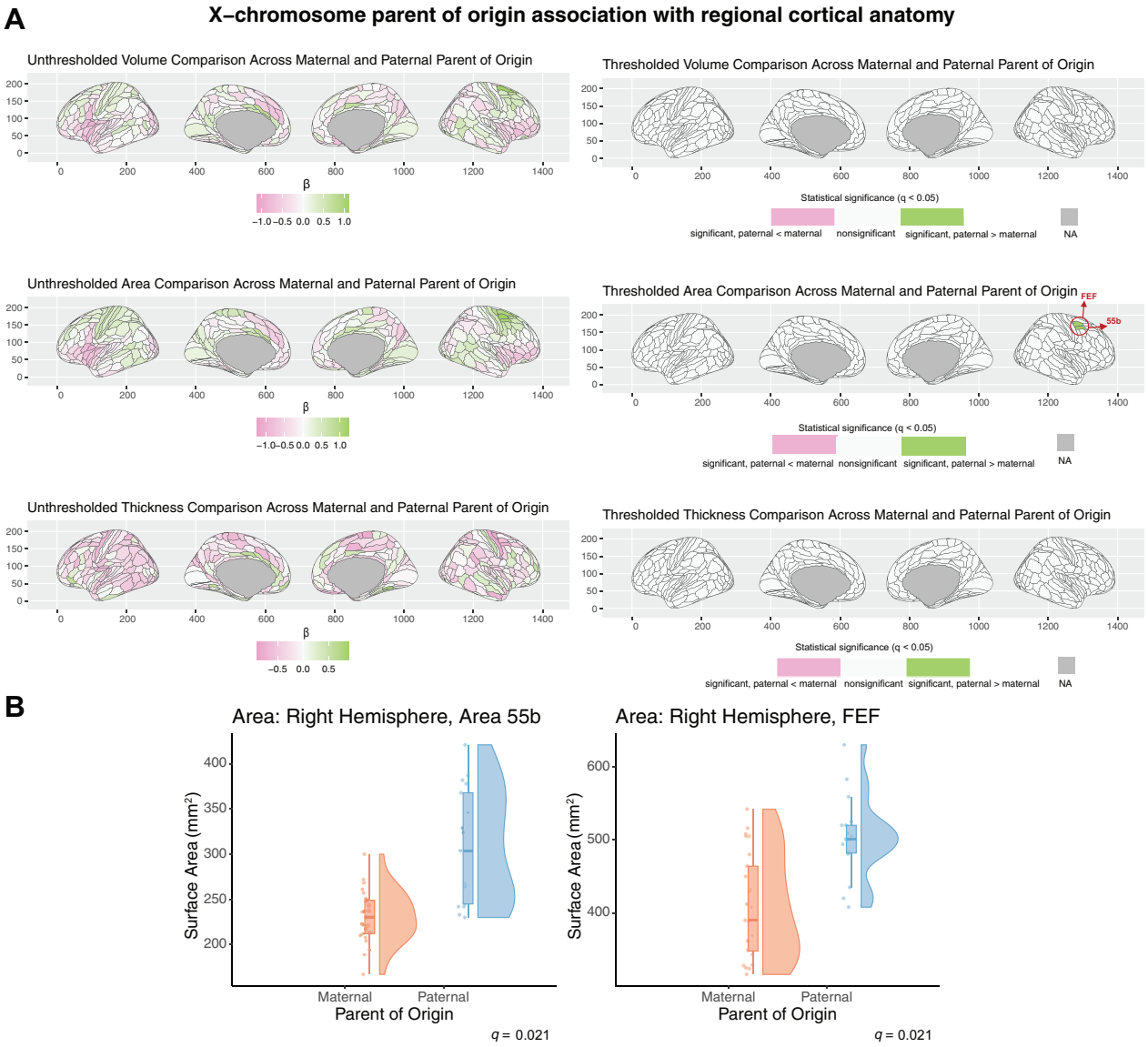

Figure 3. Mass univariate results from linear regressions for each of 360 Glasser regions adjusted for age and total tissue volume. **(A)** Both unthresholded (standardized β value on a continuous gradient) and thresholded (false discovery rate–adjusted p value threshold of $q < .05$) significance of parental origin differences are shown and colored according to the direction of the difference. **(B)** Surface area distribution of the 2 statistically significant ($q < .05$) regions, area 55b and the FEF. FEF, frontal eye field.

and behavior that could potentially be influenced by POX effects. Deep phenotypic measures of behavior allow for individual constructs (e.g., low mood) to be assayed by several different instruments, thereby providing a built-in test for cross-instrument reproducibility of any observed POX associations.

The frequency of POX types observed in our current report replicates previous findings for the parental source of nondisjunction events that give rise to XXY/KS (5,6). We found that 60.3% of patients had a maternally inherited supernumerary X (POXm). Although this variability in POX could theoretically provide a substantial source of phenotypic variability within XXY/KS, we found little evidence in support of this hypothesis. Specifically, our analysis did not detect any statistically significant differences in cognitive, psychopathology, or behavioral measures based on POX status in XXY/KS. This observation supports previous work that reported null findings and provides clarification surrounding previous studies that identified conflicting directions of effect based on POX (5,11,14–17).

Neuroimaging analysis identified a trend toward greater mean SA in the POXp than the POXm group, which notably is in the opposite direction of a reported significant POX effect that was identified in a smaller, previous neuroimaging study of 45X/TS (21). Neuroimaging analyses of regional anatomy that controlled for global brain measures identified 2 regions in the right hemisphere with significantly larger SA among individuals

with paternal X chromosome origin, 55b and the FEF. Previous literature suggests that area 55b, a premotor region, may play a role in language processing (68) and music perception (69), while the FEF is implicated in gaze control (70), and visual and spatial attention (71). However, our reported anatomical findings should be taken with caution, and replication is needed because these effects involve very few regions in one hemisphere in a sample that is relatively small for detecting subtle differences in brain morphology. Even if our findings were replicated, their clinical significance is uncertain in the absence of accompanying behavioral effects. Nevertheless, we do report a significant difference that supports the need for future research in this area.

Our findings should be considered in light of several limitations and caveats. First, although deep behavioral phenotyping was performed, there are domains that were not measured like subdimensions of cognition (17). However, given a broad lack of behavioral associations and the tendency for there to be stereotyped correlational structure among measures of human cognition and behavior, we would not expect to identify isolated associations between POX and unmeasured domains.

Second, our study sample size, although large compared with previous parent of origin work (5,11,15), places limits on the statistical power with which we can test for significant POX effects. However, this limitation may be offset by the use of deep phenotypic data, which allows for the detection of substantial effect sizes that are below statistical significance but consistent in direction across closely related traits. Third, we have tested for POX effects in XXY/KS, and our findings may not generalize to 45X/TS—the other SCA used in POX research. For example, the simpler POX status in individuals with 45,X may allow penetrance of effects that is obscured by the more complex sex chromosome karyotype of XXY/KS (72). Thus, definitive understanding of POX influences would benefit from future deep phenotypic studies in 45X/TS. Additionally, in our study, we did not examine other mechanisms that may act upon POX to contribute to variability, such as skewed X chromosome inactivation, which has been observed in XXY/KS (73).

Finally, the nature of our sample means that we cannot directly test whether lack of POX effects generalizes across different demographic characteristics including genetic ancestry, age, and environmental exposures.

Conclusions

Notwithstanding the above limitations, however, our study provides a thorough test for POX effects in XXY/KS, and we found little evidence of these for behavioral and cognitive traits, accompanied by weak evidence for potential POX effects on brain anatomy in isolated unilateral cortical regions. These anatomical POX associations await replication in future work, and we hope that the new tools published herein to call POX status from genome sequencing data will assist such future research. From a precision medicine perspective, however, our finding of limited evidence for POX effects helps to reprioritize other potential genomic features as sources of variability in XXY/KS including common-variant polygenic risk, rare variant burden, and variability of X inactivation.

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