Elevated Polygenic Burden for Autism Spectrum Disorder Is Associated With the Broad Autism Phenotype in Mothers of Individuals With Autism Spectrum Disorder

Supplementary Information

Table S1. Associations between mother's BAPQ phenotypes and proband phenotypes.

Mother's BAPQ Phenotype	Proband Phenotype	p-value	beta	SE
Aloof	ADOS CSS Total	2.38E-01	-0.13	0.11
	ADOS CSS RRB	4.51E-01	-0.08	0.1
	ADOS CSS SA	2.94E-01	-0.11	0.11
	ADI-R a total	1.69E-02	0.08	0.03
	ADI-R b non-verbal total	5.05E-03*	0.15	0.05
	ADI-R b verbal total	2.72E-02	0.1	0.05
	ADI-R c total	4.96E-01	0.05	0.07
	RBS-R total	1.41E-02	0.03	0.01
	SRS total	1.11E-04**	0.03	0.01
Pragmatic	ADOS CSS Total	8.32E-02	-0.15	0.09
	ADOS CSS RRB	2.76E-01	-0.09	0.08
	ADOS CSS SA	1.51E-01	-0.12	0.08
	ADI-R a total	7.10E-04*	0.09	0.03
	ADI-R b non-verbal total	4.83E-04*	0.14	0.04
	ADI-R b verbal total	1.79E-03*	0.11	0.04
	ADI-R c total	3.47E-01	0.05	0.06
	RBS-R total	2.08E-11**	0.06	0.01
	SRS total	1.09E-09**	0.03	0.01
Rigid	ADOS CSS Total	9.73E-02	-0.17	0.1
	ADOS CSS RRB	2.89E-01	-0.1	0.09
	ADOS CSS SA	1.01E-01	-0.16	0.1
	ADI-R a total	9.66E-02	0.05	0.03
	ADI-R b non-verbal total	8.10E-01	0.01	0.05
	ADI-R b verbal total	4.40E-01	0.03	0.04
	ADI-R c total	4.86E-02	0.14	0.07
	RBS-R total	5.44E-12**	0.07	0.01
	SRS total	1.06E-06**	0.03	0.01
Total	ADOS CSS Total	6.36E-02	-0.46	0.25
	ADOS CSS RRB	2.38E-01	-0.26	0.22
	ADOS CSS SA	9.25E-02	-0.4	0.24
	ADI-R a total	2.80E-03*	0.21	0.07
	ADI-R b non-verbal total	9.05E-03	0.31	0.12

Mother's BAPQ Phenotype	**Proband Phenotype**	**p-value**	**beta**	**SE**
	ADI-R b verbal total	1.49E-02	0.25	0.1
	ADI-R c total	1.39E-01	0.24	0.16
	RBS-R total	1.46E-10**	0.15	0.02
	SRS total	2.13E-09**	0.09	0.01

*Associations passing FDR testing correction.

** Associations passing Bonferroni testing correction.

Table S2. Associations between father's BAPQ phenotypes and proband phenotypes.

Father's BAPQ Phenotype	Proband Phenotype	p-value	beta	SE
Aloof	ADOS CSS Total	5.48E-01	0.07	0.12
	ADOS CSS RRB	3.70E-01	0.10	0.11
	ADOS CSS SA	2.82E-01	0.13	0.12
	ADI-R a total	2.90E-01	-0.04	0.04
	ADI-R b non-verbal	6.31E-01	-0.03	0.06
	ADI-R b verbal	1.77E-01	-0.07	0.05
	ADI-R c total	6.68E-01	0.03	0.08
	RBS-R total	7.26E-01	0.00	0.01
	SRS total	2.49E-02	0.02	0.01
Pragmatic	ADOS CSS Total	5.13E-01	-0.06	0.09
	ADOS CSS RRB	4.35E-01	-0.06	0.08
	ADOS CSS SA	8.06E-01	-0.02	0.09
	ADI-R a total	5.93E-01	-0.01	0.03
	ADI-R b non-verbal	3.10E-01	-0.04	0.04
	ADI-R b verbal	3.34E-01	-0.04	0.04
	ADI-R c total	2.23E-01	0.07	0.06
	RBS-R total	4.42E-03*	0.02	0.01
	SRS total	8.80E-05**	0.02	0.01
Rigid	ADOS CSS Total	1.64E-01	-0.15	0.11
	ADOS CSS RRB	2.76E-01	-0.11	0.10
	ADOS CSS SA	3.10E-01	-0.10	0.10
	ADI-R a total	2.07E-01	-0.04	0.03
	ADI-R b non-verbal	3.93E-01	-0.04	0.05
	ADI-R b verbal	6.48E-01	-0.02	0.04
	ADI-R c total	8.59E-02	0.12	0.07
	RBS-R total	1.61E-06**	0.05	0.01
	SRS total	1.09E-02	0.02	0.01
Total	ADOS CSS Total	6.02E-01	-0.13	0.26
	ADOS CSS RRB	7.64E-01	-0.07	0.24
	ADOS CSS SA	9.97E-01	0.00	0.25
	ADI-R a total	2.24E-01	-0.09	0.07
	ADI-R b non-verbal	3.46E-01	-0.12	0.12
	ADI-R b verbal	2.39E-01	-0.12	0.11
	ADI-R c total	1.78E-01	0.23	0.17
	RBS-R total	1.56E-03*	0.08	0.02
	SRS total	4.46E-04*	0.06	0.02

*Associations passing FDR testing correction.

** Associations passing Bonferroni testing correction.

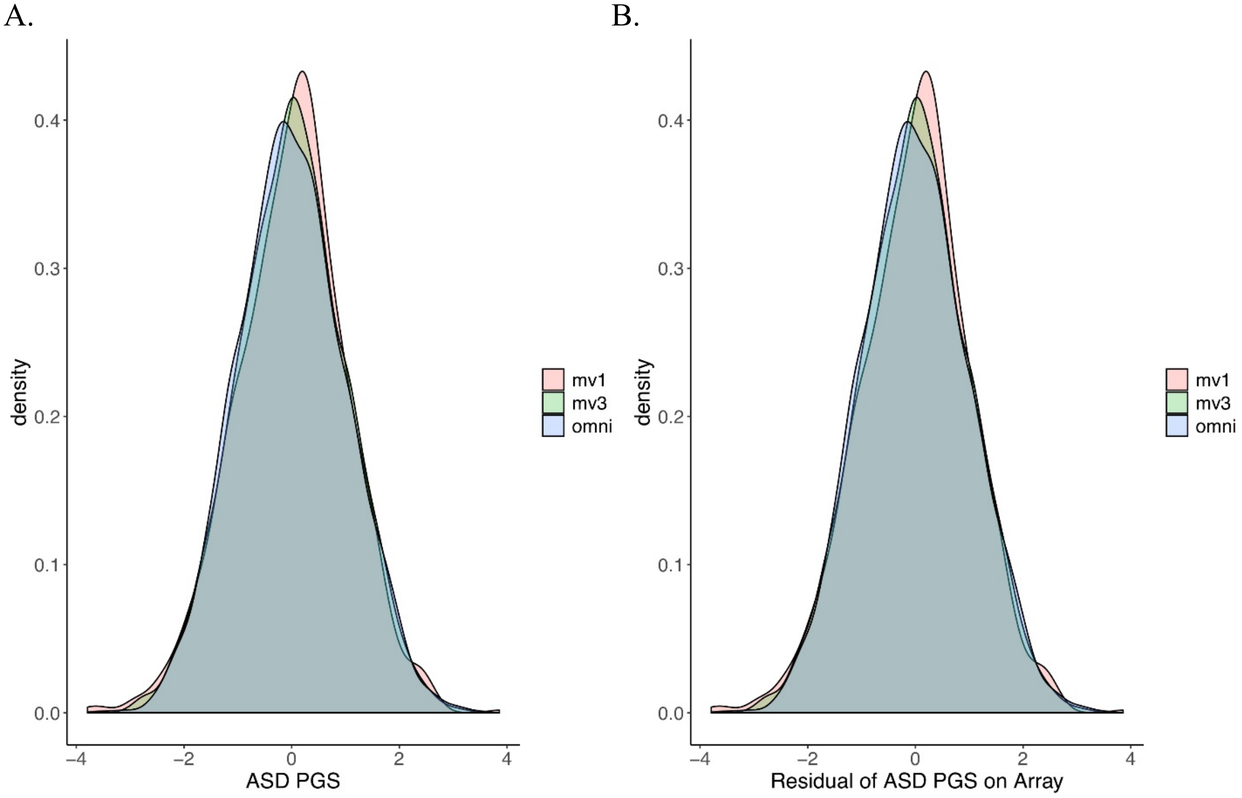

Figure S1. Distribution of ASD PGS color-coded by platform A) z-score scaled and B) residual of platform and z-score scaled.

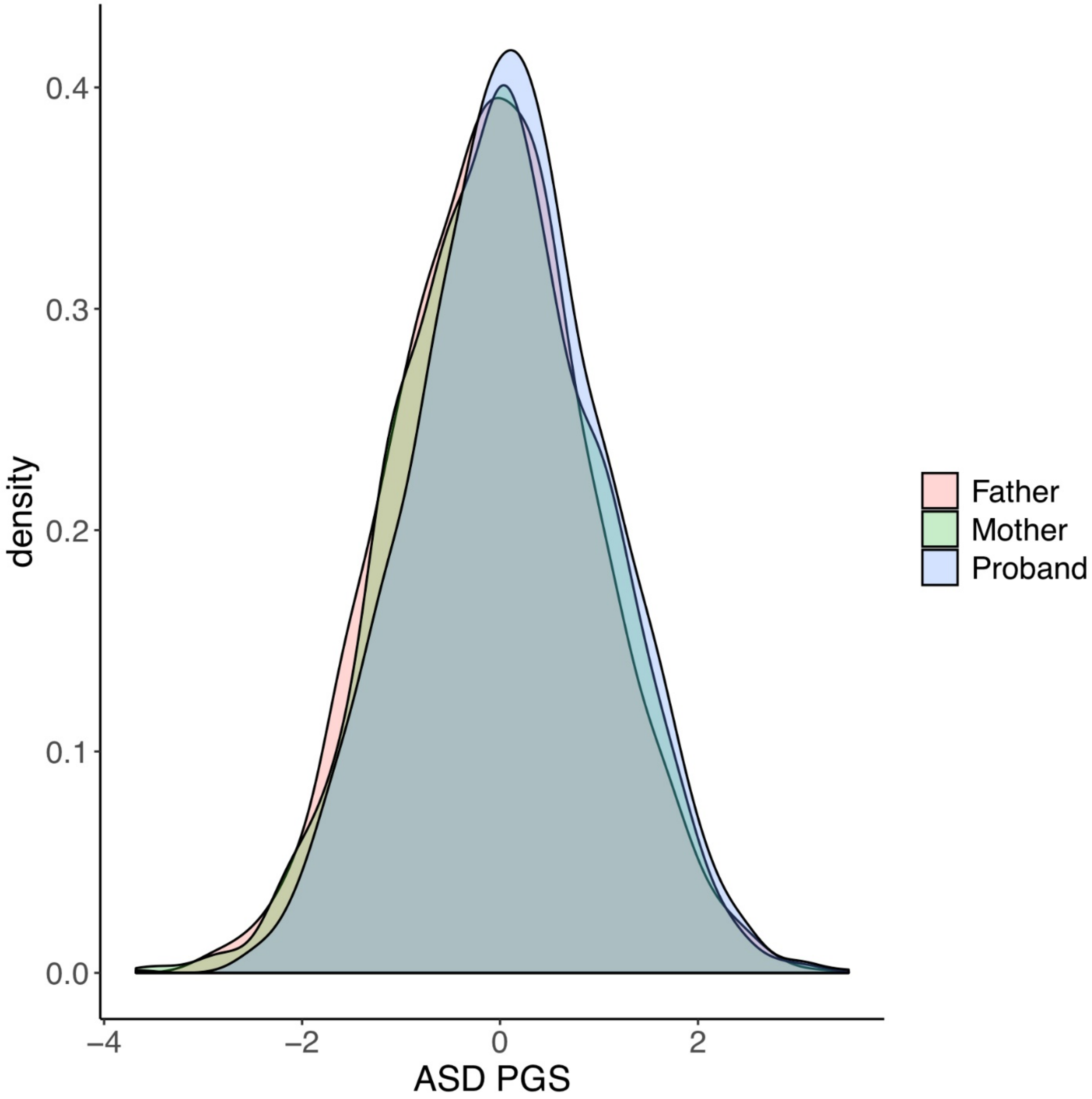

Figure S2. Distribution of ASD PGS color-coded into fathers, mothers, and probands.

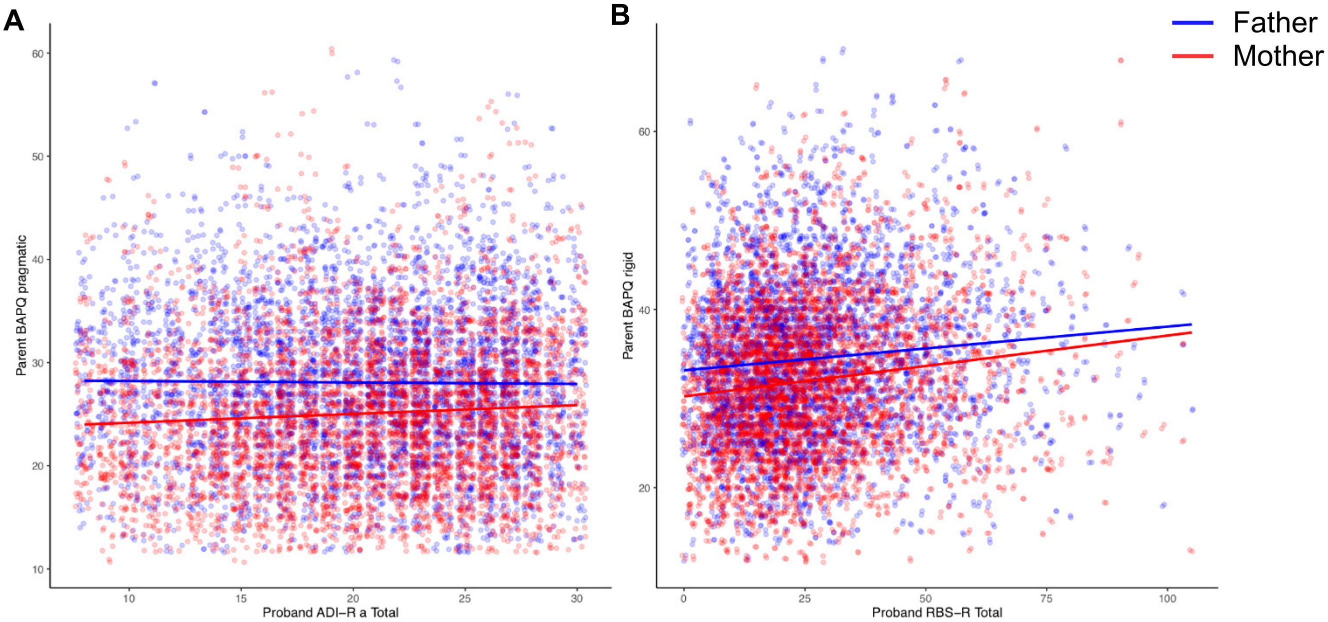

Figure S3. (A) Relationship between proband ADI-R a total scores and parent BAPQ pragmatic scores; and (B) proband RBS-R total scores and parent BAPQ rigid scores.

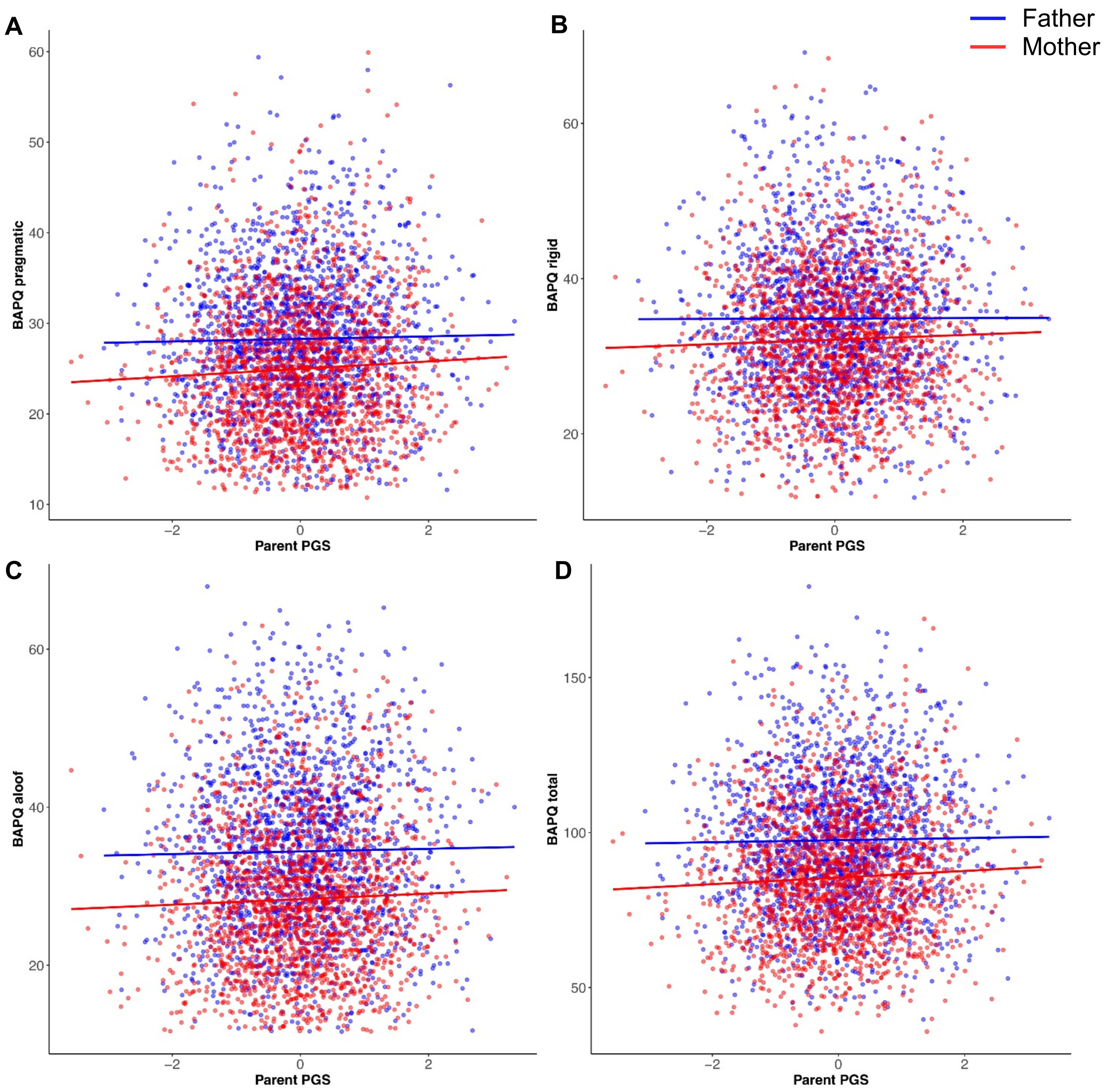

Figure S4. Relationships between maternal and paternal PGS of ASD, and BAPQ (A) pragmatic (B) rigid, (C) aloof, and (D) total scores. Linear regression lines are plotted for mothers and fathers separately.

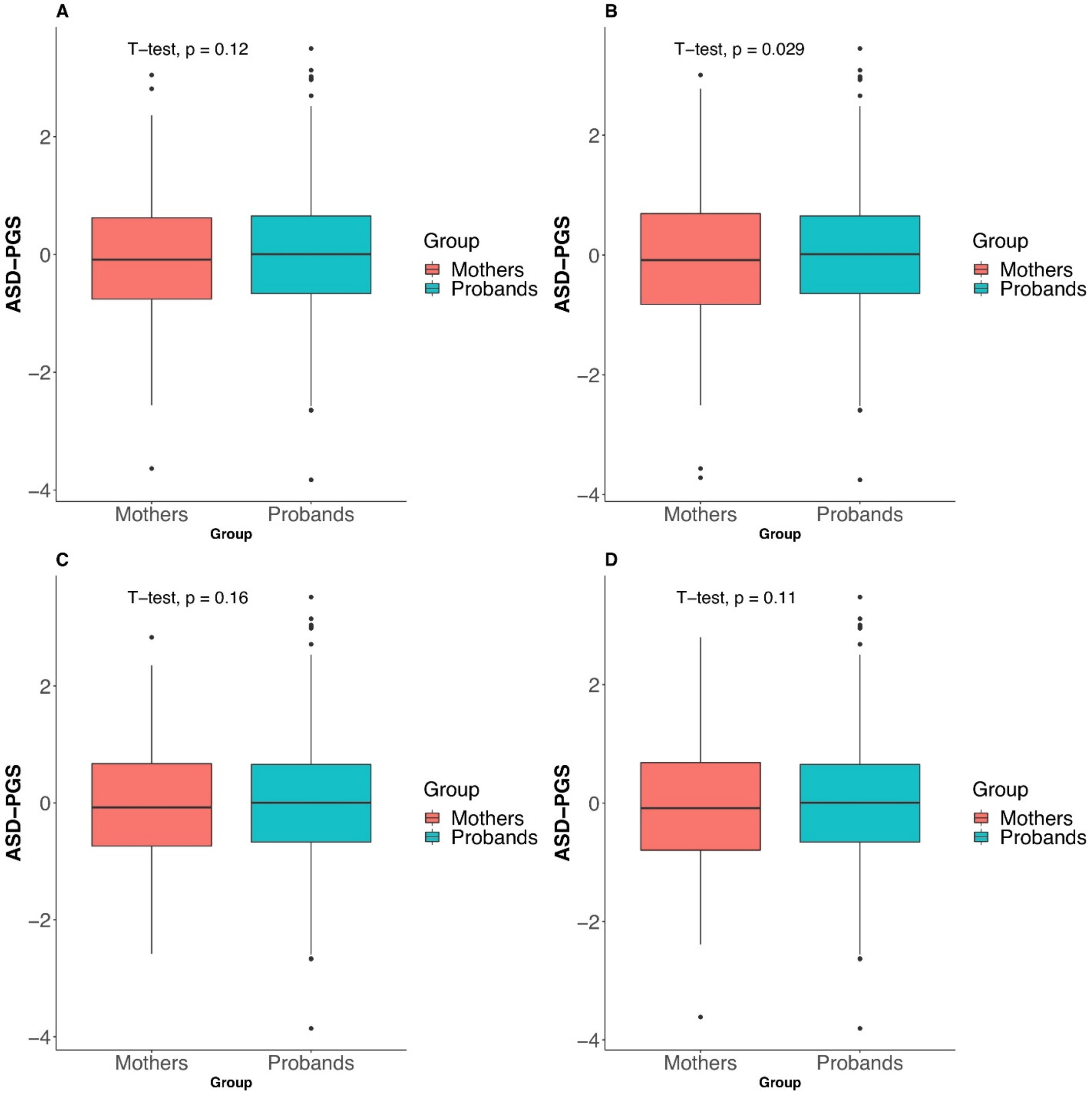

Figure S5. Comparison of ASD-PGS between 4th Quartile BAPQ-mothers and male ASD probands. A) ASD PGS of 4th Quartile BAPQ-total mothers compared to male ASD probands, B) 4th Quartile BAPQ-aloof, C) 4th Quartile BAPQ-pragmatic, and D) 4th- Quartile BAPQ-rigid. All polygenic scores were z-score scaled and significance was determined using t-tests.

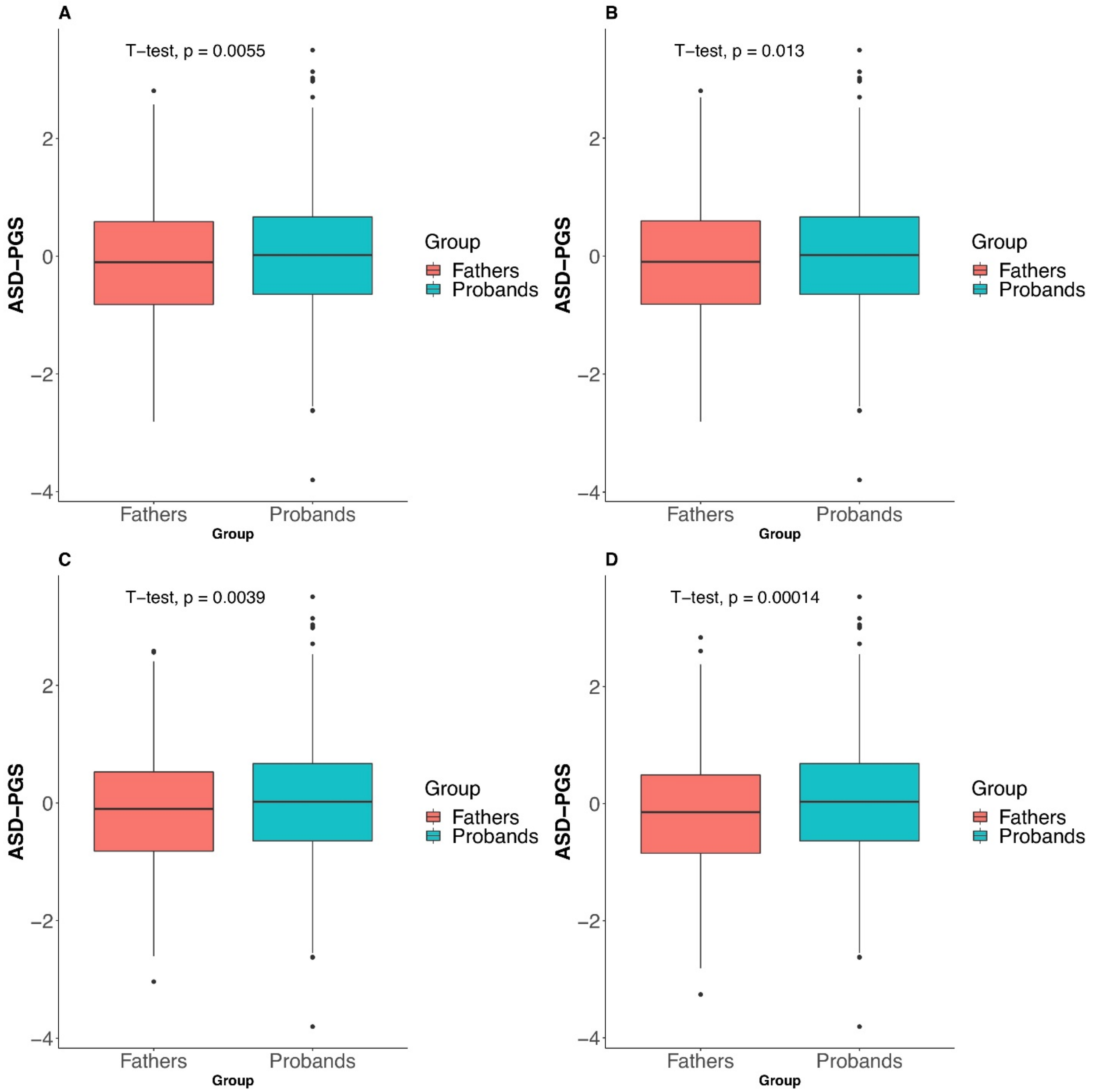

Figure S6. Comparison of ASD-PGS between 4th Quartile BAPQ-fathers and male ASD probands. A) ASD PGS of 4th Quartile BAPQ-total fathers compared to male ASD probands, B) 4th Quartile BAPQ-aloof, C) 4th Quartile BAPQ-pragmatic, and D) 4th- Quartile BAPQ-rigid. All polygenic scores were z-score scaled and significance was determined using t-tests.

Assessing use of parent BAP measures

An *a priori* decision to focus on the BAPQ (versus the Social Responsiveness Scale – Adult Version (1) (SRS-A) as the primary index of BAP features in parents was made because the BAPQ was developed specifically to measure particular subclinical personality and language characteristics of the BAP among clinically *unaffected* relatives of individuals with ASD (2–4), whereas the SRS was developed primarily as a measure of ASD symptoms (and SRS-A to capture ASD symptoms in adults), requiring a higher level of severity to meet cut-offs (2). However, as a secondary analysis, the SRS-A was examined in parents, in relationship to proband phenotypic variables. Significant associations were detected between maternal and paternal SRS-A scores and proband SRS total and RBS-R total scores. No other significant associations emerged that withstood the Bonferroni corrected *p*-value of < .0003; therefore, models reported below focused on BAPQ associations only.

SSC IQ measurements

Assessments with the largest sample size were prioritized, followed by those with lower sample sizes: Wechsler Intelligence Scale for Children (WISC), followed by the Wechsler Abbreviated Scale of Intelligence (WASI), followed by the Differential Ability Scales *School-Age* (DAS-SA), followed by Differential Ability Scales *Early Years (*DAS-EY). To compute full-scale IQ, the Perceptual Reasoning Index (PRI) and Verbal Comprehension Index (VCI) were included for the WISC, Verbal IQ (VIQ) and Performance IQ (PIQ) were included for the WASI, and Special Nonverbal Composite (SNC) and Verbal scores were included for the DAS-SA and DAS-EY (note: given that the DAS EY *Upper Level* included an Special Nonverbal Composite (SNC) score while the *Lower Level* included a non-verbal reasoning score, a combined SNC score was generated to equate the two). There was no minimum or maximum IQ set for these analyses, particularly given the heterogeneity observed in cognitive abilities in ASD (5).

Genotyping Quality Control and Imputation

First, SNPs were filtered at a call rate of 0.95. Individuals were filtered if their genotype missingness rate was greater than 0.02 ($n_{removed}$ = 8), heterozygosity above 0.2 or below -0.2

($n_{removed}$ = 3), or if there were discrepancies between the number of sex chromosomes and reported sex ($n_{removed}$ = 49). Next, SNPs were filtered more stringently for call rates below 0.98 and differential missingness above 0.02. We then used LiftOver (6) to convert the platforms from genome build hg18 to hg19 to match the 1000 Genomes Project (7) sample and the summary statistics used for polygenic scoring. Genotype Harmonizer (6) was used to align SNPs with strand information from 1000 Genomes Project Phase 3. We performed principal component analysis on parental controls using Eigenstrat (8,9). To check for platform effects, we performed logistic regressions between each platform using controls and covarying for top 10 principal components of ancestry and sex. We removed SNPs significantly associated with platform ($p < 0.001$). To define the European individuals, we performed PCA with a combined sample of self-reported white parents and the 1000 Genomes Project Phase 3 sample, and selected individuals around the Northern Europeans from Utah (CEU) cluster. For probands and siblings to be included, both parents were required to be of European genetic ancestry. We filtered the European parental sample for SNPs out of Hardy-Weinberg Equilibrium ($p < 1e^{-6}$) and removed the same SNPs from probands and siblings. Genotypes were imputed to the 1000 Genomes panel using the Michigan Imputation Server. Imputed genotypes were converted to hardcalls and filtered for SNPs with an imputation R^2>0.3. Next, SNPs with call rates lower than 98%, individuals with call rates lower than 98%, and SNPs with minor allele frequency less than 1% were removed. Finally, SNPs out of Hardy-Weinberg Equilibrium ($p < 1e^{-6}$).

IQ-Stratified Phenotype-Phenotype Associations

ADOS: There were no significant associations between maternal or paternal BAPQ scores and proband ADOS total CSS or ADOS subscales in the high or low IQ groups, showing no impact of IQ on reported overall relationships.

ADI-R: There was a significant positive association between maternal BAPQ-pragmatic and proband ADI-R reciprocal social interaction and non-verbal communication total scores in the low IQ group (*Estimates* > .09, *p*s < .0007), but not for the high IQ group, showing that overall differences reported above were true for the low IQ group only. . While there were no significant associations with paternal BAPQ scores and proband ADI-R scores overall, elevated paternal aloof scores significantly related to higher ADI-R non-verbal communication scores for probands in the high IQ group only (*Estimates* = .19, $p < .0003$).

SRS: Relationships with maternal BAPQ scores and proband SRS total scores, regardless of proband IQ, remained largely consistent with initial overall findings, demonstrating significant positive associations across all BAPQ domains and proband SRS total scores in both the low (*Estimates* > .02, *p*s < .0003) and high IQ groups (*Estimates* > .02, *p*s < .0003). Paternal BAPQ-pragmatic and total scores associated with proband SRS in low (*Estimates* > .02, *p*s < .0003) and high IQ groups (*Estimates* > .05, *p*s < .0003), revealing no differences from overall reported findings. Additionally, while no associations were observed between paternal BAPQ rigid or aloof and proband SRS scores overall, associations were evident among the high IQ proband group only (*Estimates* > .03, *p*s < .0003).

RBS-R: Relationships remained unchanged from overall associations reported, wherein maternal BAPQ-pragmatic, -rigid, and -total scores were all associated with proband RBS-R total scores among the low (*Estimates* > .05, *p*s < .0003) and high IQ groups (*Estimates* > .04, *p*s < .0003). In contrast, for fathers, only paternal BAPQ total and rigid scores remained significantly associated with proband RBS-R total scores within the high IQ group only (*Estimates* > .04, *p*s < .0003).

Fisher's z transformation tests assessing the strength of phenotypic associations.

Fisher's *z* transformation tests revealed significant differences in the strength of parent-child associations, such that mother-child associations were stronger than father-child associations (BAPQ-aloof and ADI R non-verbal communication total $z = 4.38$, $p = .00001$; BAPQ-pragmatic and ADI-R social reciprocity total $z = 2.90$, $p = .004$; BAPQ-pragmatic and ADI-R non-verbal communication total $z = 3.65$, $p = .0003$; BAPQ-pragmatic and ADI-R verbal communication total $z = 2.55$, $p = .01$; BAPQ-total and ADI-R social reciprocity total $z = 4.45$ $p = .000009$; BAPQ-total and RBS-R total $z = 2.57$, $p = .01$).

Associations Between High BAPQ Parents and Male Probands Polygenic Scores (also see Supplementary Figures S5-S6)

When restricting to mothers in the highest quartile of BAPQ-rigid, -pragmatic, or -total scores, we observed no difference between the mothers' ASD-PGS and the probands' ASD-PGS. In contrast, the ASD-PGS of high BAPQ fathers were significantly lower than male probands across all domains (total $p = 0.006$, aloof $p = 0.013$, pragmatic $p = 0.004$, rigid $p = 0.0001$). Only mothers in the highest quartile of BAPQ scores on the aloof subdomain, demonstrated significantly lower ASD-PGS from male probands ($p = .03$).

Supplemental References

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