Inherited Risk for Autism Through Maternal and Paternal Lineage

Supplemental Information

Table of Contents

Supplemental Notes

Covariates

Diagnoses were recorded using the International Classification of Diseases version 8, 9 and 10 (ICD-8, ICD-9 and ICD-10) in Sweden. Besides outcome ASD, including its subtypes, we also included any psychiatric disorder, intellectual disability, depression, anxiety disorders, substance use disorders, Bipolar disorder, compulsive disorder, Attention-Deficit Hyperactive Disorder (ADHD), affective disorders, schizophrenia, and schizoid personality disorder as covariates. Psychiatric diagnosis codes included in the study was presented in Table S1.

Model assumptions

In our analyses the measurement units can be considered as pairs: The offspring paired with each of their aunts and uncles. The main reason of using the pair-level data is to address 'family size'. We treat each pair as separate 'family', or a potential representative for the family, and consider them time-varying with ascertainment and exposure data being aggregated over time. Figure 1 shows an example of a family with two children (C1, C2) and three maternal aunts (mA1, mA2, mA3). The 1st maternal aunt, mA1, is diagnosed with ASD while the 2nd and 3rd aunts mA2 and mA3 are free from ASD diagnosis. This family will enter the analyses as two exposed children (C1 | exposed to mA1) and (C2 | exposed to mA1) and four unexposed children (C1 | mA2), (C1 | mA3), (C2 | mA2) and (C2 | mA3). This procedure will automatically weight the data by family size and amount of exposure and will treat exposure from the aunts and uncles time varying. For a similar family as above, but more exposed to ASD so all three aunts were ASD-affected then all 6 representatives ('children') will enter the analyses as exposed.   Note: There is a technical problem: The 6 children will be correlated so our usual standard errors do not apply. For this reason, we applied bootstrap to obtain 95% confidence intervals adjusting for this potential correlation.

An alternative approach is to use individuals with family exposure. This approach assumes that family size does not play a role and assumes that families where only one of several uncle(s)/aunt(s) are ASD-affected carry the same information as families where all uncle(s)/aunt(s) are affected by ASD. Following this approach, the maternal family in Figure 1 will consist of two exposed offspring: C1 and C2.

Both approaches have their problems. To address the problem with family size we also performed a supplementary analysis where we only consider the first-born child and the oldest aunt/uncle (see Table S6). Under this approach we are truncating the families to the first-born only which solve the problems above by brute force and with the cost of reduced precision (wider confidence intervals).

Lastly, and related, to allow us to formally compare (i.e. calculate a statistical test) aunt-exposure from uncle-exposure we perform yet another analysis where we are sorting each family into one of maternal families (1) ASD-affected aunt; (2) ASD-affected uncle free from ASD-affected uncle or (3) Aunt(s) and/or uncle(s) free from ASD (Table S5).

Summary of results

A series of complementary models were fitted and summarized as following:

(1) To test if ASD severity modifies the maternal silencing we estimate the 9 RRs described above but for uncles and aunts diagnosed with AD only. The RRs were slightly elevated, but 95% CIs were overlapping with the main results (Table S2a-Exposure: AD). Similar results were obtained for paternal lineage, only with wider confidence interval due to smaller number (Table S2b-Exposure: AD).

(2) For participants, as well as parents and their siblings, the measurement errors from miss-classifications of diagnoses increase with earlier birth year. To address these measurement errors, we will repeat the analyses above after extending the diagnostic classifications of maternal uncle/aunt to not only include ASD from ICD-10 and ICD-9 but also include a diagnosis of schizophrenia in the parental generation (aunt or uncle) or a diagnosis of intellectual disability or schizoid personality disorder. These outcomes may have lower specificity but increased sensitivity for the diagnosis of ASD. The RRs were slightly diluted, but 95% CIs were overlapping with the main results for both maternal and paternal lineage (Table S2a and Table S2b-Exposure ASD/SZ/ID/SPD).

(3) To test the specificity of the 9 RRs described above we repeated the analyses using AD in the offspring instead of ASD. The results were consistent with ASD in the offspring for both maternal and paternal lineage (Table S3a and Table S3b).

(4) We considered sex-specific risk associations for ASD by repeated the analyses of ASD in male and female offspring separately. The RRs were larger in females than in males with overlapping 95% CIs (Table S4).

(5) To make direct comparison for the ASD risk among offspring of ASD-affected maternal aunt(s), ASD-affected maternal uncle(s), and ASD-free maternal aunt(s) or uncle(s), we purposed an 'alternative reference group' model where the all participants with maternal aunt(s) or uncle(s) were categorized into three groups:

- if the participant has ASD-affected maternal aunt(s) => Maternal aunt(s) group

- if the participant doesn't have ASD-affected maternal aunt(s) AND has ASD-affected maternal uncle(s) => Maternal uncle(s) group
- if the participant has maternal aunt(s)/uncle(s) AND the maternal aunt(s)/uncle(s) are ASD-free => Reference group

We prioritized maternal aunt(s) to get more participants in Maternal aunt(s) group, considering ASD prevalence is only one third in females than in males.

Same model was fitted, and RRs were presented for: Maternal aunt(s) vs. Reference, Maternal uncle(s) vs. Reference, and Maternal uncle(s) vs. Maternal aunt(s). The results remained consistent with the main models (Table S5), and the difference between Maternal uncle(s) vs. Maternal aunt(s) were not statistically different (for adjusted model: RR=1.18, 95% CI: 0.78-1.78).

(6)   In our analyses, data were at offspring-aunt/uncle pair level, where some individuals are included in more than one cousin-uncle/aunt comparison (either exposed or unexposed). In such case, individuals from larger families will contribute more to the sample size. Therefore, as a test of robustness, we fitted additional models where the oldest cousin pairs was drawn within 'families' (children and grandchildren of the same maternal grandparents) and the results remained robust (Table S6).

(7) To minimize the impact of the psychiatric history in the maternal family (parental generation), we repeated analyses on the subgroup of families with no psychiatric history other than ASD and the results remained robust (Table S7).

(8) While adjusting for covariates, instead of parameters for 'any mental health', we included indicators for specific psychiatric disorders (intellectual disability, depression, anxiety disorders, substance use disorders, Bipolar disorder, compulsive disorder, Attention-Deficit Hyperactive Disorder (ADHD), affective disorders, schizophrenia, schizoid personality disorder) of the mother and the uncle/aunt[1] ASD and the results remained robust (Table S8).

(9) During the analytic sample construction, we found there were 344 mothers with ASD diagnosis, we decided to stick to our exclusion and inclusion criteria by focusing the exposure on ASD diagnosis of uncle(s)/aunt(s) only. However, we conducted a sensitivity analysis by excluding offspring of ASD-affect mothers, and the results remained robust (Table S9).

(10) We also conducted complementary analysis separately on children of those ASD-affected mothers where the RR of ASD in offspring with an uncle or aunt compared to offspring with uncle or aunt without an ASD diagnosis was estimated at RR=5.23 (1.94, 14.11) (Table S10).

(11) To verify that our results were not biased due to sparse data which potentially can result in biased estimates, we performed a supplementary analysis using the Firth correction for monotone likelihood to adjust for the case-control imbalance. The point estimates and 95% CIs were consistent with our primary results, showing robustness of our modelling (Table S11).

Table S1. Psychiatric diagnosis codes included in the study.

Psychiatric illness subgroup	ICD-10 codes	ICD-9 codes	ICD-8 codes
ASD	F84.0, F84.1, F84.5, F84.8, F84.9	299A	299A
AD	F84.0	299A	299A
Asperger	F84.5	NA	NA
PDD-NOS	F84.1, F84.8, F84.9	NA	NA
Any psychiatric disorder	F00*-F99*	295*-315* (not including: 302, 302.*, 305, 305.*, 307, 307.*, 309, 309.*)	295*-315* (not including: 302, 302.*, 305, 305.*, 307, 307.*, 309, 309.*)
Intellectual disability	F70*-F79*	317*-319*	310*-315*
Depression	F32*, F33*, F34.1*, F34.8*, F34.9*, F43.21*	296.2*, 296.3*, 296.82, 300.4*, 301.12*, 309.1*, 311*	298.0*, 300.4*
Anxiety disorders	F40*, F41*	300.0*, 300.2*	300.0*, 300.2*
Substance use disorders	F10*, F11*, F12*, F13*, F14*, F15, F16*, F17*, F18*, F19*	303*, 304*,305*	303*, 304*
Bipolar disorder	F30*, F31*, F34.0*	296.0*, 296.1*, 296.4*, 296.5*, 296.6*, 296.7*, 296.80, 296.81, 296.89, 298B	296.*, 298.1*
Compulsive disorder	F42*	300.3	300.3
ADHD	F90*	314*	NA
Affective disorder	F38*, F39*	NA	NA
Schizophrenia	F20*, F22*, F23*, F24*, F25*, F28*, F29*	295*, 297*, 298*, 298C, 298E, 298W, 298X	295*, 297*, 298.2*, 298.3*, 298.9*, 299*
Schizoid Personality Disorder	F60.1	301.2*	NA

Notes: ICD: International Classification of Diseases; ASD: autism spectrum disorder; AD: autistic disorder; PDD-NOS: pervasive developmental disorder-not otherwise specified; ADHD: attention deficit hyperactivity disorder; NA, not available; * at the end of a diagnosis code indicates all sub-categories.

Table S2a.   Relative risks for **ASD** among participants with exposed **maternal** uncle(s)/aunt(s) compared to participants with unexposed **maternal** uncle(s)/aunt(s): secondary exposures.

	Person-years of follow-up		Rates of ASD per 100,000 person-years		RR (95% confidence interval)[#]		
	Exposed	Unexposed	Exposed	Unexposed	Crude	Adjusted 1	Adjusted 2
Exposure: AD							
Affected maternal uncle(s)	6,577	6,688,618	547.32	151.42	4.24 (2.91-5.71)	3.59 (2.47-4.82)	2.56 (1.74-3.48)
Affected maternal aunt(s)	3,523	6,344,076	510.97	152.80	3.84 (2.16-5.76)	3.43 (1.93-5.12)	2.59 (1.48-3.97)
Affected maternal uncle(s)/aunt(s)	10,100	13,032,695	534.64	152.09	4.10 (3.12-5.21)	3.55 (2.71-4.49)	2.61 (1.95-3.34)
Exposure: ASD/SZ/ID/SPD							
Affected maternal uncle(s)	85,999	6,609,197	265.12	150.34	1.90 (1.66-2.16)	1.79 (1.56-2.04)	1.27 (1.10-1.48)
Affected maternal aunt(s)	60,903	6,286,696	249.58	152.07	1.74 (1.48-2.04)	1.67 (1.41-1.95)	1.25 (1.05-1.48)
Affected maternal uncle(s)/aunt(s)	146,902	12,895,893	258.68	151.18	1.83 (1.66-2.02)	1.74 (1.58-1.92)	1.27 (1.13-1.41)

Notes: ASD: autism spectrum disorder; AD: autistic disorder; SZ: schizophrenia; ID: intellectual disability; SPD: schizoid personality disorder; RR: relative risk; CI: confidence interval; Adjusted 1: Adjusted for birth year of the participant, the mother, and the maternal uncle/aunt; Adjusted 2: Adjusted for covariates in Adjusted 1 and any mental illness (yes/no) of the mother and the maternal uncle/aunt; [#]2.5%-97.5% percentiles of estimates from 1000 bootstrapped samples.

Table S2b.   Relative risks for **ASD** among participants with exposed **paternal** uncle(s)/aunt(s) compared to those with unexposed **paternal** uncle(s)/aunt(s): secondary exposures.

	Person-years of follow-up		Rates of ASD per 100,000 person-years		RR (95% confidence interval) [#]		
	Exposed	Unexposed	Exposed	Unexposed	Crude	Adjusted 1	Adjusted 2
Exposure: AD							
Affected paternal uncle(s)	4,624	6,731,914	302.74	153.42	2.33 (1.21-3.64)	2.06 (1.08-3.23)	1.62 (0.84-2.53)
Affected paternal aunt(s)	2,153	6,297,597	278.63	151.99	2.04 (0.64-3.74)	1.86 (0.57-3.36)	1.48 (0.45-2.73)
Affected paternal uncle(s)/aunt(s)	6,778	13,029,512	295.08	152.73	2.24 (1.32-3.33)	2.00 (1.18-2.96)	1.59 (0.94-2.36)
Exposure: ASD/SZ/ID/SPD							
Affected paternal uncle(s)	81,827	6,654,711	219.98	152.7	1.52 (1.32-1.75)	1.46 (1.26-1.68)	1.15 (0.99-1.34)
Affected paternal aunt(s)	62,370	6,237,381	240.5	151.15	1.65 (1.39-1.93)	1.60 (1.35-1.88)	1.33 (1.10-1.58)
Affected paternal uncle(s)/aunt(s)	144,197	12,892,092	228.85	151.95	1.58 (1.41-1.75)	1.52 (1.36-1.69)	1.23 (1.08-1.39)

Notes: ASD: autism spectrum disorder; AD: autistic disorder; SZ: schizophrenia; ID: intellectual disability; SPD: schizoid personality disorder; RR: relative risk; CI: confidence interval; Adjusted 1: Adjusted for birth year of the participant, the father, and the paternal uncle/aunt; Adjusted 2: Adjusted for covariates in Adjusted 1 and any mental illness (yes/no) of the father and the paternal uncle/aunt; [#] 2.5%-97.5% percentiles of estimates from 1000 bootstrapped samples.

Table S3a. Relative risks for **AD** among participants with exposed **maternal** uncle(s)/aunt(s) compared to those with unexposed **maternal** uncle(s)/aunt(s).

	Person-years of follow-up		Rates of ASD per 100,000 person-years		RR (95% confidence interval)[#]		
	Exposed	Unexposed	Exposed	Unexposed	Crude	Adjusted 1	Adjusted 2
Exposure: ASD							
Affected maternal uncle(s)	19,729	6,688,867	293.99	97.10	3.53 (2.64-4.47)	2.76 (2.07-3.49)	1.92 (1.43-2.44)
Affected maternal aunt(s)	10,031	6,350,325	209.36	97.54	2.53 (1.48-3.69)	1.99 (1.17-2.90)	1.46 (0.83-2.11)
Affected maternal uncle(s)/aunt(s)	29,759	13,039,192	265.46	97.31	3.20 (2.52-3.94)	2.52 (1.99-3.10)	1.80 (1.42-2.24)
Exposure: AD							
Affected maternal uncle(s)	6,608	6,701,988	408.60	97.37	4.77 (3.05-6.67)	3.74 (2.41-5.29)	2.65 (1.73-3.80)
Affected maternal aunt(s)	3,533	6,356,823	396.30	97.55	4.57 (2.34-7.26)	3.79 (1.93-6.00)	2.89 (1.46-4.54)
Affected maternal uncle(s)/aunt(s)	10,141	13,058,811	404.31	97.46	4.70 (3.37-6.22)	3.79 (2.70-5.02)	2.77 (1.99-3.72)
Exposure: ASD/SZ/ID/SPD							
Affected maternal uncle(s)	86,233	6,622,363	186.70	96.52	2.05 (1.73-2.42)	1.87 (1.59-2.21)	1.33 (1.11-1.60)
Affected maternal aunt(s)	61,071	6,299,285	178.48	96.93	1.93 (1.56-2.33)	1.81 (1.46-2.18)	1.38 (1.11-1.70)
Affected maternal uncle(s)/aunt(s)	147,304	12,921,647	183.29	96.72	2.00 (1.75-2.25)	1.85 (1.61-2.08)	1.37 (1.19-1.55)

Notes: ASD: autism spectrum disorder; AD: autistic disorder; SZ: schizophrenia; ID: intellectual disability; SPD: schizoid personality disorder; RR: relative risk; CI: confidence interval; Adjusted 1: Adjusted for birth year of the participant, the mother, and the maternal uncle/aunt; Adjusted 2: Adjusted for covariates in Adjusted 1 and any mental illness (yes/no) of the mother and the maternal uncle/aunt; [#] 2.5%-97.5% percentiles of estimates from 1000 bootstrapped samples.

Table S3b.   Relative risks for **AD** among participants with exposed **paternal** uncle(s)/aunt(s) compared to participants with unexposed **paternal** uncle(s)/aunt(s).

	Person-years of follow-up		Rates of ASD per 100,000 person-years		RR (95% confidence interval) [#]		
	Exposed	Unexposed	Exposed	Unexposed	Crude	Adjusted 1	Adjusted 2
Exposure: ASD							
Affected paternal uncle(s)	13,314	6,736,933	210.30	97.64	2.53 (1.65-3.52)	2.01 (1.31-2.78)	1.49 (0.98-2.12)
Affected paternal aunt(s)	7,356	6,305,409	122.35	96.03	1.49 (0.54-2.40)	1.18 (0.42-1.92)	0.94 (0.35-1.50)
Affected paternal uncle(s)/aunt(s)	20,670	13,042,342	179.00	96.86	2.17 (1.47-2.91)	1.72 (1.17-2.31)	1.32 (0.90-1.78)
Exposure: AD							
Affected paternal uncle(s)	4,644	6,745,603	172.26	97.81	2.01 (0.74-3.55)	1.62 (0.60-2.84)	1.21 (0.45-2.17)
Affected paternal aunt(s)	2,164	6,310,601	138.61	96.04	1.58 (0.00-3.63)	1.34 (0.00-3.06)	1.05 (0.00-2.39)
Affected paternal uncle(s)/aunt(s)	6,808	13,056,204	161.56	96.96	1.88 (0.69-3.06)	1.54 (0.57-2.51)	1.18 (0.45-1.93)
Exposure: ASD/SZ/ID/SPD							
Affected paternal uncle(s)	82,018	6,668,230	160.94	97.09	1.74 (1.45-2.04)	1.62 (1.35-1.90)	1.24 (1.01-1.50)
Affected paternal aunt(s)	62,548	6,250,217	163.08	95.39	1.76 (1.45-2.11)	1.69 (1.39-2.04)	1.39 (1.12-1.69)
Affected paternal uncle(s)/aunt(s)	144,566	12,918,447	161.86	96.27	1.75 (1.52-1.97)	1.65 (1.44-1.87)	1.31 (1.13-1.50)

Notes: ASD: autism spectrum disorder; AD: autistic disorder; SZ: schizophrenia; ID: intellectual disability; SPD: schizoid personality disorder; RR: relative risk; CI: confidence interval; Adjusted 1: Adjusted for birth year of the participant, the father, and the paternal uncle/aunt; Adjusted 2: Adjusted for covariates in Adjusted 1 and any mental illness (yes/no) of the father and the paternal uncle/aunt; [#] 2.5%-97.5% percentiles of estimates from 1000 bootstrapped samples.

Table S4. **Sex specific** relative risks for **ASD** among participants with ASD-affected **maternal** uncle(s)/aunt(s) compared to participants with **maternal** uncle(s)/aunt(s) free from ASD diagnosis.

	Person-years of follow-up		Rates of ASD per 100,000 person-years		RR (95% confidence interval)[#]		
	Exposed	Unexposed	Exposed	Unexposed	Crude	Adjusted 1	Adjusted 2
ASD affected maternal uncle(s)							
Male	10,390	3,426,835	539.00	223.88	2.87 (2.16-3.61)	2.48 (1.86-3.11)	1.76 (1.30-2.25)
Female	9,272	3,248,699	248.05	74.28	4.22 (2.71-5.98)	3.50 (2.22-4.94)	2.29 (1.47-3.39)
ASD affected maternal aunt(s)							
Male	4,914	3,255,759	427.31	225.75	2.29 (1.38-3.33)	1.95 (1.18-2.82)	1.46 (0.88-2.14)
Female	5,068	3,081,856	256.43	75.54	4.41 (2.38-7.05)	3.92 (2.11-6.13)	2.66 (1.43-4.31)
ASD affected maternal uncle(s)/aunt(s)							
Male	15,304	6,682,594	503.14	224.79	2.68 (2.13-3.31)	2.30 (1.83-2.86)	1.69 (1.34-2.11)
Female	14,342	6,330,555	251.01	74.89	4.29 (3.00-5.76)	3.67 (2.53-4.95)	2.45 (1.71-3.32)

Notes: ASD: autism spectrum disorder; RR: relative risk; Adjusted 1: Adjusted for birth year of the participant, the mother, and the uncle/aunt; Adjusted 2: Adjusted for covariates in Adjusted 1 and any mental illness (yes/no) of the mother and the uncle/aunt; [#] 2.5%-97.5% percentiles of estimates from 1000 bootstrapped samples.

Table S5.   Relative risks for **ASD** among participants with ASD-affected **maternal** uncle(s)/aunt(s) compared to participants with **maternal** uncle(s)/aunt(s) free from ASD diagnosis: alternative reference group.

Groups compared	Person-years of follow-up	Rates of ASD per 100,000 person-years	Model RR (95% confidence interval)		
			Crude	Adjusted 1	Adjusted 2
Maternal ASD-affected aunt(s) versus Reference[##]	9,866 vs. 7,293,063	324.36 vs. 149.57	2.68 (1.90-3.80)	2.29 (1.62-3.24)	2.10 (1.49-2.98)
Maternal ASD-affected uncle(s) versus Reference[##]	18,868 vs. 7,293,063	386.89 vs. 149.57	3.19 (2.53-4.01)	2.71 (2.15-3.41)	2.48 (1.97-3.12)
Maternal uncle(s) versus Maternal aunt(s)	9,866 vs. 18,868	386.89 vs. 324.36	1.19 (0.78-1.80)	1.18 (0.78-1.79)	1.18 (0.78-1.78)

Notes: Maternal aunt(s): participants have ASD affected maternal aunt(s); Maternal uncle(s): participants who don't have ASD affected maternal aunt(s) but have ASD affected maternal uncle(s); [##]Reference: participants have maternal aunt(s)/uncle(s) free from ASD diagnosis.

Table S6.   Relative risks for **ASD** among participants with ASD-affected **maternal** uncle(s)/aunt(s) compared to participants with **maternal** uncle(s)/aunt(s) free from ASD diagnosis: oldest participant-uncle/aunt pair.

	Person-years of follow-up		Rates of ASD per 100,000 person-years		RR (95% confidence intervals)		
	Exposed	Unexposed	Exposed	Unexposed	Crude	Adjusted 1	Adjusted 2
ASD affected maternal uncle(s)	13,388	4,796,935	343.58	151.37	3.46 (2.53, 4.72)	2.83 (2.07, 3.86)	2.02 (1.46, 2.79)
ASD affected maternal aunt(s)	6,005	4,494,164	366.38	153.27	3.52 (2.19, 5.67)	2.80 (1.74, 4.52)	1.99 (1.23, 3.23)
ASD affected maternal uncle(s)/aunt(s)	13,832	7,202,898	354.26	151.56	3.55 (2.61, 4.82)	2.82 (2.07, 3.84)	2.0 (1.46, 2.74)

Notes: ASD: autism spectrum disorder; RR: relative risk; Adjusted 1: Adjusted for birth year of the participant, the mother, and the uncle/aunt; Adjusted 2: Adjusted for covariates in Adjusted 1 and any mental illness (yes/no) of the mother and the uncle/aunt.

Table S7.   Relative risks for **ASD** among participants with a sibling with ASD-affected **maternal** uncle(s)/aunt(s) compared to participants with **maternal** uncle(s)/aunt(s) free from ASD diagnosis: families with no psychiatric history (other than ASD).

	Person-years of follow-up		Rates of ASD per 100,000 person-years		RR (95% confidence interval)	
	Exposed	Unexposed	Exposed	Unexposed	Crude	Adjusted 1
ASD affected maternal uncle(s)	3,929	5,790,496	305.39	136.46	2.62 (1.49, 4.61)	2.22 (1.26, 3.92)
ASD affected maternal aunt(s)	1,129	5,413,052	265.63	138.85	2.34 (0.76, 7.24)	2.07 (0.67, 6.39)
ASD affected maternal uncle(s)/aunt(s)	5,059	11,203,548	296.51	137.62	2.58 (1.56, 4.26)	2.19 (1.32, 3.64)

Notes: ASD: autism spectrum disorder; RR: relative risk; Adjusted 1: Adjusted for birth year of the participant, the mother, and the uncle/aunt.

Table S8. Relative risks for **ASD** among participants with exposed **maternal** uncle(s)/aunt(s) compared to participants with unexposed uncle(s)/aunt(s): adjusted for birth year of the participant, the mother, and the maternal uncle/aunt; and specific psychiatric disorders (intellectual disability, depression, anxiety disorders, substance use disorders, Bipolar disorder, compulsive disorder, ADHD, affective disorders, schizophrenia, and schizoid personality disorder) of the mother and the maternal uncle/aunt.

Exposure	Offspring	RR (95% confidence interval)[#]		
		Affected maternal uncle(s)	Affected maternal aunt(s)	Affected maternal uncle(s)/aunt(s)
ASD	All	2.07 (1.63, 2.63)	1.58 (1.10, 2.26)	1.89 (1.54, 2.32)
AD	All	3.14 (2.26, 4.38)	2.98 (1.87, 4.75)	3.08 (1.90, 3.37)
ASD/SZ/ID/SPD	All	1.56 (1.36, 1.79)	1.45 (1.22, 1.71)	1.51 (1.36, 1.68)

Notes: ASD: autism spectrum disorder; RR: relative risk; [#]2.5%-97.5% percentiles of estimates from 1000 bootstrapped samples.

Table S9.   Relative risks for **ASD** among participants with ASD-affected **maternal** uncle(s)/aunt(s) compared to participants with **maternal** uncle(s)/aunt(s) free from ASD diagnosis: participants with ASD-free mothers.

	Person-years of follow-up		Rates of ASD per 100,000 person-years		RR (95% confidence interval)		
	Exposed	Unexposed	Exposed	Unexposed	Crude	Adjusted 1	Adjusted 2
ASD affected maternal uncle(s)	19,486	6,672,585	390.03	151.34	3.12 (2.49, 3.91)	2.68 (2.14, 3.36)	1.88 (1.49, 2.38)
ASD affected maternal aunt(s)	9,863	6,334,336	294.02	153.23	2.37 (1.65, 3.41)	2.05 (1.43, 2.95)	1.51 (1.04, 2.18)
ASD affected maternal uncle(s)/aunt(s)	29,349	13,006,922	357.76	152.26	2.87 (2.37, 3.47)	2.47 (2.04, 2.99)	1.78 (1.46, 2.17)

Notes: ASD: autism spectrum disorder; RR: relative risk; Adjusted 1: Adjusted for birth year of the participant, the mother, and the uncle/aunt; Adjusted 2: Adjusted for covariates in Adjusted 1 and any mental illness (yes/no) of the mother and the uncle/aunt.

Table S10.   Relative risks for **ASD** among participants with ASD-affected **maternal** uncle(s)/aunt(s) compared to participants with **maternal** uncle(s)/aunt(s) free from ASD diagnosis: participants with ASD-affected mothers.

	Person-years of follow-up		Rates of ASD per 100,000 person-years		RR (95% confidence interval)		
	Exposed	Unexposed	Exposed	Unexposed	Crude	Adjusted 1	Adjusted 2
ASD affected maternal uncle(s)	176	2949	1,701.74	780.03	2.71 (0.81, 9.15)	3.01 (0.86, 10.56)	3.82 (0.74, 19.75)
ASD affected maternal aunt(s)	121	3279	4,142.50	670.99	6.42 (2.42, 17.02)	5.56 (2.07, 14.91)	6.41 (1.83, 22.42)
ASD affected maternal uncle(s)/aunt(s)	297	6227	2,693.69	722.62	4.39 (2.05, 9.36)	4.28 (1.99, 9.23)	5.23 (1.94, 14.11)

Notes: ASD: autism spectrum disorder; RR: relative risk; Adjusted 1: Adjusted for birth year of the participant, the mother, and the uncle/aunt; Adjusted 2: Adjusted for covariates in Adjusted 1 and any mental illness (yes/no) of the mother and the uncle/aunt.

Table S11.   Relative risks for **ASD** among participants with ASD-affected uncle(s)/aunt(s) compared to participants with uncle(s)/aunt(s) free from ASD diagnosis: using Firth correction.

	RR (95% Confidence interval) [#]		
	Crude	Adjusted 1	Adjusted 2
Maternal lineage			
ASD-affected uncle(s)	3.24 (2.6, 4.04)	2.77 (2.22, 3.45)	1.94 (1.54, 2.43)
ASD-affected aunt(s)	2.77 (1.99, 3.87)	2.39 (1.71, 3.34)	1.75 (1.25, 2.46)
ASD-affected uncle(s)/aunt(s)	3.07 (2.55, 3.69)	2.63 (2.19, 3.17)	1.89 (1.56, 2.28)
Paternal lineage			
ASD-affected uncle(s)	2.42 (1.78, 3.29)	2.12 (1.55, 2.89)	1.65 (1.2, 2.26)
ASD-affected aunt(s)	1.58 (0.94, 2.63)	1.37 (0.82, 2.3)	1.11 (0.66, 1.86)
ASD-affected uncle(s)/aunt(s)	2.1 (1.61, 2.74)	1.84 (1.41, 2.4)	1.46 (1.11, 1.91)

Notes: ASD: autism spectrum disorder; RR: relative risk; CI: confidence interval; Adjusted 1: Adjusted for birth year of the participant, the mother, and the uncle/aunt; Adjusted 2: Adjusted for covariates in Adjusted 1 and any mental illness (yes/no) of the mother and the uncle/aunt; [#] CIs using the Firth correction for monotone likelihood

Figure S1   Example pedigree for analytic sample identification
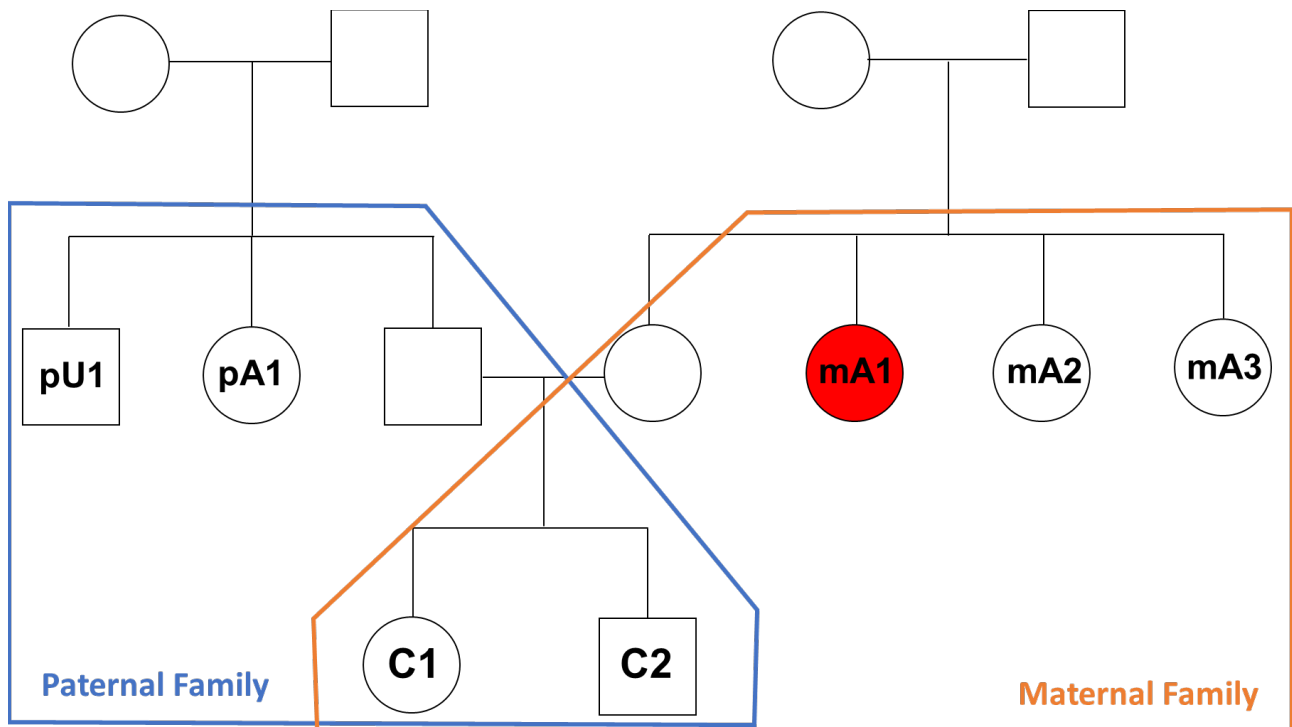
Notes:

Circle denotes for female and square denotes for male;

Red item denotes for individual diagnosed with ASD;

C1, C2 are cohort children born between 2003-2012;

Paternal family (in the blue pentagon) and maternal family (in the orange pentagon): mA1, mA2, mA3-maternal aunts, pA1-paternal aunt; pU1-paternal uncle

Figure S2   Age specific ASD prevalence estimated through inverse Kaplan-Meier survival curves
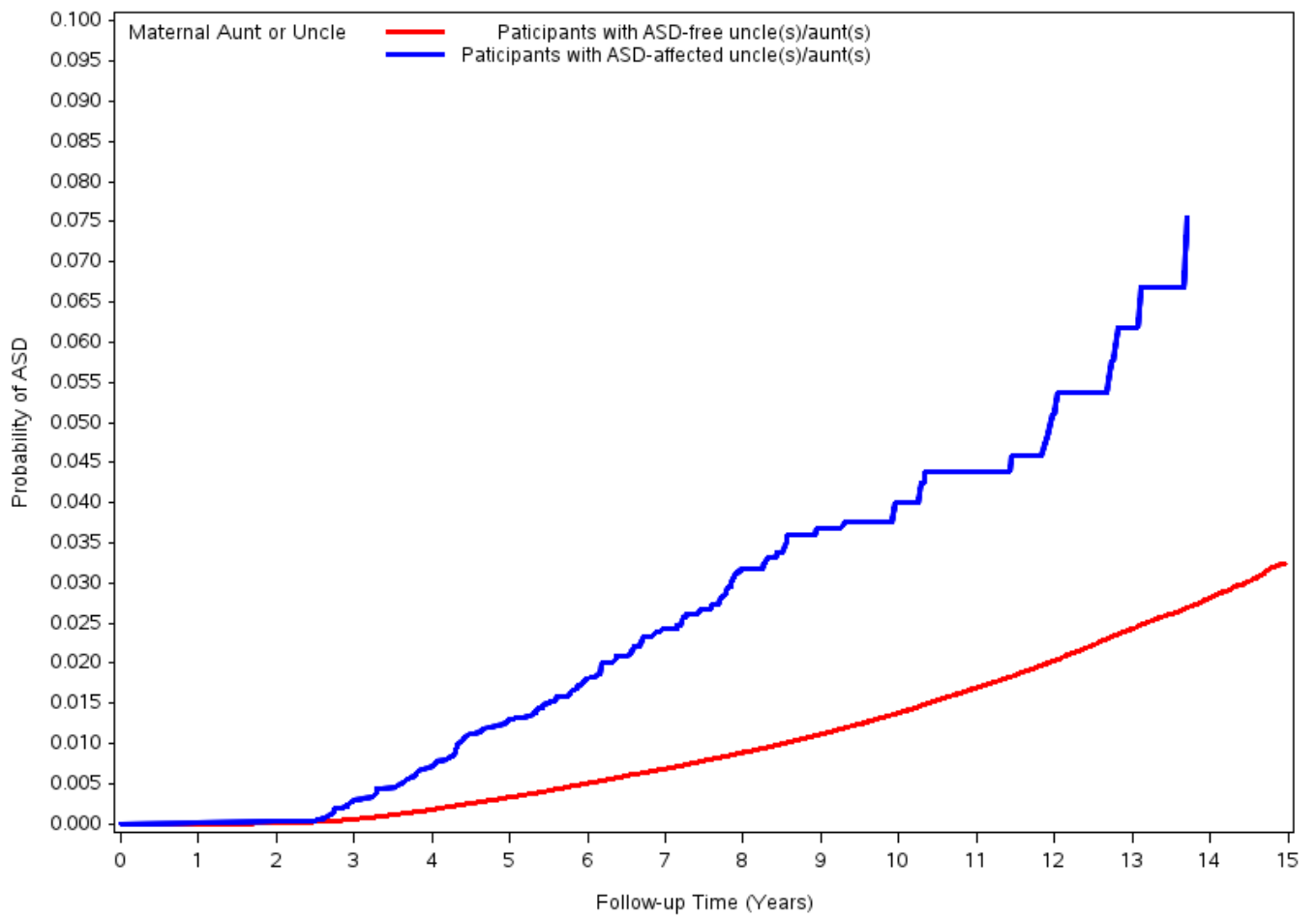
Pseudo SAS Program for Primary Analysis

```
*----------------------------------------------------------------------;
* Pseudo SAS codes for main analysis: Cox model, on offspring-uncle/aunt pair level ;
*----------------------------------------------------------------------;


**---------------------------------------------------------------------;
** Example on maternal lineage:                                        ;
** data name: maternal (including maternal uncles and aunts)           ;
** cid - ID of offspring                                               ;
** mid - ID of mother of specific offspring                            ;
** eid - ID of specific maternal uncle/aunt                            ;
** outcome - ASD (primary), AD (secondary)                             ;
** years - years to outcome diagnosis                                  ;
** event - diagnosis with outcome (ASD/AD): 1=yes, 0=no                ;
** censored - indicator of censoring (1=censor, 0=event)               ;
** mexposure - for specific uncle/aunt: 1=with ASD diagnosis, 0=without ASD diagnosis  ;
** kid_birthyr - birth year of offspring                               ;
** mom_birthyr - birth year of mother                                  ;
** exp_birthyr - birth year of specific uncle/aunt                     ;
** mom_psych - for mother at birth date of specific offspring: 1=with any psychiatric ;
**             disorder, 0=without any psychiatric disorder            ;
** exp_psych - for specific uncle/aunt at birth date of specific offspring: 1=with any ;
**             psychiatric disorder, 0=without any psychiatric disorder ;
**---------------------------------------------------------------------;


**---------------------------------------------------------------------------;
** Example lines of data 'maternal':                                        ;
** cid mid eid outcome years event censored mexposure kid_birthyr mom_birthyr exp_birthyr mom_psych exp_psych ;
** *** *** *** ASD     7.89 1     0        1          2004        1974        1983        0         0         ;
** *** *** *** ASD     8.95 0     1        0          2006        1977        1985        0         0         ;
** *** *** *** AD      9.01 0     1        0          2005        1977        1975        0         0         ;
**---------------------------------------------------------------------------;
```

```
**--------------------------------------------------------------------------;
**   Calculate person-years and ASD/AD rate by exposure: ASD maternal aunt/uncle         ;
**--------------------------------------------------------------------------;

proc sort data=maternal; by outcome mexposure; run; /* sort data by outcome AD/ASD and exposure*/
ods output summary=personyr_ASD_maternal;
proc means data=maternal sum;
class mexposure;
var years event; /* event=1 for ASD diagnosis , years=time to ASD diagnosis*/
by outcome mexposure;
run;
data personyr_maternal;
drop vname_years vname_event ;
length exposure $30.;
set personyr_ASD_masib(rename=(mexposure=exposed) drop=mexposure2);
exposure='ASD Maternl Aunt/Uncle';
yr_fu=put(years_sum, comma15.2); /* Total years of follow up */
ASD_N=put(event_sum, comma8.); /* Total N of ASD diagnosed participants */
ASD_100k_py=put(event_sum/years_sum*100000, comma8.2); /* prevalence per 100k person years */
run;


**--------------------------------------------------------------------------;
** Cox Model: 1) Crude HR                                                    ;
**            2) Adjusted: birth year of participant (offspring), mother, uncle/aunt  ;
**            3) Adjusted: 2)+ uncle/aunt's any psych + mother's any psych           ;
**--------------------------------------------------------------------------;

/* Model 1: Crude */
ods output estimates=crude;
proc phreg data=maternal nosummary;
class outcome / param=glm order=internal;
model years*censored(1) = mexposure / alpha=0.05 risklimit=wald NODUMMYPRINT;
by outcome; /* Output by outcome AD/ASD */
estimate "Model1_Crude" mexposure -1 1 / exp alpha=0.05; /* Output point estimate of HR */
run;
```

```sas
/* Model 2: Adjusted 1 */
ods output estimates=adjust1;
proc phreg data=maternal nosummary;
  baseline /method=EMP;
  class  outcome /order=internal;
  effect spl = spline(kid_birthyr mom_birthyr exp_birthyr/ naturalcubic); /* use natural cubic spline
for birth year */
  model years * censored(1) = mexposure kid_birthyr mom_birthyr exp_birthyr spl/NODUMMYPRINT; /*
Adjusted for birth year of participant, mother and uncle/aunt */
  by outcome; /* Output by outcome AD/ASD */
  estimate "Model2_Adjusted1" mexposure -1 1/exp alpha=0.05; /* Output point estimate of HR */
run;


/* Model 3: Adjusted 2 */
ods output estimates=adjust2;
proc phreg data=maternal nosummary;
  baseline /method=EMP;
  class  outcome mom_psych exp_psych/order=internal;
  effect spl = spline(kid_birthyr mom_birthyr exp_birthyr/ naturalcubic); /* use natural cubic spline
for birth year */
  model years * censored(1) = mexposure kid_birthyr mom_birthyr exp_birthyr mom_psych exp_psych
spl/NODUMMYPRINT;
/* Adjusted for birth year of participant, mother and uncle/aunt; mother's psychiatry condition at
participant's birth, uncle/aunt's psychiatry condition at participant's birth */
  by outcome; /* Output by outcome AD/ASD */
  estimate "Model2_Adjusted1" mexposure -1 1/exp alpha=0.05; /* Output point estimate of HR */
run;
```