Supplementary Materials

The phenotypic variability of 16p11.2 distal BP2-3 deletion in a transgenerational family and in neurodevelopmentally ascertained samples

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Supplementary Note

Clinical workup of family 1-0616

The second-born was followed through his early years and no neurodevelopmental diagnosis was given. He was reassessed at 8 years and 6 months and had Autism Diagnostic Observation Schedule-2 (ADOS-2) scores consistent with an ASD diagnosis (calibrated severity score (CSS) = 5). The third-born male (-005) was followed from birth to toddlerhood and assessed for ASD and other neurodevelopmental conditions in 6-month intervals. His assessment data are available from 6 months to 2 years of age (May 2012 –2013) and 4.4 years (2016). Given the associated risk for obesity in 16p11.2 distal deletion syndrome, the second-born (-004) and third-born (-005) sons were followed by SickKids Team Obesity Management Program (STOMP)[1]. Annual data from this clinic are available from 5.5 years (2013) to 9 years (2017) for the second-born son (-004) and 5 years (2014) to 7 years (2019) for the third-born son (-005).

ASD-related assessments

In addition to the ADOS [2 3], the proband (-003) and siblings (-004, -005) were evaluated using the Social Responsiveness Scales (SRS), Social Communication Questionnaire-Lifetime (SCQ-Lifetime)[4] and the Eyes Test [5]. The proband was additionally administered the Autism Diagnostic Interview-Revised (ADI-R)[6] and the NEuroPSYchological (NEPSY)[7] developmental assessment.

Cognitive and behavioral assessments

The family underwent cognitive assessments using the age appropriate version of the Wechsler scale (WPPSI-III, WISC-V) or the Stanford-Binet Test. Language skills were assessed with the Oral and Written Language Scales (OWLS-II)[8] and Child's Communication Checklist-2 (CCC-2)[9]; adaptive functioning was assessed using the Vineland Adaptive Behavior Scales-II (VABS-II), by way of interview with their mother.

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First (-003) and third-born (-005) males were evaluated for anxiety using the parent-reported versions of the Revised Children's Anxiety and Depression Scale[10] and Spence Children's Anxiety Scale[11], respectively. Attention deficits were evaluated using the Strength and Weaknesses of Attention-Deficit/Hyperactivity-symptoms and Normal-behaviors (SWAN)[12] in the proband and the Conners Comprehensive Behavior Rating Scales[51] (teacher report) and Conners Continuous Performance Test-3[13] in the middle child (-004). General behaviour and the possibility of psychiatric comorbidity were measured using the Child Behavior Checklist (CBCL)[14]. Body measurements, including height, weight and head circumference, were recorded using standard procedures.

Both parents also completed measures of cognition (WASI-II), social function and communication (Broad Autism Phenotype Questionnaire [15]; Social Communication Questionnaire; Communication Checklist-Adult[16], Social Phobia and Anxiety Inventory[17], Wisconsin Card Sorting Test [18], Toronto Alexithymia Scale-20 [19 20]). Based upon self-reported significant social impairments, the mother was also assessed for ASD by a detailed diagnostic assessment completed by an expert clinician (MWS) and facilitated completion of the ADOS module 4. In addition to formal measures, parents were also asked of their own and their children's medical and psychiatric history. Where appropriate, family consent was sought to consult medical records.

Methods: microarray and whole genome sequencing analysis

All genetic analyses were conducted according to standard procedures, as previously described [21 22]. Briefly, microarray was performed using CytoscanHD®[23]. Copy number variants were called using four algorithms: Affymetrix Chromosome Analysis Suite (ChAS; Thermo Fisher Scientific, Inc.; Waltham, MA, USA), iPattern[24], Nexus[25], and Partek[26]. A stringent set of CNVs was defined as those detected by one or both of ChAS or iPattern, and, if detected by only one of these, then also by one of Nexus or Partek. Algorithms ChAS and iPattern were used to identify stringent calls on chromosome X. To reduce false positives, only

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CNVs called with a minimum of 5 probes were analyzed, with a minimum length cutoff of 15 kb. We also removed all CNVs that had >70% overlap with a known segmental duplication. We further restricted our list to those with more than 75% overlap with copy number stable regions, according to the stringent CNV map of the human genome[27]. CNVs were filtered to prioritize rare variants that occurred with a frequency of <0.1% in control samples (N=10,851). All CNVs described in the index family have been validated using the TaqMan© copy number assay. The genomic coordinates presented in this paper are based on the February 2009 Human Genome Build (GRCh37/hg19).

The proband, his siblings and both parents were sequenced on the Illumina HiSeq X, as described previously[1] (Supplementary Tables 1 & 2). We prioritized rare (≤0.1% minor allele frequency), putatively damaging ASD-relevant variants for reporting. A maximum minor allele frequency was ascertained from 5 datasets from the MSSNG portal: ExAC 65000, from Genome Aggregation Database (gnomAD) for exome and genome, subjects sequenced on the Complete Genomics platform in the MSSNG dataset and 1000 genomes phase 3. Rare stop-gain, frameshift, high-impact splice-site (+/- 2 bp from exon boundary) and high-impact *de novo* missense sequence variants were classified as putatively damaging. Missense variants were classified as high-impact if they met four of the following criteria: SIFT[28] ≤0.05, Polyphen-2[29] ≥0.95, CADD[30] ≥15, MutationAssessor[31] score ≥2, placental mammal PhyloP ≥2.4 and vertebrate PhyloP ≥4[32 33]. Rare, deleterious small variants (single-nucleotide and insertions/deletions <50 bp) and CNVs were identified as ASD-relevant, and subsequently prioritized for reporting, if they overlapped known ASD-risk loci as previously reported in [21] (full list in Supplementary Table 3), which includes gene lists collated from large-scale sequencing efforts of individuals with ASD[21 34-38]. Guidelines from the American College of Human Genetics were used to classify variants[39 40]. Polygenic risk scores for all three male siblings were derived as previously described[22].

Supplementary Table 4. Summary genomic and phenotypic findings for family 1-0616

Family 1-0616	-001	-002	-003	-004	-005
Sex	Female (XX)	Male (XY)	Male (XY)	Male (XY)	Male (XY)
Pathogenic genetic findings (microarray + whole genome sequencing)	16p11.2, 227 kb, del[mat]/+	16p11.2, +/+	16p11.2, 227 kb, del[mat]/+	16p11.2, 227 kb, del[mat]/+	16p11.2, 227 kb, del[mat]/+
Morphology	HC = 57.2 cm; Ht = 156.9 cm; Wt = 60.3 kg;	HC = 58 cm; Ht = 184 cm; Wt = 93.3 kg;	HC = 53 cm; Ht = 149 cm; Wt = 31 kg;	HC = 54 cm; Ht = 142 cm; Wt = 55.6 kg;	HC = 53 cm; Ht = 120.5 cm; Wt = 30.7 kg;
Medical	Nil	Nil	Nil	Obesity	Obesity
IQ[1] measures: FSIQ; VIQ; NVIQ	WASI-II: 109; 113; 92	WASI-II: 122; 122; 117	Stanford-Binet: 78; 91; 68	WISC-V: 92; 106; 85	WPPSI-III: 117; 115; 113
Autism Spectrum diagnostic measures					
ADOS, module Domain scores	NA	NA	ADOS, Module 3 Com = 5 RSI = 12 Com + RSI = 17 Imag/Creat = 1 SB/RI=4 ADOS Dx = autism	ADOS-2, Module 3 Soc Affect = 6 RRB = 2 Overall total = 8 Comparison score = 5 (moderate) ADOS Dx = autism spectrum	ADOS-2, Module 3 Soc Affect = 3 RRB = 3 Overall total = 6 Comparison score = 3 (low) ADOS Dx = non-spectrum
SRS version Scores	SRS-2 Adult RRB = 51 SCI = 61 Total T-score = 59	NA	SRS Total T-score = 73	SRS RRB = 43 SCI = 51 Total T-score = 49	SRS Total T-score = 57
SCQ version Score	NA	NA	SCQ-Lifetime Total: 19 (above cut-off)	SCQ-Lifetime Total: 1 (below cut-off)	SCQ-Lifetime Total: 1 (below cut-off)

Eyes test Score	Eyes Test (Adult) Total correct: 25	Eyes Test (Adult) Total correct: 31	Eyes Test (Child) Total correct: 15	Eyes Test (Child) Total correct: 15	NA
ADI-R Subdomain scores	NA	NA	ADI-R Social domain = 24 Verbal com = 20 RRSB = 8 ADI-R Dx = Autism	NA	NA
BAP-Q Subscale mean	BAP-Q (self-report) Aloof = 4.42 Pragmatic = 2.42 Rigidity = 3.12 Total = 3.32	BAP-Q (self-report) Aloof = 3.25 Pragmatic = 3.92 Rigidity = 3.08 Total = 3.42	NA	NA	NA
Academic					
Wechsler Individual Achievement Test Score	NA	NA	NA	WIAT-III Basic reading = 80 (word read = 86; pseudoword = 72; spell = 101) Basic Math = 93 (num op = 97; prob solv = 91)	NA
Adaptive					
Vineland Adaptive Behavior Scales – II Score	NA	NA	VABS-II	VABS- II	NA

			Adaptive Behaviour Composite = 82 Communication = 94 Daily Living Skills = 78 Socializations = 80	Adaptive Behavior Composite = 88 Communication = 96 Daily Living Skills = 83 Socializations = 92	
Adaptive Behaviour Assessment System Scores	NA	NA	ABAS - II General Adaptive Composite = 68 Conceptual = 83 Social = 61 Practical = 78	NA	NA
Anxiety					
Test	Social Phobia and Anxiety Inventory	Social Phobia and Anxiety Inventory	Revised Children's Anxiety and Depression Scale (Parent)	NA	Spence Children's Anxiety Scale (Parent)
Scores, subscales and/or commentary	Panic unlikely Possible social phobia	Panic unlikely Possible social phobia	Anxiety total = 64 (T-score) Depression total = 65 (T-score) Separation anxiety total = 77 (T-score)		Generalized Anxiety/Overanxious Disorder total = 4 Separation Anxiety total = 3 Obsessive-Compulsive total = 0 Panic Attack and Agoraphobia total = 0 Physical Injury Fears total = 3 Total score = 12

					Spence Dx = within normal limits; Generalized Anxiety category slightly elevated
Attention measure					
Test Scores, subscale and/or commentary	NA	NA	Strengths and Weaknesses of Attention-Deficit / Hyperactivity - Symptoms and Normal-behaviours (SWAN) ADHD hyperactive Impulsive total = 1 ADHD inattentive total = 5 ODD total = 2 ADHD clinical range = below ODD clinical range = below	Conners Comprehensive Behaviour Rating Scales (Teacher) Responder comments: weight, low endurance, lack of mobility, kind, compliant, tries his best	NA
Test Commentary	NA	NA	NA	Conners Continuous Performance Test - 3 speed over accuracy; moderate likelihood of having a diagnosis characterized by attention deficits;	NA

				inattentiveness (strong indication); vigilance (strong indication); no indication of impulsivity	
Executive					
Wisconsin Card Sorting Test Score	WCST Number errors total = 103 Perseverative Responses = 87 Perseverative Errors total = 87 Nonperseverative Errors = 105 Conceptual Level Responses = 101 Number of Categories Completed = 4 Trials to Complete First Category = 12 Failure to Maintain Set = 1 Learning to Learn = >16th %ile	WCST Number errors total = 103 Perseverative Responses = 97 Perseverative Errors total = 99 Nonperseverative Errors = 102 Conceptual Level Responses = 106 Number of Categories Completed = 5 Trials to Complete First Category = 11 Failure to Maintain Set = 0 Learning to Learn = <16th %ile	WCST Number errors total = 76 Perseverative Responses = 81 Perseverative Errors total = 81 Nonperseverative Errors = 78 Conceptual Level Responses = 81 Number of Categories Completed = 2 Trials to Complete First Category = 11 Failure to Maintain Set = 8 Learning to Learn = 2 - 5th%ile	WCST Number errors total = 69 Perseverative Responses = 88 Perseverative Errors total = 87 Nonperseverative Errors = 65 Conceptual Level Responses = 72 Number of Categories Completed = 0 Trials to Complete First Category = 65 Failure to Maintain Set = 0 Learning to Learn = n/a	NA
A Developmental NEuroPSYchological Assessment	NA	NA	NEPSY - II	NA	NA

			Speeded naming: completion time = 7 (Borderline) Inhibition (naming/inhibition/ switch): completion time = average		
Language measures					
Test Scores	NA	NA	OWLS Receptive = 50 Expressive = 78 Total = 62	OWLS-II Receptive = 98 Expressive = 88 Total = 92	NA
Test Scores and commentary	CC-A Lang Structure = 7 Pragmatic Diff = 13 Engagement = 4 Total = (10%ile)	CC-A Lang Structure = 14 Pragmatic Diff = 9 Engagement = 10 Total = (>50%ile)	NA	CCC-2 Social interaction Deviance Composite = -6 General communication Composite = 93 Communication profile suggestive of ASD	CCC-2 Social interaction Deviance Composite = 10 General communication Composite = 92
Comprehensive Test of Phonological Processing Score	NA	NA	NA	CTOPP-2 Phonological Awareness = 92	NA

				Phonological Memory = 85 Alt. Phonological Awareness = 95 *difficulty blending words	
General behaviour measures					
Child Behaviour Checklist (CBCL) version Domain T-scores	CBCL Adult	NA	CBCL for ages 6 - 18: 2001 Withdrawn/depressed total = 66 Somatic complaints total = 57 Externalizing problems total = 46 Internalizing problems total = 63 Anxious/depressed total = 59 Social problems total = 58 Total competence total = 26 Total problems total = 53 Aggressive behaviour total = 50	CBCL for ages 6 - 18: 2001 Withdrawn/depressed total = 66 Somatic complaints total = 64 Externalizing problems total = 65 Internalizing problems total = 68 Anxious/depressed total = 66 Social problems total = 56 Total competence total = NA Total problems total = 62 Aggressive behaviour total = 67	CBCL for ages 1 - 5: 2000 Withdrawn total = 51 Emotionally reactive total = 59 Somatic complaints total = 58 Externalizing problems total = 46 Internalizing problems total = 56 Anxious/depressed total = 52 Stress problems total = 50 Total problems total = 51 Aggressive behaviour total = 50

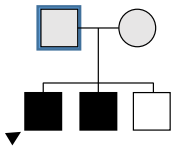
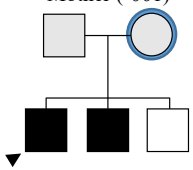
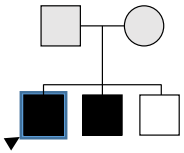
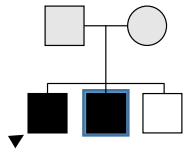
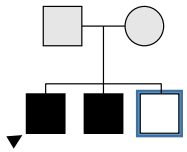
	General reported concerns: anxious depressed; on stress leave from work		Rule-breaking behaviour total = 51 Attention problems total = 57 Thought problems total = 58 Borderline concerns: NA General reported concerns: NA Description: NA	Rule-breaking behaviour total = 57 Attention problems total = 52 Thought problems total = 54 Borderline concerns reported by parents: anxious/depressed, aggressive behaviour, total problems, affective problems, oppositional defiant disorder General reported concerns: NA Description: NA	Attention problems total = 53 Sleep problems total = 53 Borderline concerns: NA General reported concerns: social skills, chewing on items, drooling Description: adorable, loving, smart
Symptom Checklist 90 Date Scores	SCL-90 April 2016 / Aug 2016 Somatization = 55/51 Obsessive-compulsive dimension = 65/62 Interpersonal	SCL-90 Somatization = 55 Obsessive-compulsive dimension = 55 Interpersonal Sensitivity = 58			

	Sensitivity = 81/74 Depression = 72/68 Anxiety = 61/59 Hostility = 60/60 Phobic Anxiety = 44/44 Paranoid Ideation = 71/62 Psychoticism = 72/65 Global Severity Index = 69/64 Positive Symptom Distress Index = 73/68 Positive Symptom total = 61/59 *Mom was more distressed in April - off work (transition)	Depression = 60 Anxiety = 60 Hostility = 65 Phobic Anxiety = 47 Paranoid Ideation = 59 Psychoticism = 58 Global Severity Index = 59 Positive Symptom Distress Index = 47 Positive Symptom total = 61			
Other Questionnaires:					
Toronto Alexithymia Scale Score	TAS-20 Total factor 1 score = 19 Total factor 2 score = 23 Total factor 3 score = 17 Total overall score = 59	TAS-20 Total factor 1 score = 8 Total factor 2 score = 10 Total factor 3 score = 18 Total overall score = 36			
Test					Relationship Questionnaire

Commentary					No close friends; somewhat shy when it comes to meeting new friends; gets along well with parents and teachers

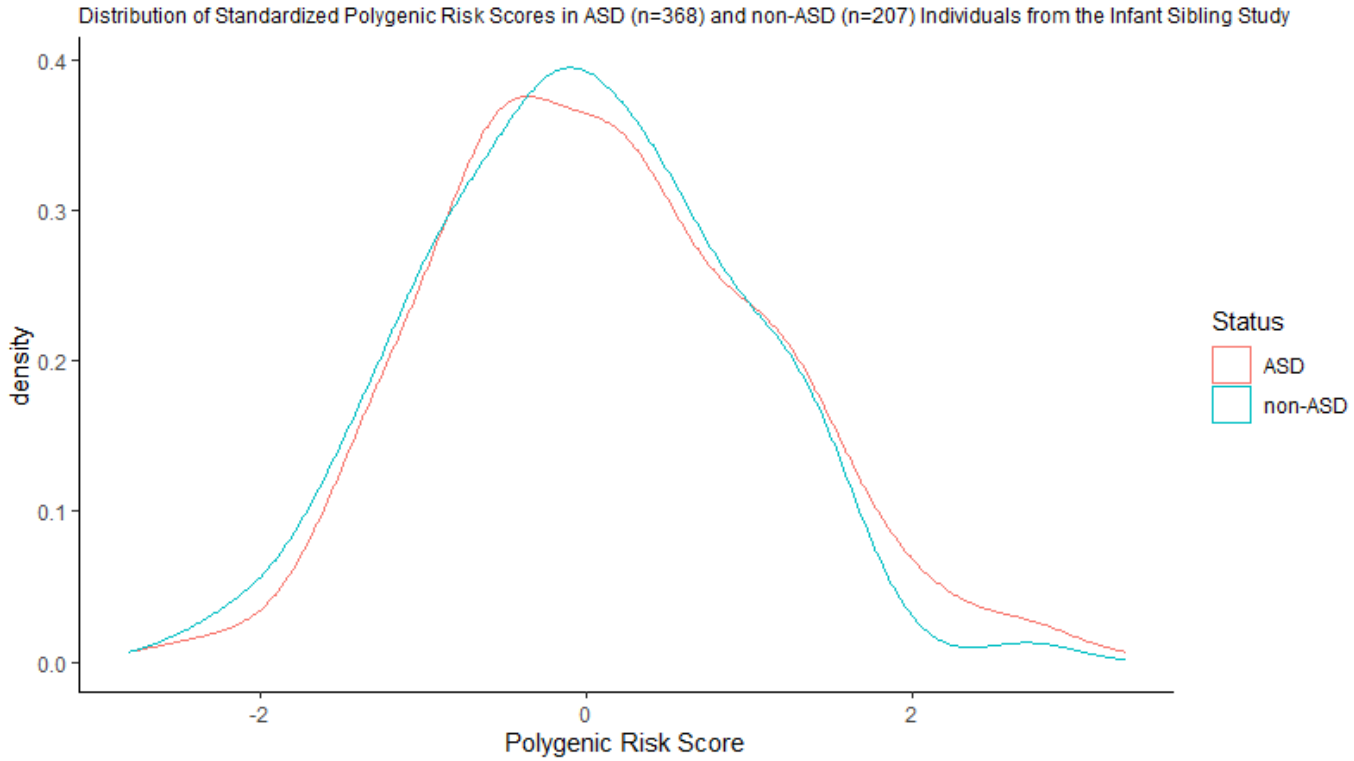
NA = not available; Nil = none; HC = head circumference; Ht = height; Wt = weight; Dx = diagnosis; ADOS = Autism Diagnostic Observation Schedule; Com = communication; RSI = reciprocal social interaction; Imag/Creat = imagination/creativity; SB/RI = social behavior/restricted interests; SRS = Social Responsiveness Scales; SCQ = Social Communication Questionnaire; ADI-R = Autism Diagnostic Interview; BAP = Broader Autism Phenotype; [1] IQ test administered is in parenthesis (see text for references); OWLS = Oral and Written Language Scales – II; CC-A = Communication Checklist-Adult; CCC-2 = Children's Communication Checklist-2.

Supplementary Table 5: Summary of genetic findings in family 1-0616

Family 1-0616	Pathogenic/Likely Pathogenic	Variants of Unknown Significance/Benign*
Father (-002) 	• 16p11.2 copy neutral +/+	• 2q21.2, *ANKRD30BL* and *GPR39* genes, gain, 637 kb, -/+
Mother (-001) 	• 16p11.2 distal deletion, 227 kb, del/+	• Xp22.31, KAL1, 107 kb gain,-/+ • *CACNA2D4*:NM_172364:exon19:c.C1882T:p.R628X, -/+
Proband (-003) 	• 16p11.2 distal deletion, 227 kb, del[mat]/+	• *ATF7IP2* (NM_001352120):c.-10998G>C, -/+, *de novo* • *CCAR1* (NM_001282959):exon16:c.G2083C:p.E695Q, -/+, *de novo* • Xp22.31, *KAL1,* 107 kb gain, -/+ • 2q21.2, *ANKRD30BL* and *GPR39* genes, gain, 637 kb, -/+ • *CACNA2D4* (NM_172364):exon19:c.C1882T:p.R628X, -/+
Sib 1 (-004) 	• 16p11.2 distal deletion, 227 kb, del[mat]/+	• Xp22.31, *KAL1,* 107 kb gain, -/+ • 2q21.2, *ANKRD30BL* and *GPR39* genes, gain, 637 kb, -/+
Sib 2 (-005) 	• 16p11.2 distal deletion, 227 kb, del[mat]/+	• 5p15.33 gain, 192 kb, -/+, *de novo* • *DXO* (NM_005510):exon2:c.G28A:p.A10T, -/+, *de novo* • *APPBP2* (NM_006380):exon11:c.G1162T:p.E388X, -/+, *de novo* • Xp22.31, *KAL1*, 107 kb gain, -/+

*Listed *de novo* variants include SNVs that are not ASD-relevant.

Supplementary Figure 1. Distribution of Polygenic Risk Scores in ASD (n=368) and non-ASD (n=207) Individuals from the Infant Sibling Study and Scores for Offspring in Family 1-0616



Individual ID	Standardized Polygenic Risk Score
1-0616-003	0.2402635
1-0616-004	0.8407516
1-0616-005	-0.0343805

Supplementary Table 6. Cases and controls with 16p11.2 BP2-BP-3 distal deletion and duplications

Sample	Relation	Disorder	Other disorder	Sex	Chr.	Start.hg19	End.hg19	Size (bp)	CNV	# Genes Impacted	Cohort	Inheritance
Cases												
1-0173-004	Affected sibling	ASD	ID, Comm	F	chr16	28,823,927	29,032,293	208,366	Loss	12	MSSNG	Paternal
- A136	Proband	ASD	NA	F	chr16	28,753,451	29,043,875	290,424	Loss	13	TCAG	NA
AU4188302	Proband	ASD	NA	F	chr16	28,678,522	29,054,721	376,199	Loss	17	MSSNG	Maternal
MSSNG00104-003	Proband	ASD	Nil	F	chr16	28,747,322	29,062,921	315,599	Loss	13	MSSNG	*de novo*
SSC07716	Proband	ASD	NA	M	chr16	28,683,322	29,055,121	371,799	Loss	14	SSC	*de novo*
1-0193-003	Proband	ASD	ADHD	M	chr16	28,812,322	29,078,321	269,999	Gain	12	MSSNG	Maternal
1-0782-004	Affected sibling	ASD	NA	M	chr16	28,722,322	29,080,321	357,999	Gain	17	MSSNG	NA
7-0049-003	Proband	ASD	ADHD	F	chr16	28,464,322	29,550,321	1,085,999	Gain	42	MSSNG	Paternal
Controls												
110036021749	Participant	Control	NA	M	chr16	28,814,098	29,083,503	269,405	Loss	12	ONC	NA

*Subject had life-threatening blood clots at birth, premature birth, slow and variable reaction time, learning disabilities
TCAG = The Centre for Applied Genomics; ONC = Ontario Nicotine Cohort; SSC = Simons Simplex Collection. M = male; F = female;
ID = intellectual disability; Comm = communication vulnerabilities (see text); NA = information not available

Supplementary Table 7: Summary of ASD-ascertained cohorts

Cohort	Sample count (males/females)	Platform (total # ASD/non-ASD subjects)
The Centre for Applied Genomics		
ASD cases	2,887 (2,453/434)	Agilent 1M (136/32) Affymetrix 6.0 (188/14) Affymetrix CytoScan HD (469/131) Illumina 1M (1,223/32) Illumina 1M-Duo (933/6) Illumina 2.5M (71/27) ≥2 platforms (122/13)
Non-ASD siblings	229 (114/115)	
MSSNG[1]		
ASD cases	4,807 (3,825/982)	Complete Genomics (788) Illumina HiSeq 2000 (261) Illumina HiSeq 2500 (24) Illumina HiSeqX (4,359)
Non-ASD siblings	157 (85/72)	
Children with no phenotype flag[2]	468 (339/129)	
Simons Simplex Collection (SSC)		
ASD cases	2,419 (2,094/325)	Illumina HiSeq 2500 (40/40) Illumina HiSeq X (2,379/1,927)
Non-ASD siblings	1,967 (928/1,039)	
Total # cases	12,934 (9,838/3,096)	

[1] MSSNG database as described in Merico, Yuen et al. (2017) and accessed at https://www.mss.ng/.
[2] Cases from external cohorts integrated into MSSNG database with no phenotype designation in metadata file.
ASD = Autism Spectrum Disorder

Supplementary Table 8: Control cohort summary

Control Cohort	Sample Size (males/females)	Platform
Ontario Population Genomics Platform (OPGP)	873 (477/396)	Affymetrix CytoScanHD array
Health, Aging, and Body Composition (Health ABC) Study	4,763 (2,020/2,743)	Affymetrix CytoScanHD array
SAGE Consortium Controls		
ONC		
KORA	2,884 (1,313/1,571)	Illumina 1M array
COGEND		
Ottawa Heart Institute Controls	2,331 (1,177/1,154)	Illumina 2.5M array
POPGEN		
Total	10,851 (4,987/5,864)	

Supplementary Table 9. Smaller deletions in genes within the 16p11.2 BP2-BP3 interval

Sample	Relation	Disorder	Chr.	Start (hg19)	End (hg19)	Exons impacted	Size (bp)	Gene	CNV	Cohort	Inheritance
2-0119-003	Proband	ASD	chr16	28898057	28901860	7-9	3,804	*ATP2A1*	Loss	MSSNG	NA
1024	Proband	ASD	chr16	28907687	28911255	13-14	3,569	*ATP2A1*	Loss	MSSNG	Maternal
SSC11925	Proband	ASD	chr16	28969291	28972486	3-4	3,196	*NFATC2IP*	Loss	SSC	Maternal
SSC12583	Proband	ASD	chr16	28969291	28972486	3-4	3,196	*NFATC2IP*	Loss	SSC	Maternal
SSC11987	Unaffected sibling	Non-ASD	chr16	28969291	28972486	3-4	3,196	*NFATC2IP*	Loss	SSC	Maternal
3-0707-000	Proband	ASD	chr16	28855875	28856051	5	177	*TUFM*	Loss	MSSNG	Maternal

ASD = Autism Spectrum, Disorder; SSC = Simons Simplex Collection; NA = not available.

Supplementary Table 10: Copy number losses in children referred to Genetic Diagnostics at Hospital for Sick Children

Subject ID	Sex	CNV Type	Coordinates (hg19)	Size (bp)	Inheritance	ASD	DD	ADHD	Language delay	Motor delay	Phenotype	Other Variants Reported
495885	M	Loss	chr16:28,371,467-29,342,589	971,122	NA						Bilateral club feet, VSD, microcephaly	None
497945	M	Loss	chr16:28,404,177-30,190,593	1,786,416	*De Novo*		+				Pulmonary, stenosis	None
201837	F	Loss	chr16:28,478,725-29,570,618	1,091,893	Paternal		+				Dysmorphic features	None
381442	F	Loss	chr16:28,478,725-29,470,979	992,254	NA		+					None
108594	F	Loss	chr16:28,496,676-29,274,699	778,023	NA			+			Learning disability, ODD	None
443980	M	Loss	chr16:28,503,824-30,190,593	1,686,769	*De Novo*		+				Partial agenesis of corpus collosum	None
435219	M	Loss	chr16:28,526,934-29,342,589	815,655	Paternal						Failure to thrive	None
402375	M	Loss	chr16:28,689,085-29,051,191	362,106	*De Novo*		+					None
322670	M	Loss	chr16:28,689,085-29,051,191	362,106	*De Novo*	+			+			VUS Maternal 2q37.2(236742448_236845166)x1
389774	F	Loss	chr16:28,696,825-29,076,252	379,427	NA						Hypotonia, cleft lip and palate, deafness	None
309165	M	Loss	chr16:28,763,834-29,070,000	306,166	NA	+	+					None
371908	M	Loss	chr16:28,763,834-29,051,191	287,357	*De Novo*				+		Hypotonia	None
126511	F	Loss	chr16:28,819,028-29,051,191	232,163	NA		+				>97th% weight and height, Congenital central hypoventilation syndrome	None
220085	F	Loss	chr16:28,819,028-29,051,191	232,163	NA		+					None
216036	F	Loss	chr16:28,819,028-29,051,191	232,163	*De Novo*		+				Hypotonia	None

469957	M	Loss	chr16:28,819,028-29,051,191	232,163	NA		+			+	Hypotonia	VUS 5q14.1(79,298,416-79,574,532)x1, Pathogenic 16p13.11(14,906,533-16,327,961)x1
185640	M	Loss	chr16:28,819,028-29,051,191	232,163	*De Novo*			+		+	Upper limb abnormality	None
259638	M	Loss	chr16:28,819,028-29,051,191	232,163	N/a				+	+		None
419238	F	Loss	chr16:28,819,028-29,051,191	232,163	NA			+			Obesity, anxiety, learning disability, diabetes, basal ganglia calcifications	None
314016	F	Loss	chr16:28,819,028-29,051,191	232,163	Maternal						Moderate ID, weight >97%, head >97%	None
282521	F	Loss	chr16:28,819,028-29,051,191	232,163	NA			+			Obesity, Mild ID,	None
312371	F	Loss	chr16:28,819,028-29,051,191	232,163	NA						VSD, Mild face retraction, premature birth, IUGR, <3% weight, height, head	None
169725	M	Loss	chr16:28,819,028-29,051,191	232,163	NA	+	+					None
353009	F	Loss	chr16:28,824,490-29,051,191	226,701	*De Novo*	+	+				Learning disability, seizures	None
330222	M	Loss	chr16:28,824,490-29,051,191	226,701	*De Novo*			+	+	+		None
412305	M	Loss	chr16:28,824,490-29,051,191	226,701	NA				+		Learning disability, schizophrenia	None
397784	M	Loss	chr16:28,824,770-29,056,973	232,203	NA		+				Seizures	None
426607	M	Loss	chr16:28,837,514-29,043,972	206,458	Paternal	+			+	+		None

ASD= Autism Spectrum Disorder; ADHD = Attention Deficit Hyperactivity Disorder; DD & Dev Delay = developmental delay; DORV = double outlet right ventricle; ID = intellectual disability; IUGR = intrauterine growth restriction; ODD = Oppositional Defiant Disorder; VSD = ventricular septal defect; VUS = variant of unknown significance

Supplementary Table 11: Copy number gains in children referred to Genetic Diagnostics at Hospital for Sick Children

Subject ID	Sex	CNV Type	Coordinates (hg19)	Size (bp)	Inheritance	ASD	DD	ADHD	Language delay	Motor delay	Phenotype	Other Variants Reported
126344	M	Gain	chr16:28,371,467-29,426,399	1,054,932	N/A						Weight <3%, height <3%, head <3%	None
250703	M	Gain	chr16:28,466,730-29,351,826	885,096	N/A		+	+			undescended testes	None
350709	M	Gain	chr16:28,802,397-29,051,191	248,794	N/A		+					None
189954	M	Gain	chr16:28,802,397-29,051,191	248,794	Maternal		+					None
255111	M	Gain	chr16:28,802,397-29,051,191	248,794	NA		+				hypotonia	None
311682	F	Gain	chr16:28,802,397-29,051,191	248,794	N/A				+		learning disability, tetralogy of fallot	None
162016	M	Gain	chr16:28,802,397-29,043,862	241,465	N/A	+	+					None
325855	M	Gain	chr16:28,802,397-29,043,862	241,465	N/A	+	+					None
478766	M	Gain	chr16:28,807,417-29,051,191	243,774	Paternal		+					None
285266	F	Gain	chr16:28,819,028-29,051,191	232,163	N/A	+						None
483570	M	Gain	chr16:28,819,028-29,051,191	232,163	N/A		+	+			ID, tremor, weight <3%	None
337455	M	Gain	chr16:28,819,028-29,043,450	224,422	*De Novo*		+					None
229915	M	Gain	chr16:28,819,028-29,051,191	232,163	N/A	+	+	+				VUS 2p15p14(62,931,037-64,743,430)x3, VUS 7q36.2(154,110,418-154,159,225)x3
409393	F	Gain	chr16:28,819,028-29,051,191	232,163	N/A		+				Dev Delay	Xq13.3(74,447,

												410-74,654,324)x3
115509	M	Gain	chr16:28,819,028-29,051,191	232,163	Paternal						Speech regression, brachycephaly	None
277521	M	Gain	chr16:28,819,028-29,051,191	232,163	N/A	+	+		+	+	learning disability, ID, psychiatric disorders	None
249180	F	Gain	chr16:28,824,490-29,043,450	218,960	N/A		+					None
150329	F	Gain	chr16:28,824,770-29,043,450	218,680	Maternal		+					None
276035	M	Gain	chr16:28,824,857-29,051,191	226,334	N/A				+		Seizures	None
124229	M	Gain	chr16:28,824,857-29,043,450	218,593	N/A		+				Dev Delay	None
356026	M	Gain	chr16:28,898,507-29,622,695	724,188	N/A				+		learning disability	

ASD= Autism Spectrum Disorder; ADHD = Attention Deficit Hyperactivity Disorder; Dev Delay = developmental delay; ID = intellectual disability; IUGR = intrauterine growth restriction; VSD = ventricular septal defect; VUS = variant of unknown significance

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