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The Phenotypic variability of 16p11.2 distal BP2–BP3 deletion in a transgenerational family and in neurodevelopmentally ascertained samples

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ABSTRACT

Background We present genomic and phenotypic findings of a transgenerational family consisting of three male offspring, each with a maternally inherited distal 220 kb deletion at locus 16p11.2 (BP2–BP3). Genomic analysis of all family members was prompted by a diagnosis of autism spectrum disorder (ASD) in the eldest child, who also presented with a low body mass index.

Methods All male offspring underwent extensive neuropsychiatric evaluation. Both parents were also assessed for social functioning and cognition. The family underwent whole-genome sequencing. Further data curation was undertaken from samples ascertained for neurodevelopmental disorders and congenital abnormalities.

Results On medical examination, both the second and third-born male offspring presented with obesity. The second-born male offspring met research diagnostic criteria for ASD at 8 years of age and presented with mild attention deficits. The third-born male offspring was only noted as having motor deficits and received a diagnosis of developmental coordination disorder. Other than the 16p11.2 distal deletion, no additional contributing variants of clinical significance were observed. The mother was clinically evaluated and noted as having a broader autism phenotype.

Conclusion In this family, the phenotypes observed are most likely caused by the 16p11.2 distal deletion. The lack of other overt pathogenic mutations identified by genomic sequencing reinforces the variable expressivity that should be heeded in a clinical setting. Importantly, distal 16p11.2 deletions can present with a highly variable phenotype even within a single family. Our additional data curation provides further evidence on the variable clinical presentation among those with pathogenic 16p11.2 (BP2–BP3) mutations.

INTRODUCTION

Autism spectrum disorder (ASD) is a common neurodevelopmental disorder hallmarked by symptoms in two core diagnostic domains: social communication and restrictive and repetitive interests and behaviours.^{1 2} Twin and family studies were the first to earmark the familial nature of the disorder, with estimates as high as 90% in monozygotic twins and 10% in dizygotic twins.^{3–5} Large-scale genome-wide linkage studies to identify genetic loci

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ The contribution of the proximal 16p11.2 deletion (BP4–BP5) to autism spectrum disorder (ASD) is well established, but while the distal deletion (BP2–BP3) is broadly linked with obesity, less is known about neurodevelopmental and psychiatric sequelae. Given the frequency of such deletions, a further understanding of their neuropsychiatric and other clinical manifestations is needed.

WHAT THIS STUDY ADDS

⇒ Here, we summarise in detail a family with the transgenerational segregation of a distal 16p11.2 deletion. The findings, along with our wider curation of 16p11.2 BP2–BP3 CNVs, provide evidence for its contribution to ASD with incomplete penetrance and variable expressivity.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The findings in this study demonstrate the contribution of the 16p11.2 distal deletion to ASD development and further reinforces the fact that ASD-relevant CNVs often present with variable phenotypes even within a family and that this should be considered when counselling a family in a clinical genetics context.

shared between affected family members have been conducted, but the results were largely negative.^{6 7} It has since become apparent that there are 100 or more ASD-implicated loci affected by rare (<0.1% of control populations) or inherited point mutations or smaller deletions and insertions, as well as larger CNV or structural variants, each associated with <1% of ASD cases.^{8–10} Consequently, a multitude of variants are seen to contribute to the autism spectrum phenotype, even among affected individuals from the same nuclear family.^{11 12}

This notwithstanding, some mutations/loci are seen more frequently, and sometimes are observed to segregate with phenotype in multiple affected families. In such instances, it may be possible to examine the relationship between gene and phenotype more precisely, as, to some extent, the



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influence of background genetic variation and environment are attenuated. Notable examples of recurrent variants are 15q11-13¹³ duplications and 1p36¹⁴ and 16p11.2¹⁵ proximal deletions.

The 16p11.2 locus, which encompasses a series of segmental duplications which mediate the formation of multiple, recurrent CNVs, is one of the most common genetic aetiologies identified in ASD (0.5%–1%).^{16–18} Approximately 20%–25% of individuals with the ~600 kb 16p11.2 proximal deletion (BP4–BP5) have a diagnosis of autism.¹⁹ Additional phenotypes associated with the proximal deletion are obesity, usually fully penetrant by adulthood (75%), deficits in motor coordination (60%) and language, speech and phonological impairment (70%).¹⁹ The less common recurrent CNVs in this region are the adjacent 16p11.2 distal deletion/duplication (BP2–BP3), both of which also exhibit reduced penetrance in relation to cognition.^{20–21} Similar to its proximal counterpart, the distal deletion syndrome is highly associated with obesity,^{22–23} increased head circumference (HC) and similar rates of ASD (26%)²⁴ as observed in 16p11.2 proximal deletion carriers.¹⁹ Similarly, a

high prevalence of developmental delay (DD)^{23–25} and increased risk of schizophrenia have been described.²⁶

Of the nine protein-coding and three non-coding genes within the distal deletion, neuronal expression of adapter protein Src homology 2 B adapter protein 1 (*SH2B1*) has been shown to positively regulate leptin and insulin signalling through the Janus kinase pathway, making this gene a strong candidate mediating the obesity phenotype.^{27–28} Transmembrane adapter protein linker for activation of T cells (*LAT*) is a candidate gene responsible for the neurodevelopmental phenotype, with overexpression leading to microcephaly in zebrafish and brain volumetric changes in mice.²⁹

In this paper, we profile a family of five who was referred to the clinical genetics service at the Hospital for Sick Children (SickKids, Toronto, Ontario, Canada) and the Holland Bloorview Kids Rehabilitation Hospital (Toronto, Ontario, Canada) as part of the POND Network (<https://pond-network.ca/>) because of a diagnosis of ASD in the first-born male offspring (figure 1). Both microarray and whole-genome sequencing (WGS) were

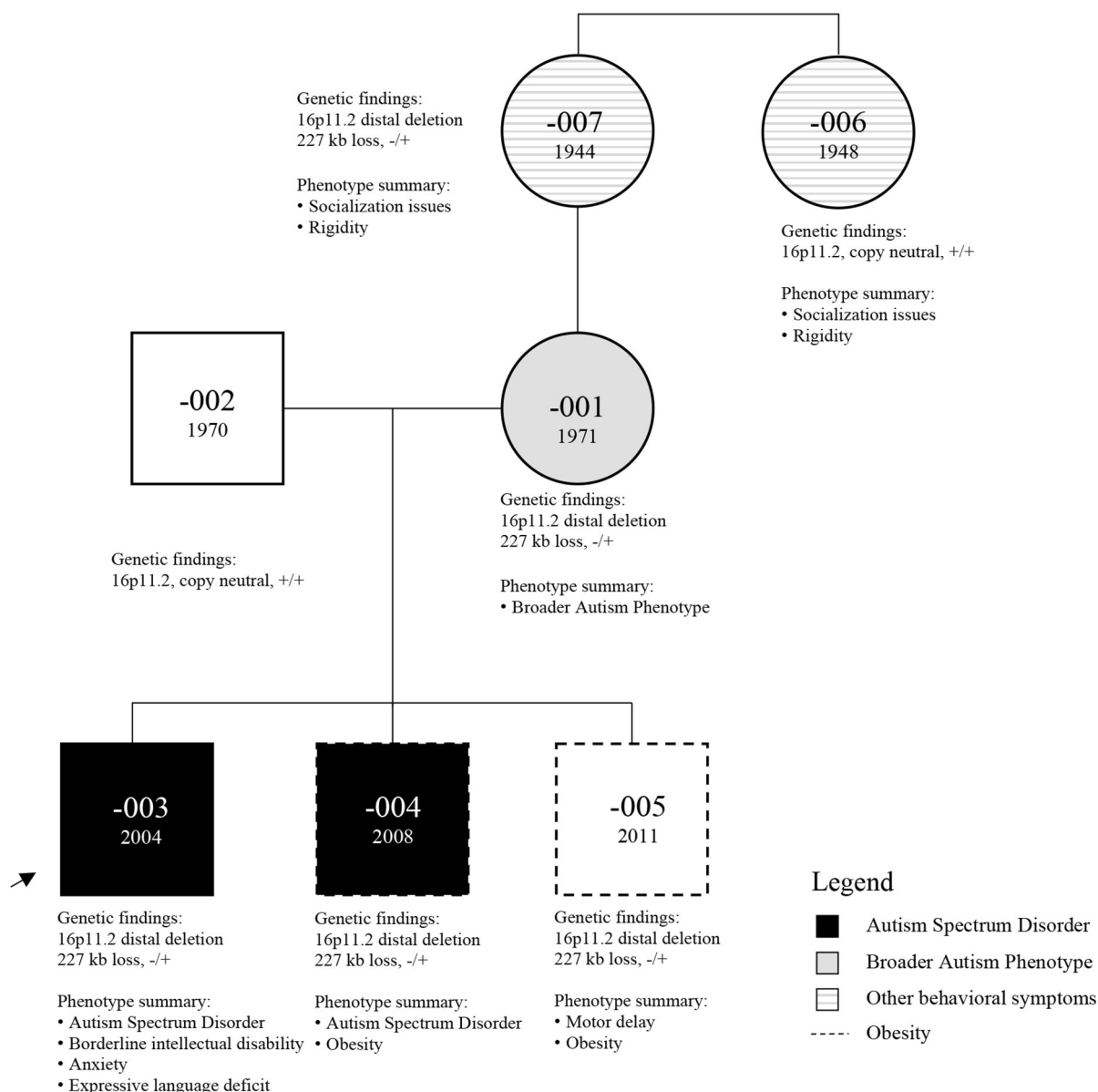


Figure 1 Pedigree of family 1-0616. Arrow indicates proband.

performed on the family, and a canonical, maternally inherited 227 kb 16p11.2 distal deletion was identified in all male offspring. All family members subsequently underwent medical and psychiatric assessments. Further data curation was undertaken from samples ascertained for neurodevelopmental disorders and congenital abnormalities.

METHODS

The family profiled in this study (ID: 1-0616) was first referred to the clinic for suspected DD of the first-born male offspring (-003, proband) at 20 months of age. At 4.5 years of age (August 2008), he received a clinical diagnosis of ASD, after being referred to a developmental paediatrician during the prior year. All five family members subsequently enrolled in SickKids' ASD research studies, which included WGS analysis as part of the Autism Speaks MSSNG project.^{30–32}

WGS data are available through a data access committee at the MSSNG site (<https://research.mss.ng>). The microarray data are accessible in dbGaP via accession number phs001876.v1.p1. Full details of clinical, behavioural and cognitive assessments undertaken are available in the online supplemental note. For the purpose of this paper, the family also provided specific written consent for their data to be shared in the scientific literature in the form of this case report.

Microarray and WGS data were analysed as previously described.^{32–34} For additional information, please consult the online supplemental note. The genomic coordinates presented in this paper are based on the February 2009 Human Genome Build (GRCh37/hg19).

RESULTS

Clinical profile of family 1-0616

The male proband was born at 38 weeks' gestation following a medically uneventful pregnancy and with birth weight of 6 lb 11 oz. The mother and father were aged 32 and 33, respectively, at the time of conception. No medical concerns were noted at birth. He was first referred to a paediatrician because of DD in language and motor milestones at 20 months, which included concerns about reduced motor tone and lack of babbling. He was then diagnosed with ASD at 4.5 years by a developmental

paediatrician. As part of the ASD research study, he subsequently underwent a series of investigations at age 9 years. His full-scale IQ (FSIQ) was 78 (Stanford-Binet test: verbal IQ=91, non-verbal IQ=68), and adaptive behaviour was consistent with this (Vineland Adaptive Behaviour Scale, VABS-II: composite: 82, communication: 94, daily living: 78, socialisation: 80). Despite an average verbal IQ of 91, performance on the Oral and Written Language Scales (OWLS)-II revealed overall impairment in language (total language standard score: 62) and in expression (standard score: 50). Standard score in receptive language revealed borderline impairment (standard score: 78). Both Autism Diagnostic Interview - Revised (ADI-R, age 12 years) and Autism Diagnostic Observation Schedule (ADOS-G) were also performed. The ADOS (age 8 years) and ADI-R (age 12 years) were consistent with a diagnosis of autism across all domains (online supplemental table 4, Summary genomic and phenotypic findings for family 1-0616). At age 9 years, no concerns were expressed by the family regarding psychiatric comorbidity, and at this same age, weight was below average (weight: 31 kg, less than the first percentile), and he was noted to be tall for his age (height: 149 cm, 99th percentile). His HC was normal (age 9 years, HC: 53 cm, 64th percentile) (table 1 and online supplemental table 4)

The proband's younger siblings (male siblings -004 and -005) were subsequently enrolled in the Canadian Infant Siblings Study for ASD,³³ and both underwent detailed neurodevelopmental and clinical assessment. The older of the two siblings (-004) was born at 38 weeks' gestation following an uneventful pregnancy and labour with a birth weight of 6 lb 14 oz. At the time of assessment at the age of 5.5 years, he was noted to be obese (body mass index (BMI) >99th percentile) and to have difficulties with socialisation and communication with his peers. However, both Nipissing Developmental Screen³⁵ and NutriSTEP screening³⁶ were normal. A formal ASD evaluation by way of ADOS-2 was consistent with a research diagnosis of ASD, although a formal clinical diagnosis was never given. Unlike the proband, -004 did not have intellectual vulnerabilities (Wechsler Intelligence Scale for Children, WISC-V: Full Scale IQ, FSIQ: 92, Verbal IQ, VIQ: 106 and Non-verbal IQ, NVIQ: 85), and communication assessments and adaptive functioning were within normal limits

Table 1 Summary of phenotypical and genomic findings in family 1-0616

Family 1-0616	-001	-002	-003	-004	-005
Family member	Mother	Father	Proband	Second-born	Third-born
Sex	Female (XX)	Male (XY)	Male (XY)	Male (XY)	Male (XY)
Clinically relevant genetic findings‡	16p11.2 distal deletion, 227 kb, del(mat)/+	16p11.2 copy neutral+/+	16p11.2 distal deletion, 227 kb, del(mat)/+	16p11.2 distal deletion, 227 kb, del(mat)/+	16p11.2 distal deletion, 227 kb, del(mat)/+
Medical history:					
Obesity	–	–	–	+	+
Epilepsy	–	–	–	–	–
Other	–	–	–	–	–
ASD	–	–	+	+	–
BAP	+	–	NA	NA	NA
IQ*	109	122	78	92	117
Adaptive deficits†	NA	NA	82	88	NA
Anxiety	Moderate	NA	Severe	Severe	Mild
Other neurodevelopmental	Communication deficits	NA	NA	Attentional deficits	Motor deficits

*Full-scale IQ determined by multiple tests; see online supplemental table 4.
†Adaptive Behaviour Composite as measured by VABS-II.
‡CNV findings initially made by microarray (Affymetrix CytoScan HD) and subsequently called from whole-genome sequencing data (Illumina HiSeqX); see online supplemental table 5.
ASD, autism spectrum disorder; BAP, broader autism phenotype; NA, not applicable/phenotype not assessed.

(online supplemental table 4). Despite these relative patterns of strength, a number of externalising behaviours were reported by parents and teachers, both aggressive and oppositional/defiant in nature, as well as internalising problems such as anxiety and mood symptoms. However, none were consistent with a formal diagnosis of a psychiatric disorder. This sibling was followed up at the Hospital for Sick Children due to his obesity. At age 11 years, his BMI remained high (height 156.8 cm, 97th percentile; weight 66.3 kg, BMI 26.97, >99th percentile, $Z=2.75$).

Unlike his older siblings, the youngest brother (-005) was not reported to have any social and communication vulnerabilities, but did receive a diagnosis of developmental coordination disorder and dyslexia at age 6 years and was obese. He too was born following an uneventful pregnancy and delivery at 38 weeks. During his first year, gross motor vulnerabilities were noted and by formal assessment identified in the very low range in contrast to relatively intact fine motor development. By 24 months, however, both gross and fine motor developments were recorded as being delayed. He completed the Wechsler Preschool and Primary Scale of Intelligence, WPPSI-III at 4 years and 5 months of age as part of his enrolment in the Canadian Infant Sibling Study and scored above average in all domains (>113; see online supplemental table 4). Screening using the SRS and SCQ were within normal limits, and ADOS was not consistent with a diagnosis of ASD. However, vulnerabilities in the communicative domain were suggested by CCC-2 scores falling in the 14th percentile. No other developmental concerns were noted at this time. At 7 years, this sibling was also noted to be clinically overweight (height 132.5 cm, 98th percentile, weight 45.1 kg, BMI 25.69 kg/m², >99th percentile, $Z=3.83$).

Both the mother (-001) and father (-002) completed the Wechsler Abbreviated Scale of Intelligence (WASI-II) with scores consistent with average (mother, FSIQ: 103) and high average skills (father, FSIQ: 122). Results on the Broader Autism Phenotype Questionnaire (BAP-Q) and expert clinical judgement deemed the mother to have a broader autism phenotype (BAP). The father was not formally assessed for BAP, although his total BAP-Q score and subscale scores did indicate vulnerabilities.

Genetic findings

The proband and his two younger siblings all possessed a distal deletion at 16p11.2 (NC_000016.9:g.28824503_29051191del) detected by both microarray and WGS (figure 1 and online supplemental table 5). This 227 kb variant impacted the genes *ATXN2L*, *TUFM*, *SH2B1*, *ATP2A1*, *ATP2A1-AS*, *RABEP2*, *CD19*, *NEATC21P*, *SPNS1* and *LAT* and two microRNAs (MIR4517 and MIR4721) and is consistent with the 16p11.2 distal deletion region.²² The gene *SH2B1* has been proposed to be associated with obesity.^{22,23} This CNV was inherited from the mother, who, in turn, had inherited it from her mother (subject -007, figure 1). We were unable to gather phenotypic information on -007, although an anecdotal description by -001 suggests she may have had some social and communication vulnerabilities. The maternal aunt also was reported to have social vulnerabilities but did not possess the deletion.

While the 16p11.2 distal deletion in this family is the only genetic finding clinically deemed to contribute to the expression of the observed phenotypes, there were additional rare variants of unknown significance (VUSs) identified in the study subjects, given their rare, de novo status and/or possible, although tenuous, association with neurodevelopmental disorder (NDD) or neuropsychiatric phenotypes (see online supplemental table 5 for all rare CNVs and small variants derived from WGS). The

third-born son possessed a de novo 192 kb duplication CNV at locus 5p15.33 involving four genes classified as a VUS. The proband and his siblings also inherited a rare 107 kb duplication at Xp22.31, impacting *KAL1*, a gene associated with Kallman syndrome, from their mother and maternal grandmother. Finally, the proband and one of his siblings (-004) inherited a 637 kb duplication at 2q21.2 from their father. This CNV impacted the *ANKRD30BL* and *GPR39* genes. Copy number detection via WGS did not detect any additional clinically relevant CNVs that were missed by microarray.

In the case of single-nucleotide variants (SNVs) and small insertions and deletions, the proband and mother possessed a stop-gain mutation in exon 19 of the calcium-channel *CACNA2D4* (NM_172364.5:c.1882C>T, p.(Arg628*)), one in a family of similar genes associated with NDDs and psychiatric disorders. Subjects -003 and -005 each had two rare de novo SNVs, none of which would be classified as pathogenic in the context of NDDs or ASD (online supplemental table 5). Polygenic risk scores (online supplemental figure 1) were calculated for all three male offspring as part of a larger study assessing the predictive utility of CNV detection in the diagnosis of ASD and neurodevelopmental vulnerabilities in a prospectively recruited sibling cohort.³³ The Pragmatic Rating Scale (PRS) for both male offspring with ASD (-003=0.24 and -004=0.84), reported as standardised values, was close to the mean for ASD cases (0.028, $SD\pm 1.024$). The PRS of the youngest son (-005=-0.034) was also close to the mean of that of non-ASD sibs (-0.048, $SD\pm 0.952$). While the general directionality of the scores aligned with ASD status, they did not correlate with clinical severity.

Curation of 16p11.2, BP2–BP3 CNVs in children ascertained for neurodevelopmental disorders and congenital anomalies

We also investigated the prevalence of 16p11.2 BP2–BP3 CNVs among families in the internal CNV database from The Centre for Applied Genomics, the Simons Simplex Collection (SSC) and the MSSNG database (table 2, extended form online supplemental tables 6–8) ($n=10\,113$ ASD cases, 8372 male and 1741 female). A total of five individuals with ASD had a 16p11.2 distal deletion (one male and four female) (5/10 113=0.05%). We identified an affected female (1-0173-004) of European descent who had a paternally inherited deletion at this locus. The deletion was initially identified via microarray (NC_000016.9:g.28823927_29043875del). She was subsequently sequenced and included in the MSSNG database. A clinical assessment at 9.6 years of age led to an ASD diagnosis (ADOS calibrated severity score: 8, ADI algorithm total=43). Additionally, she had an FSIQ of >1 SD below the mean (Leiter-R full IQ=79). She was additionally noted to have communication deficits, as observed through the OWLS, with an oral composite score of 67 (>2 SD below the mean). At the same age, physical development metrics obtained from the medical clinic noted that her weight was average (29 kg, 12th percentile), accounting for age and sex. HC was 54 cm (93rd percentile). A second female (MSSNG00104-003) of East Asian ancestry with ASD had a 16p11.2 distal deletion (NC_000016.9:g.28747322_29062921del), which was de novo. No other medical or psychiatric history was available for this subject. A third female (AU4188302) of European ancestry with a maternally inherited deletion (NC_000016.9:g.286785_22_29054721del) received an ASD diagnosis at 10.5 years of age. No additional history was available for this subject. There was an additional female participant with ASD who possessed a 16p11.2 distal deletion (-A136, NC_000016.9:g.28753451_29043875del). The origin of this deletion is unknown. Lastly,

Table 2 Cases and controls with 16p11.2 BP2–BP3 distal deletion and duplications

Sample	Sex	Chr.	Start.hg19	End.hg19	Size (bp)	CNV	Genes impacted (n)	Inheritance
Cases								
1-0173-004	F	chr16	28 823 927	29 032 293	208 366	Loss	12	Paternal
- A136	F	chr16	28 753 451	29 043 875	290 424	Loss	13	NA
AU4188302	F	chr16	28 678 522	29 054 721	376 199	Loss	17	Maternal
MSSNG00104-003	F	chr16	28 747 322	29 062 921	315 599	Loss	13	De novo
SSC07716	M	chr16	28 683 322	29 055 121	371 799	Loss	14	De novo
1-0193-003	M	chr16	28 812 322	29 078 321	269 999	Gain	12	Maternal
1-0782-004	M	chr16	28 722 322	29 080 321	357 999	Gain	17	NA
7-0049-003	F	chr16	28 464 322	29 550 321	1 085 999	Gain	42	Paternal
Controls								
110036021749	M	chr16	28 814 098	29 083 503	269 405	Loss	12	NA

F, female; M, male; NA, information not available.

an autistic male proband from the SSC (SSC07716) with a de novo deletion (NC_000016.9:g.28683322_29055121del) was found.

In our queries, we also found three ASD cases with 16p11.2 distal duplications at this locus (two male and one female) (3/10 113=0.03%). One male proband (1-0193-003) of European descent had a duplication spanning 12 genes (NC_000016.9:g.28812322_29078321dup). The variant was maternally inherited. The parent did not report any psychiatric or medical diagnoses. The male was diagnosed with ASD at 12 years of age (ADOS calibrated severity metric=8). He had an average Leiter FSIQ of 97. In addition to ASD, the male proband was noted to have comorbid attention deficit hyperactivity disorder (ADHD) and clinically significant adaptive behaviour deficits evident through VABS-II subscale standard scores (socialisation=66, communication=48, daily living skills=39; 0.6–2.4 SD below the mean). Oral expression ability was slightly below average as assessed by the OWLS-II (oral expression standard score: 69). A second autistic male offspring (1-0784-004) was found to carry a 358 kb duplication (NC_000016.9:g.28722322_29080321dup). He has an older autistic sibling who does not have the variant. He was diagnosed with ASD at 38 months of age. The inheritance status of this CNV could not be ascertained. Lastly, a female offspring (7-0049-003) was identified with a 1.1 Mb paternally inherited duplication (NC_000016.9:g.28464322_29550321dup), corresponding to BP1-4 at the 16p11.2 locus. She was diagnosed with ASD at 11 years of age, with comorbid ADHD (Swan Rating Scale 2010: ADHD inattentive subscale=5, oppositional defiant disorder subscale=5, ADHD hyperactive subscale=4) and adaptive behaviour deficits (Adaptive Behavior Assessment System (ABAS-II): general adaptive composite=56, >2SD below the mean).

We further identified CNVs of >100 bp, which could reliably be detected from short-read WGS data,³⁷ and small variants in all genes within this interval (table 3, extended form

online supplemental table 9) in ASD subjects. Deletions of <5 kb were noted in the following genes: *ATP2A1*, *NFATC2IP* and *TUFM*. Two rare loss of function (LoF) mutations in different probands were found in *ATP2A1*, one impacting exons 7–9 (NC_000016.9:g.28898057_28901860del) and the other exons 13–14 (NC_000016.9:g.28907687_28911255del). An ASD proband and unaffected sibling carried a maternally inherited rare LoF mutation in *NFATC2IP* impacting exons 3–4. Lastly, a 177 bp deletion of exon 5 of the *TUFM* gene (NC_000016.9:g.28855875_28856051del) was found in an ASD proband.

We examined the number of patients with deletions and duplications overlapping the 16p11.2 BP2–BP3 region (<2 Mb in size) identified in the Genome Diagnostics Clinical Laboratory at the Hospital for Sick Children (Toronto, Canada), which has 31 932 patients referred for DD, ASD and/or congenital anomalies. These diagnostic labels represent the reason for referral rather than confirmed diagnoses assigned to each child. Of note, a total of 28 (0.09%) distal deletions (16 male offspring and 12 female offspring) were identified (online supplemental table 10) in subjects with ages ranging from 9 months to 30 years. The most common reason for referral was the presence of DD (13/28=46.4%). Among the 16p11.2 distal deletions, five (17.9%) cited ASD as a reason for referral, three of whom also were described with DD. Additionally, four (14.3%) subjects were reported to have obesity. A number of individuals were also described as having dysmorphic features and other growth abnormalities such as clubfoot or cleft lip/palate on the clinical requisition. A total of 21 (0.07%) distal duplications (16 male offspring and 5 female offspring) overlapping this locus were found in children with ages ranging from 1 year to 12 years (online supplemental table 11). Two duplications were maternally inherited; two were paternally inherited; and one was de novo. The inheritance status of the remaining variants could not be ascertained. Of the 21 cases referred for genetic testing, 4 (19%) presented with ASD, 3 had ADHD (14.3%) and 15 (71%)

Table 3 Smaller deletions in genes within the 16p11.2 BP2–BP3 interval

Sample	Chr.	Start (hg19)	End (hg19)	Exons impacted	Size (bp)	Gene	CNV	Inheritance
2-0119-003	chr16	28 898 057	28 901 860	7–9	3804	<i>ATP2A1</i>	Loss	NA
1024	chr16	28 907 687	28 911 255	13–14	3569	<i>ATP2A1</i>	Loss	Maternal
SSC11925	chr16	28 969 291	28 972 486	3–4	3196	<i>NFATC2IP</i>	Loss	Maternal
SSC12583	chr16	28 969 291	28 972 486	3–4	3196	<i>NFATC2IP</i>	Loss	Maternal
SSC11987*	chr16	28 969 291	28 972 486	3–4	3196	<i>NFATC2IP</i>	Loss	Maternal
3-0707-000	chr16	28 855 875	28 856 051	5	177	<i>TUFM</i>	Loss	Maternal

*Unaffected sibling.

had a DD diagnosis. Unlike individuals with the distal deletion, two (9.5%) were underweight (less than the third percentile) and none reported obesity. This finding supports results from previous studies which demonstrate the mirrored phenotypes associated with 16p11.2 distal CNVs, with the deletion being primarily linked to elevated BMI.^{20 21 38 39}

DISCUSSION

In this study, we describe a family in which a deletion impacting the 16p11.2 distal locus is transmitted across three generations. Despite sharing an identical CNV, the phenotype varies quite substantially between family members carrying it. Most significantly, there was no shared diagnosis between all three siblings, and only the two younger siblings were diagnosed with obesity and the proband and middle child diagnosed with ASD, although the middle child's ASD diagnosis was a research and not a clinical diagnosis. Of course, it is not unusual for ASD symptoms to manifest in middle childhood and beyond, and the same is true of obesity. Despite the 16p11.2 distal deletion being associated with early-onset obesity, both the mother and first-born son were not obese, with the latter in the first percentile for weight. Consequently, this individual may yet develop symptoms, as he was last seen in the clinic at 9 years of age. The third-born male offspring may equally qualify for an ASD diagnosis later in childhood. Indeed, the assessment of communication did earmark the presence of some early vulnerabilities. Notably, the three siblings' mother also met the criteria for the BAP and had no medical history, including the absence of obesity. There is much to support the possibility, therefore, that in this family the CNV has high penetrance yet variable expressivity from individual to individual.

Also of interest is the pattern of other vulnerabilities. For example, although the proband had relative deficits in non-verbal IQ, all other family members had a fairly 'flat' intellectual profile in the average range. No other genetic abnormalities were identified that would offer an explanation for the proband's non-verbal deficits, and so we believe it is most likely related to the 16p11.2 distal deletion. It would appear, therefore, that this may be a more variable aspect of phenotype, given that more family members had ASD/BAP than low IQ. Moreover, given the fact that the middle sibling (-004) had research-diagnosed ASD in association with normal IQ, it seems reasonable to conclude, for this family at least, that ASD is not simply an epiphenomenon and secondary to impaired intellectual function. Similarly, comorbidity for externalising behaviour was seen in the middle sibling but not the proband, again indicating that this is not simply the result of either impaired intellectual function or ASD. It is also important to note that the third-born child (-005) received intensive behavioural intervention from toddlerhood, specifically in the form of an assistant in the classroom, and help with school-related tasks at home. This stands in contrast to his older siblings. We postulate that this vigilance at least with respect to ASD may have contributed to improved developmental trajectory.

Our curation of data from the genome diagnostics lab at Sick-Kids (online supplemental tables 8 and 9) indicates that neurodevelopmental phenotypes are common in the presence of the 16p11.2 distal deletion. Large-scale phenotype studies assessing pathogenic CNV frequencies in the general population estimate prevalence of 16p11.2 distal deletions between 0.014% and 0.02% and reciprocal duplications at 0.016%–0.038%, not unlike the association of distal deletions and, to a lesser extent, duplications, to neuropsychiatric phenotypes.^{21 39 40} The

reported penetrance for cognitive performance are 23% and 11% for the distal deletion and distal duplication, respectively.²¹

No pattern in phenotype overlap is evident from these clinical data, although there are biases inherent in data curated from different clinical sources. Specifically, cases referred through mental health services will be enriched for neuropsychiatric information, whereas paediatric services are more likely to comprise a broader range of medical characteristics. That being said, our assessment of this locus earmarks the importance of potential early detection using CNVs for ASD as biomarkers, given the strong likelihood of subsequent developmental vulnerabilities, as well as the potential for impact of behavioural interventions.⁴¹

Our curation from our internal CNV database also identified three autistic individuals with distal 16p11.2 duplications. Fernandez and colleagues previously described the clinical features of three children with 16p11.2 duplications. Although co-ordinates are not provided, they too observed a variable phenotype including ASD and DD.¹⁵ There is in fact very little literature on neuropsychiatric phenotypes among patients with distal 16p11.2 duplications. Given our findings, this does highlight the need to also study duplications in this region in more detail.

The CNV in this family does impact *SH2B1*, which is implicated in obesity. However, although both siblings were clinically obese, neither the proband nor the mother had any history of obesity at the time of assessment. At present, the dietary habits of the individual members of the family are similar. The pattern of obesity observed in this family, therefore, may be indicative of variable expressivity for obesity, as neither of the other genetic abnormalities identified in this family are associated with growth or obesity. There may, of course, be other more common variants that impact risk through interaction with *SH2B1*.

It is unclear which genes confer risk of neurodevelopmental diagnoses. The deletion in this family overlaps a number of genes that are known to be brain expressed, particularly in the cerebellum, and so there are several candidates. For example, *ATXN2L* is widely expressed in tissue, including the central nervous system, but its ataxin type 2 protein product, although a member of a neurodegenerative family of proteins, is of unknown function.⁴² The other impacted genes are largely also of undetermined function, although two are immune related (*CD19*⁴³ and *LAT*).⁴⁴ *LAT* was previously shown to mediate brain size changes in a murine knockout model, although it is the only gene in this interval to be implicated in this phenotype. We also considered other CNVs in the literature impacting this locus to determine whether there is an area of minimal overlap, but the small number of cases identified and similar CNV size precluded this. Given that our study has demonstrated that this locus has a reasonably high penetrance for ASD, it is important to pursue further investigation of these genes as this may shed unique insight on the pathogenesis of ASD, given that none of the genes fall into existing functional domains, such as synaptic, that are known to be important in the disorder's pathogenesis.⁴⁵

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Contributors MW-S, LD'A, SWS and JH conceived the study. MW-S and LD'A wrote the manuscript and revisions according to input from all authors. EA, ID, NH and AI collected phenotypical data. LD'A, DJS, MZ and BT undertook genome analyses and data curation. MW-S is the guarantor.

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Competing interests SWS is on the Scientific Advisory Committee of Population Bio, serves as a highly cited academic advisor for the King Abdulaziz University,

and intellectual property from aspects of his research held at the Hospital for Sick Children are licensed to Athena Diagnostics and Population Bio. DJS contributed to initial development of Phenotips and holds equity in the company.

Patient consent for publication Not applicable.

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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 Test⁵. 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.

First (-003) and third-born (-005) males were evaluated for anxiety using the parent-reported versions of the Revised Children's Anxiety and Depression Scale¹⁰ and Spence Children's Anxiety Scale¹¹, 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-3¹³ 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 Questionnaire¹⁵; Social Communication Questionnaire; Communication Checklist-Adult¹⁶, Social Phobia and Anxiety Inventory¹⁷, Wisconsin Card Sorting Test¹⁸, 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), iPattern²⁴, Nexus²⁵, and Partek²⁶. 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

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 genome²⁷. 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 ($\leq 0.1\%$ 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: SIFT²⁸ ≤ 0.05 , Polyphen-2²⁹ ≥ 0.95 , CADD³⁰ ≥ 15 , MutationAssessor³¹ 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 in²¹ (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 described²².

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 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	NA	NA	ADOS, Module 3	ADOS-2, Module 3	ADOS-2, Module 3
Domain scores			Com = 5 RSI = 12 Com + RSI = 17 Imag/Creat = 1 SB/RI=4 ADOS Dx = autism	Soc Affect = 6 RRB = 2 Overall total = 8 Comparison score = 5 (moderate) ADOS Dx = autism spectrum	Soc Affect = 3 RRB = 3 Overall total = 6 Comparison score = 3 (low) ADOS Dx = non- spectrum
SRS version	SRS-2 Adult	NA	SRS	SRS	SRS
Scores	RRB = 51 SCI = 61 Total T-score = 59		Total T-score = 73	RRB = 43 SCI = 51 Total T-score = 49	Total T-score = 57
SCQ version	NA	NA	SCQ-Lifetime	SCQ-Lifetime	SCQ-Lifetime
Score			Total: 19 (above cut-off)	Total: 1 (below cut-off)	Total: 1 (below cut-off)

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

			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	NA	NA	ABAS - II	NA	NA
Scores			General Adaptive Composite = 68 Conceptual = 83 Social = 61 Practical = 78		
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	NA	NA	Strengths and Weaknesses of Attention-Deficit / Hyperactivity - Symptoms and Normal-behaviours (SWAN)	Conners Comprehensive Behaviour Rating Scales (Teacher)	NA
Scores, subscale and/or commentary			ADHD hyperactive Impulsive total = 1 ADHD inattentive total = 5 ODD total = 2 ADHD clinical range = below ODD clinical range = below	Responder comments: weight, low endurance, lack of mobility, kind, compliant, tries his best	
Test	NA	NA	NA	Conners Continuous Performance Test - 3	NA
Commentary				speed over accuracy; moderate likelihood of having a diagnosis characterized by attention deficits;	

				inattentiveness (strong indication); vigilance (strong indication); no indication of impulsivity	
Executive					
Wisconsin Card Sorting Test	WCST	WCST	WCST	WCST	NA
Score	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	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	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	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	
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	NA	NA	OWLS	OWLS-II	NA
Scores			Receptive = 50 Expressive = 78 Total = 62	Receptive = 98 Expressive = 88 Total = 92	
Test	CC-A	CC-A	NA	CCC-2	CCC-2
Scores and commentary	Lang Structure = 7 Pragmatic Diff = 13 Engagement = 4 Total = (10%ile)	Lang Structure = 14 Pragmatic Diff = 9 Engagement = 10 Total = (>50%ile)		Social interaction Deviance Composite = -6 General communication Composite = 93 Communication profile suggestive of ASD	Social interaction Deviance Composite = 10 General communication Composite = 92
Comprehensive Test of Phonological Processing	NA	NA	NA	CTOPP-2	NA
Score				Phonological Awareness = 92	

				Phonological Memory = 85 Alt. Phonological Awareness = 95 *difficulty blending words	
General behaviour measures					
Child Behaviour Checklist (CBCL) version	CBCL Adult	NA	CBCL for ages 6 - 18: 2001	CBCL for ages 6 - 18: 2001	CBCL for ages 1 - 5: 2000
Domain T-scores			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	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	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

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

	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	TAS-20	TAS-20			
Score	Total factor 1 score = 19 Total factor 2 score = 23 Total factor 3 score = 17 Total overall score = 59	Total factor 1 score = 8 Total factor 2 score = 10 Total factor 3 score = 18 Total overall score = 36			
Test					Relationship Questionnaire

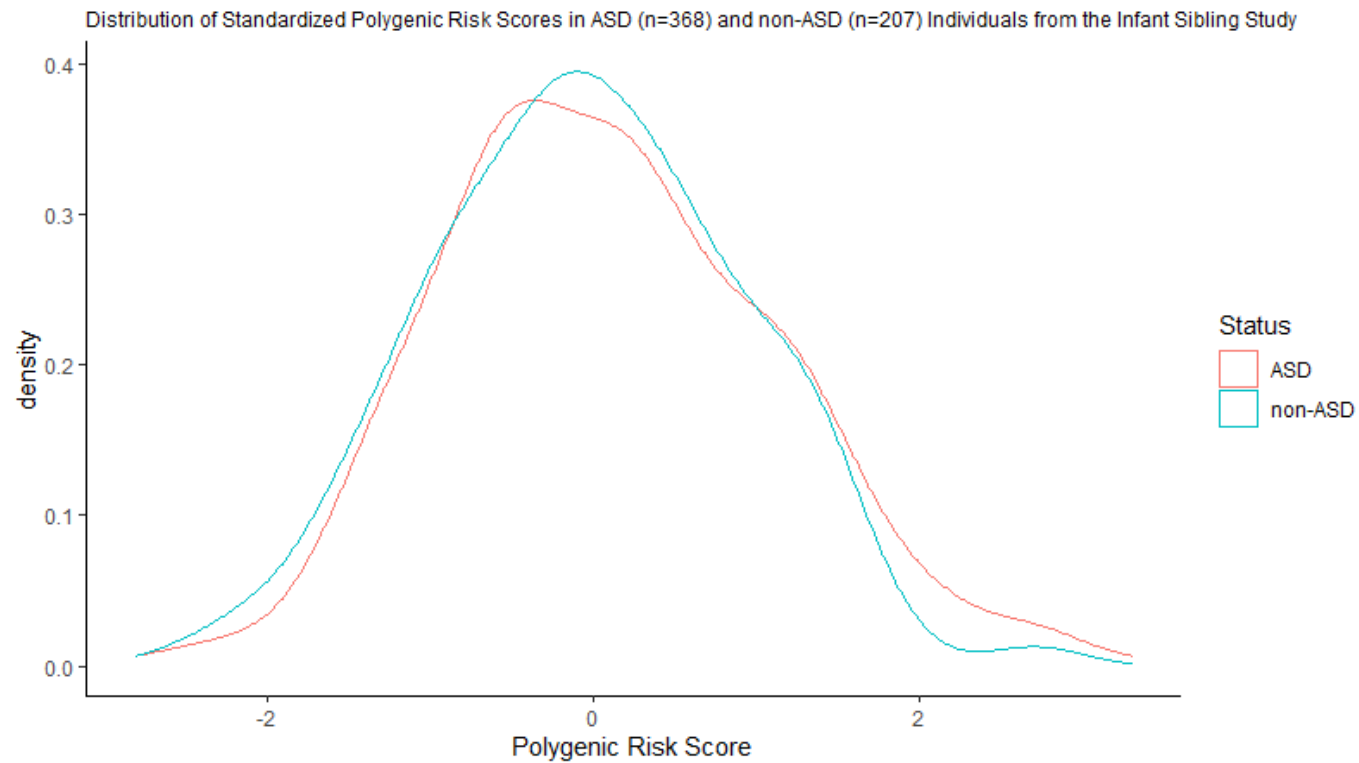
Commentary					No close friends; somewhat shy when it comes to meeting new friends; gets along well with parents and teachers
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NA = not available; Nil = none; HC = head circumference; Ht = height; Wt = weight; Dx = diagnosis; ADOS = Autism Diagnostic 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*
<p>Father (-002)</p>	<ul style="list-style-type: none"> 16p11.2 copy neutral +/- 	<ul style="list-style-type: none"> 2q21.2, <i>ANKRD30BL</i> and <i>GPR39</i> genes, gain, 637 kb, +/-
<p>Mother (-001)</p>	<ul style="list-style-type: none"> 16p11.2 distal deletion, 227 kb, del/+ 	<ul style="list-style-type: none"> Xp22.31, <i>KALI</i>, 107 kb gain, +/- <i>CACNA2D4</i>:NM_172364:exon19:c.C1882T:p.R628X, +/-
<p>Proband (-003)</p>	<ul style="list-style-type: none"> 16p11.2 distal deletion, 227 kb, del[mat]/+ 	<ul style="list-style-type: none"> <i>ATF7IP2</i> (NM_001352120):c.-10998G>C, +/-, <i>de novo</i> <i>CCAR1</i> (NM_001282959):exon16:c.G2083C:p.E695Q, +/-, <i>de novo</i> Xp22.31, <i>KALI</i>, 107 kb gain, +/- 2q21.2, <i>ANKRD30BL</i> and <i>GPR39</i> genes, gain, 637 kb, +/- <i>CACNA2D4</i> (NM_172364):exon19:c.C1882T:p.R628X, +/-
<p>Sib 1 (-004)</p>	<ul style="list-style-type: none"> 16p11.2 distal deletion, 227 kb, del[mat]/+ 	<ul style="list-style-type: none"> Xp22.31, <i>KALI</i>, 107 kb gain, +/- 2q21.2, <i>ANKRD30BL</i> and <i>GPR39</i> genes, gain, 637 kb, +/-
<p>Sib 2 (-005)</p>	<ul style="list-style-type: none"> 16p11.2 distal deletion, 227 kb, del[mat]/+ 	<ul style="list-style-type: none"> 5p15.33 gain, 192 kb, +/-, <i>de novo</i> <i>DXO</i> (NM_005510):exon2:c.G28A:p.A10T, +/-, <i>de novo</i> <i>APPBP2</i> (NM_006380):exon11:c.G1162T:p.E388X, +/-, <i>de novo</i> Xp22.31, <i>KALI</i>, 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	<i>de novo</i>
SSC07716	Proband	ASD	NA	M	chr16	28,683,322	29,055,121	371,799	Loss	14	SSC	<i>de novo</i>
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)
Non-ASD siblings	229 (114/115)	Affymetrix CytoScan HD (469/131) Illumina 1M (1,223/32) Illumina 1M-Duo (933/6) Illumina 2.5M (71/27) ≥2 platforms (122/13)
MSSNG¹		
ASD cases	4,807 (3,825/982)	Complete Genomics (788)
Non-ASD siblings	157 (85/72)	Illumina HiSeq 2000 (261) Illumina HiSeq 2500 (24)
Children with no phenotype flag ²	468 (339/129)	Illumina HiSeqX (4,359)
Simons Simplex Collection (SSC)		
ASD cases	2,419 (2,094/325)	Illumina HiSeq 2500 (40/40)
Non-ASD siblings	1,967 (928/1,039)	Illumina HiSeq X (2,379/1,927)
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	<i>ATP2A1</i>	Loss	MSSNG	NA
1024	Proband	ASD	chr16	28907687	28911255	13-14	3,569	<i>ATP2A1</i>	Loss	MSSNG	Maternal
SSC11925	Proband	ASD	chr16	28969291	28972486	3-4	3,196	<i>NFATC2IP</i>	Loss	SSC	Maternal
SSC12583	Proband	ASD	chr16	28969291	28972486	3-4	3,196	<i>NFATC2IP</i>	Loss	SSC	Maternal
SSC11987	Unaffected sibling	Non-ASD	chr16	28969291	28972486	3-4	3,196	<i>NFATC2IP</i>	Loss	SSC	Maternal
3-0707-000	Proband	ASD	chr16	28855875	28856051	5	177	<i>TUFM</i>	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	<i>De Novo</i>		+				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	<i>De Novo</i>		+				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	<i>De Novo</i>		+					None
322670	M	Loss	chr16:28,689,085-29,051,191	362,106	<i>De Novo</i>	+			+			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	<i>De Novo</i>				+		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	<i>De Novo</i>		+				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	<i>De Novo</i>			+		+	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	<i>De Novo</i>	+	+				Learning disability, seizures	None
330222	M	Loss	chr16:28,824,490-29,051,191	226,701	<i>De Novo</i>			+	+	+		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	<i>De Novo</i>		+					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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