Autism multiplex family with 16p11.2p12.2 microduplication syndrome in monozygotic twins and distal 16p11.2 deletion in their brother

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Supplementary Material

Supplementary Note



Inset of Figure 2.

The segmental duplications in the 16p11.2-p12.2 region have been assigned the alphanumeric IDs A1-A2, B1-B3, C1-C2, D1-D6, E1-E3 and F1-F3. Segmental duplications A1 and A2 are 110 kb in size, are in the same orientation and share >98% sequence identity. The distal (telomeric) breakpoint of the duplication in the twins (Patients 2 and 3) is located at A1, whereas the proximal (centromeric) breakpoint of the deletion in their non-twin brother (Patient 1) and the father is located at A2. Segmental duplications B1-B3 are ~55 kb in size, are oriented in the same way and share >99% sequence identity. As A1 and A2, segmental duplications B1 and B2 are located at the distal breakpoint of the duplication and the proximal breakpoint of the deletion, respectively, whereas B3 is located at the proximal breakpoint of the duplications C1 and C2 are ~42 kb in size, are oriented in the same way and share >98% sequence identity. The proximal and distal breakpoints of the duplication are located at segmental duplications C1 and C2, respectively. Segmental duplications D1-D6 are 15-20 kb in size, are oriented in the same way and share >98% sequence identity. D1-D6 are present at all distal and proximal breakpoints of the deletion and the duplication. Segmental duplication. Segmental duplications E1-E3 are 15 kb in size, are in the same orientation and share >98% sequence identity. D1-D6 are present at all distal and proximal breakpoints of the deletion and the duplication. Segmental duplications E1-E3 are 15 kb in size, are in the same orientation and share >98% sequence identity.

	Patient 1	Patient 2	Patient 3
Cox	Mole		
Sex Conversion beneficial and a			
Copy number impaiance	(28.40-29.25 Mb)	(21.28-30.23 Mb)	(21.28-30.23 Mb)
Inheritance	Paternal	de novo	de novo
Clinical and research diagnosis	Autism	Autism	Autism
ADI-R			
Age at evaluation	5 y 1 mo	3 y 2 mo	3 y 2 mo
Social (cutoff 10)	17	23	22
Communication (cutoff 7)	14 (non verbal)	14 (non verbal)	13 (non verbal)
Repetitive behavior (cutoff 3)	7	4	3
Onset (cutoff 1)	5	4	4
ADI-R diagnosis	Autism	Autism	Autism
ADOS (module 1)			
Age at evaluation	—	18 y 5 mo	18 y 5 mo
Communication (cutoff 4)	—	6	6
Social Interaction (cutoff 7)	—	14	13
Total (cutoff 12)	_	20	19
Play	—	4	4
Interests and Behavior	_	8	8
ADOS diagnosis	—	Autism	Autism
IQ measure			
Scale	WISC-III	RCPM	RCPM
Age at evaluation	16 y	18 y 4 mo	18 y 4 mo
Full scale IQ	47	<1st centile	<1st centile
Verbal IQ	50	—	—
Performance IQ	50	—	—
VABS II ¹			
Age at evaluation	5 y 2 mo	18 y 5 mo	18 y 5 mo
Communication	13	11	9
Daily living	20	23	21
Socialization	15	1	1
Maladaptive behaviors	38	20	23
CARS			
Age at evaluation	_	10 y 3 mo	10 y 3 mo
Composite score		37	38
Severity		Mild-moderate autism	Severe autism

Supplementary Table 1. Psychiatric, cognitive and developmental evaluations of the siblings

¹ Age equivalent scores, expressed in months. Abbreviations: CARS, Childhood Autism Rating Scale; MZ, monozygotic; RCPM, Raven's Colored Progressive Matrices; VABS II, Vineland Adaptive Behavior Scales, second edition; WISC-III, Wechsler Intelligence Scale for Children, third edition

Subject	Chr	Start ¹	End ¹	Size	Algorithms	CNV	Inheritance	DGV ³
Patient 1	2	4191253	4199818	8565	CNV partition	deletion	paternal	yes
	3	74185282	74280502	95220	CNV partition, PennCNV	deletion	maternal	partial
	4	69097539	69163188	65649	CNV partition	deletion	maternal [∠]	yes
	6	79029649	79090197	60548	CNV partition	deletion	paternal	yes
	6	170222030	170223555	1525	CNV partition	deletion	paternal ²	yes
	8	39356825	39497557	140732	CNV partition, PennCNV	deletion	paternal	yes
	9	370095	400312	30217	CNV partition, PennCNV	duplication	maternal	yes
	11	55124465	55149014	24549	CNV partition	deletion	paternal ²	yes
	11	80663196	80692622	29426	CNV partition, PennCNV	deletion	maternal	yes
	12	7891603	8014573	122970	CNV partition, PennCNV	deletion	paternal	yes
	13	56659471	56665094	5623	CNV partition	deletion	paternal	yes
Patient 2	3	164004033	164085280	81247	CNV partition	deletion	maternal	yes
	4	69097539	69163188	65649	CNV partition	deletion	maternal ²	yes
	4	161271979	161291569	19590	CNV partition	deletion	de novo?	yes
	6	67075448	67104015	28567	CNV partition	deletion	paternal	yes
	6	79029649	79090197	60548	CNV partition	deletion	paternal	yes
	6	170222030	170223555	1525	CNV partition	deletion	paternal	yes
	8	39356825	39497557	140732	CNV partition, PennCNV	deletion	paternal	yes
	11	55124465	55149014	24549	CNV partition	deletion	paternal	yes
	11	80663196	80692622	29426	CNV partition, PennCNV	deletion	maternal	yes
	12	7891603	8014573	122970	CNV partition, PennCNV	deletion	paternal	yes
	14	18531500	19387587	856087	CNV partition, PennCNV	duplication	paternal	yes
	19	20413668	20507068	93400	CNV partition	deletion	maternal	yes
Father	1	103941535	104107839	166304	CNV partition, PennCNV	deletion		yes
	2	4191253	4199818	8565	CNV partition	deletion		yes
	4	115398433	115401739	3306	CNV partition	deletion		yes
	6	67075448	67104015	28567	none (manual inspection)	deletion		yes
	6	79029649	79090197	60548	CNV partition	deletion		yes
	6	170222362	170223555	1193	CNV partition	deletion		yes
	8	39356825	39497557	140732	CNV partition, PennCNV	deletion		yes
	10	66981540	66983475	1935	CNV partition	deletion		yes
	11	55124465	55171592	4/12/	CNV partition	deletion		yes
	12	7891603	8014573	122970	CNV partition, PennCNV	deletion		yes
	13	50040795	50005094	18299	CINV partition	deletion		yes
	14	18531500	1938/58/	856087	none (manual inspection)	duplication		yes
	10	81590327	81706426	116099	CNV partition, PennCNV	deletion		no
Mother	18	30515599	30519440	3847	CNV partition	deletion		yes
wouner	2	02/94//2	02091020	90204		deletion		yes
	2	159007833	159679609	11//6	CNV partition	deletion		partial
	<u> </u>	122104645	14200002	95220	CNV partition	deletion		partial
	3	164004022	164101570	07546	CNV partition	deletion		yes
	-3	60064675	60163199	09513	CNV partition	deletion		yes
	4	70164519	70246977	90313	CNV partition	deletion		yes
	5	1/8738220	1/0240077	583252	CNV partition PennCNV	deletion		nartial
	5	150167998	150104802	26894	CNV partition	deletion		ves
	<u>a</u>	370095	400312	30217	CNV partition PennCNV	duplication		Ves
	a	43515795	43720352	204557	CNV partition	deletion		Ves
	11	80663196	80692622	29426	none (manual inspection)	deletion		ves
	15	19777876	19967366	189490	CNV partition	deletion		ves
	18	36514913	36519446	4533	CNV partition	deletion		ves
	19	20404485	20507068	102583	CNV partition PennCNV	deletion		ves
	19	53898415	53900677	2262	CNV partition	deletion		ves
	10	00000110		v	e paration			,00

Supplementary	Table 2	Other	CNVs	identified	in the	family
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HumanCNV370-Duo BeadChip (Illumina) data were analyzed using CNV partition and PennCNV. In addition, the results were inspected manually to determine inheritance of genomic variants in the children. Because none of the CNVs detected in Patients 1 and 2 appeared to be rare, no attempt was made to validate these findings with an independent method. ¹Genomic coordinates correspond to the hg18 genome assembly (Build 36). ² Apparently inherited CNV with one breakpoint differing from the parent. ³DGV, Database of Genomic Variants (http://projects.tcag.ca/variation/).

	Engelen et al. (2002) Patient 1 (mother)	Engelen et al. (2002), Finelli et al. (2004) Patient 2 (daughter)	Finelli et al. (2004) Patient 1	Ballif et al. (2007) Patient 5 ¹	Present study Patient 2 (MZ twin)	Present study Patient 3 (MZ twin)
Duplicated region	16p11.2p12.2 (21.24-29.41 Mb)	16p11.2p12.2 (21.24-29.41 Mb)	16p11.2p12.2 (21.24-29.41 Mb)	trp(16)(p11.2p12.1)dup(16)(p12. 1p12.2) (21.4-28.2 Mb) (5.7 Mb trp;1.1 Mb dup)	16p11.2p12.2 (21.28-30.23 Mb)	16p11.2p12.2 (21.28-30.23 Mb)
Molecular studies	FISH (WCP16 and specific BAC probes)	FISH (WCP16 and specific BAC probes)	FISH (WCP16 and specific BAC probes)	array-CGH, SNP array 250K	SNP array 370K	SNP array 370K
Inheritance	unknown	inherited from mother (Patient 1)	mother's karyotype normal; father's karyotype not available	de novo	de novo	de novo
Gender	F	F	M	F	Μ	М
Age	40 y	5 v	25 v	10 y 11 mo	17 y 6 mo	18 y 4 mo
Weight	NA	16.3 kg (50 th centile)	42 kg (<3 rd centile)	-2 ŚD	49 kg (-2 SD)	55 kg (-1 SD)
Stature	158 cm (<3 rd centile)	103 cm (3 rd centile -2 cm)	151 cm (<3 rd centile)	 -3 to -4 SD, growth hormone deficiency 	173 cm (normal range)	172 cm (normal range)
HC	52.5 cm (<3 rd centile)	49 cm (3 rd centile)	54 cm (25 th centile)	-1 SD	55 cm (-1 SD)	55 cm (-1 SD)
Development	learning difficulties	normal in the first year of life	severe neurodevelopmental delay	significant neurodevelopmental delay	normal development followed by regression at 26 mo	normal development followed by regression at 24 mo
IQ	IQ 83 (PIQ 64, VIQ 100, WAIS)	IQ 80	25 <iq<39 3="" at="" td="" y<=""><td>IQ 42 (WISC-IV at age 9)</td><td>severe ID</td><td>severe ID</td></iq<39>	IQ 42 (WISC-IV at age 9)	severe ID	severe ID
ASD	autism	ASD	autism	_ ` ` ` ` ` `	autism	autism
Language	automatic speech, echolalia	poor expressive language, echolalia	first words at 10 y, no functional language	12 th percentile for reading, <1 st percentile for spelling and math	language regression at 26 mo; non verbal	language regression at 24 mo; non verbal
Other behavior	ADHD, borderline psychotic symptoms, flat affect, anxiety disorder, obsessive-compulsive symptoms	stubbornness	never achieved continence, needs help in eating, dressing and self care; hyperactive, poor attention	friendly and talkative, ADHD, anxiety and nervousness with nail biting and skin picking	not fully toilet trained, hyperactivity, short and agitated sleep	not fully toilet trained, hyperactivity, aggressive behavior, self-injurious behavior, severe sleep disturbance
Dysmorphism	Symptomo		attornion			
face	-	-	asymmetric, squared, bitemporal constriction, sparse hair	round face with full cheeks	triangular face, broad forehead, prominent orbital ridge	triangular face, broad forehead, prominent orbital ridge
eyes	_	upslanting palpebral fissures	hypertelorism	narrow and slightly short palpebral fissures, relative hypertelorism, ptosis, strabismus, hyperopia	deep set eyes, upslanting palpebral fissures, thick upslant eyebrows, myopia, strabismus	deep set eyes, upslanting palpebral fissures, thick upslant eyebrows, myopia, strabismus
nose	-	-	broad nasal bridge, broad nasal tip, prominent columella	short nose, wide nasal bridge and round tip, anteverted nares	large, prominent nasal bridge, wide, bulbous tip, anteverted nares	large, prominent nasal bridge, wide, bulbous tip, anteverted nares
mouth and jaw	—	—	large mouth, thick lips, large irregular teeth, short philtrum	wide mouth, high-arched palate, thin upper lip, long philtrum, retrognathia	everted lower lip, tented philtrum wide cupid's bow, overbite, retrognathia with marked chin	everted lower lip, tented philtrum wide cupid's bow, overbite, retrognathia with marked chin
ears	_	_	long	low and posteriorly rotated, hyperacusis	backward tilted	backward tilted
Other malformations	_	_	bilateral club foot	bridged palmar creases, short fifth fingers, prominent finger tip pads, bilateral hallux valgus, minimal 2,3 toe syndactyly	ventricular septal defect, slender habitus, sternum deformity, mild scoliosis, cubitus valgus, long fingers and toes, bilateral single palmar creases and sandal gap	no heart defect, slender habitus, sternum deformity, mild scoliosis, cubitus valgus, long fingers and toes, bilateral single palmar creases and sandal gap
Infections	_	_	recurrent bronchitis, otitis, and gastroenteritis in childhood	_	recurrent otitis, nose and throat infections in childhood	recurrent otitis, nose and throat infections in childhood
Neurological	distal coreoathetotic movements, cervical dystonia	_	walked unassisted at 8 y, ataxic gait	hypotonia	hyporeflexia, no motor deficit	absent reflexes, no motor deficit
Epilepsy	_	_	yes	_	_	_
Brain imaging	brain CT normal	NA	MRI: slight ventricular dilatation, moderate diffuse cortical atrophy	NA	MRI: white matter hyperintensities, moderate cortical atrophy and thin corpus callosum	MRI: white matter hyperintensities, moderate cortical atrophy and thin corpus callosum

Supplementary Table 3. Clinical features of individuals with duplications of the 16p11.2p12 region

¹ The patient reported by Ballif et al. (2007) has a complex duplication and triplication involving the 16p11.2p12.1 region. Abbreviations: ADHD, attention deficit-hyperactivity disorder; ASD, autism spectrum disorder; CT, computed tomography; F, female; HC, head circumference; ID, intellectual disability; IQ, intellectual quotient; M, male; MRI, magnetic resonance imaging; MZ, monozygotic; NA, not available (not reported); PIQ, performance IQ; SD, standard deviation; VIQ, verbal IQ; WCP16, whole chromosome painting 16

Supplementary Table 4. Genes within the 220-kb minimal region of overlap of distal 16p11.2 deletions (28.73-28.95 l

Symbol	Name	Coordinates (hg 18)	Function	Associated disease
ATXN2L	Ataxin 2 related protein	28 741 915- 28 756 059	ATXN2L is a member of the spinocerebellar ataxia family, suggested to be a component of the cytokine signaling system	No associated disease
TUFM	Tu translation elongation factor, mitochondrial	28 761 233- 28 765 230	TUFM participates in protein translation in mitochondria	An homozygous missense mutation in <i>TUFM</i> was described in a baby with mitochondrial encephalopathy (combined oxidative phosphorylation deficiency 4, OMIM 610678)
SH2B1	SH2B adaptor protein 1	28 782 725- 28 793 027	SH2B1 is an adaptor protein involved in leptin, insulin, cytokine and growth factor receptor signaling. In addition to its role in central nervous system-mediated obesity, SH2B1 facilitates neurite outgrowth	Severe early-onset obesity (OMIM 613444)
ATP2A1	ATPase, Ca++ transporting, cardiac muscle, fast twitch 1	28 797 310- 28 823 331	ATP2A1 is a magnesium-dependent enzyme involved in muscular excitation and contraction	Autosomal recessive Brody myopathy (OMIM 601003), characterized by impaired muscular relaxation during exercise
RABEP2	RAB GTPase binding effector protein 2	28 823 243- 28 844 033	RABEP2 plays a role in membrane trafficking, regulating endosomal vesicle docking and fusion	No associated disease
CD19	CD19 antigen	28 850 761- 28 858 164	CD19 is a cell surface molecule that regulates the antigen receptor-dependent stimulation of B lymphocytes	Antibody-deficiency syndrome (OMIM 613493), autosomal recessive
NFATC2IP	Nuclear factor of activated T-cells	28 869 819- 28 885 268	NFATC2IP plays a role in the inducible expression of cytokine genes in T cells	No associated disease
SPNS1	Spinster homolog 1	28 893 650- 28 904 339	SPNS1 appears to be involved in programmed cell death. The homologue of <i>SPNS1</i> , <i>SPNS2</i> , encodes a transporter of the sphingolipid metabolite sphingosine 1-phosphate (S1P), which plays a central role in neural, cardiovascular, and immunological development and function	No associated disease
LAT	Linker for activation of T cells	28 903 648- 28 909 605	LAT is a transmembrane protein that when phosphorylated leads to the recruitment of multiple signaling molecules implicated in T cell activation	No associated disease

SNP	Position	Father		Mother		Patient 1		Patient 2 (twin)	
		B all freq	Genotype	B all freq	Genotype	B all freq	Genotype	B all freq	Genotype
rs11865131	103667	0.5	AB	0.9	BB	0.5	AB	0.5	AB
rs11248914	233563	0.5	AB	1.0	BB	0.5	AB	0.5	AB
rs9921222	315783	0.5	AB	1.0	BB	0.4	AB	0.5	AB
rs11645697	435663	0.6	AB	0.0	AA	0.0	AA	0.0	AA
rs12446663	5906038	0.5	AB	1.0	BB	1.0	BB	1.0	BB
rs6500827	6764824	0.5	AB	1.0	BB	0.5	AB	0.5	AB
rs4261521	7872048	0.6	AB	0.0	AA	0.4	AB	0.5	AB
rs1058967	8854537	0.5	AB	1.0	BB	0.5	AB	0.6	AB
rs8064140	9576189	0.6	AB	1.0	BB	0.5	AB	0.5	AB
rs6498229	11607016	0.5	AB	0.0	AA	0.5	AB	0.5	AB
rs1794302	12498592	0.5	AB	1.0	BB	0.5	AB	0.5	AB
rs1438701	13173810	0.6	AB	1.0	BB	0.5	AB	0.5	AB
rs7405176	14365847	0.6	AB	0.0	AA	0.6	AB	0.6	AB
rs4780577	15675498	0.5	AB	0.0	AA	0.5	AB	0.5	AB
rs2100401	16867138	0.4	AB	0.0	AA	0.0	AA	0.0	AA
rs4781952	17616369	0.6	AB	0.5	AB	0.5	AB	0.0	AA
rs9937539	18941493	0.5	AB	1.0	BB	1.0	BB	1.0	BB
rs739565	19624006	0.5	AB	1.0	BB	1.0	BB	1.0	BB
rs2214195	20406898	0.5	AB	0.0	AA	0.0	AA	0.0	AA
rs858205	20979000	0.6	AB	1.0	BB	0.6	AB	0.6	AB
rs9933675	21161736	0.6	AB	0.0	AA	0.0	AA	0.0	AA
rs2238478	21190256	0.5	AB	0.0	AA	0.5	AB	0.5	AB
rs226035	21202238	0.5	AB	1.0	BB	0.5	AB	0.5	AB
rs194548	21515973	0.5	AB	0.5	AB	1.0	BB	0.7	AB
rs226005	21528048	0.5	AB	1.0	BB	0.5	AB	0.3	AB
rs238547	23267700	0.6	AB	1.0	BB	0.5	AB	0.3	AB
rs8048821	23886863	0.5	AB	0.0	AA	0.5	AB	0.6	AB
rs4787697	25111249	0.6	AB	0.0	AA	0.5	AB	0.7	AB
rs4592663	25810187	0.5	AB	0.5	AB	0.0	AA	0.3	AB
rs321441	26611178	0.5	AB	0.0	AA	0.0	AB	0.6	AB
rs10400960	26738119	0.5	AB	1.0	BB	0.5	AB	0.4	AB
rs17200687	26743810	0.5	AB	0.0	AA	0.0	AA	0.0	AA
rs4787393	26857335	0.6	AB	1.0	BB	0.6	AB	1.0	BB
rs4238945	26882508	0.6	AB	0.0	AA	0.5	AB	0.0	AA
rs7206078	26955737	0.6	AB	1.0	BB	1.0	BB	0.3	AB
rs205432	27992496	0.6	AB	0.0	AA	0.5	AB	0.0	AA
rs205355	28012030	0.5	AB	1.0	BB	0.5	AB	1.0	BB
rs4075049	28150790	0.5	AB	1.0	BB	0.5	AB	1.0	BB
rs2650492	28240912	0.6	AB	1.0	BB	0.5	AB	1.0	BB
rs1064886	28396413	1.0	BB	10	BB	1.0	BB	1.0	BB
rs26528	28425210	1.0	BB	0.0	AA	0.0	AA	0.7	AB
rs153107	28432806	1.0	BB	0.0	AA	0.0	AA	0.7	AB
rs151228	28470527	1.0	BB	0.0	AA	0.0	AA	0.6	AB
rs151230	28490716	0.0	AA	1.0	BB	1.0	BB	0.3	AB
rs7201929	28779467	0.0	AA	1.0	BB	1.0	BB	0.3	AB
rs4072401	28844776	1.0	BB	0.0	AA	0.0	AA	0.7	AB
rs7184953	29223380	1.0	BB	1.0	BB	1.0	BB	1.0	BB
rs4787478	29256578	0.4	AB	0.6	AB	1.0	BB	0.0	AA
rs8054172	29563365	0.5	AB	0.0	AA	0.6	AB	0.0	AA
rs11150564	29574237	0.5	AB	1.0	BB	0.5	AB	1.0	BB
rs7202714	30085308	0.5	AB	1.0	BB	0.5	AB	1.0	BB
rs9938125	30195224	1.0	BB	10	BB	10	BB	1.0	BB
rs4238961	30287987	0.6	AR	0.0	<u>A</u> A	0.6	AR	0.0	A A
rs1064524	30400324	0.5	AR	1.0	RR	0.5	AR	1.0	BR
rs8058961	30716564	0.5	AR	0.0	AA	0.6	AR	0.0	AA
rs8048583	31187037	0.5	AR	1.0	BB	1.0	BB	0.5	AR
rs12927233	32045466	0.6	AR	0.0	AA	0.5	AR	0.0	AA
rs8047974	34079200	0.5	AR	1.0	BR	0.5	AR	1.0	BR
Contromoro	5-07 5200	0.0	AD	1.0	00	0.0	AD	1.0	00

Supplementary Table 5. B allele frequency and genotypes of the short arm of chromosome 16

Informative SNPs were selected to illustrate the rearrangements and the parental origin. The 16p11.2p12.2 region duplicated in Patient 2 (and in his twin brother, Patient 3) is highlighted in blue, the 16p11.2 region deleted in Patient 1 and his father is highlighted in red. Informative SNPs for which Patient 1 and Patient 2 share the same paternal allele are shown in blue font, those for which they inherited a different allele are shown in red font. A crossing-over in the father's germline inherited by Patient 1 occurred between 26 743 810 bp and 26 857 335 bp. Non-informative SNPs at the start and end of the duplication and the deletion are shown in black. Genomic coordinates correspond to NCBI Build 36 (hg18).