Supplementary Data

The clinical significance of small copy number variants in neuro-developmental disorders

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Supplementary Table 1. List of all CNVs <500 kb detected in 714 patients with Affymetrix high resolution arrays

Patient ID	Gender	CNV	Chr	Band	hg, chr:start-end	Size (kbp)	Confidence value (%)	Marker count	Affected gene(s)	MLPA target (s)	MLPA result	Inheritance	Pathogenicity	Affymetrix array	Remarks
59484	M	Del	4	q12	hg18, 4:52554386-52556739	2	96	7	LRRC66	LRRC66 e4	Confirmed	Paternal	no evidene	2.7	
61587	M	Del	2	q31.1	hg18, 2:170957153-170959604	2	96	6	MYO3B	MYO3B e15, e16	Confirmed	Paternal	no evidene	2.7	
60984	F	Del	16	p12.1	hg18, 16:24793142-24797112	4	96	6	SLC5A11	SLC5A11 e6, e7	Confirmed	Maternal	no evidene	2.7	Sanger sequencing of the gene was negative. SLC5A11 is a cotransporter. Cotransporters represent a major class of proteins that make use of ion gradients to drive active transport for the cellular accumulation of nutrients, neurotransmitters, osmolytes, and ions. Many members of this protein family are involved in genetic disorders.
617	F	Del	17	p13.3	hg18, 17:2519114-2523280	4	96	6	PAFAH1B1	PAFAH1B1 e6, e7	Confirmed	Parents N/A	Pathogenic	2.7	PAFAH1B1 is OMIM gene for incomplete lissencephaly.
9229	M	Del (homo)	1	p36.31	hg18, 1:6361008-6368114	7	98	8	ACOT7	ACOT7 e1, i1-2	Confirmed	Parental (het)	Likely pathogenic	2.7	He is the only child in the family with homozygous deletion and is the only affected. Details are available in the descripton of the patient.
58822	M	Del	7	q11.22	hg18, 7:69233202-69240841	8	93	16	AUTS2	AUTS2 i3-4, e4	Confirmed	Paternal	Pathogenic	2.7	Father is affected. Sanger sequencing of the gene was negative. Microdeletions of the gene in patients with variable neurodevelopmental features have been reported.
68629	M	Del	11	q13.4	hg19, 11:74569938-74579385	9	92	19	XRRA1	XRRA1 i11-12, e12	Confirmed	Maternal	no evidene	Cytoscan	
61825	F	Del	19	q13.11	hg18, 19:39379364-39389949	11	92	14	LSM14A	LSM14A e3, i3-4	Confirmed	Paternal	no evidene	2.7	
62610	M	Del	2	p16.2	hg18, 2:54411976-54424374	12	91	9	C2orf73	C2orf73 e2, e3	Confirmed	Paternal	no evidene	2.7	Only exon 2 of C2orf73 was deleted.

62611	F	Del	4	q22.1	hg18, 4:88438703-88452522	13	90	14	HSD17B13	HSD17B13 i6-7, e7	Confirmed	Paternal	Likely benign	2.7	Heterozygous de novo mutation in SCN2A: c.4025T>C (p.L1342P) was found in this patient. There are DGV variations 30208 (2/485 ctls, Jakobsson 2008), 51479 (3/2026 ctls, Shaikh 2009) and 36311 (1/1 ctl, Kidd 2008) with deletions encompassing some other exon(s) of HSD17B13.
56366	F	Del	12	q24.21	hg18, 12:115158648-115175505	17	87	18	MED13L	MED13L i1-2, e2	Confirmed	*de novo*	Pathogenic	2.7	Sanger sequencing of the gene was negative. Whole-exome sequenicng showed no evidence for a non-allelic second hit. This patient and further cases were described elsewhere (EJHG 2013).
45333	M	Del	20	q13.32	hg19, 20:57556968-57575495	19	94	28	TH1L (NELFCD), CTSZ	TH1L (NELFCD) e2, e10; CTSZ e5	Confirmed	*de novo*	Likely benign	Cytoscan	Pathogenic mosaic heterozygous mutation in PIK3CA: c.2740G>A (p.G914R) was found in the patient by whole-exome sequencing.
68738	M	Del	7	q35	hg19, 7:144509480-144534911	25	93	35	TPK1	TPK1 i1-2, i2-3	Confirmed	Father N/A	VOUS	Cytoscan	This deletion was absent in the mother and a healthy sister but present in the likewise affected sister. Father's DNA was not available. Sanger sequencing of the gene in available samples was negative. TPK1 encodes thiamin pyrophosphokinase 1 which catalyzes the conversion of thiamine to thiamine pyrophosphate. It is expressed in brain, and a de novo missense mutation of TPK1 has been reported in an autism cohort using WES by Sanders et al.
31553	M	Del	21	q22.3	hg18, 21:43169759-43201708	32	92	22	NDUFV3, WDR4	NDUFV3 e3	Confirmed	Paternal	VOUS	2.7	Sanger sequencing of the gene was negative. A de novo missense mutation of WDR4 has been reported in an autism cohort using WES and mutations of NDUFV3 have been reported in complex I deficiency. WDR4 is a candidate for some disorders mapped to 21q22.3 as well as for the development of Down syndrome phenotypes. NDUFV3 encodes NADH dehydrogenase (ubiquinone) flavoprotein 3 which is one of the subunits that make up the NADH-ubiquinone oxidoreductase complex.

70886	M	Dup	2	q33.1	hg19, 2:200278502-200310272	32	89	68	SATB2	SATB2 i3-4, e4	Confirmed	*de novo*	Pathogenic	Cytoscan	Deletions and truncating mutations of SATB2 have been implicated as causative for cleft palate and ID.
64035	M	Del	8	q24.3	hg18, 8:141650841-141693661	43	92	43	EIF2C2 (AGO2)	EIF2C2 (AGO2) e2, e3	Confirmed	Maternal	no evidene	2.7	
60407 & 66928 & 60324	F (all)	Del	4	q12	hg18, 4:52579180-52624947	46	94	52	SGCB, SPATA18	SGCB e2; SPATA18 e4	Confirmed in all	Maternal	no evidene	2.7	They are triplet. Exome sequencing did not reveal any obvious Mendelian cause.
43552	M	Del	16	p13.3	hg18, 16:4986264-5046682	60	89	53	NAGPA, C16orf89, SEC14L5	NAGPA e4, SEC14L5 e12	Confirmed	*de novo*	VOUS	2.7	A smaller deletion including only SEC14L5 and NAGPA is present in 1/1038 of a world wide control cohort by Affymetrix.
63660	M	Del	12	q24.31	hg18, 12:120240193-120307177	67	88	45	ANAPC5	ANAPC5 e4, e10	Confirmed	Maternal	no evidene	2.7	
70229	M	Del	12	q24.33	hg19, 12:132552537-132623611	71	90	48	EP400, EP400NL, DDX51	EP400 e51, EP400NL e3	Confirmed	*de novo*	VOUS	Cytoscan	There is patient 262376 in Decipher database with a duplication of unknown inheritance encompassing the same genes.
62915	F	Del	X	p22.2	hg18, X:16531546-16605488	73	91	57	S100G, CTPS2	CTPS2 e11, e12, e16	Confirmed	Maternal	no evidene	2.7	
50286 & 53032	F & M	Del (homo)	2	p21	hg18, 2:44435674-44519612	83	99	93	PREPL, C2orf34 (CAMKMT)	PREPL e1, C2orf34 e1, e2, e3	Confirmed	Parental (het)	Pathogenic	2.7	Two siblings were affected. It is overlapping hypotonia-cystinuria syndrome. These patients are described elsewhere (AJMG 2013).
54949	M	Del	6	q26	hg18, 6:162072914-162156999	84	92	93	PARK2	N/A	array in parents	Maternal	VOUS	2.7	This deletion was within PARK2. CNVs of PARK2 have been shown by Glessner et al. 2009 and Girirajan et al. 2013 to be significantly enriched in ASD cases versus controls.
72125	F	Dup	10	p14	hg19, 10:7932363-8033508	101	90	108	TAF3	TAF3 e3	Confirmed	*de novo*	VOUS	Cytoscan	TAF3 encodes TAF3 RNA polymerase II, TATA box binding protein (TBP)-associated factor. TAFs contribute to promoter recognition and selectivity and act as antiapoptotic factors.
62563	F	Del	5	p15.2	hg18, 5:11431816-11545236	113	NA	99	CTNND2	CTNND2 e5, e6	Confirmed	*de novo*	Pathogenic	6.0	Sanger sequencing of the gene was negative. CTNND2 is implicated in the ID phenotype of cri-du-chat syndrome. Further patients are discussed in this paper.
60045	M	Dup	7	q36.1	hg18, 7:148278728-148391821	113	89	60	PDIA4	PDIA4 e7, e8	Confirmed	Paternal	no evidene	2.7	
45679	M	Dup	20	q11.21-q11.22	hg18, 20:31442246-31556613	114	88	72	SNTA1, CDK5RAP1, CBFA2T2	SNTA1 e2, e4	Confirmed	Paternal	no evidene	2.7	

62848	F	Dup	12	q24.23	hg18, 12:117061815-117183853	122	NA	71	TAOK3, PEBP1	TAOK3 e9, e10, e18, e21	Confirmed	*de novo*	Likely benign	6.0	Pathogenic heterozygous mutation in SHANK2: c.2514_2515insG (p.P841Sfs*32) was found in this patient by whole-exome sequencing.
64926	M	Dup	1	p35.1	hg18, 1:33599961-33727852	128	90	100	ZSCAN20, PHC2	ZSCAN20 e2, e3	Confirmed	Maternal	no evidene	2.7	
62709	M	Del	17	q25.3	hg18, 17:73412776-73547163	134	90	90	TNRC6C	TNRC6C e2	Confirmed	Paternal	no evidene	2.7	
63930	M	Del	5	q15	hg18, 5:95753227-95900228	147	92	162	PCSK1	PCSK1 e3, e11	Confirmed	Paternal	no evidene	2.7	This patient has balanced translocation 5/12.
71156	F	Del	16	p13.3	hg19, 16:3788867-3935836	147	91	353	CREBBP	MLPA Kit P245	Confirmed	*de novo*	Pathogenic	Cytoscan	This deletion was within CREBBP which is the OMIM gene for Rubinstein-Taybi syndrome.
56761	M	Del	X&Y	q28	hg18, X:154686877-154843251	156	NA	15	VAMP7	VAMP7 e4, e5	Confirmed	Paternal	Likely benign	6.0	Sanger sequencing of the gene was negative. Patient 272246 in Decipher database with similar inherited deletion, has an additional 10Mb de novo deletion.
68422	M	Dup	1	q43	hg19, 1:236964009-237124627	161	90	236	MTR	N/A	array in parents	Maternal	no evidene	Cytoscan	
55113	M	Del	3	p14.1	hg18, 3:67592633-67762247	170	93	198	SUCLG2	SUCLG2 e5, e9	Confirmed	Maternal	VOUS	2.7	Sanger sequencing of the gene was negative. Patient 265229 in Decipher database with deletion within SUCLG2 (inherited) and patient 259685 with duplication of the whole gene (inherited) have different phenotypes. SUCLG2 encodes a GTP-specific beta subunit of succinyl-CoA synthetase.
62155	M	Del	9	p22.1	hg18, 9:19379757-19555588	176	NA	113	ASAH3L (ACER2), SLC24A2	ASAH3L (ACER2) e2, e4	Confirmed	Maternal	Likely benign	6.0	Sanger sequencing of the gene was negative. Pathogenic hemizygous 4 bp del in RPS6KA3 (RSK2): IVS10+4_+7delAGTA was found in this patient confirming the clinical diagnosis of Coffin-Lowry syndrome.
60245	F	Del	16	q23.3	hg18, 16:81560769-81736858	176	93	259	CDH13	CDH13 e3, e4	Confirmed	Maternal	Likely benign	2.7	Pathogenic heterozygous mutation in PTPN11: c.922A>G (p.N308D) was found in this patient confirming the diagnosis of Noonan syndrome. There are two duplications and five deletions within CDH13 (inherited or undefined) in Decipher database.

68942	M	Dup	X	q25	hg19, X:122803456-122986898	183	94	516	THOC2	THOC2 e1, e3	Confirmed	Maternal	no evidene	Cytoscan	This duplication was also present in maternal grandfather.
60482	M	Dup	1	p33	hg18, 1:46605120-46799318	194	NA	110	DMBX1, KNCN, FAAH, MKNK1	FAAH e3, e11; DMBX1 i1-2, e3	Confirmed	Paternal	no evidene	6.0	
57650	M	Del	X	p21.1	hg18, X:31598556-31801270	203	99	257	DMD	MLPA Kit P034	Confirmed	Parents N/A	Pathogenic, incidental finding	2.7	This deletion within DMD gene, in a male patient with facial dysmorphysim, developmental delay and ptosis but no sign of muscular dystrophy and normal muscle enzymes, was considered as an incidental finding with prognostic value. The deletion does not affect Dp71 isoform.
63153	F	Dup	1	p34.3	hg18, 1:38974598-39179543	205	90	168	GJA9, RRAGC, MYCBP, RHBDL2	GJA9 e2; RRAGC e7	Confirmed	Paternal	Likely benign	2.7	There are three partially overlapping deletions and one duplication in DGV database.
61006	M	Dup	4	q25	hg18, 4:113767097-113982110	215	89	133	LARP7, C4orf21, ANK2	LARP7 e2; C4orf21 e1; ANK2 e1	Confirmed	Maternal	no evidene	2.7	
62789	F	Dup	20	p13	hg18, 20:2635422-2864768	229	NA	136	FAM113A, CPXM1, VPS16, PCED1A, C20orf141, EBF4, PTPRA	VPS16 e22	Confirmed	Father N/A	VOUS	6.0	This dup was absent in the mother but present in the likewise affected sister. Father's DNA was not available. There are patients 254264 and 272274 in Decipher database with similar inherited duplications and ID.
60045	M	Dup	6	p21.1	hg18, 6:42147766-42383555	236	90	212	GUCA1B, GUCA1A, MRPS10, TRERF1, TAF8	GUCA1A e5, e6	Confirmed	Maternal	no evidene	2.7	
60984	F	Dup	X	q22.3	hg18, X:110044420-110289813	245	91	301	PAK3	PAK3 e1, e2	Confirmed	Paternal	Likely benign	2.7	PAK3 is a known X-linked recessive disease gene. In our case, it is a duplication and it is inherited from the healthy father.
59700	M	Dup	19	p13.11	hg18, 19:17283794-17537339	254	NA	81	11 genes	FAM125A (MVB12A) e2; SLC27A1 e2	Confirmed	Maternal	Likely benign	6.0	An overlapping duplication is present in 1/1038 of a world wide control cohort by Affymetrix.
53983	M	Del	7	q31.33	hg18, 7:125890040-126149790	260	93	301	GRM8	GRM8 e7, e8	Confirmed	Paternal	Likely pathogenic	2.7	Sanger sequencing of the gene was negative. Several CNVs of GRM8 have been reported in patients with ADHD compared to controls.
57614	M	Del	3	q12.3	hg18, 3:103671524-103941739	270	NA	141	ZPLD1	ZPLD1 e10, e11	Confirmed	Maternal	no evidene	6.0	

57028	F	Del	4	q22.3	hg18, 4:98745657-99078956	333	93	304	C4orf37 (STPG2)	C4orf37 (STPG2) e10	Confirmed	Paternal	VOUS	2.7	Sanger sequencing of the gene was negative. There are patients 249670 (maternally inherited) with partially overlapping phenotype to our patient and 256952 (inherited from normal parent) with seizure in Decipher database who have intragenic STPG2 deletions.
59248	F	Del	1	q24.3	hg18, 1:170135864-170505998	372	NA	244	DNM3	N/A	confirmed by FISH	*de novo*	VOUS	6.0	Sanger sequencing of the gene was negative. DNM3 encodes dynamin 3, a member of a family of guanosine triphosphate (GTP)-binding proteins that associate with microtubules and are involved in vesicular transport. WES of the patient and healthy parents revealed a heterozygous de novo missense mutation in ADAM7: c.190A>G (p.K64E). Since no germline mutation of ADAM7 has been reported so far, the relevance of this finding remains also unclear.
63409	F	Del	X	q13.1	hg18, X:70006030-70385683	380	93	209	10 genes	IL2RG i4-5, e6; MED12 e9, e13	Confirmed	Maternal	Pathogenic (in males), incidental finding	2.7	This patient was later diagnosed with Opitz-Bohring syndrome with pathogenic mutation in ASXL1:c.2197C>T (p.Gln733X). The deletion contains several known X-linked recessive disease genes and is likely lethal in males. The patient, carrier mother and grandmother showed 98% skewing of X-inactivation.
64717	F	Dup	16	p11.22	hg18, 16:29594946-30038054	443	88	287	25 genes	MLPA Kit P297	Confirmed	Maternal	Likely pathogenic	2.7	Recurrent 16p11.22 microduplications syndrome.
71264	M	Del	17	q21.31	hg19, 17:43703800-44163085	459	90	857	9 genes	MLPA Kit P245	Confirmed	*de novo*	Pathogenic	Cytoscan	Recurrent 17q21.31 microdeletion syndrome.
48459	M	Del	14	q24.3-q31.1	hg18, 14:78321869-78784675	462	92	586	NRXN3	NRXN3 e9, e10	Confirmed	Paternal	Likely pathogenic	2.7	Sanger sequencing of the gene was negative. A mixture of inherited and de novo deletions within NRXN3 has been reported in ASD.
69234	F	Del	X	p11.4	hg19, X:41339667-41811516	472	91	1040	CASK, GPR34, GPR82	CASK e23, e24	Confirmed	*de novo*	Pathogenic	Cytoscan	Phenotypic spectrum associated with CASK loss-of-function has been described.
52253	M	Del	17	q21.31	hg18, 17:41049320-41522088	473	NA	329	9 genes	MLPA Kit P245	Confirmed	Parents N/A	Pathogenic	6	Recurrent 17q21.31 microdeletion syndrome.

57305	M	Dup	21	q21.1	hg18, 21:21541563-22029191	488	NA	331	NCAM2	NCAM2 e10, e12	Confirmed	Paternal	Likely benign	6.0	There is an almost overlapping deletion in Decipher population CNVs (singleton).
62848	F	Del	3	q22.1	hg18, 3:132148497-132640489	492	NA	263	NEK11, ASTE1, NUDT16, NUDT16P, ATP2C1	ATP2C1 e23; NEK11 e16	Confirmed	Paternal	no evidene	6.0	
69551	M	Dup	X	q25	hg19, X:123586540-123589505	3	88	16	ODZ1 (TENM1)	ODZ1 (TENM1) e23	Not Confirmed			Cytoscan	
66314	F	Dup	22	q13.2	hg 19, 22:41530715-41533822	3	94	16	EP300	MLPA Kit P333	Not Confirmed			Cytoscan	
57123	F	Del	5	q12.3	hg18, 5:64893125-64897683	5	93	8	CENPK, PPWD1	CENPK i1-2; PPWD1 e1	Not Confirmed			2.7	It was only detected by annotation 29.
69551	M	Dup	X	p11.4	hg19, X:41392871-41396522	4	91	11	CASK	CASK e23, e24	Not Confirmed			Cytoscan	
56894	F	Del	1	q42.2	hg19, 1:231999534-232003661	4	92	12	DISC1	DISC1 i9-10, e10	Not Confirmed			Cytoscan	
67354	F	Del	9	p24.3	hg19, 9:332170-336058	4	92	12	DOCK8	DOCK8 e11, i11-12	Not Confirmed			Cytoscan	
67674	M	Del	11	p14.2	hg19, 11:26535654-26539603	4	92	12	ANO3	ANO3 i5-6, i6-7	Not Confirmed			Cytoscan	
65010	M	Del	5	q12.1	hg18, 5:61926273-61935437	9	93	12	IPO11	IPO11 i27-28, e28	Not Confirmed			2.7	It was only detected by annotation 29.
63935	M	Del	X	q28	hg18, X:147733407-147741983	9	95	10	AFF2	AFF2 e6, e7, i7-8	Not Confirmed			2.7	
60984	F	Del	4	p14	hg18, 4:39021615-39032347	11	89	12	RFC1	RFC1 e2, e3	Not Confirmed			2.7	It was only detected by annotation 29.
66075	F	Del	11	q14.1	hg18, 11:77960543-77971422	11	88	14	NARS2	NARS2 e1, e2	Not Confirmed			2.7	
64622	M	Del	11	q21	hg18, 11:93862415-93873098	11	88	15	MRE11A, ANKRD49	MRE11A e1, ANKRD49 i2-3	Not Confirmed			2.7	
38048	M	Del	10	q22.1	hg18, 10:71953731-71965051	11	90	12	KIAA1274 (PALD1)	KIAA1274 (PALD1) e2, e3, e5, e6	Not Confirmed			2.7	This patient was later diagnosed with acrocallosal syndrome with pathogenic compound heterozygous mutation in KIF7.
38004	F	Del	1	p31.3	hg18, 1:66862092-66873951	12	90	15	SGIP1	SGIP1 e2, e3, e4	Not Confirmed			2.7	

67116	F	Del	5	q33.1	hg18, 5:151746458-151759934	13	86	12	NMUR2	NMUR2 e2, e3	Not Confirmed	2.7	
66075	F	Del	4	q25	hg18, 4:113765627-113779348	14	88	17	LARP7, C4orf21	LARP7 e1; C4orf21 e1	Not Confirmed	2.7	It was only detected by annotation 29.
64103	M	Del	14	q21.31	hg18, 14:41498859-41512482	14	87	19	KIAA1267 (KANSL1)	KIAA1267 (KANSL1) e4, e5	Not Confirmed	2.7	
68198	M	Del	1	p35.1	hg18, 1:32918077-32933954	16	89	17	SYNC, RBBP4	SYNC e1, e2, e3	Not Confirmed	2.7	
62783	M	Del	2	q36.1	hg18, 2:223459673-223479127	19	88	19	ACSL3	ACSL3 i2-3, e3	Not Confirmed	2.7	
65880	M	Dup	X	q25	hg19, X:123541092-123559735	19	85	44	ODZ1 (TENM1)	ODZ1 (TENM1) e23, e24	Not Confirmed	Cytoscan	
60439	M	Del	15	q26.3	hg18, 15:97948427-97967301	19	88	21	MEF2A	MEF2A i1-2, i2-3	Not Confirmed	2.7	
67687	F	Del	9	q21.11	hg18, 9:72418692-72446917	28	88	30	TRPM3	TRPM3 e11, e12	Not Confirmed	2.7	
61186	M	Del	1	p36.21	hg18, 1:15829278-15860394	31	86	33	DDI2, RSC1A1	DDI2 e6, e9	Not Confirmed	2.7	
62075	F	Del	7	p12.2	hg18, 7:49959248-49994235	35	87	42	ZPBP	ZPBP e7, i7-8	Not Confirmed	2.7	
56985	M	Dup	6	q16.3	hg18, 6:102585885-102621667	36	89	48	GRIK2	GRIK2 e14, e15	Not Confirmed	2.7	It was only detected by annotation 29.
58404	F	Del	1	p34.3	hg18, 1:36197451-36234202	37	86	40	EIF2C3 (AGO3)	EIF2C3 (AGO3) i3-4, e5, i5-6	Not Confirmed	2.7	
66075	F	Del	3	q29	hg18, 3:197974381-198016767	42	85	51	PAK2	PAK2 e2, e3	Not Confirmed	2.7	
60407 & 66928 & 60324	F (all three)	Del	18	q21.1	hg18, 18:43620216-43668188	48	88	31	SMAD2	SMAD2 e3, e6	Not Confirmed	2.7	They are triplet.
62924	M	Dup	11	q13.5	hg18, 11:76468150-76561055	93	88	63	OMP, MYO7A, CAPN5	MYO7A e14, e15; CAPN5 e5, e9	Not Confirmed	2.7	
48307	M	Dup	4	p16.1	hg18, 4:8994976-9095173	100	NA	52	DEFB131, LOC650293	DEFB131 e1	Not Confirmed	6.0	

48307	M	Del	5	p15.33	hg18, 5:723744-826556	102	NA	34	TPPP	TPPP e2	Not Confirmed	6.0
60106	M	Dup	4	q13.3	hg18, 4:72549816-72662940	113	90	142	SLC4A4	SLC4A4 e19, e25	Not Confirmed	2.7
61186	M	Dup	18	q22.3	hg18, 18:70252255-70369954	118	88	73	C18orf51 (FAM69C), CNDP2, CNDP1	C18orf51 (FAM69C) e3, CNDP2 e6	Not Confirmed	2.7
62783	M	Dup	8	q24.3	hg18, 8:141587386-141711847	124	88	112	EIF2C2 (AGO2), CHRAC1	EIF2C2 (AGO2) e2, e3	Not Confirmed	2.7
64170	F	Dup	X	p11.22	hg18, X:50147702-50277014	129	88	131	DGKK	DGKK e1, e4	Not Confirmed	2.7
66075	F	Dup	X	p22.11	hg18, X:23210130-23347055	137	88	132	PTCHD1	PTCHD1 e2, e3	Not Confirmed	2.7
62075	F	Dup	X	q22.1	hg18, X:101379750-101531945	152	88	131	NXF2, NXF2B	NXF2 e3, e23	Not Confirmed	2.7
61098	M	Dup	19	p13.3	hg18, 19:2096867-2277988	181	NA	44	10 genes	DOT1L e5, e6	Not Confirmed	6.0

Pathogenic or likely pathogenic inherited heterozygous CNVs

patient 58822, 8 kb deletion within *AUTS2*, paternally inherited (pathogenic)

AUTS2 (autism susceptibility candidate 2, MIM *607270) was first discovered as a candidate gene when it was found to be disrupted by a balanced translocation in a pair of monozygotic twins with autism, developmental delay and epilepsy.[1] Despite some functional studies indicating its nuclear location, brain expression in various cell types as well as in regions implicated in autism spectrum disorder (ASD) and its potential role in neuronal development,[2-3] exact function of the gene and its pathways are unknown.

We found an 8 kb in-frame deletion encompassing exon 4 of *AUTS2* in a male patient with mild developmental delay, growth deficiency, microcephaly, cryptorchidism, bilateral inguinal and umbilical hernia, and minor anomalies including clinodactyly of the 5th finger, mild interdigital webbing and joint laxity. At the age 5 years 10 months height was 103 cm (<3rd centile), weight 16 kg (3rd centile), OFC 49 cm (<3rd centile) and eruption of teeth was delayed with first tooth at the age of 1 year. He was speaking in full sentences but was shy and had some mild motor delay with balancing problems and reduced pain sensitivity. This deletion was paternally inherited and the father was likewise affected with relative short stature (168.2 cm (10th-25th centile), OFC 55cm (10th centile)) in comparison to his parents, brother and sisters who had a height of about 180 cm.

Various aberrations disrupting *AUTS2* have been linked to ID, developmental delay and ASD. In addition, many of the reported patients have had additional manifestations such as epilepsy, microcephaly, facial dysmorphisms and short stature.[4-6] A recent study reported 24 exonic microdeletions of the gene in patients with variable neuro-developmental features, growth and feeding problems, skeletal abnormalities, and congenital malformations introducing an AUTS2 syndrome. They indicated that dysmorphic features and ID were more pronounced in individuals with 3' deletions because these affect also the alternative transcript of exons 9-19, which is expressed in brain.[4] The finding of the mild phenotype in our family with exon 4 deletion, which is 5' of the alternative transcription start, further supports this genotype-phenotype correlation and emphasises mild short stature as a major clinical finding.

Possible regulatory function of AUTS2 is in line with the presence of several protein interaction motifs for SH2, SH3, and WW domains, as well as the existence of numerous phosphorylation sites.[3] Since no functional domains were reported for exon 4, protein interaction motifs and phosphorylation sites were predicted using the eukaryotic linear motif (ELM) databank.[7] Phosphorylation sites were additionally verified using the NetPhosK

server.[8] The absence of exon 4 resulting in the deletion of amino acids 209-220 affects a sequence stretch that contains an unusually high number of residues that are predicted to become phosphorylated: S207, S209, S210, S213, S214, T217, and Y219. Interestingly, the spacing of the serine residues meets exactly the preferences for the action of the hierarchical protein kinases CK1 and GSK3.[9] Residues T217 and Y129 are part of the typical TGY dual-phosphorylation motif of MAP-kinase p38, which becomes phosphorylated by kinases of the MKK family during cell cycle regulation.[10]

patient 64717, 443 kb duplication in 16p11.2, maternally inherited (likely pathogenic)

Recurrent reciprocal 16p11.2 CNVs are characterised by a spectrum of neuro-cognitive and psychiatric phenotypes that are subject to incomplete penetrance and variable expressivity.[11-12]

CMA revealed a 443 kb maternally inherited duplication on 16p11.2 (MIM #614671) encompassing 25 genes in the girl referred for global developmental delay, muscular hypotonia, mild unsteady gate, and overgrowth. She was born spontaneously at term with weight 4500 g (>97th centile) and length 50 cm (25th centile). Developmental milestones were delayed (walking age 2 years, first words at ~4 years). At the age of 6 years and 8 months, her weight, height and head circumference were 31.9 kg (>97th centile), 131 cm (>97th centile; father: 180 cm, mother: 170 cm) and 50 cm (10th-25th centile). Physical examination revealed brachycephaly, relatively short neck, round face with prominent cheeks, narrow eyebrows, hypertelorism, broad philtrum, high-arched palate and large ears (6 cm; 98th centile). Since recurrent 16p11.2 CNVs have been associated with developmental delay, ID, autism, ADHD, seizures, and psychiatric problems and the spectrum of abnormal phenotypes has been expanded to include congenital abnormalities,[12] it is very likely that the duplication is pathogenic in this patient with incomplete penetrance or variable expressivity since the mother's cognition was not formally tested.

patient 48459, 462 kb deletion within *NRXN3*, paternally inherited (likely pathogenic)

NRXN3 (MIM *600567) encodes neurexin 3 in alpha- and beta isoforms expressed at variable levels throughout the brain. The neurexins are highly expressed in presynaptic terminals and have been shown to have important roles in synaptic cell adhesion and neurotransmitter secretion.[13]

CMA revealed a 462 kb paternally inherited deletion encompassing exons 6-12 of the alpha isoform of *NRXN3* and reaching very closely to the transcription start of the beta isoform

(Figure S2). Sanger sequencing of the gene in the patient revealed no pathogenic mutation. This boy was referred due to mild learning difficulties, minor motor problems, dilated cardiomyopathy, some facial features and anal atresia. Deletions of *NRXN3* have been recently reported in patients with ASD (one de novo, one inherited from the father with borderline autism phenotype, and two inherited from apparently healthy parents) with reduced penetrance and variable expressivity.[14] Therefore, the *NRXN3* deletion likely contributes to the learning difficulty in our patient, but the cause of his physical problems remains unknown.

patient 53983, 260 kb deletion within *GRM8*, paternally inherited (likely pathogenic)

GRM8 (MIM *601116) encoding glutamate receptor, metabotropic 8 is a group III metabotropic glutamate receptor which is linked to the inhibition of the cyclic AMP cascade with high expression in human fetal and adult brains.[15]

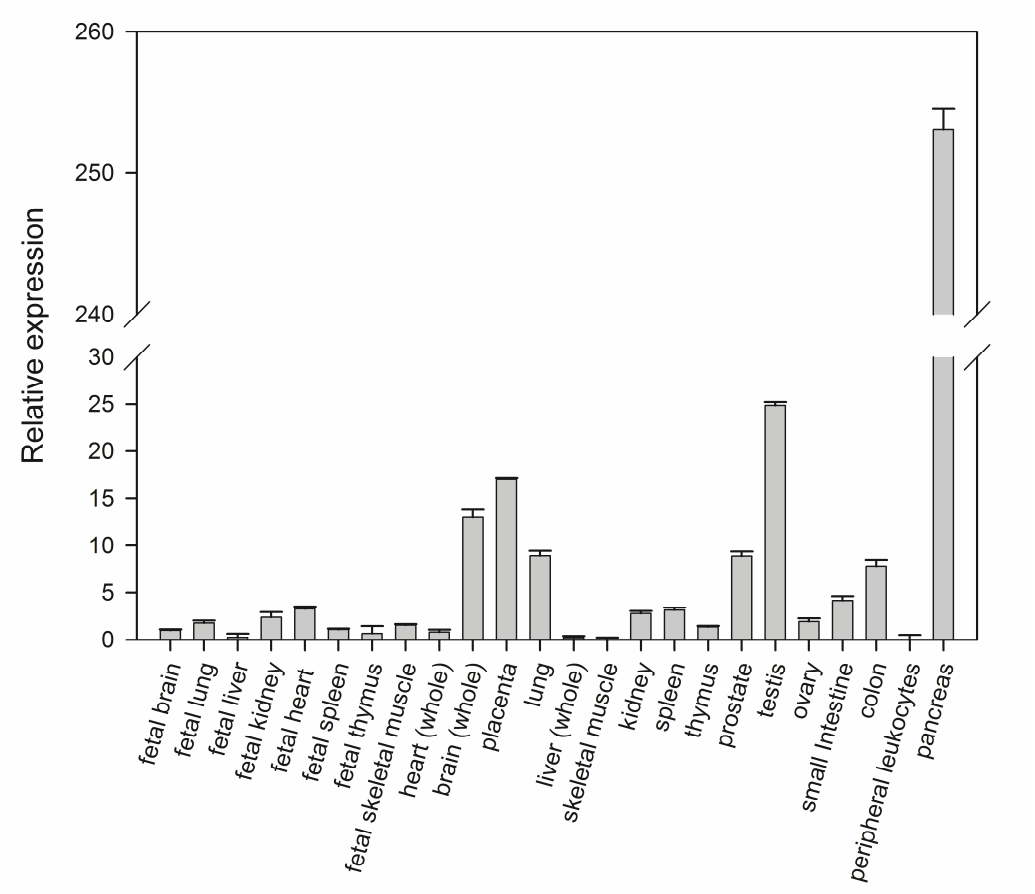
CMA showed a 260 kb paternally inherited deletion within the gene *GRM8*. This deletion encompasses exons 8 and 9 of the major isoforms a and b which causes a frame-shift introducing a premature stop codon at aa 516 and resulting in the removal of all transmembrane domains.[15] Concerning the minor, probably secreted isoform c without transmembrane domains,[15] the deletion affects exons 7-9 and causes an addition of 64 aminoacids and stop at amino acid 516 . Sequencing of the gene in the patient for a second hit revealed no mutation.

The boy was born spontaneously at 40 weeks of gestation without complications. Birth weight was 3680 g (50th-75th centile), length 52 cm (25th-50th centile) and head circumference was 34.5 cm (10th-25th centile). Apgar scores were 8/9/10. At the age of 1 year, he was diagnosed with a mixed spastic-ataxic movement disorder and a convergent strabismus with reduced vision on the left eye. He was hypotonic and his development was globally retarded with speech delay and unaided walking at age 3 years. Due to his long face with prominent forehead and periorbital fullness Fragile X syndrome was suspected but molecular testing revealed a normal repeat length. At age 6 10/12 years he showed hypotonia and fine and gross motor clumsiness, but there was no evidence for a movement disorder. He was distractible and hyperactive but was speaking fluently with some deficits and formal Kaufman ABC testing revealed a low normal IQ of about 80 with special strength in the puzzle subtest (level 96). His weight, height, and head circumference were 20.5 kg (19th centile), 121 cm (40th centile) and 49.5 cm (2nd centile), respectively. He received physical, occupational and speech therapy and attended a special school. Physical examination revealed mild left sided strabismus, anteverted nostrils, flat philtrum, large mouth, wide tooth spacing, high-arched

palate, retrognathia, clinodactyly of toes 4 and 5 and joint laxity. The older sister who does not carry the deletion also had a mild cerebral movement disorder, low normal head circumference (3rd-10th centile) and low normal cognition with an average HWAIK IV testing result of 88. Their non-carrier mother has had learning disability and developed epilepsy and hearing loss in adulthood. The carrier father attended regular school and completed an apprenticeship in a drug store, but functioned on a low social level.

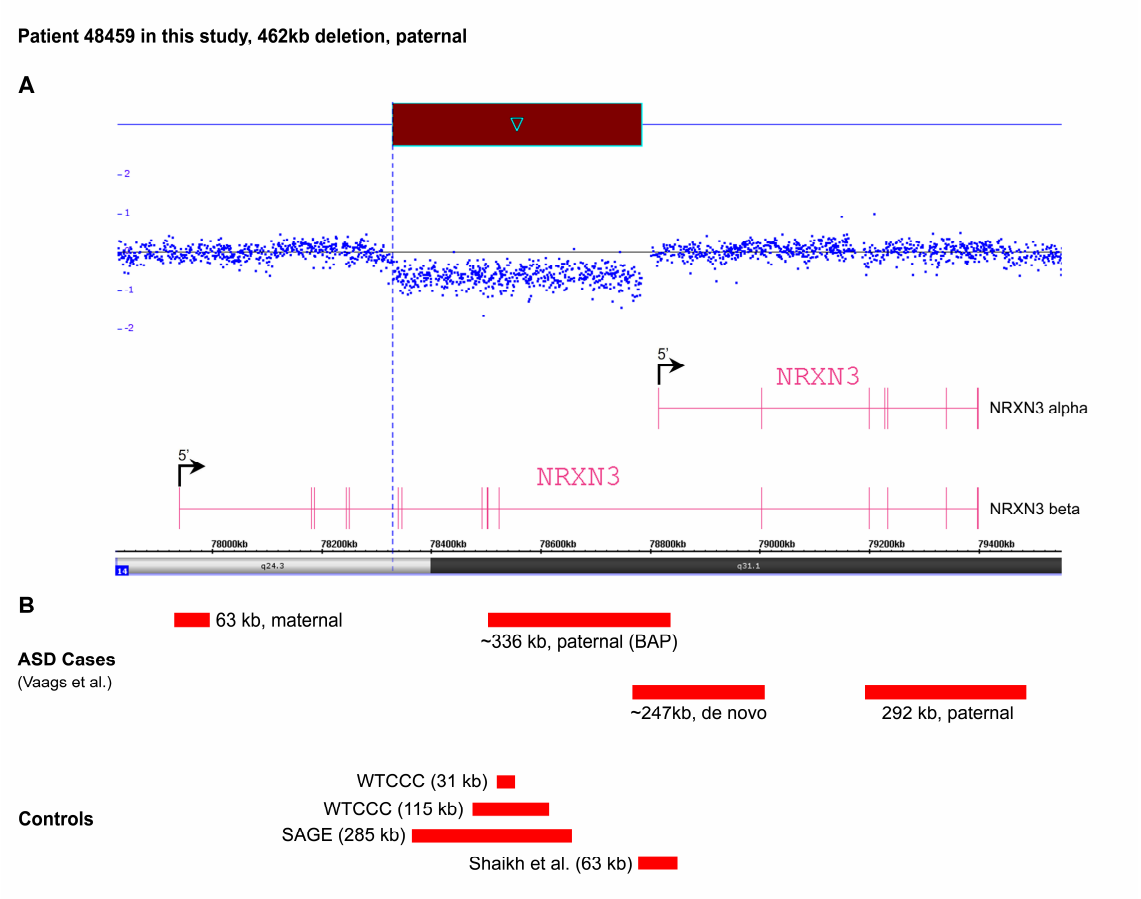
CNVs of glutamate receptor genes have been associated with ADHD including eight patients out of 2493 with deletions in *GRM8* but none in controls.[16] One patient with a paternally inherited 34.7 kb exonic duplication within *GRM8* and two maternally inherited intronic CNVs have been reported among rare CNVs in individuals with ASD.[17] Thus in accordance with the literature findings attention deficit and hyperactivity of the patient could be well explained by *GRM8* defect, but additional unidentified familial factors may contribute to his phenotype.

Figure S1. Expression analysis of *ACOT7* (isoform ENST00000377855) in cDNA panels from fetal and adult human tissues.



Expression analysis was performed using customized SYBR green qPCR for exons 1 & 2 of *ACOT7* (specific for isoform ENST00000377855). Relative expression levels normalized to GAPDH were set into relation to the mean expression value of this isoform in fetal brain. The highest levels were found in adult pancreas, testis, brain, lung, prostate and colon. No expression was detected in peripheral leukocytes.

Figure S2. Screenshot of the array finding in patient 48459 compared to ASD cases reported by Vaags et al.[14] and controls.



(A) Affymetrix 2.7 array derived plots of copy number and log2 ratio values of the *NRXN3* region in patient 48459 indicating a 462 kb deletion encompassing exons 6-12 of the alpha isoform and reaching very closely to the transcription start of the beta isoform. (B) ASD cases with inherited or de novo deletions of *NRXN3* reported by Vaags et al[14] and controls from the Wellcome Trust Case Control Consortium (WTCCC), the Study of Addiction: Genetics and Environment (SAGE) consortium and Shaikh et al.[18] Father of the affected child with the 336 kb paternal deletion has had clinical diagnosis of broader autism phenotype (BAP).

Supplementary Table 2. Clinical and genetic features of patients with inherited heterozygous or homozygous candidate CNVs ≤ 500 kb sorted by descending size

Patient ID	Age* (yrs)	Gender	Phenotype	Aberration	Chromosome band	Genome coordinates	Size (kb)	Confidence value (%)	Marker count	Affected gene(s)	Validation	Sanger sequencing of the affected genes	Pathogenicity
57305	4	M	muscular hypotonia, microcephaly, chorioretinal dysplasia, and lymphedema	duplication	21q21.1	hg18, chr21: 21541563-22029191	488	N/A	331	*NCAM2*	MLPA/ paternally inherited	N/A	likely benign (there is an almost overlapping deletion in Decipher population CNVs (singleton))
48459	7	M	mild learning difficulties, motor problems, facial dysmorphic features, dilated cardiomyopathy, and anal atresia	deletion	14q24.3-q31.1	hg18, chr14: 78321869-78784675)	462	92	586	*NRXN3*	MLPA/ paternally inherited	negative	likely pathogenic, reduced penetrance (a mixture of inherited and de novo deletions within *NRXN3* has been reported in ASD)[14]
64717	6	F	developmental delay, and overgrowth	duplication	16p11.2	hg18, chr16: 29594946-30038054	443	88	287	25 genes on 16p11.2	MLPA/ maternally inherited	N/A	likely pathogenic, reduced penetrance (recurrent microduplication syndrome)[19]
57028	1	F	developmental delay, sever speech delay, microcephaly, facial dysmorphic features, hallux valgus, and short stature	deletion	4q22.3	hg18, chr4: 98745657-99078956	333	93	304	C4orf37 (STPG2)	MLPA/ paternally inherited	negative	VOUS (there are patients 249670 (maternally inherited) similar to our patient and 256952 (inherited from normal parent) with seizure in Decipher database who have intragenic *STPG2* deletions)
53983	1	M	developmental delay, hypotonia, and strabismus	deletion	7q31.33	hg18, chr7: 125890040-126149790	260	93	301	*GRM8*	MLPA/ paternally inherited	negative	likely pathogenic, reduced penetrance (several CNVs of *GRM8* have been reported in ADHD compared to controls)[16]
59700	7	M	global developmental delay, congenital cerebellar ataxia, and sensorineural hearing loss	duplication	19p13.11	hg18, chr19: 17283794-17537339	254	N/A	81	11 genes	MLPA/ maternally inherited	N/A	likely benign (an overlapping duplication is present in 1/1038 of a world wide control cohort by Affymetrix)
60984	4	F	developmental delay, growth hormone deficiency, celiac disease, hypoplastic left kidney	duplication	Xq22.3	hg18, chrX: 110044420-110289813	245	91	301	*PAK3*	MLPA/ paternally inherited	N/A	likely benign (*PAK3* is a known X-linked recessive disease gene. In our case, it is a duplication and it is inherited from the healthy father)
62789	14	F	developmental delay, truncal ataxia, generalized epilepsy, and tall stature	duplication	20p13	hg18, chr20: 2635422-2864768	229	N/A	136	*VPS16*, *FAM113A (PCED1A)*, *C20orf141*, *CPXM1*, *EBF4*, and *PTPRA*	MLPA/- (absent in the mother, but father was N/A, (likely paternal)	N/A	VOUS (this duplication was absent in the mother but present in the likewise affected sister. There are patients 254264 and 272274 in Decipher database with inherited similar duplications and ID)
63153	2	F	developmental delay, hypotonia, epilepsy, and strabismus	duplication	1p34.3	hg18, chr1: 38974598-39179543	205	90	168	*GJA9*, *RRAGC*, *MYCBP*, and *RHBDL2*	MLPA/ paternally inherited	N/A	likely benign (there are three partially overlapping deletions and one duplication in DGV database)
62155	1	M	developmental delay, hypotonia, microcephaly, complex heart defect, short stature, and urether stenosis; follow-up clinical diagnosis of Coffin-Lowry syndrome	deletion	9p22.1	hg18, chr9: 19379757-19555588	176	N/A	113	*ASAH3L (ACER2)*, and *SLC24A2*	MLPA/ maternally inherited	negative	likely benign (pathogenic hemizygous 4 bp del in RPS6KA3 (RSK2): IVS10+4_+7delAGTA was found in this patient confirming the diagnosis of Coffin-Lowry syndrome)

60245	3	F	mild developmental delay, borderline short stature, and peripheral pulmonary stenosis	deletion	16q23.3	hg18, chr16: 81560769-81736858	176	93	259	*CDH13*	MLPA/ maternally inherited	N/A	likely benign (pathogenic heterozygous mutation in PTPN11: c.922A>G (p.N308D) was found in this patient confirming the diagnosis of Noonan syndrome. There are two duplications and five deletions within CDH13 (inherited or undefined) in Decipher database)
55113	18	M	ADHD, microcephaly, short stature, and myopia	deletion	3p14.1	hg18, chr3: 67592633-67762247	170	93	198	*SUCLG2*	MLPA/ maternally inherited	negative	VOUS (there are patients 265229 with inherited intragenic deletion of *SUCLG2* and 259685 with inherited duplication of the whole gene in Decipher database presenting with different phenotypes)
56761	5	M	global developmental delay with prominent speech delay, and cryptorchidism	deletion	Xq28 (pseudoautosomal)	hg18, chrX: 154686877-154843251	156	N/A	15	*VAMP7*	MLPA/ paternally inherited	negative	likely benign (patient 272246 in Decipher database with similar inherited deletion, has an additional 10 Mb de novo deletion. *VAMP7* (*synaptobrevin 1*) has been associated with bipolar affective disorder)[20]
54949	7	M	global developmental delay, muscular hypotonia, ataxia intermittent strabismus convergens, mild thoracic scoliosis, and pes planovalgus,	deletion	6q26	hg18, chr6: 162072914-162156999	84	92	93	*PARK2*	array in parents/ maternally inherited	N/A	VOUS (CNVs of *PARK2* have been shown to be significantly enriched in ASD cases versus controls)[21-22]
50286 and 53032 (siblings)	7.4 and 5	F and M	developmental delay, hypotonia, cleft palate, growth failure, and genital abnormalities (details reported elsewhere)[23]	deletion (homozygous)	2p21	hg18, chr2: 44435674-44519612	83	99	93	*PREPL* and *C2orf34* (*CAMKMT*)	MLPA/ parental	N/A	pathogenic (overlapping Hypotonia Cystinuria- syndrome, described elsewhere)[23]
31553	14	M	autistic features, motor problems, and macrocephaly	deletion	21q22.3	hg18, chr21: 43169759-43201708	32	92	22	*NDUFV3*, and *WDR4*	MLPA/ paternally inherited	negative	VOUS (a de novo missense mutation of *WDR4* has been reported in an autism cohort using WES and mutations of *NDUFV3* have been reported in complex I deficiency)[24-25]
68738	5	M	global developmental delay	deletion	7q35	hg19, chr7: 144509480-144534911	25	93	35	*TPK1*	MLPA/- (absent in the mother, but father	negative	VOUS (this deletion was absent in the mother and a healthy sister but present in the

											was N/A, (likely paternal)		likewise affected sister. A de novo missense mutation of *TPK1* has been reported in an autism cohort using WES)[24]
62611	3	F	developmental delay, muscular hypotonia, and epilepsy	deletion	4q22.1	hg18, chr4: 88438703-88452522	13	90	14	*HSD17B13*	MLPA/ paternally inherited	N/A	likely benign (heterozygous de novo mutation in *SCN2A*: c.4025T>C (p.L1342P) was found in this patient. There are DGV variations 30208 (2/485 ctls), 51479 (3/2026 ctls) and 36311 (1/1 ctl) with deletions encompassing some other exon(s) of *HSD17B13*)
58822	3	M	mild developmental delay, microcephaly, growth deficiency, cryptorchidism, and bilateral inguinal and umbilical hernia	deletion	7q11.22	hg18, chr7: 69233202-69240841	8	93	16	*AUTS2*	MLPA/ paternally inherited	negative	pathogenic (the father is similarly affected. Microdeletions of the gene in patients with variable neuro-developmental features have been reported)[4]
9229	27	M	moderate intellectual disability, epilepsy, and abnormal behaviour	deletion (homozygous)	1p36.31	hg18, chr1: 6361008-6368114 (this position is intronic, however, the nearby exon 1 of *ACOT7* (isoform ENST00000377855) with no markers in the array was confirmed to be deleted by MLPA)	7	98	8	*ACOT7*	MLPA/parental	N/A	likely pathogenic (segregation of the homozygous deletion, function of the gene, its expression pattern, and overlap with the KO mice phenotype are all in favour of its pathogenicity, however, further patients are needed)

* Age at the time of array.

VOUS: variant of uncertain significance, N/A: not available, WES: whole-exome sequencing.

Supplementary Table 3. SNPs of the affected genes in selected patients with inherited heterozygous candidate CNVs ≤ 500 kb detected by Sanger sequencing

Patient ID	Gender	Phenotype	Aberration	Genome coordinates	Size (kb)	Affected gene(s)	Validation	Sanger sequencing of the affected genes	Pathogenicity
48459	M	mild learning difficulties, motor problems, facial dysmorphic features, dilated cardiomyopathy, and anal atresia	deletion	hg18, chr14: 78321869-78784675)	462	*NRXN3*	MLPA/ paternally inherited	1 SNP: c.669C>T (het), p.T 223T, rs1004212, MAF: 0.17	likely pathogenic, reduced penetrance (a mixture of inherited and de novo deletions within *NRXN3* has been reported in ASD)
57028	F	developmental delay, sever speech delay, microcephaly, facial dysmorphic features, hallux valgus, and short stature	deletion	hg18, chr4: 98745657-99078956	333	C4orf37 (STPG2)	MLPA/ paternally inherited	8 SNPs: c.-78T>C (het, 5'UTR), rs4699605, MAF: 0.30 (C) c.222+18a>g (homo), rs13328005, MAF: 0.35 (g) c.373T>C (homo), p.Y125H, rs17558193, MAF: 0.35 (C) c.501-3a>g (homo), rs2903151, MAF:0.35 (g) c.532A>G (homo), p.I178V, rs2903150, MAF: 0.35 (g) c.557A>G (het), p.Y186C, rs28403003, MAF: 0.05 (G) c.579A>G (homo), p.L193L, rs2865979, MAF: 0.35 (G) c.1204+16c>t (het), rs202103504, MAF: < 0.01 (t)	VOUS (there are patients 249670 (maternally inherited) similar to our patient and 256952 (inherited from normal parent) with seizure in Decipher database which have intragenic STPG2 deletions)
53983	M	developmental delay, hypotonia, and strabismus	deletion	hg18, chr7: 125890040-126149790	260	*GRM8*	MLPA/ paternally inherited	2 SNPs: c.1018+14A>G (het), rs769199, MAF: 0.04 (A) c.31T>C (het, 3' UTR), rs712723, MAF: 0.41	likely pathogenic, reduced penetrance (several CNVs of *GRM8* have been reported in ADHD compared to controls)
62155	M	developmental delay, hypotonia, microcephaly, complex heart defect, short stature, and urether stenosis; follow-up clinical diagnosis of Coffin-Lowry syndrome	deletion	hg18, chr9: 19379757-19555588	176	*ASAH3L (ACER2)*, and *SLC24A2*	MLPA/ maternally inherited	2 SNPs in *SLC24A2*: c.960A>G (het), p.P320P, rs4977308, MAF: 0.21 (A) c.1201A>C (het), p.R401R, rs2383101, MAF: 0.15 (A)	likely benign (pathogenic hemizygous 4 bp del in RPS6KA3 (RSK2): IVS10+4_+7delAGTA was found in this patient confirming the clinical diagnosis of Coffin-Lowry syndrome)
55113	M	ADHD, microcephaly, short stature, and myopia	deletion	hg18, chr3: 67592633-67762247	170	*SUCLG2*	MLPA/ maternally inherited	1 SNP: c.45G>A (homo, 3' UTR), rs1065399, MAF: 0.32 (G)	VOUS (there are patients 265229 with inherited intragenic deletion of SUCLG2 and 259685 with inherited duplication of the whole gene in Decipher database presenting with different phenotypes)
56761	M	global developmental delay with prominent speech delay, and cryptorchidism	deletion	hg18, chrX: 154686877-154843251	156	*VAMP7*	MLPA/ paternally inherited	1 SNP: c.595-10G>C (homo), rs143821247, MAF: 0.310	likely benign (patient 272246 in Decipher database with similar inherited deletion, has an additional 10Mb de novo deletion. *VAMP7* (*synaptobrevin 1*) has been associated with bipolar affective disorder)

31553	M	autistic features, motor problems, and macrocephaly	deletion	hg18, chr21: 43169759-43201708	32	*NDUFV3*, and *WDR4*	MLPA/ paternally inherited	3 SNPs in *WDR4*: c.213G>C (het), p.K71N, rs2248490, MAF: 0.494 (G) c.796C>T (het), p.P266S, rs15736, MAF: 0.35 c.1169G>A (het), p.R390Q, rs6586250, MAF: 0.2	VOUS (a de novo missense mutation of *WDR4* has been reported in an autism cohort using WES and mutations of *NDUFV3* have been reported in complex I deficiency)
68738	M	global developmental delay	deletion	hg19, chr7: 144509480-144534911	25	*TPK1*	MLPA/- (absent in the mother, but father was N/A, (likely paternal)	-	VOUS (this deletion was absent in the mother and a healthy sister but present in the likewise affected sister. A de novo missense mutation of *TPK1* has been reported in an autism cohort using WES)
58822	M	mild developmental delay, microcephaly, growth deficiency, cryptorchidism, and bilateral inguinal and umbilical hernia	deletion	hg18, chr7: 69233202-69240841	8	*AUTS2*	MLPA/ paternally inherited	1 silent variant: c.1158C>T (het), p.S386S	pathogenic (the father is similarly affected. Microdeletions of the gene in patients with variable neurodevelopmental features have been reported)
60984	F	developmental delay, growh hormon deficiency, left hypoplastic kidney, and celiac disease	deletion	hg18, chr16: 24793142-24797112	3	*SLC5A11*	MLPA/ maternally inherited	1 SNP: c.669T>C (homo), p.F223F, rs274081, MAF: <0.01 (T)	no evidence (many members of Solute Carrier Family are involved in genetic disorders)

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