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Original Article

Recurrent copy number variations as risk factors for autism spectrum disorders: analysis of the clinical implications

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Chromosomal microarray analysis (CMA) is currently considered a first-tier diagnostic assay for the investigation of autism spectrum disorders (ASD), developmental delay and intellectual disability of unknown etiology. High-resolution arrays were utilized for the identification of copy number variations (CNVs) in 195 ASD patients of Greek origin (126 males, 69 females). CMA resulted in the detection of 65 CNVs, excluding the known polymorphic copy number polymorphisms also found in the Database of Genomic Variants, for 51/195 patients (26.1%). Parental DNA testing in 20/51 patients revealed that 17 CNVs were *de novo*, 6 paternal and 3 of maternal origin. The majority of the 65 CNVs were deletions (66.1%), of which 5 on the X-chromosome while the duplications, of which 7 on the X-chromosome, were rarer (22/65, 33.8%). Fifty-one CNVs from a total of 65, reported for our cohort of ASD patients, were of diagnostic significance and well described in the literature while 14 CNVs (8 losses, 6 gains) were characterized as variants of unknown significance and need further investigation. Among the 51 patients, 39 carried one CNV, 10 carried two CNVs and 2 carried three CNVs. The use of CMA, its clinical validity and utility was assessed.

Conflict of interest

All authors declare no conflict of interest.

V. Oikonomakis[a], K. Kosma[a], A. Mitrakos[a], C. Sofocleous[a,b], P. Pervanidou[c], A. Syrmou[a], A. Pampanos[a,d], S. Psoni[a], H. Fryssira[a], E. Kanavakis[a,b], S. Kitsiou-Tzeli[a] and M. Tzetis[a]

[a]Department of Medical Genetics, Medical School, National and Kapodistrian University of Athens, Athens, Greece, [b]Research Institute for the Study of Genetic and Malignant Diseases in Childhood, "Aghia Sophia" Children's Hospital, Athens, Greece, [c]1st Department of Pediatrics, Medical School, National and Kapodistrian University of Athens, Athens, Greece, and [d]Department of Genetics, "Alexandra" University Maternal Hospital, Athens, Greece

Key words: autism – autism spectrum disorder – chromosome microarray analysis – copy number variations

Corresponding author: Konstantina Kosma, MD, MSc, Department of Medical Genetics, Aghia Sophia Children's Hospital, Thivon & Levadias, 11527 Athens, Greece.
Tel.: 0030 210 746 7464;
fax: 0030 210 779 5553;
e-mail: kokosma@med.uoa.gr

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Autism spectrum disorder (ASD) identified in about 1% of children and is four times more common in males than in females (1), is a group of lifelong neurodevelopmental condition with features of impaired communication, social interaction and repetitive behavior with a narrow range of interests, while over 70% of individuals with ASD have intellectual disability (ID) (2).

Strong evidence for genetic background in ASD has been provided from family and twin studies, with reported concordance of 60–90% in monozygotic twins, 0–10% in dizygotic twins, while sibling recurrence risk is between 3% and 10% (3, 4).

Rare medical or genetic conditions and syndromes are associated with autism, such as Joubert, Smith Lemli Opitz, fragile X and tuberous sclerosis, although none account for more than 1% of cases, and most are even rarer (2). The genomic regions most commonly reported include 2q37, 5p14, 5p15, multiple locations on chromosome 7, 11q25, 15q11-q13, 16q22.3, 17p11.2, 18q21.1, 18q23, 22q11.2, 22q13.3, and Xp22.2-p22.3.

Cytogenetically visible chromosomal anomalies are found in approximately 7–8% of patients with autism (5).

Recent studies based on copy number variations (CNV) and single nucleotide variations put the number of ASD-implicated genes between 200 and 1000 (6). The majority of these are genes code for key neurological molecules functioning in the synaptic junctions of neurons including members of the neurexin–neuroligin complex suggesting that synapse development and function represents a major pathogenetic hub for ASD and related disorders (7).

Array comparative genomic hybridization (aCGH) and single nucleotide polymorphism (SNP) genotyping arrays, collectively referred to as chromosomal microarray analysis (CMA) is a molecular cytogenetic technique that overcomes the limited resolution of conventional cytogenetics and allows genome-wide scanning for both microscopic and submicroscopic chromosomal aberrations. It is commonly applied as a clinical diagnostic tool for patients with multiple congenital anomalies (MCA), intellectual disabilities/developmental delay (ID/DD) and ASD (8) offering a much higher diagnostic yield (15–20%) for these individuals than G-banded karyotype (9). Truly balanced rearrangements and low-level mosaicism are generally not detectable by CMA, but these are relatively infrequent (<1%) (10). The International Collaboration for Clinical Genomics (ICCG), also known as International Standard for Cytogenomic Array (ISCA; www.ncbi.nlm.nih.gov/dbvar/studies/nstd37/) Consortium, has recommended CMA over karyotyping as the first-tier cytogenetic diagnostic test for patients with ID/DD and ASD.

The aim of this study was to investigate the clinical application of CMA in ASD patients and attempt a correlation of phenotype and genotype.

Material and methods

Patients

One hundred and ninety five patients with ASD of unknown etiology were evaluated with CMA, from 2008 to 2015, consisting of 126 males and 69 females (male/female ratio: 1.82), from 1 to 38 years old (5 adult patients). All patients were examined by clinical geneticists and neurodevelopmental pediatricians and assessed for ASD according to the behavioral criteria listed in the 1994 American Psychiatric Association Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV). The study was approved by the Ethics Committee of 'Aghia Sophia' Children's Hospital', and informed consent for genetic studies was obtained directly from all family members included in the study.

Genetic studies

Isolation of genomic DNA

DNA was extracted from 3 ml peripheral blood lymphocytes from patients and parental samples (when available), using the commercially available QiAmp DNA Blood Mini kit (Qiagen, Hilden, Germany). The quality and quantity of the DNA samples were determined using the NanoDrop ND-1000 UV–Vis spectrophotometer.

Chromosomal microarray analysis

High-resolution commercial Agilent technologies arrays (Santa Clara, CA, USA): the 244K, 4 × 180K and the 4 × 180K CGH + SNP platforms (SurePrint G3 arrays; design ID: 014693, 022060 and 029830, respectively) were used. The experimental procedures were performed according to the manufacturer's description and as previously described (11).

Data analysis

The findings were assessed by searching the literature and the following databases: Database of Genomic Variants (DGV; http://projects.tcag.ca/variation/), to exclude the common polymorphic CNVs or otherwise named Copy Number Polymorphisms (CNPs), University of California Santa Cruz (UCSC) (http://genome.ucsc.edu/), Online Mendelian Inheritance in Man (OMIM) (www.ncbi.nlm.nih.gov/OMIM), ISCA and DECIPHER (http://www.sanger.ac.uk/PostGenomics/decipher/) for information on the genes included in the CNVs and on patients with similar CNVs reported in the ISCA and DECIPHER databases. In addition for ASD, three databases were used Simons Foundation for Autism Research Initiative (SFARI) (https://gene.sfari.org/autdb), Autism DataBase (AUTDB) (http://autism.mindspec.org/autdb) and Autism Chromosome Rearrangement Database (ACRD; http://projects.tcag.ca/autism/). The aberration calls were grouped in three categories: benign (CNPs) listed in the DGV database, uncertain (variants of uncertain significance, VOUS) or pathogenic. CNVs were considered pathogenic if they overlapped with the critical regions of well-characterized duplication/deletion syndromes or pathogenic regions as reported in ISCA, DECIPHER and Autism databases; were relatively large and encompassing many genes, or were referred as pathogenic in the literature.

Results

CMA resulted in a total detection of 65 causal and VOUS CNVs, not included in DGV and therefore not CNPS, in 51/195 patients (29 males and 22 females,) representing a diagnostic yield of 26.1%. Parental DNA tested for 20/51 patients revealed 18 *de novo*, 6 paternal and 3 of maternal origin CNVs. Forty-three of 65 CNVs were deletions (66.1%), while 22 were duplications (33.8%). From the total of 65 CNVs, 50 were of diagnostic significance and well described in the literature, the rest were VOUS and need further investigation (Tables 1 and 2).

The assignment of CNV pathogenicity as described in the materials and methods section, was based on previous publications, the report of similar aberrations in ASD patients in DECIPHER, ISCA and the Autism specific databases and the gene content of the aberration.

Table 1. ASD patients with one CNV

Case/array platform/hg	Age (years), sex	Clinical characteristics other than ASD	Nomenclature according to ISCN	Size	Important genes
91/244K/NCBI36/hg18	8, male	Seizures, antimogoloid palpebral fissures, low nuchal hairline, hyperactivity	2p14 (68539032–68583521)x1 ***[VOUS]***	44 Kb	*FBXO48*, *APLF*
88/244K/NCBI36/hg18	4, female	Hypotonia, speech delay, behavioral problems	2p16.3 (51,090,504–51,167,934)x1pat	77 Kb	*NRXN1* (signal peptide)
324/180K/NCBI36/hg18	6, female	Hyperactivity, isolated behavior	2p16.3 (50850691–51100471)x1	249.8 Kb	*NRXN1* (exonic)
621/180K/GRCh37/hg19	3, male	Macrocephaly	2q36.3 (230,719,378–230,900,186)x3 ***[VOUS]***	180.81 Kb	*TRIP12*, *FBXO36*
301/180K/NCBI36/hg18	5, male	ID	5p15.32-p14.3 (4633545–18945364)x3	14.3 Mb	*MED10*, *SEMA5A*, *CTNND2*, *FBXL7*, *ZNF622*, *MYO10*, *BASP1*
586/180K/GRCh37/hg19	11, female	Seizures , isolated behavior	5p14.3 p14.2 (22,308,420–24,492,723)x3	2.184 Mb	*CDH12*, *CDH10*
41/244K/NCBI36/hg18	12, female	Seizures, hypotonia, increased tendon reflexes	6p22.1-p21.33 (29,677,271–30,302,130)x1 mat	624.8 Kb	*GABBR1*
433/244K/NCBI36/hg18	2, female	Epicanthal folds, flat nasal bridge, full checks, prominent ears, pointed chin, ataxic gait	7p22.2-p22.1 (4497389–7104105) x3	2.6 Mb	*FOXK1*, *RBAK*, *WIPI2*, *TRIAD3*, *MMD2*, *SLC29A4*, *ZDHHC4*, *ZNF12*, *ACTB*
480/244K/NCBI36/hg18	5, female	Obesity, short stature, synophrys, antimogoloid-narrow palpebral fissures, hypertelorism	7p22.2-p22.1 (4340544–7025368) x3	2.7 Mb	*FOXK1*, *RBAK*, *WIPI2*, *TRIAD3*, *MMD2*, *SLC29A4*, *ZDHHC4*, *ZNF12*, *ACTB*
211/180K/NCBI36/hg18	2, female	Hypertelorism, high-arched palate, prominent ears, stereotypic movements, hypoplasia of corpus callosum	9p24.3p24.2 (194193–> 4585541)x1 dn	4.4 Mb	*DOCK8*, *KANK1*, *DMRT1*, *DMRT3*, *DMRT2*, *SMARCA2*, *GLIS3*
350/180K/GRCh37/hg19	38, male	ID, microotia, retrognathia, cryptorchidism	9p24.3 (288161–625573)x3 dn	337 Kb	*DOCK8* (exonic), *KANK1* (exonic)
475/180K/NCBI36/hg18	7, female	Hearing impairment, antimogoloid palpebral fissures, epicanthal folds, agenesis of VIII and VII cranial nerve	12q24.33 (132118942–> 132257380) x1 dn ***[VOUS]***	140 Kb	*ZNF84*, *ZNF140*, *ZNF10*

Table 1. Continued

Case/array platform/hg	Age (years), sex	Clinical characteristics other than ASD	Nomenclature according to ISCN	Size	Important genes
59/244K/NCBI36/hg18	4, female	Hypotonia, obesity, microstomia, long eyelashes, hirsutism, microretrognathia, short nose, seizures, partial corpus callosum dysplasia	15q11.2q14 (19109124–36837570)x3 dn (maternal origin)	17.73 Mb	*CYFIP1*, *NIPA1*, *NIPA2*, *SNRPN*, *SNURF*, *IPW*, *UBE3A*, *GABRG3*, *CHRNA7*, *SLC12A6*
22/244K/NCBI36/hg18	22, female	Obesity	15q11.2(21,982,746–22,396,107)x1pat	413.362 Kb	*PWRN3*, *PWRN1*
79/244K/NCBI36/hg18	31, male	Seizures, hypotonia, ataxia	15q11.2q13.1 (21213950–26208646)x1 dn (maternal origin)	5 Mb	*PWRN2*, *PWRN1*, *SNRPN*, *SNURF*, *IPW*, *UBE3A*, *GABRG3*, *CHRNA7*, *SLC12A6*
665/180K/GRCh37/hg19	1, male	Arthrogryposis	15q11.2 (22,765,628–23,082,821)x1pat	317 Kb	*TUBGCP5*, *CYFIP1*, *NIPA2*, *NIPA1*
709/180K/GRCh37/hg19	4, male	Arthrogryposis	15q11.2 (22,765,628–23,165,915)x1pat	400.3 Kb	*TUBGCP5*, *CYFIP1*, *NIPA2*, *NIPA1*
673/180K/GRCh37/hg19	4, male	Hypospadias	15q13.2 (30,819,465–31,077,833)x1	257.8 Kb	*FAM7A1*, *FAM7A2*, *ARHGAP11B*
258/180K/NCBI36/hg18	3, female	Hypotonia, arched eyebrowns, low set ears, microotia, macroglossia, open mouth, drooling	15q21.3-q22.2 (51161898–59890048)x1	8.7 Mb	*WDR72*, *AQP9*, *RNF111*, *MYO1E*, *LDHAL6B*, *FOXB1*, *ANXA2*, *NARG2*, *RORA*, *MIR628*
444/180K/GRCh37/hg19	6, male	Dolichochephaly, obesity	16p11.2 (29652999–30198600)X1	545.5 Kb	*SPN*, *C16orf54*, *ZG16*, *KIF22*, *MAZ*, *PRRT2*, *C16orf53*, *SEZ6L2*, *ASPHD1*, *KCTD13*, *DOC2A*, *C16orf92*, *ALDOA*, *TBX6*, *GDPD3*, *MAPK3*, *CORO1A*
578/180K/GRCh37/hg19	12, male	Seizures, hyperactivity, long philtrum, thin upper lip	16p13.11 (15,048,751–15,496,452)x3 dn ***[VOUS]***	447.7 Kb	*PDXDC1*, *NTAN1*, *RRN3*, *MIR3180-4*, *MPV17L*
119/244K/NCBI36/hg18	5, female	ID, frontal bossing, long eyelashes, large prominent ears, high-arched palate, full checks, prominent lower lip, hypotonia, stereotypic movements	16q24.2-q24.3 (85681658–88690615)x1	3 Mb	*ANKRD11*, *ZNF778*, *CDH15*

Table 1. Continued

Case/array platform/hg	Age (years), sex	Clinical characteristics other than ASD	Nomenclature according to ISCN	Size	Important genes
186/244K/NCBI36/hg18	7, male	Seizures, low set ears, bulbous nose, anterverted nares, thin upper lip, short philtrum, micrognathia, obesity,	17q21.31q21.32 (41,390,000–41,980,000) X1 dn	590 Kb	*MAPT*, *STH*, *KANSL1*
76/244K/NCBI36/hg18	5, male	Moderate ID	17q21.31 (41577520–41700815)x1	123 Kb	*KANSL1*
32/244K/NCBI36/hg18	15, male	Macrocephaly, narrow downslanting palpebral fissures, macrostomia, thin upper lip, large prominent ears, happy behavior, good communication skills	17q21.31-q21.32(41331503–42142422)x1 dn	810 Kb	*MAPT*, *STH*, *KANSL1*
447/180K/GRCh37/hg19	17, female	Seizures	17q21.31 (44188441–44345038)x1	156.6 Kb	*KANSL1* (exonic)
145/244K/NCBI36/hg18	2, female	ID, frontal bossing, flat nasal bridge, high-arched palate, long philtrum, hypotonia	20q13.33 (61433519–> 62419593)x1	99 Kb	*CHRNA4*, *KCNQ2*, *EEF1A2*, *ZBTB46*, *SOX18*
102/244K/NCBI36/hg18	36, male	Psychotic behavior	22q11.21 (17274835–> 17390508)x1 ***[VOUS]***	116 Kb	*DGCR6*, *PRODH*, *DGCR5*, *DGCR9*, *DGCR10*
715/180K/GRCh37/hg19	11, female	Obesity	22q11.21 (18,706,001–19,010,508)x3 ***[VOUS]***	305 Kb	*GGT3P*, *DGCR6*, *PRODH*, *DGCR5*, *DGCR9*, *DGCR10*
707/180K/GRCh37/hg19	3, male	Hyperactive	22q11.22 (22,313,381–22,556,733)x3pat ***[VOUS]***	243 Kb	*TOP3B*
120/244K/NCBI36/hg18	5, male	Strabismus	21q22.3 (44484360–45790945)X1 ***[VOUS]***	1.31 Mb	*ICOSLG*, *DNMT3L*, *UBE2G2*, *SUMO3*, *ITGB2*
342/180K/NCBI36/hg18	5, male	Echolalia, stereotypic movements	Xp11.3 (43573288–3580109)x2	180 Kb	*MAOA* (intronic)
244/180K/NCBI36/hg18	2, male	Seizures, facial and body asymmetry, low nuchal hairline	Xp22.31 (6477006–> 8091810)x2	1.61 Mb	*HDHD1A*, *STS*, *VCX*, *PNPLA4*, *PNPLA4*, *VCX2*, *hsa-mir-651*
69/244K/NCBI36/hg18	2, male	Antimogoloid palpebral fissures, high-arched palate, hypertelorism, carp mouth	Xp22.31(6,467,006–8,131,810)x0 mat	1.65 Mb	*HDHD1A*, *STS*, *VCX*, *PNPLA4*, *PNPLA4*, *VCX2*
687/180K/GRCh37/hg19	11, male	Speech delay, isolated behavior, dysplasia of right pectoralis major	Xp22.31 (6,488,721–8,097,511)x2	1.7 Mb	*HDHD1*, *STS*, *VCX*, *PNPLA4*, *MIR651*

Table 1. Continued

Case/array platform/hg	Age (years), sex	Clinical characteristics other than ASD	Nomenclature according to ISCN	Size	Important genes
473/244K/NCBI36/hg18	6, male	Epicanthal folds, flat nasal bridge, micrognathia, small teeth, long philtrum, high-arched palate	Xp22.32 (5818688–5918660)X2	99.9 Kb	*NLGN4X* (exonic)
288/180K/NCBI36/hg18	5, male	Speech delay, dolichochephaly	Xp22.33 (1691671–1706762)x2	15.1 Kb	*ASMT*
36/244K/NCBI36/hg18	8, male	Hypotonia, antimogoloid palpebral fissures, anterverted nares	Xq27.3-q28 (146486322–147109041) x0 dn	622.72 Kb	*ASFMR1*, *FMR1*
47/244K/NCBI36/hg18	13, male	Seizures, antimogoloid palpebral fissures, low set ears, short philtrum, cryptorchidism, stereotypic movements	Xq28 (102,641, 391–102,961, 998)x0 dn	87 Kb	*FLNA*, *RPL10*, *GDI1*

ASD, autism spectrum disorders; CNV, copy number variation; dn, *de novo*; ID, intellectual disability; Kb, kilobase; mat, maternal; Mb, megabase; pat, paternal; VOUS, variant of unknown significance.
[VOUS] means Variant of unknown significance- it is a term used for variants that are not pathogenic but their pathologic significance is not yet determined due to the rarity of reported cases.

Thirty nine of the 51 patients carried one CNV either causal (32 CNVs) or VOUS (7 CNVs) (Table 1). Ten patients carried two CNVs: both causal (five patients) or one causal and the other VOUS (four patients) or both VOUS (one patient). Two patients carried three CNVs, one with three causal CNVs and the other two causal and one VOUS (Table 2). Of the 51 causal aberrations, 39 were on autosomes [2p16.3 (2x), 2p12, 5p15.3p14.3, 5p14.3p14.2, 6p22.1p21.33, 7p22.2p22.1 (2x), 8q24.3, 9p21.1p11.2, 9p24 (2x), 10p15.3, 12p13, 12q24 (2x), 15q11q14 (9x), 15q21.3q22.2, 16p11.2 (2x), 16p13.11, 16q24.2q24.3, 17q21.31 (8x), 20p12.1, 20q13.33] and 12 on the X-chromosome [Xp11.23 (2x), Xp11.22, Xp11.3, Xp22.31 (3x), Xp22.32, Xp22.33, Xq21.31, Xq27.3q28 and Xq28] (Tables 1 and 2). The 14 VOUS were all located on autosomes [2p14, 2q14.2, 2q36.3, 3q22.3, 6q24.3, 12q24.33, 16p13.11, 20p12.1 (2x), 21q22.3, 22q11.21 (3x), 22q11.22] (Tables 1 and 2).

The commonest aberration 17q21.31 (Koolen De Vries syndrome) (12) was identified as a single causal CNV in four patients (Table 1) and in combination with 15q13.3, 16p11.2, 20p12.1 (VOUS), Xp21.31 and Xp11.23 in four other patients (Table 2). The other common locus was 15q11.2q14 encompassing large duplications of the whole interval or smaller deletions of the 15q11.2 interval as a single causal CNV (Table 1). In patients with two or more CNVs, the following were detected: a *de novo* deletion of 15q11.2 in combination with 3q22.3, a 15q13.3 duplication with 17q21.3 deletion, and a combination of 15q13.2 and 15q13.3 deletions (Table 2).

Discussion

We report on the genetic basis of ASD using high resolution CMA, carried out in a Greek cohort of 195 patients resulting in a diagnostic yield of 26.1% (51/195 ASD patients).

The 15q11q14 locus was detected in 8/51 cases (7 deletions and 2 duplications) showing variability in size from 258 Kb containing only one ASD causative gene (*ARHGAP11B*) to two large aberrations with well-characterized neurodevelopmental pathway genes such as *GABRB3*, *CHRNA7*, *CYFIP1*, *UBE3A*, *TUBGCP5*, *ARHGAP11B*, *PWRN3* and *PWRN1* (13–18) (Tables 1 and 2). The most severe phenotypes including hypotonia and seizures were observed in two patients (59 and 79), one with a 17.7 Mb duplication and the other with a 5-Mb deletion, both of maternal origin. Two other patients (665 and 709) both with a paternal 15q11.2 deletion had ASD in combination with arthrogryposis which has been previously mentioned as a coincidental finding (19).

The 17q21.31 microdeletion was found in 8/51 patients (Tables 1 and 2) (12, 20–22). The critical region contains two causative genes *MAPT* and *KANSL1* contributing to the major phenotypic features (DD, hypotonia, facial dysmorphisms and a friendly/amiable behavior) (12, 22). Our patients with a single 17q21.3 CNV had a phenotype agreeing with the published cases whereas two interesting cases (4, 23) with a combination of three CNVs (17q21.3, 20p12.1 and Xq21.3) or two (17q21.3 and Xp11.23), respectively, showed differing characteristics such as isolated behavior and hyperactivity, while case 4 also had absence of speech.

Table 2. ASD patients with two or more CNVs

Case/array platform/hg	Age (years), sex	Clinical characteristics other than ASD	Nomenclature according to ISCN	Size	Important genes
477/180K/GRCh37/hg19	5, female	ID	2p12 (80272577–80492820)x1	220 Kb	*CTNNA2* (*intron7*), *LRRTM1*
			20p12.1 (13610726–14190591)x3 ***[VOUS]***	579 Kb	*MACROD2*
218/244K/NCBI36/hg18	10, female	Flat nasal bridge, high-arched palate, short philtrum	2q14.2 (120958377–121473628)x1 ***[VOUS]***	515 Kb	*GLI2*
			6q24.3 (145971958–146619406)x3	615 Kb	*EPM2A*, *GRM1* ***[VOUS]***
3/244K/NCBI36/hg18	8, female	Microotia, microretrognathia, deep philtrum, strabismus, narrow palbebral frissures, hypotonia, contractures	3q22.3(136072761–136108079) x1 dn ***[VOUS]***	35.32 Kb	*EPHB1*
			15q11.2(22718000–22727519)x1 dn	9.6 Kb	*SNRPN*
472/180K/GRCh37/hg19	6, male	ID, low set ears, hypertelorism, antimogoloid palpebral fissures, broad base to nose, short philtrum, high-arched palate	8q24.3 (141977927–146106695)x1	4.129 Mb	>98 genes
			20p12.1 (14849501–15134216)x1 ***[VOUS]***	284.7 Kb	*MACROD2*
615/180K/GRCh37/hg19	1, female	Hyperactive	9p21.1-p11.2 (30,694,198–47,199,169)x3	16.5 Mb	>50 genes
			22q11.21 (18,894,835–19,010,508)x3 ***[VOUS]***	115.7 Kb	*DGCR6*, *PRODH*, *DGCR5*, *DGCR9*, *DGCR10*
595/180K/GRCh37/hg19	3, female	Strabismus	10p15.3 (102,539–1,352,270)x1	1.25 Mb	*ZMYND11*, *DIP2C*, *IDI2*, *IDI2-AS1*, *IDI1*, *WDR37*, *ADARB2*
			12p13.33 (316,832–2,162,750)x1	1.8 Mb	*SLC6A12*, *SLC6A13*, *KDM5A*, *WNK1*, *RAD52*, *ERC1*, *FBXL14*, *ADIPOR2*, *CACNA2D4*, *LRTM2*, *CACNA1C*
			12q24.33 (132,264,736–133,773,528)x1	1.51 Mb	*NOC4L*, *GALNT9*, *FBRSL1*, *P2RX2*, *POLE*, *PXMP2*, *PGAM5*, *ANKLE2*, *ZNF605*, *ZNF26*, *ZNF84*, *ZNF140*, *ZNF10*, *ZNF268*

Table 2. Continued

Case/array platform/hg	Age (years), sex	Clinical characteristics other than ASD	Nomenclature according to ISCN	Size	Important genes
449/180K/GRCh37/hg19	18, female	Hearing impairment	15q13.3 (32051233–32510863)x3	459.6 Kb	*CHRNA7*
			17q21.31 (44188441–44345038)x1	156.6 Kb	*KANSL1*(exonic)
543/180K/GRCh37/hg19	3, female	Growth deficiency, stereotypic movements, frontal bossing, arched upper lip, macrostomia, open mouth, microcephaly	15q13.2 (30,390,021–30,888,853)x1	498.8 Kb	*CHRFAM7A*, *FAM7A2*, *FAM7A1*
			15q13.3 (32,438,884–33,263,643)x1	824.8 Kb	*CHRNA7*, *FAM7A3*, *FAM7A2*, *FAM7A1*, *ARHGAP11A*, *SCG5*, *FMN1*
40/244K/NCBI36/hg18	5, male	Seizures, strabismus, iris colobomata, atrial septal defects	16p11.2 (29572030–30106101)x1	534.1 Kb	*SPN*, *PRRT2*, *MVP*, *CDIPT*, *SEZ6L2*, *ASPHD1*, *KCTD13*, *TBX6*, *GDPD3*, *MAPK3*, *CORO1A*
			17q21.31 (41577520–41700815)x1	123.3 Kb	*KANSL1*
4/244K/NCBI36/hg18	4, male	Seizures, behavioral problems, hyperactivity, MRI: atrophy of hippocampus on	17q21.31 (41577520–41841246)x1 dn	263.7 Kb	*KANSL1* (*KIAA1267*)
			20p12.1(14710983–15125728)x1 pat ***[VOUS]***	414.75 Kb	*MACROD2*
			Xq21.31(90925106–91012127)x0 dn	87.02 Kb	*PCDH11X*
35/244K/NCBI36/hg18	12, male	Narrow downslanting palpebral fissures, thin upper lip, large prominent ears, deep philtrum, delayed myelinosis, hyperactivity, isolated behavior	17q21.31 (41458670–41706929)x1 dn	248.3 Kb	*MAPT*, *STH*, *KANSL1*
			Xp11.23(48540936–48584007)X0 mat	43.1 Kb	*HDAC6*, *ERAS*, *PCSK1N*
562/180K/GRCh37/hg19	4, male	No speech	Xp11.23 (48,204,101–48,760,198)x2 dn	556.1 Kb	*SSX3*, *SSX4B*, *SSX4*, *FTSJ1*, *PORCN*, *GATA1*, *HDAC6*, *ERAS*, *PCSK1N*, *TIMM17B*, *PQBP1*
			Xp11.22 (53,449,448–53,459,515)x2 dn	10.1 Kb	*SMC1A*, *RIBC1*, *HSD17B10*

ASD, autism spectrum disorders; ISCN, International System for Human Cytogenetic Nomenclature; CNV, copy number variation; dn, *de novo*; ID, intellectual disability; mat, maternal; pat, paternal; Mb, megabase; Kb, kilobase; MRI, magnetic resonance imaging; VOUS, variant of unknown significance.

Two 2p16 microdeletions (one paternally inherited) (88 and 324, Table 1), harbored the well-known *NRXN1* gene (24, 25) showing ASD, speech delay and behavioral problems with variable expressivity.

The CNV in the 2p12 region (477, Table 2) resulted in a simultaneous deletion of intron 7 of the *CTNNA2* and the *LRRTM1* gene, with *LRRTM1* haploinsufficiency implicated in schizophrenia and autism (26). We are reporting the smallest possible causal deletion resulting in typical autism without dysmorphic features despite the additional VOUS (20p12.1 duplication), possibly not contributing to the phenotype.

Regarding other chromosome 2 aberrations, two deletions (2p14 and 2q14.2, 91 and 218) and a duplication 2q36.3 (621) are considered VOUS (Tables 1 and 2). Haploinsufficiency of two genes *APLF* and *FBXO48* for 2p14, (27, 28) probably did not solely contribute to case 91 dysmorphic features and ASD. Case 218 with the 2q14.2 microdeletion affecting the *GLI2* gene (29) and 6q24.3 duplication (30) presents the smallest critical region of 2q14.2 among the four reported DECIPHER cases. The 2q36 duplication contains among others the *TRIP12* gene for which *de novo* mutation carriers were found to have ASD and ID (5, 31). Case 621 is among eight other similar cases in DECIPHER (UAM292836).

A large duplication in the 5p15.3p14.3 region was found in case 301 with isolated ASD/ID (Table 1). The duplication on its own has not been previously reported, however, complex chromosome rearrangements have been found in single patients with severe phenotypes (32). The region contains several genes involved in axonal guidance and neurite outgrowth plasticity (*NSUN2*, *SEMA5A*, *BASP1*) and implicated in autism and ID (33, 34).

Case 586 with 5p14.3p14.2 microduplication (Table 1) had seizures and autism without dysmorphic features. The cadherin genes *CDH10* and *CDH12* have been associated with epilepsy/ID, autism and schizophrenia (35).

The 6p22.1-21.33 deletion of maternal origin (41, Table 1) included the *GABBR1* gene (23). The patient had seizures and hypotonia while the mother was healthy indicating reduced penetrance or variable expressivity.

The 7p22 locus hosts OMIM genes that participate in neurodevelopmental pathways and more than 60 cases of duplications and deletions have been reported with DD, ID, behavioral problems, abnormal speech development, ASD and hypotonia with the majority also showed additional syndromic features (36) as was the case for the two female patients (433 and 480, Table 1).

Case 472 (Table 2) has a 8q24.3 deletion encompassing over 90 genes, in combination with VOUS 20p12.1. A similar maternally inherited deletion has been reported in combination with a paternal 14q23.3 deletion, in a family with autism and hypotonia but no other dysmorphic features (37). Our case has ASD has severe dysmorphic features, confirming the pathogenicity of 8q24.3 deletion.

Two *de novo* 9p24 aberrations, (211 and 350, Table 1) define the smallest critical region containing two genes *DOCK8* and *KANK1* responsible for ASD/ID (5, 38). The additional genes (as in case 211) contribute to dysmorphic features and corpus callosum hypoplasia.

Case 615 (Table 2) with the 9p21.1p11.2 deletion (38) in combination with VOUS 22q11.21, and presenting only with ASD confirms the variable expressivity of the deletion.

The rare 15q21.3-q22.2 deletion containing the causative genes *NARG*, *RORA* has been associated with growth retardation, hypotonia and moderate to severe ID including autistic features (39). Case 258, (Table 1) has hypotonia and coarse facial features.

One case (119, Table 1) of 16q24.2-q24.3 microdeletion with two autism-related genes (*CDH15* and *ANKRD11*) (40) was detected in a female with ASD/ID, hypotonia and severe facial dysmorphism, agreeing with previously reported phenotypes.

Locus 16p11.2 (29–30 Mb) has been reported as influencing susceptibility to autism when it is either deleted or duplicated (41) (444 and 40, Tables 1 and 2). Case 40 is indicative of a second CNV (17q21.3 deletion) contributing to a more severe phenotype including seizures, atrial septal defects and iris colobomata.

Case 578, (Table 1) with ASD and seizures, long philtrum and thin upper lip, carried a *de novo* duplication in 16p13.11. Deletions have been associated with ID and MCA with phenotypic variability, however, duplications have been characterized as common variants in the population (5/1682, 0.29%) (42). This case adds knowledge regarding the 16p13.11 duplication.

Case 145 (Table 1) defines the smallest critical region of 20q13.33 containing only *CHRNA4* and *KCNQ2* genes, both expressed in the brain (43) and furthermore underscores the possibility of dysmorphic features.

Case 595 (Table 2) with three microdeletions (10p15.3, 12p13.33 and 12q24.33) presents only with ASD and strabismus, although previously reported patients with one of the three CNVs, especially the 10p terminal deletion, presented also with severe mental retardation (44), while the 12p13.33 deletion encompassing the *KDM5A* and *FBXL14* genes, has been associated with ASD, along with the 12q24 locus (45). In addition, case 475 (Table 1) carries the smallest reported 12q24.33 deletion that includes genes *ZNF84*, *ZNF140* and *ZNF10* perhaps implicating them in ASD (46).

3q22.3 (47) (case 3), 20p12.1 (48) detected in three cases (477, 472 and 4), (Table 2) were found in combination with a pathogenic CNV, and 21q22.3 (49) (case 120, Table 1) is referred in decipher.

Three duplications and one deletion (715, 707, 615 and 102, Tables 1 and 2) were found in the 22q11.21 locus where deletions and duplications are associated with autism, ID and schizophrenia (50). Causative genes include *PRODH*, *DGCR6* and *TOP3B*. Case 615 was found in combination with a pathogenic CNV. Paternally inherited duplication 22q11.22 containing the *TOP3B* gene (707, Table 1) has been associated in a previously reported maternally inherited case with mild ID and generalized overgrowth (51).

X-chromosome CNVs

One of the first genes involved in X-linked Intellectual Disability (XLID) was FMR1 (OMIM 309550)

responsible for fragile X syndrome and accounting for about 1–2% of all ID cases (52). For case 36 (Table 1), the initial suspected clinical diagnosis was for FRAX, but *FMR1* mutation analysis displayed normal CGG-expansion pattern (29 repeats) for both the patient and his mother. He was therefore referred for aCGH analysis that revealed a mosaic *de novo* deletion in Xq27.3q28, encompassing *ASFMR1* and *FMR1* genes. The aCGH mosaic finding, confirmed by immunocytochemical detection of FMRP protein from DNA extracted from hair roots, probably, contributed to a distinct and less severe phenotype, compared with typical FRAX (FXS, OMIM300624).

The Xq28 locus containing *GDI1*, *FLNA* and *RPL10* genes, was found deleted in case 47, with ASD, seizures and dysmorphic features (Table 1) (5).

A deletion in the Xq21.31 region containing the *PCDH11X* gene (53) was found in case 4, (Table 2) along with two other CNVs, one causal (17q21.3), contributing to the severe phenotype.

Case 342 with severe ASD (Table 1) has a small intronic duplication, in the Xp11.3 containing the *MAOA* gene (54).

Case 35, carrying the smallest reported deletion in Xp11.22, along with case 562 (two *de novo* pathogenic gains at neighboring loci Xp11.23 and Xp11.22) (Table 2) establish that *HSD17B10*, *PQBP1* and *HDAC6* genes can lead to ASD (55, 56).

Two microduplications and one maternally inherited deletion in the Xp22.31 locus in cases 244, 687 and 69 (Table 1) empower the hypothesis that both microdeletions and microduplications of this locus cause at least ASD (57, 58). Patient 473 (Table 1) with cognitive deficits carries a partial duplication of *NLGN4X* gene (Xp22.32) (59, 60).

The *ASMT* gene (Xp22.33) was found duplicated in case 288 (Table 1) with ASD, speech delay and dolichocephaly. Genetic variants in *ASMT* have been associated with autism caused by low *ASMT* activity and melatonin levels (61).

A high heritability for ASD has long been recognized. However, the phenotypic heterogeneity associated with a particular CNV makes data interpretation challenging. The same CNV may confer risk for variable phenotypic expressions and in some cases other additional risk factors may be required for the development of a specific disease outcome. This has led to the 'two hit' model hypothesis in which a second modifying CNV or point mutation explains the phenotype (62, 63) and is the case for patients in our cohort that harbor more than one pathological CNV (Table 2).

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References

1. Bill BR, Geschwind DH. Genetic advances in autism: heterogeneity and convergence on shared pathways. Curr Opin Genet Dev 2009: 19: 271–278.
2. Baird G, Simonoff E, Pickles A et al. Prevalence of disorders of the autism spectrum in a population cohort of children in South Thames: the Special Needs and Autism Project (SNAP). Lancet 2006: 368: 210–215.
3. Ozonoff S, Young GS, Carter A et al. Recurrence risk for autism spectrum disorders: a Baby Siblings Research Consortium study. Pediatrics 2011: 128: e488–e495.
4. Posthuma D, Polderman TJ. What have we learned from recent twin studies about the etiology of neurodevelopmental disorders? Curr Opin Neurol 2013: 26: 111–121.
5. Pinto D, Delaby E, Merico D et al. Convergence of genes and cellular pathways dysregulated in autism spectrum disorders. Am J Hum Genet 2014: 94: 677–694.
6. Berg JM, Geschwind DH. Autism genetics: searching for specificity and convergence. Genome Biol 2012: 13: 247.
7. Cristino AS, Williams SM, Hawi Z et al. Neurodevelopmental and neuropsychiatric disorders represent an interconnected molecular system. Mol Psychiatry 2014: 19: 294–301.
8. Xu J, Zwaigenbaum L, Szatmari P et al. Molecular cytogenetics of autism. Curr Genomics 2004: 5: 347–364.
9. Feuk L, Carson AR, Scherer SW. Structural variation in the human genome. Nat Rev Genet 2006: 7: 85–97.
10. Miller DT, Adam MP, Aradhya S et al. Consensus statement: chromosomal microarray is a first-tier clinical diagnostic test for individuals with developmental disabilities or congenital anomalies. Am J Hum Genet 2010: 86: 749–764.
11. Tzetis M, Kitsiou-Tzeli S, Frysira H et al. The clinical utility of molecular karyotyping using high-resolution array-comparative genomic hybridization. Expert Rev Mol Diagn 2012: 12: 449–457.
12. Koolen DA, Kramer JM, Neveling K et al. Mutations in the chromatin modifier gene KANSL1 cause the 17q21.31 microdeletion syndrome. Nat Genet 2012: 44: 639–641.
13. Bacchelli E, Battaglia A, Cameli C et al. Analysis of CHRNA7 rare variants in autism spectrum disorder susceptibility. Am J Med Genet A 2015: 167: 715–723.
14. Castronovo C, Crippa M, Bestetti I et al. Complex *de novo* chromosomal rearrangement at 15q11-q13 involving an intrachromosomal triplication in a patient with a severe neuropsychological phenotype: clinical report and review of the literature. Am J Med Genet A 2015: 167a: 221–230.
15. Chen CH, Huang CC, Cheng MC et al. Genetic analysis of GABRB3 as a candidate gene of autism spectrum disorders. Mol Autism 2014: 5: 36.
16. Kitsiou-Tzeli S, Tzetis M, Sofocleous C et al. *De novo* interstitial duplication of the 15q11.2-q14 PWS/AS region of maternal origin: Clinical description, array CGH analysis, and review of the literature. A J Med Genet A 2010: 152a: 1925–1932.
17. Tropeano M, Andrieux J, Vassos E et al. Clinical utility gene card for: 15q13.3 microdeletion syndrome. Eur J Hum Genet 2014: 22.
18. Buiting K, Nazlican H, Galetzka D et al. C15orf2 and a novel noncoding transcript from the Prader-Willi/Angelman syndrome region show monoallelic expression in fetal brain. Genomics 2007: 89: 588–595.
19. Usrey KM, Williams CA, Dasouki M et al. Congenital arthrogryposis: an extension of the 15q11.2 BP1-BP2 microdeletion syndrome? Case Rep Genet 2014: 2014: 127258.
20. Grisart B, Willatt L, Destree A et al. 17q21.31 microduplication patients are characterised by behavioural problems and poor social interaction. J Med Genet 2009: 46: 524–530.
21. Kitsiou-Tzeli S, Frysira H, Giannikou K et al. Microdeletion and microduplication 17q21.31 plus an additional CNV, in patients with intellectual disability, identified by array-CGH. Gene 2012: 492: 319–324.
22. Zollino M, Orteschi D, Murdolo M et al. Mutations in KANSL1 cause the 17q21.31 microdeletion syndrome phenotype. Nat Genet 2012: 44: 636–638.
23. Fatemi SH, Reutiman TJ, Folsom TD et al. mRNA and protein levels for GABAAalpha4, alpha5, beta1 and GABABR1 receptors are altered in brains from subjects with autism. J Autism Dev Disord 2010: 40: 743–750.
24. Imitola J, Walleigh D, Anderson CE et al. Fraternal twins with autism, severe cognitive deficit, and epilepsy: diagnostic role of chromosomal microarray analysis. Semin Pediatr Neurol 2014: 21: 167–171.
25. Gregor A, Albrecht B, Bader I et al. Expanding the clinical spectrum associated with defects in CNTNAP2 and NRXN1. BMC Med Genet 2011: 12: 106.
26. Rocca MS, Fabretto A, Faletra F et al. Contribution of SNP arrays in diagnosis of deletion 2p11.2-p12. Gene 2012: 492: 315–318.

27. Xiu X, Song Z, Gao K et al. Genetic analysis of the *FBXO48* gene in Chinese Han patients with Parkinson disease. Neurosci Lett 2013: 541: 224–226.
28. Iles N, Rulten S, El-Khamisy SF et al. APLF (C2orf13) is a novel human protein involved in the cellular response to chromosomal DNA strand breaks. Mol Cell Biol 2007: 27: 3793–3803.
29. Tichy J, Zinke J, Bunz B et al. Expression profile of sonic hedgehog pathway members in the developing human fetal brain. Biomed Res Int 2015: 2015: 494269.
30. Davarniya B, Hu H, Kahrizi K et al. The role of a novel *TRMT1* gene mutation and rare *GRM1* gene defect in intellectual disability in two Azeri families. PLoS One 2015: 10: e0129631.
31. O'Roak BJ, Stessman HA, Boyle EA et al. Recurrent *de novo* mutations implicate novel genes underlying simplex autism risk. Nat Commun 2014: 5: 5595.
32. Vera-Carbonell A, Bafalliu JA, Guillen-Navarro E et al. Characterization of a *de novo* complex chromosomal rearrangement in a patient with cri-du-chat and trisomy 5p syndromes. Am J Med Genet A 2009: 149A: 2513–2521.
33. Kantor DB, Chivatakarn O, Peer KL et al. Semaphorin 5A is a bifunctional axon guidance cue regulated by heparan and chondroitin sulfate proteoglycans. Neuron 2004: 44: 961–975.
34. Abbasi-Moheb L, Mertel S, Gonsior M et al. Mutations in NSUN2 cause autosomal-recessive intellectual disability. Am J Hum Genet 2012: 90: 847–855.
35. Redies C, Hertel N, Hubner CA. Cadherins and neuropsychiatric disorders. Brain Res 2012: 1470: 130–144.
36. Goitia V, Oquendo M, Stratton R. Case of 7p22.1 microduplication detected by whole genome microarray (REVEAL) in workup of child diagnosed with autism. Case Rep Genet 2015: 2015: 212436.
37. Hu J, Sathanoori M, Kochmar S et al. A novel maternally inherited 8q24.3 and a rare paternally inherited 14q23.3 CNVs in a family with neurodevelopmental disorders. Am J Med Genet A 2015: 167: 1921–1926.
38. Pedurupillay CR, Misceo D, Gamage TH et al. Post-zygotic breakage of a dicentric chromosome results in mosaicism for a telocentric 9p marker chromosome in a boy with developmental delay. Gene 2014: 533: 403–410.
39. Lalani SR, Sahoo T, Sanders ME et al. Coarctation of the aorta and mild to moderate developmental delay in a child with a *de novo* deletion of chromosome 15(q21.1q22.2). BMC Med Genet 2006: 7: 8.
40. Willemsen MH, Fernandez BA, Bacino CA et al. Identification of ANKRD11 and ZNF778 as candidate genes for autism and variable cognitive impairment in the novel 16q24.3 microdeletion syndrome. Eur J Hum Genet 2010: 18: 429–435.
41. Weiss LA, Shen Y, Korn JM et al. Association between microdeletion and microduplication at 16p11.2 and autism. N Engl J Med 2008: 358: 667–675.
42. Hannes FD, Sharp AJ, Mefford HC et al. Recurrent reciprocal deletions and duplications of 16p13.11: the deletion is a risk factor for MR/MCA while the duplication may be a rare benign variant. J Med Genet 2009: 46: 223–232.
43. Bena F, Bottani A, Marcelli F et al. A *de novo* 1.1-1.6 Mb subtelomeric deletion of chromosome 20q13.33 in a patient with learning difficulties but without obvious dysmorphic features. Am J Med Genet A 2007: 143A: 1894–1899.
44. Lindstrand A, Malmgren H, Verri A et al. Molecular and clinical characterization of patients with overlapping 10p deletions. Am J Med Genet A 2010: 152a: 1233–1243.
45. Silva IM, Rosenfeld J, Antoniuk SA et al. A 1.5Mb terminal deletion of 12p associated with autism spectrum disorder. Gene 2014: 542: 83–86.
46. Wilson C, Bonnet C, Guy C et al. Tsc1 haploinsufficiency without mammalian target of rapamycin activation is sufficient for renal cyst formation in Tsc1+/- mice. Cancer Res 2006: 66: 7934–7938.
47. Robichaux MA, Chenaux G, Ho HH et al. EphB1 and EphB2 intracellular domains regulate the formation of the corpus callosum and anterior commissure. Dev Neurobiol 2015. DOI 10.1002/dneu.22323
48. Jones RM, Cadby G, Blangero J et al. MACROD2 gene associated with autistic-like traits in a general population sample. Psychiatr Genet 2014: 24: 241–248.
49. Saha T, Dutta S, Rajamma U et al. A pilot study on the contribution of folate gene variants in the cognitive function of ADHD probands. Neurochem Res 2014: 39: 2058–2067.
50. Crespi BJ, Crofts HJ. Association testing of copy number variants in schizophrenia and autism spectrum disorders. J Neurodev Disord 2012: 4: 15.
51. Tarsitano M, Ceglia C, Novelli A et al. Microduplications in 22q11.2 and 8q22.1 associated with mild mental retardation and generalized overgrowth. Gene 2014: 536: 213–216.
52. Healy A, Rush R, Ocain T. Fragile X syndrome: an update on developing treatment modalities. ACS Chem Neurosci 2011: 2: 402–410.
53. Durand CM, Kappeler C, Betancur C et al. Expression and genetic variability of PCDH11Y, a gene specific to *Homo sapiens* and candidate for susceptibility to psychiatric disorders. Am J Med Genet B Neuropsychiatr Genet 2006: 141b: 67–70.
54. Bortolato M, Godar SC, Alzghoul L et al. Monoamine oxidase A and A/B knockout mice display autistic-like features. International J Neuropsychopharmacol 2013: 16: 869–888.
55. Bonnet C, Gregoire MJ, Brochet K et al. Pure de-novo 5 Mb duplication at Xp11.22-p11.23 in a male: phenotypic and molecular characterization. J Hum Genet 2006: 51: 815–821.
56. Kicka S, Bonnet C, Sobering AK et al. A mitotically inheritable unit containing a MAP kinase module. Proc Natl Acad Sci USA 2006: 103: 13445–13450.
57. Ben Khelifa H, Soyah N, Ben-Abdallah-Bouhjar I et al. Xp22.3 interstitial deletion: a recognizable chromosomal abnormality encompassing VCX3A and STS genes in a patient with X-linked ichthyosis and mental retardation. Gene. 2013 Sep 25;527(2):578–83.
58. Li F, Shen Y, Kohler U et al. Interstitial microduplication of Xp22.31: causative of intellectual disability or benign copy number variant? Eur J Med Genet 2010: 53: 93–99.
59. Chiurazzi P, Schwartz CE, Gecz J et al. XLMR genes: update 2007. Eur J Hum Genet 2008: 16: 422–434.
60. Bassani S, Zapata J, Gerosa L et al. The neurobiology of X-linked intellectual disability. Neuroscientist. 2013 Oct;19(5):541–52.
61. Jonsson L, Anckarsater H, Zettergren A et al. Association between ASMT and autistic-like traits in children from a Swedish nationwide cohort. Psychiatr Genet 2014: 24: 21–27.
62. Girirajan S, Rosenfeld JA, Coe BP et al. Phenotypic heterogeneity of genomic disorders and rare copy-number variants. N Engl J Med 2012: 367: 1321–1331.
63. Girirajan S, Rosenfeld JA, Cooper GM et al. A recurrent 16p12.1 microdeletion supports a two-hit model for severe developmental delay. Nat Genet 2010: 42: 203–209.