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Chromosomal Microarray Analysis in Turkish Patients with Unexplained Developmental Delay and Intellectual Developmental Disorders

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ABSTRACT

Introduction: Aneuploids, copy number variations (CNVs), and single nucleotide variants in specific genes are the main genetic causes of developmental delay (DD) and intellectual disability disorder (IDD). These genetic changes can be detected using chromosome analysis, chromosomal microarray (CMA), and next-generation DNA sequencing techniques. Therefore; In this study, we aimed to investigate the importance of CMA in determining the genomic etiology of unexplained DD and IDD in 123 patients.

Method: For 123 patients, chromosome analysis, DNA fragment analysis and microarray were performed. Conventional G-band karyotype analysis from peripheral blood was performed as part of the initial screening tests. FMR1 gene CGG repeat number and methylation analysis were carried out to exclude fragile X syndrome.

Results: CMA analysis was performed in 123 unexplained IDD/DD patients with normal karyotypes and fragile X screening, which were evaluated by conventional cytogenetics. Forty-four CNVs were detected in 39 (39/123=31.7%) patients. Twelve CNV variant of unknown significance (VUS) (9.75%) patients and 7 CNV benign (5.69%) patients were reported. In 6 patients, one or more pathogenic CNVs were determined. Therefore, the diagnostic efficiency of CMA was found to be 31.7% (39/123).

Conclusion: Today, genetic analysis is still not part of the routine in the evaluation of IDD patients who present to psychiatry clinics. A genetic diagnosis from CMA can eliminate genetic question marks and thus alter the clinical management of patients. Approximately one-third of the positive CMA findings are clinically intervenable. However, the emergence of CNVs as important risk factors for multiple disorders increases the need for individuals with comorbid neurodevelopmental conditions to be the priority where the CMA test is recommended.

Keywords: Copy number variations, chromosomal microarray, developmental delay, intellectual developmental disorder, mental retardation, genetic testing

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INTRODUCTION

Developmental delay (DD) affects 1–3% of children, and the frequency of intellectual disability disorder (IDD) has been estimated to be 18.3 in every 1000 people in the child/adolescent population. IDD is a clinically heterogeneous group of disorder in which genetic and environmental factors play causative roles (1, 2). Developmental delay occurs when a child does not achieve developmental milestones in comparison to peers of the same age range. A significant delay is defined as performance that is two or more standard deviations below the mean on age-appropriate standardised norm-referenced testing. DD occurs in people who are 5 years and younger and exhibits several significant delays in the following areas: cognitive, speech, social/personal, and good/gross motor and daily activities. Intellectual disability (ID), according to Diagnostic and Statistical Manual of Mental Disorders (DSM-5) diagnostic criteria, is a chronic condition that involves impairments in intellectual functioning, present during developmental periods, and deficits in adaptive functioning. IDD can be diagnosed at the age of 5 years or later when a person's intelligence quotient is determined to be less than 70, and persistent mental disability results in a general impairment in adaptive behaviour (3). The American Association on Intellectual and Developmental Disability defines ID by using measures of 3 domains: intelligence (IQ), adaptive behavior, and systems of supports afforded the individual.

Aneuploids, copy number variations (CNVs), and single nucleotide variants in specific genes are the main genetic causes of DD and IDD. During developmental and regular lifetime functions, a large number of proteins must be functionally active at the right amount and at the right time. Therefore, it should be noted that an unexpected mutation, deletion, or rearrangement affecting any of the genes encoding these proteins can have serious consequences for brain development or cognitive functionality. These genetic alterations can be detected using

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chromosome analysis, chromosomal microarray (CMA) and next-generation DNA sequencing techniques. These tests are crucial for clinical diagnostic tools that can identify genetic abnormalities and provide better disease management in individuals with unexplained DD and IDD (for those without a specific hereditary disorder) (4–6). Choosing the right method is also important at this point. Karyotype generates both numerical and structural information of the underlying genome, while CMA mainly provide numerical information. For example, karyotype analysis may show that a piece of 12p is moved to 21q in all the cells in a sample, but since there is no net gain or loss, the CMA analysis of the same sample will not show any changes. Similarly, CMA may show gain of several genes on 8q but it cannot determine where the extra DNA is located (i.e., still attached to 8q or moved to somewhere else). Karyotype data produce genomic information with single cell resolution, while CMA provide an "average" copy number change for all the cells present in a sample. For example, karyotype analysis may show half of the cells in a sample have a loss of one copy of chromosome 12 and the other half have a gain of one copy of chromosome 12, but the CMA of the same sample will show no net gain or loss. Karyotype analysis has a resolution at the G-banding level (generally >10 Mb for tumor samples) while CMA offer gene-or exon-level resolution. Finally, karyotyping is a manual process requiring years of training and acquisition of great skill not only in analysis but also in interpretation, whereas CMA assays are largely automated. Typical karyotype analysis also provides insight into approximately only 20 metaphase cells. These comparisons illustrate the relative advantages and disadvantages of CMA and karyotype/FISH, supporting the notion that these are complementary technologies best used together to assess the numerical and structural features

Currently, genetic analysis is still not part of the routine evaluation of IDD patients presenting to psychiatric clinics. However, the emergence of CNVs as important risk factors for multiple anomalies increases the need to prioritize individuals with comorbid neurodevelopmental conditions where the CMA test is recommended.

Systematic reviews and meta-analyses carried out with thousands of patients have reported that a 3% diagnosis rate is obtained with karyotyping, while with CMA, a diagnosis rate of 15% is obtained (7–9). However, conventional karyotype analysis cannot detect cryptic deviations smaller than 5–10 Mb in size, which may be clinically significant. Considering the increased diagnostic yield of CMA compared to that of conventional karyotyping, the American Academy of Pediatrics (AAP), the American Medical Genetics and Genomic Society (ACMG) and the American Academy of Neurology (AAN) are recommending CMA in the first-line tests for the diagnosis of DD/IDD (10–12). A genetic diagnosis from CMA can eliminate genetic question marks and thus alter the clinical management of patients. Approximately one-third of the positive CMA findings can be intervened clinically.

Although CMA is a very robust and reliable technique, it has limitations: it is unable to detect balanced genomic abnormalities such as inversions, repeats, and Robertsonian translocations. Depending on the platform used, low-level mosaicism and some polyploidies cannot be detected. Finally, CMA does not give information on rearrangement, and FISH is often used as a complementary method to identify possible rearrangement with implications for genetic counselling. Thus, karyotypes remain more suitable to evaluate potential carriers of chromosomal rearrangements, couples with recurrent miscarriage, or patients with a distinctive aneuploidy phenotype. However, FISH is more suitable if a specific microdeletion syndrome is highly suspected.

In this study, we aimed to determine the diagnostic yield of CMA in 123 patients with unexplained DD and IDD.

METHODS

Participants

Patients who presented to the Child and Adolescent Mental Health Clinic and were diagnosed with intellectual developmental disorder (IDD) according to DSM-V diagnostic criteria, or those who were diagnosed with DD were included in this study. Between August 2015 and May 2018, we collected data on 123 participants. All patients were evaluated both by a child psychiatrist and a clinical geneticist. Participants with a history of drug exposure during pregnancy and neonatal hypoxic-ischemic brain injury had been excluded. Investigators identified eligible participants from their caseloads based upon the study inclusion criteria, namely that the participants should be aged 18 years or younger with idiopathic ID, one or more psychiatric diagnoses and/or significant challenging behaviours. In these patients, neurological examination revealed developmental delay or intellectual disability such as language or motor impairment, muscular hypotonia, or autistic traits. General observations for dysmorphic features were made and measurements of height and head circumference were collected by the clinician or researcher.

Patients with DD/IDD their parents were informed about the CMA study and the risks, benefits and limitations of the CMA test. Informed consent was obtained by clinical geneticists or researchers. Detailed physical examination and pedigree drawing were performed in all patients.

Conventional G-band karyotype analysis from peripheral blood was performed as part of the initial screening tests. FMR1 gene CGG repeat number and methylation analysis were carried out to exclude fragile X syndrome. A total of 123 patients (80 males and 43 females) (Table 1) between 1 year and 18 years of age with normal karyotypes and without fragile X syndrome were accepted for CMA analysis. The mean age at the time of diagnosis was 8.49 years (range: 10 months to 18 years). Ten patients were diagnosed with DD, 113 patients had only IDD or minor congenital findings in addition to IDD.

Chromosome analysis

The study included peripheral lymphocyte culture by a standard method using the Leishman-banding technique, centromere-banding (C-banding) and nucleolar organizing region staining performed as needed. Peripheral blood lymphocytes were cultured for 72 hours at approximately 0.5–1 mL. Lymphocyte cultures were treated with hypotonic KCl solution to attain metaphase and then harvested by the addition of colcemid for 45 min (0.075 M), treatment for 4 min and fixation using standard fixative prepared with methanol and acetic acid (3:1) (8, 9). At least 25 metaphases were scored for each patient. The best metaphases were karyotyped, and the total chromosome count was usually determined in 25 cells. If mosaicism was suspected, then 100 or more cell counts were carried out for documentation for abnormal cases. Fluorescent in situ hybridization (FISH) was used if needed, and the International System for Human Cytogenetic Nomenclature (ISCN) was used for the nomenclature of human chromosomes.

DNA preparation

Pure genomic DNA was isolated from peripheral blood collected into EDTA tubes by the MagNA Pure LC DNA Isolation Kit (Roche) or manually.

Table 1. Summary of CMA findings in patients

Case	Age	Sex	Clinical features	Fragment analysis	Microarray	Size (kb)	OMIM genes (N)	Classification
1	9	M	IDD	N	2q37.2 duplication	398 kb	AGAP1	VOUS
2	13	M	IDD, Minor Congenital Anomaly, Marfanoid Appearance	N	Xq21.31-q21.32 duplication	538 kb	PCDH11X	Likely benign
3	8	M	MR	N	4q32.3 duplication	1410 kb	SPOCK3, ANXA10,DOX60	VOUS
4	17	M	Attention Deficit and Hyperactivity Disorder, Mild IDD	N	10q21.3 deletion	127 kb	CTNNA3	VOUS
5	13	M	Severe IDD, hearing loss	N	2q37.3 del.	2983 kb	HDAC4, MGC16025, MIR4269, LOC150935, NDUFA10, OR6B2, PRR21, OR6B3, MYEOV2, OTOS, GPC1, PP14571, MIR149, ANKMY1, DUSP28, RNPEPL1, CAPN10, GPR35, AQP12B, AQP12A, KIF1A, AGXT, C2orf54, LOC200772, SNED1, MTERFD2, PASK, PPP1R7, ANO7, HDLBP, SEPT2, FARP2, STK25, BOK-AS1, BOK, THAP4, ATG4B, DTYMK, ING5, D2HGDH, GAL3ST2, NEU4, PDCD1, C2orf85, LOC728323	Pathogenic
					20q13.2-q13.33 dup.	6363 kb	C20orf85, PPP4R1L, RAB22A, VAPB, APCDD1L, LOC149773, STX16, STX16-NPEPL1, NPEPL1, MIR296, MIR298, GNAS-AS1, GNAS, TH1L, CTSZ, TUBB1, ATP5E, SLMO2-ATP5E, SLMO2, ZNF831, EDN3, PHACTR3, SYCP2, C20orf177, PPP1R3D, CDH26, C20orf197, CDH4, MIR1257, TAF4, LSM14B, PSMA7, SS18L1, GTPBP5, HRH3, OSBPL2, ADRM1, LAMA5, RPS21, CABLES2, C20orf151, GATA5, C20orf200, C20orf166, MIR1-1, MIR133A2, SLCO4A1, LOC100127888, NTSR1, C20orf20, OGFR, COL9A3, TCFL5, DPH3P1, DIDO1, C20orf11, SLC17A9, BHLHE23, LOC63930, NCRNA00029, LOC100144597, HAR1B, HAR1A, MIR124-3, YTHDF1, BIRC7, MIR3196, NKAIN4, FLJ16779, ARFGAP1, MIR4326, COL20A1, CHRNA4, KCNQ2, EEF1A2, PPDPF, PTK6, SRMS, C20orf195, PRIC285, GMEB2, STMN3, RTEL1, RTEL1-TNFRSF6B, TNFRSF6B, ARFRP1, ZGPAT, LIME1, SLC2A4RG, ZBTB46, ABHD16B, TPD52L2, DNAJC5, MIR941-1, MIR941-3, MIR941-2, UCKL1, MIR1914, MIR647, UCKL1-AS1, ZNF512B, SAMD10, PRPF6, NCRNA00176, SOX18, TCEA2, RGS19, OPRL1, C20orf201, NPBWR2, MYT1, PCMTD2	
6	12	M	Attention Deficit and Hyperactivity Disorder, IDD	N	16p11.2 deletion	534kb	SPN, QPRT, C16orf54, ZG16, KIF22, MAZ, PRRT2, C16orf53, MVP, CDIPT, LOC440356, SEZ6L2, ASPHD1, KCTD13, TMEM219, TAOK2, HIRIP3, INO80E, DOC2A, C16orf92, FAM57B, ALDOA, PPP4C, TBX6, YPEL3, GDPD3, MAPK3, LOC100271831	Pathogenic
7	17	M	Borderline IDD, DD	N	19q13.41 duplication	294 kb	FPR3, ZNF577, ZNF649, ZNF613, ZNF350, ZNF615, ZNF614, ZNF432, ZNF841	Likely benign

Continuation of Table 1

Case	Age	Sex	Clinical features	Fragment analysis	Microarray	Size (kb)	OMIM genes (N)	Classification
8	9	F	IDD	N	20q11.21-q12 duplication	11706 kb	DEFB115, DEFB116, DEFB118, DEFB119, DEFB121, DEFB122, DEFB123, DEFB124, REM1, NCRNA00028, HM13, PSIMCT-1, ID1, MIR3193, COX4I2, BCL2L1, TPX2, MYLK2, FOXS1, DUSP15, TTLL9, PDRG1, XKR7, C20orf160, HCK, TM9SF4, TSPY26P, PLAGL2, POFUT1, KIF3B, ASXL1, C20orf112, LOC149950, C20orf203, COMMD7, DNMT3B, MAPRE1, SUN5, BPIL1, BPIL3, C20orf185, C20orf186, C20orf70, BASE, C20orf71, PLUNC, C20orf114, CDK5RAP1, SNTA1, CBFA2T2, NECAB3, C20orf144, C20orf134, E2F1, PXMP4, ZNF341, CHMP4B, RALY, EIF2S2, ASIP, AHCY, ITCH, MIR644, DYNLRB1, MAP1LC3A, PIGU, TP53INP2, NCOA6, HMGB3P1, GGT7, ACSS2, GSS, MYH7B, MIR499, TRPC4AP, EDEM2, PROCR, MMP24, EIF6, FAM83C, UQCC, GDF5, CEP250, C20orf173, ERGIC3, FER1L4, SPAG4, CPNE1, RBM12, NFS1, ROMO1, RBM39, PHF20, SCAND1, C20orf152, LOC647979, EPB41L1, C20orf4, DLGAP4, MYL9, TGIF2, TGIF2-C20ORF24, C20orf24, SLA2, NDRG3, DSN1, KIAA0889, C20orf118, SAMHD1, RBL1, C20orf132, RPN2, GHRH, MANBAL, SRC, BLCAP, NNAT, CTNNBL1, VSTM2L, TTI1, RPRD1B, TGM2, KIAA1755, BPI, LBP, LOC388796, SNORA71B, SNORA71A, SNORA71C, SNORA71D, SNHG11, SNORA39, SNORA60, RALGAPB, ADIG, ARHGAP40, SLC32A1, ACTR5, PPP1R16B, FAM83D, DHX35, LOC339568, MAFB, TOP1, PLCG1, ZHX3, LPIN3, EMILIN3, CHD6, PTPRT	Pathogenic
					20q13.31 deletion	331 kb	MIR4325, SPO11, RAE1, MTRNR2L3, RBM38, CTCFL, PCK1	
9	15	M	IDD, operated inguinal hernia, undescended testis	N	Chromosome 1 LOH	-	LOH	VOUS
10	11	M	IDD	N	Xp21.1 duplication	850 kb	DMD	VOUS
11	1	M	IDD	N	15q11.2 del.	602 kb	GOLGA8DP, GOLGA6L1, TUBGCP5, CYFIP1, NIPA2, NIPA1, WHAMML1, GOLGA8IP, HERC2P2	Likely benign
12	1	F	IDD + Brachycephaly + epicanthus	N	3q22.2 -q22.3 deletion	3567 kb	PP2R3A, MSL2, PCCB, STAG1, TMEM22, NCK1, IL20RB, SOX14, CLDN18, DZIP1L, A4GNT, DBR1, ARMC8, TXNDC6, MRAS, ESYT3, CEP70, FAIM, PIK3CB, FOXL2	Pathogenic
					16p11.2 duplication	534 kb	SPN, QPRT, C16orf54, ZG16, KIF22, MAZ, PRRT2, C16orf53, MVP, CDIPT, LOC440356, SEZ6L2, ASPHD1, KCTD13, TMEM219, TAOK2, HIRIP3, INO80E, DOC2A, C16orf92, FAM57B, ALDOA, PPP4C, TBX6, YPEL3, GDPD3, MAPK3, LOC100271831	
13	1	M	5p Microdeletion Syndrome?	N	5p15.33 - p14.3 deletion	18975 kb	PLEKHG4B, LRRC14B, CCDC127, SDHA, PDCD6, AHRR, LOC100310782, C5orf55, EXOC3, LOC25845, SLC9A3, CEP72, TPPP, ZDHHC11, BRD9, TRIP13, NKD2, SLC12A7, SLC6A19, SLC6A18, TERT, CLPTM1L, SLC6A3, LPCAT1, SDHAP3, LOC728613, MIR4277, MRPL36, NDUFS6, IRX4, IRX2, C5orf38, LOC285577, IRX1, LOC340094, ADAMTS16, KIAA0947, FLJ33360, MED10, UBE2QL1, LOC255167, NSUN2, SRD5A1, PAPD7, MIR4278, LOC442132, ADCY2, C5orf49, FASTKD3, MTRR, SEMA5A, SNORD123, TAS2R1, LOC285692, FAM173B, CCT5, CMBL, MARCH6, ROPN1L, ANKRD33B, DAP, CTNND2, TAG, DNAH5, TRIO, FAM105A, FAM105B, ANKH, LOC100128508, FBXL7, MARCH11, ZNF622, FAM134B, MYO10, LOC285696, BASP1, LOC401177	Pathogenic

Continuation of Table 1

Case	Age	Sex	Clinical features	Fragment analysis	Microarray	Size (kb)	OMIM genes (N)	Classification
14	2	F	Angelman Syndrome?	N	15q11.2-q13.1	5998 kb	LOH	Pathogenic
15	3	M	IDD	N	2q21.1-q22.1 del, 2q31 del	10966 kb	CCDC115, IMP4, PTPN18, CFC1B, CFC1, LOC150527, LOC646743, C2orf14, GPR148, FAM123C, ARHGEF4, FAM168B, PLEKHB2, POTEE, LOC440910, LOC150786, LOC389043, LOC401010, TUBA3D, MZT2A, LOC150776, CCDC74A, POTEKP, C2orf27A, C2orf27B, ANKRD30BL, MIR663B, GPR39, LYPD1, NCKAP5, MIR3679, MGAT5, LOC151162, TMEM163, ACMSD, LOC100129961, CCNT2, YSK4, RAB3GAP1, ZRANB3, R3HDM1, MIR128-1, UBXN4, LCT, MCM6, DARS, CXCR4, THSD7B, HNMT, SPOPL, NXPH2, LOC647012, LRP1B	Pathogenic
16	3	F	Mild IDD + ADHD	N	2q21.1 del.	216 kb	SMPD4, MZT2B, TUBA3E, CCDC115, IMP4, PTPN18	Benign
17	7	F	Mild IDD + ADHD	N	2q21.1 del.	216 kb	SMPD4, MZT2B, TUBA3E, CCDC115, IMP4, PTPN18	Benign
18	7	F	IDD	N	1q22.1q21.2 del	2725 kb	LOC728989, PRKAB2, PDIA3P, FMO5, CHD1L, BCL9, ACP6, GJA5, GJA8, GPR89B, GPR89C, PDZK1P1, NBPF11, NBPF24, FLJ39739, PPIAL4B, PPIAL4A, NBPF14, PPIAL4D, PPIAL4F, NBPF15, NBPF16, PPIAL4E, LOC645166	Pathogenic
19	6	M	46,XY,der(21) add(21)(q11.2)	N	Chr7q33q36.3 dup.	21981 kb	NDUFB2, BRAF, MRPS33, LOC100131199, AGK, KIAA1147, FLJ40852, WEE2, SSBP1, TAS2R3, TAS2R4, TAS2R5, PRSS37, OR9A4, CLEC5A, TAS2R38, MGAM, LOC93432, LOC100124692, MOXD2P, PRSS58, LOC730441, MTRNR2L6, PRSS1, TRY6, PRSS2, EPHB6, TRPV6, TRPV5, C7orf34, KEL, OR9A2, OR6V1, OR6W1P, PIP, TAS2R39, TAS2R40, GSTK1, TMEM139, CASP2, CLCN1, FAM131B, ZYX, EPHA1, LOC285965, TAS2R60, TAS2R41, CTAGE15P, FAM115C, CTAGE6P, LOC154761, FAM115A, OR2F2, OR2F1, OR6B1, OR2A5, OR2A25, OR2A12, OR2A2, OR2A14, CTAGE4, ARHGEF35, OR2A42, OR2A1, OR2A9P, OR2A20P, OR2A7, LOC728377, ARHGEF5, NOBOX, TPK1, CNTNAP2, MIR548I4, MIR548F4, MIR548F3, MIR548T, C7orf33, CUL1, EZH2, PDIA4, ZNF786, ZNF425, ZNF398, ZNF282, ZNF212, ZNF783, LOC155060, ZNF777, ZNF746, ZNF767, KRBA1, ZNF467, SSPO, ZNF862, LOC401431, ATP6V0E2, ACTR3C, LRRC61, C7orf29, RARRES2, REPIN1, ZNF775, LOC728743, LOC285972, GIMAP8, GIMAP7, GIMAP4, GIMAP6, GIMAP2, GIMAP1, GIMAP1-GIMAP5, GIMAP5, LOC100128542, TMEM176B, TMEM176A, ABP1, KCNH2, NOS3, ATG9B, ABCB8, ACCN3, CDK5, SLC4A2, FASTK, TMUB1, AGAP3, GBX1, ASB10, ABCF2, CHPF2, MIR671, SMARCD3, NUB1, WDR86, LOC100131176, CRYGN, MIR3907, RHEB, PRKAG2, GALNTL5, GALNT11, MLL3, FABP5P3, LOC100128822, XRCC2, ACTR3B, DPP6, LOC100132707, PAXIP1, LOC202781, HTR5A, INSIG1, EN2, CNPY1, RBM33, SHH, NCRNA00244, C7orf13, RNF32, LMBR1, NOM1, MNX1, UBE3C, DNAJB6, PTPRN2, MIR153-2, MIR595, NCAPG2, ESYT2, WDR60, LOC154822, VIPR2	Pathogenic

Continuation of Table 1

Case	Age	Sex	Clinical features	Fragment analysis	Microarray	Size (kb)	OMIM genes (N)	Classification
					Chr21q22.2q22.3 del	7865 kb	SMG1, BRWD1, NCRNA00257, HMGN1, WRB, LCA5L, SH3BGR, C21orf88, B3GALT5, IGSF5, PCP4, DSCAM, C21orf130, MIR3197, BACE2, PLAC4, FAM3B, MX2, MX1, TMPRSS2, NCRNA00111, C21orf129, NCRNA00112, RIPK4, PRDM15, C2CD2, ZNF295, C21orf121, UMODL1, C21orf128, ABCG1, TFF3, TFF2, TFF1, TMPRSS3, UBASH3A, RSPH1, SLC37A1, PDE9A, WDR4, NDUFV3, PKNOX1, CBS, U2AF1, CRYAA, SIK1, C21orf125, C21orf84, HSF2BP, RRP1B, PDXK, CSTB, RRP1, LOC284837, AGPAT3, TRAPPC10, PWP2, C21orf33, ICOSLG, DNMT3L, AIRE, PFKL, C21orf2, TRPM2, LRRC3, TSPEAR, C21orf90, KRTAP10-1, KRTAP10-2, KRTAP10-3, KRTAP10-4, KRTAP10-5, KRTAP10-6, KRTAP10-7, KRTAP10-8, KRTAP10-9, KRTAP10-10, KRTAP10-11, KRTAP12-4, KRTAP12-3, KRTAP12-2, KRTAP12-1, KRTAP10-12, UBE2G2, SUMO3, PTTG1IP, ITGB2, C21orf67, C21orf70, NCRNA00163, NCRNA00162, C21orf122, ADARB1, POFUT2, LOC642852, COL18A1, NCRNA00175, SLC19A1, PCBP3, COL6A1, COL6A2, FTCD, C21orf56, LSS, MCM3AP-AS1, MCM3AP, YBEY, C21orf58, PCNT, DIP2A, S100B, PRMT2	
20	6	M	Williams Syndrome?	N	7q11.23 deletion	1.4 Mb	RIM50, FKBP6, FZD9, BAZ1B, BCL7B, TBL2, MLXIPL, VPS37D, DNAJC30, WBSCR22, STX1A, MIR4284, WBSCR26, ABHD11, CLDN3, CLDN4, WBSCR27, WBSCR28, ELN, LIMK1, EIF4H, MIR590, LAT2, RFC2, CLIP2, GTF2IRD1, GTF2I	Pathogenic
21	10	M	IDD	N	22q11.2 deletion	781 kb	ZNF74, PI4KA, SERPIND1, SNAP29, CRKL, LZTR1, SLC7A4, SCARF2, KLHL22, MED15, POM121L4P, TMEM191A, AIFM3, THAP7, FLJ39582, MGC16703, P2RX6, P2RX6P, LOC400891	Pathogenic
22	2	F	Williams Syndrome?	N	7q11.23 deletion	1407 kb	RIM50, FKBP6, FZD9, BAZ1B, BCL7B, TBL2, MLXIPL, VPS37D, DNAJC30, WBSCR22, STX1A, MIR4284, WBSCR26, ABHD11, CLDN3, CLDN4, WBSCR27, WBSCR28, ELN, LIMK1, EIF4H, MIR590, LAT2, RFC2, CLIP2, GTF2IRD1, GTF2I	Pathogenic
23	12	F	IDD	N	8p23.3 deletion	567 kb	ZNF596, FBXO25, C8orf42, ERICH1	Pathogenic
					16q23.2-q24.3 dup.	8657 kb	CMIP, PLCG2, SDR42E1, HSD17B2, MPHOSPH6, CDH13, MIR3182, HSBP1, MLYCD, OSGIN1, NECAB2, SLC38A8, MBTPS1, HSDL1, LRRC50, TAF1C, ADAD2, KCNG4, WFDC1, ATP2C2, KIAA1609, COTL1, KLHL36, USP10, CRISPLD2, ZDHHC7, KIAA0513, FAM92B, LOC400548, KIAA0182, GINS2, C16orf74, MIR1910, COX4NB, COX4I1, IRF8, LOC732275, LOC400550, FOXF1, MTHFSD, FLJ30679, FOXC2, FOXL1, LOC100506581, FBXO31, MAP1LC3B, ZCCHC14, JPH3, KLHDC4, SLC7A5, CA5A, BANP, ZNF469, ZFPM1, ZC3H18, IL17C, CYBA, MVD, MGC23284, SNAI3, RNF166, CTU2, FAM38A, CDT1, APRT, GALNS, TRAPPC2L, PABPN1L, CBFA2T3, ACSF3, C16orf81, CDH15, ZNF778, ANKRD11, SPG7, RPL13, SNORD68, CPNE7, DPEP1, CHMP1A, C16orf55, CDK10, SPATA2L, C16orf7, LOC100128881, ZNF276, FANCA, SPIRE2, TCF25, MC1R, TUBB3, DEF8, CENPBD1, AFG3L1P, DBNDD1, GAS8, C16orf3, LOC100130015, PRDM7	

Continuation of Table 1

Case	Age	Sex	Clinical features	Fragment analysis	Microarray	Size (kb)	OMIM genes (N)	Classification
24	5	M	IDD + macroglossia	N	15q11.2q13.1 deletion	4813 kb	MKRN3, MAGEL2, NDN, PWRN2, PWRN1, C15orf2, SNRPN, SNURF, SNORD107, PAR-SN, PAR5, SNORD64, SNORD108, SNORD109B, SNORD109A, SNORD116-1, SNORD116-2, SNORD116-3, SNORD116-9, SNORD116-4, SNORD116-5, SNORD116-7, SNORD116-6, SNORD116-8, SNORD116-10, SNORD116-11, SNORD116-12, SNORD116-13, SNORD116-14, SNORD116-15, SNORD116-16, SNORD116-19, SNORD116-17, SNORD116-18, SNORD116-20, SNORD116-21, SNORD116-22, SNORD116-23, SNORD116-24, SNORD116-25, SNORD116-26, SNORD116-27, SNORD116-28, SNORD116-29, IPW, PAR1, SNORD115-1, SNORD115-2, SNORD115-3, SNORD115-4, SNORD115-5, SNORD115-9, SNORD115-10, SNORD115-12, SNORD115-6, SNORD115-7, SNORD115-8, SNORD115-11, SNORD115-29, SNORD115-36, SNORD115-43, SNORD115-13, SNORD115-14, SNORD115-16, SNORD115-17, SNORD115-18, SNORD115-19, SNORD115-20, SNORD115-15, SNORD115-21, SNORD115-22, PAR4, SNORD115-23, SNORD115-24, SNORD115-25, SNORD115-26, HBII-52-27, HBII-52-28, SNORD115-30, SNORD115-31, SNORD115-32, SNORD115-33, SNORD115-34, SNORD115-35, SNORD115-37, SNORD115-38, SNORD115-39, SNORD115-40, SNORD115-41, SNORD115-42, SNORD115-44, HBII-52-45, HBII-52-46, SNORD115-48, UBE3A, ATP10A, GABRB3, GABRA5, GABRG3, OCA2, HERC2	Pathogenic
25	2	F	1p36 microdeletion syndrome	N	1p36.33p36.32 deletion	2176 kb	NCRNA00115, LOC643837, FAM41C, FLJ39609, SAMD11, NOC2L, KLHL17, PLEKHN1, C1orf170, HES4, ISG15, AGRN, LOC401934, C1orf159, MIR200B, MIR200A, MIR429, TTLL10, TNFRSF18, TNFRSF4, SDF4, B3GALT6, FAM132A, UBE2J2, SCNN1D, ACAP3, PUSL1, CPSF3L, GLTPD1, TAS1R3, DVL1, MXRA8, AURKAIP1, CCNL2, LOC148413, MRPL20, LOC441869, TMEM88B, VWA1, ATAD3C, ATAD3B, ATAD3A, C1orf70, SSU72, MIB2, MMP23B, MMP23A, CDK11B, SLC35E2B, CDK11A, SLC35E2, NADK, GNB1, CALML6, TMEM52, KIAA1751, GABRD, PRKCZ, C1orf86, LOC100128003, SKI, MORN1, LOC100129534, RER1, PEX10, PLCH2, PANK4, HES5, LOC115110, LOC100133445, TNFRSF14, C1orf93, MMEL1	Pathogenic
26	10	F	IDD	N	15q26.1-q26.3 duplication	8816 kb	MCTP2, LOC400456, LOC145820, NR2F2, MIR1469, SPATA8, LOC91948, ARRDC4, FAM169B, IGF1R, PGPEP1L, SYNM, TTC23, LRRC28, MEF2A, LYSMD4, DNM1P46, ADAMTS17, FLJ42289, LASS3, LINS, ASB7, ALDH1A3, LRRK1, CHSY1, SELS, SNRPA1, PCSK6, TM2D3, TARSL2, OR4F6, OR4F15, GPCRLTM7, OR4F4	Pathogenic
					22q13.31-q13.33 deletion	4361 kb	CELSR1, GRAMD4, CERK, TBC1D22A, FLJ46257, MIR3201, FAM19A5, C22orf34, BRD1, LOC90834, ZBED4, ALG12, CRELD2, PIM3, IL17REL, MLC1, MOV10L1, PANX2, TRABD, SELO, TUBGCP6, HDAC10, MAPK12, MAPK11, PLXNB2, FAM116B, PPP6R2, SBF1, ADM2, MIOX, LMF2, NCAPH2, SCO2, TYMP, ODF3B, KLHDC7B, C22orf41, CPT1B, CHKB-CPT1B, CHKB, LOC100144603, MAPK8IP2, ARSA, SHANK3, ACR, RPL23AP82, RABL2B	

Continuation of Table 1

Case	Age	Sex	Clinical features	Fragment analysis	Microarray	Size (kb)	OMIM genes (N)	Classification
27	8	M	IDD	N	3p25.2 duplication	72 kb	MKRN2, RAF1	Likely benign
28	8	F	IUGR, IDD	N	15q11.1-q11.2 duplication	4170 kb	CHEK2P2, HERC2P3, GOLGA6L6, GOLGA8CP, NBEAP1, MIR3118-4, MIR3118-3, MIR3118-2, POTEB3, POTEB, POTEB2, NF1P2, MIR5701-3, MIR5701-1, MIR5701-2, LINC01193, LOC646214, CXADRP2, LOC101927079, LOC727924, OR4M2, OR4N4, OR4N3P, MIR1268A, REREP3, MIR4509-3, MIR4509-2, MIR4509-1, GOLGA8DP, GOLGA6L1, GOLGA6L22, TUBGCP5, CYFIP1, NIPA2, NIPA1, LOC283683, WHAMMP3, GOLGA8IP, HERC2P2, HERC2P7, GOLGA8EP, GOLGA8S, GOLGA6L2, MIR4508, MKRN3, MAGEL2, NDN, PWRN4, PWRN2	Pathogenic
29	9	M	IDD	N	6p25.1 deletion	103 kb	FARS2	VOUS
30	7	M	ADHD + IDD	N	4q13.3 duplication	225 kb	COX18, ANKRD17	VOUS
31	12	M	IDD	N	1p36.11 duplication	1244,11 kb	-	VOUS
32	9	F	IDD	N	2q14.2 duplication	861421 kb	-	VOUS
33	3	M	IDD	N	14q21.2 deletion	269464 kb	FSCB	VOUS
34	10	M	Williams Syndrome	N	7q11.23 deletion	1407 kb	TRIM50, FKBP6, FZD9, BAZ1B, BCL7B, TBL2, MLXIPL, VPS37D, DNAJC30, WBSCR22, STX1A, MIR4284, WBSCR26, ABHD11, CLDN3, CLDN4, WBSCR27, WBSCR28, ELN, LIMK1, EIF4H, MIR590, LAT2, RFC2, CLIP2, GTF2IRD1, GTF2I	Pathogenic
35	11	F	IDD	N	5q22.3-q23.1 duplication	719087	-	VOUS
36	5	M	Williams Syndrome	N	7q11.23 delesyonu	1407 kb	TRIM50, FKBP6, FZD9, BAZ1B, BCL7B, TBL2, MLXIPL, VPS37D, DNAJC30, WBSCR22, STX1A, MIR4284, WBSCR26, ABHD11, CLDN3, CLDN4, WBSCR27, WBSCR28, ELN, LIMK1, EIF4H, MIR590, LAT2, RFC2, CLIP2, GTF2IRD1, GTF2I	Pathogenic
37	11	F	IDD	N	18p11.22 duplication	193031 kb	RAB12, GACAT2, MTCL1	VOUS
38	14	M	Prader-Willi Syndrome?	N	1p12 duplication	195613 kb	WDR3, SPAG17	Benign
39	13	F	IDD, DD	N	22q11.1-q11.21del	257494 kb	XKR3,IL17RA, CECR1, SLC25A18, ATP6V1E1, BID, MICAL3, MIR648, PEX26, TUBA8, USP18, DGCR6, PRODH, DGCR2, TSSK2, GSC2, SLC25A1, CLTCL1, HIRA, MRPL40, UFD1L, CLDN51	Pathogenic

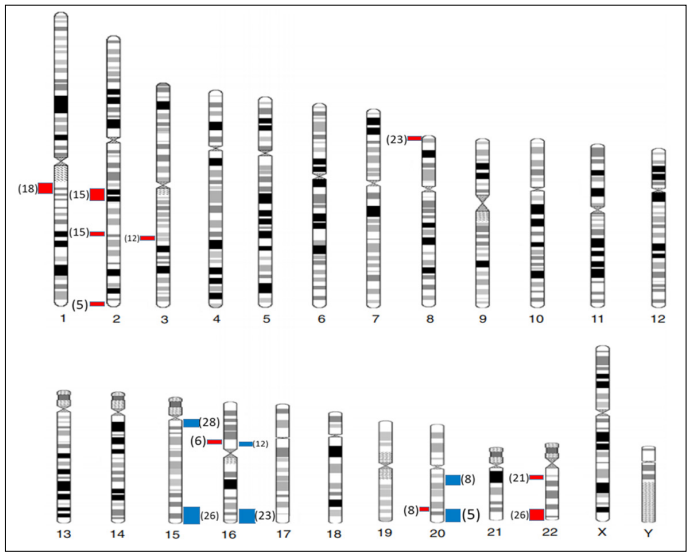


Figure 1. An overview of the aCGH-detected genomic losses and gains in this study. Losses are shown as red *(left)* and gains in blue *(right)*, respectively. The numbers in parentheses are the case numbers indicated in the table. The areas covered by the bar indicate the size of each aberrant region.

Fragment analysis

Two millilitres of peripheral venous blood samples from patients were collected in EDTA tubes. Genomic DNA was extracted according to the protocol of the kit used (EZ1 Advanced Instruments, Qiagen, Hilden, Germany). The concentration and purity values of the DNA samples were measured on a NanoDrop device (Nanodrop 2000C, ThermoScientific, USA) Bisulfite DNA was extracted according to the protocol of the kit (EpiTect Bisulfite conversion kit, Qiagen, Hilden, Germany). As an alternative method for the diagnosis of fragile X syndrome, fluorescent-labelled methylation-specific PCR was performed. The fragmentation analysis of fluorescent-labelled PCR fragments was performed with a AB 3130xl analyser (ABI 3130×l AppliedBiosystems, USA) and analysed with the GeneMapper v5.0 software program.

Chromosomal microarray

The DNA obtained from peripheral blood samples was then investigated for genetic aberrations by aCGH using the CytoSure 4×180K oligonucleotide array (Agilent Technologies, Inc.). For each sample, 20.2 µl of genomic DNA was used. The CytoSure Genomic DNA aCGH labelling kit (Agilent Technologies, Inc.) was used to label the reference and patient DNA with Cy-3 and Cy-5, respectively. The labelled targets were then purified according to the manufacturer's protocol with a minimum labelling efficiency of 250 pmol/µg DNA for Cy3 and 200 pmol/µg DNA for Cy-5. The purified Cy3-and Cy5-labelled genomic DNA was then mixed with human Cot-1 DNA, blocking agent and hybridization buffer and then was denatured and incubated at 37°C for 30 min. A total of 100 µl of the sample mixture was then pipetted into the hybridization chamber containing the microarray and placed in a rotisserie hybridization oven at 65°C for 40 h. Subsequent to hybridization, the slides were washed with 2 wash buffers: wash buffer 1 for 5 min at room temperature and wash buffer 2 for 1 min at 37°C. The slides were then scanned using the Agilent DNA scanner to produce the raw image data. These data were further processed using the Agilent Feature Extraction software (Agilent Technologies, Inc.), and the results were analysed with CytoSure Interpret software v3.4.2 (Agilent Technologies, Inc.).

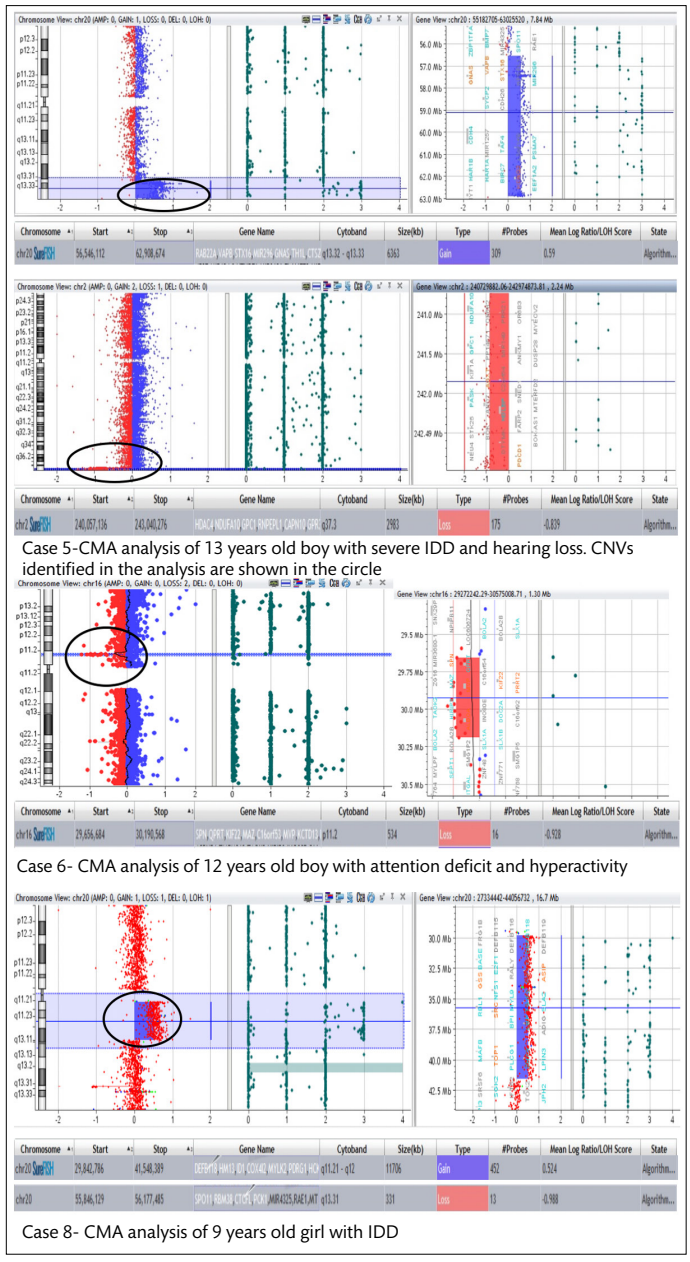
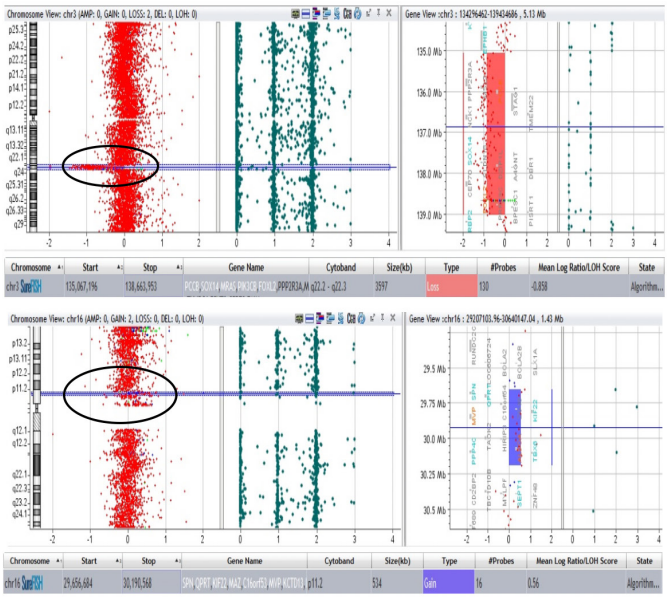
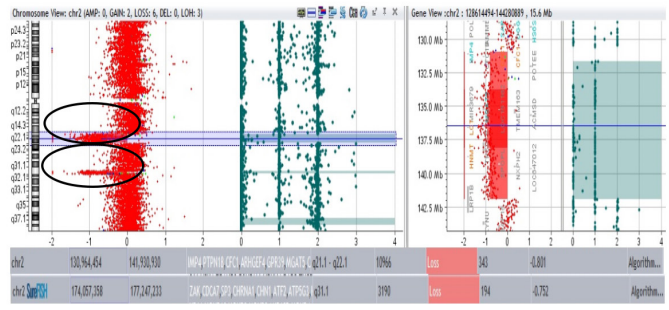


Figure 2. Case 5, 6, 8 CMA image

CNVs were categorized as pathogenic, benign, and variants of uncertain significance (VUS) by considering gene content, size, inheritance pattern, and previously reported microdeletion or microduplication regions. dbVar (https://www.ncbi.nlm.nih.gov/dbvar) and the Database of Chromosomal Imbalance and Phenotype in Humans using Ensembl Resources (https://decipher.sanger.ac.uk/) were consulted to check clinically relevant structural variations and to determine whether the observed CNVs were of clinical significance. We considered CNVs to be causative if the variant was responsible for a known syndrome; encompassed a gene (s) of known function; or occurred de novo or, if inherited, the parent was variably affected or if the involved gene (s) had been reported to be associated with ID/DD/ (autism spectrum disorder) ASDs in public databases and the scientific literature. The term VUS was used if the variants affected a gene (s) of unknown clinical significance and if, at the same time, the size was substantially less than 100 kb (the average resolution of the probes used) and when the family studies were inconclusive or unavailable.

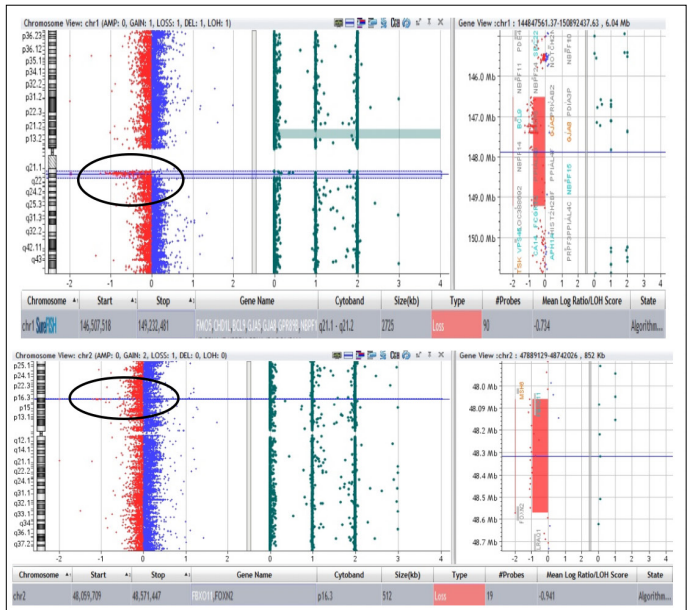


Case 12- CMA analysis of 1 years old girl with IDD, hypotonia, brachycephaly, bilateral epicanthus, ptosis and speech disorder. CNVs identified in the analysis are shown in the circle.

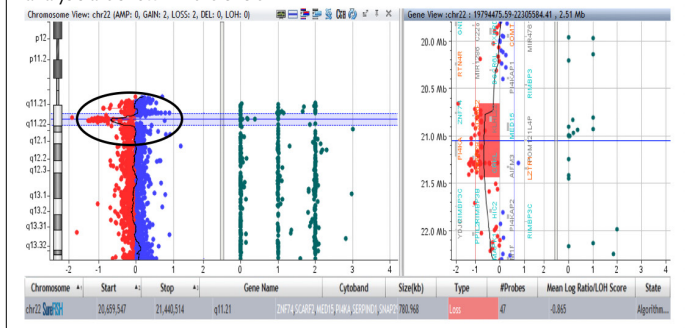


Case 15- CMA analysis of 3 years old boy with IDD and motor retardation. CNVs identified in the analysis are shown in the circle.

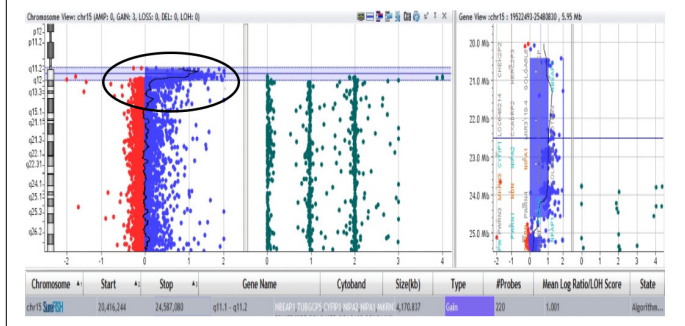
Figure 3. Case 12,15 CMA image



Case 18- CMA analysis of 7 years old boy with isolated IDD. CNVs identified in the analysis are shown in the circle



Case 21- CMA analysis of 10 years old boy with IDD. CNVs identified in the analysis are shown in the circle.



Case 28- CMA analysis of 8 years old girl with DD and motor retardation. CNVs identified in the analysis are shown in the circle

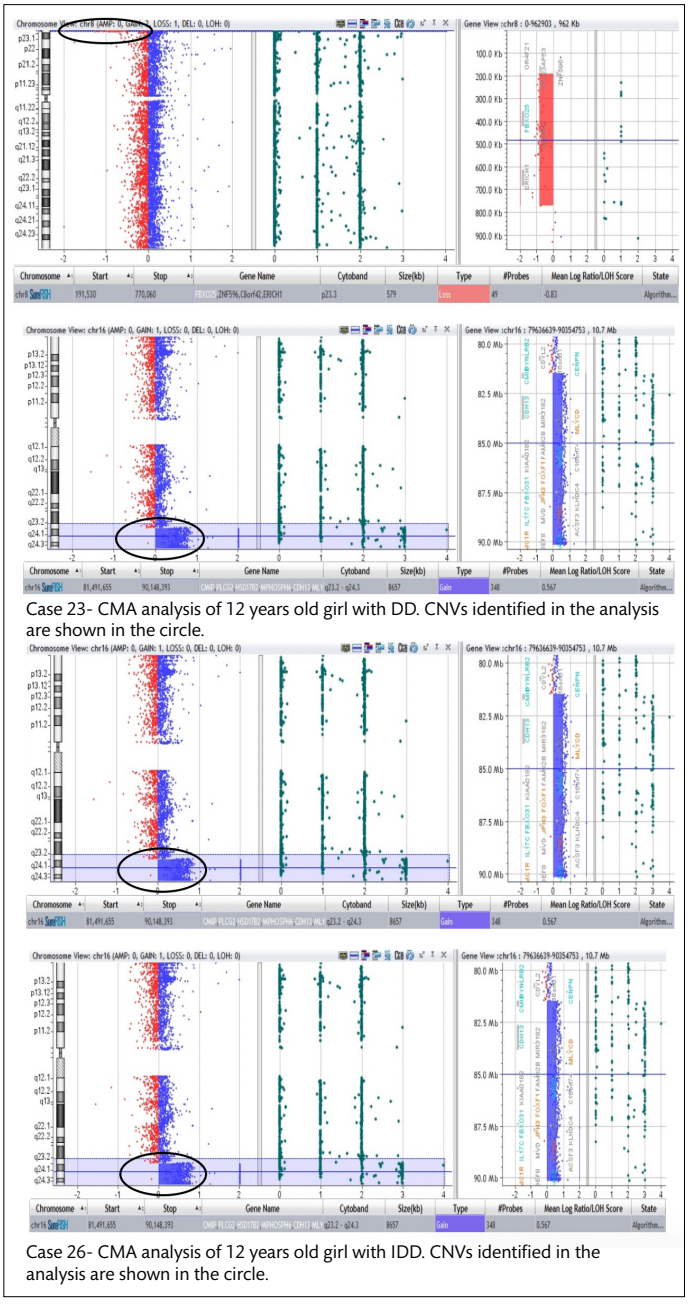
Figure 4. Case 18,21,28 CMA image.

RESULTS

CMA analysis was performed in 123 unexplained IDD/DD patients with normal karyotypes and fragile X screening, which were evaluated by conventional cytogenetics. Forty-four CNVs were detected in 39 (39/123=31.7%) patients. Twelve CNV VUS (9.75%) patients and 7 CNV benign (5.69%) patients were reported. Two pathogenic CNVs were determined together in six patients. Therefore, the CMA detection rate was found to be 31.7% (39/123).

Four out of 7 benign variants ranged in size from 72 kb to 195613 kb. The other 3 variants were deletion (loss) with sizes between 216 kb and 602 kb. Twelve detected VUS had a size between 103 kb and 861421 kb. The sizes of 20 pathogenic variants that were also detected were between 534 kb and 257494 kb. Among our patients, four patients (4/123, 3.25%) were diagnosed with Williams syndrome. One patient was diagnosed with 5p microdeletion syndrome, one patient with 1p36 microdeletion syndrome, one patient with Angelman syndrome and one patient with DiGeorge syndrome (4/123, 0.813).

The 195613 kb duplication in chromosome 1p12, which was classified as benign, was the largest CNV and encompassed the WDR3 and SPAG17 gene regions. In another patient, a 72 kb benign CNV duplication in chromosome 3p25.2, which spanned the MKRN2 and RAF1 gene regions, was found. Another benign CNV located on chromosome 22q21.1 was detected in two of our patients; this CNV was a 216 kb deletion and encompassed the SMPD4, MZT2B, TUBA3E, CCDC115, IMP4, and PTPN18 gene regions. This CNV was maternally inherited by patients. The 602 kb deletion that we detected in a patient in the chromosome 15q11.2 region included the GOLGA8DP, GOLGA6L1, TUBGCP5, CYFIP1, NIPA2, NIPA1, WHAMML1, GOLGA8IP, and HERC2P2 genes and was reported as benign. One of the benign duplications detected in the patients was located on chromosome 19 and spanned a 294 kb region corresponding to the 19q13.41 band. This region contained 9 genes (FPR3, ZNF577, ZNF649, ZNF613, ZNF350, ZNF615, ZNF614, ZNF432, ZNF841). Finally, another variation considered benign was a 538 kb duplication of the Xq21.31-q21.32 regions on the X chromosome and contained only



Case 23- CMA analysis of 12 years old girl with DD. CNVs identified in the analysis are shown in the circle.

Case 26- CMA analysis of 12 years old girl with IDD. CNVs identified in the analysis are shown in the circle.

Figure 5. Case 23,26 CMA image.

one gene (PCDH11X). It was found that the benign variations that were detected and the genes covered by these variations had no effect on the phenotype of the patients. We classified 12 CNVs (8 CNV duplications, 4 CNV deletions) in the patients included in the study as a variance of uncertain significance (VUS). There were no reported data in the literature or databases regarding variations considered VUS. The 1244.11 kb duplication on chromosome 1p36.11 and the 86441 kb duplication on chromosome 2q14.2 did not contain genes. The AGAP1, SPOCK3, ANXA10, DOX60, DMD, COX18, ANKRD17, RAB12, GACAT2, and MTCL1 genes were mapped in other regions with duplication and evaluated as VUS, and their effects on patient phenotypes were not reported.

Among the patients with pathogenic variations, 2 different regions were detected in 6 patients. Although the chromosomal size of the pathogenic variation regions was different, the gene content of these variation regions was high. One patient from the study group was pre-diagnosed with IDD and a 5p microdeletion. As a result of the analysis, a 18975 kb deletion covering the band of 5p15.33-p14.3 was confirmed. Another patient had a preliminary diagnosis of Angelman syndrome and IDD. The analysis of the patient revealed a loss of heterozygosity (LOH) in the 15q11.2-q13.1 band. Four patients that were included in the study had a preliminary diagnosis of Williams syndrome. A 1.[4] Mb deletion containing 27 genes was detected in the 7q11.23 band supporting the pre-diagnosis. A deletion in band 1p36.33p36.32 was detected as a result of the CMA study in a patient with an indication of 1p36 microdeletion syndrome. This deletion region was 2176 kb in size and contained 73 genes. Among 13 patients with pathogenic variations, minor congenital anomalies (hearing loss, attention deficit, hyperactivity, operated inguinal hernia, undescended testis, brachycephaly, epicanthus, macroglossia) were observed in addition to the preliminary diagnosis of IDD and DD. Chromosomal changes in two different regions were generally detected in patients with additional findings of IDD and DD with pathogenic variations.

DISCUSSION

We conducted CMA analysis in 123 unexplained DD and IDD patients with normal karyotypes as assessed by conventional cytogenetics. According to the literature, chromosomal microarray detects abnormalities in approximately 15% of patients with developmental delay or intellectual disability. CNV size is known to be important in the interpretation of pathogenicity (13–16). The improved diagnostic yield (approximately 31.7%) noted in our experience is likely related to the fact that we undertook array comparative genomic hybridization studies only after an in-depth clinical evaluation by a skilled clinical geneticist who was able to recognize a well-known genetic condition in many cases. Thus, chromosomal microarray analysis was performed in carefully selected patients. (15–18).

In our 13-year-old patient, CMA analysis revealed a 6363 kb duplication between the 20q13.2-q13.33 band regions in addition to a 2983 kb deletion in the 2q37.3 region. Aberration in zone 2q37.3 has been reported as "Chromosome 2q37 deletion syndrome, MIM: 600430" and "Albright Hereditary Osteodystrophy-Like Syndrome, Brachydactyly Mental Retardation Syndrome". Chromosome 2q37 Microdeletion Syndrome has been reported with mild to moderate growth deficiency brachymetaphalangy in 3–5 fingers (>50%), short stature, obesity, hypotonia, characteristic facial appearance (frontal bossing, wide-round face, midface hypoplasia, deeply located eyes, high arched eyebrow structure, hypoplastic alae nasi, prominent columella, thin upper lip, minor ear anomalies), sensorineural hearing loss, autism or autism spectrum disorder (30%), joint hypermobility/dislocation, scoliosis, seizures (20–35%), congenital heart disease, central nervous system abnormalities (hydrocephalus, dilated ventricle), umbilical/inguinal hernia, tracheomalacia, situs abnormalities, gastrointestinal abnormalities and renal malformations. Among these findings, severe intellectual disability along with hearing loss was also present in our patient.

The duplication in chromosome 20q13.32-q13 (an increase in the number of copies) that we detected in the patient has been associated with normal growth during the intrauterine period, craniofacial abnormalities, skeletal defects, cardiac malformations and neurodevelopmental retardation, by Blanc P, et al. and Igleasias A, et al. (19, 20) Our patient had presented with neurodevelopmental disorder

A 12-year-old patient with a 534 kb deletion in chromosome 16p11.2 was reported to have attention deficit and hyperactivity disorder in the clinical pre-diagnosis. A microdeletion on chromosome 16p11.2 that was detected in the patient as a result of CMA analysis was reported as

Chromosome 16p11.2 deletion syndrome, MIM: 611913 in the literature. Chromosome 16p11.2 deletion syndrome is associated with growth retardation (cognitive and motor dysfunction, reduced language ability), mental disability and/or autism spectrum disorder, and obesity. Seizures, macrocephaly, structural brain abnormalities (Chiari malformation, cerebellar ectopia) and vertebral abnormalities have been reported in 20% of affected individuals (21–25). Intellectual developmental disorder was detected in our patient, which is line with the literature. Hyperactivity disorder was reported for the first time in our case.

We detected a 20q11.21-q12 duplication and a 20q13.31 deletion in our 9-year-old IDD patient who had 2 different aberrations of the 20th chromosome. The duplication detected in the patient contained 11706 kb and 150 gene regions. Chromosome 20q11.21-q12 duplication (gain) in different studies was associated with trigonocephaly, growth retardation, facial dysmorphic findings (low ears, epicanthus, hypertelorism, flat philtrum), short hands, cardiovascular anomalies (muscular VSD, postductal stenosis in the aorta) and oncogenic transformation (26–28). In the same patient, the deletion was 331 kb in size on the 20q13.31 chromosomal region and contained 7 genes. Although there is no literature directly related to the chromosome 20q13.31 deletion that was detected in the patient, Butler MG. et al. reported that 20q13.2-q13.33 deletion syndrome was associated with intellectual retardation, a lack of speech, hypotonia, pre-and postnatal growth retardation, and abnormal facial structure (29). Among the findings reported in the literature, it was intellectual developmental disorder which we demonstrated in our patient

In our 1-year-old patient with IDD, hypotonia, brachycephaly, bilateral epicanthus, ptosis and speech disorder, we found a 3567 kb deletion in the chromosome 3q22.2-q22.3 region and a 534 kb duplication in the chromosome 16p11.2 region. The deletion site encompassed 20 genes, including the FOXL2 gene, and the duplication site encompassed 25 genes. At this time, chromosome 3q22.2-q22.3 deletion with chromosome 16p11.2 duplication has not been reported in the literature. Interstitial 3q deletions have been associated with a broad phenotype, including dysmorphic facial features, ear and finger abnormalities, hypogonadism, intracranial malformations, cardiac and renal abnormalities, intellectual disability and developmental delay. Blepharophimosis, ptosis and epicanthus inversus syndrome were found in most of the reported cases. A strong association has been established in the literature between blepharophimosis-ptosis-epicanthus inversus syndrome and deletions in the 3q22 region that cause the haploinsufficiency of the FOXL2 gene (OMIM: 605597). It has been reported in the literature that the general features of 16p11.2 microduplication are low weight, microcephaly, developmental delay in speech and language, and an increased risk of behavioural problems in affected individuals. It has been reported that approximately one-third of children with 16p11.2 microduplication experienced delays in the development of physical skills such as sitting and walking. Speech or language problems have been reported in approximately 80% of people with 16p11.2 microduplication. Both expressive language skills (vocabulary and the production of speech) and comprehensive language skills can be affected. One of the most common behavioural problems related to this chromosomal variation is attention deficit hyperactivity disorder. Autism spectrum disorders affecting communication and social skills are diagnosed in one in five individuals with microduplication 16p11.2 (30–35).

In another 3-year-old patient who was referred with the diagnosis of IDD and motor retardation, a deletion was detected in two different regions of chromosome 2. We detected a 10966 kb deletion on chromosome 2q21.1-q22.1 and a 3190 kb deletion in chromosome 2q31.1. The deletion sites encompassed 53 and 29 genes, respectively. Shanske AL, et al., Porfirio MC, et al., and Gimelli S, et al. have associated the deletions spanning the 2q21.1-q22.1 chromosomal regions with central nervous system abnormalities, eating problems, weight gain, other congenital malformations and Hirschsprung's disease (36–38). Losses involved in the chromosome 2q31.1 region are described in the studies of Puvabanditsin S, et al., and Dimitrov B, et al. as 2q31.1 microdeletion syndrome, and these losses were associated with short stature, extremity abnormalities [syndactyly, brachydactyly, mild finger abnormalities such as camptodaktyly, severe malformations such as cleft foot and/or hand, monodactyly], microcephaly, moderate-severe developmental delay, mild facial dysmorphology, scoliosis and growth retardation, ocular and genital abnormalities, and cardiac defects (39–41). Microcephaly, developmental delay, motor retardation were the findings detected in our patient.

We detected an aberration on the first and second chromosomes in a 7-year-old patient with isolated IDD. In addition to a 2725 kb deletion on chromosome 1q21.1-q21.2, a 512 kb deletion on 2p16.3 was found. The deletion region on chromosome 1 encompassed 24 genes, and the deletion region on chromosome 2 contained 2 genes. In the OMIM database, deletions involving chromosome 1q21.1 have been reported as "#612474 Chromosome 1q21.[1] Deletion Syndrome" (42). Chromosome 1q21.1 microdeletion increases the risk of delayed development, mental disability, physical abnormalities, and neurological and psychiatric problems. Approximately 75% of all children with microdeletions in 1q21.1 show developmental delays, particularly those affecting the development of motor skills such as sitting, standing and walking. The intellectual disability and learning problems associated with this genetic variation are usually mild. Pronounced facial features in 1q21.1 microdeletion syndrome are a thin and pronounced forehead; a large, round nose tip; a long space between the nose and the upper lip; and a high, arched palate of the mouth. Other common signs and symptoms of 1q21.1 microdeletions include eye problems such as cataracts, short stature, and microcephaly. Rarely, microdeletion in 1q21.1 is associated with heart defects, abnormalities in the genital or urinary system, bone abnormalities (especially in the hands and feet), and hearing loss. Psychiatric or behavioural problems occur in a small percentage of people with this genetic change. These problems include developmental conditions such as autistic spectrum disorders, attention deficit hyperactivity disorder (ADHD), and sleep disorders that affect communication and social interaction. Studies have suggested that a deletion of genetic material from region 1q21.1 may be a risk factor for schizophrenia (43). ADHD was present in our patient. In addition, mild IDD was detected in our patient.

In another 10-year-old IDD patient, we detected a 781 kb deletion on chromosome 22q11.2, including 19 genes. In the OMIM database, deletions involving the 22q11.21 region have been reported as "#188400 Chromosome 22q11.2 Deletion Syndrome, DiGeorge Syndrome" and "#192430 Velocardiofacial Syndrome; VCFS" (44, 45). Chromosome 22q11.21 deletion syndrome is characterized by congenital heart disease (74% of individuals), especially conotruncal malformations (Fallot tetralogy, aortic arch cut, ventricular septal defect and trunk artery arteriosus); palatal abnormalities (69%), especially velopharyngeal insufficiency, submucosal cleft palate, bifid uvula and cleft palate; characteristic facial features; learning difficulties (70%-90%); immunodeficiency (77%); hypocalcaemia (50%); significant feeding and swallowing problems; constipation with or without structural gastrointestinal abnormalities (intestinal malrotation, imperforate anus and Hirschsprung's disease); renal anomalies (31%); hearing loss (conductive and sensorineural); laryngeal tracheoesophageal abnormalities; growth hormone deficiency; autoimmune disorders;

seizures (idiopathic or hypocalcaemia) associated); CNS anomalies, including spinal cord and skeletal abnormalities (scoliosis with or without vertebral anomalies, polydactyly and craniosynostosis); ophthalmologic abnormalities (strabismus, posterior embryotoxon, curved retinal vessels, sclerocornea and anophthalmos); enamel hypoplasia; and malignancies (rare). Developmental delay (especially delay in the emergence of language), intellectual disability and learning disparities (nonverbal learning disability in which oral IQ is significantly higher than performance IQ) are common. Autism or autistic spectrum disorder can be seen in approximately 20% of children, and psychiatric disorders (especially schizophrenia) are found in 25% of adults. However, attention deficit disorder, anxiety and difficulties with social interactions are also common (46). Intellectual disability, learning disability, anxiety and difficulties with social interactions were observed in our patient.

In another 12-year-old patient with DD, we detected a deletion in chromosome 8p23.3 and a duplication in chromosome 16q23.2-q24.3. The chromosome 8p23.3 deletion was 567 kb in size and encompassed 4 genes, while the chromosome 16q23.2-q24.3 duplication was 8657 kb in size and encompassed 99 genes. Nucaro A, et al., and Chien WH, et al. have reported in their studies that deletions involving the chromosome 8p23.2-pter region were associated with autism spectrum disorders (ASD) (47, 48). Richer J, et al. and Xiang B, et al. have reported that duplications in the chromosome 16q23.2-q24.3 region were associated with mental retardation, dysplastic kidneys, growth-developmental retardation, recurrent upper respiratory tract infections, marked forehead structure, thin lower lip, and hypermetropia (49, 50). Among these findings reported in the literature, intellectual disability was observed in our patient.

In another 10-year-old patient who was diagnosed with IDD, we detected CNVs with different sizes on 2 different chromosomes. In addition to the duplication (gain) of 34 genes in the chromosome 15q26.1-q26.3 region, we identified a loss of 88 genes in the region of chromosome 22q13.31-q13.33 containing 37 genes of 4361 kb. In the literature, an increase in the number of copies (gain, duplication), including the chromosome 15q26.1-q26.3 region and especially the IGF1R gene (macrosomia at birth), has been reported to be associated with macrocephaly; mild developmental delay; learning disability; mental and psychomotor delay; impaired speech capacity; a long, thin face structure with jaw and nose abnormalities; and renal anomalies (renal agenesis, horseshoe kidney, hydronephrosis) (51–54). Phelan K, et al. have indicated in their studies that a loss of copy number (loss, deletion) in chromosome 22, especially covering the SHANK3 gene, has been associated with Phelan-McDermid syndrome (55). Phelan-McDermid syndrome is a neurogenetic disorder characterized by global growth retardation, mental disability (moderate-severe), the delay or absence of speech, and neonatal hypotonia. In addition, approximately 50% of patients manifest autism or autism-like behaviour (51, 56). Among these findings reported, learning disability, mental and psychomotor delay have been observed.

In an 8-year-old patient with intrauterine growth retardation, DD and motor retardation, CMA analysis revealed a duplication of 4170 kb in chromosome 15q11.1-q11.2 that spanned 47 genes. Brenda M, et al. indicated that duplications involving chromosome 15q11.1-q11.2 were related to severe hypotonia and motor delays during infancy, intellectual disability and/or delays in speech and language development, ASD and infantile spasms, including epilepsy (57). The duplication (gain) covering the chromosome 15q11.1-q11.2 region detected in our patient was reported as "#608636 Chromosome 15q11-q13 Duplication Syndrome" in the OMIM database (58).

According to the literature, CMA detects abnormalities in approximately 15% of patients with developmental delay or intellectual disability (17, 18). The improved diagnostic yield (approximately 30%) noted in our experience is likely related to the fact that we undertook CMA only after an in-depth clinical evaluation by a skilled clinical geneticist, who was able to recognize a well-known genetic condition in many cases. Thus, CMA was performed in carefully selected cases. This is of paramount relevance, considering that CMA is a sophisticated, demanding, and expensive laboratory technique. Despite these, CMA has established its place among molecular cytogenetic technologies as a valuable addition to the identification and molecular characterization of known and novel microdeletions and other chromosome abnormalities.

CMA has provided a comprehensive and high-resolution test that can be directed at particular genomic locations or used to screen populations to discover novel regions involved in alterations.

CONCLUSION

Our findings support the necessity of implementing CMA as a routine diagnostic test in the Turkish population. Moreover, first-tier use of CMA for the clinical genetic evaluation of unexplained DD and IDD in the Turkish population could be beneficial for patients, considering the cost-effectiveness of CMA compared with that of current conventional cytogenetics and MLPA or FISH analysis.

The clinical application of CMA on 123 pediatric cases demonstrated an increased analytical resolution and an improved abnormal detection rate. Although CMA demonstrated a higher analytical resolution, chromosome analysis is still the method of choice in detecting balanced rearrangements and marker chromosomes, and FISH is still a powerful tool in confirming array findings and detecting mosaic patterns. We recommend the use of a CMA test and a complement chromosome study as the first line of genetic evaluation. The complement chromosome study will be performed by analysing 5 metaphases in at least 550-band level and counting at least 25 metaphases to rule out balanced structural rearrangements and to screen for marker chromosomes or mosaic patterns. Cell pellets will be preserved for FISH mapping of detected genomic aberrations.

Follow up parental CMA analysis could be considered to resolve familial variants and pathogenic aberrations. To interpret the array findings, many online resources such as Database of Genomic Variants (http://projects. tcag. ca/variation/), Human Genome Browser (http://genome.ucsc.edu/), Online Mendelian Inheritance in Man (OMIM) and Database of Chromosomal Imbalance and Phenotype in Humans using Ensembl Resources (http://www.sanger.ac.uk/postgenomics/decipher/) have provided useful information for determining disease-causing imbalances or copy number variants. Recently published ACMG standards and guidelines on microarray analysis for constitutional cytogenetic abnormalities will help to ensure high quality genomic screening to pediatric patients with IDD, DD, and multiple congenital disorders (59).

The implementation of the so called "next generation sequencing" technologies (that allow the analysis of whole-genomes, transcriptomes and interactomes) could lead to detect single base mutations and structural variations, further broadening the possibility of diagnosis IDD/DD. Understanding the pathological pathways underlying unexplained forms of IDD/DD represent a future challenge to increase both prevention and possible therapies.

Ethics Committee Approval: Ethical committee approval for the study was obtained from Trakya University Medical Faculty Ethical Board.

Informed Consent: Informed consent was obtained from the parents or legal carers of all patients participating in the study. All procedures were performed in accordance with the Declaration of Helsinki.

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