

Genomewide Array Comparative Genomic Hybridization in 55 Japanese Normokaryotypic Patients with Non-Syndromic Intellectual Disability

Miki Asahina^{1*}, Yusaku Endoh², Tomoko Matsubayashi¹, Koichi Hirano², Tokiko Fukuda¹ and Tsutomu Ogata¹

¹Department of Pediatrics, Hamamatsu University School of Medicine, Hamamatsu, Japan

²Department of Pediatrics, Hamamatsu City Welfare and Medical Center for Development, Hamamatsu, Japan

Abstract

Background: Genomewide array comparative genomic hybridization (aCGH) has widely been utilized as the diagnostic tool in patients with non-syndromic intellectual disability (ID). Indeed, aCGH has identified pathogenic copy number variants (pCNVs), as well as variants of uncertain clinical significance (VsUS) and benign CNVs (bCNVs), in such patients.

Aims: To examine the frequencies of various CNVs and clinical findings in patients with non-syndromic ID.

Patients and methods: We studied 55 Japanese normokaryotypic patients (35 males, 20 females) with apparently non-syndromic ID. Genomewide aCGH was performed using leukocyte genomic DNA samples. Clinical findings were compared among patients with pCNVs (group 1), those with VsUS (group 2), and those with bCNVs or no CNVs (group 3).

Results: Nine patients had pCNVs: one had 5p deletion syndrome, two had 22q11.2 deletion syndrome, one had 17q23.1q23.2 microdeletion syndrome, three had CNVs involving known pathogenic genes, and the remaining two had CNVs overlapping with previously described CNVs in patients with ID (one with duplication at 1q36 and the other with deletion at 12q42). Furthermore, 11 patients had VsUS, and nine patients had bCNVs. Clinical findings were grossly comparable among groups 1-3.

Conclusions: The results provide further support for the usefulness of aCGH in the identification of underlying genetic factor(s) for ID, although there was no clinical finding indicative of the presence of pCNVs or VsUS. Furthermore, our data are expected to serve to identify pathogenic genes on chromosomes 1q36 and 12q42, as well as those on several VsUS.

Keywords: Array comparative genomic hybridization; Intellectual disability; Copy number variants; Pathogenic gene; Clinical finding

Introduction

Intellectual disability (ID) is a highly heterogeneous condition occurring in 1–3% of the general population [1]. ID is divided into syndromic and non-syndromic forms, with the former accounting for roughly one-third of affected patients [2]. The syndromic form is associated with a constellation of clinical features characteristic of known syndromes, and is usually caused by specific genetic factors such as mutations of causative genes, copy-number variants (CNVs) involving relevant genes, and aneuploidies [3]. By contrast, non-syndromic form, though it may be accompanied by non-specific multiple congenital anomalies (MCA), is free from diagnostic clinical manifestations, and is usually subject to multiple (epi) genetic and environmental factors such as mutations of genes for non-specific ID, various usually non-recurrent CNVs, central nervous infections, and environmental chemicals [4].

Recently, genomewide array comparative genomic hybridization (aCGH) has widely been utilized as the diagnostic tool in patients with non-syndromic ID. Indeed, aCGH has identified pathogenic copy number variants (pCNVs) in 5–35% of such patients (average 12.2%) [5]. In addition, aCGH has also detected multiple variants of uncertain clinical significance (VsUS) that could be relevant to ID, and benign CNVs (bCNVs) that are irrelevant to ID [6].

Here, we examined the frequencies of various CNVs and clinical finding in patients with apparently non-syndromic ID.

Patients and Methods

Patients

This study consisted of 55 Japanese patients (35 males, 20 females) with apparently non-syndromic ID with or without non-specific MCA. The ages at examination ranged from 0.8 to 42 years (median, 4.0 years). All patients had normal karyotype in the 50 lymphocytes examined by the conventional 400–550 level G-banding analysis. The ID was assessed as extremely severe (developmental quotient (DQ)/intelligence quotient (IQ), < 20) in 21 patients, severe (DQ/IQ, 21–34) in 11 patients, moderate (DQ/IQ, 35–49) in six patients, and mild (DQ/IQ, 50–69) in 17 patients, by the DSM-IV method [7].

Ethical approval and samples

This study was approved by the Institutional Review Board Committees of Hamamatsu University School of Medicine, and was performed after obtaining written informed consent for the molecular

***Corresponding author:** Miki Asahina, Department of Pediatrics, Hamamatsu University School of Medicine, Hamamatsu 431-3192, Japan, Tel: +81-53-435-2310; Fax: +81-53-435-2310; E-mail: miki.a@hama-med.ac.jp

Received October 05, 2016; **Accepted** October 28, 2016; **Published** November 07, 2016

Citation: Asahina M, Endoh Y, Matsubayashi T, Hirano K, Fukuda T, et al. (2016) Genomewide Array Comparative Genomic Hybridization in 55 Japanese Normokaryotypic Patients with Non-Syndromic Intellectual Disability. *Neonat Pediatr Med S1*: 008. doi: [10.4172/2572-4983.S1-008](https://doi.org/10.4172/2572-4983.S1-008)

Copyright: © 2016 Asahina M, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

analysis and the publication of genetic and clinical data after removing information for personal identification.

Genomewide aCGH analysis: Genomewide aCGH was performed with a catalog human array (4×180K format, ID G4449A) (Agilent Technologies) using leukocyte genomic DNA samples of all the patients, the parents who agreed to genetic analysis, and sex-matched control subjects. The procedure was as described in the manufacturer's instructions. For autosomes and female X chromosomes, log₂ signal ratios of around -1.0 and around +0.5 were regarded as indicative of heterozygous deletions and duplications, respectively. For male sex chromosomes that appear in a heterogametic condition, log₂ signal ratios of -∞ and around +1.0 were interpreted as hemizygous deletions and duplications, respectively. When ≥three consecutive probes showed abnormal log₂ ratios, the corresponding region was regarded as CNVs. Minimum and maximum sizes of CNVs were obtained as the regions between two distal ends of signals indicative of deletions or duplications and those between two proximal ends of signals indicative of normal copy numbers. The genomic position was based on human GRCh37/hg19 (<http://genome.ucsc.edu/>).

CNVs were regarded as pCNVs, when they were identical to those of established ID-positive syndromes with causative or candidate genes, they included known pathogenic genes in which intragenic mutations or CNVs involving the genes alone have been identified in patients with ID, or they shared an overlapping region with previously described plural CNVs in patients with ID [1-3]. By contrast, CNVs were interpreted as bCNVs, when (1) they were inherited from either of the healthy parents, or (2) they have been registered as normal variants in the public databases such as Database of Genomic Variants (<http://dgv.tcag.ca/dgv/app/home>) and ClinVar (<http://www.ncbi.nlm.nih.gov/clinvar/>) [1,2]. Other CNVs were regarded as VsUS, most of which contained candidate genes for ID or were formed as de novo events.

Clinical assessment: Multiple clinical features were examined in all the patients by two clinicians (M.A. and Y.E.), except for ophthalmologic, cardiac, and renal features which were evaluated by professional doctors in each field. We summarized clinical findings in patients with pCNVs (group 1), those with VsUS (group 2), and those with bCNVs or no CNVs (group 3), and compared them among different groups. Statistical significance of the median was examined by the Mann-Whitney's *U*-test, and that of the frequency by the Fisher's exact test. *P* < 0.05 was considered significant.

Results

Genomewide aCGH analysis

Representative aCGH findings are shown in Figure 1. The data of groups 1 and 2 are summarized in Table 1, and the pathogenic and candidate genes on the identified pCNVs and VsUS are shown in Supplementary Table 1. CNVs were identified in 29 of the 55 patients. Of the 29 patients, nine patients (cases 1-9) were assessed to have pCNVs, because: (1) case 2, and cases 7 and 8, had deletions for 5p deletion syndrome (Cri Du Chat syndrome) and 22q11.2 deletion syndrome (Di George syndrome) with known pathogenic genes, respectively; (2) case 6 had a deletion typical of 17q23.1q23.2 microdeletion syndrome with candidate genes; (3) cases 3, 4, and 9 had CNVs involving known pathogenic genes, as well as candidate genes; and (4) cases 1 and 5 had CNVs partially overlapping with previously described CNVs in patients with ID (Figures 2 and 3) [8-10]. Furthermore, of case 1-9, cases 1-3, 5, 6, and 9 had de novo CNVs. By contrast, nine of the 22

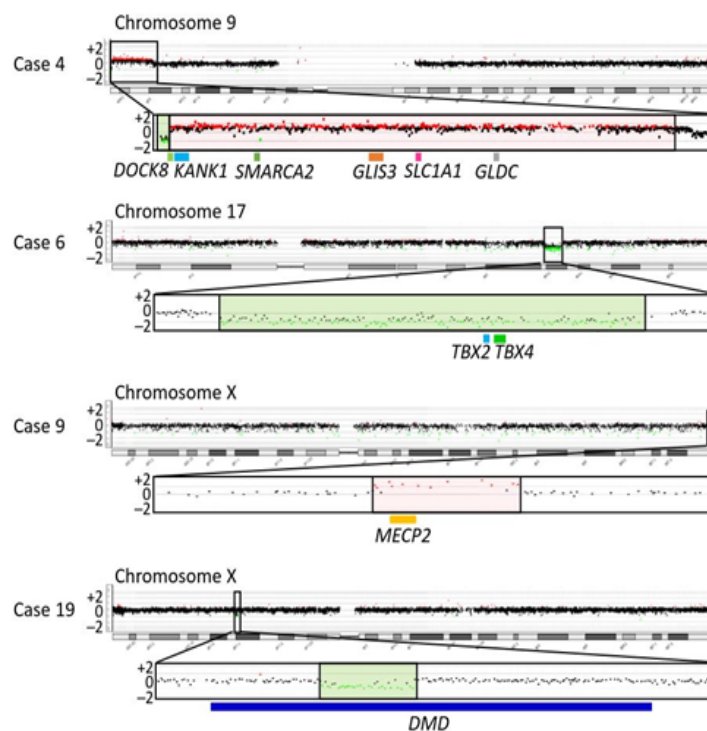


Figure 1: Representative results of aCGH analysis. The black, the red, and the green dots denote signals indicative of the normal, the increased (log₂ signal ratio >+0.4), and the decreased (log₂ signal ratio <-0.8) copy numbers, respectively. The rectangles highlighted with light green and light red denote the deleted and the duplicated regions, respectively.

Case	Age (y)	Sex	Chromosomal location	CNV	Inheritance	Start (max) · Start (min)	End (max) · End (min)	Size (max) · Size (min)	Protein coding genes	Pathogenic gene(s) for ID	Candidate gene(s) for ID	Clinical features	ID (DQ/IQ)
<Patients with pathologic copy number variations (pCNVs)>													
1	18	F	1p36.21-p36.12	Duplication	<i>De novo</i>	13,178,528 13,791,307	22,364,327 22,352,713	9,185,799 8,561,406	65	–	<i>ARHGEF10L</i> , <i>EMC1</i>	Epilepsy, Pulmonary artery stenosis, Syndactyly, Congenital dislocation of the hip joint, Hyperphosphatasia [†] , Strabismus, Amenorrhea	Most severe
2	5	F	5p15.33-p15.2 [†]	Deletion	<i>De novo</i>	1 26,142	13,897,399 13,882,896	13,897,398 13,856,755	40	<i>CTNND2</i> <i>TERT</i>	–	Sleep disorder, Bushy eyebrow, Astigmatism	Moderate (40)
3	4	F	5q14.3	Duplication	<i>De novo</i>	87,556,055 87,574,384	88,110,678 88,100,645	554,623 526,261	3	<i>MEF2C</i>	–	Motor delay, Microcephaly, Choreoathetosis, Hyperopia, Sleep disorder, Eating disorder	Most severe
4	2	M	9p24.2	Deletion	Unknown	1 20,4193	373,868 356,138	373,867 151,945	4	<i>DOCK8</i>	–	Short stature, Macrocephaly, Deafness,	Mild (61)
			9p23-p24.2	Duplication	Unknown	356,138 373,868	9,781,684 9,766,294	9,425,546 9,392,426	31	–	<i>KANK1</i> , <i>SLC1A1</i> <i>SMARCA2</i>	Hypertelorism, Epicanthus, Low-set ears, Sleep disorder, Eating disorder	
			Xp 22.2	Duplication	Unknown	16,997,317 16,997,258	17,733,918 17,725,454	736,601 728,196	2	–	<i>NHS</i>		
			Xp 21.3	Duplication	Unknown	28,817,193 28,829,765	28,919,846 28,902,121	102,653 72,356	1	<i>IL1RAPL1</i> [#] (Intragenic duplication)	–		
5	6	F	12q24.31-q24.32	Deletion	<i>De novo</i>	121,496,723 121,530,401	127,569,632 127,537,641	6,072,909 6,007,240	49	–	<i>KDM2B</i>	Motor delay, Short stature, Epilepsy, Tetralogy of Fallot	Most severe
6	2	M	17q23.1-q23.2 [‡]	Deletion	<i>De novo</i>	58,066,851 58,120,809	60,316,690 60,251,568	2,249,839 2,130,759	12	–	<i>TBX2</i> , <i>TBX4</i>	Motor delay, Short stature, Deafness, Camptodactyly, Congenital aural atresia, Cryptorchidism, Sleep disorder	Mild (60)
7	4	M	22q11.21 [§]	Deletion	Unknown	18,651,673 18,894,835	20,719,112 20,311,763	2,067,439 1,416,928	28	<i>TBX1</i>	–	Motor delay, Epicanthus, Low-set ears, Sleep disorder	Mild (56)
8	6	M	22q11.21 [§]	Deletion	Unknown	18,651,673 18,894,835	20,719,112 20,311,763	2,067,439 1,416,928	28	<i>TBX1</i>	–	Motor delay, Hypertelorism, Epicanthus, Low-set ears	Mild (56)
9	4	M	Xq28	Duplication	<i>De novo</i>	153,190,720 153,197,498	153,630,137 153,609,163	439,417 411,665	13	<i>MECP2</i>	–	Motor delay, Autism, Sleep disorder	Severe (27)
<Patients with variants of uncertain clinical significance (VsUS)>													
10	7	M	3q29	Duplication	<i>De novo</i>	193,201,737 193,220,394	193,353,202 193,345,836	151,465 125,442	2	–	<i>ATP13A4</i> , <i>OPA1</i>	Motor delay, Short stature, Low-set ears, Macrocephaly	Severe (30)
			21q21.1	Duplication	<i>De novo</i>	17,196,538 17,205,639	17,385,656 17,360,796	189,118 155,157	1	–	–		
11	39	M	5q23.3-q31.1	Duplication	Unknown	130,594,844 130,616,397	130,740,811 130,728,922	145,967 112,525	1	–	<i>CDC42SE2</i>	Motor delay, Epilepsy, Hypertelorism, Epicanthus, Bushy eyebrow, Down-slanted palpebral Fissure, Wide mouth, Extrapyramidal symptoms	Most severe

12	42	M	5q31.1	Duplication	Unknown	130,405,344 130,476,155	130,808,141 130,797,583	402,797 321,428	1	–	<i>CDC42SE2</i>	Motor delay, Epilepsy, Hypertelorism, Epicanthus, Bushy eyebrow, Down-slanted palpebral Fissure, Wide mouth, Extrapyrmidal symptoms	Most severe
			9p23	Duplication	Unknown	9,679,865 9,762,182	9,972,017 9,957,044	292,152 194,862	1	–	<i>PTPRD</i>		
13	1.5	F	6q25.1	Duplication	Unknown	150,244,525 150,263,131	150,294,629 150,281,545	50,104 18,414	1	–	–	Motor delay, High arched palate, Short stature, Strabismus, Eating disorder, Sleep disorder	Mild (50)
			20q11.21	Deletion	Unknown	29,842,845 29,857,590	30,167,165 30,157,286	324,320 299,696	8	–	–		
14	1.8	M	8p23.3	Duplication	<i>De novo</i>	1,507,333 1,522,999	1,833,763 1,815,984	326,430 292,985	3	–	<i>CLN8, ARHGEF10</i>	Microcephaly, Highly arched eyebrow, Short fingers, Macrota, Eating disorder	Mild
			8p23.2	Duplication	<i>De novo</i>	2,701,555 2,725,834	3,006,205 2,993,737	304,650 267,903	1	–	<i>CSMD1</i>		
15	15	M	18q12.2	Duplication	Unknown	34,439,213 34,447,971	34,685,037 34,662,565	245,824 214,594	1	–	<i>KIAA1328</i>	Motor delay, Scoliosis, Dandy-walker variants, Hypercholesterolemia	Most severe
16	3	M	14q23.1	Deletion	Unknown	61,503,826 61,518,515	61,543,918 61,536,213	40,092 17,698	1	–	<i>SLC38A6</i>	Short stature, Autism, Ventricular septal defect, Blepharophimosis	Moderate (42)
17	2	F	20q11.21	Deletion	<i>De novo</i>	29,638,422 29,652,452	30,212,322 30,193,658	573,900 541,206	8	–	–	Epicanthus, Blepharophimosis, Protruding ear, Brachydactyly of the hand, Strabismus	Mild
18	1	M	21q22.11	Deletion	<i>De novo</i>	34,911,504 34,925,956	35,109,693 35,103,544	198,189 177,588	4	–	<i>ITSN1</i>	Motor delay, Epilepsy, Down-slanted palpebral Fissure, Right macrota, Plagiocephaly, Heterotopic gray matter, Eating disorder	Most severe
19	16	F	Xp21.1	Deletion	Unknown	31,575,837 31,608,431	31,980,038 31,979,979	404,201 371,548	1	–	<i>DMD</i> (Intragenic deletion)	Motor delay, Scoliosis	Moderate
20	3	M	Xq25	Deletion	Unknown	127,083,788 127,144,318	127,232,612 127,222,241	148,824 77,923	1	–	<i>ACTRT1</i>	Motor delay, Epilepsy, Hypertelorism, Blepharoptosis, Cryptotia, High-arched palate, Microcephaly, Overlapping finger, Short stature, Inguinal hernia, Cryptorchidism, Microphthalmia, Eating disorder	Most severe

According to GRCh37/hg19.

†The 5p deletion syndrome (Cri Du Chat syndrome).

‡The 17q23.1q23.2 microdeletion syndrome.

§The 22q11.2 deletion syndrome (Di George syndrome).

¶Hyperphosphatasia may be due to duplication of *ALPL*.

*This intragenic duplication is predicted to have a loss-of-function effect.

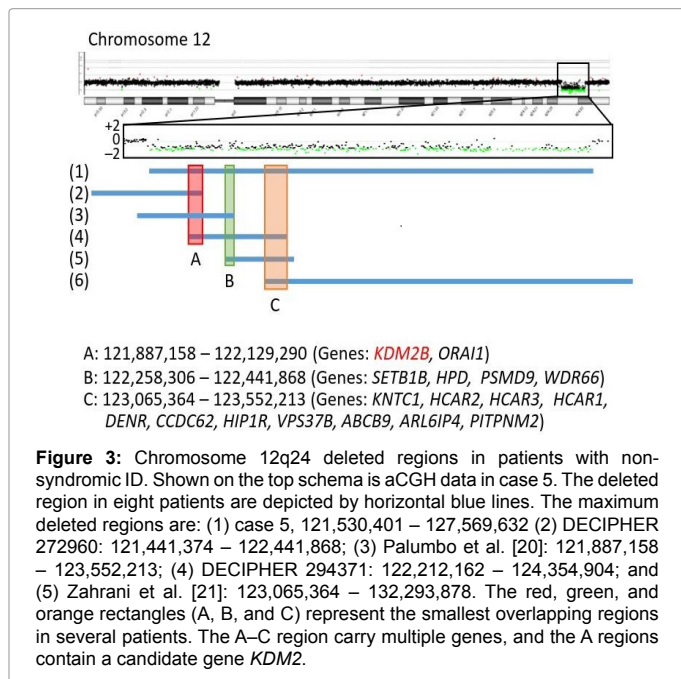
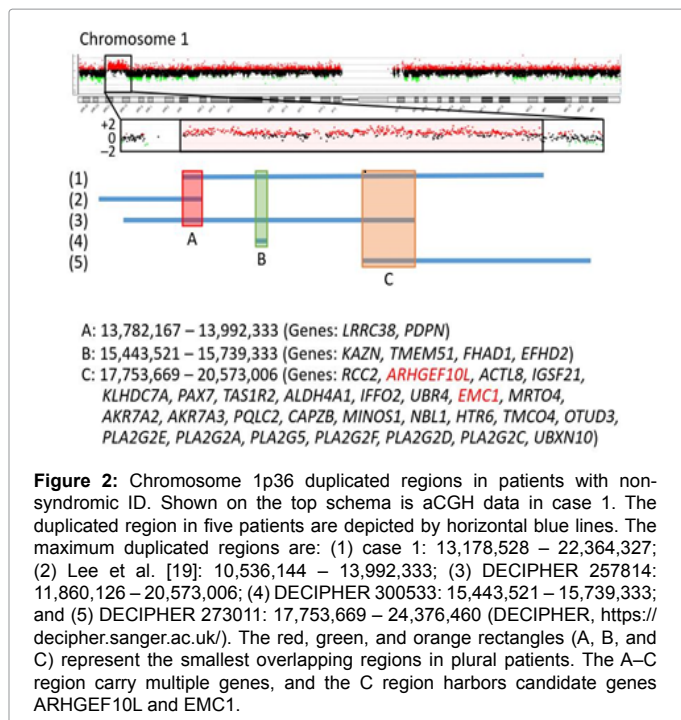
Abbreviations: y, years; F, female; M, male; CNV, copy number variant; ID, intellectual disability; DQ, developmental quotient; and IQ, intelligence quotient.

Table 1: Summary of array CGH data and clinical findings in 20 patients with pCNVs or VsUs.

patients were evaluated to have bCNVs, because they were present in either of the healthy parents. The remaining 11 patients (cases 10-20) were assessed to have VsUS with or without candidate genes. Of cases 10-20, cases 10, 14, 17, and 18 had de novo CNVs. Notably, cases 4, 10, and 12-14 had plural CNVs. In particular, the co-existing two VsUS in cases 10 and those in case 14 were found to be generated as de novo CNVs, although parental samples were not available in cases 4, 12, and 13.

Clinical assessment

Clinical features in patients of groups 1 and 2 are described in Table 1. Clinical features were highly variable with no pathognomonic features. Indeed, while case 2 had 5p deletion for Cri Du Chat syndrome, she showed no characteristic mewing cry. Similarly, while case 7 and 8 had 22q11.2 deletion for Di George syndrome, they were free from cardiovascular anomalies, abnormal calcium metabolism,



and immune deficiency. In addition, while case 6 had a deletion typical of 17q23.1q23.2 microdeletion syndrome, his overall clinical features remained rather non-specific.

Detailed clinical findings in groups 1-3 are summarized in Table 2. Male-dominant sex ratio was common to groups 1-3 as well as total patients, and the examined age and the degree of ID were similar among groups 1-3. Furthermore, the frequencies of clinical features were similar among groups 1-3, except for significantly high frequency of eating disorder in group 2 and that of sleep disorder in group 1.

Discussion

This study identified pCNVs in nine of 55 patients with apparently non-syndromic ID. The frequency (16%) is grossly similar to that reported previously [5]. The results provide further support for the usefulness of aCGH in the clarification of underlying genetic factor(s) for ID.

Of cases 1-9 with pCNVs, cases 2-4 and 7-9 had pathogenic genes for ID on the pCNVs (Supplementary Table 1). Indeed, intragenic mutations of *CTNND2*, *DOCK8*, *IL1RAPL1*, and *TBX1*, and loss of only *TERT*, are associated with ID, as are duplication of only *MECP2* or overexpression of *MEF2C* [11-17]. Thus, copy number alterations of these genes would have played a major role in the development of ID in the six cases, while the relevance of candidate genes on the pCNVs in case 4, and probably other non-specified genes as well, would remain tenable. In addition, since the CNV in case 2 and the CNVs in cases 7 and 8 are known to cause 5p deletion syndrome and 21q22.11 deletion syndrome, respectively, this confirms their pathogenicity [8,9]. Furthermore, since the CNVs in cases 2, 3, and 9 were formed as de novo events, this would also support their pathogenicity.

The remaining cases 1, 5, and 6 were also interpreted as having pCNVs. Actually, they had *de novo* CNVs, although there were no definite pathogenic genes on the identified CNVs. In particular, the ~2.2 Mb deletion in case 6 is identical to that reported as chromosome 17q23.1-q23.2 microdeletion syndrome with ID, and *TBX2* and *TBX4* have been regarded as candidate genes for ID because of their biological functions and expression pattern [18]. Since this 17q23.1-q23.2 microdeletion is known to be generated by non-allelic homologous recombination mediated by low-copy repeats [10], this would explain the recurrence of the same deletion in multiple unrelated subjects. For the ~9 Mb duplication on chromosome 1p36.21-p36.12 in case 1 and the ~6 Mb deletion on chromosome 12q24.31-q24.32 in case 5, previous studies have revealed similar duplications and deletions in patients with ID (Figures 2 and 3) [19-21]. Notably, although there is no segment shared by all the duplications and deletions, the duplicated region in case 1 and the deleted region in case 5 encompass three different smallest regions of overlaps (SROs-A–C) common to plural patients, and SRO-C on chromosome 1p36 and SRO-A on chromosome 12q24 harbor candidate genes for ID. Thus, it is likely that the duplication in case 1 and the deletion in case 5 are pathogenic, and that different genes are involved in the development of ID in patients with duplications involving 1p36 and in those with deletions affecting 12q24.

Cases 10-20 were evaluated to have VsUS rather than pCNVs. Indeed, since similar CNVs have not been reported in patients with ID, their pathogenicity remains uncertain. However, the VsUS in cases 10, 14, 17, and 18 were de novo CNVs, and those in cases 10–13, 14-16, and 18-20 harbor candidate genes (Supplementary Table 1). Thus, some of the VsUS would actually be pCNVs that harbor hitherto unrecognized pathogenic genes for ID. One may argue that the intragenic deletion of *DMD* identified in female case 19 is unlikely to explain her phenotype, although it could have phenotypic effects depending on the X-inactivation patterns in target tissues. However, since it is a disease-causing pCNV and could lead to ID in affected males [22], we categorized this intragenic deletion in female case 19 as a VUS rather than a pCNV or a bCNV.

Unexpectedly, plural pCNVs or VsUS were found in cases 4, 10, and 12–14. In particular those in cases 10 and 14 were generated as de novo abnormalities. Such co-existence of plural CNVs has been

	Total	pCNV	VOUS	bCNV and no CNV
		Group 1	Group 2	Group 3
	(n=55)	(n=9)	(n=11)	(n=35)
Male	35	5	8	22
Female	20	4	3	13
Examined age (median [range]) (years)	4.0 [0.8–18]	4.0 [1.9–18]	3.0 [1.0–42]	4.0 [0.8–30]
Paternal age at birth (median [range]) (years)	33 [21–42]	33 [24–40]	32 [21–42]	32.5 [23–42]
Maternal age at birth (median [range]) (years)	30 [21–44]	31 [25–37]	28 [21–33]	30 [21–44]
Assisted reproductive technology	5/55	0/9	2/11	3/35
Premature birth	8/55	0/9	3/11	5/35
Small for gestational age	8/55	0/9	1/11	7/35
Short stature	24/55	3/9	5/11	16/35
Intellectual disability	55/55	9/9	11/11	35/35
Most severe (DQ/IQ <20)	21/55	4/9	3/11	14/35
Severe (DQ/IQ, 21–34)	11/55	1/9	2/11	8/35
Moderate (DQ/IQ, 35–49)	6/55	1/9	1/11	4/35
Mild (DQ/IQ, 50–69)	17/55	3/9	5/11	9/35
Speech delay	51/55	8/9	10/11	33/35
No single word	31/55	4/9	5/11	22/35
Motor delay	42/55	6/9	8/11	28/35
Unable to walk without support	19/55	2/9	6/11	11/35
Hypotonia	29/55	5/9	5/11	19/35
Neurodevelopmental disorders	10/55	1/9	1/11	8/35
Autism spectrum disorder	10/55	1/9	1/11	8/35
Attention-deficit/hyperactivity disorder	1/55	0/9	0/11	1/35
Eating disorders	12/55	2/9	5/11*	5/35*
Sleep disorders	16/55	6/9†	1/11	9/35†
Magnetic resonance imaging abnormalities	19/55	1/9	6/11	12/35
Epilepsy	25/55	2/9	4/11	19/35
Craniofacial features	33/55	7/9	8/11	18/35
Macrocephaly	5/55	1/9	1/11	3/35
Microcephaly	9/55	1/9	2/11	6/35
Hypertelorism	7/55	2/9	3/11	2/35
Strabismus	13/55	2/9	2/11	9/35
Microphthalmus	2/55	0/9	1/11	1/35
Palpebral fissure deformity	17/55	3/9	6/11	8/35
Eyebrow deformity	6/55	1/9	3/11	2/35
Ear deformity	16/55	4/9	5/11	7/35
Deafness	6/55	2/9	0/11	4/35
Cleft palate	3/55	0/9	0/11	3/35
High-arched palate	6/55	0/9	2/11	4/35
Abnormal teeth	3/55	0/9	1/11	2/35
Congenital heart defect	8/55	2/9	1/11	5/35
Congenital renal anomaly	1/55	0/9	0/11	1/35
Skeletal abnormality	18/55	3/9	5/11	10/35
Ambiguous genitalia (male)	5/35	1/5	1/8	3/22

Each feature has been examined in all the patients.
*P=0.043.
†P=0.044.

Table 2: Summary of clinical findings.

reported previously [23]. This would imply that de novo CNVs can occur with a certain frequency.

Several findings are noteworthy with regard to the clinical findings. First, the patient number was larger in males than in females. This would primarily be due to the presence of a large number of genes for X-linked non-syndromic ID [11]. It is predicted that a substantial fraction of male patients have hidden mutations of such genes. Furthermore, identification of pCNVs involving such X-linked genes in cases 4 (*IL1RAPL1*) and 9 (*MECP2*) suggests that X-chromosomal pCNVs are also more frequent in males than in females. Second, the

age at examination, the degree of ID, and the frequencies of various features were grossly similar among groups 1-3. This suggests lack of a clinical indication for pCNVs as well as VsUS in patients with non-syndromic ID.

In summary, we performed aCGH in 55 patients with non-syndromic ID. The results provide further support for the usefulness of aCGH in the identification of underlying genetic factor(s) for ID, although there was no clinical finding indicative of the presence of pCNVs or VsUS. Furthermore, our data are expected to serve to identify pathogenic genes on chromosomes 1q36 and 12q42, as well as those on several VsUS.

References

1. Roeleveld N, Zielhuis GA, Gabreëls F (1997) The prevalence of mental retardation: a critical review of recent literature. *Developmental Medicine and Child Neurology* 39: 125-132.
2. Hunter AG (2000) Outcome of the routine assessment of patients with mental retardation in a genetics clinic. *Am J Med Genet* 90: 60-68.
3. Rauch A, Hoyer J, Guth S, Zweier C, Kraus C, et al. (2006) Diagnostic yield of various genetic approaches in patients with unexplained developmental delay or mental retardation. *Am J Med Genet* 140: 2063-2074.
4. Kaufman L, Ayub M, Vincent JB (2010) The genetic basis of non-syndromic intellectual disability: a review. *J Neurodev Disord* 2: 182-209.
5. Miller DT, Adam MP, Aradhya S, Biesecker LG, Brothman AR, et al. (2010) Consensus statement: chromosomal microarray is a first-tier clinical diagnostic test for individuals with developmental disabilities or congenital anomalies. *Am J Hum Genet* 86: 749-764.
6. Choucair N, Ghoch JA, Corbani S, Cacciagli P, Mignon-Ravix C, et al. (2015) Contribution of copy number variants (CNVs) to congenital, unexplained intellectual and developmental disabilities in Lebanese patients. *Mol Cytogenet* 8: 26.
7. American Psychiatric Association (1994) *DSM-IV: Diagnostic and Statistical Manual of Mental Disorders*. American Psychiatric Association Publishing, Arlington.
8. Overhauser J, Huang X, Gersh M, Wilson W, McMahon J, et al. (1994) Molecular and phenotypic mapping of the short arm of chromosome 5: sublocalization of the critical region for the cri-du-chat syndrome. *Hum Mol Genet* 3: 247-252.
9. Driscoll DA, Budarf ML, Emanuel BS (1992) A genetic etiology for DiGeorge syndrome: consistent deletions and micro deletions of 22q11. *Am J Hum Genet* 50: 924-933.
10. Ballif BC, Theisen A, Rosenfeld JA, Traylor RN, Gastier FJ, et al. (2010) Identification of a recurrent micro deletion at 17q23.1q23.2 flanked by segmental duplications associated with heart defects and limb abnormalities. *Am J Hum Genet* 86: 454-461.
11. Honda S, Hayashi S, Imoto I, Toyama J, Okazawa H, et al. (2010) Copy-number variations on the X chromosome in Japanese patients with mental retardation detected by array-based comparative genomic hybridization analysis. *J Hum Genet* 55: 590-599.
12. Griggs BL, Ladd S, Saul RA, DuPont BR, Srivastava AK (2008) Deducator of cytokinesis 8 is disrupted in two patients with mental retardation and developmental disabilities. *Genomics* 91: 195-202.
13. Nawara M, Klapecki J, Borg K, Jurek M, Moreno S, et al. (2008) Novel mutation of IL1RAPL1 gene in a nonspecific X-linked mental retardation (MRX) family. *Am J Med Genet* 146: 3167-3172.
14. Chieffo C, Garvey N, Gong W, Roe B, Zhang G, et al. (1997) Isolation and characterization of a gene from the DiGeorge chromosomal region homologous to the mouse Tbx1 gene. *Genomics* 43: 267-277.
15. Nguyen JM, Qualmann KJ, Okashah R, Reilly A, Alexeyev MF, et al. (2015) 5p deletions: Current knowledge and future directions. *Am J Med Genet C Semin Med Genet* 169: 224-238.
16. Ramocki MB, Vayev YJ, Peters SU (2010) The MECP2 duplication syndrome. *Am J Med Genet* 152: 1079-1088.
17. Le MN, Holder EM, Jaillard S, Goldenberg A, Joriot S, et al. (2010) MEF2C haploinsufficiency caused by either microdeletion of the 5q14.3 region or mutation is responsible for severe mental retardation with stereotypic movements, epilepsy and/or cerebral malformations. *J Med Genet* 47: 22-29.
18. Ballif BC, Theisen A, Rosenfeld JA, Traylor RN, Gastier-Foster J, et al. (2010) Identification of a recurrent microdeletion at 17q23.1q23.2 flanked by segmental duplications associated with heart defects and limb abnormalities. *Am J Hum Genet* 86: 454-461.
19. Lee BH, Kasparis C, Chen B, Mei H, Edelmann L, et al. (2015) Setleis syndrome due to inheritance of the 1p36.22p36.21 duplication: evidence for lack of penetrance. *J Hum Genet* 60: 717-722.
20. Palumbo O, Palumbo P, Delvecchio M, Palladino T, Stallone R, et al. (2015) Microdeletion of 12q24.31: report of a girl with intellectual disability, stereotypies, seizures and facial dysmorphisms. *Am J Med Genet* 167: 438-444.
21. Al-Zahrani J, Al-Dosari N, Abudheim N, Alshidi TA, Colak D, et al. (2011) Chromosome 12q24.31-q24.33 deletion causes multiple dysmorphic features and developmental delay: First mosaic patient and overview of the phenotype related to 12q24qter defects. *Mol Cytogenet* 4: 9.
22. Daoud F, Angeard N, Demerre B, Martie I, Benyaou R, et al. (2009) Analysis of Dp71 contribution in the severity of mental retardation through comparison of Duchenne and Becker patients differing by mutation consequences on Dp71 expression. *Hum Mol Genet* 18: 3779-3794.
23. Hayashi S, Imoto I, Aizu Y, Okamoto N, Mizuno S, et al. (2011) Clinical application of array-based comparative genomic hybridization by two-stage screening for 536 patients with mental retardation and multiple congenital anomalies. *J Hum Genet* 56: 110-124.