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メタデータ	言語: eng 出版者: OMICS International 公開日: 2018-03-06 キーワード (Ja): キーワード (En): 作成者: Asahina, Miki メールアドレス: 所属:
URL	http://hdl.handle.net/10271/3224

Genomewide array comparative genomic hybridization in 55 Japanese normokaryotypic patients with non-syndromic intellectual disability

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1 **Abstract**

2 ***Background:*** Genomewide array comparative genomic hybridization (aCGH) has widely been
3 utilized as the diagnostic tool in patients with non-syndromic intellectual disability (ID).
4 Indeed, aCGH has identified pathogenic copy number variants (pCNVs), as well as variants of
5 uncertain clinical significance (VsUS) and benign CNVs (bCNVs), in such patients.
6 ***Aims:*** To examine the frequencies of various CNVs and clinical findings in patients with
7 non-syndromic ID.
8 ***Patients and Methods:*** We studied 55 Japanese normokaryotypic patients (35 males, 20
9 females) with apparently non-syndromic ID. Genomewide aCGH was performed using
10 leukocyte genomic DNA samples. Clinical findings were compared among patients with
11 pCNVs (group 1), those with VsUS (group 2), and those with bCNVs or no CNVs (group 3).
12 ***Results:*** Nine patients had pCNVs: one had 5p deletion syndrome, two had 22q11.2 deletion
13 syndrome, one had 17q23.1q23.2 microdeletion syndrome, three had CNVs involving known
14 pathogenic genes, and the remaining two had CNVs overlapping with previously described
15 CNVs in patients with ID (one with duplication at 1q36 and the other with deletion at 12q42).
16 Furthermore, 11 patients had VsUS, and nine patients had bCNVs. Clinical findings were
17 grossly comparable among groups 1–3.
18 ***Conclusions:*** The results provide further support for the usefulness of aCGH in the
19 identification of underlying genetic factor(s) for ID, although there was no clinical finding
20 indicative of the presence of pCNVs or VsUS. Furthermore, our data are expected to serve to
21 identify pathogenic genes on chromosomes 1q36 and 12q42, as well as those on several VsUS.
22

23 ***Keywords:*** Array comparative genomic hybridization; Intellectual disability; Copy number
24 variants; Pathogenic gene; Clinical finding
25

1 ## Introduction

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

12 Recently, genomewide array comparative genomic hybridization (aCGH) has widely
13 been utilized as the diagnostic tool in patients with non-syndromic ID. Indeed, aCGH has
14 identified pathogenic copy number variants (pCNVs) in 5–35% of such patients (average
15 12.2%) [5]. In addition, aCGH has also detected multiple variants of uncertain clinical
16 significance (VsUS) that could be relevant to ID, and benign CNVs (bCNVs) that are
17 irrelevant to ID [6].

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

20

21 ## Patients and Methods

22 ### Patients

23 This study consisted of 55 Japanese patients (35 males, 20 females) with apparently
24 non-syndromic ID with or without non-specific MCA. The ages at examination ranged from
25 0.8 to 42 years (median, 4.0 years). All patients had normal karyotype in the 50 lymphocytes
26 examined by the conventional 400–550 level G-banding analysis. The ID was assessed as
27 extremely severe (developmental quotient (DQ) / intelligence quotient (IQ), < 20) in 21
28 patients, severe (DQ/IQ, 21–34) in 11 patients, moderate (DQ/IQ, 35–49) in six patients, and
29 mild (DQ/IQ, 50–69) in 17 patients, by the DSM-IV method [7].

1

2 **Ethical approval and samples**

3 This study was approved by the Institutional Review Board Committees of Hamamatsu
4 University School of Medicine, and was performed after obtaining written informed consent
5 for the molecular analysis and the publication of genetic and clinical data after removing
6 information for personal identification.

7

8 **Genomewide aCGH analysis**

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

22 CNVs were regarded as pCNVs, when (1) they were identical to those of established
23 ID-positive syndromes with causative or candidate genes, (2) they included known pathogenic
24 genes in which intragenic mutations or CNVs involving the genes alone have been identified
25 in patients with ID, or (3) they shared an overlapping region with previously described plural
26 CNVs in patients with ID. By contrast, CNVs were interpreted as bCNVs, when (1) they were
27 inherited from either of the healthy parents, or (2) they have been registered as normal
28 variants in the public databases such as Database of Genomic Variants
29 (http://dgv.tcag.ca/dgv/app/home) and ClinVar (http:// http://www.ncbi.nlm.nih.gov/clinvar/).

1 Other CNVs were regarded as VsUS, most of which contained candidate genes for ID or were
2 formed as *de novo* events.
3
4 **Clinical assessment**
5 Multiple clinical features were examined in all the patients by two clinicians (M.A. and Y.E.),
6 except for ophthalmologic, cardiac, and renal features which were evaluated by professional
7 doctors in each field. We summarized clinical findings in patients with pCNVs (group 1),
8 those with VsUS (group 2), and those with bCNVs or no CNVs (group 3), and compared
9 them among different groups. Statistical significance of the median was examined by the
10 Mann-Whitney′s *U*-test, and that of the frequency by the Fisher's exact test. $P < 0.05$ was
11 considered significant.
12

13 Results

14 **Genomewide aCGH analysis**
15 Representative aCGH findings are shown in Figure 1. The data of groups 1 and 2 are
16 summarized in Table 1, and the pathogenic and candidate genes on the identified pCNVs and
17 VsUS are shown in Supplementary Table 1. CNVs were identified in 29 of the 55 patients. Of
18 the 29 patients, nine patients (cases 1–9) were assessed to have pCNVs, because: (1) case 2,
19 and cases 7 and 8, had deletions for 5p deletion syndrome (Cri Du Chat syndrome) [8] and
20 22q11.2 deletion syndrome (Di George syndrome) [9] with known pathogenic genes,
21 respectively; (2) case 6 had a deletion typical of 17q23.1q23.2 microdeletion syndrome with
22 candidate genes [10]; (3) cases 3, 4, and 9 had CNVs involving known pathogenic genes, as
23 well as candidate genes; and (4) cases 1 and 5 had CNVs partially overlapping with
24 previously described CNVs in patients with ID (Figure 2 and Figure 3). Furthermore, of case
25 1–9, cases 1–3, 5, 6, and 9 had *de novo* CNVs. By contrast, nine of the 20 patients were
26 evaluated to have bCNVs, because they were present in either of the healthy parents. The
27 remaining 11 patients (cases 10–20) were assessed to have VsUS with or without candidate
28 genes. Of cases 10–20, cases 10, 14, 17, and 18 had *de novo* CNVs. Notably, cases 4, 10, and
29 12–14 had plural CNVs. In particular, the co-existing two VsUS in cases 10 and those in case

1 14 were found to be generated as *de novo* CNVs, although parental samples were not
2 available in cases 4, 12, and 13.
3
4 **Clinical assessment**
5 Clinical features in patients of groups 1 and 2 are described in Table 1. Clinical features were
6 highly variable with no pathognomonic features. Indeed, while case 2 had 5p deletion for Cri
7 Du Chat syndrome, she showed no characteristic mewing cry. Similarly, while case 7 and 8
8 had 22q11.2 deletion for Di George syndrome, they were free from cardiovascular anomalies,
9 abnormal calcium metabolism, and immune deficiency. In addition, while case 6 had a
10 deletion typical of 17q23.1q23.2 microdeletion syndrome, his overall clinical features
11 remained rather non-specific.
12 Detailed clinical findings in groups 1–3 are summarized in Table 2. Male-dominant sex
13 ratio was common to groups 1–3 as well as total patients, and the examined age and the
14 degree of ID were similar among groups 1–3. Furthermore, the frequencies of clinical features
15 were similar among groups 1–3, except for significantly high frequency of eating disorder in
16 group 2 and that of sleep disorder in group 1.
17
18 **Discussion**
19 This study identified pCNVs in nine of 55 patients with apparently non-syndromic ID. The
20 frequency (16%) is grossly similar to that reported previously [5]. The results provide further
21 support for the usefulness of aCGH in the clarification of underlying genetic factor(s) for ID.
22 Of cases 1–9 with pCNVs, cases 2–4 and 7–9 had pathogenic genes for ID on the pCNVs
23 (Supplementary Table 1). Indeed, intragenic mutations of *CTNND2* [11], *DOCK8* [12],
24 *IL1RAPL1* [13], and *TBX1* [14], and loss of only *TERT* [15], are associated with ID, as are
25 duplication of only *MECP2* [16] or overexpression of *MEF2C* [17]. Thus, copy number
26 alterations of these genes would have played a major role in the development of ID in the six
27 cases, while the relevance of candidate genes on the pCNVs in case 4, and probably other
28 non-specified genes as well, would remain tenable. In addition, since the CNV in case 2 and
29 the CNVs in cases 7 and 8 are known to cause 5p deletion syndrome and 21q22.11 deletion

1 syndrome, respectively [8,9], this confirms their pathogenicity. Furthermore, since the CNVs
2 in cases 2, 3, and 9 were formed as *de novo* events, this would also support their
3 pathogenicity.

4 The remaining cases 1, 5, and 6 were also interpreted as having pCNVs. Actually, they
5 had *de novo* CNVs, although there was no definitive pathogenic genes on the identified CNVs.
6 In particular, the ~ 2.2 Mb deletion in case 6 is identical to that reported as chromosome
7 17q23.1-q23.2 microdeletion syndrome with ID, and *TBX2* and *TBX4* have been regarded as
8 candidate genes for ID because of their biological functions and expression pattern [18]. Since
9 this 17q23.1-q23.2 microdeletion is known to be generated by non-allelic homologous
10 recombination mediated by low-copy repeats [10], this would explain the recurrence of the
11 same deletion in multiple unrelated subjects. For the ~ 9 Mb duplication on chromosome
12 1p36.21–p36.12 in case 1 and the ~ 6 Mb deletion on chromosome 12q24.31–q24.32 in case 5,
13 previous studies have revealed similar duplications [19] and deletions [20,21] in patients with
14 ID (Figure 2 and Figure 3). Notably, although there is no segment shared by all the
15 duplications and deletions, the duplicated region in case 1 and the deleted region in case 5
16 encompass three different smallest regions of overlaps (SROs-A–C) common to plural
17 patients, and SRO-C on chromosome 1p36 and SRO-A on chromosome 12q24 harbor
18 candidate genes for ID. Thus, it is likely that the duplication in case 1 and the deletion in case
19 5 are pathogenic, and that different genes are involved in the development of ID in patients
20 with duplications involving 1p36 and in those with deletions affecting 12q24.

21 Cases 10–20 were evaluated to have VsUS rather than pCNVs. Indeed, since similar
22 CNVs have not been reported in patients with ID, their pathogenicity remains uncertain.
23 However, the VsUS in cases 10, 14, 17, and 18 were *de novo* CNVs, and those in cases 10–13,
24 14–16, and 18–20 harbor candidate genes (Supplementary Table 1). Thus, some of the VsUS
25 would actually be pCNVs that harbor hitherto unrecognized pathogenic genes for ID. One
26 may argue that the intragenic deletion of DMD identified in female case 19 is unlikely to
27 explain her phenotype, although it could have phenotypic effects depending on the
28 X-inactivation patterns in target tissues. However, since it is a disease-causing pCNV and
29 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 have been 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.

Disclosure

The authors have nothing to declare.

Funding

This work was supported in part by Grant from National Center for Child Health and Development (26-11), Grant from the Japan Agency for Medical Research and Development (16ek0109166h0002, 16ek0109067h0003), and Grant from Hamamatsu University School of Medicine.

1 **Acknowledgements**

2 We thank the patients and the parents for participating to this study.

3

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15

Figure legends

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.

Figure 2. Chromosome 1p36 duplicated regions in patients with non-syndromic ID. Shown on the top schema is aCGH data in case 1. The duplicated region in five patients are depicted by horizontal blue lines. The maximum duplicated regions are: (1) case 1: 13,178,528 – 22,364,327; (2) Lee et al. [19]: 10,536,144 – 13,992,333; (3) DECIPHER 257814: 11,860,126 – 20,573,006; (4) DECIPHER 300533: 15,443,521 – 15,739,333; and (5) DECIPHER 273011: 17,753,669 – 24,376,460 (DECIPHER, https://decipher.sanger.ac.uk/). The red, green, and orange rectangles (A, B, and C) represent the smallest overlapping regions in plural patients. The A–C region carry multiple genes, and the C region harbors candidate genes *ARHGEF10L* and *EMC1*.

Figure 3. Chromosome 12q24 deleted regions in patients with non-syndromic ID. Shown on the top schema is aCGH data in case 5. The deleted region in eight patients are depicted by horizontal blue lines. The maximum deleted regions are: (1) case 5, 121,530,401 – 127,569,632 (2) DECIPHER 272960: 121,441,374 – 122,441,868; (3) Palumbo et al. [20]: 121,887,158 – 123,552,213; (4) DECIPHER 294371: 122,212,162 – 124,354,904; and (5) Zahrani et al. [21]: 123,065,364 – 132,293,878. The red, green, and orange rectangles (A, B, and C) represent the smallest overlapping regions in several patients. The A–C region carry multiple genes, and the A regions contain a candidate gene *KDM2*.