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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 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 $-\infty$ and around + 1.0 were interpreted as hemizygous deletions and duplications, respectively. When $\geq$ 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 (1) they were identical to those of established ID-positive syndromes with causative or candidate genes, (2) they included known pathogenic genes in which intragenic mutations or CNVs involving the genes alone have been identified in patients with ID, or (3) they shared an overlapping region with previously described plural CNVs in patients with ID. 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:// http://www.ncbi.nlm.nih.gov/clinvar/).

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) [8] and 22q11.2 deletion syndrome (Di George syndrome) [9] with known pathogenic genes, respectively; (2) case 6 had a deletion typical of 17q23.1q23.2 microdeletion syndrome with candidate genes [10]; (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 (Figure 2 and Figure 3). Furthermore, of case 1–9, cases 1–3, 5, 6, and 9 had *de novo* CNVs. By contrast, nine of the 20 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* [11], *DOCK8* [12], *IL1RAPL1* [13], and *TBX1* [14], and loss of only *TERT* [15], are associated with ID, as are duplication of only *MECP2* [16] or overexpression of *MEF2C* [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 [8,9], this confirms their pathogenicity. 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 was no definitive 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 [19] and deletions [20,21] in patients with ID (Figure 2 and Figure 3). 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 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.

Acknowledgements

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

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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*.