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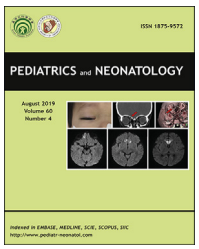

Original Article

Array-CGH increased the diagnostic rate of developmental delay or intellectual disability in Taiwan

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Background: Unexplained developmental delay or intellectual disability (DD/ID) has an estimated prevalence of about 3%–5% in the general population of Taiwan. Array comparative genomic hybridization (array-CGH) is a high-resolution tool that can detect about 50 Kb chromosome aberrations. A previous study has reported a detection rate of 10%–20% for this array.[1] This study aimed to investigate and compare the diagnosis rate for DD/ID using array-CGH and conventional chromosome study in DD/ID patients in Taiwan.
Methods: We enrolled 177 patients with DD/ID who underwent array-CGH examination at the MacKay Memory Hospital between June 2010 and September 2017. The copy number variants (CNV) were classified into the following three groups: pathogenic (potential pathologic variant), benign (normal genomic variant), and uncertain clinical significance (variance of uncertain significance, VOUS), according to the ACMG guideline.[2]
Results: Of the 177 enrolled patients, 100 (56.5%) were men and 77 (43.5%) were women. Ages ranged from 3 months to 50 years, with a median age of 5.2 years. Total 32.0% (32/100) male patients had pathogenic CNV, and 32.5% (25/77) female patients had pathogenic CNV. The ratio of pathogenic CNV in male and female patients was not significantly different ($p = 0.379$). The proportions of pathogenic CNV at <3 years, 3–6 years, 6–12 years, 12–18 years, and >18 years of age were 32.3% (31/96), 19.4% (6/31), 34.8% (8/23), 16.7% (2/12), and 66.7% (10/15), respectively. The overall diagnosed rate of DD/ID with pathogenic CNV was 27.7% (49/177) using array-CGH in this study. There were 105 patients with conventional karyotyping and array-CGH data at the same time. Nineteen (18.1%) patients had visible chromosomal abnormality. Total 32/105 (30.5%) patients could find at least one pathogenic CNVs. The array-CGH had a higher diagnosed rate than the conventional karyotyping in clinical application.
Conclusions: Although array-CGH could not detect point mutation, balanced translocations, inversions, or low-level mosaicism, the diagnosis rate in clinical application was up to 46.3% and 2.5 times that of conventional karyotyping analysis (18.1%). This study demonstrated that array-CGH is a powerful diagnostic tool and should be the first genetic test instead of conventional karyotyping analysis for patients with unexplained DD/ID.

1. Introduction

For patients with intellectual disabilities or developmental delay (ID/DD), autism spectrum disorders (ASD), and multiple congenital anomalies (MCA), array comparative genomic hybridization (array-CGH) and single nucleotide polymorphism (SNP) genotyping array, as chromosomal microarray analysis (CMA), are the first cytogenetic diagnostic test.[3] In the general population, 3% people experience ID/DD and ~1:150 individuals experience ASD.[4–6] The detection rates for Asia, Europe, and Northern America are similar.[7–10] Before the development of CMA, karyotyping was the gold standard for diagnosing patients with ID/DD and ASD. However, it can only detect large, microscopically visible chromosomal changes (>5–7 Mb in size). This diagnostic rate is only about 3%–5% of the cases.[11] The advent of fluorescence in situ hybridization (FISH) that targets known submicroscopic deletions and duplications could increase the diagnostic yield by ~2%–3%.[12,13] However, in many individuals, it is difficult to ascribe a well-known syndrome.

CMA development overcomes these technical limitations. It is able to interrogate the entire genome at a much higher resolution than conventional karyotyping and FISH. The International Collaboration for Clinical Genomics (ICCG), also known as the International Standard for Cytogenomic Array (ISCA) Consortium has recommended CMA as the first-tier cytogenetic diagnostic test for patients with ID/DD and MCA.[14] The American College of Medical Genetics (ACMG) has also published standards and guidelines of CMA using.[15,16] Two studies have described abnormal CMA findings with predicted clinical impact. Ellison et al. reviewed 46,298 patients tested using CMA and found pathogenic abnormalities associated with 151 disorders.[17] Total 35% of the pathogenic abnormalities were found in all patients. Riggs et al. described 28,526 cases and 146 clinically actionable phenotypes in the International Standards for Cytogenomics Arrays Consortium database and concluded that about 46% of all pathogenic or likely pathogenic CNVs submitted (1908/4125) and 7% of all cases potentially have the indications for clinical management.[18]

Many studies have described that CNVs play a causative role in DD/ID and ASD,[19] congenital heart diseases,[14] epilepsy,[20] and congenital kidney malformation.[21] However, these studies also illustrated that the same CNVs may cause multiple diseases, and other additional risk factors are needed for the development of a specific disease. This is the "two-hit" theory.[22,23] The clinical diagnosis, genetic counseling, and management become challenging because of this condition.

Few studies have been conducted on this subject; therefore, we investigated the diagnostic rate using

array-CGH for DD/ID patients in Taiwan. Moreover, we analyzed the CNV characteristic and feature for which patients have clinical significance and have discussed it in the context of the published literature. Finally, we compared the diagnostic rate between array-CGH and conventional chromosome study, an issue studied in few previous reports.

2. Materials and methods

2.1. Patients

Total 252 patients willingly underwent the array-CGH examination at MacKay Memory Hospital between June 2010 and September 2017. All of them had one or more clinical indications of multiple congenital anomaly, developmental delay or intellectual disability, and neurologic disorder. Multiple congenital anomaly was diagnosed when the patient had obvious dysmorphic features. Neurologic disorders included epilepsy with abnormal electroencephalography (EEG) finding, CNS malformation detected using brain computed tomography or brain magnetic resonance imaging, and autism spectrum disorder diagnosed by a psychiatrist. Developmental delay and intellectual disability were in a single group because maternal retraction was mostly presented as developmental delay at first and because there were no valid instrument for measuring intelligence before 5 years of age. We enrolled 177 of the 252 above patients with DD/ID. Among them, 105 patients had also previously undergone conventional karyotyping analysis with G-banding resolution of about 550 bands. Nineteen patients had a visible chromosomal abnormality, and others had a normal karyotype. This study was approved by the Institutional Review Board of the MacKay Memory Hospital (IRB approval number: 13MMHIS076), and written informed consent was obtained from the parents or guardians of all patients.

2.2. Array comparative genomic hybridization

Genomic DNA was isolated from the peripheral blood according to standard protocols and sent to two different laboratories. One was the National Center for Genome Medicine in Taiwan that used the Affymetrix GeneChip Genome-Wide Human SNP array 6.0 (Affymetrix, Santa Clara, CA, USA) and another was the Gene Biodesign that applied the NimbleGen ISCA Plus Cytogenetic Array (Roche NimbleGen, Madison, WI, USA). The Affymetrix GeneChip Genome-Wide Human SNP array 6.0 contained 750,000, 950,000, and 2,700,000 probes with a resolution ranging from 100 kb to 200 Kb across the entire genome for the detection of copy number variation. The array data were analyzed using the Affymetrix Genotyping Console™ version 3.0.1. The NimbleGen ISCA Plus Cytogenetic Array has 630,000 and 1,400,000 probes with resolutions of about 15 kb−30 kb throughout the whole genome. The related data were represented using Nexus 6.1 (BioDiscovery, Hawthorne, CA, USA). All samples were handled according to the manufacturers' instructions.

2.3. Data analyses

The CNVs were classified into the following three groups: pathogenic (potential pathologic variant), benign (normal genomic variant), and VOUS (variance of uncertain significance) as outlined elsewhere. The detailed definition of CNV was as follows: (1) Pathogenic CNVs were those that overlap with well-established as well as recently recognized microdeletion and microduplication syndromes, contained morbid OMIM genes, large deletions or duplications (usually $>$ 3 Mb in size) involving many OMIM genes, inherited from an affected parent, and greater than 1 copy number amplification. (2) Benign CNVs included those well documented in the normal population or the public databases, not previously reported but inherited from a health parent, without any morbid OMIM genes, and duplication with no known dosage-sensitive gene. (3) VOUS CNVs were assigned when insufficient evidence was available to conclude if the CNV was pathogenic or benign. Whenever possible, the blood samples of the patient's parents were obtained and tested to investigate the CNV inheritance. The publicly available databases DGV (Database of Genomic Variants), DECIPHER (Database of Chromosomal Imbalance and Phenotype in Humans Using Ensembl Resources), OMIM (Online Mendelian Inheritance in Man), PubMed, ClinVar and the UCSC Genome Browser were used to compare the present findings with previous reports and evaluate the morbidity of the candidate gene. All genomic coordinates are based on the February 2009 assembly of Genome Reference Consortium build 37 (GRCh37)/UCSC hg19.

3. Results

Of the 177 patients, 100 (56.5%) males and 77 (43.5%) females were enrolled in this study. Ages ranged from 2 days to 50 years, with a median age of 5.2 years. In addition, the Affymetrix GeneChip Genome-Wide Human SNP array 6.0 was used in 101 patients, and the NimbleGen ISCA Plus Cytogenetic Array was used in 76 patients.

After data analyses, total 101 CNVs were found in 82 patients. The average size of CNVs was 9.27 $\pm$ 7.14 Mb, with range 0.002 Mb−33.83 Mb. Among them, 72 were loss and 29 were gain. These CNVs were classified into the following three groups according to the clinical interpretation: 62.4% (63/101) CNVs were classified as pathogenic, 19.8% (20/101) as VOUS, and 17.8% (18/101) as benign (Fig. 1). The number of CNVs with loss and gain in pathogenic, VOUS, and benign groups were 51:12, 9:11, and 11:7, respectively. The prevalence of pathogenic CNVs in loss (71.8%, 51/71) was more than that in gain (40.0%, 12/30) (Fig. 2). The percentage of pathogenic CNVs in different sizes was as follows: (1) 57.7% (15/26) loss and 25.0% (4/16) gain CNVs were $<$1 Mb; (2) 66.7% (10/15) loss and 33.3% (1/3) gain CNVs were between 1 Mb and 3 Mb; (3) 89.4% (17/19) loss and 50% (3/6) gain CNVs were between 3 Mb and 10 Mb; (4) 100% (12/12) loss and 75% (3/4) gain CNVs were $>$10 Mb. The larger size of the CNV regions was found to be pathogenic. The array-CGH and short tandem repeat analysis from patients and patients' parents showed that 61 CNV

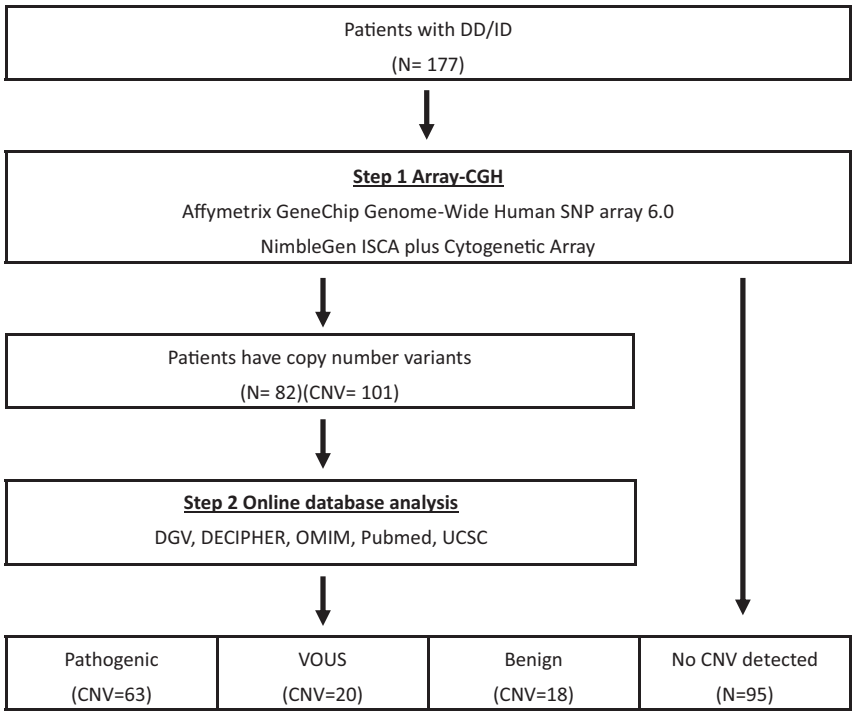
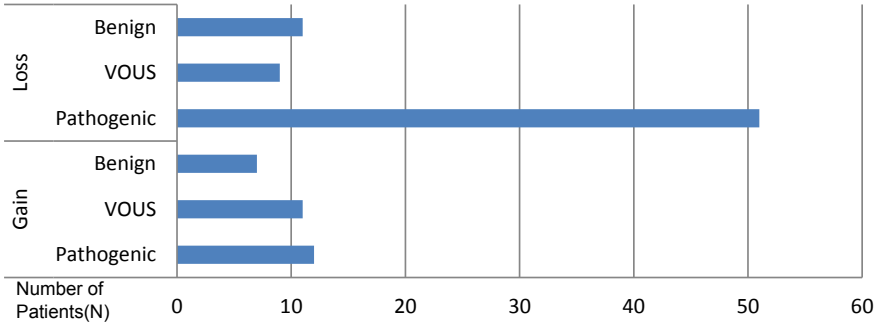


Figure 1 Diagnostic work-up of patients with developmental delay or intellectual disability (DD/ID) (n = 177). All four scores increased progressively with age ($p < 0.01$).



	Gain			Loss		
	Pathogenic	VOUS	Benign	Pathogenic	VOUS	Benign
N 1	12	11	7	51	9	11

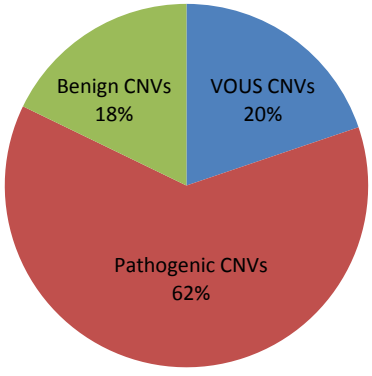


Figure 2 The class of the CNV in clinical significance (N = the number of patients).

regions in 51 patients could confirm the inherited status. Total 78.7% (48/61) CNVs were de novo and 21.3% (13/61) of them were inherited. Among them, 37 de novo and 5 inherited CNVs (4 from paternal and 1 from maternal origin) were causative. All inherited pathogenic CNVs descended from a healthy parent.

The chromosome structural aberration could be classified into the following three types: deletion, duplication, and complex rearrangements that had more than one CNV region. Of the 82 patients in whom CNV was detected using array-CGH, the CNVs were deletion type in 53 patients, duplication type in 10 patients, and complex rearrangements type in 19 patients. Fifty-seven of the 82 patients could find at least one casual CNV among which, 38 were deletion type, 4 were duplication type, and 15 were complex rearrangement type (Fig. 3). There was high risk to have pathogenic CNV in the patients who had the types of complex rearrangement (78.9%, 15/19).

Further analyses in these 57 patients with casual CNV for sex, age, and clinical pictures are shown in Table 1. The ratio of pathogenic CNV in male and female patients was not significantly different ($p = 0.379$). There were 32.0% (32/100) male patients and 32.5% (25/77) female patients. The proportions of those aged <3 years, those age 3–6 years, those aged 6–12 years, those aged 12–18 years, and those >18 years were 32.3% (31/96), 19.4% (6/31), 34.8% (8/23), 16.7% (2/12), and 66.7% (10/15), respectively. The overall diagnostic rate of DD/ID was 32.2% (57/177) using array-CGH in this study.

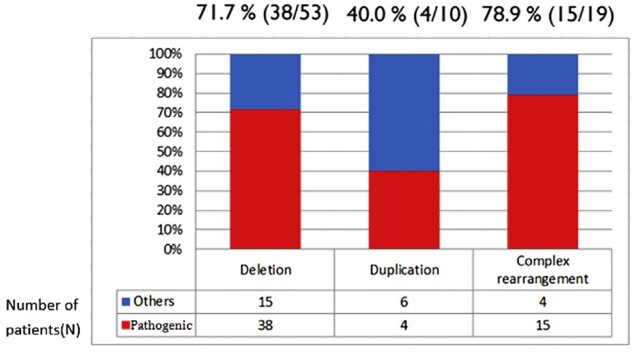


Figure 3 The class of pathogenic CNV (N = the number of patients).

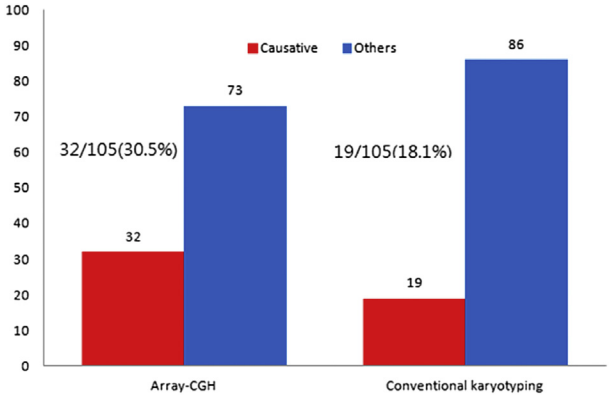


Figure 4 The diagnosed rate between conventional karyotyping and array-CGH data.

There were 105 patients with conventional karyotyping and array-CGH data at the same time (Fig. 4). For a part of conventional karyotyping, 19 (18.1%) patients had visible chromosomal abnormality. Others had a normal karyotype, including the following three normal variants: 46,XX,inv(9)(p12q13), 46,XX,9ph+, and 46,XY,Yqh+. For another part of the array-CGH, 32 (30.5%) patients could find at least one pathogenic CNVs. CNVs were not detected in 51 patients. Six patients had benign CNVs, and 9 patients had the VOUS region. Thus, the array-CGH had a higher diagnostic rate than conventional karyotyping in clinical application.

4. Discussion

To our knowledge, this is the first study and the largest investigation for assessing the diagnostic rate of array-CGH for DD/ID in Taiwan. Total 177 patients were enrolled in this study, and all of them were evaluated, if necessary, using array-CGH by the clinical geneticists of the pediatric tertiary hospital. Therefore, these findings could decrease unnecessary array-CGH examinations for patients with obvious clinical signs of a specific genetic disorder.

In this study, the overall diagnostic yield of DD/ID was 32.2% (57/177) using array-CGH. Only pathogenic CNV was classified as having clinical significance. This value was higher than that reported in previous studies, with a detection rate of 10%–20%. The detection rate of array-

Table 1 Demographic and clinical features of patients.

	Pathogenic	VOUS	Benign	No CNV detected	Total
Gender					
Male	32 (32.0%)	11	6	51	100 (56.5%)
Female	25 (32.5%)	5	3	44	77 (43.5%)
Age					
<3 y/o	31 (32.3%)	7	4	54	96 (54.2%)
3–6 y/o	6 (19.4%)	2	1	22	31 (17.5%)
6–12 y/o	8 (34.8%)	3	2	10	23 (13.0%)
12–18 y/o	2 (16.7%)	2	2	6	12 (6.8%)
>18 y/o	10 (66.7%)	2	0	3	15 (8.5%)
Total	57 (32.2%)	16	9	95	177

CGH was strongly influenced by the selection bias of patients, and the enrollment criteria were more discriminating than previously applied. In addition, the detected ratio of array-CGH and conventional karyotyping was 30.5% and 18.1%, respectively. This result was also similar to those of previous studies.[24–33] Consequently, array-CGH was confirmed as the first-line test for diagnosing chromosome aberration especially in the microdeletion and microduplication region for DD/ID.[3,34]

Among the 101 CNVs in this study, 70.3% (71/101) were loss and 29.7% (30/101) were gain. It has been reported that the frequency of random gain in the human genome may be lower than that of loss. Further, greater CNV loss (71.8%, 51/71) was detected among patients with clinically significant CNV. Less gain CNVs (40%, 12/30) were found in patients with pathogenic CNV, suggest that gain of the human genome had better tolerance than loss. In others words, the loss CNV identified using the array-CGH were more likely to be pathogenic than the gain. This study also found that the larger size CNV region tends to be pathogenic.

The average size of the pathogenic CNV was >3 Mb (5.6 ± 2.1 Mb in loss and 25.2 ± 17.8 Mb in gain). In contrast, the average size of the benign CNV was <1 Mb (0.6 ± 0.3 Mb in loss and 0.4 ± 0.2 Mb in gain). In fact, a greater CNV size has a higher chance to include the pathogenic OMIM genes. Moreover, increasing evidence shows that the phenotype did not correspond only to a single gene but was affected by multiple genes (contiguous gene syndrome). Although, inherited CNV has a 38.5% (5/13) chance of being pathogenic CNV, most of them were inherited from a healthy parent.

With respect to chromosomal structural aberration, complex rearrangement type (78.9%, 15/19) had a higher proportion of pathogenic CNV than the deletion type (71.7%, 38/53) and the duplication type (40%, 4/10). In the past, balanced translocation in conventional karyotyping studies was considered without apparent clinical significance. Currently, array-CGH has demonstrated that partial balance translocation in karyotype was actually unbalanced. Several studies have revealed that about 20% of the patients with balanced translocation (de novo or familial) have deletion or duplication of genetic material, as per array-CGH.[35]

Total 105 patients in the present study underwent conventional karyotyping and array-CGH examination at the same time. Nineteen (18.1%) patients had visible chromosomal abnormality and 32 (30.5%) had at least once pathogenic CNVs. It was noteworthy that 4 patients had balanced translocation in conventional karyotyping. After array-CGH examination, all of them had pathogenic CNVs; one patient had duplication type and others had deletion type. It revealed that the normal variant result in conventional karyotyping could be pathogenic in the array-CGH examination because of higher resolution. Array-CGH could be a powerful tool for patients with unexplained causes using conventional karyotyping.

In this study, there was no difference in the ratio of pathogenic CNV between male and female subjects ($p = 0.379$). The range of diagnosed age was wide, from 4 days to 24 years and 10 months, using array-CGH. In addition, older patients ended to have a higher chance of casual CNV. There were two possible reasons for this trend. One was that the array-CGH has been used in clinical settings only for this decade and is not the first-tier test for DD/ID. Some chromosome deletion or duplication syndromes had already been diagnosed using FISH or MLPA that were excluded in this study. Another was that the patient was older and the clinical picture was clear with respect to congenital anomalies. Therefore, some single gene disorders may have been misdiagnosis using array-CGH in younger patients.

5. Limitation

Although, to our knowledge, this study enrolled the highest number of study subjects from among all similar studies conducted in Taiwan, the sample size was insufficient to observe the phenotype–genotype relation comprehensively. The inability to study the clinical interpretation of many CNVs was a major challenge. Benign or VOUS CNVs may be postulated as being pathogenic in some patients even if they were inherited from healthy parents owing to epigenetic effects, position effects, or gene dosage effects.[36] Therefore, further studies on CNVs that include detailed clinical phenotype assessment are warranted.

The array-CGH is a widely used, high-resolution, and dosage-dependent detection tool for the whole human genome. Therefore, it has the technologic limitation of being unable to detect point mutations, balanced translocations, inversions, or low-level mosaicism. Conventional cytogenetic analyses should be performed if the array-CGH is negative.[37] New and developing technology, such as next generation sequencing (NGS), may overcome these limitations of array-CGH in the future. However, NGS has certain technical, clinical, and economic shortcomings.[38] For example, not all target DNA segments could be completely copied sometimes; these may be misinterpreted as deletions or variant conditions. There was insufficient clinical data and evidence to confirm all the phenotype–genotype relationships. Finally, most importantly, NGS is considerably more expensive (US$1000 per genome) than array-CGH (US$600 per patient) in clinical practice.

Most previous studies on array-CGH were required to perform FISH or MLPA to check CNV identity, prenatal data analyses for decided inheritance, and identification of the clinical significance of the pathogenic CNVs.[39] In the present study, few patients (12.2%, 10/82) detected with CNV using array-CGH underwent FISH or STR (short tandem repeat) analyses to conform CNV identity; however, all results were the same as the array-CGH results. In addition, 73.2% (60/82) of the patients with CNV who had undergone array-CGH and those data could assist determine inheritance and decide clinical significance. Genetic laboratory study is an expensive test that is not covered by health insurance in Taiwan currently; therefore, only highly uncertain cases are prescribed FISH or STR analyses to recheck the CNV identity because of limited research funding.

6. Conclusion

This study has demonstrated that array-CGH is a powerful diagnostic tool that should be the first genetic test rather than conventional karyotyping analysis for patients with

unexplained DD/ID. Although array-CGH could not detected point mutation, balanced translocations, inversions, and low-level mosaicism, the diagnosis rate in clinical application was up to 32.2% and 1.8 times higher than that using conventional karyotyping analyses. In addition, the CNV loss region, large region, and complex rearrangement type has a high potential of having clinical pathological significance.

Conflict of interest

The authors have no conflict of interest.

Acknowledgements

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