ity that *LMNA* mutations may cause active inflammation in skeletal muscle during infancy by a certain mechanism. In support of this notion, three of 15 L-CMD patients report by Quijano-Roy et al. had inflammatory cell infiltration [2]. In Patients 4, 7, 9, 10 and 11, muscle biopsies were done at the age of 2 years or later and inflammatory changes were relatively milder compared to the other earlier biopsies. These findings suggest that severities of inflammation may be related to the age of biopsies.

Inflammatory myopathy manifesting with muscle weakness starting during infancy is a poorly defined muscle disorder and limited number of patients were described in the literature [4,17–20]. Thompson emphasized that responsiveness to corticosteroid is one of the crucial findings that define the infantile myositis [17]. However, this is unlikely to be always the case as some of our laminopathy patients, who were initially diagnosed as infantile-onset inflammatory myopathy also showed some clinical improvement by corticosteroid therapy. Good response to steroids is not only a feature of myositis but can also be seen in other muscular dystrophies including Duchenne muscular dystrophy. Therefore, the possibility of laminopathy should not be excluded solely based upon steroid responsiveness. Interestingly, all steroid-responsive patients were ambulant whereas non-responsive patients could not walk, which might imply some genotype–phenotype correlation. Nonetheless, the correlation between genotype and steroid responsiveness cannot be discussed at this moment as all patients for whom steroid was used had distinct mutations. In any case, corticosteroid therapy could be considered for infantile striated muscle laminopathy patients as some patients respond, although its long-term efficacy is still unknown.

The p.Arg249Trp mutation found in this study was previously reported in L-CMD patients [2], but not in AD-EDMD or LGMD1B. In contrast, p.Glu358Lys mutation has also been reported with extremely variability of phenotypes, including AD-EDMD, LGMD1B, or L-CMD [10]. Thus, the same mutation can result in different phenotypes and severities. These findings raise a possibility that other unknown factor(s) may play a role in the development of laminopathy phenotype.

Muscle imaging demonstrated selective muscle involvement in all eight patients examined. Vastus lateralis and intermedius were markedly affected, while involvement of adductor magnus was minimal. In addition, medial head of the gastrocnemius was remarkably involved while lateral head was relatively spared in most patients. This selective muscle involvement is basically identical to that observed in AD-EDMD/LGMD1B patients [21] and may be helpful for the diagnosis of laminopathy in children.

Cardiomyopathy with conduction defects is a common serious clinical problem in patients with EDMD and LGMD1B [1]. In the present study, 8 of 11 patients developed cardiac complications such as arrhythmia and heart failure in their childhood and two died due to arrhythmia and heart failure, respectively. These findings clearly demonstrate that accurate diagnosis followed by periodic examination of cardiac function including electrocardiogram, holter electrocardiogram and echocardiogram, and appropriate implantation of defibrillators is necessary to avoid unexpected sudden death [22,23].

Our results expand clinical and pathological variation of striated muscle laminopathy and the inflammatory histology is an important diagnostic clue to the *LMNA* related myopathy patients. Further analysis is needed to elucidate the role of mutant A-type lamins in inducing inflammatory process during infancy.

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COMMENTARY

Going BAC or oligo microarray to the well: A commentary on Clinical application of array-based comparative genomic hybridization by two-stage screening for 536 patients with mental retardation and multiple congenital anomalies

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In this issue of the *Journal of Human Genetics*, Hayashi *et al.* document the results of their originally designed study of a 'two-stage screening' method that uses array-based comparative genomic hybridization for diagnosing patients who present with both multiple congenital anomalies and mental retardation (MCA/MR).[1] They collected DNA samples from 536 patients with MCA/MR by multicenter cooperation throughout Japan (from Hokkaido to Okinawa). They first screened all samples using the 'MCG Genome Disorder Array,' which covers subtelomeric regions and well-known disease-causing regions using 550 or 660 bacterial artificial chromosome (BAC)-based arrays that were originally constructed by them. Next, samples that did not show copy number variation (CNV) in the first stage of screening were screened again using 'MCG Whole Genome Array-4500,' which minutely covers all human chromosomes using 4523 bacterial artificial chromosomes at intervals of 0.7 Mb. In the first stage of screening, 54 (10.1%) patients showed CNVs that were confirmed by fluorescence *in situ* hybridization. In the second stage of screening, 63 (18.0%) of 349 patients demonstrated CNVs, of which 60 cases were confirmed by fluorescence *in situ* hybridization.

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The authors classified CNVs found in the second stage of screening into three categories: pathogenic, benign or variant of uncertain clinical significance). Initially, pathogenic CNVs were classified according to the following six criteria: (1) CNVs identified in recently established syndromes; (2) CNVs containing pathogenic gene(s); (3) recurrent CNVs in the same regions; (4) CNVs reported as pathogenic in previous studies; (5) large/gene-rich CNVs or CNVs containing morbid OMIM genes; or (6) *de novo* CNVs or CNVs that are maternally inherited through the X chromosome. CNVs that did not meet any of these criteria were classified as benign if they were inherited from a parent or as a variant of uncertain clinical significance if parental samples were not available. Consequently, 48 (13.8%) of 349 patients had pathogenic CNVs, 9 (2.6%) had benign CNVs and 6 (1.7%) had a variant of uncertain clinical significance.

MR is a highly heterogeneous condition and nearly 2500 syndromes of various congenital abnormalities are associated with MR[2] (http://becomerich.lab.u-ryukyu.ac.jp/). It is very difficult to determine the etiology of MR unless characteristic combinations of features can be accurately described, such as upslanted palpebral fissures in Down syndrome, overgrowth in Sotos syndrome, overeating in Prader–Willi syndrome or stereotypical hand movements in Rett syndrome, or unless specific and abnormal findings on laboratory or neuroimaging examinations are found, such as a metabolic screening indicative of phenylketonuria or lysosomal diseases, or brain magnetic resonance imaging indicative of polymicrogyria or lissencephaly. G-banded karyotyping has also been used to diagnose specific syndromes in patients with MCA/MR, and fluorescence *in situ* hybridizationis also useful for detecting microdeletion or microduplication syndromes; however, it is not easy for general practitioners or even pediatric neurologists to diagnose rare syndromes, such as Potocki–Lupski syndrome (17p11.2 duplication syndrome), Smith–Magenis syndrome (17p11.2 deletion syndrome) or 1p36 deletion syndrome. On the other hand, clinical applications of chromosomal microarrays are rapidly increasing for the diagnosis of congenital anomalies, hematological and solid tumors, and neuropsychological disorders, including MR and autism. In particular, chromosomal microarrays are used to diagnose MCA/MR. The diagnostic yields of chromosomal microarrays for detecting chromosomal aberrations among patients with MCA/MR or MR are only 7–15% in patients with normal G-banded karyotyping, depending on the probe coverage. These yields are much higher than G-banded karyotyping, which shows a yield of less than 3% if Down syndrome and other recognizable chromosomal syndromes are excluded.[3] The International Standard Cytogenomic Array Consortium and other groups support the consensus that chromosomal microarray is a first-tier clinical

diagnostic test and should be used before routine G-banded karyotyping for diagnosing individuals with unexplained developmental disabilities and/or congenital anomalies.[3–5] The 'two-stage screening' method by Hayashi *et al.* shows a diagnostic yield of 10.1% for the first targeted array and 13.8% for the second array capable of analyzing the whole genome. The total yield of their study was at least 18.1% (97 of 536 cases), which is comparable to the recent reports on higher-resolution oligonucleotide arrays. Unfortunately, G-banded karyotyping is still the first diagnostic tool for diagnosing MCA/MR in Japan because public health insurance currently covers only G-banded karyotyping and fluorescence *in situ* hybridization tests. Although chromosomal microarrays are much more expensive than G-banded cytogenetic analysis, the cost has reduced and is now less than the total cost of both traditional tests.[3] Thus, we now stand at the crossroads of genetic testing.

The study by Hayashi *et al.* used bacterial artificial chromosome-based arrays, while the expanded commercial availability of high-density oligonucleotide and single-nucleotide polymorphism arrays facilitates their use. In addition to good resolution, oligonucleotide arrays can detect regions of loss of heterozygosity and uniparental disomy (UPD), which are clinically important for the diagnosis of Silver–Russell syndrome and Beckwith–Wiedemann syndrome. Although major diseases caused by loss of heterozygosity or UPD, such as Prader–Willi syndrome and Angelman syndrome, can be clinically suspected by their characteristic features and UPD, most chromosomes show no phenotypic effects.[6] Physicians should know the limitations of each microarray in order to prevent the misdiagnosis of unfamiliar but important UPD disorders, such as maternal or paternal UPD chromosome 14.[7]

G-banded cytogenetic analysis still has the advantage over microarrays in terms of cost and ability to identify balanced rearrangements. Recognizable chromosomal syndromes, such as Down syndrome, trisomy 13, Turner syndrome, Klinefelter syndrome and MCA/MR with a family history of recurrent miscarriage or reproductive loss, all of which may be caused by balanced translocations, can be more efficiently diagnosed by traditional karyotyping.[3]

The application of microarrays to clinical testing is widening the scope of genomic medicine. Microarrays have accelerated the discovery of new syndromes and the causative genes of sporadic diseases, such as epileptic syndromes[8,9] and highly complex neuropsychological diseases.[10] However, the increasing number of variant of uncertain clinical significance cases makes definitive diagnosis difficult. No matter how far the tools for genetic analysis progress, clinical diagnosis based on medical history and examinations will remain pivotal. Future collaborations between basic scientists and trained clinicians, like the one performed in the study by Hayashi *et al.*,[1] will help to advance this new field.

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Progressive Atrophy of the Cerebrum in 2 Japanese Sisters with Microcephaly with Simplified Gyri and Enlarged Extraaxial Space

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Key words
- microcephaly
- simplified gyri
- enlarged extraaxial space
- atrophy

Abstract

This is a case report that describes 2 sisters with microcephaly, simplified gyri, and enlarged extraaxial space. Clinical features of the cases include dysmorphic features, congenital microcephaly, failure of postnatal brain growth, neonatal onset of seizures, quadriplegia, and severe psychomotor delay. Neuroradiological imaging demonstrated hypoplasia of bilateral cerebral hemispheres with enlarged extraaxial spaces, simplified gyral patterns without a thickened cortex, hypoplastic corpus callosum, and enlarged lateral ventricles, with a reduction in gray and white matter volume during the prenatal and neonatal periods. Repeat MRI revealed progressive atrophy of the cerebral gray and white matter, with enlarged lateral ventricles, although the sizes of the bilateral basal ganglia, thalamus, and infratentorial structures were relatively preserved. These neuroradiological findings imply that this disease is caused by the gene involved in neuronal and glial proliferation in the ventricular zone and in tangential neuronal migration from the ganglionic eminence. The nature of the progressive degeneration of the hemispheric structures should be clarified.

Introduction

From medical records and brain images in 237 patients with brain malformations characterized as microcephaly with simplified gyri, Basel-Vanagaite and Dobyns classified patients into 4 major groups: microcephaly with simplified gyri only, microcephaly with simplified gyri and pontocerebeller hypoplasia, microcephaly with simplified gyri and enlarged extraaxial space, and microcephaly with simplified gyri and both pontocerebeller hypoplasia and enlarged extraaxial space [1]. One of these groups, microcephaly with simplified gyri and enlarged extraaxial space is clinically characterized by severe developmental failure, feeding difficulty, spastic quadriplegia, and dyskinesia, with postnatal or congenital brain growth failure [occipital frontal circumference (OFC) below − 3 SD]. MRI findings typically show microcephaly, simplified gyri, enlarged extraaxial space and relatively preserved pontocerebeller structures [1]. In this case study, we describe 2 Japanese sisters with microcephaly with simplified gyri and enlarged extraaxial space. In one of the sisters, repeat MRI findings showed progressive atrophy of the cerebral hemispheres.

Case Report

Patient 1

The older sister, the first child of unrelated parents, was born after 38 weeks gestation by spontaneous delivery following a normal pregnancy. Microcephaly was noted during fetal ultrasonographic examination in the last trimester. The patient's birth weight was 2400 g (−1.5 SD), length 45.0 cm (−1.7 SD), and OFC 30 cm (−2.2 SD). She temporally showed clonic seizure activity on day 0. Upon admission at the age of 1 month, her general condition was unremarkable in spite of microcephaly and feeding difficulties. Dysmorphic features including a sloping forehead, arched and thick eyebrows, blephalophimosis, a saddle nose, triangular mouth, and micrognathia were observed. She began having complex partial seizures with right facial clonic seizures at 2 months of age. The seizures were controlled with valproic acid. The patient had spastic quadriplegia without obvious spontaneous movements and gastroesophageal reflux disease (GERD) beginning at 3 months of age. She died suddenly at 4 years and 8 months of age.

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Laboratory examinations were normal including blood NH_3, blood gas analysis, serum lactate, blood glucose, cerebrospinal fluid (CSF) glucose, CSF lactate, CSF white cell count, blood amino acid analysis, urine organic acid analysis, and plasma very long-chain fatty acid (VLCFA). Chromosome analysis and fluorescent in situ hybridization (FISH) studies for the LIS1 specific deletion at 17p13.3 revealed no abnormalities.

Her electroencephalogram (EEG) showed low amplitude and irregular waking background without obvious epileptic discharges on day 0. The ictal EEG of complex partial seizures at 3 months of age revealed right fronto-central spike bursts. Auditory evoked potentials (ABRs) and visual evoked potentials (VEPs) both showed a flat pattern. Brain magnetic resonance imaging (MRI) on day 0 revealed hypoplasia of bilateral cerebral hemispheres with enlarged extraaxial space, a simplified gyral pattern without a thickened cortex, a relatively spared volume of the bilateral basal ganglia and thalamus, a mildly flattened brain stem, and a hypoplastic corpus callosum (◎ **Fig. 1a–c**).

Patient 2

The microcephaly of the younger sister was recognized at a gestational age (GA) of 28 weeks by means of ultrasonography. She was born after 37 weeks gestation by spontaneous delivery following a normal pregnancy. The patient's birth weight was 2566 g (−0.5 SD), length 46.0 cm (−0.7 SD), and OFC 27 cm (−4.0 SD). Her Apgar score was 8 at 1 min, and 9 at 5 min. She developed generalized tonic seizures at 3 months of age. Her seizures were well controlled with valproic acid beginning when she was 2 years old.

She was able to bottle feed through the first 12 months, but her feeding skills deteriorated beginning at 18 months of age. At 2 years and 6 months, she was also diagnosed with GERD and required the use of a duodenal feeding tube. She also had spastic quadriplegia and visual impairment from early infancy. No developmental progress was observed.

Clinical examination performed at 3 years and 1 month of age showed microcephaly of OFC 41.5 cm (−4.2 SD), and other growth parameters were between −1 and −2 SD. Her dysmorphism was similar to that of her older sister. She had marked scoliosis, with hypertonic extremities and a posture characterized by asymmetrical tonic neck reflex. Deep tendon reflexes were exaggerated, and ankle clonus appeared bilaterally. Erratic myoclonus in the bilateral orbicular muscles and systemic myoclonus easily induced by sounds were often seen. There was no spontaneous movement of the extremities.

Laboratory examinations were normal including blood chemistry, creatinine kinase, intrauterine infection screen, blood NH_3, blood gas analysis, serum lactate, serum glucose, CSF glucose, CSF lactate, CSF white cell count, blood amino acid analysis, urine organic acid analysis, and plasma VLCFA. Chromosome analysis (G band) was 46XX; FISH for the LIS1 specific deletion at 17p13.3 was negative. Array-based comparative genomic hybridization (array-CGH) was performed using the Agilent Human Genome Microarray kit 244A (Agilent Technologies, Santa Clara, CA, USA), and it showed no apparent deletions or duplication.

The brain MRIs were performed at a GA of 30 weeks via intrauterine imaging, at day 0, and at 3 years and 1 month (◎ **Fig. 2a–g**). The former 2 MRI findings were almost identical to those of the older sister. Cerebellar white matter around the dentate nucleus had high T_2 signal intensity, showing unmyelinated cerebellar white matter. The MRI at 3 years and 1 month of age demonstrated marked dilatation of the posterior and inferior horns of the lateral ventricles and severe volume reduction of whole hemispheric gray and white matter, which was most dominant in the frontal lobes, whereas the volumes of bilateral basal ganglia, thalamus, and infratentorial structures were relatively preserved. The patient's EEG at 4 months of age and 3 years and 1 month of age demonstrated almost continuous spikes in the mid-frontal to right frontal regions. ABR and VEP were normal.

Discussion

There have been only 3 reports describing patients with microcephaly with simplified gyri and enlarged extraaxial space [1,2,8]. None of these reports included repeat MRI studies. As in the previous reports, our patients suggested an autosomal recessive trait of inheritance. Alternatively, an autosomal dominant or X-linked dominant inheritance with gonadal mosaisism is also possible. The genes responsible for microcephaly with simplified gyri only have been identified as *MCPH1, ASPM, CDK5RAP2, CENPJ,* and *WDR62* [1, 9]. However, it is not clear whether microcephaly with simplified gyri and enlarged extraaxial space with this phenotypic presentation can be explained by different mutation patterns of the already identified genes or whether it represents a distinct disease entity caused by still unknown genes. The extraaxial space enlargement described previously was less severe as compared to the present cases [1]. Dysmorphic features as observed in the present patients have not been described previously, although multiple anomalies, eye defects and jejunal atresia have been reported in patients with microcephaly with simplified gyri [1]. It remains to be clarified whether those phenotypic and neuroradiological features suggest distinctive clinical entity. Moreover, there may be overlap in the MRI findings between patients with microcephaly with simplified gyri and enlarged extraaxial space and those with microcephaly with simplified gyri and both enlarged extraaxial space

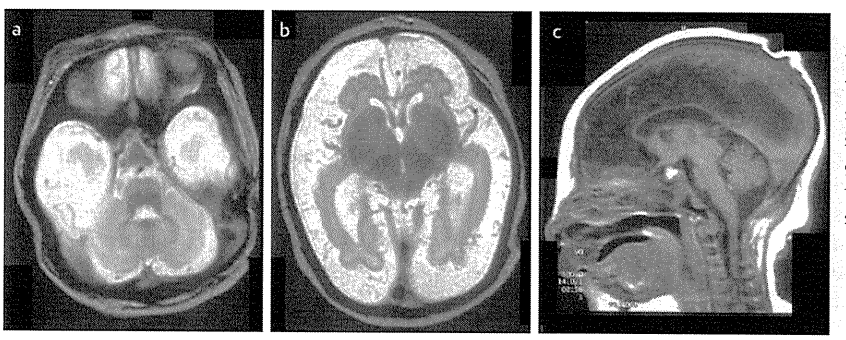

Fig. 1 Brain MRI of older sister at age of day 0. The MRI (**a** and **b**: T_2-weighted image [TR 4000, TE 132], **c**: T_1-weighted image [TR 500, TE 14.0]) showing hypoplasia of bilateral cerebral hemispheres with enlarged extraaxial space, a simplified gyral pattern without a thickened cortex, hypoplastic corpus callosum, and a mildly flattened brain stem.

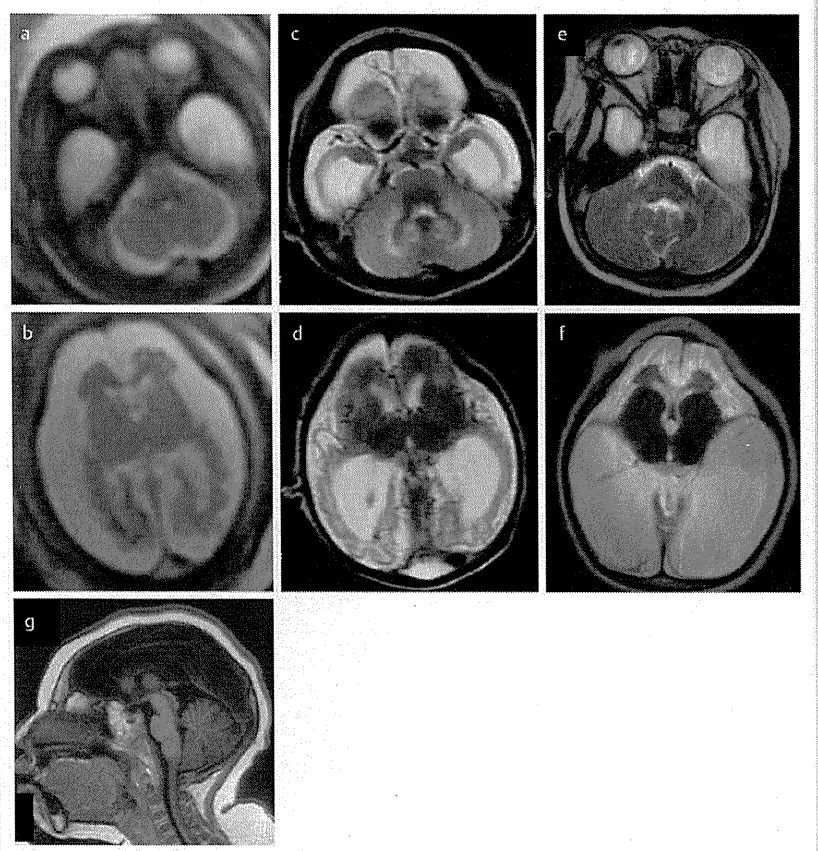

Fig. 2 Brain MRI with T_2-weighted images [TR 4500, TE 90] (**a–f**) and T_1-weighted images [TR 500, TE 14.0] (**g**) of the younger sister at 30 weeks gestational age (**a**, **b**), day 0 (**c**, **d**), and 3 years and 1 month of age (**e–g**). The MRI at 30 weeks gestational age and day 0 (**a–d**) revealed hypoplasia of bilateral cerebral hemispheres, particularly in the frontal regions, with enlarged extraaxial space, a simplified gyral pattern without thickened cortex, and enlarged lateral ventricles, especially in the posterior and temporal horns, with a reduction in the surrounding white matter. There was no change in the findings between GA 30 weeks and day 0. High signal intensity was observed in the lateral sides of the dentate nucleus (**c**). The MRI at 3 years and 1 month of age (**e–g**) demonstrated progressive dilatation of the posterior and inferior horns of the lateral ventricles, with a volume reduction in the surrounding hemispheric structures, especially in the frontal lobe. Some extent of myelination in the cerebellar hemisphere was observed (**e**). The size of the basal ganglia and thalamus, as well as of the infratentorial structures, was relatively preserved (**g**).

and pontocerebeller hypoplasia, because the older sister in our study had a mildly flattened brain stem at age of day 0. On the other hand, pontocerebeller hypoplasia may be the result of extensive cerebral pathology, as seen in the pontocerebeller hypoplasia in preterm infants [7].

A striking finding in these patients was progressive atrophy of the cerebral gray and white matter, with enlarged lateral ventricles, which was evident in the younger sister. Neurodegenerative processes such as accelerated apoptosis may be estimated from the MRI findings described in this report and the clinical deterioration observed in the younger sister. Basel-Vanagaite and Dobyns also described a rapid decrease in OFC postnatally in the subgroup of patients without congenital microcephaly but with enlarged extraaxial space [1]. Similar progressive changes in the cerebrum have also been reported in a patient most likely categorized as microcephaly with simplified gyri and pontocerebeller hypoplasia [4].

In spite of remarkable volume reductions in cerebral hemisphere cortices and white matter, the size of the bilateral basal ganglia, thalamus, and infratentorial structures was relatively preserved in these cases. As a cortical ribbon was formed and periventricular nodular heterotopia or band heterotopia was not observed, migration of cortical neurons from the ventricular zone may not be involved, but the proliferation process of neuronal and glial cells in the ventricular zone may be altered. On the other hand, the proliferation of neuronal cells in the lateral ganglionic eminence that generates the striatum and in the medial ganglionic eminence that mostly generates the globus pallidus and septum [3,6] may not be involved, although tangential migration of cortical GABAergic interneurons from the ganglionic eminence may have been altered [5].

In conclusion, it is believed that the genes responsible for microcephaly with simplified gyri and enlarged extraaxial space are involved in the neuronal and glial proliferation in the ventricular zone as well as in tangiential neuronal migration. Moreover, the nature of progressive degeneration of the hemispheric structures should be clarified in the near future.

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ORIGINAL ARTICLE

Clinical application of array-based comparative genomic hybridization by two-stage screening for 536 patients with mental retardation and multiple congenital anomalies

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Recent advances in the analysis of patients with congenital abnormalities using array-based comparative genome hybridization (aCGH) have uncovered two types of genomic copy-number variants (CNVs); pathogenic CNVs (pCNVs) relevant to congenital disorders and benign CNVs observed also in healthy populations, complicating the screening of disease-associated alterations by aCGH. To apply the aCGH technique to the diagnosis as well as investigation of multiple congenital anomalies and mental retardation (MCA/MR), we constructed a consortium with 23 medical institutes and hospitals in Japan, and recruited 536 patients with clinically uncharacterized MCA/MR, whose karyotypes were normal according to conventional cytogenetics, for two-stage screening using two types of bacterial artificial chromosome-based microarray. The first screening using a targeted array detected pCNV in 54 of 536 cases (10.1%), whereas the second screening of the 349 cases negative in the first screening using a genome-wide high-density array at intervals of approximately 0.7 Mb detected pCNVs in 48 cases (13.8%), including pCNVs relevant to recently established microdeletion or microduplication syndromes, CNVs containing pathogenic genes and recurrent CNVs containing the same region among different patients. The results show the efficient application of aCGH in the clinical setting.
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Keywords: array-CGH; congenital anomaly; mental retardation; screening

INTRODUCTION

Mental retardation (MR) or developmental delay is estimated to affect 2–3% of the population.[1] However, in a significant proportion of cases, the etiology remains uncertain. Hunter[2] reviewed 411 clinical cases of MR and reported that a specific genetic/syndrome diagnosis was carried out in 19.9% of them. Patients with MR often have congenital anomalies, and more than three minor anomalies can be useful in the diagnosis of syndromic MR.[2,3] Although chromosomal aberrations are well-known causes of MR, their frequency determined by conventional karyotyping has been reported to range from 7.9 to 36% in patients with MR.[4–8] Although the diagnostic yield depends on the population of each study or clinical conditions, such studies

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suggest that at least three quarters of patients with MR are undiagnosed by clinical dysmorphic features and karyotyping.

In the past two decades, a number of rapidly developed cytogenetic and molecular approaches have been applied to the screening or diagnosis of various congenital disorders including MR, congenital anomalies, recurrent abortion and cancer pathogenesis. Among them, array-based comparative genome hybridization (aCGH) is used to detect copy-number changes rapidly in a genome-wide manner and with high resolution. The target and resolution of aCGH depend on the type and/or design of mounted probes, and many types of microarray have been used for the screening of patients with MR and other congenital disorders: bacterial artificial chromosome (BAC)-based arrays covering whole genomes,[9,10] BAC arrays covering chromosome X,[11,12] a BAC array covering all subtelomeric regions,[13] oligonucleotide arrays covering whole genomes,[14,15] an oligonucleotide array for clinical diagnosis[16] and a single nucleotide polymorphism array covering the whole genome.[17] Because genome-wide aCGH has led to an appreciation of widespread copy-number variants (CNVs) not only in affected patients but also in healthy populations,[18–20] clinical cytogenetists need to discriminate between CNVs likely to be pathogenic (pathogenic CNVs, pCNVs) and CNVs less likely to be relevant to a patient's clinical phenotypes (benign CNVs, bCNVs).[21] The detection of more CNVs along with higher-resolution microarrays needs more chances to assess detected CNVs, resulting in more confusion in a clinical setting.

We have applied aCGH to the diagnosis and investigation of patients with multiple congenital anomalies and MR (MCA/MR) of unknown etiology. We constructed a consortium with 23 medical institutes and hospitals in Japan, and recruited 536 clinically uncharacterized patients with a normal karyotype in conventional cytogenetic tests. Two-stage screening of copy-number changes was performed using two types of BAC-based microarray. The first screening was performed by a targeted array and the second screening was performed by an array covering the whole genome. In this study, we diagnosed well-known genomic disorders effectively in the first screening, assessed the pathogenicity of detected CNVs to investigate an etiology in the second screening and discussed the clinical significance of aCGH in the screening of congenital disorders.

MATERIALS AND METHODS

Subjects

We constructed a consortium of 23 medical institutes and hospitals in Japan, and recruited 536 Japanese patients with MCA/MR of unknown etiology from July 2005 to January 2010. All the patients were physically examined by an expert in medical genetics or a dysmorphologist. All showed a normal karyotype by conventional approximately 400–550 bands-level G-banding karyotyping. Genomic DNA and metaphase chromosomes were prepared from peripheral blood lymphocytes using standard methods. Genomic DNA from a lymphoblastoid cell line of one healthy man and one healthy woman were used as a normal control for male and female cases, respectively. All samples were obtained with prior written informed consent from the parents and approval by the local ethics committee and all the institutions involved in this project. For subjects in whom CNV was detected in the first or second screening, we tried to analyze their parents as many as possible using aCGH or fluorescence *in situ* hybridization (FISH).

Array-CGH analysis

Among our recently constructed in-house BAC-based arrays,[22] we used two arrays for this two-stage survey. In the first screening we applied a targeting array, 'MCG Genome Disorder Array' (GDA). Initially GDA version 2, which contains 550 BACs corresponding to subtelomeric regions of all chromosomes except 13p, 14p, 15p, 21p and 22p and causative regions of about 30 diseases already reported, was applied for 396 cases and then GDA version 3, which contains 660 BACs corresponding to those of GDA version 2 and pericentromeric regions of all chromosomes, was applied for 140 cases. This means that a CNV detected by GDA is certainly relevant to the patient's phenotypes. Subsequently in the second screening we applied 'MCG Whole Genome Array-4500' (WGA-4500) that covers all 24 human chromosomes with 4523 BACs at intervals of approximately 0.7 Mb to analyze subjects in whom no CNV was detected in the first screening. WGA-4500 contains no BACs spotted on GDA. If necessary, we also used 'MCG X-tiling array' (X-array) containing 1001 BAC/PACs throughout X chromosome other than pseudoautosomal regions.[12] The array-CGH analysis was performed as previously described.[12,23]

For several subjects we applied an oligonucleotide array (Agilent Human Genome CGH Microarray 244K; Agilent Technologies, Santa Clara, CA, USA) to confirm the boundaries of CNV identified by our in-house BAC arrays. DNA labeling, hybridization and washing of the array were performed according to the directions provided by the manufacturer. The hybridized arrays were scanned using an Agilent scanner (G2565BA), and the CGH Analytics program version 3.4.40 (Agilent Technologies) was used to analyze copy-number alterations after data extraction, filtering and normalization by Feature Extraction software (Agilent Technologies).

Fluorescence *in situ* hybridization

Fluorescence *in situ* hybridization was performed as described elsewhere[23] using BACs located around the region of interest as probes.

RESULTS

CNVs detected in the first screening

In the first screening, of 536 cases subjected to our GDA analysis, 54 (10.1%) were determined to have CNV (Figure 1; Tables 1 and 2).

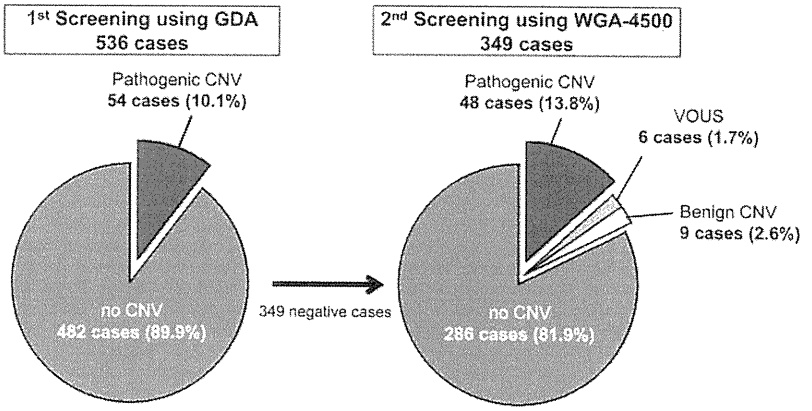

Figure 1 Percentages of each screening in the current study.

Table 1 A total of 40 cases with CNV at subtelomeric region(s) among 54 positive cases in the first screening

	Position where CNV detected				
Gender	*Loss*	*Gain*	*Corresponding disorder*[a]	*OMIM or citation*	*Parental analysis*[b]
M	1p36.33		Chromosome 1p36 deletion syndrome	#607872	
M	1p36.33p36.32		Chromosome 1p36 deletion syndrome	#607872	
M	1p36.33p36.32		Chromosome 1p36 deletion syndrome	#607872	
M	1p36.33p36.32		Chromosome 1p36 deletion syndrome	#607872	
M	1q44		Chromosome 1q43-q44 deletion syndrome	#612337	
F	2q37.3		2q37 monosomy[c]	Shrimpton *et al.*[24]	
F	2q37.3		2q37 monosomy[c]	Shrimpton *et al.*[24]	
M	3q29		Chromosome 3q29 deletion syndrome	#609425	
F	5p15.33p15.32		Cri-du-chat syndrome	#123450	
M	5q35.2q35.3		Chromosome 5q subtelomeric deletion syndrome	Rauch *et al.*[25]	
F	6p25.3		Chromosome 6pter-p24 deletion syndrome	#612582	
M	7q36.3		7q36 deletion syndrome[d]	Horn *et al.*[26]	
F	7q36.3		7q36 deletion syndrome[d]	Horn *et al.*[26]	
M	9p24.3p24.2		Chromosome 9p deletion syndrome	#158170	
F	9q34.3		Kleefstra syndrome	#610253	
F	10q26.3		Chromosome 10q26 deletion syndrome	#609625	
F	16p13.3		Chromosome 16p13.3 deletion syndrome	#610543	
F	22q13.31		Chromosome 22q13 deletion syndrome	#606232	
M	22q13.31q13.33		Chromosome 22q13 deletion syndrome	#606232	
M		15q26.3	15q overgrowth syndrome[c]	Tatton-Brown *et al.*[27]	
F		15q26.3	15q overgrowth syndrome[c]	Tatton-Brown *et al.*[27]	
M		21q22.13q22.3	Down's syndrome (partial trisomy 21)	#190685	
M		Xp22.33	A few cases have been reported; e.g. V5-130 in Lu *et al.*[28]		
M		Xq28	Chromosome Xq28 duplication syndrome	#300815	
F	1q44		Chromosome 1q43-q44 deletion syndrome	#612337	
		8p23.2p23.3			
M	3p26.3		3p deletion syndrome[d]	Fernandez *et al.*[29]	
		12p13.33p11.22			
F	3p26.3		3p deletion syndrome[d]	Fernandez *et al.*[29]	
		16p13.3	Chromosome 16p13.3 duplication syndrome	#613458	
F	4q35.2		4q− syndrome[d]	Jones *et al.*[30]	
		7q36.3			
M	5p15.33		Cri-du-chat syndrome	#123450	
		20p13			
M	5p15.33p15.32		Cri-du-chat syndrome	#123450	
		2p25.3			
F	6q27		6q terminal deletion syndrome[d]	Striano *et al.*[31]	
		11q25			
F	6q27		6q terminal deletion syndrome[d]	Striano *et al.*[31]	
		8q24.3			
M	7q36.3		7q36 deletion syndrome[d]	Horn *et al.*[26]	*dn*
		1q44			
M	9p24.3p24.2		Chromosome 9p deletion syndrome	#158170	
		7q36.3			
F	10p15.3p15.2		Chromosome 10p terminal deletion[d]	Lindstrand *et al.*[32]	*pat*
		7p22.3p22.2			
M	10p15.3		Chromosome 10p terminal deletion[d]	Lindstrand *et al.*[32]	
		2p25.3			
M	10q26.3		Chromosome 10q26 deletion syndrome	#609625	
		2q37.3	Distal trisomy 2q[d]	Elbracht *et al.*[33]	
M	18q23		Chromosome 18q deletion syndrome	#601808	
		7q36.3			
F	22q13.31q13.33		Chromosome 22q13.3 deletion syndrome	#606232	*pat*
		17q25.3	One case was reported	Lukusa *et al.*[34]	
M	Xp22.33/Yp11.32		Contiguous gene–deletion syndrome on Xp22.3[d]	Fukami *et al.*[35]	
		Xq27.3q28	Chromosome Xq28 duplication syndrome	#300815	

Abbreviations: F, female; CNV, copy-number variant; M, male; OMIM, Online Mendelian Inheritance in Man; *dn, de novo* CNV observed in neither of the parents.
[a]The name of disorder is based on entry names of OMIM, expect for entry names in DECIPHER and description in each cited article.
[b]*pat*, father had a balanced translocation involved in corresponding subtelomeric regions.
[c]Entry names in DECIPHER.
[d]Description in each cited article.

All the CNVs detected in the first screening were confirmed by FISH. Among the positive cases, in 24 cases one CNV was detected. All the CNVs corresponded to well-established syndromes or already described disorders (Table 1). In 16 cases, two CNVs, one deletion and one duplication, were detected at two subtelomeric regions, indicating that one of parents might be a carrier with reciprocal translocation involved in corresponding subtelomeric regions, and at least either of the two CNVs corresponded to the disorders. We also performed parental analysis by FISH for three cases whose parental samples were available, and confirmed that in two cases the subtelomeric aberrations were inherited from paternal balanced translocation and in one case the subtelomeric aberrations were *de novo* (Table 1). In the other 14 cases, CNVs (25.9%) were detected in regions corresponding to known disorders (Table 2).

Table 2 Other cases among 54 positive cases in the first screening

	Position where CNV detected			
Gender	*Gain*	*Loss*	*Corresponding disorder*	*OMIM*
F		4p16.3	Ring chromosome	
		4q35.2		
M		3q22.323	BPES	#110100
M		2q22.3	ZFHX1B region	*605802
M		4q22.1	Synuclein (SNCA) region	*163890
F		7p21.1	Craniosynostosis, type 1	#123100
F		7q11.23	Williams syndrome	#194050
F		8q23.3q24.11	Langer–Giedion syndrome	#150230
M	15q11.2q13.1		Prader–Willi/Angelman	#176270/ #105830
F		17p11.2	Smith–Magenis syndrome	#182290
M		17q11.2	Neurofibromatosis, type I	+162200
M	22q11.21		DiGeorge syndrome	#188400
F		22q11.21	DiGeorge syndrome	#188400
F	Xp22.31		Kallmann syndrome 1	+308700
F	Whole X		Mosaicism	

Abbreviations: CNV, copy-number variant; F, female; M, male; OMIM, Online Mendelian Inheritance in Man.

CNVs detected in the second screening and assessment of the CNVs

Cases were subject to the second screening in the order of subjects detected no CNV in the first screening, and until now we have analyzed 349 of 482 negative cases in the first screening. In advance, we excluded highly frequent CNVs observed in healthy individuals and/or in multiple patients showing disparate phenotypes from the present results based on an internal database, which contained all results of aCGH analysis we have performed using WGA-4500, or other available online databases; for example, Database of Genomic Variant (http://projects.tcag.ca/variation/). As a result, we detected 66 CNVs in 63 cases (Figure 1; Table 3). Among them, three patients (cases 36, 42 and 44) showed two CNVs. All the CNVs detected in the second screening were confirmed by other cytogenetic methods including FISH and/or X-array. For 60 cases, we performed FISH for confirmation and to determine the size of each CNV. For five cases, cases 13, 36, 48, 57 and 63, with CNVs on the X chromosome, we used the X-array instead of FISH. For cases 4, 6, 16–19 and 34, we also used Agilent Human Genome CGH Microarray 244K to determine the refined sizes of CNVs. The maximum and minimum sizes of each CNV determined by these analyses are described in Table 3.

Well-documented pCNVs emerged in the second screening

CNVs identified for recently established syndromes. We assessed the pathogenicity of the detected CNVs in several aspects (Figure 2).[21,37,38] First, in nine cases, we identified well-documented pCNVs, which are responsible for syndromes recently established. A heterozygous deletion at 1q41–q42.11 in case 2 was identical to patients in the first report of 1q41q42 microdeletion syndrome.[39] Likewise a CNV in case 3 was identical to chromosome 1q43–q44 deletion syndrome (OMIM: #612337),[40] a CNV in case 4 was identical to 2q23.1 microdeletion syndrome,[41] a CNV in case 5 was identical to 14q12 microdeletion syndrome[42] and a CNV in case 6 was identical to chromosome 15q26-qter deletion syndrome (Drayer's syndrome) (OMIM: #612626).[43] Cases 7, 8 and 9 involved CNVs of different sizes at 16p12.1-p11.2, the region responsible for 16p11.2-p12.2 microdeletion syndrome.[44,45] Although an interstitial deletion at 1p36.23-p36.22 observed in case 1 partially overlapped with a causative region of chromosome 1p36 deletion syndrome (OMIM: #607872), the region deleted was identical to a proximal interstitial 1p36 deletion that was recently reported.[46] Because patients with the proximal 1p36 deletion including case 1 demonstrated different clinical characteristics from cases of typical chromosome 1p36 deletion syndrome, in the near term their clinical features should be redefined as an independent syndrome.[46]

CNVs containing pathogenic gene(s). In four cases we identified pCNVs that contained a gene(s) probably responsible for phenotypes. In case 10, the CNV had a deletion harboring *GLI3* (OMIM: *165240) accounting for Greig cephalopolysyndactyly syndrome (GCS; OMIM: 175700).[47] Although phenotypes of the patient, for example, pre-axial polydactyly of the hands and feet, were consistent with GCS, his severe and atypical features of GCS, for example, MR or microcephaly, might be affected by other contiguous genes contained in the deletion.[48] Heterozygous deletions of *BMP4* (OMIM: *112262) in case 11 and *CASK* (OMIM: *300172) in case 13 have been reported previously.[49,50] In case 12, the CNV contained *YWHAE* (OMIM: *605066) whose haploinsufficiency would be involved in MR and mild CNS dysmorphology of the patient because a previous report demonstrated that haploinsufficiency of *ywhae* caused a defect of neuronal migration in mice[51] and a recent report also described a microdeletion of *YWHAE* in a patient with brain malformation.[52]

Recurrent CNVs in the same regions. We also considered recurrent CNVs in the same region as pathogenic; three pairs of patients had overlapping CNVs, which have never been reported previously. Case 16 had a 3.3-Mb heterozygous deletion at 10q24.31–q25.1 and case 17 had a 2.0-Mb deletion at 10q24.32–q25.1. The clinical and genetic information will be reported elsewhere. Likewise, cases 14 and 15 also had an overlapping CNV at 6q12–q14.1 and 6q14.1, and cases 18 and 19 had an overlapping CNV at 10p12.1–p11.23. Hereafter, more additional cases with the recurrent CNV would assist in defining new syndromes.

CNVs reported as pathogenic in previous studies. Five cases were applicable to these criteria. A deletion at 3p21.2 in case 20 overlapped with that in one case recently reported.[53] The following four cases had CNVs reported as pathogenic in recent studies: a CNV at 7p22.1 in case 21 overlapped with that of patient 6545 in a study by Friedman *et al.*,[14] a CNV at 14q11.2 in case 22 overlapped with those of patients 8326 and 5566 in Friedman *et al.*,[14] a CNV at 17q24.1–q24.2 in case 23 overlapped with that in patient 99 in Buysse *et al.*[54] and a CNV at 19p13.2 in case 24 overlapped with case P11 in Fan *et al.*[55]

Large or gene-rich CNVs, or CNVs containing morbid OMIM genes. In cases inapplicable to the above criteria, we assessed CNVs

Table 3 Sixty-three cases with CNV in the 2nd screening

Case	Gender	Clinical diagnosis	Remarkable clinical features	CNV	Position	WGA-4500[b]	FISH[b]	Base position and size of the identified CNV[a]						Parental analysis	Protein-coding genes[c]	CNV assessment[d]	Corresponding or candidate gene(s)
								Start (max)	Start (min)	End (min)	End (max)	Size (min)	Size (max)				
1	M	MCA/MR		del	1p36.23p36.22	arr cgh 1p36.23p36.22 (RP11-81J7→RP11-199O1)x1	ish del(1)(p36.23p36.22) (RP11-462M3+, RP11-106A3-, RP11-28P4+)dn	8585127	8890860	10561097	11143717	1670237	2558590	*dn*	32	P	
2	M	MCA/MR		del	1q41q42.11	arr cgh 1q41 (RP11-135J2→RP11-239E10)x1	ish del(1)(q41q42.11) (RP11-706L9+, RP11-224O19-, RP11-367O4-)dn	215986492	216532600	221534398	222467931	5001798	6481439	*dn*	35	P	
3	F	MCA/MR	Epilepsy	del	1q44	arr cgh 1q44 (RP11-156E8)x1	ish del(1)(q44) (RP11-56O19+, RP11-156E8-)	241996973	243177632	243251660	244141010	74028	2144037		11	P	
4	F	MCA/MR		del	2q22	arr cgh 2q23.1 (RP11-72H23)x1	ish del(2)(q23.1) (RP11-375H16-)	147651472	147688255	149855826	149879891	2167571	2228419		7	P	
5	F	MCA/MR		del	14q12q13.2	arr cgh 14q12q13.2 (RP11-369O9→RP11-26M6)x1	ish del(14)(q13.2) (RP11-831F6-)	28768137	29297829	34689412	35489337	5391583	6721200		25	P	
6	M	MCA/MR	CHD	del	15q26.2	arr cgh 15q26.2q26.3 (RP11-79C10→RP11-80F4)x1	ish del(15)(q26.2) (RP11-308P12-)	93199415	93214053	96928421	96942334	3714368	3742919		6	P	
7	M	MCA/MR	CHD	del	16p12.1p11.2	arr cgh 16p12.1p11.2 (RP11-309I14→RP11-150K5)x1	ish del(16)(p11.2) (RP11-75J11-)dn	25795340	27008538	29825404	31443492	2816866	5648152	*dn*	138	P	
8	M	MCA/MR	CHD	del	16p11.2	arr cgh 16p12.1p11.2 (RP11-360L15→RP11-150K5)x1	ish del(16)(p11.2) (RP11-360L15-, RP11-388M20+, RP11-75J11+)dn	27184508	28873631	29825404	31443492	951773	4258984	*dn*	134	P	
9	F	MCA/MR		del	16p11.2	arr cgh 16p11.2 (RP11-368N21→RP11-499D5)x1	ish del(16)(p11.2) (RP11-388M20-, RP11-75J11-)	28873841	29408698	32773200	34476095	3364502	5602254		125	P	
10	M	MCA/MR		del	7p14.2p13	arr cgh 7p14.2p13 (RP11-138E20→RP11-52M17)x1	ish del(7)(p14.1p13) (RP11-258I11+, RP11-2J17-, RP11-346F12-)dn	35621006	36470190	44657334	45508196	8187144	9887190	*dn*	70	P	*GLI3*
11	F	MCA/MR	Corneal opacity	del	14q22.1q22.3	arr cgh 14q22.1q22.3 (RP11-122A4→RP11-172G1)x1	ish del(14)(q22.1) (RP11-122A4-, RP11-316L15+)dn	51964774	51983834	54730496	55054754	2746662	3089980	*dn*	18	P	*BMP4*
12	M	MCA/MR	Idiopathic leukodystrophy	del	17q13.3	arr cgh 17p13.3 (RP11-294J5→RP11-357O7)x1	ish del(17)(p13.3) (RP11-4F24-, RP11-26N6+)dn	1008128	1146211	2077151	2026967	930940	1018839	*dn*	22	P	*YWHAE*
13	M	MCA/MR		del	Xp11.4p11.3	arr cgh Xp11.3p11.4 (RP11-1069J5→RP11-245M24)x1	ish del(X)(p11.4p11.3) (RP11-95C16-, RP11-829C10-)dn	41392291	41385453	45419624	45495709	4034171	4103418	*dn*	9	P	*CASK*

Table 3 Continued

Case	Gender	Clinical diagnosis	Remarkable clinical features	CNV	Position	WGA-4500[b]	FISH[b]	Base position and size of the identified CNV[a]						Parental analysis	Protein-coding genes[c]	CNV assessment[d]	Corresponding or candidate gene(s)
								Start (max)	Start (min)	End (min)	End (max)	Size (min)	Size (max)				
14	M	MCA/MR		del	6q12q14.1	arr cgh 6q12q14.2(RP11-502L6→RP11-232L4)x1	ish del(6)(q13)(RP11-28P18-)dn	69 029 871	69 731 888	83 926 178	85 101 718	14 194 290	16 071 847	*dn*	56	P	
15	M	ZLS		del	6q14.1	arr cgh 6q14.1(RP11-343P23→RP11-217L13)x1	ish del(6)(q14.1)(RP11-5N7-,RP11-990K4-,RP11-116+)	75 484 004	76 145 436	79 474 428	79 851 528	3 328 992	4 367 524		10	P	
16	F	MCA/MR	CHD	del	10p12.1p11.23	arr cgh 10p12.1p11.23(RP11-89D1→91A23)x1	ish del(10)(p12.1p11.23)(RP11-164A7-,RP11-110B21-)	27 045 285	27 054 002	29 057 401	29 088 950	2 003 399	2 043 665		18	P	
17	M	MCA/MR		del	10p12.1p11.23	arr cgh 10p12.1p11.23(RP11-218D6→RP11-RP11-181I11)x1	ish del(10)(p11.23)(RP11-15H10-)	28 121 596	28 131 608	30 559 024	30 577 807	2 427 416	2 456 211		12	P	
18	M	MCA/MR	CHD	del	10q24.31q25.1	arr cgh 10q24.31q25.1(RP11-108L7→RP11-108L7)x1	ish del(10)(q24.33)(RP11-416N2-)dn	102 560 783	102 568 462	105 914 057	105 929 608	3 345 595	3 368 825	*dn*	66	P	
19	M	MCA/MR		del	10q24.32q25.1	arr cgh 10q24.32q25.1(RP11-21N23→RP11-99N20)x1	ish del(10)(q24.33)(RP11-416N2-)dn	103 917 900	103 928 189	106 005 827	106 011 522	2 077 638	2 093 622	*dn*	41	P	
20	F	MCA/MR		del	3p21.31p21.2	arr cgh 3p21.31p21.2(RP11-24F11→RP11-89F17)x1	ish del(3)(p21.31)(RP11-3B7-)	46 150 261	46 359 965	51 390 597	52 571 544	5 030 632	6 421 283		175	P	
21	M	MCA/MR		del	7p22.1	arr cgh 7p22.1(RP11-90J23→RP11-2K20)x1	ish del(7)(p22.1)(RP11-2K20-)dn	3 185 609	5 892 225	6 233 987	6 409 277	341 762	3 223 668	*dn*	28	P	
22	F	MCA/MR	Corneal opacity, CHD	dup	14q11.2	arr cgh 14q11.2(RP11-152G22→RP11-84D12)x3	ish dup(14)(q11.2)(RP11-152G22++)	20 070 731	20 306 624	20 534 929	21 264 945	228 305	1 194 214		>30	P	
23	M	MCA/MR		del	17q24.1q24.2	arr cgh 17q24.1q24.2(RP11-89L7→RP11-79K13)x1	ish del(17)(q24.1q24.2)(RP11-93E5-,RP11-89L7-,RP11-79K13-)	60 576 365	60 936 391	64 592 701	64 587 782	3 656 310	4 011 417		29	P	
24	M	SMS susp.		del	19p13.2	arr cgh 19p13.2(RP11-197O4→RP11-164D24)x1	ish del(19)(p13.2)(91O21-)	9 248 377	10 248 853	11 968 772	12 553 279	1 719 919	3 304 902	*dn*		P	
25	M	MCA/MR	Epilepsy	dup	2q11.2q13	arr cgh 2q11.2q13(RP11-90G13→RP11-79K7)x3	ish dup(2)(q11.2)(RP11-542D13++)	88 273 220	91 696 986	109 869 691	112 714 666	18 172 705	24 441 446		>30	P	
26	M	MCA/MR	CHD	dup	4p16.1	arr cgh 4p16.1(RP11-17I9)x3	ish dup(4)(p16.1)(RP11-301J10++)	8 202 790	8 520 479	9 793 705	10 638 054	1 273 226	2 435 264		17	P	

Table 3 Continued

Case	Gender	Clinical diagnosis	Remarkable clinical features	CNV	Position	WGA-4500[b]	FISH[b]	Base position and size of the identified CNV[a]						Parental analysis	Protein-coding genes[c]	CNV assessment[d]	Corresponding or candidate gene(s)
								Start (max)	Start (min)	End (min)	End (max)	Size (min)	Size (max)				
27	F	MCA/MR		del	7q22.1q22.2	arr cgh 7q22.1q22.2 (RP11-10D8→ RP11-72J24)x1	ish del(7)(q22.1q22.2) (RP11-124G15+,RP11-188E1-,RP11-95P19-)	97 314 215	98 261 079	105 604 920	106 451 506	7 343 841	9 137 291		135	P	
28	F	MCA/MR	Epilepsy	del	12q13.13	arr cgh 12q13.13 (RP11-74I8→ RP11-624J6)x1	ish del(12)(q13.13) (RP11-624J6-)	50 987 232	51 016 427	51 956 291	52 180 088	939 864	1 192 856		44	P	
29	M	MCA/MR		dup	16q22.3	arr cgh 16q22.3 (RP11-90L19→ RP11-89K4)x3	ish dup(16)(q22.3) (RP11-115E3++, RP11-90L19++)	70 355 260	70 848 592	72 328 913	73 785 124	1 480 321	3 429 864		25	P	
30	M	RTS susp.		dup	16q24.1	arr cgh 16q24.1 (RP11-140K16→ RP11-44201)x3	ish dup(16)(q24.1) (RP11-770B4++, RP11-140K16++)	82 699 729	82 797 548	83 749 375	84 123 857	951 827	1 424 128		16	P	
31	M	MCA/MR	Epilepsy	del	2q24.2q24.3	arr cgh 2q24.2 (RP11-89L13→ RP11-79L13)x1	ish del(2)(q24.2) (RP11-638N12-)	160 407 234	161 072 815	162 883 584	166 923 475	1 810 769	6 516 241		28	P	*TBR1*
32	M	MCA/MR		del	3p26.2	arr cgh 3p26.2 (RP11-32F23)x1	ish del(3)(p26.2) (RP11-32F23-)	3 943 353	4 016 797	4 198 468	4 329 970	181 671	386 617		2	P	*SUMF1*
33	M	MCA/MR	IgA deficiency	del	7q21.11	arr cgh 7q21.11 (RP11-22M18)x1	ish del(7)(q21.11) (RP11-115M2+, RP11-35304-, RP11-22M18-)	83 597 839	83 601 541	84 549 609	84 788 160	948 068	1 190 321		3	P	*SEMA3A*
34	M	MCA/MR		dup	14q32.2	arr cgh 14q32.2 (RP11-128L1)x3	ish dup(14)(q32.2) (RP11-177F8++)	99 330 486	99 337 358	99 841 558	99 845 472	504 200	514 986		7	P	*EML1, YY1*
35	M	MCA/MR	Epilepsy	dup	16p13.3	arr cgh 16p13.3 (RP11-349I11)x3	ish dup(16)(p13.3) (RP11-349I11++)	4 851 459	5 678 447	5 906 909	6 165 923	228 462	1 314 464		9	P	*A2BP1*
36	M	MCA/MR		dup	Xp22.2p22.13	arr cgh Xp22.2p22.13 (RP11-2K15→ RP11-115I10)x3	not performed (X-tiling array)	16 874 735	16 952 121	17 596 600	17 638 351	644 479	763 616		2	P	
				dup	Xp21.3	arr cgh Xp21.3 (RP11-438J7)x3	not performed (X-tiling array)	28 704 076	28 704 076	28 868 075	28 868 075	163 999	163 999		1	P	*IL1RAPL1*
37	F	MCA/MR		del	1p34.3	arr cgh 1p34.3 (RP11-89N10→ RP11-416A14)x1	ish del(1)(p34.2) (RP11-195A8+, RP11-166F21-)dn	37 830 131	38 338 265	39 466 349	39 583 645	1 128 084	1 753 514	*dn*	7	P	
38	M	MCA/MR	Hyper IgE	dup	1q25.2	arr cgh 1q25.2 (RP11-177A2→ RP11-152A16)x3	ish dup(1)(1q25.2) (RP11-177A2++, RP11-152A16++)	177 088 480	177 196 858	177 535 659	177 859 828	338 801	771 348	*dn*	9	P	
39	M	MCA/MR		del	2p24.1p23.3	arr cgh 2p24.1p23.3 (RP11-80H16→ RP11-88F6)x1	ish del(2)(p23.3) (RP11-88F6-, RP11-373D23+)dn	20 037 821	23 094 244	26 815 794	28 414 457	3 721 550	8 376 636	*dn*	86	P	
40	F	MCA/MR	CHD	del	3p26.1p25.3	arr cgh 3p26.1p25.3 (RP11-128A5→ RP11-402P11)x1	ish del(3)(p26.1p25.3) (RP11-936E1-, RP11-402P11-, RP11-1079H21+) dn	8 190 557	8 497 949	9 930 973	10 026 217	1 433 024	1 835 660	*dn*	18	P	

Table 3 Continued

Case	Gender	Clinical diagnosis	Remarkable clinical features	CNV	Position	WGA-4500[b]	FISH[b]	Base position and size of the identified CNV[a]						Parental analysis	Protein-coding genes[c]	CNV assessment[d]	Corresponding or candidate gene(s)
								Start (max)	Start (min)	End (min)	End (max)	Size (min)	Size (max)				
41	M	MCA/MR		del	3p22.1p21.31	arr cgh 3p22.1p21.31 (RP11-241P3→ RP11-88B8)x1	ish del(3)(p22.1) (RP11-61H16+, RP11-241P3-, RP11-78O10+)dn	41 365 663	42 284 365	48 177 538	49 198 542	5 893 173	7 832 879	*dn*	123	P	
42	M	MCA/MR	Corneal opacity	del	3p14.3p14.2	arr cgh 3p14.3p14.2 (RP11-80H18→ RP11-79J9)x1	ish del(3)(p14.2) (RP11-79J19-, RP11-230A22+)mat	57 370 434	58 149 199	58 742 633	58 887 574	593 434	1 517 140	*mat*	11	B	
				del	8q21.11q21.13	arr cgh 8q21.11q21.13 (RP11-225J6→ RP11-214E11)x1	ish del(8) (q21.11q21.13) (RP11-225J6-, RP11-48B3+)dn	75 722 961	75 821 163	81 110 557	81 493 446	5 289 394	5 770 485	*dn*	12	P	
43	M	MCA/MR		del	3q26.31q26.33	arr cgh 3q26.31-q26.33 (RP11-292L5→ RP11-355N16)x1	ish del(3)(q26.32) (RP11-300L9+, RP11-105L6-)dn	175 650 310	176 531 688	180 613 203	181 653 281	4 081 515	6 002 971	*dn*	12	P	
44	M	MCA/MR	CHD	del	13q13.2q13.3	arr cgh 13q13.2 (RP11-269G10→ 90F5)x1	ish del(13)(q13.2) (RP11-142E9+, RP11-381E21-, RP11-98D3+)dn	33 451 136	33 895 560	34 813 379	34 909 905	917 819	1 458 769	*dn*	1	P	
				del	22q11.21	arr cgh 22q11.21 (RP11-155F20→ 54C2)x1	ish del(22)(q11.21) (RP11-155F20-, RP11-590C5-, RP11-54C2-)pat	19 310 307	19 310 307	19 590 642	19 590 642	280 335	280 335	*pat*	15	B	
45	F	aRS		del	18q21.2	arr cgh 18q21.2 (RP11-89B14)x1	ish del(18)(q21.2) (RP11-159D14+, RP11-186B13-, RP11-111C17-)dn	48 218 621	49 166 752	51 288 665	51 861 143	2 121 913	3 642 522	*dn*	9	P	
46	M	MCA/MR		dup	19p13.3	arr cgh 19p13.3 (RP11-49M3→ RP11-268O21)x3		1 095 485	2 418 857	3 499 581	4 460 252	1 080 724	3 364 767	*dn*	113	P	
47	F	MCA/MR	Autism	del	19p13.3	arr cgh 19p13.3 (RP11-30F17→ RP11-330I7)x1	ish del(19)(p13.3) (RP11-330I7-)dn	4 844 383	6 043 505	6 859 584	6 881 792	816 079	2 037 409	*dn*	23	P	
48	M	MCA/MR		del	Xp11.3	arr cgh Xp11.3 (RP11-151G3→ RP11-48J14)x0	ish del(X)(p11.3) (RP11-203D16-)mat	44 403 077	44 433 162	46 795 584	46 795 588	2 362 422	2 392 511	*mat*	18	P	
49	M	MCA/MR		dup	3p26.3	arr cgh 3p26.3 (RP11-630I)x3	ish dup(3)(p26.3) (RP11-630I++)pat	2 377 366	2 443 357	2 619 407	2 628 216	176 050	250 850	*pat*	1	B	
50	M	MCA/MR		dup	5p14.3	arr cgh 5p14.3 (RP11-91A5)x3	ish dup(5)(p14.3) (RP11-91A5++)pat	19 046 234	19 485 530	19 656 108	20 798 445	170 578	1 752 211	*pat*	1	B	
51	M	MCA/MR		dup	5q13.3	arr cgh 5q13.1 (RP11-40N8→ RP11-91C10)x3	ish dup(5)(q13.1) (RP11-105A11++)mat	66 417 271	66 481 371	67 501 700	67 838 977	1 020 329	1 421 706	*mat*	3	B	

Table 3 Continued

Case	Gender	Clinical diagnosis	Remarkable clinical features	CNV Position	WGA-4500[b]	FISH[b]	Base position and size of the identified CNV[a]						Parental analysis	Protein-coding genes[c]	CNV assessment[d]	Corresponding or candidate gene(s)
							Start (max)	Start (min)	End (min)	End (max)	Size (min)	Size (max)				
52	M	MCA/MR		dup 7p22.3	arr cgh 7p22.3 (RP11-23D23)x3	ish dup(7)(p22.3) (RP11-23D23++, RP11-1133D5+)mat	1	954016	954584	1101944	568	1101943	*mat*	12	B	
53	F	MCA/MR		dup 8p23.2	arr cgh 8p23.2 (RP11-79I19→ RP11-89I12)x3	ish dup(8)(p23.2) (RP11-89I19++, RP11-89I12++)pat	3324954	3726061	4564671	5973493	838610	2648539	*pat*	1	B	
54	M	MCA/MR		dup 9q33.1	arr cgh 9q33.1 (RP11-150L1)x3	ish dup(9)(q33.1) (RP11-150L1++)pat	118980752	119452372	119614984	120011559	162612	1030807	*pat*	2	B	
55	F	MCA/MR		dup 10q22.3	arr cgh 10q22.3 (RP11-79M9)x3	ish dup(10)(q22.3) (RP11-79M9++)mat	77356915	77718484	77873148	78230039	154664	873124	*mat*	1	B	
56	M	MCA/MR	ELBW, hepato-blastoma	dup 12q21.31	arr cgh 12q21.31 (RP11-91C4)x3	ish dup(12)(q21.31) (RP11-91C4++, RP11-142L2+)pat	80924954	82678148	82830190	85768388	152042	4843434	*pat*	3	B	
57	M	GS		del Xp11.23	arr cgh Xp11.23 (RP11-876B24) x0 mat	not performed (X-tiling array)	47752808	47747918	47852109	47868412	104191	115604	*mat*	3	B	
58	M	MCA/MR		dup 8q11.23	arr cgh 8q11.23 (RP11-221P7)x3	ish dup(8)(q11.23) (RP11-221P7++, RP11-26P22++)	53665974	53717675	54235229	54576654	517554	910680		3	VOUS	
59	F	MCA/MR	Micro-cephaly	dup 10q11.21	arr cgh 10q11.21 (RP11-178A10)x3	ish dup(10)(q11.21) (RP11-178A10++)	41986946	42197693	42320775	43603027	123082	1616081		15	VOUS	
60	M	MCA/MR		dup 11p14.2p14.1	arr cgh 11p14.2p14.1 (RP11-1L12)x3	ish dup(11) (p14.2p14.1) (RP11-1L12++)	26723462	27033270	27213374	27445504	180104	722042		4	VOUS	
61	F	MCA/MR		dup 12p11.1	arr cgh 12p11.1 (RP11-88P4)x3	ish dup(12)(p11.1) (RP11-472A10++)	33333493	33359944	33572956	33572956	213012	239463		2	VOUS	
62	F	aRS		dup 12q21.31	arr cgh 12q21.31 (RP11-91I24→ RP11-91C4)x3	ish dup(12)(q21.31) (RP11-91C4++, RP11-142L2++)	79949648	82172368	83968319	85768388	1795951	5818740		12	VOUS	
63	F	MR	Congenital myopathy	dup Xq12	arr cgh Xq12 (RP11-90P17→ RP11-383C12)x3	Not performed (X-tiling array)	66212661	66216353	66921699	66948538	705346	735877		1	VOUS	

Abbreviations: aRS, atyplical Rett syndrome; B, benign; CNV, copy-number variant; *dn*: *de novo* CNV observed in neither of the parents; ELBW, extremely low birth weight; FISH, fluorescence *in situ* hybridization; GS, Gillespie syndrome; *mat*: CNV identified also in mother; P, pathogenic; *pat*: CNV identified also in father; RTS, Rubinstein–Taybi syndrome; SMS, Smith–Magenis syndrome; VOUS, variant of uncertain clinical significance; ZLS, Zimmermann–Laband syndrome.
[a]The sizes were estimated by WGA-4500, X-array, FISH or Agilent Human Genome CGH microarray 244K.
[b]The notation systems is based on ISCN2005.[36]
[c]The number of protein-coding genes contained in the respective CNVs.
[d]The result of CNV assessment.

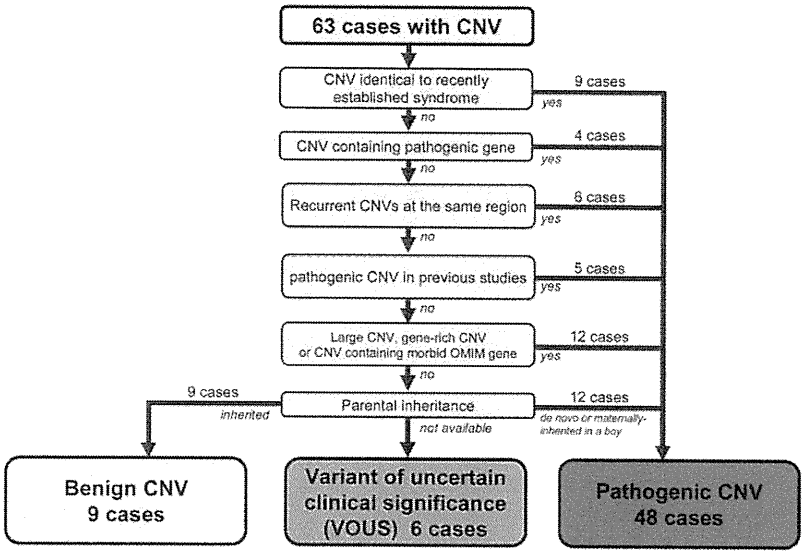

Figure 2 A flowchart of the assessment of CNVs detected in the second screening.

from several aspects. A CNV that contains abundant genes or is large (>3 Mb) has a high possibility to be pathogenic.[21] The CNVs in cases 25–30 probably correspond to such CNVs. Also, we judged a CNV containing a morbid OMIM gene as pathogenic:[21] *TBR1* (OMIM: *604616) in case 31,[56] *SUMF1* (OMIM: *607939) in case 32,[57,58] *SEMA3A* (OMIM: *603961) in case 33,[59] *EML1* (OMIM: *602033) and/or *YY1* (OMIM: *600013) in case 34,[60,61] *A2BP1* (OMIM: *605104) in case 35[62] and *IL1RAPL1* (OMIM: *300206) in case 36.[63] Several previous reports suggest that these genes are likely to be pathogenic, although at present no evidence of a direct association between these genes and phenotypes exists.

CNVs de novo or X maternally inherited. Among the remaining 27 cases, 12 cases had CNVs considered pathogenic as their CNVs were *de novo* (cases 37–47) or inherited del(X)(p11.3) from the mother (case 48). In the second screening we performed FISH for 36 CNVs of the 34 cases whose parental samples were available to confirm that 24 cases had *de novo* CNVs, which were probably pathogenic. A CNV in case 48, a boy with a nullizygous deletion at Xp11.3 inherited from his mother, was also probably relevant to his phenotype (Tables 3 and 4). Meanwhile, although case 57 was a boy with a deletion at Xp11.23 inherited from his mother, he was clinically diagnosed with Gillespie syndrome (OMIM: #206700) that was reported to show an autosomal dominant or recessive pattern,[64] thus we judged that the deletion was not relevant to his phenotype. As a result, cases 49–57 had only CNVs inherited from one of their parents which are likely to be unrelated to the phenotypes; that is, bCNV (Table 4).

As a result, we estimated that 48 cases among 349 analyzed (13.8%) had pCNV(s) in the second screening (Table 3; Figure 2). The CNVs of the remaining six cases, cases 58–63, were not associated with previously reported pathogenicity and their inheritance could not be evaluated, thus we estimated they were variants of uncertain clinical significance (VOUS).[38]

DISCUSSION

Because aCGH is a high-throughput technique to detect CNVs rapidly and comprehensively, this technique has been commonly used for analyses of patients with MCA and/or MR.[38,65–68] However, recent studies of human genomic variation have uncovered surprising properties of CNV, which covers 3.5–12% of the human genome even in healthy populations.[18–20,69] Thus analyses of patients with uncertain clinical phenotypes need to assess whether the CNV is pathogenic or unrelated to phenotypes.[21] However, such an assessment may diminish the rapidness or convenience of aCGH.

In this study, we evaluated whether our in-house GDA can work well as a diagnostic tool to detect CNVs responsible for well-established syndromes or those involved in subtelomeric aberrations in a clinical setting, and then explored candidate pCNVs in cases without any CNV in the first GDA screening. We recruited 536 cases that had been undiagnosed clinically and studied them in a two-stage screening using aCGH. In the first screening we detected CNVs in 54 cases (10.1%). Among them, 40 cases had CNV(s) at subtelomeric region(s) corresponding to the well-established syndromes or the already described disorders and the other 14 cases had CNVs in the regions corresponding to known disorders. Thus about three quarters of cases had genomic aberrations involved in subtelomeric regions. All the subtelomeric deletions and a part of the subtelomeric duplications corresponded to the disorders, indicating that especially subtelomeric deletions had more clinical significance compared to subtelomeric duplications, although the duplication might result in milder phenotypes and/or function as a modifier of phenotypes.[70] Moreover, parental analysis in three cases with two subtelomeric aberrations revealed that two of them were derived from the parental balanced translocations, indicating that such subtelomeric aberrations were potentially recurrent and parental analyses were worth performing. Recently several similar studies analyzed patients with MCA/MR or developmental delay using a targeted array for subtelomeric regions and/or known genomic disorders and detected clinically relevant CNVs in 4.4–17.1% of the patients.[28,65,70,71] Our detection rate in the first screening was equivalent to these reports. Although such detection rates depend on the type of microarray, patient selection criteria and/or number of subjects, these results suggest that at least 10% of cases with undiagnosed MCA/MR and a normal karyotype would be detectable by targeted array.

Table 4 Parental analysis of 34 cases in the second screening

Case	Gender	Clinical diagnosis	CNV		Size of CNV (bp)		Protein-coding genes	Parental analysis	Pathogenicity
			del/dup	Position	Min.	Max.			
1	M	MCA/MR	del	1p36.23p36.22	1 670 237	2 558 590	32	*de novo*	P
2	M	MCA/MR	del	1q41q42.11	5 001 798	6 481 439	35	*de novo*	P
7	M	MCA/MR	del	16p12.1p11.2	2 816 866	5 648 152	138	*de novo*	P
8	M	MCA/MR with CHD	del	16p11.2	951 773	4 258 984	134	*de novo*	P
10	M	MCA/MR	del	7p14.2p13	8 516 513	9 421 233	70	*de novo*	P
11	F	MCA/MR	del	14q22.1q22.3	2 746 662	3 089 980	18	*de novo*	P
12	M	MCA/MR	del	17q13.3	930 940	1 018 839	22	*de novo*	P
13	M	MCA/MR	del	Xp11.4p11.3	4 034 171	4 103 418	9	*de novo*	P
14	M	MCA/MR	del	6q12q14.1	14 194 290	16 071 847	56	*de novo*	P
18	M	MCA/MR	del	10q24.31q25.1	3 345 595	3 368 825	66	*de novo*	P
19	M	MCA/MR	del	10q24.32q25.1	2 077 638	2 093 622	41	*de novo*	P
21	M	MCA/MR	del	7p22.1	341 762	3 223 668	28	*de novo*	P
24	M	SMS susp.	del	19p13.2	1 719 919	3 304 902	23	*de novo*	P
37	F	MCA/MR	del	1p34.3	1 128 084	1 753 514	7	*de novo*	P
38	M	MCA/MR	dup	1q25.2	338 801	771 348	9	*de novo*	P
39	M	MCA/MR	del	2p24.1p23.3	3 721 550	8 376 636	86	*de novo*	P
40	F	MCA/MR	del	3p26.1p25.3	1 433 024	1 835 660	18	*de novo*	P
41	M	MCA/MR	del	3p22.1p21.31	5 893 173	7 832 879	123	*de novo*	P
42[a]	M	MCA/MR	del	8q21.11q21.13	5 289 394	5 770 485	12	*de novo*	P
42[a]	M	MCA/MR	del	3p14.3p14.2	593 434	1 517 140	11	Maternal	B
43	M	MCA/MR	del	3q26.31q26.33	4 081 515	6 002 971	12	*de novo*	P
44[b]	M	MCA/MR	del	13q13.2q13.3	917 819	1 458 769	1	*de novo*	P
44[b]	M	MCA/MR	del	22q11.21	917 819	1 458 769	15	Paternal	B
45	F	Rett syndrome	del	18q21.2	2 121 913	3 642 522	9	*de novo*	P
46	M	MCA/MR	dup	19p13.3	2 041 395	2 404 096	113	*de novo*	P
47	F	MCA/MR	del	19p13.3	816 079	2 037 409	23	*de novo*	P
48[c]	M	MCA/MR	del	Xp11.3	2 362 422	2 392 511	18	Maternal	P
49	M	MCA/MR	dup	3p26.3	176 050	250 850	1	Paternal	B
50	M	MCA/MR	dup	5p14.3	170 578	1 752 211	1	Paternal	B
51	M	MCA/MR	dup	5q13.3	1 020 329	1 421 706	3	Maternal	B
52	M	MCA/MR	dup	7p22.3	568	1 101 943	12	Maternal	B
53	F	MCA/MR	dup	8p23.2	838 610	2 648 539	1	Paternal	B
54	M	MCA/MR	dup	9q33.1	162 612	1 030 807	2	Paternal	B
55	F	MCA/MR	dup	10q22.3	154 664	873 124	1	Maternal	B
56	M	MCA/MR	dup	12q21.31	152 042	4 843 434	3	Paternal	B
57	M	Gillespie syndrome	del	Xp11.23	104 191	115 604	3	Maternal	B

Abbreviations: B, benign; CNV, copy-number variant; F, female; M, male; MCA/MR, multiple congenital anomalies and mental retardation; P, pathogenic.
[a]Two CNVs were detected in case 42.
[b]Two CNVs were detected in case 44.
[c]Nullizygous deletion inherited from his mother probably affected the phenotype.

Another interesting observation in the first screening was that subtelomeric rearrangements frequently occurred even in patients with MCA/MR of uncertain whose karyotype had been diagnosed as normal. This result may be consistent with a property of subtelomeric regions whose rearrangements can be missed in conventional karyotyping,[72] and in fact other techniques involving subtelomeric FISH or MLPA also identified subtelomeric abnormalities in a number of patients with MCA and/or MR in previous reports.[70,73,74] Our result may support the availability of prompt screening of subtelomeric regions for cases with uncertain congenital disorders.

In the second screening we applied WGA-4500 to 349 cases to detect 66 candidate pCNVs in 63 cases (18.1%), and subsequently assessed the pathogenicity of these CNVs. The pCNVs included nine CNVs overlapping identical regions of recently recognized syndromes (cases 1–9; deletion at 1p36.23–p36.22, 1q41–q42.11, 1q43–q44, 2q23.1, 14q12, 15q26-qter and 16p11.2–p12.2, respectively), four CNVs containing disease-associated genes (cases 10–13; *GLI3*, *BMP4*, *YWHAE* and *CASK*, respectively), three pairs of CNVs of recurrent deletions (cases 14, 15: at 6q12–q14.1 and 6q14.1; case 16, 17: at 10p12.1–p11.23 and case 18, 19: at 10q24.31–q25.1 and 10q24.32–q25.1), five CNVs identical to pCNVs in previous studies (cases 20–24), six large and/or gene-rich CNVs (cases 25–30) and six CNVs containing a morbid OMIM gene (cases 31–36). For the remaining cases, we estimated the pathogenicity of the CNVs from a parental analysis (Table 4). We judged the 11 *de novo* CNVs (cases 37–47) and 1 CNV on chromosome Xp11.3 inherited from

the mother (case 48) as probably pathogenic. And nine inherited CNVs (cases 49–57) were probably benign. The clinical significance of CNVs in the other six cases, cases 58–63, remains uncertain (VOUS). As a result we estimated CNVs as pathogenic in 48 cases among 349 cases (13.8%) analyzed in the second screening. None of the pCNVs corresponded to loci of well-established syndromes. This may suggest that our two-stage screening achieved a good balance between rapid screening of known syndromes and investigation of CNV of uncertain pathogenicity.

Table 5 Summary of parental analyses

		Average size (bp)		*The average number of protein-coding genes*
		Min.	*Max.*	
Pathogenic CNVs[a]				
del	23	3 309 267	4 597 689	43
dup	2	1 190 098	1 587 722	61
Total	25	3 139 733	4 356 892	44
Benign CNVs[b]				
del	3	538 481	1 030 504	10
dup	8	334 432	1 740 327	3
Total	11	390 082	1 546 739	5

Abbreviation: CNV, copy-number variant.
[a]Twenty-four *de novo* CNVs and case 48.
[b]Eleven inherited CNVs other than case 48.

Among the cases with parental analyses, the 25 pCNVs had larger sizes and contained more protein-coding genes (average size, 3.1 Mb at minimum to 4.4 Mb at maximum; average number of genes, 44) as compared with the 11 inherited bCNVs that were probably unrelated to phenotypes (average size, 0.39 Mb at minimum to 1.5 Mb at maximum; average number of genes, 5) (Table 5). Although all of the 25 pCNVs except 2 were deletions, about three quarters (8 of 11 cases) of the inherited bCNVs were duplications (Table 5). These findings are consistent with previously reported features of pCNVs and bCNVs.[21,38]

We also compared our current study with recent aCGH studies meeting the following conditions: (1) a microarray targeted to whole genome was applied; (2) patients with MCA and/or MR of uncertain etiology, normal karyotype and the criteria for patients selection were clearly described; (3) pathogenicity of identified CNVs were assessed. On the basis of the above criteria, among studies reported in the past 5 years, we summarized 13 studies (Table 6).[10,14,15,17,54,55,75–81] Diagnostic yield of pCNVs in each study was 6.3–16.4%, and our current diagnostic yield of the second screening was 13.8%. Though cases with subtelomeric aberration detected in the first screening had been excluded, our diagnostic yield was comparable to those of the reported studies. It is not so important to make a simple comparison between diagnostic yields in different studies as they would depend on the conditions of each study, for example, sample size or array resolution,[38,82] however it seems interesting that the higher resolution of a microarray does not ensure an increase in the rate of detection of pCNVs. One recent study showed data that may explain the discrepancy between the resolution of microarray and diagnostic yield.[54,83] The authors analyzed 1001 patients with MCA and/or MR using one

Table 6 Previous studies of analyzing patients with MCA and/or MR using aCGH targeted to whole genome

	Applied array			*Patients*		*Pathogenic CNV*	
Author (year)	*Type*	*Number*[a]	*Distribution*[b]	*Number*	*Type of disorders*	*Number*	*%*
Schoumans *et al.*[75]	BAC	2600	1.0 Mb*	41	MCA and MR	4	9.8
de Vries *et al.*[76]	BAC	32 477	Tiling	100	MCA and/or MR	10	10.0
Rosenberg *et al.*[77]	BAC	3500	1.0 Mb*	81	MCA and MR	13	16.0
Krepischi-Santos *et al.*[78]	BAC	3500	1.0 Mb*	95	MCA and/or MR	15	15.8
Friedman *et al.*[14]	SNP	Affymetrix 100K	23.6 kb**	100	MR	11	11.0
Thuresson *et al.*[79]	BAC		1.0 Mb*	48	MCA and MR	3	6.3
Wagenstaller *et al.*[80]	SNP	Affymetrix 100K	23.6 kb**	67	MR	11	16.4
Fan *et al.*[55]	Oligo	Agilent 44K	24 kb–43 kb**	100[c]	MCA and MR, Autism	15[d]	15.0
Xiang *et al.*[15]	Oligo	Agilent 44K	24 kb–43 kb**	40[e]	MR, DD and autism	3	7.5
Pickering *et al.*[10]	BAC	2600	1 Mb*	354[f]	MCA and/or MR	36[g]	10.2
McMullan *et al.*[17]	SNP	Affymetrix 500K	2.5 kb–5.8 kb**	120	MCA and/or MR	18	15.0
Bruno *et al.*[81]	SNP	Affymetrix 250K	2.5 kb–5.8 kb**	117	MCA and/or MR	18	15.4
Buysse *et al.*[54]	BAC	3431	1 Mb*	298	MCA and/or MR	26	8.7
	Oligo	Agilent 44K	24 kb–43 kb**	703	MCA and/or MR	74	10.5
Our current study	BAC	4523	0.7 Mb	349	MCA and MR	48	13.8
Total				2613		305	11.7

Abbreviations: BAC, bacterial artificial chromosome; CNV, copy-number variant; DD, developmental delay; MCA, multiple congenital anomalies; MR, mental retardation; SNP, single nucleotide polymorphism.
[a]The number of clones or name of array is described.
[b]Each distribution referred to each article (*) or manual of each manufacturer (**).
[c]All cases were analyzed by both a targeted array and a genome-wide array.
[d]In five cases, CNVs were also identified by a targeted array.
[e]Ten cases with an abnormal karyotype were excluded.
[f]Only cases studied with an array throughout the genome are described. Ninety-eight cases were also analyzed by a targeted array.
[g]Seventeen cases with an abnormal karyotype were excluded.