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Genome-Wide Oligonucleotide Array Comparative Genomic Hybridization for Etiological Diagnosis of Mental Retardation

A Multicenter Experience of 1499 Clinical Cases

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To assess the clinical utility of genome-wide oligonucleotide arrays in diagnosis of mental retardation and to address issues relating to interpretation of copy number changes (CNCs), we collected results on a total of 1499 proband patients from five academic diagnostic laboratories where the same 44K array platform has been used. Three of the five laboratories achieved a diagnostic yield of 14% and the other two had a yield of 11 and 7%, respectively. Approximately 80% of the abnormal cases had a single segment deletion or duplication, whereas the remaining 20% had a compound genomic imbalance involving two or more DNA segments. Deletion of 16p11.2 is a common microdeletion syndrome associated with mental retardation. We classified pathogenic CNCs into six groups according to the structural changes. Our data have demonstrated that the 44K platform provides a reasonable resolution for clinical use and a size of 300 kb can be used as a practical cutoff for further investigations of the clinical relevance of a CNC detected with this platform. We have discussed in depth the issues associated with the clinical use of array CGH and provided guidance for interpretation, reporting, and counseling of test results based on our experience. ***(J Mol Diagn 2010, 12:204–212; DOI: 10.2353/jmoldx.2010.090115)***

Mental retardation (MR) and developmental delay (DD) occurs in ~1% of the general population.[1,2] Chromosomal abnormalities are detected in about 3.7% children with global DD using conventional G-banding analysis.[3] Fluorescence *in situ* hybridization (FISH) has enhanced the detection rate but can only target well-characterized microdeletion syndromes and subtelelomeric regions. Genome-wide analysis using array-based comparative genomic hybridization (aCGH) has shown an ~10% of additional diagnostic yield in patients referred for unexplained MR or DD.[4,5]

A number of array platforms are commercially available, and each of them has some unique features in terms of coverage, resolution, and utility.[6] It is now well-known that copy number variants (CNVs) occur with a high frequency in normal human population.[7–9] A higher resolution of an array platform may lead to a higher diagnostic yield but is also associated with a higher detection rate of benign CNVs in patients,[10–12] and this can make interpretation of the aCGH results challenging at a clinical laboratory setting. Currently available databases have been built with information generated from different aCGH platforms, and each laboratory may have developed its own standard for confirmation and reporting.

The platform of Agilent 44K oligonucleotide array has been validated for clinical use in our early studies.[11,13] To further assess the utility of this platform for clinical testing on developmental disorders, we have collected results of 1499 consecutive cases tested from five laboratories. Here we summarize the overall detection rate and the spectrum of pathogenic copy number changes with further discussions on the issues relating to the clinical applications of aCGH.

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Materials and Methods

Patients

Clinical information and aCGH results of 1499 patients were collected from five clinical laboratories at University of Miami Miller School of Medicine (M), Children's Hospital Boston and Harvard Medical School (H), Yale University School of Medicine (Y), Tulane University School of Medicine (T), and University of South Alabama (A). All patients were referred by physicians as part of clinical assessment of unexplained MR or DD with or without dysmorphic features. Conventional chromosome analysis was performed in most of these cases with a normal karyotype reported. Patients with an indication of autism, pervasive developmental disorder or Asperger syndrome have not been included and will be dealt with separately.

The aCGH Analysis

The standard Agilent 44K platform (Agilent Technologies, Santa Clara, CA) has been used by all five laboratories. This platform is composed of 44,290 60-mer oligonucleotide probes for the mapped genes or unique DNA sequences with an average spatial resolution of ~30–35 kb. It was noted that this platform lacks coverage for the pseudoautosomal regions of the X and the Y chromosomes. An aCGH test was performed using the standardized protocol as recommended by the manufacturer as described previously.[11] Briefly, patient and reference DNA samples were differentially labeled and cohybridized to Agilent 4 × 44K arrays. Reference DNA samples were prepared either from apparently healthy individual (mixture of four to eight same gender individuals) or purchased (Promega, Madison, WI). Hybridized slides were scanned with microarray scanner (Agilent G2505B or Axon Genepix 4200A), and the image data were extracted and converted to text file with Feature Extraction (Agilent Technologies). DNA Analytics 4.0 (Agilent Technologies) was used to plot the $\log_2$ ratio of signal intensity of each probe across the whole genome. The copy number data were visualized along each chromosome with correspondent National Center for Biotechnology Information annotated gene information for each probe. For copy number change (CNC) identification, we used DNA Analytics build-in aberration detection algorithm followed by visual inspection. Each lab performed technical validation of the platform before using it as a diagnostic tool. A consensus cutoff for recording an alteration was a CNC involving ≥3 consecutive probes based on our previous validation studies.[11]

Criteria for Result Interpretation

We used the term "CNC" to describe a DNA copy alteration observed, the term "pathogenic CNC" to describe a CNC associated with abnormal phenotypes and the term "CNV" for a benign CNC. These labs have used the following consensus criteria for interpretation of pathogenic CNCs versus benign variants: A CNC is considered likely to be pathogenic if 1) it involves a region known to be associated with a microdeletion or microduplication syndrome, 2) it is inherited from a similarly affected parent, 3) it involves known dosage sensitive gene(s), or 4) it is a multigene imbalance, either *de novo* or inherited from a parent as a product of segregation of a balanced translocation/insertion or recombination of an inversion. In contrast, a CNC is considered likely to be benign if 1) it is a known CNV in normal populations, 2) it is inherited from a normal parent, or 3) it does not involve the regions associated with known microdeletion or microduplication syndromes or dosage-sensitive genes. Possible exceptions are described in *Discussion*. We have used Database of Genome Variants (http://projects.tcag.ca/variation/, July 24, 2009) as a reference for currently known CNVs in normal individuals, DECIPHER (DatabasE of Chromosomal Imbalances and PHenotypes using Ensembl Resources) (www.sanger.ac.uk/PostGenomics/decipher/, July 24, 2009) for currently known microdeletion and microduplication syndromes and Online Medelian Inheritance in Man (www.ncbi.nlm.nih.gov/Omim/getmorbid.cgi, July 24, 2009) for currently known disease-causing genes, gene functions, and inheritance patterns. Literature search on a particular CNC was also an important step to determine its clinical significance. A final result was reported as abnormal when a CNC was interpreted as pathogenic or normal when no alteration was detected or observed alterations were judged as benign. The term "variant of unknown clinical significance" was used when an imbalance was >300 kb involving multiple genes, but the significance of the imbalance could not be determined based on available knowledge and family studies.

Table 1. Array CGH Results from Five Labs

Lab	No. of patients	No. patients with pathogenic CNCs	Diagnostic yield (%)
M	545	74	14
Y	275	38	14
H	319	21	7
T	227	25	11
A	133	19	14
Total	1499	177	12

M, University of Miami Miller School of Medicine; H, Children's Hospital Boston and Harvard Medical School; Y, Yale University School of Medicine; T, Tulane University School of Medicine; and A, University of South Alabama.

Table 2. Size Distribution of Pathogenic Imbalances

Size of imbalance	No. cases with imbalances	Percentage
>10 Mb	50	28.25
5 to ~10 Mb	33	18.64
1 to ~5 Mb	71	40.11
0.5 to ~1 Mb	15	8.47
0.3 to ~0.5 Mb	7	3.95
<0.3 Mb	1	0.56
Total	177	100

Table 3. Pathogenic CNCs Involving a Single DNA Segment

Patient	Imbalance	Size (kb)	ISCN description	NCBI build
A0024	del	1,060	1q21.1(143789559-144849923)x1	hg18
M0894	del	1,170	1q21.1(145031367-146201635)x1	hg18
A0021	dup	14,762	1q42.13q43(225784048-240546387)x3	hg18
M0516	dup	1,883	1 q42.2q43(233617930-235500555)x3	hg18
M1006	del	837	2p14p14(67241932-68078842)x1	hg18
M0099	del	13,769	2q14.1q14.3(115776113-129545173)x1	hg17
Y0008	del	13,698	2q24.3q31.1(162106534-175804618)x1	hg17
M1060	del	17,759	2q31.12q32.2(171993989-189752539)x1	hg18
T0074	del	12,456	3p26.3p25.1(224527-12680213)x1	hg17
Y0295	dup	40,927	3p22.1(224727-41152166)x3	hg17
H0243	del	2,090	3p21.31(45998019-48087642)x1	hg18
A0005	del	10,147	3q22.3q24(138095979-148242751)x1	hg18
M0773	dup	51,470	4q31.1q35.2(139651136-191121344)x3	hg18
Y0068	dup	6,457	4q35.1q35.2(184033371-190490075)x3	hg17
Y0173	del	3,781	5p15.33(148243-3929457)x1	hg17
M0717	dup	964	5p13.3(31244617-32209107)x3	hg18
A0027	dup	5,069	5q13.1q13.3(68203607-73272937)x3	hg18
M0254	dup	5,025	5q14.3q15(87529305-92554625)x3	hg18
H0181	del	8,761	5q23.1q23.3(119295079-128055733)x1	hg18
M0381	del	5,299	6q14.3q15(86137153-91436233)x1	hg18
T0018	del	10,481	6q16.1q21(95641152-106121873)x1	hg17
M0109	del	19,086	6q22.1q22.32(107058287-126144058)x1	hg17
M0840	dup	20,097	6q23.3q25.3(135720981-155817943)x3	hg18
Y0078	dup	14,601	6q24.1q25.3(142510586-157111635)x3	hg17
Y0004	del	9,452	6q26q27(161357458-170809875)x1	hg17
Y0014	del	7,953	6q26q27(162903508-170856715)x1	hg17
M0866	del	903	6q27(169830830-170734227)x1	hg18
Y0033	del	7,880	7p21.1p15.2(18809907-26689559)x1	hg17
M0975	del	3,048	7p21.1p15.3(19151860-22199856)x1	hg18
A0008	del	19,584	7p21.3p15.2(6275894-25859565)x1	hg17
Y0519	del	946	7p15.1(29959761-30905557)x1	hg17
M0531	del	11,444	7p15.3p14.3(20567604-32011280)x1	hg18
T0009	del	3,976	7q11.22q11.23(69801305-73777467)x1	hg17
Y0148	dup	457	7q11.23(72171229-72628014)x3	hg17
Y0431	dup	392	7q11.23(72723421-73115544)x3	hg17
Y0298	del	5,058	7q22.1q31.1(103693910-108751427)x1	hg17
M0979	dup	1,397	7q36.3(157384149-158781397)x3	hg18
M0300	del	2,995	8p23.3p23.2(181530-3176742)x1	hg18
M0798	del	4,602	8q22.3q23.1(102281230-106883502)x1	hg18
A0016	del	7,565	9p24.3p24.1(204367-7769314)x1	hg18
M0112	del	3,598	9q33.3q34.11(124897698-128495943)x1	hg17
Y0059	del	506	9q34.3(137723586-138229984)x1	hg17
M0266	del	5,773	10q25.3q25.2(112249547-118022746)x1	hg18
Y0534	del	13,168	10q26.12q26.3(122125789-135293404)x1	hg17
M0650	dup	1,574	10q26.3(132780691-134355172)x3	hg18
M0681	dup	376	11p15.4(6239388-6615806)x3	hg18
M1015	dup	1,823	11p15.5p15.4(1515185-3338575)x3	hg18
M0522	del	6,836	11p15.4p15.1(9967639-16803929)x1	hg18
M1003	dup	2,591	11q22.1q22.3(100828925-103420043)x3	hg18
Y0207	del	5,708	12p13.2p12.3(11678312-17386429)x1	hg17
M0702	del	12,758	13q33.1q34(101319059-114077122)x1	hg18
Y0039	del	13,239	13q33.1q34(100838094-114077122)x1	hg17
A0006	del	1,091	14q21.1q21.2(41116021-42206812)x1	hg18
M0152	del	1,150	14q32.2q32.31(99330427-100480657)x1	hg17
Y0048	dup	5,791	15q11.2q13.1(21208344-26999801)x3	hg17
Y0528	dup	7,891	15q11.2q13.1(19109124-26999801)x3	hg17
M0028	del	3,042	15q24.1q24.2(70764625-73807021)x1	hg17
T0001	del	3,292	15q26.1q26.2(89197342-92489641)x1	hg17
T0059	del	2,234	16p13.12p13.11(13949643-16183616)x1	hg17
H0221	del	166	16p13.3(2005613-2171887)x1	hg18
M0742	dup	3,392	16p13.11p12.3(15062188-18454260)x3	hg18
H0195	del	446	16p11.2(29581455-30027260)x1	hg18
H0264	del	446	16p11.2(29581455-30027260)x1	hg18
M0961	del	523	16p11.2(29581455-30104842)x1	hg18
M0480	del	525	16p11.2(29581455-30106101)x1	hg18
M0611	del	525	16p11.2(29581455-30106101)x1	hg18
H0338	dup	544	16p11.2(29560500-30104842)x3	hg18
H0367	del	546	16p11.2(29560500-30106852)x1	hg18
H0240	dup	659	16p11.2(29581455-30240082)x3	hg18

(*table continues*)

Table 3. *Continued*

Patient	Imbalance	Size (kb)	ISCN description	NCBI build
T0008	dup	16,113	16q22.3q24.3(72538455-88651780)x3	hg17
H0301	del	5,077	16q23.1q23.3(76508445-81585846)x1	hg18
H0376	del	1,207	17p13.3(48539-1255546)x1	hg18
T0017	del	4,132	17p13.3p13.2(116343-4248317)x1	hg17
M0481	dup	547	17p13.3(183662-730351)x3	hg18
Y0313	dup	2,493	17p13.2p13.1(6277211-8769730)x3	hg17
M0280	dup	319	17p13.1(9989893-10308758)x3	hg18
H0302	del	1,383	17p12(14040843-15423597)x1	hg18
M0855	del	1,380	17p12(14052497-15432473)x1	hg18
Y0273	del	3,590	17p11.2(16543855-20133761)x1	hg17
H0113	del	3,618	17p11.2(16543855-20162287)x1	hg18
H0194	dup	3,590	17p11.2(16644378-20234630)x3	hg18
H0074	dup	3,590	17p11.2(16543855-20133761)x3	hg18
H0371	del	1,288	17p11.2(16723271-18010992)x1	hg18
Y0494	dup	1,351	17q12(31891535-33242217)x3	hg17
M0761	del	627	17q21.3(41073486-41700815)x1	hg18
A0028	dup	14,749	18 p11.32p11.2(170229-14918854)x3	hg18
M0673	del	7,267	18q12.3q21.1(37556785-44824255)x1	hg18
M0482	del	4,391	18q12.3q21.2(38169137-42560421)x1	hg18
Y0489	del	2,719	18q21.2(48686656-51405390)x1	hg17
M0523	del	4,818	18q22.3q23(71264994-76083117)x1	hg18
M0262	del	511	19p13.3(737550-1248499)x1	hg18
M0015	del	3,459	19p13.2(10291150-13749674)x1	hg17
M0308	dup	733	19p13.2(10128082-10861364)x3	hg18
M0680	del	8,231	19q12q13.13(34854071-43085470)x1	hg18
Y0005	del	7,284	21q22.3(39608300-46892294)x1	hg17
M0054	del	6,182	21q21.3q22.12(28802339-34984201)x1	hg17
T0062	dup	14,789	21q22.11q22.3(32103127-46892494)x3	hg17
T0050	dup	2,268	22q11.21(17021209-19289184)x3	hg17
M0541	del	1,118	22q11.21q11.22(20128705-21246612)x1	hg18
A0029	dup	1,994	22q11.22q11.23(21322838-23316556)x3	hg18
T0014	del	8,003	22q13.2q13.33(40993242-48996488)x1	hg17
M0491	dup	3,144	Xp22.33(000000-3144100)x3*	hg18
M0958	dup	151,784	Xp22.33q28(2710316-154494649)x3	hg18
T0049	del	1,431	Xp22.31(6560955-7992261)x1	hg17
Y0268	del	1,525	Xp22.31(6317139-7841856)x1	hg17
Y0497	del	1,431	Xp22.31(6410891-7841856)x1	hg17
T0038	del	1,431	Xp22.31(6560955-7992261)x1	hg17
M0849	dup	4,705	Xp11.23p11.22(48005240-52710691)x3	hg18
Y0357	del	5,224	Xp11.2(43850835-49074718)x1	hg17
A0013	dup	9,370	Xp22.11p21.1(22924079-32294279)x3	hg18
H0121	del	2,734	Yp11.2(6457810-9191638)x1	hg18

NCBI, National Center for Biotechnology Information; ISCN, International System for Human Cytogenetic Nomenclature (2009).
The cases with common microdeletion syndromes involving 1p36, 4p16, 7q11.23, 15q11.2, and 22q11.2 have been excluded from this table.
*Duplication of the terminal region. Upper breakpoint could not be assigned due to lack of probe coverage for the region.

Results

Results on a total of 1499 consecutive proband patients including previously published 150 cases[11,13] were collected (Table 1). Pathogenic CNCs were reported in 177 patients representing an overall diagnostic yield of 12% with a variation from 7 to 14%. variant of unknown clinical significance was reported ~5% of cases (Supplemental Table S1, see *http://jmd.amjpathol.org*). One or more benign CNVs have been reported in ~48% of the patients. The sizes of the pathogenic imbalances varied from 166 Kb to152 Mb (Tables 2–4). For the compound imbalances, this size represented the total size of two or more DNA segments. The imbalances were >500 kb in the vast majority of abnormal cases (95.5%). Pathogenic CNCs <500 kb but >300 kb were detected in a small number of patients (4.5%). It is very important to find out the functions of the genes involved in a small CNC, particularly when it is not a known CNV and parental studies cannot be completed. For example, we reported a CNC <300 kb as pathogenic in a patient because of deletion of the *TSC2* gene. Pathogenic imbalances >10 Mb were detected in 28% of patients, and most of them did not have a conventional chromosome study before aCGH. We noticed that deletions or duplications >10 Mb were missed by chromosome karyotyping in several patients. A significant proportion (19%) of pathogenic CNCs had a size of 5 to ~10 Mb (Table 2). However, we could not determine the percentage of the cases missed by chromosome analysis, because in many cases, the initial karyotyping was performed outside of the five laboratories. A few cases with a chromosomal aneuploidy were observed but were not included in this article.

We classified the 177 cases with pathogenic CNCs into two groups. The first group had a gain or loss of a single DNA segment (141 of 177, 80%; Table 3). Among them, 46 patients had one of the previously well-known

Table 4. Pathogenic CNCs Involving Two or More DNA Segments

Patient	Imbalance	Size (kb)	ISCN description	NCBI build	Classification
M099	del/dup	4,134/2,164	1p36.33p36.32(799622-4933181)x1/ Xq28(152241308-154405159)x3	hg18	2
A011	del/dup	9,275/5,885	1q43q44(236147009-245422419)x1/ 9p24.3p24.1(204367-6089101)x3	hg17	2
M183	del/dup	1,496/1,718	2p25.3(29193-1525513)x1/ 4q13.2q13.3(69802468-71520579)x3	hg18	3
M127	del/dup	3,471/6,495	4p16.3p16.2(62447-3533180)x1/ 7p22.3p22.1(149268-6644183)x3	hg18	2
M564	del/dup	4,349/1,397	4p16.3p16.2(62447-4411346)x1/ 7q36.3(157384149-158781397)x3	hg18	2
M192	del/del	2,132/1,431	4q31.21q31.22(144878812-147011076)x1/ Xp22.31(6561155-7992120)x1	hg18	6
T013	del/dup	28,400/11,947	4q32.2q35.2(162914399-191314689)x1/ 3q27.2q29(187378521-199325500)x3	hg17	2
M470	del/dup	5,739/9,993	5p15.33p15.32(148243-5887664)x1/ 16q23.2q24.3(78658713-88651780)x3	hg18	2
T037	del/dup	1,872/27,999	6p25.3(204328-2076297)x1/ 9p24.3p21.1(204167-28203565)x3	hg17	2
M411	del/dup	9,362/2,984	6p25.2p24.1(4014025-13376010)x1/ 6p24.1p22.3(13419730-16403770)x3	hg18	4
M347	del/dup	3,181/5,277	7p22.3p22.2(149268-3330301)x1/ 9q34.13q34.3(134852111-140128736)x3	hg18	3
Y451	del/dup	14,193/3,283	7p21.3p15.3(7407526-21600331)x1/ 7p22.3p22.2(149268-3431999)x3	hg17	4
M119	del/dup	7,702/6,748	7q36.1q36.3(150706898-158409214)x1/ 22q13.31q13.33(42720092-49468408)x3	hg18	2
M449	del/dup	3,918/5,822	7q36.2q36.3(154684956-158602499)x1/ 9q34.13q34.3(134251761-140073968)x3	hg18	2
M479	del/dup	6,720/17,530	8p23.1p23.3(181530-6901486)x1/ 8p12p23.1(12627630-30157579)x3	hg18	4
Y222	dup/dup	42,685/40,034	8p23.31p11.2(181530-42866112)x3/ 9p24.3p12(204367-40238102)x3	hg17	2
Y006	del/dup	7,109/10,669	8p23.3p23.1(181530-7290597)x1/ 8p21.3p23.1(12285464-22954412)x3	hg17	4
M323	del/dup	4,366/38,156	9p24.2p24.1(4142060-8508353)x1/ 9q13q31.2(70225166-108381595)x3	hg18	5
T019	del/del	14,426/470	9p22.3p24.3(204367-14629971)x1/ 9q34.11(129926621-130396376)x1	hg17	6
A018	del/dup	2,683/58,847	10q26.3(132610756-135293404)x1/ 4q28.3q35.2(132273948-191121344)x3	hg18	2
A002	del/dup	1,422/5,589	10q26.3(133871004-135293404)x1/ 17q25.3(73034631-78623230)x3	hg17	2
Y279	dup/dup	17,723/4,253	11q23.3q25(116228648-133951370)x3/ 22q11.1q11.2(14433473-18686317)x3	hg17	6
M613	del/dup	5,607/18,843	12p13.33p13.31(179323-5786793)x1 15q25.2q26.3(81325482-100168718)x3	hg18	2
Y017	del/dup	10,269/3,107	13q33.2q34(103855141-114123908)x1/ 1p36.3(807922-3914685)x3	hg17	2
M160	del/dup	1,300/1,178	14q13.2q13.3(35053035-36352972)x1/ 9p22.1p22.2(17492885-18671089)x3	hg18	3
A014	dup/dup	9,791/11,936	15q11.2q13.3(18362555-28153357)x3/ 15q11.2q13.3(18362555-30298096)x3	hg17	6
T058	del/dup	86/49,036	18p11.32(170029-255680)x1/ 18q12.11q23(27047211-76083258)x3	hg17	5
H177	del/dup	13,535/1,714	18p11.32p11.21(121700-13656290)x1/ 18p11.21(13656231-153706830)x3	hg18	4
T074	del/dup	2,943/8,234	18q23(73139833-76083258)x1/ 7p22.3p21.3(1490689-9724287)x3	hg17	3
H158	del/dup	5,545/20,681	18q22.3q23(70565916-76110964)x1/ 13q31.3q34(93433835-114114568)x3	hg18	2
Y199	del/dup	2,477/44,675	21q21.1(13926078-16402867)x1/ 13q11.1q21.31(18650699-63325724)x3	hg17	3
M483	del/dup	11,797/1,121	21q22.12q22.3(35084120-46880878)x1/ 21q22.11q22.12(33918888-35040082)x3	hg18	4
M295	del/dup	7,830/1,137	22q13.2q13.33(41219395-49049035)x1/ 22q13.2(40003772-41140907)x3	hg18	4

(*table continues*)

Table 4. *Continued*

Patient	Imbalance	Size (kb)	ISCN description	NCBI build	Classification
Y001	del/dup/dup	35,770/3,767/2,165	Xp22.33p11.4(2693677-38463601)x1/ 9q34.13q34.3(134517453-138284752)x3/ 22q11.2(17539511-19704512)x3	hg17	6
M111	del/dup	3,735/61,522	Xq28(150669991-154405159)x1/ 7q21.3q36.3(96887100-158409214)x3	hg18	2
T040	del/dup	4,020/25,202	Yq11.222q11.223(19263493-23283748)x1/ 7q33q36.3(133400864-158602499)x3	hg17	2

NCBI, National Center for Biotechnology Information; ISCN, International System for Human Cytogenetic Nomenclature (2009).

microdeletion/duplication syndromes involving 1p36, 4p16, 7q11, 15q11, 17p11, 17p13, 22q11, and Xp22 region. The remaining cases of the group had recently recognized syndromes or novel pathogenic imbalances, including the imbalances of the16p11.2 region in eight patients (six deletions and two duplications, 0.5% of the total patients) with a size ranging from ~450 to 660 kb. The second group (36 of 177, 20%) had an imbalance involving two DNA segments except for a single case with more complex alterations (Table 4). These two groups were further divided into six classes: 1) deletion or duplication of a single DNA segment; 2) a deletion of the terminal region of one chromosome and a duplication of the terminal region of another chromosome; 3) a deletion in one chromosome and a duplication in another with at least one interstitial alteration; 4) a deletion and a duplication involving a contiguous genomic region within the same chromosome arm; 5) a deletion and a duplication on different arms of the same chromosome; and 6) others including more complex structural changes and mosaicism (Figure 1). We noted that four of these patients had both a chromosomally visible alteration and a submicroscopic imbalance <4 Mb (cases T036, Y451, T019, and A018; Table 4).

Discussion

Our results derived from a cohort of 1499 proband patients with MR showed an overall diagnostic yield of 12% (Table 1). With use of the same platform and consensus analytical criteria, a diagnostic yield of 14% was achieved in three of five laboratories. One lab had a diagnostic yield of 11%. A molecular genetics lab had a significantly lower detection rate (7%), most likely due to less stringent referring criteria and exclusion of chromosomal abnormalities. A review[5] on the results of a total of 1364 patients collected from 17 published reports, including 973 patients studied with genome-wide BAC arrays (11 reports) and 391 patients studied with genome-wide oligonucleotide arrays (6 reports) showed that oligonucleotide-based arrays appear to give a higher diagnostic yield than the BAC-based arrays (14.83 versus 9.76%). Our results are consistent with the reported data on genome-wide oligonucleotide arrays.

We have categorized the pathogenic imbalances detected in 177 patients into two major groups: one (141 patients) had a simple single DNA segment deletion or duplication that can be terminal or interstitial (Table 3); the second group (36 patients) had compound imbalances involving two or more DNA segments (Table 4). Forty-six patients in the first group had imbalances associated with common microdeletion or microduplication syndromes, and 33 patients in the second group had imbalances involving the terminal regions. All these genomic imbalances (79 of 177, 45%) are detectable by FISH using commercially available probes. In a report on 1500 consecutive patients tested with an array targeting the common microdeletion syndromes and the subtelomeric regions, 5.6% showed clinically relevant genomic alterations.[14] A detection rate of 9.8% was achieved

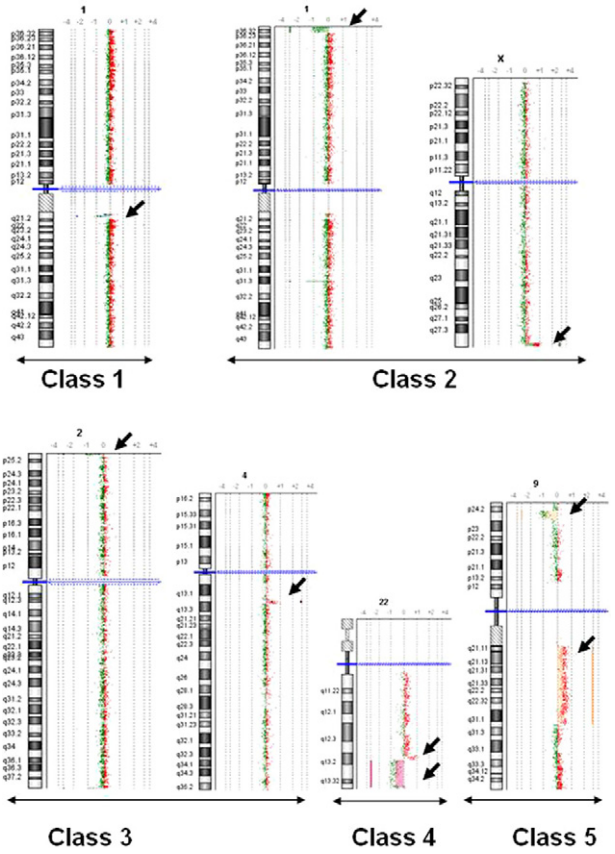

Figure 1. Exemplary array CGH plots of pathogenic CNCs. Class 1: a deletion or duplication of a single DNA segment; class 2: a deletion of the terminal region of one chromosome and a duplication of the terminal region of another chromosome; class 3: a deletion in one chromosome and a duplication in another with at least one interstitial alteration; class 4: a deletion and a duplication involving a contiguous genomic region within the same chromosome arm; class 5: a deletion and a duplication on different arms of the same chromosome; and class 6: represents a heterogeneous group of compound imbalances, not included in this figure.

when a targeted array was used in conjunction with a low-resolution BAC clone-based genome-wide array in a cohort of 1176 patients.[15] Our collective data have clearly demonstrated the diagnostic efficacy of the 44K genome-wide olionucleotide array. Recently, the 44K oligonucleotide array has been customized with enriched probes for the genes or regions known to be associated with phenotypic anomalies.[16] The customized array may reveal subtle copy number changes in the targeted regions and therefore enhances detection rate. This platform has been used in one of the five laboratories recently, and deletion of a single gene, *UBE3A*, has been detected in 1 of 16 patients studied (data not included). A larger study population to assess the clinical utility of the customized 44K platform is desirable.

The diagnostic yield is also determined by other variables such as patient selection, array resolution, and the genomic coverage of the array used. As aCGH is used widely in genetics laboratories, the criteria for patient selection will be less stringent as we have experienced in subtelomere FISH studies in the past years. Arrays have been designed with spatial resolutions ranging from 1 Mb to <1 kb,[6] and the latest oligonucleotide array platforms made by major commercial manufacturers have ranged from 13 kb to 700 bp with multiple sets and formats.[17,18] The Agilent 44K array used by our laboratories has a spatial resolution of 30 to 35 kb. Previous studies have used oligonucleotide arrays with a resolution of 30 kb, and the vast majority of reported pathogenic CNCs were >500 kb.[11,12,19,20] Similarly, our current large cohort has shown that 95.5% of confirmed pathogenic CNCs are >500 kb; 3.95% are 300 to ~500 kb and a single case (0.56%) with a size <300 kb (Table 2). Our data and the previous reports provide good evidence that a size of 300 kb can be used as a cutoff for further investigations of the clinical relevance of a CNC observed with this platform although a smaller imbalance can be pathogenic. The use of a genome-wide array with an overall spatial resolution <30 kb may increases diagnostic yield but also reveals many more benign CNVs. A reliable database for benign CNVs and software for screening these CNVs appear to be prerequisite for clinically implementing genome-wide arrays with a resolution >30 kb.

The pathogenic CNCs detected in this cohort were unevenly distributed throughout the genome with clusters in 1p36, 4p16, 7pter-q11, 15q12-13, 16p, 17p, 18q, 22q11, and Xp. Approximately 45% of the pathogenic CNCs involved the regions associated with the previously known common microdeletion or microduplication syndromes and the subtelomeric regions. The remaining pathogenic CNCs may represent emerging recurrent syndromes or novel pathogenic genomic imbalances. Of particular interests, we observed two cases (A0024 and M0894; Table 3) with a 1q21.1deletion that has been recently described as a recurrent genomic aberration associated with variable phenotypes.[21] We had one case (M0761; Table 3) with a 17q21.3 deletion that was reported as a recurrent microdeletion associated with a common inversion polymorphism.[22,23] One case had a deletion of 15q24 (M0028; Table 3) that was previously detected by FISH in several cases.[24] Most significantly, we have detected imbalances of the16p11.2 region in eight patients (0.5%) with a size ranging from ~450 to 660 kb. Deletion of 16p11.2 was reported to be associated with variable phenotypes including MR or DD and autism spectrum disorders.[25–27] Our results showed that the imbalance of 16p11.2 represents one of the most frequent microdeletion/microduplication syndromes associated with MR or DD.

Approximately 80% of the pathogenic CNCs involved a deletion or duplication of a single DNA segment (Table 3). The remaining 20% had alterations of two DNA segments or occasionally more than two DNA segments or chromosomes (Figure 1). Similar to conventional cytogenetics, a single deletion or duplication could be the result of the segregation of a balanced insertion. A deletion and a duplication in the same patient could be derived from a balanced translocation. The finding of both deletion and duplication on the same chromosome can be caused by an inversion in a parent. For genetic counseling purposes, we have recommended a conventional karyotyping for the parents to rule out a balanced translocation, inversion, or insertion when an alteration was likely to be chromosomally visible or FISH when an alteration involved subtelometic regions or known microdeletion regions. Parental studies are critical in determining the clinical significance of novel CNCs. Classification of the pathogenic CNCs and discussion of the possible mechanism in the report can provide useful information for genetic counseling. On the other hand, our results also shown that a submicroscopic deletion or duplication may exist and contribute to abnormal phenotypes in the cases with structural abnormalities detected with G-banding analysis. Further characterization of the submicroscopic genomic variants may help us to better understand the architecture of the genome such as inversions or presents of low copy number repeats.[28]

All five laboratories have used the same criteria and process, which are similar to those previously described[11,29] for interpreting the clinical relevance of CNCs. However, clinical correlation can be challenges in some patients, such as 1) the inheritance of a CNC may not be determined due to lack of parental samples; 2) a CNC inherited from a normal parent can be pathogenic due to incomplete penetrance or variable expressivity; 3) a small deletion or duplication can be pathogenic when a critical gene is involved; 4) a variant deletion in normal individuals can be pathogenic by unmasking a recessive gene mutation in a patient; and 5) a CNC involving an autosomal dominant gene may not be pathogenic depending on the nature of the function of the gene. For example, one of our patients had a 4.8 Mb of deletion in 18q22.2q23 (M0523; Table 3) with global DD, growth retardation, MR, hypotonia, genitourinary anomalies, and dysmorphic features. The same deletion was found in her mother who had a graduate degree in teaching and music and had no dysmorphic features except for bifid uvula. We reported this deletion as pathogenic on the bases of the gene content and patient's phenotypes, which were consistent with the 18q deletion syndrome. We hypothesized that a mosaicism might be the explanation for the normal mother but could not confirm it. This

case has illustrated the complex nature in genotype-phenotypes correlation. The phenotype variability was also described in the patients with 1q21 deletion[21,30] and 16p11.2 deletions.[26]

In summary, our collective data has shown that genome-wide oligonucleotide array Agilent 44K has provided reasonable resolution for clinical use while an array with a higher resolution particularly for the regions containing known disease genes is desirable. Using 300 kb as a cutoff for further investigation of the clinical relevance appears to be a practical approach when a CNC is observed using this platform. We have confirmed that 16p11.2 deletion is a frequent cause of MR or DD. We stress that appropriate interpretation of array CGH results is critical and a discussion on the structural type of a pathogenic CNC is useful for counseling and patient management. Understanding of the complex genotype-phenotype relationship and reviewing currently available literatures on particular genomic alterations are necessary for appropriate clinical correlation of genomic alterations observed.

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