

Variability in interpreting and reporting copy number changes detected by array-based technology in clinical laboratories

Karen D. Tsuchiya, MD¹, Lisa G. Shaffer, PhD², Swaroop Aradhya, PhD³, Julie M. Gastier-Foster, PhD⁴, Ankita Patel, PhD⁵, M. Katharine Rudd, PhD⁶, Julie Sanford Biggerstaff, PhD⁷, Warren G. Sanger, PhD⁸, Stuart Schwartz, PhD^{9,10}, James H. Tepperberg, PhD⁹, Erik C. Thorland, PhD¹¹, Beth A. Torchia, PhD², and Arthur R. Brothman, PhD¹²

Purpose: The purpose of this study was to assess the variability in interpretation and reporting of copy number changes that are detected by array-based technology in the clinical laboratory. **Methods:** Thirteen different copy number changes, detected by array comparative genomic hybridization, that have not been associated with an abnormal phenotype in the literature were evaluated by directors from 11 different clinical laboratories to determine how they would interpret and report the findings. **Results:** For none of the thirteen copy number changes was there complete agreement in the interpretation of the clinical significance of the deletion or duplication. For some cases, the interpretations ranged from normal to abnormal. **Conclusion:** There is a need for more specific guidelines for interpreting and reporting copy number changes detected by array-based technology to clearly and more consistently communicate the clinical significance of these findings to ordering providers. *Genet Med* 2009;11(12):866–873.

Key Words: array CGH, deletion, duplication, interpretation, reporting

The use of microarrays for the assessment of copy number changes (CNCs) in individuals with constitutional disorders is becoming widespread in clinical cytogenetic laboratories. Although this technology has significantly increased the detection of chromosome imbalances that are known to be associated with abnormal phenotypes, the finding of CNCs in healthy individuals (also known as copy number variants or CNVs) has created new challenges for laboratorians and clinicians who must interpret the clinical significance of previously undescribed CNCs. In clinical laboratories using high-density oligo-nucleotide or single nucleotide polymorphism arrays, multiple CNCs can be detected in every patient. Although some of these CNCs are clearly not responsible for a patient's abnormal phenotype based on the frequency with which they are detected

in normal control populations, determination of the clinical significance of individually rare CNCs can be much more difficult.

Recently, approaches for evaluating the pathogenicity of CNCs in the diagnostic setting have been proposed.^{1–3} One of the most useful criteria for distinguishing a benign from a pathogenic CNC is whether it is de novo or inherited. In general, with the exception of CNCs that demonstrate variable expressivity and decreased penetrance, de novo CNCs are more likely to be pathogenic compared with CNCs inherited from a phenotypically normal parent. However, in a clinical setting, an initial decision regarding the likely pathogenicity of a CNC must be rendered to determine if parental testing should even be pursued. If a CNC is likely benign based on other criteria, parental testing may be deemed unnecessary. Also, it may be impractical to delay reporting until parental specimens are received, and one or both parents may never be available for testing. Therefore, it is often necessary to make an initial categorization of a CNC based on other criteria. These other criteria that have been previously discussed include cross-referencing the change to internal or publicly available databases of known benign CNCs, the size and gene content of the CNC, and whether the CNC is a duplication or deletion.^{1–6} Even when these criteria are used, there is likely to be variability between laboratories not only in terms of which CNCs they decide to report but also in how these CNCs are reported. Internal databases of benign CNCs will vary depending on the volume of testing that a laboratory performs and the platform that the laboratory is using. There will also be subjectivity in evaluating whether specific genes may contribute to a phenotype, in determining the importance of the size of a CNC, and in how much emphasis to place on duplications versus deletions.

From the ¹Department of Laboratories, Seattle Children's Hospital, and Department of Laboratory Medicine, University of Washington School of Medicine, Seattle, Washington; ²Signature Genomics Laboratories, Spokane, Washington; ³GeneDx, Gaithersburg, Maryland; ⁴Department of Pathology and Laboratory Medicine, Nationwide Children's Hospital and Departments of Pathology and Pediatrics, The Ohio State University, Columbus, Ohio; ⁵Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, Texas; ⁶Department of Human Genetics, Emory University School of Medicine, Atlanta, Georgia; ⁷Idaho Cytogenetics Diagnostic Laboratory, Boise, ID and Providence Associates Medical Laboratories, Spokane, Washington; ⁸Department of Pediatrics/Munroe-Meyer Institute, University of Nebraska Medical Center, Omaha, Nebraska; ⁹Laboratory Corporation of America, Research Triangle Park, North Carolina; ¹⁰Previous affiliation, Department of Human Genetics, University of Chicago, Chicago, Illinois; ¹¹Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota; and ¹²ARUP Laboratories, Department of Pathology, University of Utah, Salt Lake City, Utah.

Karen Tsuchiya, MD, Department of Laboratories, A-6901, P.O. Box 5371, Seattle, WA 98105-0371. E-mail: karen.tsuchiya@seattlechildrens.org.

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (www.geneticsinmedicine.org).

Disclosure: Lisa G. Shaffer, PhD is co-founder, sits on the Members' Board and has ownership in Signature Genomic Laboratories, LLC. Swaroop Aradhya, PhD is a director of clinical cytogenetics at GeneDx, a subsidiary of BioReference laboratories.

Submitted for publication July 7, 2009.

Accepted for publication September 11, 2009.

Published online ahead of print November 6, 2009.

DOI: 10.1097/GIM.0b013e3181c0c3b0

Variability in the interpretation of sequence variants became apparent after the introduction of full gene sequencing in the clinical laboratory. This variability has led to the development of recommendations for standards for interpretation and reporting of sequence variations by the American College of Medical Genetics and other working groups.^{7,8} Array-based copy number assessment is still relatively new to clinical cytogenetics, and a recent proficiency survey implemented by the College of American Pathologists (CAP) indicates that interpretation of CNCs detected by array-based technology may be just as problematic as sequence variants.⁹ This proficiency survey involved copy number assessment of an individual with autism spectrum disorder. While participants were asked to list only clinically significant abnormalities in their responses, 76% reported only a gain on 15q and 21% reported both a gain on 15q and a loss on 22q. The duplication in 15q consisted of the 15q11.2q13 region that has been reported in approximately 3% of patients with autism spectrum disorder. The deletion in 22q11.2, which is distal to the DiGeorge/velocardiofacial syndrome critical region, was designated a benign CNV by the majority (75%) of participants in a supplemental question. Therefore, although the 22q11.2 CNC was considered to be benign by the majority of participating laboratories, a fairly high percentage of participants reported it as a clinically significant CNC. The purpose of this study was to better document the variability between laboratories in the interpretation of CNCs detected using array-based technology and to ascertain the specific criteria that different laboratories are using for determining the clinical significance of CNCs.

MATERIALS AND METHODS

Information from bacterial artificial chromosome (BAC) and oligonucleotide array cases was provided to clinical laboratories that perform array-based CNC assessment for diagnostic purposes. Information on a single CNC from each case was given to participants who were asked to assess the CNC for pathogenicity as they normally would in their daily practice. All of the cases were originally received and processed in a single laboratory for diagnostic testing. The CNCs that were chosen for this study did not represent a random sampling of results from this laboratory, but rather, they were chosen because they potentially posed an interpretive challenge in terms of their clinical significance. The CNCs consisted of both duplications and deletions and represented a range of sizes. Some of the CNCs were not visualized by fluorescence in situ hybridization (FISH), either because there was not a probe available within the region in question or because the CNC was most likely below the level of resolution of FISH. Cases that were not FISH confirmed were thought to represent true positive array findings, as opposed to false-positive results due to technical reasons, based on criteria for detection of a CNC in the laboratory performing the arrays.

Participating laboratories

Survey materials were sent to 11 different laboratories, not including the laboratory that originally generated the results. Ten laboratories returned responses. Participants were from both academic and commercial laboratories that handle a range of array volume. For some laboratories, responses came from only one director, whereas at least one laboratory returned consensus responses that were reached between more than one director.

Array CGH and FISH

All specimens were received for routine clinical testing in a single laboratory. DNA was obtained from peripheral blood for testing on either a BAC (6 cases) or an oligonucleotide (7 cases) array at the request of the ordering provider. The BAC arrays contained 4685 clones covering 1543 loci (SignatureSelect™ v. 2.0). The oligonucleotide arrays consisted of approximately 105 K features (88,953 autosomal probes, 6369 X chromosome, and 1677 Y chromosome probes) printed by Agilent Technologies (SignatureChipOS™).¹⁰ Some of the patients had other concurrent or previously normal conventional cytogenetic or molecular test results. Criteria for detection of a CNC identified on a BAC array consisted of a single clone, or multiple adjacent clones, demonstrating a log₂ ratio of at least 0.3 and -0.3 in two dye-reversed hybridizations. A CNC on an oligonucleotide array consisted of at least five adjacent probes that demonstrated a gain or loss in a single hybridization experiment. FISH was performed for all of the CNCs detected by BAC array. FISH was not attempted in four oligonucleotide array cases, either because of lack of an available FISH probe in the region in question or because the original laboratory that performed the testing decided that the change was most likely clinically benign. Examples of representative array and FISH results from two of the cases used in the survey are shown in Figure 1. These images were not provided to participating laboratories at the time of the survey. Parental follow-up information was available for some of the cases at the time the survey was performed, but it was not provided to participants to simulate a real clinical testing situation in which an initial assessment of CNCs must often be made before obtaining parental specimens.

Information provided to participating laboratories

Table 1 lists information that was provided to participating laboratories. Participants were given the clinical indication for array testing, as well as results of previous or concurrent cytogenetic or molecular testing from each case. For each case, nucleotide coordinates (UCSC 2006, hg18 assembly) for one CNC detected in the case, including any adjacent gaps in array coverage, were provided. A gap was defined as the distance between a probe showing an imbalance and the nearest adjacent probe with a normal copy number. The CNCs consisted of both duplications (7 cases) and deletions (6 cases) and were from a variety of autosomes as well as the X chromosome. All of the CNCs were 1 Mb or less in size, with the exception of two duplications that could possibly be bigger than 1.0 Mb, depending on the involvement of adjacent gaps in coverage on the arrays. The single CNC given for each case was not necessarily the only CNC detected in the case, although participants were told that none of the cases were found to have other CNCs in critical regions of the genome known to be associated with well-documented syndromes or abnormal phenotypes. In addition, information on FISH confirmation of the CNC was provided.

Case questionnaire

For each case, participants were asked to fill out a questionnaire (Fig. 2).

RESULTS

Survey responses

All questionnaire responses for each case from each participant are provided in Table, Supplemental Digital Content 1, <http://links.lww.com/GIM/A89>. For none of the thirteen cases

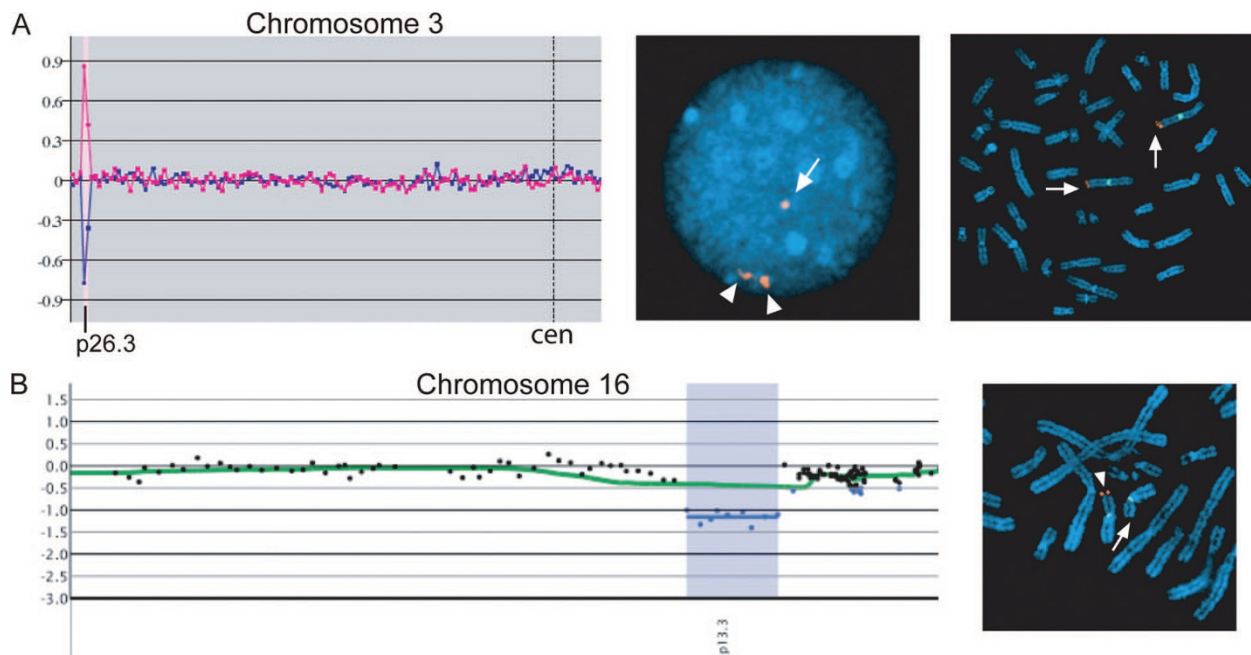


Fig. 1. Array CGH and FISH results from two representative cases used in this survey. A, Partial Chromosome 3 array plot (left panel), interphase FISH image (middle panel), and metaphase FISH image (right panel) from Case 1. The array plot shows a two clone duplication in the short arm of Chromosome 3, at band p26.3, detected on a BAC array (cen = centromere). The interphase FISH image is representative of the signal pattern observed in 62% of nuclei from the patient when hybridized with one of the duplicated clones (RP11-624H2) detected by array CGH, compared with this signal pattern observed in 7% of nuclei from a mixture of normal control individuals hybridized with the same probe (arrowheads = signals from homologue with tandem duplication, arrow = signal from homologue without duplication). The metaphase FISH image is consistent with a tandem duplication in this patient (RP11-624H2 probe in orange indicated by arrows; D3Z1 centromere probe in green) and excludes insertion of the duplicated material into a different region of the genome. B, Partial Chromosome 16 array plot (left) and metaphase FISH image (right) from Case 9. The array plot shows a loss of nine adjacent probes detected on an oligonucleotide array. The metaphase FISH image shows absence of signal from the short arm of one homologue of Chromosome 16 (arrow) using a probe from within the deleted region detected by array CGH (WI2-2909M10 probe in orange indicated by arrowhead; D16Z2 centromere probe in green).

was there 100% concordance in how a result was classified (Table 2). Responses for classification of a CNC ranged from normal to abnormal in some cases. Some participants appeared to use a binary classification system (normal versus abnormal), whereas other participants used additional categories (normal, likely benign, uncertain clinical significance [UCS], likely pathogenic/abnormal). Some participants had a lower threshold for designating CNCs in the UCS or abnormal categories than others (for example, in Table 2, participants 4 and 11 were compared with 3 and 8.). All but one of the BAC array cases was designated abnormal by at least one participant, whereas none of the oligonucleotide cases were classified as abnormal. This observation may be due to the presence of larger gaps in coverage adjacent to the CNC in some of the BAC array cases, potentially causing ambiguity in the sizing and determination of the gene content of the region in question.

Duplications appeared just as likely to be categorized as UCS when compared with deletions (Table 3). There also did not appear to be good correlation between the size of a CNC and the frequency with which it was categorized as normal, as small CNCs containing multiple genes were just as likely to be categorized as UCS compared with larger CNCs (see Cases 8 and 9; Table 3). A CNC was just as likely to be categorized as abnormal or UCS regardless of whether it was confirmed by FISH; however, FISH visualization of array findings can still be

useful, especially when duplicated material is inserted into another chromosome. In the case of insertion duplications, the possibility exists that even if the duplicated material itself is benign, there could be disruption or alteration of a gene or genes at the site of insertion.

The duplication on the X chromosome in Case 10 was categorized as UCS by four participants, although one participant noted that the female control DNA used in their laboratory has a duplication in this region, and another participant also noted that this duplication is frequently seen in their laboratory (Table, Supplemental Digital Content 1, <http://links.lww.com/GIM/A89>). The presence of known benign CNCs in either public or personal databases was also useful to participants for categorizing CNCs in other cases (see Cases 11 and 12; Table 3), although the presence of benign CNCs did not always correlate with a participant categorizing a CNC as normal (Table 3 and Table, Supplemental Digital Content 1, <http://links.lww.com/GIM/A89>). The latter phenomenon may be due to variables pertaining to public databases, such as the frequency with which a benign CNC appears in public databases, and ambiguities in sizing of CNCs in public databases due to different array platforms.

This survey did not address the specific databases that were used by individual participants to decide whether a CNC is benign. That information has already been collected from a larger number of clinical laboratories through the

Table 1 Clinical indication, other test results, copy number change, gaps in probe coverage, and FISH confirmation for cases

Case	Array	Clinical indication	Other test results	Copy number change	Gaps	FISH
1	BAC	Omphalocele, mega cisterna magna, hypoglycemia	NA	2 overlapping clone gain, 3p26.3: 955368→1149577 (0.19 Mb)	Tel: 0.44 Mb Cen: 0.24 Mb	Dup visualized by IP analysis
2	BAC	DD, family history learning disabilities	NA	5 clone loss, 8p23.2: 3367998→4176886 (0.81 Mb)	Tel: none Cen: 0.08 Mb	Del visualized
3	BAC	Severe DD, seizures, autism	FISH—neg 22q11.2 and subtel screen	Single clone loss, 3p26.2: 4158950→4329972 (0.17 Mb)	Tel: 0.22 Mb Cen: none	Del visualized
4	BAC	Duane anomaly, delayed milestones	Normal karyotype, FISH negative for 4p-	3 clone loss, 6p11.2: 57668543→58131862 (0.46 Mb)	Tel: 0.25 Mb Cen: 0.27 Mb	Del visualized
5	BAC	DD, dysmorphic	Normal karyotype	2 overlapping clone gain, 6q26: 163220837→163413238 (0.19 Mb)	Tel: none Cen: 2.7 Mb	Dup not visualized
6	BAC	DD, seizures	NA	3 clone gain, 6q27: 168674979→168938075 (0.26 Mb)	Tel: 0.34 Mb Cen: 0.32 Mb	Dup visualized by IP analysis
7	Oligo	Autism	NA	10 adjacent probe gain, 3p12.3: 80499218→81126137 (0.63 Mb)	Tel: 0.09 Mb Cen: 0.07 Mb	Not done
8	Oligo	Congenital heart anomalies, hypoparathyroidism, vitiligo	Normal karyotype, FISH—neg 22q11.2, 10p13p14, subtel screen	6 adjacent probe loss, 22q11.21: 80499218→81126137 (0.04 Mb)	Tel: 0.28 Mb Cen: 0.012 Mb	Not done
9	Oligo	Language delay, dysmorphic, dwarfism	Normal karyotype, FISH—neg Williams and subtel screen	9 adjacent probe loss, 16p13.3: 1942265→2015940 (0.07 Mb)	Tel: 0.01 Mb Cen: 0.005 Mb	Del visualized
10	Oligo	Female with ataxia, cataracts, seizures, DD, hemiparesis	46,XX,t(2;20)(p21;p12.3)mat	16 adjacent probe gain, Xp11.23: 47655252→47937597 (0.28 Mb)	Tel: 0.005 Mb Cen: 0.07 Mb	Dup visualized by IP analysis
11	Oligo	DD, autism	NA	6 adjacent probe loss, 7q11.23: 75977217→76395961 (0.42 Mb)	Tel: 0.07 Mb Cen: 0.09 Mb	Del visualized
12	Oligo	Female with DD, macrocephaly/somia, hypotonia, dysmorphic, cleft palate	Normal karyotype, FISH—neg 22q11.2 and subtel screen, neg for NSD1 mutation	154 probe gain, Xp22.33: 1789761→2328419 (0.54 Mb)	Tel: 0.008 Mb Cen: 0.002 Mb	Not done
13	Oligo	DD, corpus callosum agenesis	NA	15 adjacent probe gain, 5q21: 103846273→104851861 (1.0 Mb)	Tel: 0.07 Mb Cen: 0.05 Mb	Not done

DD, developmental delay; Tel, telomeric; Cen, centromeric; IP, interphase; Dup, duplication; Del, deletion.

CAP CYCGH proficiency surveys. In a recent CAP survey⁹ that included 34 participants, 97% reported using the Database of Genomic Variants (<http://projects.tcag.ca/variation/>),¹¹ 56% used an internal database, 47% used the Ens-

embl Genome Browser (<http://www.ensembl.org>), and 18% used the Human Structural Variation Database (<http://humanparalogy.gs.washington.edu/structuralvariation/>). It is clear from this information that many laboratories use

Case questionnaire:

Assuming no known clinically significant CNCs were detected in this case, how would you report the finding? (check answer that applies)

- Normal
- Abnormal
- Copy number change of uncertain clinical significance (UCS)
- Other – specify _____

Which of the following criteria were used to determine how you chose to report this finding? Check all that apply

- Size of gain/loss
- Size of gaps in coverage adjacent to gain/loss
- Presence or absence of gene(s) in region of gain/loss
- Presence of specific gene(s) in region of gain/loss – which one(s) _____
- Presence of genes within gaps in coverage adjacent to gain/loss
- Clinical indication
- Gain/loss was confirmed by FISH
- Gain/loss was not confirmed by FISH
- Presence of benign copy number variants in public or personal databases
- Lack of benign copy number variants in public or personal databases

Ancillary questions:

1. Is there an absolute size cut-off below which you would not report a copy number change, regardless of other variables (presence/absence of genes in region, ability to FISH confirm, etc)?
2. If the answer to 1. is yes, what is your size cut-off for:
 - a. Deletions
 - b. Duplications
3. Do you perform array CGH testing on prenatal specimens?
4. If the answer to 3. is yes, do you use the same criteria for reporting copy number changes in prenatal vs. postnatal cases?

Fig. 2. Questionnaire for each case and ancillary questions.

multiple databases to assist in the interpretation of CNCs. In response to another question in the CAP CYCGH survey, 88% of participants stated that they do not list all CNCs considered to be benign in their clinical reports. Although not included as a formal question in either this survey or the CAP survey, some participants in this survey noted that they use other databases, such as Online Mendelian Inheritance in Man (OMIM), DECIPHER, and PubMed searches, in addition to the databases of benign variants cited earlier, to evaluate CNCs for pathogenic effects.

Responses to ancillary questions

Some laboratories have absolute size cutoffs below which CNCs are not reported. These size cutoffs range anywhere from 50 Kb to 500 Kb for deletions and 150 Kb to 500 Kb for duplications. Some laboratories will potentially report any size CNC as a finding other than normal, depending on variables such as gene content. Five participating laboratories perform array testing for CNCs on prenatal specimens. Their criteria for classifying CNCs are the same for prenatal and postnatal cases, but some laboratories use different arrays for prenatal cases than for postnatal cases (targeted arrays for prenatal and whole-genome arrays for postnatal).

DISCUSSION

We report the results of a survey to examine the variability among laboratories for interpretation of gains and losses de-

tected by microarray analysis. We prefer the use of CNC over CNV because of confusion over the meaning of “variant” itself. The term variant has often been used to imply a normal population heteromorphism,¹² which would be consistent with benign CNCs, but not with pathogenic CNCs. However, variant has also been applied to pathogenic CNCs, or has been used to refer to both benign and pathogenic CNCs.¹⁻³

The results of this survey demonstrate that while laboratories may have similar approaches for attempting to determine the clinical significance of a CNC that has not been previously associated with an abnormal phenotype, the final interpretation between different laboratories can vary significantly. Thus, different laboratories could detect the same CNC, but it may be considered benign and not reported by some laboratories, whereas others may report it as a finding of clinical significance or possible clinical significance. There were some potential limitations of this survey that may have exaggerated the differences in interpretation that were observed for certain cases. Participants were asked to evaluate the CNCs as they normally would in their daily practice, although the information provided to the participants may not have exactly duplicated all of the information that a laboratory would have available to them if they processed the case in their own laboratory. Also, some of the participants may not have experience with the specific platforms that were used to generate the array findings for this survey. Some participants may have limited diagnostic reporting categories but may indicate in the text of the report the level of uncertainty regarding the clinical significance of a CNC. The

Table 2 Reporting responses from participants for copy number changes

Case	Copy number change	Participants										
		1	2	3	4	5	6	7	8	9	10	11
1	0.19/0.87 ^a Mb duplication, 3p26.3	NL	NL	NL	UCS	ABN	NL	NL	UCS	NL	UCS	UCS
2	0.81/0.89 Mb deletion, 8p23.2	UCS	UCS	NL	ABN	ABN	UCS	ABN	NL	UCS	NL	UCS
3	0.17/0.39 Mb deletion, 3p26.2	NL	UCS	NL	UCS	NL	NL	NL	UCS	UCS	UCS	UCS
4	0.46/1.0 Mb deletion, 6p11.2	UCS	NL	NL	UCS	NL	NL	ABN	NL	NL	UCS	LB
5	0.19/2.9 Mb duplication, 6q26	NL	NL	UCS	UCS	UCS	UCS	ABN	NL	UCS	UCS	UCS
6	0.26/0.92 Mb duplication, 6q27	UCS	NL	ABN	UCS	NL	NL	NL	NL	UCS	UCS	UCS
7	0.63/0.79 Mb duplication, 3p12.3	UCS	UCS	NL	NL	NL	NL	NL	NL	NL	NL	LB
8	0.04/0.33 Mb deletion, 22q11.21	NL	UCS	NL	UCS	NL	UCS	NL	NL	NL	UCS	UCS
9	0.07/0.09 Mb deletion, 16p13.3	UCS	UCS	NL	UCS	NL	LB	NL	NL	UCS	UCS	UCS
10	0.28/0.36 Mb duplication, Xp11.23 ^b	NL	NL	NL	UCS	UCS	UCS	NL	NL	NL	UCS	LB
11	0.42/0.58 Mb deletion, 7q11.23	NL	NL	NL	UCS	NL	NL	NL	NL	NL	NL	NL
12	0.54/0.55 Mb duplication, Xp22.33 ^b	LB	NL	NL	NL	NL	NL	NL	NL	NL	UCS	LB
13	1.0/1.1 Mb duplication, 5q21	UCS	UCS	NL	UCS	LB	NL	NL	NL	LB	UCS	UCS

Cases 1 through 6 are BAC array cases; 7 through 13 are oligonucleotide array cases.

^aMinimum/maximum size.

^bFemale.

ABN, abnormal; NL, normal; UCS, uncertain clinical significance; LB, likely benign.

latter information may not have been captured in the responses, even though an option for “Other-specify” was given in the questionnaire. Despite these potential limitations, the results still indicate that there is a lack of consensus between laboratories in the interpretation and reporting of CNCs.

Variability in the interpretation of CNCs could be due to differences in the content of internal databases containing benign CNC information between laboratories, differing opinions as to the importance of deletion or duplication of specific genes within or overlapping a CNC, and the comfort level of a director in reporting or not reporting a CNC. The content of internal benign CNC databases may vary not only because of the volume of array cases that a laboratory experiences but also by the criteria that a laboratory uses to determine that a given CNC is benign. For example, how many times should a CNC be found to be inherited from a phenotypically normal parent before it can be considered benign? Given the finding of variable expressivity and incomplete penetrance associated with other CNCs in the genome,^{13–16} the argument could be made that inheritance from a phenotypically normal parent needs to be documented more than once before a CNC is deemed benign; however, the specific number will vary at the discretion of the laboratory. For example, one laboratory requires that a CNC is seen five or more times as inherited from a phenotypically normal mother and five or more times inherited from a phenotypically normal father before their reporting criteria are adjusted to reflect this change as a benign CNC. Also, before designating a CNC as benign, the laboratory should have a high degree of confidence that the parents have been properly evaluated for subtle abnormal phenotypic features.

The observation in this survey of at least one CNC (Case 10; Table 3) that could easily be categorized as benign by at least two laboratories based on internal database information, but that was classified as a finding of UCS by four other participants,

emphasizes the necessity for reliable, well-annotated, publicly available databases that contain CNC information. Toward this end, recent studies that have used high-density platforms to screen large cohorts of apparently phenotypically normal individuals provide invaluable information to assist in the interpretation of CNCs in the clinical laboratory.^{6,17} Interestingly, the study performed by Shaikh et al.⁶ specifically addresses difficulties in interpreting one of the CNCs included in the present survey. Case 2 is from a patient with developmental delay who has a deletion of ~800 Kb that is entirely contained within the *CSMD1* gene (OMIM #608397), but involves multiple exons. This gene is a putative tumor suppressor and is expressed in fetal and adult brain, although little additional information is available on its function. This deletion was considered normal, of UCS, or abnormal by different participants (Table 3). Shaikh et al. detected hundreds of CNCs within *CSMD1* in phenotypically normal individuals; however, these CNCs were much smaller than those previously reported in this gene, and only 0.8% of them disrupted exonic sequence, whereas 24.5% of previously reported CNCs were predicted to disrupt one or more exons. These results suggest that the size of previously reported CNCs could be overstated, or that the larger imbalances represent rare variants that may or may not confer disease risk. Additional studies of this type are needed to resolve these ambiguities, so that decisions regarding the likely pathogenicity of CNCs are based on reliable information.

Although the ISCN (2009) provides a standardized system for reporting CNCs, it does not aid in their interpretation.¹⁸ The development of interpretation recommendations analogous to those proposed for sequence variants identified by sequence-based testing would be extremely useful for providing guidance to clinical laboratories performing array-based copy number assessment. A categorization system that provides enough granularity to convey the degree of uncertainty in the clinical sig-

Table 3 Characteristics and reporting of copy number changes

Case	Copy number change	Genes in region/gaps	FISH	Benign CNCs (No. laboratories) ^a		Result (No. laboratories)			
				Yes	No	NL	LB	UCS	ABN
1	0.19/0.87 ^b Mb duplication, 3p26.3	<i>CNTN6</i>	Dup visualized by IP analysis	9	1	6	0	4	1
2	0.81/0.89 Mb deletion, 8p23.2	<i>CSMD1</i>	Del visualized	4	1	3	0	5	3
3	0.17/0.39 Mb deletion, 3p26.2	<i>SETMAR</i>	Del visualized	7	1	5	0	6	0
4	0.46/1.0 Mb deletion, 6p11.2	None/ <i>PRIM2</i> , <i>GUSBL2</i>	Del visualized	7	1	6	1	3	1
5	0.19/2.9 Mb duplication, 6q26	<i>PACRG</i> /8 OMIM genes	Dup not visualized	1	5	3	0	7	1
6	0.26/0.92 Mb duplication, 6q27	<i>SMOC2</i> / <i>DACT2</i>	Dup visualized by IP analysis	6	2	5	0	5	1
7	0.63/0.79 Mb duplication, 3p12.3	None	Not done	3	2	8	1	2	0
8	0.04/0.33 Mb deletion, 22q11.21	<i>TUBA8</i> , <i>USP18</i> / <i>GGTP3</i> , <i>DGCR6</i> , <i>PRODH</i>	Not done	7	0	6	0	5	0
9	0.07/0.09 Mb deletion, 16p13.3	7 OMIM genes, 6 other/ 2 OMIM	Del visualized	5	1	4	1	6	0
10	0.28/0.36 Mb duplication, Xp11.23 ^c	4 OMIM genes, 2 other	Dup visualized by IP analysis	7	0	6	1	4	0
11	0.42/0.58 Mb deletion, 7q11.23	<i>UPK3B</i> , <i>POMZ3</i>	Del visualized	10	0	10	0	1	0
12	0.54/0.55 Mb duplication, Xp22.33 ^c	<i>DHRXY</i>	Not done	8	1	8	2	1	0
13	1.0/1.1 Mb duplication, 5q21	<i>RAB9PI</i>	Not done	6	1	4	2	5	0

^aPersonal or public databases.^bMinimum/maximum size.^cFemale.

IP, interphase; Dup, duplication; Del, deletion.

nificance of a CNC would give useful information for genetic counseling, including the need for parental carrier testing. In addition to normal and abnormal categories, such a classification system could further subdivide UCS cases into “likely benign,” “uncertain,” and “likely pathogenic.” The abnormal category should be reserved for those CNCs for which there is well-documented evidence of an association with an abnormal phenotype, but that do not display significant variability between different individuals or incomplete penetrance. The “likely pathogenic” category could be used for those CNCs for which there is well-documented evidence of association with an abnormal phenotype, but with known variability in phenotypic expression. This category could also be used for large CNCs that contain genes for which there is evidence of copy-dependent pathogenicity and rare case reports, but no well-documented syndrome. The “likely benign” category could be reserved for CNCs that are relatively small and that lack known genes, or for which there is fairly convincing evidence of lack of pathogenicity based on independent database entries of benign CNCs that completely overlap the CNC in question. CNCs in this category may not warrant parental follow-up testing. The

“UCS” category could include those CNCs that fall between the “likely pathogenic” and the “likely benign” categories.

A standardized classification system would not completely eliminate reporting discrepancies between laboratories, but it would alleviate markedly discrepant results such as one laboratory not reporting a CNC at all and another reporting it as abnormal. A standardized classification system would also give the ordering provider a better idea of the level of uncertainty of the clinical significance of a CNC. A weighted scoring system that attempts to quantitate different variables that are used to assess pathogenicity could even be considered to assist laboratories in placing CNCs into different categories. For example deletions, which typically are more deleterious, could be given a higher point value than duplications. Weighted scores could also be based on the size of a CNC. In a recent study that examined the frequency of CNCs based on size, 65% to 80% of apparently phenotypically normal individuals harbor a CNC of at least 100 kb in size, 5% to 10% of normal individuals carry a CNC of at least 500 kb in length, and ~1% of normal individuals carry a variant of at least 1 Mb.¹⁷ Although these findings confirm that size alone cannot be used as a predictor of

pathogenicity, the difference in the frequency of small versus large CNCs could justify assigning increasing point scores to CNCs of increasing size.

In addition to the findings presented here, other variability in clinical reports undoubtedly exists. Consensus recommendations from working groups could also provide laboratories with guidance regarding content of reports. For example, should clinical reports include a list of all genes within a CNC? Should they include information on gene expression patterns and information on the potential clinical relevance of all genes within the region in question, even though this information can be accessed by anyone from publicly available databases? Should reports state the size of gaps in array coverage adjacent to CNCs? How much clinical interpretation of a CNC should be provided by the laboratory, in light of the limited clinical information on patients that is usually given to the laboratory?

The findings presented in this study demonstrate that our ability to interpret the significance of some CNCs is lagging behind our ability to detect them. As the number of clinical laboratories that perform array-based copy number assessment increases, both laboratorians and providers may be increasingly asked to interpret reports that are generated outside their own institution. Clinical laboratories, and the providers who order these tests and communicate the results to patients, could greatly benefit from more uniform classification of CNCs and report content between laboratories.

ACKNOWLEDGMENTS

The authors thank Devon Lamb Thrush, Nationwide Children's Hospital, and Bassem Bejjani, Allen Lamb, Roger Schultz, Trilochan Sahoo, and Gail Wenger, Signature Genomics Laboratories for participating in the survey. The authors also thank Andrew Wilson, ARUP laboratories, for statistical advice.

REFERENCES

1. Lee C, Iafate AJ, Brothman AR. Copy number variations and clinical cytogenetic diagnosis of constitutional disorders. *Nat Genet* 2007;39:S48–S54.
2. Fan YS, Jayakar P, Zhu H, et al. Detection of pathogenic gene copy number variations in patients with mental retardation by genomewide oligonucleo-

3. Koolen DA, Pfundt R, de Leeuw N, et al. Genomic microarrays in mental retardation: a practical workflow for diagnostic applications. *Hum Mutat* 2009;30:283–292.
4. Vermeesch JR, Fiegler H, de Leeuw N, et al. Guidelines for molecular karyotyping in constitutional genetic diagnosis. *Eur J Hum Genet* 2007;15:1105–1114.
5. Buchanan JA, Scherer SW. Contemplating effects of genomic structural variation. *Genet Med* 2008;10:639–647.
6. Shaikh TH, Gai X, Perin JC, et al. High-resolution mapping and analysis of copy number variations in the human genome: a data resource for clinical and research applications. *Genome Res* 2009;19:1682–1690.
7. Richards CS, Bale S, Bellissimo DB, et al. ACMG recommendations for standards for interpretation and reporting of sequence variations: revisions 2007. *Genet Med* 2008;10:294–300.
8. Plon SE, Eccles DM, Easton D, et al. Sequence variant classification and reporting: recommendations for improving the interpretation of cancer susceptibility genetic test results. *Hum Mutat* 2008;29:1282–1291.
9. CAP Cytogenetics Resource Committee. *Comparative Genomic Hybridization Microarray Participant Summary Report*. Survey CYCGH-B 2008. Northfield, IL: College of American Pathologists, 2008.
10. Ballif BC, Theisen A, McDonald-McGinn DM, et al. Identification of a previously unrecognized microdeletion syndrome of 16q11.2q12.2. *Clin Genet* 2008;74:469–475.
11. Iafate AJ, Feuk L, Rivera MN, et al. Detection of large-scale variation in the human genome. *Nat Genet* 2004;36:949–951.
12. Brothman AR, Schneider NR, Saikovich I, et al. Cytogenetic heteromorphisms: survey results and reporting practices of giemsa-band regions that we have pondered for years. *Arch Pathol Lab Med* 2006;130:947–949.
13. Ullmann R, Turner G, Kirchhoff M, et al. Array CGH identifies reciprocal 16p13.1 duplications and deletions that predispose to autism and/or mental retardation. *Hum Mutat* 2007;28:674–682.
14. Hannes FD, Sharp AJ, Mefford HC, et al. Recurrent reciprocal deletions and duplications of 16p13.11: the deletion is a risk factor for MR/MCA while the duplication may be a rare benign variant. *J Med Genet* 2009;46:223–232.
15. Brunetti-Pierri N, Berg JS, Scaglia F, et al. Recurrent reciprocal 1q21.1 deletions and duplications associated with microcephaly or macrocephaly and developmental and behavioral abnormalities. *Nat Genet* 2008;40:1466–1471.
16. Mefford HC, Sharp AJ, Baker C, et al. Recurrent rearrangements of chromosome 1q21.1 and variable pediatric phenotypes. *N Engl J Med* 2008;359:1685–1699.
17. Itsara A, Cooper GM, Baker C, et al. Population analysis of large copy number variants and hotspots of human genetic disease. *Am J Hum Genet* 2009;84:148–161.
18. Shaffer LG, Slovak ML, Campbell LJ, editors. *ISCN (2009): an international system for human cytogenetic nomenclature*. Basel: S. Karger, 2009.

ERRATUM

Technical standards and guidelines: Prenatal screening for Down syndrome that includes first-trimester biochemistry and/or ultrasound measurements: Erratum

In the article that appeared on page 669 of volume 11, issue 9, the authors' disclosures were omitted mistakenly. The authors' disclosures should have appeared as follows: Glenn E. Palomaki reports that he has no personal conflicts of interest to disclose, but his employer has received project grants from Beckman Coulter, PerkinElmer and DSL. Jo Ellen S. Lee reports that she is a consultant for Genzyme Genetics. Jacob A. Canick reports that, with others, he holds patents on the use of unconjugated estriol in prenatal screening for Down syndrome and is a paid consultant to Beckman Coulter Inc. Geraldine A. McDowell declares no conflicts of interest. Alan E. Donnenfeld is an employee of Genzyme Genetics.

This error has been noted in the online version of the article, which is available at www.geneticsinmedicine.com.

REFERENCE

- Palomaki GE, Lee JES, Canick JA, McDowell GA, Donnenfeld AE; for the ACMG Laboratory Quality Assurance Committee. Technical standards and guidelines: Prenatal screening for Down syndrome that includes first-trimester biochemistry and/or ultrasound measurements. *Genet Med* 2009;11:669–681.