Commentary

Diagnostic Genome Profiling

Unbiased Whole Genome or Targeted Analysis?

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This commentary appears in conjunction with the Review Article by Bejjani et al.¹ In these articles, *The Journal of Molecular Diagnostics* explores array comparative genomic hybridization (CGH) and offers two perspectives on the question of testing using whole-genome arrays or targeted arrays.

Unbiased Whole-Genome Array CGH

For several decades, cytogenetic analysis has relied on chromosome banding by karyotyping for the genome-wide detection of structural and/or numerical chromosomal anomalies in patients with various disorders and/or disabilities.^{2,3} High-resolution targeted fluorescence *in situ* hybridization (FISH) analysis was developed as an additional tool for the identification of common microdeletion syndromes, which cannot be identified by karyotyping because of its limited resolution. The FISH technology is predominantly used to confirm a clinical diagnosis because it can only screen for a limited number of genomic targets in a single hybridization experiment. Recent developments in genomic microarray technologies have resulted in a novel profiling approach called microarray-based comparative genomic hybridization or array CGH.^{4,5} Array CGH combines the advantages of karyotyping with that of the FISH technology, ie, a whole genomic view with high resolution. Bejjani and Shaffer,¹ in this issue of *The Journal of Molecular Diagnostics*, review the potential of this technology in clinical

diagnosis, with a focus on targeted genomic arrays. We agree with the authors in that array CGH technology is revolutionizing cytogenetics and, as such, is providing clinicians with a powerful diagnostic tool that can be used in their daily practice. We disagree, however, with another conclusion in the article that specifically designed arrays targeting genomic regions of known clinical significance offer the best option for diagnostics at present. Bejjani and Shaffer state that "genome-wide dense arrays that are currently available for research are not appropriate to use in a clinical diagnostic setting as these arrays raise a number of medical, technical, and financial concerns."¹ In contrast to Bejjani and Shaffer, based on our experience in a clinical setting,^{6,7} we are strongly convinced of the diagnostic power of whole-genome profiling by array CGH and believe that the whole-genome approach offers numerous advantages over targeted arrays. These advantages will be discussed below. In addition, we will briefly touch on alternative microarrays being developed and elaborate on the future prospects of genomic microarrays in clinical diagnostics. Although we use mental retardation as an example, the discussion can be seen in a wider perspective, including disorders such as autism, inborn errors of development, etc.

Genomic Microarrays: Preparation, Analysis, Validation, and Interpretation

Several microarray platforms have been developed for unbiased genome-wide DNA copy number analysis using bacterial artificial chromosome (BAC) arrays, initially with a coverage of approximately one clone per megabase^{6,8,9} and, currently, with a tiling resolution of approximately one clone per 100 kb.^{7,10} The most commonly used BAC clone sets for microarray purposes have been extensively validated using FISH and DNA fingerprinting, as well as end sequencing.^{9,11,12} Therefore, less than 1% of the BACs in these clone sets will map to incorrectly assigned genomic locations. Moreover, even when a clone has accidentally been mapped to a wrong position in the genome, this will not pose serious problems, let alone have diagnostic consequences. This is because the use of high-density microarrays, such as our tiling

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resolution genome-wide BAC arrays, allows the employment of spatial information and does not rely on single clone measurements. In our diagnostic setting, we only follow up those genomic alterations that encompass a minimum of three adjacently located BAC clones. This approach dramatically reduces the chance that a problem is caused by rare clones with an adverse position assigned to it, since it is highly unlikely that three or more of such clones are located next to each other. The intrinsic technical variability of the microarray-based method is also reduced to a minimum by using this same approach. In addition, all microarray-based findings with a potential clinical relevance (see below) are validated by an independent technique such as FISH and/or semi-quantitative polymerase chain reaction (eg, multiplex ligation-dependent probe amplification¹³). Only after an independent confirmation are the results sent to the referring clinician, who subsequently has the parents tested for *de novo* occurrence. In our experience, this does not lead to significantly raised anxiety within the family as long as its members are counseled properly. The clinical geneticists and pediatricians involved are accustomed to dealing with chromosomal aberrations in their patients. Therefore, the (subtle) chromosome abnormalities identified by genomic microarray analysis, accommodated by proper explanation, will not pose serious diagnostic challenges to these clinicians.

One important issue that has been raised in recent years is the unexpectedly high frequency of inherited submicroscopic copy number variants,^{14,15} along with the initial difficulty in discerning between a copy number alteration causing disease versus one without clinical consequences. In the period between 2004 to 2005, we were the first to test patients with unexplained mental retardation using a tiling resolution microarray in a diagnostic setting.⁷ At that time, knowledge about genomic copy number variation was limited, and therefore, extensive parental analysis was necessary to distinguish between inherited variants and *de novo* alterations. Since then, knowledge about genomic variation has increased dramatically, and it appears that a limited number of genomic variation hotspots explain the majority of variation in the human population at the BAC clone resolution level (~100 kb).^{7,16} Because these regions appear to vary both in normal controls and in patients, we have decided not to follow up genomic variations at these regions in our diagnostic setting. After having excluded these variation hotspots, it is our experience that there are either no or few regions left that display genomic copy number variation in an individual patient.⁷ As a consequence, parental testing is limited to those genomic alterations that have a high potential of being clinically relevant.

In all, we feel that genome-wide BAC arrays can effectively be used in a diagnostic setting, and this has now been demonstrated by several laboratories including ours.^{6,7,17–20} Still, one can imagine that both the workload and the costs are higher for a genome-wide microarray analysis compared with the targeted microarray analysis encompassing far less genomic clones. Therefore, the real issues include determining the advantages of the

genome-wide approach versus the targeted approach and whether specific subgroups of patients can be directed toward one or the other type of analysis.

The Added Value of Whole-Genome Microarrays over Targeted Microarrays

To answer this question, we first have to realize how targeted arrays are designed. In general, these arrays contain clones targeting genomic regions of known clinical significance, for example, all known microdeletion syndrome regions and all subtelomeric regions, which are known to be frequently affected in patients with mental retardation.^{21–23} As a consequence, these arrays are very well suited for screening these specific regions but only these regions. Still, this is an important clinical application with considerable diagnostic yield in patients submitted for a variety of developmental problems, as was demonstrated by the recent publication of Shaffer et al.²⁴

The diagnostic yield of targeted as well as genome-wide microarray analysis, however, strongly depends on the clinical selection as well as the previously performed cytogenetic analyses, and this may vary considerably between countries and even between institutions within countries. In The Netherlands, like in many other countries, most patients with congenital malformations and/or mental retardation are seen by a highly trained clinical geneticist and/or pediatrician. These professionals will recognize most known microdeletion syndromes and will request specific FISH tests to confirm their diagnosis. Patients with these recognizable syndromes will, therefore, only rarely be sent in for microarray analysis. In addition, cytogenetic analysis with chromosome banding will be performed in all patients in The Netherlands with mental retardation with or without congenital malformations. For those patients in which no chromosomal aberrations are detected, a high-throughput and relatively cheap subtelomeric screen will be performed.¹³ Genomic microarray analysis is only indicated after the clinical evaluation (and subsequent confirmation by FISH analyses), the routine chromosome analysis, and the subtelomeric analysis fail to provide a diagnosis. As a consequence, the diagnostic yield for a targeted array, which specifically targets subtelomeric regions and known regions for microdeletion syndromes, will be low. Indeed, in our first series of 100 patients with unexplained mental retardation tested on our genome-wide tiling resolution BAC array,⁷ we identified only two causative genomic alterations that contained genomic regions for known microdeletion syndromes (both atypical cases) and no subtelomeric aberrations. In addition, we identified eight submicroscopic interstitial alterations localized throughout the human genome, none of which would have been identified by the targeted microarrays currently available. Similar findings have been reported by others using either 1-Mb resolution BAC arrays^{17–20} or, more recently, microarrays containing over 100,000 single nucleotide polymorphisms.²⁵

The two main conclusions that can be drawn from these whole-genome diagnostic microarray approaches in mental retardation are that submicroscopic copy number alterations, both subtelomeric as well as interstitial, are responsible for a considerable proportion of mental retardation, varying between 5 and 20% of cases depending on clinical selection, and that these submicroscopic alterations occur all over the human genome. Until now, there were only a few recurrent alterations that have been identified. This poses a serious challenge for targeted microarrays and predicts that these arrays need to be updated regularly to stay abreast of all novel reported regions involved in mental retardation. Therefore, we conclude that genome-wide microarrays, especially those with a tiling resolution coverage, are of great value in the diagnosis of mental retardation and will continue to be so in the coming years. These genome-wide microarrays may actually replace routine chromosome banding as a first diagnostic screen in a significant number of clinical indications, such as mental retardation, given the increase in diagnostic yield. The pace of implementation, however, strongly depends on the finances involved and the expertise present in the various diagnostic laboratories.

Novel Genomic Microarray Approaches

Most currently used genome-wide copy number profiling microarrays are produced in academic settings, and the resolution of these microarrays varies depending on the type and number of genomic targets selected, the protocols used, and the data analysis tools used. In addition, aberrations below 100 kb cannot be detected with current resolution, which is limited by the size of the genomic fragments (ie, BACs) used as array elements. Only recently have private enterprises embarked on this novel genomic microarray application, and several companies are now offering microarrays for genome-wide copy number profiling. These microarrays encompass oligonucleotides targeting random genomic sequences^{26,27} or single nucleotide polymorphisms.^{25,28} The advantages of using such commercial platforms for diagnostic applications are numerous.

1. They provide a higher genome coverage than most microarrays generated in academia by placing up to half a million oligonucleotides on a single microarray, which will increasingly allow the detection of smaller aberrations linked to disease.

2. They can be produced in large quantities according to industrial quality standards.

3. They are available to all research and diagnostic laboratories and those without access to dedicated microarray facilities.

4. Their widespread use will rapidly generate large data sets of normal controls as well as patients with various disorders, thus greatly facilitating clinical interpretation of genome profiles.

Given these advantages, it can be expected that these commercially available microarrays will rapidly find their way to routine clinical practice.

Conclusion

Despite the natural caution associated with the implementation of new technologies in the clinical arena, genomic microarrays have been recognized as effective diagnostic tools in molecular cytogenetics for several years now. The ability to obtain quantitative copy number information on the entire human genome has already been shown to significantly improve the diagnostic yield in mental retardation. Further increases in resolution provided by commercially available ultra-high density microarrays are likely to increase this yield even more. We expect that, ultimately, genomic copy number scanning of all ~250,000 exons in the human genome will become the standard in genomic microarray diagnostics, thus allowing the detection of causative dosage variations up to the single exon level. The current diagnostic use of targeted microarrays will likely be surpassed by the availability of affordable whole-genome arrays combined with rapid increases in our knowledge on the clinical interpretation of these microarrays.

References

1. Bejjani BA, Shaffer LG: Application of array-based comparative genomic hybridization to clinical diagnostics. *J Mol Diagn* 2006, 8:528–533
2. Graham SM, Selikowitz M: Chromosome testing in children with developmental delay in whom the aetiology is not evident clinically. *J Paediatr Child Health* 1993, 29:360–362
3. van Karnebeek CD, Jansweijer MC, Leenders AG, Offringa M, Hennekam RC: Diagnostic investigations in individuals with mental retardation: a systematic literature review of their usefulness. *Eur J Hum Genet* 2005, 13:6–25
4. Pinkel D, Seagraves R, Sudar D, Clark S, Poole I, Kowbel D, Collins C, Kuo WL, Chen C, Zhai Y, Dairkee SH, Ljung BM, Gray JW, Albertson DG: High resolution analysis of DNA copy number variation using comparative genomic hybridization to microarrays. *Nat Genet* 1998, 20:207–11
5. Mantripragada KK, Buckley PG, de Stahl TD, Dumanski JP: Genomic microarrays in the spotlight. *Trends Genet* 2004, 20:87–94
6. Vissers LE, de Vries BB, Osoegawa K, Janssen IM, Feuth T, Choy CO, Straatman H, van der Vliet W, Huys EH, van Rijk A, Smeets D, van Ravenswaaij-Arts CM, Knoers NV, van der Burgt I, de Jong PJ, Brunner HG, Geurts van Kessel A, Schoenmakers EF, Veltman JA: Array-based comparative genomic hybridization for the genomewide detection of submicroscopic chromosomal abnormalities. *Am J Hum Genet* 2003, 73:1261–1270
7. de Vries BB, Pfundt R, Leisink M, Koolen DA, Vissers LE, Janssen IM, Reijmersdal S, Nillesen WM, Huys EH, Leeuw N, Smeets D, Sistermans EA, Feuth T, van Ravenswaaij-Arts CM, Geurts van Kessel A, Schoenmakers EF, Brunner HG, Veltman JA: Diagnostic genome profiling in mental retardation. *Am J Hum Genet* 2005, 77:606–616
8. Snijders AM, Nowak N, Seagraves R, Blackwood S, Brown N, Conroy J, Hamilton G, Hindle AK, Huey B, Kimura K, Law S, Myambo K, Palmer J, Ylstra B, Yue JP, Gray JW, Jain AN, Pinkel D, Albertson DG: Assembly of microarrays for genome-wide measurement of DNA copy number. *Nat Genet* 2001, 29:263–264
9. Fiegler H, Carr P, Douglas EJ, Burford DC, Hunt S, Scott CE, Smith J, Vetrie D, Gorman P, Tomlinson IP, Carter NP: DNA microarrays for comparative genomic hybridization based on DOP-PCR amplification of BAC and PAC clones. *Genes Chromosomes Cancer* 2003, 6:361–374
10. Ishkanian AS, Malloff CA, Watson SK, DeLeeuw RJ, Chi B, Coe BP, Snijders A, Albertson DG, Pinkel D, Marra MA, Ling V, MacAulay C, Lam WL: A tiling resolution DNA microarray with complete coverage of the human genome. *Nat Genet* 2004, 36:299–303
11. Cheung VG, Nowak N, Jang W, Kirsch IR, Zhao S, Chen XN, Furey

- TS, Kim UJ, Kuo WL, Olivier M, Conroy J, Kasprzyk A, Massa H, Yonescu R, Sait S, Thoreen C, Snijders A, Lemyre E, Bailey JA, Bruzel A, Burrill WD, Clegg SM, Collins S, Dharni P, Friedman C, Han CS, Herrick S, Lee J, Ligon AH, Lowry S, Morley M, Narasimhan S, Osoegawa K, Peng Z, Plajzer-Frick I, Quade BJ, Scott D, Sirotkin K, Thorpe AA, Gray JW, Hudson J, Pinkel D, Ried T, Rowen L, Shen-Ong GL, Strausberg RL, Birney E, Callen DF, Cheng JF, Cox DR, Doggett NA, Carter NP, Eichler EE, Haussler D, Korenberg JR, Morton CC, Albertson D, Schuler G, de Jong PJ, Trask BJ: BAC Resource Consortium: Integration of cytogenetic landmarks into the draft sequence of the human genome. *Nature* 2001, 409:953-958
12. Krzywinski M, Bosdet I, Smalil D, Chiu R, Mathewson C, Wye N, Barber S, Brown-John M, Chan S, Chand S, Cloutier A, Ginn N, Lee D, Masson A, Mayo M, Olson T, Pandoh P, Prabhu AL, Schoenmakers E, Tsai M, Albertson D, Lam W, Choy CO, Osoegawa K, Zhao S, de Jong PJ, Schein J, Jones S, Marra MA: A set of BAC clones spanning the human genome. *Nucleic Acids Res* 2004, 32:3651-3660
13. Koolen DA, Nillesen WM, Versteeg MH, Merkx GF, Knoers NV, Kets M, Vermeer S, van Ravenswaaij CM, de Kovel CG, Brunner HG, Smeets D, de Vries BB, Sistermans EA: Screening for subtelomeric rearrangements in 210 patients with unexplained mental retardation using multiplex ligation dependent probe amplification (MLPA). *J Med Genet* 2004, 41:892-899
14. Iafrate AJ, Feuk L, Rivera MN, Listewnik ML, Donahoe PK, Qi Y, Scherer SW, Lee C: Detection of large-scale variation in the human genome. *Nat Genet* 2004, 36:949-951
15. Sebat J, Lakshmi B, Troge J, Alexander J, Young J, Lundin P, Maner S, Massa H, Walker M, Chi M, Navin N, Lucito R, Healy J, Hicks J, Ye K, Reiner A, Gilliam TC, Trask B, Patterson N, Zetterberg A, Wigler M: Large-scale copy number polymorphism in the human genome. *Science* 2004, 305:525-528
16. Sharp AJ, Locke DP, McGrath SD, Cheng Z, Bailey JA, Vallente RU, Pertz LM, Clark RA, Schwartz S, Segreaves R, Oseroff VV, Albertson DG, Pinkel D, Eichler EE: Segmental duplications and copy-number variation in the human genome. *Am J Hum Genet* 2005, 77:78-88
17. Shaw-Smith C, Redon R, Rickman L, Rio M, Willatt L, Fiegler H, Firth H, Sanlaville D, Winter R, Colleaux L, Bobrow M, Carter NP: Microarray based comparative genomic hybridisation (array-CGH) detects submicroscopic chromosomal deletions and duplications in patients with learning disability/mental retardation and dysmorphic features. *J Med Genet* 2004, 41: 241-248
18. Schoumans J, Ruivenkamp C, Holmberg E, Kyllerman M, Anderlid BM, Nordenskjöld M: Detection of chromosomal imbalances in children with idiopathic mental retardation by array based comparative genomic hybridisation (array-CGH). *J Med Genet* 2005, 42:699-705
19. Menten B, Maas N, Thienpont B, Buysse K, Vandesompele J, Melotte C, de Ravel T, Van Vooren S, Balikova I, Backx L, Janssens S, De Paepe A, De Moor B, Moreau Y, Marynen P, Fryns JP, Mortier G, Devriendt K, Speleman F, Vermeesch JR: Emerging patterns of cryptic chromosomal imbalance in patients with idiopathic mental retardation and multiple congenital anomalies: a new series of 140 patients and review of published reports. *J Med Genet* 2006, 43:625-633
20. Rosenberg C, Knijnenburg J, Bakker E, Vianna-Morgante AM, Sloos W, Otto PA, Kriek M, Hansson K, Krepsich-Santos AC, Fiegler H, Carter NP, Bijlsma EK, van Haeringen A, Szuhai K, Tanke HJ: Array-CGH detection of micro rearrangements in mentally retarded individuals: clinical significance of imbalances present both in affected children and normal parents. *J Med Genet* 2006, 43:180-186
21. Bejjani BA, Saleki R, Ballif BC, Rorem EA, Sundin K, Theisen A, Kashork CD, Shaffer LG: Use of targeted array-based CGH for the clinical diagnosis of chromosomal imbalance: is less more? *Am J Med Genet A* 2005, 134:259-267
22. Wong A, Lese Martin C, Heretis K, Ruffalo T, Wilber K, King W, Ledbetter DH: Detection and calibration of microdeletions and microduplications by array-based comparative genomic hybridization and its applicability to clinical genetic testing. *Genet Med* 2005, 7:264-271
23. Cheung SW, Shaw CA, Yu W, Li J, Ou Z, Patel A, Yatsenko SA, Cooper ML, Furman P, Stankiewicz P, Lupski JR, Chinault AC, Beaudet AL: Development and validation of a CGH microarray for clinical cytogenetic diagnosis. *Genet Med* 2005, 7:422-432
24. Shaffer LG, Kashork CD, Saleki R, Rorem E, Sundin K, Ballif BC, Bejjani BA: Targeted genomic microarray analysis for identification of chromosome abnormalities in 1500 consecutive clinical cases. *J Pediatr* 2006, 149:98-102
25. Friedman JM, Baross A, Delaney AD, Ally A, Arbour L, Asano J, Bailey DK, Barber S, Birch P, Brown-John M, Cao M, Chan S, Charest DL, Farnoud N, Fernandes N, Flibotte S, Go A, Gibson WT, Holt RA, Jones SJ, Kennedy GC, Krzywinski M, Langlois S, Li H, McGillivray BC, Nayar T, Pugh TJ, Rajcan-Separovic E, Schein JE, Schnerch A, Siddiqui A, Van Allen MI, Wilson G, Yong S-L, Zahir F, Eyedoux P, Marra MA: Oligonucleotide microarray analysis of genomic imbalance in children with mental retardation. *Am J Hum Genet* 2006, 79:500-513
26. Barrett MT, Scheffer A, Ben-Dor A, Sampas N, Lipson D, Kincaid R, Tsang P, Curry B, Baird K, Meltzer PS, Yakhini Z, Bruhn L, Laderman S: Comparative genomic hybridization using oligonucleotide microarrays and total genomic DNA. *Proc Natl Acad Sci USA* 2004, 101:17765-17770
27. Selzer RR, Richmond TA, Pofahl NJ, Green RD, Eis PS, Nair P, Brothman AR, Stallings RL: Analysis of chromosome breakpoints in neuroblastoma at sub-kilobase resolution using fine-tiling oligonucleotide array CGH. *Genes Chromosomes Cancer* 2005, 44:305-319
28. Slater HR, Bailey DK, Ren H, Cao M, Bell K, Nasioulas S, Henke R, Choo KH, Kennedy GC: High-resolution identification of chromosomal abnormalities using oligonucleotide arrays containing 116,204 SNPs. *Am J Hum Genet* 2005, 77:709-726

Targeted Array CGH

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In their commentary, Drs. Veltman and de Vries¹ agree with the main conclusion of our Review,² that array CGH has irrevocably transformed and revolutionized clinical cytogenetics. As often happens with evolving concepts and technologies, differences in opinion arise, as eloquently pointed out by Drs. Veltman and de Vries¹: whereas we argue that the use of whole-genome profiling by array CGH in the clinical laboratory should await further understanding of the genome and its architecture, they convey that the time is now. Thus, our differences are chronological rather than conceptual. These differences, however, are significant and should be debated, because they have important medical implications. The *Journal of Molecular Diagnostics* is uniquely positioned to provide a platform for such valuable discussions.

In our Review we state, "The careful and prudent approach to diagnostic applications stands in sharp contrast to the more explorative world of research."² We believe that whole-genome arrays are explorative by design and by purpose. This is evidenced by the continuous stream of discoveries of new syndromes and of unexpected genomic complexities that emanate from the use of these outstanding research instruments, the latest being the discovery of the 17q21.31 syndrome by Drs. Veltman and de Vries and by others.³⁻⁵ This latest breakthrough is exciting to us because a new and potentially important cause for mental retardation is discovered, a peculiarity of the genomic sequence that is responsible for the deletion is revealed, and because these reports validate our contention that whole-genome arrays are still instruments of exploration and discovery.

In relation to the quality of the BAC clones that are available for building arrays for clinical applications, Drs.

Veltman and de Vries state that "... less than 1% of the BACs in these clone sets will map to incorrectly assigned genomic locations."¹ Our experience, in contrast, shows that 7% of the BACs map to the wrong location and 16% hybridize to multiple locations in the genome.⁶ More recently, while designing a BAC array targeted to the pericentromeric areas, we showed that 2.2% of the BACs we selected were mismapped, and 22% cross-hybridized to locations other than the primary chromosomal locations assigned by the University of California, Santa Cruz genome browser (B. C. Ballif, S. A. Horner, S. G. Sulpizio, R. M. Lloyd, S. L. Minier, E. A. Rorem, A. Theisen, B. A. Bejjani, L. G. Shaffer, unpublished data). Whereas a large number of the first generation of BACs was FISH-mapped, fingerprinted, and end-sequenced, the BACs that are currently being used have been through many cycles of freezing, thawing, culturing, shipping, DNA isolation, DNA amplification, etc. All of these operations are performed in research and commercial laboratories across the globe with varying standards, protocols, and procedures to check for errors. When one is handling more than 32,000 BACs, error rates even in the single digits have significant diagnostic implications.

In all, Drs. Veltman and de Vries "feel that genome-wide BAC arrays can effectively be used in a diagnostic setting, and this has been demonstrated by several laboratories,"¹ including theirs. This is a very instructive statement, as all four laboratories they reference are European.⁷⁻¹⁰ This may reflect an historic, regulatory, and perhaps cultural difference in the adoption of new medical technologies and newly developed drugs between Europe and the United States.

Although we agree that whole-genome arrays will uncover more abnormalities of the genome than targeted arrays, we differ in the way we balance the improved detection rate with the exponential increase in uninterpretable results due to copy number variants (CNVs) of unclear clinical significance, with the additional burden on clinicians and families who have to struggle with ambiguous information, and with the increased cost of health care as a result of performing additional tests on most parents. We also differ in our assessment of the performance of the targeted arrays compared with whole-genome arrays. When one looks closely at the results of the study of 100 cases with unexplained mental retardation,¹¹ the diagnostic yield of targeted arrays would not have been as low as they suggest in their commentary.¹ Indeed, our targeted array would have identified four of the 10 anomalies detected by the whole-genome array (the two microdeletion syndromes and two of eight remaining anomalies). Perhaps more importantly, a targeted array would not have compelled follow-up on as many as 200 parents to determine whether the abnormalities were significant or benign CNVs. In the reality of a busy diagnostic laboratory, tripling the number of cases adds undue burden and cost. Furthermore, in the United States (and presumably in The Netherlands too, since in this cohort of 100 study subjects, only 72 parents were collected instead of the necessary 200¹¹), it is often impossible to obtain both parents to evaluate whether an abnormality is benign or causative. In our clinical labora-

tory, we are able to obtain both parental samples on approximately 42% of patients on whom such study is clinically indicated. Accurate interpretation in a diagnostic laboratory is not always possible without parents. Of course, as Drs. Veltman and de Vries indicate,¹ our understanding of these CNVs has grown significantly, and many of the CNVs are now clearly benign variants of no clinical significance and do not necessitate follow-up on parents. However, as Drs. Veltman and de Vries explain,¹ experience with CNVs is only 2 years old. There is much more to be learned before using these data in the daily routine of a clinical laboratory. In contrast to their statement that after excluding a limited number of genomic hot-spots for CNVs, "there are either no or few regions that display genomic copy number variation in an individual patient,"¹ our experience with >6000 patients with a targeted array shows that we continue to uncover unique ("novel" or "private"), presumably benign, CNVs (present also in a clinically normal parent) that occur in regions that are traditionally considered not to contain benign CNV but are associated with abnormal phenotypes, such as the telomeres.

A recent report by Friedman et al¹² that Drs. Veltman and de Vries reference is also telling. In this study of 100 individuals with mental retardation with a dense oligonucleotide array, 3125 putative CNVs were identified. These were present in all individuals, with a range of 19 to 43 and a median of 30 CNVs per study subject. Of these 3125 putative CNVs, only 12 were confirmed both in the child and in one of the parents, and 2669 occurred only once in this data set. Furthermore, the authors state that most CNVs they identified "are unique and have not been independently confirmed."¹² This is a staggering number of ambiguous and unhelpful results that a clinical diagnostic laboratory has to understand and interpret.

In conclusion, we agree with Drs. Veltman and de Vries that targeted arrays will continue to require regular updating to stay abreast of all reported novel regions involved in mental retardation. As these targeted arrays evolve to reflect a better understanding of the genome, they may eventually expand into whole-genome arrays. However, we think that diagnostic laboratories should remain at least one step behind the cutting edge of research. After all, we serve patients, not study subjects. With the dizzying pace of this revolution, it is our duty to do our best to protect our patients from the inevitable mistakes that will occur in the explorative world of research while striving to provide the latest advancements in diagnostics. Attaining this delicate balance is a challenge. With hope, open discussions such as these will help us all serve our patients better.

References

1. Veltman JA, de Vries BB: Diagnostic genome profiling: unbiased whole genome or targeted analysis? *J Mol Diagn* 2006, 8:534-537
2. Bejjani BA, Shaffer LG: Application of array-based comparative genomic hybridization to clinical diagnostics. *J Mol Diagn* 2006, 8:528-533
3. Koolen DA, Vissers LE, Pfundt R, de Leeuw N, Knight SJ, Regan R, Kooy RF, Reyniers E, Romano C, Fichera M, Schinzel A, Baumer A,

- Anderlid BM, Schoumans J, Knoers NV, van Kessel AG, Sistermans EA, Veltman JA, Brunner HG, de Vries BB: A new chromosome 17q21.31 microdeletion syndrome associated with a common inversion polymorphism. *Nat Genet* 2006, 38:999–1001
4. Shaw-Smith C, Pittman AM, Willatt L, Martin H, Rickman L, Gribble S, Curley R, Cumming S, Dunn C, Kalaitzopoulos D, Porter K, Prigmore E, Krepischi-Santos AC, Varela MC, Koiffmann CP, Lees AJ, Rosenberg C, Firth HV, de Silva R, Carter NP: Microdeletion encompassing MAPT at chromosome 17q21.3 is associated with developmental delay and learning disability. *Nat Genet* 2006, 38:1032–1037
5. Sharp AJ, Hansen S, Selzer RR, Cheng Z, Regan R, Hurst JA, Stewart H, Price SM, Blair E, Hennekam RC, Fitzpatrick CA, Segraves R, Richmond TA, Guiver C, Albertson DG, Pinkel D, Eis PS, Schwartz S, Knight SJ, Eichler EE: Discovery of previously unidentified genomic disorders from the duplication architecture of the human genome. *Nat Genet* 2006, 38:1038–1042
6. Bejjani BA, Saleki R, Ballif BC, Rorem EA, Sundin K, Theisen A, Kashork CD, Shaffer LG: Use of targeted array-based CGH for the clinical diagnosis of chromosomal imbalance: is less more? *Am J Med Genet A* 2005, 134:259–267
7. Shaw-Smith C, Redon R, Rickman L, Rio M, Willatt L, Fiegler H, Firth H, Sanlaville D, Winter R, Colleaux L, Bobrow M, Carter NP: Microarray based comparative genomic hybridisation (array-CGH) detects submicroscopic chromosomal deletions and duplications in patients with learning disability/mental retardation and dysmorphic features. *J Med Genet* 2004, 41:241–248
8. Schoumans J, Ruivenkamp C, Holmberg E, Kyllerman M, Anderlid BM, Nordenskjöld M: Detection of chromosomal imbalances in children with idiopathic mental retardation by array based comparative genomic hybridisation (array-CGH). *J Med Genet* 2005, 42:699–705
9. Menten B, Maas N, Thienpont B, Buysse K, Vandesompele J, Melotte C, de Ravel T, Van Vooren S, Balikova I, Backx L, Janssens S, De Paepe A, De Moor B, Moreau Y, Marynen P, Fryns JP, Mortier G, Devriendt K, Speleman F, Vermeesch JR: Emerging patterns of cryptic chromosomal imbalance in patients with idiopathic mental retardation and multiple congenital anomalies: a new series of 140 patients and review of published reports. *J Med Genet* 2006, 43:625–633
10. Rosenberg C, Knijnenburg J, Bakker E, Vianna-Morgante AM, Sloos W, Otto PA, Kriek M, Hansson K, Krepischi-Santos AC, Fiegler H, Carter NP, Bijlsma EK, van Haeringen A, Suzhai K, Tanke HJ: Array-CGH detection of micro rearrangements in mentally retarded individuals: clinical significance of imbalances present both in affected children and normal parents. *J Med Genet* 2006, 43:180–186
11. de Vries BB, Pfundt R, Leisink M, Koolen DA, Vissers LE, Janssen IM, Reijmersdal S, Nillesen WM, Huys EH, Leeuw N, Smeets D, Sistermans EA, Feuth T, van Ravenswaaij-Arts CM, Geurts van Kessel A, Schoenmakers EF, Brunner HG, Veltman JA: Diagnostic genome profiling in mental retardation. *Am J Hum Genet* 2005, 77:606–616
12. Friedman JM, Baross A, Delaney AD, Ally A, Arbour L, Asano J, Bailey DK, Barber S, Birch P, Brown-John M, Cao M, Chan S, Charest DL, Farnoud N, Fernandes N, Flibotte S, Go A, Gibson WT, Holt RA, Jones SJ, Kennedy GC, Krzywinski M, Langlois S, Li H, McGillivray BC, Nayar T, Pugh TJ, Rajcan-Separovic E, Schein JE, Schnerch A, Siddiqui A, Van Allen MI, Wilson G, Yong S-L, Zahir F, Eyedoux P, Marra MA: Oligonucleotide microarray analysis of genomic imbalance in children with mental retardation. *Am J Hum Genet* 2006, 79:500–513