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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.

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