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COMMENTARY

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

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

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

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

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

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

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

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

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