The Impact of New Genomic Technologies in Reproductive Medicine

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Abstract: Recent advances in the clinical application of genomic technologies have significantly impacted the field of prenatal diagnosis. Central to these advances has been the implementation of chromosomal microarray analysis (CMA). Microdeletions and microduplications, undetectable by traditional karyotyping, have recently been confirmed to play a role in altered neurocognitive development. CMA is now recommended for fetuses with structural anomalies. However, CMA comes with an increased need and role for genetic counseling, because the potential genomic information available is exponentially increased. CMA also can be performed on a small number of preimplantation embryonic cells for assessment of the embryo’s reproductive potential. Implementation of these new genomic techniques in an in vitro fertilization setting has already demonstrated significant improvements in reproductive outcome. Techniques are now being developed to eliminate the necessity for invasive prenatal diagnosis procedures. Currently in its infancy, noninvasive prenatal testing using cell-free fetal DNA from maternal blood has already improved the sensitivity for detection of the common aneuploidies and current efforts are focused on identifying select microdeletions. The explosion of new genomic technologies continues to offer great benefits. However, each needs critical assessment prior to adoption in a clinical setting. [Discovery Medicine 17(96):313-318, June 2014]

Chromosomal Microarray Technology

As opposed to karyotyping, which analyzes the number and structure of chromosomes under the light microscope, CMA uses molecular technologies to identify smaller genomic imbalances (Aradhya and Cherry, 2007). There are two approaches to identifying these submicroscopic imbalances. One approach is based on comparative genomic hybridization (CGH), in which a sample of DNA from the patient is compared to a normal control DNA sample (Snijders et al., 2001). The
two DNA samples are cut into fragments, labeled with different fluorescent colors (usually red and green), and then mixed in equal proportions. The DNA mixture is then placed onto a target (glass slide) containing probes from representative sequences from across the human genome. The number of probes on an array typically ranges from tens of thousands to a few million. Each DNA fragment, from either the patient or the control, will hybridize (bind) only to a probe on the array with an exact complimentary sequence. After hybridization, digital imaging systems are used to quantify the relative fluorescence intensities of each of the hybridized regions. The ratio of the fluorescence intensities at any one location (locus) indicates the relative contribution of the test and reference genomes. If equal contributions are present, the ratio would be equal to 1 and the patient is said to have a normal copy number at that probe location. When the patient has excess DNA (a duplication) at a specific region, they would have more of their DNA hybridizing compared to the control which would result in a fluorescence intensity ratio that is significantly greater than 1. Conversely, if the patient had a deletion, there would be more of the control DNA hybridizing and this would be reflected by a fluorescence intensity ratio that is significantly less than 1. The extent and size of the gain/loss can be determined by the number of consecutive probes that show a ratio above/below 1.

More recently, SNP (single nucleotide polymorphism) arrays have been used in which the target probes are chosen from DNA locations known to vary between individuals by a single base pair (Beaudet and Belmont, 2008). The general principal is the same as with CGH arrays, but only the patient’s DNA is labeled and hybridized to the array. The absolute intensity, rather than the comparison of intensities between the patient and a control, is measured to determine the copy number. The genotype information from the SNPs can be used to determine clinically useful information beyond copy number such as uniparental disomy, zygosity, parent of origin, and maternal cell contamination. In addition, triploidy, which cannot be detected by CGH arrays, can be identified by assessment of the allele patterns on a SNP array.

**Comparison of CMA to Karyotype in Prenatal Diagnosis**

To transition CMA into prenatal diagnostic testing, the NICHD (National Institute of Child Health and Human Development) supported a large, prospective blinded comparison of CMA and karyotype to evaluate the aspects of CMA testing that are unique to prenatal diagnosis. This evaluation along with work from other centers (Bejiani et al., 2005; Rickman et al., 2006; Schaeffer et al., 2004; Shaffer, 2006) included scientific questions, such as the ability to achieve accurate fetal results using direct sources such as uncultured amniotic fluid or uncultured chorionic villi, the impact of maternal cell contamination and confined placental mosaicism, and the appropriate design of a prenatal array, which must balance the ability to detect clinically relevant copy number variants against the identification of findings of unknown clinical significance (Check, 2005). Similarly, counseling and interpretive issues required evaluation (Bernhardt et al., 2013).

Compared to standard cytogenetic analysis of G-banded karyotypes, which requires 5 to 7 days of tissue culture, CMA was able to be performed on uncultured samples, hence reducing turnaround time, an important advantage in prenatal testing. CMA proved capable of identifying all the common trisomies identified by karyotype and most importantly identified additional clinically relevant microdeletions and microduplications. Table 1, shows data from the largest prenatal CMA series performed to date and quantifies the incremental value of CMA testing in pregnancies with normal karyotypes. The NICHD study found that overall 2.5% of cases with a normal karyotype had a clinically significant copy number change identified by CMA (Wapner et al., 2012b). In women referred for standard indications such as advanced maternal age and positive Down syndrome screening 1.7% of cases with a normal karyotype had a clinically relevant copy number variant, many of which are associated with neurocognitive alterations (Wapner et al., 2012a). When looking specifically at patients with fetal structural abnormalities, approximately 6% were found to have a clinically significant copy number change not seen on standard karyotyping (Wapner et al., 2012a), leading the American College of Obstetricians and Gynecologists and the Society for Maternal-Fetal Medicine to recommend in December 2013 that CMA become the first tier prenatal cytogenomic test for pregnancies with one or more major fetal structural abnormalities identified by ultrasound (American College of Obstetricians and Gynecologists Committee on Genetics, 2013). The joint statement also recognized CMA as an appropriate alternative to karyotyping for the evaluation of a structurally normal fetus undergoing invasive prenatal diagnostic testing for standard indications (American College of Obstetricians and Gynecologists Committee on Genetics, 2013).

**Counseling Issues with CMA**

Given the multitude of prenatal testing options now
available and the varying information each test can reveal, comprehensive pre- and post-test counseling from qualified personnel such as a genetic counselor or geneticist is imperative. This is increasingly important with the introduction of the newer high resolution technologies like CMA that, in addition to clear-cut pathogenic genomic abnormalities, have the potential to identify findings of uncertain significance, adult-onset diseases, parental pre-symptomatic disorders, nonpaternity, and unsuspected consanguinity. These added complexities highlight the necessity of obtaining informed consent concomitant with a thorough discussion of the patients’ threshold for uncertain or unsuspected information (American College of Obstetricians and Gynecologists Committee on Genetics, 2013).

Transitioning counseling to clinical care has not been simple and has demonstrated that as new genomic technologies are introduced, practitioner education specific to the clinical scenario is necessary (Bernhardt et al., 2013). Two counseling issues are particularly challenging when dealing with CMA results: (1) findings of uncertain clinical significance and (2) the variable expressivity of certain copy number variants (CNVs).

CNVs are a normal part of the human condition and are found covering approximately 12% (360 million base pairs) of our genome (Redon et al., 2006). Most of these CNVs are not associated with any obvious pathology, and they are thought to contribute to normal phenotypic variation. While the majority of benign CNVs tend to be on the smaller size (<100 Kb), gene content is also a key factor in the clinical consequence of a given copy number change (Feuk et al., 2006). Over the last 7 years, databases of both benign and pathogenic CNVs have been developed to help facilitate clinical interpretation (Table 2). The difficulty comes when encountering a copy number variant not previously seen or seen only rarely. This in-utero discovery of a copy number variant of uncertain significance, when the complete phenotype is unknown and pregnancy termination is still an option, is distressing for the patient and frustrating for the provider and requires skilled counseling and interpretation. Case and repository review is valuable but will not always provide sufficient information to guide management. Identification of the gene content of the CNVs by searching the University of California Santa Cruz Genome Browser (Kent et al., 2002) can give further insight into the potential pathogenicity of the finding but unfortunately, in many cases, can only give limited prediction of the phenotype. Fortunately, as databases and the literature continue to expand, findings of uncertain clinical significance are less frequent. For example, the initial interpretation of CNV pathogenicity from the NICHD study found that uncertain CNVs occurred in approximately 2.5% of cases. When

| Table 1. Recent Estimates of Detectable Pathogenic CNVs in High- and Low-Risk Pregnancies. |
|---------------------------------|----------------|----------------|-----------------|----------------|----------------|----------------|----------------|
| Indication                      | Lee et al., 2012 (n = 3171) | Armengol et al., 2012 (n = 906) | Other Studies a (n = 1000) | Fiorentino et al., 2013 (n = 3000) | Wapner et al., 2012 (n = 3822) | Combined (n = 11899) |
| All High Risk Pregnancies (Primarily abn US) | # Samples | # Pathogenic CNVs (%) | # Samples | # Pathogenic CNVs (%) | # Samples | # Pathogenic CNVs (%) | # Samples | # Pathogenic CNVs (%) | # Samples | # Pathogenic CNVs (%) |
| All Low Risk Pregnancies | 2926 | 15 (0.5) | 733 | 8 (1.1) | 493 | 5 (1.0) | 2880 | 17 (0.6) | 3067 | 50 (1.6) | 10099 | 95 (0.9) |
| Low Risk Subgroups |
| AMA | 1911 | 10 (0.5) | 273 | 3 (1.1) | 253 | 1 (0.4) | 1118 | 6 (0.5) | 1966 | 33 (1.7) | 5521 | 53 (1.0) |
| Parental Anxiety | 989 | 5 (0.5) | 60 | 1 (1.7) | 93 | - | 1675 | 11 (0.7) | 372 | 5 (1.3) | 3189 | 23 (0.7) |
| Abn Serum Screen | 26 | - | 235 | 1 (0.4) | 11 | 1 (9.1) | 29 | - | 729 | 12 (1.6) | 1030 | 14 (1.4) |
| Total | 3171 | 34 (1.1) | 906 | 14 (1.5) | 1000 | 18 (1.8) | 3000 | 24 (0.8) | 3822 | 95 (2.5) | 11899 | 185 (1.6) |

Note: a, compiled by Fiorentino et al., 2013; including Coppinger et al., 2009; Shaffer et al., 2008; Maya et al., 2010; Van den Veyver et al., 2009; Sahoo et al., 2006.
the same CNVs were reclassified 5 years later, only 1.5% remained uncertain (Wapner et al., 2012b). In addition, design of arrays specifically for prenatal use could potentially minimize the occurrence of uncertain findings.

Variable expressivity, in which an identical genetic alteration can be associated with significant variation in the phenotype, is a well-known phenomenon in clinical genetics. For example, the well-described 22q11.2 deletion associated with DiGeorge syndrome is associated with a host of clinical anomalies that may or may not be present in a given patient: palatal abnormalities in 76%, cardiac defects in 75%, hypoparathyroid in 60%, genitourinary defects in 36%, and psychosis/behavioral problems in 25% (Driscoll et al., 1993; Lee et al., 2014). Similarly, some CNVs occur in patients with multiple phenotypes including schizophrenia, autism, or epilepsy (Mefford et al., 2012). When discovered on a prenatal test, the inability to precisely predict how the genomic imbalance will actually affect the child (i.e., which end of the clinical spectrum the child will present with), can be distressful for parents and extensive counseling by individuals skilled in array interpretation is required (Bernhardt et al., 2013). Nonetheless, this information found during prenatal testing can be of significant value in childhood management allowing early intervention and treatment for affected children.

### Use of CMA in Preimplantation Genetic Diagnosis (PGD) and In Vitro Fertilization (IVF)

The ability to perform progressive genetic diagnostic procedures on increasingly smaller samples now allows prenatal diagnosis to be performed by testing a handful of cells (1-10) from a developing preimplantation embryo retrieved during an IVF procedure and then selectively implanting those that are normal. Originally developed for patients at high risk for fetal Mendelian genetic disorders and for whom termination of a fetus was not an option, the technology, termed preimplantation genetic diagnosis/screening (PGD/S), is now used relatively frequently to improve outcomes for patients with multiple repetitive miscarriages, those undergoing IVF, and for women of advanced maternal age (Thornhill et al., 2005).

The blend of whole-genome amplification, multiplex-PCR and CMA provides the ability to perform comprehensive 24-chromosome aneuploidy analysis as part of PGD (Treff et al., 2010; 2012). While these methods represent exciting and important advances for the field of PGD, only recently has the potential value of comprehensive chromosome screening on IVF embryos been fully evaluated (Treff and Scott, 2012). The results from these recent studies demonstrate the capability to predict the reproductive potential of human embryos prior to implantation to increase clinical pregnancy rates (Scott et al., 2012; 2013a). These data suggest that

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Table 2. List of Online Resources Used in CMA Results Interpretation.

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<thead>
<tr>
<th>Name</th>
<th>Abbreviation</th>
<th>Website</th>
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<tbody>
<tr>
<td>Database of Genomic Variants</td>
<td>DGV</td>
<td><a href="http://projects.tcag.ca/variation">http://projects.tcag.ca/variation</a></td>
</tr>
<tr>
<td>Database of Chromosomal Imbalance and Phenotype in Humans using Ensembl Resources</td>
<td>DECIPHER</td>
<td><a href="https://decipher.sanger.ac.uk/application">https://decipher.sanger.ac.uk/application</a></td>
</tr>
<tr>
<td>European Cytogeneticists Association Register of Unbalanced Chromosome Aberrations</td>
<td>ECARUCA</td>
<td><a href="http://www.ECARUCA.net">http://www.ECARUCA.net</a></td>
</tr>
<tr>
<td>Genomic Oligoarray and SNP array evaluation tool</td>
<td></td>
<td><a href="http://www.ccs.miami.edu/cgi-bin/ROH/ROH_analysis_tool.cgi">http://www.ccs.miami.edu/cgi-bin/ROH/ROH_analysis_tool.cgi</a></td>
</tr>
<tr>
<td>International Standards for Cytogenomic Arrays Consortium</td>
<td>ISCA</td>
<td><a href="https://www.iscaconsortium.org">https://www.iscaconsortium.org</a></td>
</tr>
<tr>
<td>USCS Genome Browser</td>
<td>UCSC</td>
<td><a href="http://genome.ucsc.edu/cgi-bin/hgGateway">http://genome.ucsc.edu/cgi-bin/hgGateway</a></td>
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<tr>
<td>Prenatal Array</td>
<td></td>
<td><a href="http://prenatalarray.org">http://prenatalarray.org</a></td>
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blastocyst biopsy coupled with comprehensive aneuploidy screening of all chromosomes, and fresh/frozen embryo transfer may optimize embryo selection and eventually facilitate the practical application of single embryo transfer, reducing the need to transfer multiple embryos resulting in less IVF induced higher order multiple gestations (Forman et al., 2013; Scott et al., 2013a; 2013b). With the development of comprehensive chromosome screening, blastocyst vitrification, and trophectoderm biopsy techniques, older women have the opportunity of elective single-embryo transfer with live birth rates as high as those reported for younger good-prognosis infertility patients (Schoolcraft and Katz-Jaffe, 2013).

CMA and Non-invasive Prenatal Testing

Non-invasive screening for Down syndrome has been a major focus in low risk women of reproductive age for the past 40 years. This has primarily been accomplished by a combination of ultrasound and serial detection of maternal serum biochemical markers in the first and second trimesters, with follow-up diagnosis by invasive procedures such as amniocentesis or chorionic villus sampling (CVS). These traditional approaches have detection rates that range from only 75% to 96% (depending on the screening approach utilized) and have false-positive rates ranging from 1.9% to 5.2% and false-negative rates ranging from 12% to 23% (Levy and Norwitz, 2013). More recently, advances in non-invasive prenatal testing (NIPT) using cell-free fetal DNA from maternal circulation has dramatically improved the accuracy (as high as 99.9% for some aneuploidies) and the scope of fetal aneuploidies that can be detected, with false positive and false negative rates far below 1% (Levy and Norwitz, 2013). It is now common practice in various parts of the U.S.A. to order NIPT for the common trisomies (13, 18, and 21) to minimize the number of invasive procedures (amniocentesis or CVS) being performed (Levy and Norwitz, 2013). These tests are still regarded in the realm of “screening” and not as a replacement for current cytogenetic testing and CMA following CVS and amniocentesis.

With the recent observation that microdeletions/microduplications occur relatively frequently and are a significant cause of neurocognitive alterations, there have been attempts at developing non-invasive approaches to uncover their presence in the fetus. In the past, invasive prenatal testing has targeted pregnancies at high risk for aneuploidy, such as those in women of advanced maternal age. However, microdeletions/duplications have no age associated risk. A young mother may have up to a ten times higher likelihood of having a child with a clinically significant microdele-
ation or microduplication than she does to have a child with Down syndrome. Recently the ability to test fetal cell free DNA in the maternal plasma for fetal microdeletions has been described (Bianchi et al., 2014) and in the near future may lead to routine screening for affected pregnancies. While this information will be clinically valuable, counseling and ethical questions similar to those with CMA on invasive samples will require thoughtful answers.

Conclusion/Moving Forward

One of the major advantages of CMA is the incremental information it provides. However, with this increased and sometimes uncertain information available, the role of genetic counseling is more essential than ever (Bernhardt et al., 2013). As with other emerging genetic tests, such as sequencing, there is a critical need to evaluate the educational and psychosocial implications to better understand the best way to integrate genomic information into clinical care.

Disclosure

The authors report no conflicts of interest.

References


Coppinger J, Alliman S, Lamb AN, Torchia BS, Bejjani BA, Shaffer LG. Whole-genome microarray analysis in prenatal specimens identi-
flies clinically significant chromosome alterations without increase in results of unclear significance compared to targeted microarray. Prenat Diagn 29(12):1156-1166, 2009.


Schaeffer AJ, Chung J, Heretik K, Wong A, Ledbetter DH, Lese Martin C. Comparative genomic hybridization-array analysis enhances the detection of aneuploidies and submicroscopic imbalances in sponta-


