Array Comparative Genomic Hybridization (aCGH): A Diagnostic Test for the Prenatal Diagnosis of Chromosomal Abnormalities with Emphasis on Patients with Abnormal Ultrasounds

Paul R. McGown
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Abstract

**Background:** When abnormalities are found during the anatomy scan most patients are offered amniocentesis and conventional karyotyping, using Giemsa (G)-banding of metaphase chromosomes to detect aneuploidies and large structural changes in the prenatal diagnosis. The use of fluorescent in situ hybridization (FISH) reduces the time to obtain a result because culture is not necessary, but can only detect a limited number of prespecified targets. Small studies have shown that array comparative genomic hybridization (aCGH) can detect all unbalanced chromosomal abnormalities as well as smaller deletions and duplications that cannot be detected with routine cytogenetic analysis. Should aCGH screening be used instead of karyotyping to diagnose prenatal chromosomal abnormalities in pregnant patients with abnormal ultrasound?

**Methods:** An exhaustive search of available medical literature from the past 5 years was conducted using Medline-OVID, CINAHL, Web of Science. Key words included: comparative genomic hybridization, pregnancy, abnormal ultrasound, prenatal ultrasound and ultrasound. Relevant articles were assessed for quality using GRADE.

**Results:** Two studies met inclusion criteria and were used in this review. The first is a large prospective, comparison to gold standard (karyotyping). This study compared prenatal diagnostic samples, and found microarray (aCGH) was equivalent to standard karyotype analysis for common aneuploidies and found additional clinically relevant information when patients had abnormal ultrasounds. The second study was a prospective study of over 5000 pregnancies and again additional clinical significant findings were found using aCGH.

**Conclusion:** Array comparative genomic hybridization should be considered for all patients who wish to undergo invasive prenatal screening and should be offered to all patients with abnormal prenatal ultrasounds. Adequate genetic counseling should be provided by a trained professional in all cases. A cost analysis should be done comparing tests.

**Keywords:** Array comparative genomic hybridization, pregnancy, abnormal ultrasound, prenatal diagnosis

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Array Comparative Genomic Hybridization (aCGH): A Diagnostic Test for the Prenatal Diagnosis of Chromosomal Abnormalities with Emphasis on Patients with Abnormal Ultrasounds

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A Clinical Graduate Project Submitted to the Faculty of the School of Physician Assistant Studies Pacific University Hillsboro, OR For the Masters of Science Degree, August 10, 2013

Clinical Graduate Project Coordinator: Annjanette Sommers, PA-C, MS
Biography

[Redacted for privacy]
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**Conclusion:** Array comparative genomic hybridization should be considered for all patients who wish to undergo invasive prenatal screening and should be offered to all patients with abnormal prenatal ultrasounds. Adequate genetic counseling should be provided by a trained professional in all cases. A cost analysis should be done comparing tests.

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Acknowledgements

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List of Abbreviations

aCGH  Array Comparative Genomic Hybridization
ACOG  American Congress of Obstetricians and Gynecologists
CVS   Chorionic Villus Sampling
CNV   Copy Number Variants/Variations or Copy Number Alterations
GRADE Grading of Recommendations, Assessment, Development and Evaluations
IFS   Indication For Study
VOUS  Variant Of Uncertain Significance
SNP   Single Nucleotide Polymorphism
BACKGROUND

The decision to undergo invasive prenatal screening can be difficult. There are many factors that may lead to the decision: family or personal history of birth defects, the increased risk of chromosomal abnormalities, a future life with an affected child, and the options for earlier termination. When patients are at increased risk for chromosomal abnormalities, they are offered chorionic villus sampling (CVS) in their first trimester or amniocentesis in the second and third trimester.\(^1,2\) The standard screening test is conventional metaphase karyotype analysis of chromosomes to detect aneuploidies and large structural changes in the prenatal diagnosis, limitations include: inconsistent identification of microscopic gene defects and requires cell culture, which takes seven to fourteen days to obtain.\(^2-4\) The use of fluorescent in situ hybridization (FISH) reduces the time to obtain a result because culture is not necessary, but can only detect a limited number of prespecified targets.\(^3\)

Array comparative genomic hybridization (aCGH), also known as microarray, has been successful in the diagnosis of genomic rearrangements in children and adults with birth defects, dysmorphic features, and developmental delay or neurobehavioral abnormalities.\(^5\) This led to the use in the prenatal setting. Some smaller studies\(^2,3\) have shown some clinical benefit to using aCGH in the prenatal setting, and it has the ability to detect all unbalanced chromosomal abnormalities as well as smaller deletions and duplications that would be missed on standard karyotype. Furthermore, culture is not needed, and the entire genome can be assessed, unlike FISH. However, aCGH does
however have limitations. Several studies\(^2,^3,^6-^9\) show that aCGH testing was unable to diagnose balanced translocations, inversions and alterations, and has difficulty diagnosing all polyploidy conditions and low-level mosaicism. Additionally, studies have shown that copy number variants (CNV) of uncertain clinical significance as well as benign CNVs may be found during testing.\(^2,^3,^6-^9\) The studies cited above have shown the benefits of adding aCGH testing for prenatal diagnosis but current ACOG guidelines suggest that it be additive for patients with abnormal ultrasounds with normal karyotypes.\(^10\) The purpose of this search was to concentrate specifically on studies with a large patient population as well as large subpopulations of patients with abnormal ultrasounds and to answer the question; should aCGH screening be used instead of karyotyping to diagnose prenatal chromosomal abnormalities in pregnant patients with abnormal ultrasounds?

**METHODS**

An exhaustive search of available medical literature from the past 5 years was conducted using Medline-OVID, CINAHL, and Web of Science. Key words included: comparative genomic hybridization, pregnancy, abnormal ultrasound, prenatal ultrasound and ultrasound. Search results were narrowed eliminating articles that were not in English, non-human, articles prior to 2009 and n< 1000 when pertaining to abnormal ultrasounds. Bibliographies of the articles were also searched for relevant sources. Articles with large patient populations with abnormal prenatal ultrasounds and aCGH screening compared to standard karyotype were included. Relevant articles were critically reviewed and assessed for quality using Grading of Recommendations, Assessment, Development and Evaluation (GRADE).\(^11\)
RESULTS

The initial search yielded 29 articles for review. After assessing the articles for relevancy to the topic, eight articles were further reviewed based on the inclusion criteria. Of these, two articles met both inclusion and exclusion criteria. Both articles are large prospective studies.\textsuperscript{12,13} See Table I

**Chromosomal Microarray versus Karyotyping for Prenatal Diagnosis**

This large prospective and blinded study\textsuperscript{12} assessed the ability for microarray analysis (aCGH) to detect common chromosome abnormalities and determine what additional clinically relevant information can be provided by microarray analysis. Training, microarray kits and reagents were provided by Agilent Technologies and Affymetrix free of charge and were not involved in the actual study. Data analysis was performed by the authors. Integrated Genetics had no part in the microarray analysis but did receive funding to perform conventional cytogenetic data (karyotyping). Primary outcomes assessed microarray results as being a true positive, true negative, false positive or false negative when compared to standard karyotyping for identifying common autosomal and sex-chromosome aneuploidies. Secondary outcomes included: the number and classification of CNV identified by microarray in the presence of a normal karyotype, the success of microarray analysis, and the ability for microarray to identify uncommon cytogenetic abnormalities, such as, polyploidy, marker chromosomes or rearrangements that would normally be seen on karyotype.\textsuperscript{12}

Starting in October 2008 and finishing in July 2011, 6537 women were screened for this study. The study enrolled 4406 women with singleton gestation and utilized 29 diagnostic centers where patients received CVS or amniocentesis. Eligibility for testing
included: advanced maternal age, positive aneuploidy screening test and structural anomalies on ultrasound. Appropriate pretest genetic counseling was performed. Of this group 4282 had both karyotype and microarray analysis. One hundred and twenty four samples were excluded due to maternal contaminations, culture or karyotype analysis failure, microarray failure, or mosaic karyotypes. At least 2mg of chronic-villus tissue and approximately 7 to 10 ml of amniotic fluid were deidentified and sent with parental blood samples to laboratories at Baylor College Medicine, Columbia University, Emory University, or Signature Genomic Laboratories for microarray analysis. Integrated Genetics received all CVS and amniocentesis results and karyotype results were reported to the referring physician.

Two array platforms were used; Agilent 4-plex array and Affymetrix Genome wide Human SNP Array 6.0. The majority 71% were performed on Agilent 4-plex array. When Affymetrix was used analysis software was used to mask the data to match resolution and coverage of the Agilent platform. Initially both cultured and uncultured samples were analyzed. Acceptable uncultured results were observed in first 259 CVS and 275 amniotic fluid samples so the remaining samples were performed uncultured, which allows for a faster turnaround time. When CNV were detected parental blood samples were used to determine if these were inherited or de novo. De novo findings were checked with normal karyotype and by FISH analysis.

George Washington University Biostatistics Center was used for data coordination. This independent coordination center received both array and karyotype results from each laboratory. SAS software was used in the analysis. CNVs were assessed by the study’s clinical geneticist and all CNVs not judged as likely benign were sent to an
independent clinical advisory group for review and classification. All variants determined clinically important were reported to patients.\textsuperscript{12}

Standard karyotype revealed 317 common autosomal and 57 sex-chromosome aneuploidies. One hundred percent of these findings were also found using microarray analysis, in addition microarray revealed clinically significant segmental aneuploidies not detected on standard karyotype. Of the structural rearrangements, microarray also discovered all 22 unbalanced and rearrangements that were revealed on standard karyotype, however, did not reveal any balanced rearrangements or triploid samples. Microarray identified 1399 samples CNV of these 165 were looked at more closely and 35 were on a predetermined pathogenic list. Further analysis revealed 61 more CNVs that were clinically significant. Overall 96 of the 3822 samples with normal karyotypes had clinically significant CNVs (2.5\%; 95\% CI 2.1 -3.1).\textsuperscript{12}

Subgroups results included: advanced maternal age, positive down syndrome screening, anomaly on ultrasound, and other. Microarray found additional clinically significant findings in all categories. The largest percentage was noted in patients with anomaly on ultrasound. A total of 1109 patients were enrolled for this indication and 755 had normal karyotypes, of that group 45 were determined to have clinically significant findings on microarray (6.0\%; 95\% CI 4.5-7.9).\textsuperscript{12}

\textbf{Experience with Microarray-based Comparative Genomic Hybridizations for Prenatal Diagnosis in Over 5000 Pregnancies}

In this large prospective study\textsuperscript{13} the authors attempt to demonstrate the advantages of aCGH over karyotyping for prenatal diagnosis of clinically relevant chromosome alterations. Starting in July 2004 through December 2011 prenatal samples from amniotic
fluid, CVS, fetal blood and products of conception were sent directly to the authors’ laboratory for analysis. A total of 5003 samples were tested from the US and abroad. The indication for array testing or indication of study (ISF) included: abnormal karyotype, family history of a parent known to carry a chromosome rearrangement or imbalance, fetal demise, abnormal first or second trimester screen, abnormal ultrasound, other family history of genetic conditions, advanced maternal age, paternal anxiety, and other/not specified. The largest IFS was abnormal ultrasound with 2858 patients.13

Using the laboratory data base and Genoglyphix database, samples were identified as normal (no clinically significant CNVs or with or without benign CNVs), with variant of uncertain significance (VOUS), or abnormal (clinically significant). Each case was reviewed by the authors MPD and JAR and categorized based on the results discussed above as well as their IFS. The latter was categorized based on the patient’s most significant risk factor. Samples with unclear results were reviewed further by authors LGS and JAR and reassigned if indicated. Abnormal ultrasound were further classified according to clinical phenotype.13,14

Samples with unclear results were review by two different authors LGS and JAR and reassigned to either normal or abnormal groups. Abnormal results were then reviewed by author JAR and stratified based on the size of the alteration. An overall detection rate of clinically significant results was 5.3 % with a two-tailed p=0.024. Of the total samples 56.3 % or 2819 were referred with normal karyotypes and the detection rate increased to 5.5% among this category when the bias of family history and fetal demise were excluded (5.5%, 140/2533). In addition 71% of the abnormalities identified were < 10Mb and would likely not be seen on standard karyotype. Microarray also detected 33
samples with abnormalities > 10Mb that had normal karyotypes. When IFS was an abnormal ultrasound, the percent of additional significant clinically relevant CNVs increased to 6.5% in the presence of a normal karyotype.\textsuperscript{13}

**DISCUSSION**

Microarray-based comparative genomic hybridization has been shown in many small prospective studies and case studies to add clinically significant detection rates for chromosomal abnormalities in the prenatal setting.\textsuperscript{2,6-9,15,16} Currently ACOG supports the use of aCGH testing but not as a first line test in the prenatal setting.\textsuperscript{1,10} These two large prospective studies\textsuperscript{12,13} give additional evidence to support past findings. Additionally, the large patient population includes a larger subpopulation with abnormal ultrasound to help answer the question: Should aCGH screening be used instead of karyotyping to diagnose prenatal chromosomal abnormalities in pregnant patients with abnormal ultrasound?

When comparing aCGH with standard karyotype for the prenatal diagnosis of common aneuploidies Wapner et al\textsuperscript{12} showed equivalence. Furthermore, both studies\textsuperscript{12,13} revealed clinically relevant information that was not found on standard karyotype. In the presence of an abnormal prenatal ultrasound and normal karyotype Wapner et al\textsuperscript{12} found an additional 6% of clinically significant findings and Shaffer et al\textsuperscript{13} had similar results 6.5%. Furthermore, Wapner et al\textsuperscript{12} found that uncultured samples are just as efficacious, therefore avoiding the additional time needed for culture.\textsuperscript{12,13}

However, both studies\textsuperscript{12,13} established that there are some limitations. This includes the ability for aCGH to fined VOUS, and its inability to identify triploidy or balanced translocations. Wapner et al\textsuperscript{12} does report that a post hoc review reveled that use
single nucleotide polymorphism (SNP) probes would have identify triploid cases and thus recommends using SNP probes with testing. Mutually, these studies agree that appropriate pre and post genetic testing should be required to prepare patients for potential result.\textsuperscript{12,13}

Both studies\textsuperscript{12,13} were large prospective studies. Additionally, Wapner et al\textsuperscript{12} was a blind comparison to a gold standard (karyotype). Each study characterized CNVs into four similar categories. Wapner et al\textsuperscript{12} was a well conducted study that utilized blinding when applicable and used an independent clinical advisory group to analyze all samples that were not judged as “likely benign.” The Shaffer et al\textsuperscript{13} study did not mention blinding and all results were reviewed by the study authors, which could contribute to some bias. These limitations were taken into consideration for the GRADE analysis. See Table II

A cost analysis was not performed in either study.\textsuperscript{12,13} It would be important to determine if this would be a cost effective method when compared to standard karyotyping alone or with FISH analysis.

CONCLUSION

Array comparative genomic hybridization should be considered for all patients who wish to undergo invasive prenatal screening and should be offered to all patients with abnormal prenatal ultrasounds. This test has shown to be equivalent to standard karyotype analysis in determining common aneuploidies and is able to find clinically relevant abnormalities that would otherwise be missed on karyotype. Adequate genetic counseling should be provided by a trained professional in all cases and should include the benefits and limitations of aCGH testing. As aCGH becomes more utilized the
amount of variants of uncertain significance will decrease and thereby decreasing the challenge of genetic counseling and anxiety placed on the parents.
References


### Table I. Characteristics of Reviewed Studies

<table>
<thead>
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<th>Studies</th>
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<tr>
<td></td>
<td>Ability to identify abnormal autosomal and sex-chromosome aneuploidies</td>
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<tr>
<td></td>
<td>Karyotype</td>
</tr>
<tr>
<td>Wapner et al¹²</td>
<td>374</td>
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<tr>
<td>Shaffer et al¹³</td>
<td>*</td>
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### Table II. Summary of Findings

<table>
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<tr>
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<tr>
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<td>Wapner et al¹²</td>
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</tr>
<tr>
<td>Shaffer et al¹³</td>
<td>prospective</td>
<td>5000</td>
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Figure I:

Array CGH: The Complete Process

Steps 1-3: Patient and control DNA are labeled with fluorescent dyes and applied to the microarray.

Step 4: Patient and control DNA compete to attach, or hybridize, to the microarray.

Step 5: The microarray scanner measures the fluorescent signals.

Step 6: Computer software analyzes the data and generates a plot.

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