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Clinical experience with a targeted microarray designed for cytogenetic diagnostics. L.G. Shaffer¹, C.D. Kashork¹, B.C. Ballif¹, R. Saleki¹, F. Lacbawan², K. Chong³, M. McDonald⁴, M. Irons⁵, E.H. Zackai⁶, B.A. Bejjani¹. ¹Signature Genomic Laboratories, LLC, Spokane, WA; ²Children's National Medical Center, Washington, D.C.; ³Mount Sinai Hospital, Toronto, Canada; ⁴Duke University, Durham, NC; ⁵Children's Hospital Boston, MA; ⁶Children's Hospital of Philadelphia, PA.

Comparative genomic hybridization (CGH) on microarrays (array CGH) provides distinct advantages over conventional cytogenetic analysis. We report the study of 396 consecutive cases sent to our diagnostic laboratory for array CGH testing. The SignatureChip™ was designed to detect the common microdeletions, reciprocal microduplications, rearrangements involving the subtelomeric regions and pericentromeric regions, unbalanced translocations, and aneuploidy. The design also allows for distinguishing the common-sized deletions in DGS, WS, PWS/AS, and SMS from larger deletions through the use of flanking control loci. The array comprises 831 BACs covering over 126 clinical and 104 control loci in 3-6 clone contigs. Of the 396 cases studied, the majority had previous cytogenetic analysis, subtelomere FISH, and/or locus specific FISH. Forty-three cases were identified to have DNA copy number alterations. Of these, 10 were found in a phenotypically normal parent. These likely represent polymorphisms, but because of the genetic content of some sequences for 6 cases, clinical relevance could not be excluded. The remaining 33 cases (8.3%) were de novo alterations of genomic regions known to result in abnormal phenotypes. The following cases illustrate important advantages of the SignatureChip™. A larger deletion of the SMS region on 17p11.2 was identified that extended through the distal control contig; the reciprocal duplication of the 22q11.2 deletion in a case with heart defect and family history of heterotaxy; deletion in Dystrophin in a 30 month old with developmental delay and dysmorphic features that lead to the identification of his mother as a carrier for DMD; and deletion of 22q11.2 and deletion of 15q12 in cases with nonspecific clinical presentations. Our data demonstrate that targeted array CGH detects a substantial proportion of abnormalities even in patients who have already had extensive cytogenetic/FISH testing. Further use of this approach is likely to expand the phenotypes of recognized cytogenetic disorders, uncover the chromosomal etiology of known syndromes, and identify new syndromes.

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First Trimester Isolation and Purification of Trophoblasts from Cervical Swabs for Non-Invasive Prenatal Diagnosis. F.Z. Bischoff¹, T. Pircher², Z. Tang², H. Radisch², P. Tsinberg², J.L. Simpson¹, P. Cotter², R. Bhatt². ¹Dept OB/GYN, Baylor Col Medicine, Houston, TX; ²Biocept, Inc. San Diego, CA.

BACKGROUND: Early prenatal diagnosis to detect fetal genetic disorders is desired by both expectant mothers and physician to make informed decisions. Current methods of prenatal testing, namely amniocentesis and chorionic villus sampling (CVS), carry small but finite risk of miscarriage, and the results are rarely available before 12-16 weeks of pregnancy due to the time required for cell culture. Thus, recovery and analysis of endocervical (fetal) trophoblast cells would provide a safe alternative approach for rapid non-invasive prenatal diagnosis. **OBJECTIVE:** To determine the reliability of a novel device utilizing microelectro mechanical system (MEMS) channels to isolate, purify and characterize fetal trophoblasts from maternal transcervical mucous specimens. **METHOD:** Following IRB approval and consent for the pilot study, endocervical swab specimens were obtained from 17 pregnant women during the first trimester (8-12 weeks gestation). Samples were washed and processed for trophoblast targeted isolation using a novel MEMS device coated with proprietary reagent and trophoblast specific antibody. Immunohistochemical staining using cytokeratin 7 (CK7) and/or cytokeratin 17 (CK17) was employed to determine the purity of the recovered cell population. **RESULTS:** Following purification and cytokeratin staining, trophoblasts were detected in each of the 17 cases. However, the number of trophoblasts present in each sample depended upon the sample collection procedure. Although only 0.02% to 1.94% of the initial total cell population were trophoblasts, the recovered cell population was determined to be predominantly of trophoblast origin. Trophoblast isolation was optimal in samples not contaminated with blood which adversely affected the percent recovery. **CONCLUSION:** Recovery of highly purified trophoblast cells from early first trimester endocervical swab specimens is feasible using this novel MEMS device. Investigations are currently underway to detect fetal chromosomal aneuploidy and diagnostic potential utilizing FISH and PCR based methods.

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Prenatal diagnosis of cytogenetic abnormalities by comparative genomic hybridization on microarrays: experience of a pilot program. S. Darilek, A. Patel, P. Ward, J. Li, I. Van den Veyver, S. McAdoo, A. Burke, B. Roa, C. Shaw, T. Sahoo, C. Chinnault, S. Cheung, A. Beaudet, C.M. Eng. Dept of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

The recent development and availability of chromosome microarray analysis (CMA) for the screening and rapid detection of chromosomal aberrations has provided an important new tool for the diagnosis of genetic disorders. While CMA now has an established role in the genetic evaluation of pediatric/adult patients, the potential use of CMA has not been evaluated in the prenatal setting. The purpose of this study was to 1) assess the reliability of CMA to detect cytogenetic abnormalities in fetal samples, 2) design a program to provide patient education and support for the unique genetic counseling issues that may arise from this new testing platform, and 3) assess the acceptance of CMA among couples undergoing prenatal diagnosis. Our current microarray (v.3) contains 362 FISH-verified clones that span genomic regions implicated in over 40 known human genetic disorders as well as subtelomeric clones for all 41 relevant human chromosome telomeric regions, thereby greatly expanding the capability to detect abnormalities in these regions when compared to a conventional prenatal karyotype. Study participants were recruited from among couples undergoing either amniocentesis or CVS. Each participant had genetic counseling and the benefits and risks of CMA, including the possibility of obtaining inconclusive results, were discussed in detail prior to obtaining informed consent. Thus far, 38 couples elected to participate in CMA testing. Of the 38 samples, 23 (60%) were amniotic fluid, 14 (37%) were CVS, and 1 was a fetal blood sample. Indications for prenatal testing included advanced maternal age (22, 58%), fetal ultrasound anomalies (10, 26%), and a previous history of an affected child (5, 13%). All abnormalities detected by karyotype were also detected by CMA and consisted of three cases of trisomy 21. In addition, in 5 of 38 cases (13%), initial CMA analysis yielded inconclusive results that required study of parental samples for clarification. There was 63% acceptance of CMA testing in a consecutive series of 19 eligible participants seen by a single counselor. The most frequent reasons for accepting CMA testing included having a previous child with anomalies, an abnormal ultrasound, or a desire to learn as much about the current pregnancy as possible. Reasons for declining testing included the perception that the disorders tested were rare and concern that the test would raise anxiety. This study represents the initial use of CMA in pregnancies being monitored due to increased risk of chromosomal abnormalities and demonstrates a high level of acceptance and accuracy. Additional experience will optimize patient education and counseling and yield further insight into the degree of normal variation in these regions.

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Patient decision making regarding population screening for cystic fibrosis and fragile X syndrome in the prenatal genetic counseling setting. A. Cronister, V.J. Weinblatt. Genetic Services, Genzyme Genetics, Phoenix, AZ.

OBJECTIVE: Patients referred for genetic counseling are often offered carrier screening for disorders which range in severity and are unrelated to their referral indication. The purpose of this study was to evaluate carrier testing acceptance rates in patients referred for prenatal genetic counseling for two common disorders: cystic fibrosis (CF) and fragile X syndrome (FXS). In addition to documenting overall acceptance, other factors which might influence carrier screening acceptance rates including referral indication and acceptance of an invasive procedure were analyzed. **METHODS AND MATERIALS:** The study population included 2865 patients referred for prenatal genetic counseling between January 2002 and October 2004. Patients were referred for maternal age (MA) (N=2035), positive maternal serum screening (+MMS) (N= 679), exposure (N=114) or parental anxiety (N=37) and were offered both CF and FXS carrier testing on the basis of population screening only. Patients found to have a known or suggestive family history of either CF or FXS were excluded from the study. To support analysis of a population with comparable risks for the disorders in question, only patients of Caucasian and Ashkenazi Jewish backgrounds were included. **RESULTS:** Overall carrier testing acceptance rates were 30% for CF and 25% for FXS (p<.0001). Twenty-three percent of patients accepted CF and FXS testing, while 67% declined both tests at the time of their visit. Significantly fewer (10%; p<.0001) accepted one test but declined the other. Patients who consented to an invasive procedure were more than twice as likely to accept both screening tests (31.8%), compared to patients who declined invasive testing (14.6%)(p<.0001). We found no significant difference in carrier testing acceptance rates among MA patients compared to patients referred for + MMS. The CF acceptance rate among the two referral populations was 30.7% and 30% respectively (p = 0.7616 ns) and FXS was 25.7% and 24.7% respectively (p = 0.8880 ns). **CONCLUSION:** Caucasian and Ashkenazi Jewish patients are more likely to consent to CF screening than FXS screening and are more likely to consent to screening in general when they accept invasive prenatal diagnosis via CVS or amniocentesis. These findings suggest that there may be specific populations of patients who are uncomfortable undergoing testing in general, and other populations for whom the concept of testing is much more acceptable. This is demonstrated by the significantly fewer patients who declined one carrier test and accepted another and by the fact that patients are more likely to consent to screening when they accept an invasive procedure.

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Reporting Genetic Test Results: Format, Context, and Use of Language in Clinical Laboratory Result Reports for DNA-Based Cystic Fibrosis Testing. I.M. Lubin¹, M. Caggana^{2,5}, C. Constantin¹, D.J. Wolff^{3,5}, E. Lyon^{4,5}. ¹Div Lab Services, Centers for Disease Control and Prevention, Atlanta, GA; ²Wadsworth Center, NY Dept Public Health, Albany, NY; ³Dept Path/Lab Med, Medical University of South Carolina, Charleston, SC; ⁴Assoc Regional Univ Pathologists Laboratories and Dept Path, University of Utah, Salt Lake City, UT; ⁵Association for Molecular Pathology, Clinical Practice Committee, Bethesda, MD.

Genetic test reports must effectively communicate essential information to clinicians to meet the health needs of their patients and families. This study provides a detailed analysis of reporting practices for cystic fibrosis DNA-based testing among twenty-six U.S. laboratories (academic and private) that are geographically dispersed. While previous studies provided important data as to what critical elements, as defined by professional recommendations, are sometimes missing from reports, our intent was to systematically evaluate the 1) use of information collected from the laboratory's native requisition form in preparing the result report, and 2) variation in format, content, and the use of language in communicating results. Laboratories returned reports for two diagnostic and three carrier mock testing scenarios presented by way of their native requisition forms and provided genotypic results. Report formats varied. Genotypic results were described using differing terminologies, typically found mid-page, and minimally emphasized compared to other text. Reporting of risk estimates varied according to the case scenario. For instance, reports for carrier tests with a positive family history where no mutations were found were more likely to provide patient-specific risk estimates (81%) than when a family history was not present (54%). In addition, reports having an R117H finding were less likely to provide patient-specific risk assessments (64%). A risk assessment was most often reported relevant to the patient carrying a disease-associated mutation as opposed to risk for having an affected child. Detection rate was provided in 88% of reports but its significance was not uniformly described. For diagnostic testing scenarios, language varied in describing the clinical implications for the test result when only a single mutation was found. Recommendation for genetic counseling was consistent among reports with the exception for cases pertaining to a negative family history where no mutations were found. Overall, laboratory reports were accurate but there was significant variation in format, content, and use of language. This raises concern for confusion among clinicians not familiar with a particular laboratory's report, testing practices, or cystic fibrosis testing. Adopting common language and formatting in ordering tests and reporting results for cystic fibrosis and other genetic disorders may promote effective communication between the laboratory and clinician to the benefit of their patients and families.

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Development of a novel orally administered gene expression therapy for Gaucher Disease. E.I. Ginns, D.M. Faryna, C. Chrzanowski, M. Galdzicka, G.R. Ostroff. Molecular Diagnostics Laboratory and Pediatric Neurology, Department of Pediatrics, University of Massachusetts Medical School, Worcester, MA 01605.

A novel, orally administered yeast β -glucan particle delivery system has been developed to deliver into macrophages bioactive DNA that expresses enzyme to restore normal enzyme activities to treat lysosomal storage disorders. Enzyme replacement therapy (ERT) for Gaucher disease, the most common lysosomal storage disorder, has been available since 1991. When administered long-term, ERT improves blood counts, and reverses liver and spleen abnormalities. However, the current enzyme formulation must be intravenously administered over several hours every 2 weeks, is costly, and has not significantly reversed or retarded bone or neurological complications. As a demonstration of this technology's ability to improve upon current ERT, we applied this approach to Gaucher disease.

This orally administered DNA delivery system was used to introduce human glucocerebrosidase DNA into both the J774 murine macrophage cell line *in-vitro*, and to our long-lived Gaucher mice *in-vivo*. These Gaucher mice having either the L444P or R463C mutations were generated by gene targeting in murine embryonic stem cells. These mutations are two of the five most common mutations found in Gaucher patients. The clinical manifestations of Gaucher disease in these mice can be worsened and accelerated by short courses of conduritol- β -epoxide (CBE), a glucosidase inhibitor. Following oral particle uptake through Peyer's Patches, macrophages phagocytose the particles containing human glucocerebrosidase DNA and migrate to the organs of the reticuloendothelial system. Within the macrophage endosomal compartment the DNA encoding human glucocerebrosidase is released at acid pH, migrates to the nucleus, and is expressed to produce normal human glucocerebrosidase.

Particle-mediated DNA delivery results in high levels of human glucocerebrosidase in the J774 murine macrophage cell line. In Gaucher mice this treatment results in high levels of normal human glucocerebrosidase within splenic, liver and lung macrophages. Studies on bone and brain are in progress. Preliminary findings also suggest that this therapy provides sufficient correction of tissue enzyme activity to ameliorate the symptoms in treated mice, compared to untreated, severely affected Gaucher mice.

Our findings demonstrate that the orally administered microscopic yeast cell wall particles containing DNA encoding normal human glucocerebrosidase allow macrophages to very efficiently produce normal human glucocerebrosidase. These encouraging preclinical data, along with results from ongoing therapeutic trials with Gaucher mice, provide a strong rationale to apply this novel therapeutic approach to treat Gaucher Disease in human clinical trials.