may be useful molecular markers for monitoring the presence of pre-eclampsia.

2-5
Percentage of fetal cell-free DNA in maternal plasma: Dependence on multiple clinical factors
Xitong Li, Amy Sehnert, Richard Rava
Verinata Health, Inc., Redwood City, California, United States

OBJECTIVES: The objectives of this study were to determine the influence of multiple clinical factors on the percentage of fetal cell free DNA (cfDNA) in maternal plasma samples. Blood samples from women with both aneuploid and unaffected fetuses from a multi-center study using massively parallel DNA sequencing (MPS) were examined. METHOD: Blood samples were collected in a prospective, blinded study from 2,882 women undergoing prenatal diagnostic procedures at 60 United States sites to determine the capability of MPS to detect fetal aneuploidy. MPS of total cfDNA obtained from the maternal plasma yields millions of short sequence tags that can be aligned and uniquely mapped to sites on a reference human genome, and counted to detect fetal aneuploidy and sex. The relative fetal fraction was determined from normalized chromosome values (NCVs) obtained from MPS for chromosome X and 21, respectively. On 532 samples that were part of a case:control analytical cohort, the fraction of cfDNA was determined for samples classified as male by MPS (n=183) and trisomy 21 samples (n=93) in the cohort. The relationships between clinical factors including body mass index (BMI), gestational age (GA), maternal age (MA), maternal race, and other fetal factors with NCVs were independently determined. RESULTS: Statistically significant but weak dependence was observed for cfDNA fetal fraction and maternal BMI (range 17-59 kg/m2) using NCVs for X chromosome in male classified samples (p=3.34 x 10^-4, R2= 0.069) and chromosome 21 in samples with fetal trisomy 21 (p=0.016, R2= 0.062). The dependence of cfDNA fetal fraction on the GA range examined (10-23 weeks) was not significant (p=0.052, R2= 0.021 for Chr X analysis and p=0.065, R2= 0.037 for Chr 21 analysis). There was no dependence of cfDNA fetal fraction for any of the other factors studied (maternal race, maternal age, and other). CONCLUSIONS: The fetal fraction of cfDNA in maternal plasma depends weakly on BMI but is not significantly affected by other clinical factors.

3-1
The use of chromosome microarray analysis as a first-line test in low risk pregnancies
Francesco Fiorentino1, Stefania Napoletano1, Fiorina Caiazzo1, Sara Bono1, Letizia Spizzichino1, Silvia Michiorri1, Anthony Gordon2, Andrea Nuccitelli1, Giuseppe Rizzo1, Mariateresa Sessa1, Marina Baldi1
1GENOMA- Molecular Genetics Laboratory, Rome, Italy, 2Bluegnome Ltd, Cambridge, United Kingdom

OBJECTIVES: Although several large-scale prospective clinical trials have demonstrated the usefulness of chromosome microarray analysis (CMA) in clinical prenatal diagnosis practice, at present it is not clear yet which indications could benefit from this assay. In the absence of specific guidelines, it has been suggested that CMA should be offered to selected groups of high risk pregnancies, using the technique as a second-line test only. In this study we aimed to explore the usefulness of CMA in groups of pregnancies with a priori low risk for detection of submicroscopic chromosome abnormalities, usually not considered an indication for testing, in order the assess if CMA improves the prenatal detection rate of chromosomal aberrations. METHOD: A total of 2800 prenatal samples were processed in parallel using both CMA and G-banding for conventional karyotyping. The indications for prenatal testing included: 1033(36.9%) advanced maternal age (AMA), 27(1.0%) abnormal results of maternal serum screening tests (MSS), 90(3.2%) abnormal ultrasound findings (AUS), 24(0.9%) known abnormal fetal karyotype (AFK), 1569(56.0%) parental anxiety (PA), 24(0.9%) family history of a genetic condition (FIS), 33(1.2%) cell culture failure (CCF). RESULTS: The use of CMA resulted in an increased detection rate regardless of the indication for analysis. This became especially evident in high-risk groups (AMA, AFK), in which the percentage of detection was 6.1% (7/114), and also in low risk groups, such as AMA (7/1033, 0.7%) and PA (10/1569, 0.6%). A total of 24 fetal conditions (0.9% of the entire cohort of samples and 25.0% of the clinically relevant findings), would have remained undiagnosed if only a standard karyotype had been performed. More importantly, 17(0.6%) of such findings would have otherwise been overlooked offering CMA to high risk pregnancies only. CONCLUSIONS: The results of this study demonstrate that more widespread testing by CMA in fetuses would result in a higher detection of chromosome abnormalities prenatally, also in low risk pregnancies. Offering CMA only for selected groups of pregnancies substantially limit the diagnostic potential of this assay, missing pathological copy number
anomalies in a single organ system, CNAs were found with CNS anomalies (n=26/402, 6.5%), heart defects (n=11/267, 4.1%), dysmorphisms (n=7/108, 6.5%), diaphragmatic hernia (n=4/49, 8.2%), omphalocele (n=4/51, 7.8%), musculoskeletal anomalies (n=18/231, 7.8%), genitourinary anomalies (n=7/137, 5.1%), and cystic hygroma (n=9/212, 4.2%). In addition, the following anomalies in isolation or with multiple findings had particularly high detection rates of CNAs: holoprosencephaly (n=9/84, 10.7%), posterior fossa defects (n=21/144, 14.6%), skeletal anomalies (n=15/140, 10.7%), VSD (n=15/131, 11.5%), hypoplastic left heart (n=11/69, 15.9%), and cleft lip/palate (n=14/135, 10.4%). CONCLUSIONS: Microarray analysis identified clinically significant CNAs in 6.6% of cases referred for testing because of one or more abnormal ultrasound finding. Among the cases tested, referrals for posterior fossa defects and hypoplastic left heart were among the highest detection rates of clinically significant abnormal array findings. Larger datasets will allow for further sub-stratification of specific anomalies to determine the related risks for genomic alterations detectable by microarray analysis.

3-4
Prenatal detection of both copy number and copy neutral changes by SNP array analysis

Stuart Schwartz1, Rachel Burnside1, Inder Gadi1, Elizabeth Keitges2, Romela Pasion1, Karen Phillips1, Venkatswara Potluri1, Hiba Risheg2, Brooke Rush1, Holly Taylor1, Jim Tepperberg1, Peter Papenhausen1

1Laboratory Corporation of America, Department of Cytogenetics, Center for Molecular Biology and Pathology, Research Triangle Park, North Carolina, United States
2LabCorp/Dynacare, Seattle, Washington, United States

OBJECTIVES: Microarray analysis has become routinely integrated, as a standard protocol, for pediatric patients referred with anomalies and/or developmental delay. However, this technology has been less utilized for prenatal analysis. The overall objectives from this work include: (1) To understand the technology underlying SNP array analysis and to realize the importance in delineating small copy number changes in prenatal diagnosis; (2) To understand how SNP array technology can detect both UPD and consanguinity and to delineate why the detection of these are both important prenatally. METHOD: In this study we have utilized an Affymetrix 6.0 and Cytoscan HD SNP microarray analysis to study over 1150 prenatal specimens. This technology allows the detection of both copy number and copy neutral changes. The vast majority of our specimens were ascertained because of normal chromosome results but an abnormal ultrasound (77.3%); although some were ascertained due to the detection of a chromosome abnormality (11%) or parental anxiety (11.7%). RESULTS: Results from the approximate 839 cases with an abnormal ultrasound, but chromosomally normal, revealed that approximately 5.7% had copy number changes and 3.8% had copy neutral changes. Both of these frequencies were increased when multiple abnormalities were detected and lowered if only soft ultrasonographic findings were detected. When the abnormalities involved the brain, kidney or the heart, both copy number and copy neutral changes were detected at a higher frequency. CONCLUSIONS: These studies have important and broad implications for the utilization of microarrays prenatally. These include that: (1) SNP array analysis can effectively be used prenatally to detect both copy number and copy neutral changes; (2) By utilizing a whole genome with a targeted gene list and conservative reporting ranges, detection of familial variants can be limited to 1-2% of the patient population; (3) More copy neutral changes (especially consanguinity) were detected in patients with multiple abnormalities, consistent with the presence of an underlying recessive disorder; (4) UPD was detected in several cases consistent with phenotypic abnormalities; and (5) the patients referred with chromosomal abnormalities could be examined in more detail and their abnormalities better defined (e.g. marker chromosomes and de novo rearrangements).

3-5
Chromosome microarray analysis in routine prenatal diagnosis practice: A prospective study on 2,800 clinical cases

Francesco Fiorentino1, Fiorina Caiazzo1, Stefania Napoletano1, Letizia Spizzichino1, Sara Bono1, Silvia Michiorri1, Andrea Nuccitelli1, Anthony Gordon2, Giuseppe Rizzo1, Mariateresa Sessa1, Marina Baldi1

1GENOMA- Molecular Genetics Laboratory, Rome, Italy, 2Bluegnome Ltd, Cambridge, United Kingdom

OBJECTIVES: Although several studies have demonstrated the usefulness of chromosome microarray analysis (CMA) in clinical prenatal diagnosis practice, only limited conclusions could be drawn due to the small size of the cohorts analysed. Whilst these studies have all provided reassuringly consistent results in terms of analytical validity, clinical validity and clinical utility of the technique applied in the
prenatal diagnosis setting, their limited data has necessitated undertaking of large-scale prospective clinical trials. To assess the feasibility of offering CMA for prenatal diagnosis as a first-line diagnostic test, a prospective study was performed on a cohort of 2800 consecutive prenatal samples, with parallel processing for both CMA and conventional cytogenetic analysis. **OBJECTIVES:** To evaluate the promoter methylation status of interleukin 10 in order to investigate the role of epigenetic markers in subsequent placental hypoxia and preeclampsia. **METHOD:** A prospective case control study was conducted from November 2010 to January 2012. 100 cases of abnormal uterine artery Doppler velocimetry (UADV) and 100 age-matched controls were enrolled. Blood samples were obtained at five different time points: 11-14 weeks of gestation (NT screening), 19-23 weeks of gestation (level II ultrasound), 24-28 weeks of gestation (GDM screening), onset of preeclampsia (onset of labor for the control group), and after delivery. All patients also underwent Doppler examination to measure the pulsatility index (PI) of the bilateral uterine artery (UA) and present of notches. The methylation degree of the IL-10 promoter was measured using EpiTect MethyLight Assays and IL-10 concentration was measured with ELISA method (RandD). Tests were been done by an examiner who was blind to the clinical outcome. **RESULTS:** We observed that the methylation degree of the IL-10 promoter was higher in the abnormal UADV group at each time point (11-14 weeks’ GA: p<0.001, 19-23 weeks’ GA: p<0.01, 24-28 weeks’ GA: p<0.03, postpartum: p<0.04). Promoter methylation degree was negatively correlated with IL-10 concentration in both the abnormal UADV group and the control group. 46 patients in the abnormal UADV group had onset of preeclampsia. The difference of the methylation degree between the preeclamptic patients and the non-preeclamptic patients was not statistically significant in the first trimester (p = 0.06). However, methylation degree decreased more in the non-preeclamptic group between the 1st and 2nd trimester (15.7% vs. 9.6%). **CONCLUSIONS:** Aberrant hyper-methylation of IL-10 promoter is negatively correlated with the presence of notches. The methylation degree of the IL-10 promoter was measured using EpiTect MethyLight Assays and IL-10 concentration was measured with ELISA method (RandD). Tests were been done by an examiner who was blind to the clinical outcome. **RESULTS:** We observed that the methylation degree of the IL-10 promoter was higher in the abnormal UADV group at each time point (11-14 weeks’ GA: p<0.001, 19-23 weeks’ GA: p<0.01, 24-28 weeks’ GA: p<0.03, postpartum: p<0.04). Promoter methylation degree was negatively correlated with IL-10 concentration in both the abnormal UADV group and the control group. 46 patients in the abnormal UADV group had onset of preeclampsia. The difference of the methylation degree between the preeclamptic patients and the non-preeclamptic patients was not statistically significant in the first trimester (p = 0.06). However, methylation degree decreased more in the non-preeclamptic group between the 1st and 2nd trimester (15.7% vs. 9.6%). **CONCLUSIONS:** Aberrant hyper-methylation of IL-10 promoter is negatively correlated with IL-10 concentration, which may contribute to the interruption of angiogenesis and poor placentation, resulting in subsequent placental hypoxia and preeclampsia.

### 4-1

**Applied DNA methylation analysis on cytokine regulatory mechanism of pre-eclampsia**

Tzu Hung Lin1, Lang Yao Chen1, Chia Hui Lin1, Shin Yu Lin1, Chien Nan Lee1, Yi Ning Su2

1Department of Obstetrics and Gynecology, National Taiwan University Hospital, Taipei, Taiwan

2Department of Medical Genetics, National Taiwan University Hospital, Taipei, Taiwan

**OBJECTIVES:** To evaluate the promoter methylation status of interleukin 10 in order to investigate the role of epigenetic markers in subsequent placental hypoxia and preeclampsia. **METHOD:** A prospective case control study was conducted from November 2010 to January 2012. 100 cases of abnormal uterine artery Doppler velocimetry (UADV) and 100 age-matched controls were enrolled. Blood samples were obtained at five different time points: 11-14 weeks of gestation (NT screening), 19-23 weeks of gestation (level II ultrasound), 24-28 weeks of gestation (GDM screening), onset of preeclampsia (onset of labor for the control group), and after delivery. All patients also underwent Doppler examination to measure the pulsatility index (PI) of the bilateral uterine artery (UA) and present of notches. The methylation degree of the IL-10 promoter was measured using EpiTect MethyLight Assays and IL-10 concentration was measured with ELISA method (RandD). Tests were been done by an examiner who was blind to the clinical outcome. **RESULTS:** We observed that the methylation degree of the IL-10 promoter was higher in the abnormal UADV group at each time point (11-14 weeks’ GA: p<0.001, 19-23 weeks’ GA: p<0.01, 24-28 weeks’ GA: p<0.03, postpartum: p<0.04). Promoter methylation degree was negatively correlated with IL-10 concentration in both the abnormal UADV group and the control group. 46 patients in the abnormal UADV group had onset of preeclampsia. The difference of the methylation degree between the preeclamptic patients and the non-preeclamptic patients was not statistically significant in the first trimester (p = 0.06). However, methylation degree decreased more in the non-preeclamptic group between the 1st and 2nd trimester (15.7% vs. 9.6%). **CONCLUSIONS:** Aberrant hyper-methylation of IL-10 promoter is negatively correlated with IL-10 concentration, which may contribute to the interruption of angiogenesis and poor placentation, resulting in subsequent placental hypoxia and preeclampsia.

### 4-2

**The incidence of twin-twin transfusion syndrome in the first trimester: A systematic review of literature**

A. Cristina Rossi, Vincenzo D’Addario

University of Bari, Bari, Italy

**OBJECTIVES:** Recent literature shows that twin-twin transfusion syndrome (TTTS) may be predicted as early as 11-14 gestational weeks by assessment of crown-rump length (CRL), nuchal translucency (NT) and ductus venosus (DV). It is reasonable to assume that if signs of TTTS are present since the first trimester, also the syndrome is present. Therefore, diagnosis of first trimester TTTS (FT-TTTS)