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Spectral Karyotyping for Identification of Constitutional Chromosomal Abnormalities at a National Reference Laboratory. *B.T. Wang, A. Anguiano, S. Wang, F.Z. Boyar, L.W. Mahon, M.M. El Naggar, P.H. Kohn, M.H. Haddadin, V. Sulcova, A. Sbeiti, M. Ayad, T. Sahoo, B.J. White, C.M. Strom.* Cytogenetics Dept, Quest Diagnostics Nichols Inst, San Juan Capistrano, CA.

Spectral karyotyping is a diagnostic tool that allows visualization of chromosomes in different colors using the FISH technology and a spectral imaging system. To assess the value of spectral karyotyping analysis for identifying constitutional supernumerary marker chromosomes or derivative chromosomes at a national reference laboratory, we reviewed the results of 179 consecutive clinical samples (31 prenatal and 148 postnatal) submitted for spectral karyotyping. Over 90% of the cases were requested to identify either supernumerary marker chromosomes or chromosomal exchange material detected by G-banded chromosome analysis. We also reviewed clinical indications of those cases with marker chromosomes in which chromosomal origin was identified by spectral karyotyping. Our results showed that spectral karyotyping identified the chromosomal origin of marker chromosomes or the source of derivative chromosomal material in 158 (88%) of the 179 clinical cases; the identification rate was slightly higher for postnatal (89%) compared to prenatal (84%) cases. Cases in which the origin could not be identified had either a small marker chromosome present at a very low level of mosaicism (<10%), or contained very little euchromatic material. Supplemental FISH analysis confirmed the spectral karyotyping results in all 158 cases. Clinical indications for prenatal cases were mainly for marker identification after amniocentesis. For postnatal cases, the primary indications were developmental delay and multiple congenital anomalies (MCA). The most frequently encountered markers were of chromosome 15 origin for satellited chromosomes, and chromosomes 2 and 16 for non-satellited chromosomes. We were able to obtain pertinent clinical information for 47% (41/88) of cases with an identified marker chromosome. We conclude that spectral karyotyping is sufficiently reliable for use and provides a valuable diagnostic tool for establishing the origin of supernumerary marker chromosomes or derivative chromosomal material that cannot be identified with standard cytogenetic techniques.

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The Developmental Genome Anatomy Project (DGAP): Annotating the Genome by Cytogenetic and Sequencing Approaches. *A.M. Lindgren[1], M.E. Talkowski[2,3], C. Hanscom[2], C. Chiang[2], C. Ernst[2,3], S. Ahsan[1], B.B. Currall[1], L. Yuan[1], S. Lachke[4], I. Saadi[4], D.J. Harris[5], R.L. Maas[4], B.J. Quade[1], J.F. Gusella[2,3], C.C. Morton[1].* 1) Depts. of Ob/Gyn and Pathology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA; 2) Center for Human Genetic Research, Massachusetts General Hospital, Boston, MA; 3) Depts. of Genetics and Neurology, Harvard Medical School, Boston, MA; 4) Dept. of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA; 5) Children's Hospital Boston, Harvard Medical School, Boston, MA.

The Developmental Genome Anatomy Project (DGAP, dgap.harvard.edu) is a collaborative endeavor to identify genes critical in human development and disease. Balanced chromosomal rearrangements are the biological resource for gene discovery in DGAP as they may indicate the location of disrupted or dysregulated genes that lead to an abnormal phenotype. DGAP analyzes the correlation between genotype and phenotype through FISH-based breakpoint localization, various sequencing methods, candidate gene identification and functional analysis in model organisms. Of 235 cases enrolled to date, breakpoints are FISH mapped in 88 cases, 116 of which are localized to a single clone. Seventy-six breakpoint sequences are determined in 36 cases and 57 disrupted genes identified for which 24 animal models have been evaluated. Notable cases under active investigation include DGAP100 [46,X,t(X;5)(p11.3;q35.2)], a nonverbal 16 year-old female with septo-optic dysplasia, cleft palate, severe myopia, neuromuscular scoliosis, hearing impairment, and a history of seizures. KDM6A, a histone 3 lysine 27 demethylase, is disrupted at Xp11.3, and qRT-PCR reveals ~50% reduction in KDM6A expression compared to control lymphoblast cell lines, suggesting haploinsufficiency of KDM6A is pathogenetic in the phenotype. Zebrafish knockdowns are underway and preliminary analyses show craniofacial anomalies. DGAP120 [46,XY,t(6;11)(q24.3;q21)] is a 12 year-old male with low-to-mid frequency sensorineural hearing loss, intermittent exotropia and craniofacial defects; C6ORF103 is disrupted at 6q24.3. DGAP191 [46,XY,t(5;7)(q14.3;q21.3)], a 3 year-old male, has sensorineural hearing loss, mental retardation, hypotonia and seizures. Although no genes are directly disrupted, the 5q14.3 breakpoint is ~500 kb upstream of MEF2C and the 7q21.3 breakpoint is 2.86 kb upstream of COL1A2. Normal expression of MEF2C and over-expression of COL1A2, as determined by qRT-PCR, suggest dysregulation of COL1A2 as etiologic in the phenotype. Chromosomal rearrangements remain a rich resource for identifying genes and regulatory elements underlying human disease and traits. In conjunction with development of affordable sequencing methods, the study of balanced chromosome rearrangements in phenotypically abnormal individuals is imperative in rapid annotation of the human genome.

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A new case of azoospermic male with 46,XY,t(1;21)(p11;p12) karyotype. *E.O. Ote[1], T. Turunc[2], O. Ozer[1], Z. Yilmaz[1], F.I. Sahin[1].* 1) Department of Medical Genetics, Baskent University Faculty of Medicine, Ankara, Turkey; 2) Department of Urology, Baskent University Faculty of Medicine, Ankara, Turkey.

Infertile men have an increased prevalence of chromosome abnormalities compared to phenotypically normal newborns. Translocations involving acrocentric chromosomes regardless of the partner chromosome integrate with male meiosis. Here we report a 32 years old azoospermic man with a history of infertility for seven years. The patient consulted the infertility clinic and was evaluated for testicular biopsy. Hormone levels were detected as FSH: 7.5 mIU/ml, LH: 4.3 mIU/ml, total testosterone: 3.11 ng/ml, all in the normal range. The patient was also referred to our department for cytogenetic analysis which resulted in a 46,XY,t(1;21)(p11;p12) karyotype, pedigree analysis, although cytogenetic analysis was not performed, revealed recurrent abortions in mother and sister of the proband suggesting a familial translocation. The patient was informed about the result during genetic counseling. Patients with similar karyotypes were reported previously. The mechanism underlying the relation between translocations involving acrocentric chromosomes and male infertility is not clear yet. However, it has been postulated that interaction between the quadrivalent in pachytene of male meiosis and XY body might be the explanatory mechanism.

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Accurate detection of copy number variations in next generation sequencing data by a latent variable model. *G. Klambauer, D.A. Clevert, A. Mayr, K. Schwarzbauer, A. Mitterecker, S. Hochreiter.* Institute of Bioinformatics, Johannes Kepler University Linz, Linz, Upper Austria, Austria.

The quantitative analysis of next generation sequencing (NGS) data like the detection of copy number variations (CNVs) is still challenging. Current methods detect CNVs as changes of read densities along chromosomes, therefore they are prone to a high false discovery rate (FDR) because of technological or genomic read count variations, even after GC correction. A high FDR means many wrongly detected CNVs that are not associated with the disease considered in a study, though correction for multiple testing must take them into account and thereby decreases the study's discovery power. We propose "Copy Number estimation by a Mixture Of PoissonS" (cn.MOPS) for CNV detection from NGS data, which constructs a model across samples at each genomic position, therefore it is not affected by read count variations along chromosomes. In a Bayesian framework, cn.MOPS decomposes read variations across samples into integer copy numbers and noise by its mixture components and Poisson distributions, respectively. The more the data drives the posterior away from a Dirichlet prior corresponding to copy number two, the more likely the data is caused by a CNV, and, the larger is the informative/non-informative (I/NI) call. cn.MOPS detects a CNV in the DNA of an individual by a region with large I/NI calls. I/NI call based CNV detection gurantees a low FDR because wrong detections are less likely for large I/NI calls. We compare cn.MOPS with the five most popular CNV detection methods for NGS data at three benchmark data sets: (1) artificial, (2) NGS data from a male HapMap individual with implanted CNVs from the X chromosome, and (3) the HapMap phase 2 individuals with known CNVs. At all benchmark data sets cn.MOPS outperformed its five competitors with respect to precision (1-FDR) and recall both at gains and losses.