**Human Genetics Laboratory (HGL)**

**NEWSLETTER**

**June 2006**

General Update

**UBE3A Sequence Analysis for Angelman Syndrome**

Jennifer Sanmann, B.S., Jan Williamson, B.S., MLT(ASCP), CLSp(CG, MB), Julie Carstens, M.S., CLSp(CG, MB), & Warren Sanger, Ph.D.

Angelman syndrome (AS) is a neurobehavioral disorder with an incidence of approximately 1 in 20,000. Clinical features of individuals with AS include severe developmental delays, severe speech impairment, seizures, microcephaly, and movement and balance difficulty. Behavioral characteristics of AS patients include a short attention span, hyperactivity, and the display of inappropriate happiness. Angelman syndrome results from a lack of a functional maternal copy of the E3 ubiquitin protein ligase (UBE3A) gene, which lies within the Prader Willi/Angelman syndrome critical region (PWASCR; 15q11.2-q13). Multiple mechanisms are known to be responsible for AS, including paternal uniparental disomy (UPDpat), imprinting defects, cytogenetic rearrangements, deletions, and mutations in the UBE3A gene. In approximately 15% of AS cases, the genetic mechanism remains unknown. However, recent studies have revealed that approximately 7% of patients with an AS phenotype have a MECP2 mutation. In this subgroup, in which UBE3A studies are normal, MECP2 studies should be offered.

In the past, our laboratory has offered prometaphase chromosome analysis along with methylation and fluorescence in situ hybridization (FISH) studies to detect ~80% of patients with AS. We have recently developed and validated an assay to detect mutations within the UBE3A gene which will confirm an additional 5-7% of AS cases. This assay utilizes multiple PCR primers that flank the intron/exon junctions of the coding region of the UBE3A gene (Exons 7-16) to amplify the regions of interest. The PCR products are then analyzed by direct sequence analysis in both the forward and reverse directions utilizing fluorescence dideoxy sequencing methods. This assay will detect missense, nonsense, and frameshift mutations that cause AS. For reference, the turn around time for UBE3A and/or MECP2 sequencing analysis is 21-28 days.

**Clinical Microarray Experience & Expanded Coverage of Constitutional Array**

Warren G. Sanger, Ph.D. & Diane Pickering, M.S., CLSp(CG)

Microarray comparative genomic hybridization (aCGH) is used for the detection of subtle chromosomal deletions and duplications throughout the genome. The microarray chip utilized in our laboratory for constitutional studies includes DNA clones which span multiple regions of the genome associated with known microdeletion and microduplication syndromes as well as all subtelomeric regions. This constitutional chip has recently been expanded from 434 genomic clones to 606 clones, thus increasing the density of coverage throughout the human genome. These clones represent approximately 150 specific genetic loci which, when deleted or duplicated, are associated with specific disorders. A higher density chip is also available, which includes 2,600 gene loci. This chip is primarily utilized for those cases in which microarray is being utilized for the determination of chromosomal breakpoints. Our experience thus far has revealed the detection of chromosomal deletions or duplications in approximately 8% of those cases which had normal cytogenetic studies. The overall abnormality rate for microarray is higher when including cases where microarray was incorporated to further delineate a chromosome abnormality previously identified by high resolution chromosome analysis. Overall, aCGH has been proven to be a strong adjunct to classical high resolution chromosome analysis which enhances the detection of even a higher percentage of abnormalities and facilitates clinical diagnosis and patient management.

Our laboratory strives to provide “state-of-the-art” laboratory services. We encourage feedback regarding any new services you would like offered and/or any recommendations as to how we can improve our services. Also, if you would like any particular issues addressed in future newsletters, please let us know. We appreciate having the opportunity to provide genetics services for you and your patients.
Chromosome 14 Uniparental Disomy Methylation Assay

Julie M. Carstens, M.S., CLSp(CG, MB), Jan Williamson, B.S., MLT(ASCP), CLSp(CG, MB) Ann Olney, M.D. & Warren G. Sanger, Ph.D.

Uniparental disomy (UPD) for chromosome 14 has been detected in some patients referred for multiple congenital anomalies. Most individuals diagnosed with UPD(14) have 2 maternal copies of 14. The phenotypic characteristics associated with UPD(14)mat include IUGR, short stature, scoliosis, hypotonia, obesity, developmental delay (mental and physical), precocious puberty and a distinctive facial appearance. Paternal UPD (14) is less common. These individuals have a more severe phenotype than those with UPD(14)mat. Phenotypic features of UPD(14)pat include decreased survival due to respiratory difficulties, marked developmental delay, skeletal abnormalities (including short limb dwarfism with narrow thorax), dysmorphic facies (hirsute forehead, short palpebral fissures/blepharophimosis, small abnormal ears, protruding, long philtrum, depressed nasal bridge, short neck), abdominal muscle hypoplasia, joint contractures, scoliosis and mental retardation.

Researchers have identified 2 imprinted genes (DLK1 & MEG3) located 92 kb apart on 14q32. Regulation of imprinted expression of DLK1 and MEG3 involves a differentially methylated region (DMR) that encompasses the MEG3 promoter (Murphy et al). The critical region of 14 is methylated and inactive in the paternal homologue while the maternal homologue is unmethylated and transcriptionally active.

A method for detection of UPD(14) has been described utilizing methylation specific PCR (mPCR). This method detects maternal and paternal UPD utilizing primers complementary to sequences located in the critical region of chromosome 14. DNA is treated with sodium bisulfite which converts cytosine to uracil except when cytosine is methylated. Following modification, DNA from the UPD(14) critical region is amplified utilizing PCR and the products are visualized utilizing agarose gel electrophoresis. Primer sets used in PCR were designed specifically for both the methylated and unmethylated versions of this critical region. Two PCR products are present from patients normal for UPD(14). These include a 120 bp product from the maternal (unmethylated) homologue and a 160 bp product from the paternal (methylated) homologue. Patients with UPD(14)mat will produce only a 120 bp fragment while UPD(14)pat patients will produce only a 160 bp product.

This UPD(14) methylation assay has recently been developed and validated in our lab and is available for use upon physician referral.

MECP2 Sequence Analysis

Janet E. Williamson, B.S., MLT(ASCP),CLSp(CG, MB), Jennifer Sanmann, B.S., Julie M. Carstens, M.S., CLSp(CG, MB) & Warren G. Sanger, Ph.D.

Rett Syndrome (RTT) is a relatively common genetic cause of mental retardation which affects approximately 1/10,000 to 1/15,000 females. RTT follows an X-linked dominant inheritance pattern which is lethal in males. However, recent studies have shown occasional mutations in living males with X-chromosome aneuploidy. Patients with classical RTT develop normally until approximately 6-18 months of life, at which time they begin to show a delay in neurological development and a regression of fine motor and communication skills. These patients may develop microcephaly or macrocephaly, abnormal breathing patterns, seizures, autism, ataxia, and stereotypic hand movements.

Approximately 60-85% of known female Rett syndrome patients have mutations identified by sequencing. Our laboratory has recently developed and validated an assay that allows us to sequence the coding region (Exons 1-4) of the MECP2 gene in order to detect silent (a mutation that does not alter the amino acid sequence), missense (a mutation that alters the encoded amino acid), nonsense (a mutation that changes the amino acid into a stop codon), and frameshift (an insertion or a deletion that changes all of the amino acids downstream) mutations. This assay involves amplification of the target DNA with primers flanking the intron/exon junctions of the MECP2 coding region and analysis by direct sequencing utilizing fluorescence dideoxy sequencing methods. It is important to note that this method of analysis may not detect large insertions and deletions. The turn around time for MECP2 sequencing analysis is 21-28 days.
CUSTOMER SATISFACTION SURVEY 2006
Please read each item carefully and circle the answer you feel is appropriate.

1. The receptionist who answered the telephone, answered promptly, has been courteous and helpful to me or my patients. If No, please explain: ____________________________
   Yes  No  N/A

2. The delivery service (FedX, courier, etc.) which picks up the specimen has been prompt, courteous, convenient and met the needs of my office and/or laboratory staff. If No, please explain: ____________________________
   Yes  No  N/A

3. The test results were legible and understandable, and received in a timely manner. If No, please explain: ____________________________
   Yes  No  N/A

4. Client billing was easy to read and handled promptly. If No, please explain: ____________________________
   Yes  No  N/A

5. Billing issues were handled promptly and to my satisfaction. If No, please explain: ____________________________
   Yes  No  N/A

6. Are you satisfied with the service you received from our laboratory. If No, please explain: ____________________________
   Yes  No  N/A

7. Would your office or facility staff like to have a continuing education visit (no cost to you). If yes, what topics would you like to include? ____________________________
   Yes  No  N/A

8. Is your office aware of our genetics counseling services which provides genetic counseling for families with abnormal genetic results? ____________________________
   Yes  No  N/A

If there is anything that we can do to make our service better or more convenient for you, please let us know. Your comments or suggestions for improvement are important to us.

__________________________________________________________

Please complete the name & address portion for follow-up.

Your Name: _________________________________________________

Address: _________________________________________________

__________________________________________________________

Phone: ____________________________    Fax: ____________________________

Thank you for completing our survey. Please return by fax to (402) 559-7248.
SPECIMEN SHIPPING & HANDLING

1. Notify HGL in advance of specimen arrival and/or to arrange for specimen pickup (pickup provided within the Omaha-Council Bluffs metropolitan area only). Outside of this area, the transport of specimens by overnight express courier service is available and will be paid by HGL. Specimens should arrive within 24 hours of collection.

2. Specimens should be collected under sterile conditions and transported at room temperature unless otherwise indicated. Special arrangements are required if extremes in temperatures or if extended transport times are anticipated. DO NOT FREEZE.

3. Label specimen with patient’s full name, date of birth, and date/time of specimen collection.

4. Send a completed cytogenetics patient information form with the specimen. Include the patient’s name, date of birth, diagnosis, physician’s name and phone number, billing/insurance information and tissue type. If these forms are not available, please call us at (402) 559-5070, and we will fax these to you, or they can be retrieved by accessing our website.

More information is available on our website:  www.unmc.edu/services/geneticslab