R.C.P.U. NEWSLETTER

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R.C. Philips Research and Education Unit
Vol. XXI No. 2 A statewide commitment to the problems of mental retardation June 2010

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Chromosomes and Beyond…The new technologies for genetic testing

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Review of Genetic Testing

As we learn more about the human genome and the science of genetics our ability to perform genetic testing is increasing at a rapid pace. Genetic testing is often the best way to confirm a diagnosis in a patient with symptoms and features suggestive of a genetic disease. Today, there are hundreds of different genetic tests for disorders ranging from those that are relatively common, such as cystic fibrosis, to very rare disorders. Not all disorders will be tested for in the same way - there are many different types of tests (or techniques for genetic testing) which can be performed for various genetic diseases. A genetic test is defined as "the analysis of human DNA, RNA, chromosomes, proteins, and certain metabolites in order to detect heritable disease-related genotypes, mutations, phenotypes, or karyotypes for clinical purposes" (Hotlzman, 1999). This newsletter will review advances in our ability to perform genetic testing in one area – chromosome studies.

Chromosome analysis – The original genetic test

Cytogenetic tests are used to diagnose chromosomal disorders, in which chromosomes or chromosomal segments are duplicated, deleted, or moved (translocated) to different chromosomes. These tests make it possible to identify the basis of many different conditions which are caused by the presence of extra chromosomes, missing and incomplete chromosomes or rearranged chromosomes. The simplest and most common cytogenetic test is a chromosome analysis.

Chromosomes are made up of genes which are in turn made up of DNA. These genes serve as the instruction manual for our biological development. Thus, missing or extra genes can cause significant problems within our bodies systems and with our development. Every individual has 46 chromosomes which are inherited in pairs. One copy of each pair is inherited from the mother and one from the father; thus half of every person's genetic material comes from each parent. A chromosome analysis provides a picture or karyotype (Figure 1) that enables identification of missing or extra chromosomes, large pieces of missing or extra DNA, or rearrangements of chromosomal material (translocations). This type of testing can diagnose conditions like Down syndrome.

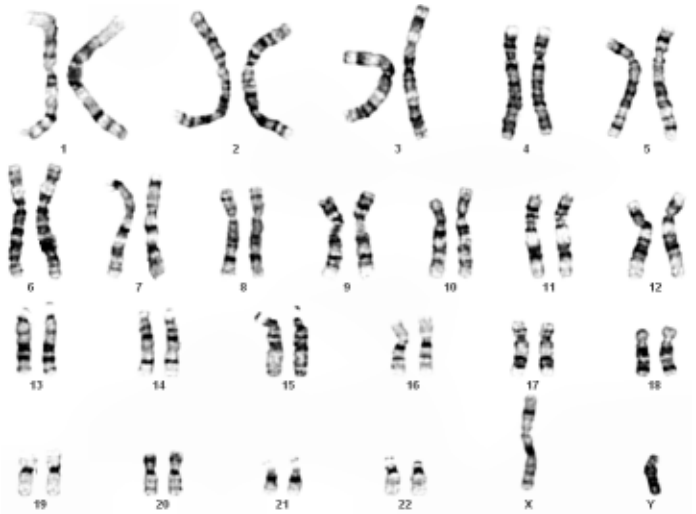

Figure 1: A normal chromosome analysis (karyotype). Notice the banding patterns of the chromosomes. Each band contains, on average, over 100 genes. The chromosomes are grouped in pairs and labeled # 1 through #22. The last two chromosomes are an X and a Y (male).

FISH analysis

One cytogenetic technique, fluorescence *in situ* hybridization (FISH), identifies specific chromosomal regions through the use of fluorescent DNA probes. In

this technique a DNA probe is labeled with fluorescent molecules so that it can be seen with a microscope. The DNA probe is added to the cell where it hybridizes (attaches) to its complementary target DNA. Once hybridized, the probe will fluoresce allowing identification of the precise location of the target DNA. If the probe does not fluoresce, it means that the target DNA is deleted (Figure 2).

FISH analysis can pinpoint small chromosomal duplications and deletions missed by conventional chromosome analysis (Burke, 2002). One common region of the genome tested with by FISH is the 22q11.2 region, known to be deleted in Velo-cardio-facial syndrome/DiGeorge syndrome (VCFS/DGS). FISH analysis is a versatile testing method because, depending upon the design of the probe DNA, it can detect many different types of genetic changes. Thus when ordering a "FISH test" one must specify which region of which chromosome the test is being performed, e.g. 22q11.2, 15q11.2, 17p11.2 etc.

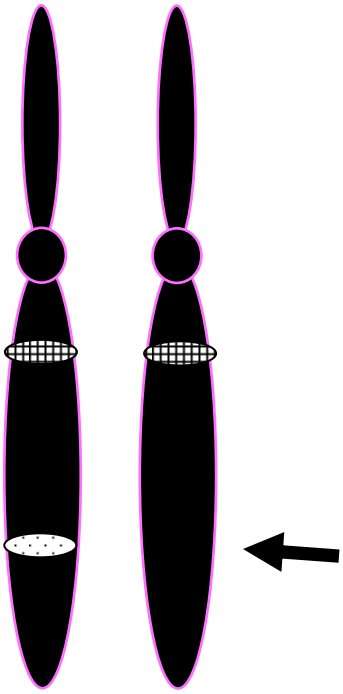

Figure 2: Depiction of a FISH probe. The hatched probe is the control probe. The dotted probe designates the region being tested. It is missing on the second chromosome and therefore a deletion is present.

Comparative Genomic Hybridization

Comparative Genomic Hybridization (CGH) is a relatively new genetic testing method which also uses the FISH technique, but on a larger scale. CGH was developed to measure alterations in dosage of DNA throughout the entire genome in a single experiment (Kallioniemi et al, 1992). CGH uses comparative hybridization of labeled DNA from an abnormal cell population ("test" genome) to a normal cell population ("reference" genome). By measuring the intensity of the hybridization, copy number variations in the test genome are able to be detected.

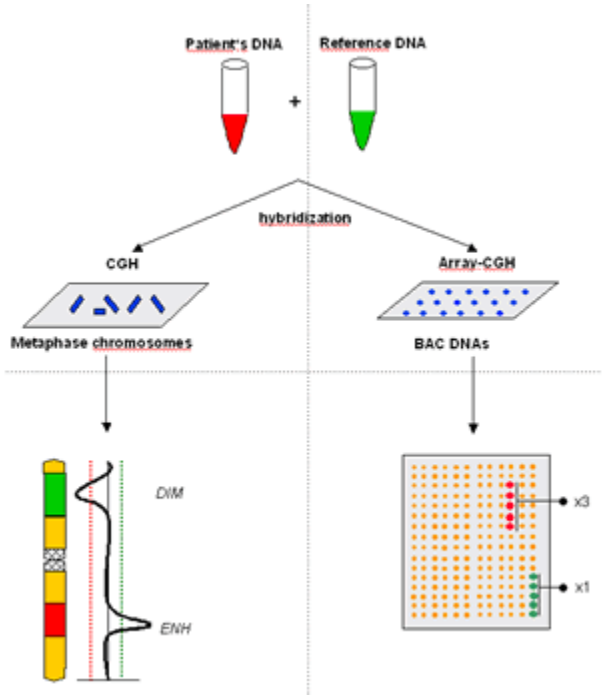

Figure 3: Comparative Genomic Hybridization showing segments deviating from the normal expression associated with a deletion at DIM and a duplication of ENH.

CGH has dramatically improved our ability to detect small duplications and deletions, and the syndromes that can be associated with them. We will review some of the newly identified syndromes later in this newsletter. However, along with the ability to identify new genetic conditions, increased use of CGH has also led us to understand that not all chromosome deletions and duplications have physical or developmental consequences, but are familial copy number variants. Additionally, there are many instances in which one individual will have a small duplication or deletion which has a clinical effect, but that other members of the family or other families who have the same duplication or deletion will apparently be clinically normal.

In Table 1 the drawbacks and benefits of the different type of cytogenetic techniques are compared and contrasted.

Table 1: Comparison of Cytogenetic Testing Techniques

	Chromosome analysis	FISH analysis	CGH Analysis
Labor Intensity	+++	+	+
Speed of Test	-	++	++
Resolution of Testing	+	++	++++
Need for prior Knowledge	–	+	–
Ability to detect Balanced Rearrangements	++	+	–
Possibility of uninterpretable results	+/-	+/-	++

Newly Identified Syndromes:

A. Deletion syndromes:

Deletion Location	Frequency	Symptoms	Additional Comments
17q21.31	0.3	Severe hypotonia, friendly affect, dysmorphia (long face, broad chin, ptosis, long fingers), feeding problems, ventriculomegaly , moderate MR	Similarities to Angelman syndrome
15q13.3	0.3	Mild to moderate MR, autism, dysmorphia (hypertelorism, everted full lower lip, short fingers), EEG abnormalities	Some inherited from normal parent
15q24	0.3	Mild to moderate MR, micrcephaly, high forehead, hypertelorism, proximal thumbs, short fingers, hypospadias, GH deficiency, IUGR	
1q41-q42	0.07	Moderate to severe MR, seizures, coarse facies, short digits, nail hypoplasia, cleft palate, diaphragmatic hernia, brain malformations	Similarities to Fryns syndrome
16p11-12.1	0.05	Severe MR, hypotonia, autistic behaviors, dysmorphic features, clefting and heart defects	Duplication syndrome also exists
2p15-q16.1	1.2	Mild to severe MR, microcephaly and FTT, autistic features, dysmorphia, optic nerve hypoplasia, renal anomalies, spasticity, brain anomalies	
9q22.3	4	Severe MR, overgrowth, trigonocephaly, small mouth, ear pits, pectus, joint laxity, delayed teeth, brain anomalies, advanced bone age, strabismus	
3q29		Mild to moderate MR, obesity, microcephaly, round face, deep hand creases, flat feet, anomalies of aortic valve	Inherited
20q13.13-q13.2		Normal devt to severe MR, autistic features, growth delays, Duane anomaly, radial aplasia, abnormal thumbs, choanal atresia, renal & cardiac anomalies, deafness	Similarities to Okihiro syndrome

B. Duplication syndromes:

Deletion Location	Frequency	Symptoms	Additional Comments
17p11.2 Potocki-Lupski		Autism, variable MR, prenatal growth failure, behavioral problems, cardiac anomalies, eye anomalies, palatal anomalies	Same region as is deleted in Smith Magenis sx
17q11.2-q12		Mild MR, anger outbursts, coarse facial features, prominent eyelashes, 5th finger clinodactyly, flattened and cone shaped epiphyses,	
11q11q13.3		Multiple suture craniosynostosis, variable MR, heart defects	
16p11p12.1		Normal devt to severe MR, microcephaly, seizures, autistic features	Some inherited from normal parent
22q11.2		Mild to severe MR, seizures, dysmorphia, hearing loss, renal and cardiac anomalies	Features similar to VCFS 22q11.2 deletion syndrome
8p23.1	0.002	Normal devt to mild MR, mild dysmorphia, cardiac anomalies, toe syndactyly	

Summary:

Chromosome microarray studies have added approximately 6-10% to our ability to detect causation of conditions involving mental retardation or congenital anomalies. However, due to the relative novelty of this technology and the variability of manifestations in affected individuals, care must be taken in the interpretation of abnormal results.

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About the RCPU

The Raymond C. Philips Research and Education Unit began in 1978 when the legislature established section 393.20, F.S., of what is now known as the "prevention" legislation. It is named after Raymond C. Philips, who was the Superintendent of Gainesville's Tacachale (formerly Sunland) Center for 38 years, and was an acknowledged state and national leader in services for mentally retarded persons. The Unit is located on the Tacachale campus and is funded through a contract with the Department of Children and Families and the Department of Health.

The purpose of the R.C.P.U. is to treat, prevent, and/or ameliorate mental retardation through medical evaluations, education and research. The unit provides direct evaluations and counseling to families and promotes service, education, and prevention projects.
Some of the conditions currently under study at the RCPU involve Angelman, Velo-Cardio-Facial, Prader-Willi, Fragile X, Williams and Smith-Lemli-Opitz syndromes.

The R.C. Philips Unit is a resource for all Floridians interested in the diagnosis, treatment and prevention of mental retardation. Staff members are available for consultation and for educational programs for health professionals and for the community at large.

Acknowledgments:

The RCPU Newsletter is funded by the Raymond C. Philips Research and Education contract with the Department of Health, Children's Medical Services.

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