



R.C.P.U. NEWSLETTER

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R.C. Philips Research and Education Unit
A statewide commitment to the problems of mental retardation

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Hunting for Genes - It isn't as easy as it looks!

In this era of the Human Genome Project and the quest to map the entire human genetic code, it seems that the identification of genes causing human diseases should be a simple matter, right? Wrong. The identification of disease causing genes often requires not only the use of the stunning array of new molecular techniques which have presented themselves over the last decade, but also requires something else - a bit of luck. Luck is something that is even more difficult to come by than NIH grant funding, and it is oftentimes more necessary to success.

This issue of the RCPU newsletter tells the story of the identification of the genes behind the expression of three different genetic disorders: Angelman syndrome, Rett syndrome and Rubinstein Taybi syndrome. The stories of these three conditions exemplify the less than straightforward road that sometimes is taken in the identification of disease causing genes.

Angelman syndrome:

Angelman syndrome is a neurobehavioral condition which is characterized by developmental delay, progressive microcephaly, ataxic gait, absence of speech, seizures and spontaneous bouts of laughter. The incidence is estimated to be between 1 in 15,000 and 1 in 20,000 live births⁷. AS was originally called the "Happy Puppet Syndrome" in its description by Approximately 70% of individuals with clinical Angelman syndrome were found to have either chromosomally or molecularly identifiable deletions in the q11-q13 region of their maternally derived chromosome 15. A further 5% of individuals with AS had been shown to have paternal uniparental disomy for chromosome 15. However, this left 25% of children with classical clinical features of AS with normal diagnostic testing, and the search was on to discover the mechanism leading to their having

Harry Angelman in 1965 in an attempt to describe the upheld hands, clumsy gait and happy demeanor of individuals with this condition.

The history of the search for the cause of Angelman syndrome (AS) is integrally linked to that of another condition; Prader-Willi syndrome (PWS). PWS is a condition which involves early muscle hypotonia and failure to thrive followed by obesity related to insatiable appetite, mild-moderate learning delays and obsessive compulsive behavior.

Prader-Willi syndrome had been associated with microdeletions of 15q11-q13 for quite some time when it was noted in 1987 that this deletion was also associated with features of Angelman syndrome^{11,14}. AS and PWS became recognized as classical examples of genomic imprinting when it was discovered that PWS results from deletions in the paternally derived chromosome 15 and AS results from similar deletions in the maternally derived 15¹³, suggesting that some genes behave differently depending upon the parent of origin.

The presence of both maternally and paternally imprinted gene(s) in this region is further evidenced by the occurrence of maternal uniparental disomy (UPD - the presence of two copies of the chromosome from the same parent) in many individuals with PWS and the discovery of paternal UPD in some individuals with Angelman syndrome¹⁵.

Angelman syndrome. A new type of diagnostic testing known as DNA methylation testing was applied to Angelman syndrome, and it was noted that methylation studies were able to detect individuals with AS as the result of a maternal deletion and as the result of paternal uniparental disomy⁸. In addition, it was noted that an additional 2% of individuals with Angelman syndrome who showed no evidence of a deletion, or of UPD 15, demonstrated a DNA methylation pattern consistent with AS. These

patients are thought to have mutations in the imprinting control center (IC) which controls imprinting and gene expression via methylation and demethylation throughout the 15q11-q13 region. IC mutations produce the clinical phenotype of AS or PWS by preventing the resetting of imprinting during gametogenesis in a parent or grandparent¹⁸. The identification of molecular deletions upstream of the AS deletion region by Buiting et al.⁶ in patients with both Angelman and Prader-Willi syndromes defined the location of the imprinting center.

In 1997, the laboratories of Joe Wagstaff and Art Beaudet identified a single gene known as UBE3A/E6-AP within the AS deletion region in which mutations appeared to result in Angelman syndrome. The gene codes for a protein known as ubiquitin protein ligase. This protein acts as an enzyme in normal protein turnover within the cell. The hypothesis is that failure to degrade certain proteins as the result of improper functioning of this gene must result in the clinical features seen in individuals with Angelman syndrome.

Ironically, the UBE3A/E6-AP gene had previously been considered as a candidate gene for Angelman syndrome, and discarded on the basis of a lack of evidence of imprinting. Later study demonstrated that it is imprinted only in some areas of the brain (where only the maternal copy is functional), but is expressed from both copies in all other tissues examined. Because of its maternal-only expression in the brain, regional deletions, paternal UPD and mutations that affect expression of the gene would all result in no protein being expressed within some areas of the brain - thereby affecting protein "clean-up" in those areas. The brain specific imprinting of this gene also fits quite well with the neurologic basis of the clinical problems in Angelman syndrome.

More recent mutation analysis of all coding regions of the UBE3A gene show that approximately 15-20% of clinically typical AS patients with negative cytogenetic, molecular and methylation studies have an identifiable mutation in this gene. Family studies performed when a mutation is identified have shown that about 50% of AS mutations are maternally inherited, and about 50% are *de novo*^{16,12}. The recurrence risk in familial UBE3A mutations is expected to be 50%, and just as with IC mutations, other matrilineal family members may be at risk to have affected children or grandchildren. UBE3A mutation analysis has recently become clinically available.

Despite the identification of the UBE3A gene and its role in AS, the group of individuals with unexplained AS remains large (approximately 15%). An undeniable presence in this group are children who do not have Angelman syndrome and have been given this diagnosis in error. The nonspecific nature of many of

the features of AS make it easily mistaken for other conditions which involve ataxia, mental retardation and lack of speech. Further delineation of this group is ongoing.

Rett syndrome

Rett syndrome (RS) is a devastating neurogenetic disorder that affects females almost exclusively. Girls with RS usually have fairly normal development for the first 6 to 18 months of life. At that time, development plateaus and the girl may regress, becoming less communicative. She begins to lose the purposeful use of her hands and speech. Then, stereotypical "hand wringing" movements and slowed head growth are noted. Scoliosis and contractures may develop later in life. Other signs commonly seen in girls with Rett syndrome are seizures, bruxism (teeth grinding) and unusual breathing patterns.

The gene that causes Rett syndrome was elusive for many years. Since nearly all individuals with RS are female, it had been speculated that the gene for RS must be on the X chromosome and likely inherited in a dominant pattern. RS is familial in less than 1% of cases, so the vast majority of the mutations must be new mutations. Genes are commonly found by linkage analysis. This is when family studies compare the DNA of affected family members to unaffected family members to determine which DNA areas differ. These areas may be close to the gene. Unfortunately, with RS, 99.5% of the time there is only one affected family member so this approach is not very helpful. Although the familial cases are few, they were key to finding the gene for RS. In these families, using exclusion mapping, the gene's location was narrowed down to a specific region of the long arm of the X chromosome by Dr. Eric Hoffman and Dr. Sakkubai Naidu. This technique was used to examine the affected family members X chromosomes to see which areas differed. These areas were unlikely to contain the gene for RS. Now the hunt was on in force. The localized region, Xq28, contained several thousand genes. Dr. Zoghbi and Dr. Francke's groups examined about 25 genes over several years while looking for the gene for RS.

After fourteen years of searching, the gene for Rett syndrome was found in September of 1999 at Baylor College of Medicine. The gene is called MeCP2. MeCP2 makes a biological switch that tells other genes when they should turn off. During development, our bodies form based on a carefully orchestrated mix of genes. It is very important that each gene activates and deactivates at the appropriate times for normal development. If a mutation is present in MeCP2, the genes it controls may activate at inappropriate times or stay on for longer than the proscribed window when they should, disrupting

normal development.

The knowledge of the gene that causes RS gives us answers to many previous questions about the inheritance of RS. Although typically only females are affected, there were reports of two males born to couples who had a girl with RS. These boys both died early in life because of severe neurological problems. Females have two X chromosomes, whereas males have one X and one Y chromosome. Therefore, if females have a mutation in MeCP2 on one chromosome, the functioning MeCP2 on the other X chromosome ensures survival, although they have RS.

On the other hand, males with a mutation of MeCP2 on their only X chromosome usually do not survive fetal life. This knowledge allows us to explain why sometimes, couples have more than one child with RS.

Since women have two X chromosomes, they turn off one X chromosome in every cell of their body. This is called X inactivation. A woman who carries a mutation in the MeCP2 gene can have skewed inactivation, with most of the X chromosomes with the mutations inactivated and more of the normal X chromosomes active. This may allow a woman to be neurologically normal, or have more mild problems than most women with RS. A woman who carries a MeCP2 mutation has a 1 in 2 (50%) chance to have a child with RS.

Thus far, mutations in MeCP2 have been found in only about 50% of girls with RS and this testing is available at several laboratories throughout the world. There are three major reasons why mutations have not been found in all patients with RS. Since RS is currently a clinical diagnosis, some girls thought to have RS may have other conditions, such as autism or cerebral palsy. Also, the MeCP2 gene is very large and it is technically difficult and expensive to look for changes in the gene. Our technology is probably not advanced enough to find every change in this gene. Finally, since the gene regulates other genes, it is possible that mutations in other genes may also cause RS.

Discovering the gene for RS not only gives us important insight into the disorder and an opportunity to offer molecular testing, but hopefully it will eventually lead to a therapy or cure for RS.

Rubinstein-Taybi Syndrome

Rubinstein-Taybi Syndrome (RTS) was first described by Dr. Jack Rubinstein and Dr. Hooshang Taybi in 1963. They reported seven children with similar features including broad thumbs and great toes, unusual facial appearance, and mental retardation¹. Since then, RTS has been reported hundreds of times around the world. It is estimated that 1 in 125,000 newborns has RTS. The characteristic facial features of RTS include a "beaked" nose, down-slanting eyes, highly arched eyebrows, and epicanthal folds. Other features, in addition to the broad thumbs/great toes

and mental retardation, include: short stature, low-set or malformed ears, and heart or kidney defects.

The search for the gene which causes RTS began in 1991, when a sporadic case of RTS was described in which the patient had a translocation involving chromosomes 2 and 16¹². Some debate occurred as to whether the gene was on chromosome 2 or chromosome 16 then, another patient with a translocation involving chromosomes 7 and 16 was described. The breakpoint on chromosome 16 involved the same region as was observed in the previously described patient. After studying other patients with translocations involving chromosome 16, it became clear that the RTS gene was located on chromosome 16 at band p13.3²⁴.

Scientists then began looking more closely at the region of chromosome 16p13.3. By using fluorescence in situ hybridization (FISH) and studying patients with RTS who had translocations, researchers showed that RTS was caused by submicroscopic deletions within 16p13.3 in approximately 25% of patients with a clinical diagnosis of RTS. These detectable deletions were either due to translocations with breakpoints in this area or to sporadic deletions⁷.

Prior to this discovery, it had been postulated that RTS was caused by teratogens, an autosomal recessive pattern of inheritance, an autosomal dominant with variable expression, or multifactorial inheritance¹¹. It is now known that RTS is an autosomal dominant condition. The majority of cases are sporadic with a recurrence risk of less than 1% based on empiric data.

In 1995, it was shown that all of the breakpoints and microdeletions that had been reported in patients with RTS were restricted to a region on chromosome 16 that was known to contain the gene for the human cyclic AMP-responsive element binding (CREB) protein (CBP). CBP is a nuclear protein which participates in permitting gene expression. Once the gene was identified, the researchers studied how the protein was affected in individuals with RTS due to microdeletions, translocations, and point mutations. The occurrence of point mutations within the gene in some patients, implied that RTS was not a contiguous-gene syndrome, as had been previously thought. The variable features associated with RTS are explained by the role of CBP as a transcriptional co-activator. Mutations in the gene for CBP are predicted to influence a large set of target genes - each with different roles, and each perhaps contributing to the features of RTS²¹.

It is suggested that two functional copies of the CBP gene are required to produce a sufficient protein product for normal development. If a mutation in the CBP gene is present in all the cells of an individual,

then this causes RTS.

At present, patients who are thought to have RTS, can first undergo FISH analysis of chromosome 16p13.3.

As stated earlier, only about 25% of patients who have clinical features of RTS will have a detectable deletion. For the remaining 75% of patients, the diagnosis is made based on clinical findings. The nature of the anatomical and physiological abnormalities in patients with RTS in relation to CBP mutations is still poorly understood and will require much more study to clarify.

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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 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.

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.

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