Using Exome Sequencing to find Primary Blepharospasm Genes

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Identification of the genetic basis of a disease is a first step toward understanding Its cause and ultimately the development of specific treatments. The genetic bases of most forms of primary dystonia remain unknown and pathogenic mechanisms are poorly understood. However, genetics seem to play a significant role in different types of primary focal dystonia like blepharospasm, as several studies have shown that between 10-30% of relatives of patients are also affected with some form of dystonia.

Blepharospasm is one of the most common forms of primary focal dystonia affecting between 12 and 133 people per million. However, identification of genes for blepharospasm is challenging because traditional methods of gene discovery are based on studying large families with multiple affected members who share the same disease-causing gene. Such families with blepharospasm are rare, since the disease occurs later in life and has a low penetrance, meaning a person can carry a disease mutation but not show any of the clinical symptoms.

The human genome is the complete set of human genetic information stored in 23 pairs of chromosomes in the nucleus of each cell in the human body and is made up of 3 billion building blocks (also called base pairs). The recently coined term "human exome" refers to the part of the genome coding for about 20,000 human proteins (this represents about 2% of the entire genome). The vast majority of mutations that cause diseases disrupt these gene coding regions or the "exome". However, until recently, researchers had no way to assess all the coding regions of the human genome simultaneously. Instead, traditional methods of disease gene discovery were based on finding a region of a chromosome that was shared by all affected family members and then sequencing all the protein coding parts within this region, a very time consuming and expensive method.

Currently, a revolution in sequencing technology, next generation sequencing, is changing the way disease genes are discovered by making it more time and cost-effective to sequence the entire exome of an individual in a single experiment. This technology is developing so rapidly that not just the exome can be sequenced from a person but also their entire genome (all 3 billion base pairs!) at $5,000/person, whereas exome sequencing is only $1,000/person. The major advantage of exome sequencing is that large families with multiple affected members are no longer needed to find a disease gene. Instead, almost every patient can be important for genetic research, even those without a family history.

Since November 2009, exome sequencing has led to the identification of over 30 new genes for human diseases. Moreover, several primary dystonia genes have been discovered using exome sequencing including CIZ1, ANO3 and one from our laboratory GNAL. Although the generation of sequence has become routine, the main challenge associated with these new techniques is identifying the disease-causing mutations among the multitude of variants discovered in each exome. Variants are the single base pair difference in the DNA between different individuals. On average, exome sequencing identifies ˜44,000 variants in African American samples and ˜40,000 variants in European American samples. More than 95% of these variants are already known as neutral genetic variants, but the remaining 5% (1000-1200) are novel and thus potentially disease causing.

We are currently working to find a causative gene for blepharospasm using exome sequencing. We have sequenced several exomes from patients and families affected by blepharospasm and detected about 1000 novel variants (i.e. not detected in the online databases) per person. We are currently trying to identify which of these are shared among the affected individuals and will use computational tools to help us to predict which of these variants are located in the protein coding portions of the genome and can disrupt the protein function and therefore cause the disease.

This research will hopefully reveal a new causative gene for blepharospasm and possibly other forms of primary dystonia which should significantly advance our understanding of disease mechanism and provide a starting point for the development of new therapeutic interventions for this disabling disease.