Genetic Sequence Variants in Primary Dystonia

Mark LeDoux, M.D., Ph.D., Professor
Department of Neurology
University of Tennessee
Health Science Center
Memphis, Tennessee

Genetics 101. The central dogma of genetics tells us that DNA (deoxyribonucleic acid) is transcribed into RNA (ribonucleic acid) and RNA is translated into protein. There are two basic types of DNA, nuclear (nDNA) and mitochondrial DNA (mtDNA), and most cells of the body can be divided into two parts, the nucleus and the cytoplasm. The nucleus of the cell harbors nDNA and mtDNA is present in the cytoplasm, within mitochondria, the energy factories for cells. Transcription takes place in the nucleus. RNA is transported out of the nucleus into the cytoplasm where translation occurs.

The four letters in the DNA alphabet (A, C, G and T) are used to carry the instructions for making a human or other organism. The order (or sequence) of these letters holds the code just like the order of letters makes words mean something. Each set of three letters corresponds to a single amino acid. There are 20 different amino acids available to produce the various proteins in our bodies. Very specific combinations of amino acids make proteins such as keratin in hair, hemoglobin in blood, and the hormone insulin.

Most human cells contain 46 chromosomes: pairs of chromosomes 1 to 22 and a pair of sex chromosomes (females have two Xs, and males have an X and a Y). Sperm and eggs contain one of each chromosome. The human genome contains about 6 billion bases and approximately 25,000 genes. Importantly, 99% of the genetic code (A, C, G and T) is identical among different people.

What makes us unique? Perhaps most importantly, approximately 1% of the genetic code does differ among individuals. DNA sequence variation comes in several types which have partially overlapping definitions: single nucleotide polymorphisms (SNPs) and rare variants, indels, and copy number variants (CNVs). A SNP is present when a single nucleotide (A, T, C or G) in the genome differs between members of the species and the least common nucleotide is present in at least 1% of chromosomes. Indels are small insertions and/or deletions of a small group of nucleotides. Any of these sequence variants may fall within coding sequences of genes, non-coding regions of genes or in the intergenic regions between genes, and may alter amino acid sequences, overall gene expression or the relative proportion of splice variants (described below). CNVs are large deletions or duplications of genes or portions of genes. Many CNVs have

no known health consequences whereas others, such as duplication of the gene that encodes -synuclein, may cause Parkinson disease.

Most genes are transcribed into several splice variants. On average, each gene has 8 exons. Exons are the coding regions of genes. Approximately, 85% of "mutations" that cause human disease are located in exons or the immediately contiguous portions of introns. The intervening introns are spliced out of primary transcripts in order to generate splice variants. Some genes contain over 100 exons and can generate hundreds of splice variants. Splicing shows temporal and spatial specificity. For illustration, certain splice variants are only present during development whereas other splice variants are only preset in certain structures of the brain. Splice variants add to the richness and complexity of the genome.

With rare exceptions, mtDNA is inherited solely from your mother. The mitochondrial genome contains 16,569 nucleotide pairs. Each cell contains hundreds of mitochondria each of which has multiple copies of its genome. mtDNA exhibits a higher mutation rate than nuclear DNA. Mutations in mtDNA can cause neurological syndromes that may include dystonia as one manifestation.

Next- or now-generation sequencing. The human genome project took 20 years to complete. Now, with the advent of new sequencing technologies, commonly known as next-generation sequencing (NGS), a human genome can be sequenced in a couple of weeks. Moreover, for a few thousand dollars, all exons in the human genome (the exome) can be sequenced in less than 48 hours. Current estimates suggest that each human exome harbors less than 150 unique sequence variants. Many of these variants may be associated with increased risk for specific medical disorders such as dystonia or breast cancer.

Are blepharospasm and other forms of adult-onset primary dystonia caused by genetic variants? Currently available clinical data suggests that primary dystonia, like many other adult-onset diseases, is due to complex interactions among genetic and environmental risk factors. In most published series, about 10% of patients with adult-onset dystonia have at least one first degree relative with dystonia. These numbers are much higher than predicted by chance alone and provide clear indication that genetic factors contribute to the development of dystonia. Furthermore, several hereditary dystonias (e.g., DYT1, DYT5, DYT6, DYT11, and DYT12) exhibit variable expressivity and incomplete penetrance. For illustration, variable expressivity could result in one family member developing blepharospasm at age 50 and another family member (e.g., sibling or cousin) manifesting writer's cramp (AKA hand-forearm dystonia) at 35 years of age.

Environmental triggers and risk factors must be included in discussions of dystonia since only a small percentage of individuals with certain genetic variants actually develop dystonia during their lifetime. For example, less than 40% of people with the classic DYT1 GAG deletion mutation actually develop dystonia. With many of the DYT6 (THAP1) genetic variants, penetrance may be less than 20%. Environmental triggers for hand-forearm dystonia may include extreme repetitive use of specific muscles during writing, painting and piano playing. Dry eye symptoms may trigger blepharospasm in genetically-predisposed persons. Many cases of jaw dystonia are precipitated by major dental procedures. Similarly, cervical dystonia (AKA spasmodic torticollis) may be preceded by neck trauma or cervical strain. Clearly, however, the commonality of these triggers points to an essential role for genetic risk factors. It is commonly stated that genetics loads the gun and environmental perturbations pull the trigger! Do genetic risk factors play a major role in the development of blepharospasm? Most researchers working in this field would respond with a resounding "YES." The relative rarity of blepharospasm among large populations of people exposed to similar environmental perturbations argues for the presence of predisposing genetic variants.

THAP1. DYT6 dystonia was initially described in Amish-Mennonites. In 2009, the responsible mutation was identified in Exon 2 of the gene THAP1. In several follow-up studies, over 35 additional THAP1 sequence variants have been identified in patients with both early- and late-onset dystonia. Of note, the term "sequence variant" is often preferred over mutation given that causality may be difficult to establish with certainty. In a significant number of patients with THAP1 sequence variants, there was no family history of dystonia. Patients with sequence variants in THAP1 typically do not manifest blepharospasm whereas the larynx, jaw and neck are commonly affected, and it appears that the arms are more commonly affected than the legs in DYT6 dystonia. Ongoing studies are trying to determine the role of non-coding variants in THAP1 on the risk of developing dystonia.

Blepharospasm: Research Directions. You must understand what is broken before you can fix it! Are certain genetic variants (SNPs, CNVs) associated with an increased risk of developing blepharospasm? Do patients with blepharospasm harbor variants in mtDNA? How are expression levels of certain genes related to particular SNPs and CNVs? Is blepharospasm due to rare sequence variants in a small number of "dystonia" or "blepharospasm" genes? Can we identify blepharospasm-specific mutations in dystonia-related genes? Answers to these important questions should unfold over the next few years.

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