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PUMILIO1 Links Epilepsy to Spinocerebellar Ataxia

A Mild PUM1 Mutation Is Associated With Adult-Onset Ataxia, Whereas Haploinsufficiency Causes Developmental Delay and Seizures

Gennarino VA, Palmer EE, McDonell LM, et al. *Cell*. 2018;172(5):924-936.e11. doi:10.1016/j.cell.2018.02.006.

Certain mutations can cause proteins to accumulate in neurons, leading to neurodegeneration. We recently showed, however, that upregulation of a wild-type protein, Ataxin1, caused by haploinsufficiency of its repressor, the RNA-binding protein Pumilio1 (PUM1), also causes neurodegeneration in mice. We therefore searched for human patients with PUM1 mutations. We identified 11 individuals with either PUM1 deletions or de novo missense variants who suffer a developmental syndrome (PUM1-associated developmental disability, ataxia, and seizure). We also identified a milder missense mutation in a family with adult-onset ataxia with incomplete penetrance (PUM1-related cerebellar ataxia). Studies in patient-derived cells revealed that the missense mutations reduced PUM1 protein levels by ~25% in the adult-onset cases and by ~50% in the infantile-onset cases; levels of known PUM1 targets increased accordingly. Changes in protein levels thus track with phenotypic severity, and identifying posttranscriptional modulators of protein expression should identify new candidate disease genes.

Commentary

Two copies of nearly every gene are inherited, one paternal and one maternal, although exceptions are made for genes present on sex chromosomes and mitochondrial genetic material.[1] Haploinsufficiency refers to a phenotype that is the result of losing one copy of a gene, with the intuition that the remaining copy cannot produce sufficient protein to prevent disease. This phenomenon underlies many disease-causing mutations that are found in proteins involved in regulating DNA transcription.[2] Recently, however, haploinsufficiency for genes encoding proteins that bind RNA and regulate translation (termed "RNA-binding proteins") have also been identified.[3]

ATAXIN1 is a protein best known for its role in causing spinocerebellar ataxia type 1 (SCA1), where a trinucleotide repeat expansion acts as a toxic "gain-of-function" mutation.[4] Researchers who study ATAXIN1 have previously observed that mutating proteins which regulate ATAXIN1 levels is sufficient to produce an SCA1-like syndrome in mice. One such protein is PUMILIO1 (PUM1), an RNA-binding protein that regulates the posttranscriptional control of mRNA expression.[4] Genetic knockout of PUM1 in mice leads to an increase in ATAXIN1 levels and ultimately produces an SCA1-like syndrome.[4] These results led Gennarino et al to the hypothesis that mutations in human *PUM1*, the gene responsible for producing PUM1, might lead to an SCA1-like syndrome in humans.

In a remarkable study, Gennarino and colleagues set out to test this hypothesis and examined a large human database in order to identify patients with *PUM1* mutations. They found 9 patients with large deletions that included *PUM1* and reasoned that the clinical presentation of these 9 patients (developmental delay, intellectual disability, ataxia, and in 3 cases seizures) was likely related to *PUM1* deletion. In addition to these large deletion mutations, the team found 2 patients with point mutations in *PUM1*. One point mutation patient exhibited chorea, ataxia, dysarthria, and spasticity, while the other had uncontrollable generalized epilepsy that began at age 5 months and has progressively worsened over time, as well as progressive ataxia and hypotonicity. In total, their screen identified 11 patients with a similar presentation and led them to define PADDAS syndrome: PUM1-associated developmental disability, ataxia, and seizure syndrome.

Beyond the 11 patients described above, Gennarino et al also described a second clinical syndrome related to *PUM1* mutations. This second cohort of patients was comprised of 4 members of a single family that had a missense mutation in *PUM1*. Rather than the early onset clinical syndrome of PADDAS, these patients had an adult onset ataxia beginning at 30 to 50 years of age and had undergone extensive (negative) genetic testing for spinocerebellar ataxias. This adult-onset syndrome was termed PUM1-related cerebellar ataxia (PRCA).

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Although the investigators had identified these patients based on mutations in *PUM1*, it remained to be seen precisely how these mutations might produce the 2 distinct clinical syndromes. To address this questions, the researchers next cultured fibroblasts from a skin biopsy of one PADDAs patient and cultured lymphoblastoid cells from a venous blood draw of 2 patients with PRCA. They found that the earlier onset PADDAS sample had ~43% of the PUM1 protein levels compared to unaffected patients, while the later onset PRCA sample had ~73% to 74% of PUM1 protein of controls. These experiments provide a simple mechanistic explanation for the difference in disease presentation: the earlier onset PADDAs has much less PUM1 protein compared to the adult-onset PRCA, but both syndromes exhibit a reduction compared to healthy controls.

One of the cardinal features of PADDAS that the authors describe is an early onset epilepsy. To confirm that this feature of the disease is causally linked to the loss of PUM1, they examined mice heterozygous for loss of PUM1. Using EEG recordings, they found that these animals indeed have seizures in an age-dependent manner, with onset around 21 weeks of age. This experiment helps strengthen the case that dysfunction of this ataxia-related pathway is indeed capable of producing epilepsy, although the relatively mild phenotype in mice compared to the severe phenotype in humans remains a mystery.

A notable question of this study is that while the team's PUM1 protein levels correlate well with the age of disease onset, the same is not true for ATAXIN1. Despite differences in the level of PUM1 protein seen in PADDAS and PRCA, samples from both groups had similar (~50%) increases in the level of ATAXIN1 protein, which is not what would be expected if ATAXIN1 was indeed the final common pathway leading to disease. A similar discrepancy between molecular results and clinical observation was also seen for nearly all of the RNA expression data, which is notable because PUM1 is an RNA-binding protein and the prediction is that different levels of PUM1 expression would produce different alterations in RNA expression. Although there are many possible explanations for this discrepancies (ie, differences in samples examined in the 2 conditions, low sample numbers, age of participants, involvement of other pathways, etc), it may in fact be the case that ATAXIN1 is not the only (or the primary) pathological mechanism for these disorders.

It remains unclear how changes in PUM1 would produce both epilepsy and cerebellar disease. Spinocerebellar ataxia type 1 does not produce epilepsy, suggesting that alterations in PUM1 leads to other pathological effects besides dysregulation of ATAXIN1 that are responsible for producing epilepsy. An intriguing possibility is that PUM1 may have a distinct function in the neocortex from its role in the cerebellum. RNA-binding proteins often regulate many pathways simultaneously, and additional work will be needed to parse these differences.

"Precision medicine" refers to the promise that genome sequencing of a patient will allow physicians to tailor treatments and diagnoses based on the patient's unique genetic background.[5] So far, however, there has been little evidence that such efforts have had a meaningful effect on mortality outside of a few high-profile successes.[6] The current study provides a window into the difficulties associated with precision medicine efforts as they relate to diagnostics and highlights the need for ongoing validation and experimentation efforts. On the one hand, the team brought to bear an armament of the latest genetic tools in order to map and validate disease-causing mutations with exquisite specificity. Despite their success, the probability of identifying an underlying mutation for a given patient who arrives in the clinic with a suspected rare disease remains low. Gennarino et al engaged in "reverse genetics" whereby a hypothesis is tested by identifying a gene and then finding mutations of that gene in order to study the effect of mutating the gene.[7] Going from clinical patient to genetic mutation underlying the syndrome can be conceived as a case of "forward genetics," whereby the clinical phenotype is known but the investigator then tries to identify the underlying genetic mutation.[7] As such, it remains challenging to unambiguously identify a disease causing mutation with just a single patient (given the myriad point mutations of uncertain significance in everyone's genome) unless other patients have already been described and reported.

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