Monogenic mysteries unravel mitochondrial mechanisms

This scientific commentary refers to ‘Biallelic loss-of-function variations in \textit{PRDX3} cause cerebellar ataxia’, by Rebelo \textit{et al}. (doi:10.1093/brain/awab071).

Cerebellar ataxia arises from dysfunction of the cerebellum or its connections. More than 200 different monogenic forms of progressive ataxia have been identified. The pathophysiological mechanisms of inherited ataxias vary, but converge on a subset of metabolic pathways and other cellular processes, one of which is mitochondrial dysfunction. Mitochondria are especially important to and abundant in cell types with high energy demands, like neurons, which need a continuous energy supply for axonal/dendritic transport, synaptic transmission and activity of ion pumps and channels. Thus, neurons are often affected in mitochondrial disorders, and the cerebellum appears to be especially vulnerable to mitochondrial damage.

Several of the genetic ataxias are associated with mitochondrial defects, including more frequent subtypes like Friedreich’s ataxia and spastic paraplegia type 7. The prevalence of hereditary ataxias varies between populations and studies, but is estimated to be around 6:100 000. The number of identified disease-causing genes has increased enormously over the last decade, in particular as a result of the increased feasibility of high-throughput sequencing, which has made it possible to sequence the entire protein-coding DNA sequence (whole exome sequencing) or even, more recently, the whole genome.\textsuperscript{1,2} This unravelling of genetic causes of ataxias has also shed light on key cellular pathways and mechanisms within the nervous system, and has revealed pathogenic pathways shared with other, more frequent neurological disorders.\textsuperscript{3} These rare, monogenic mysteries may thus serve as model systems to understand cellular mechanisms and indeed neurodegeneration in a much broader sense. In addition, monogenic disorders, where the genetic mechanism is known, provide intriguing possibilities for research on targeted treatments. However, the road to identifying a novel disease gene and understanding its precise pathophysiological mechanism is long, and requires more than a single genetic finding.

In this issue of \textit{Brain}, Rebelo and co-workers present \textit{PRDX3} as a novel ataxia gene, substantiated by meticulous clinical characterization and functional studies, and identify dysfunction of an oxidative mitochondrial pathway as the underlying pathological mechanism.\textsuperscript{4}
The authors present the results of deep phenotyping with thorough clinical characterization of five unrelated individuals with sporadic cerebellar ataxia with adolescent to young adult onset. One patient had a pure ataxia phenotype, whereas the others had additional signs from other parts of the nervous system such as mild parkinsonian features, myoclonus, dystonia or learning disabilities. Only one had ptosis, a typical sign in mitochondrial disorders. Thus, the phenotype of these patients did not point clearly towards any specific genetic ataxia.

The authors used whole exome sequencing to search for presumed disease-causing genetic variants, having first ruled out hereditary ataxias caused by repeat expansions not readily detected by this method. Whole exome sequencing revealed biallelic variants in the \textit{PRDX3} gene. \textit{PRDX3} encodes peroxiredoxin 3 (PRDX3), a mitochondrial antioxidant enzyme not previously associated with human disease.

In studies of monogenic disorders, the power of the novel genetic finding depends largely on replication of the same finding in more than one family (Fig. 1). To select patients and to analyse the data, the authors therefore took advantage of a large international consortium for the sharing of genetic and clinical data.\textsuperscript{5} The patients originated from four different countries and two different continents. The authors themselves represent at least nine different sites, five countries and two continents. This illustrates an important point in research on rare disorders: international collaboration and sharing of data are mandatory. It also shows the importance of, and the possibilities for, sharing knowledge across borders, even in a pandemic.

To assess the consequences of the \textit{PRDX3} variants further, the authors used various bioinformatic tools and structural modelling, and studied protein levels and enzyme activity in patient fibroblasts and in a knockdown cell model. They also generated a model system using fruit flies. Despite little outer resemblance to humans, fruit flies, nematode worms (\textit{Caenorhabditis elegans}), zebrafish and mice have proven efficient animal models for studying neurodegenerative disorders.\textsuperscript{6} The authors induced knockdown of the orthologue of \textit{PRDX3} in fruit flies, and these flies showed reduced locomotor activity, reduced life span when exposed to oxidative stress, and signs suggestive of neurodegeneration.

Functional analyses revealed a reduced ability to tolerate oxidative stress, with increased levels of the reactive oxygen species hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}). ROS are highly reactive molecules, created mainly as a natural byproduct of the normal aerobic metabolism of oxygen through the mitochondrial respiratory chain. Reactive oxygen species have important roles in cell signalling and homeostasis, but in excess, they can have deleterious effects as they oxidize, including...
damaging nuclear and mitochondrial DNA, and inducing lipid peroxidation, protein oxidation and apoptosis (Fig. 2).⁷ Many neurological diseases are associated with reactive oxygen species-induced neurological deterioration. Mitochondrial dysfunction has emerged as an important concept in ageing and neurodegeneration—not only for several ataxias, but also in more prevalent disorders, like Alzheimer’s disease and Parkinson’s disease.⁸ PRDX3 is a member of the peroxiredoxin family, which catalyses the reduction of H₂O₂. The protein is located entirely in the mitochondria where most H₂O₂ is generated, and plays an important role in protecting against oxidative stress.⁸

Rebelo and colleagues demonstrate that PRDX3 itself is important for detoxifying H₂O₂, and that it also seems to be part of a network of several H₂O₂-detoxifying mechanisms acting in concert. In the cellular knockdown model, not only were levels of PRDX3 reduced, but also levels of peroxiredoxin 5 (PRDX5) and glutathione peroxidase activity. Like PRDX3, PRDX5 catalyses H₂O₂ reduction, and glutathione helps protect against peroxide oxidative damage by reducing reactive oxygen species. That levels of all three were reduced suggests that these H₂O₂-detoxifying pathways may be connected and co-regulated. And indeed, reductions in glutathione have been observed in amyotrophic lateral sclerosis, Parkinson’s disease, Alzheimer’s disease and other neurodegenerative disorders.⁹

Another protein closely connected to PRDX3 is thioredoxin-2 (TXN2), which is essential for efficient cycling of PRDX3. One earlier study of a child with a homozygous variant in the TXN2 gene, and a severe phenotype including a movement disorder and cerebellar atrophy, suggested possible clinical effects of treatment with the antioxidant idebenone (a synthetic analogue of coenzyme Q₁₀).¹⁰ This study of a single patient should be interpreted with caution; however, given the shared mechanism, antioxidant treatment represents an intriguing path for clinical evaluation also in PRDX3-related disorders. Elucidating and understanding this cluster of pathways could potentially uncover multiple disease mechanisms and treatment targets.

The thorough clinical characterization performed by Rebelo and colleagues provides important information for future clinical follow-up, and PRDX3 should be included in diagnostic gene panels for hereditary ataxias. Whether this gene could also be implicated in clinical syndromes dominated by signs of basal ganglia or mitochondrial dysfunction, as was observed in some of the patients, remains to be investigated.

To conclude, Rebelo and co-workers present a novel ataxia gene, and further unravel the function of PRDX3 and the network of pathways in which it participates, thereby facilitating
future studies on targeted treatment. In an exemplary way, this study demonstrates the importance of studying rare disorders by showing that they can serve as model systems for pathways and mechanisms that are also involved in other more frequent disorders. It illustrates the long and resource-intensive process of elucidating a disease mechanism, from deep phenotyping to genetic and protein function analyses. Not least, the study also demonstrates the paramount importance of collaboration and of joining forces across borders and medical specialties in order to gain this new knowledge, thereby paving the way to better patient care.

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Competing interests

The authors report no competing interests.

Figure legends

Figure 1 Identifying a novel gene as the cause of a phenotype. Schematic overview and examples of key steps in establishing a novel genetic finding as the cause of a rare, monogenic disorder (grey boxes), exemplified by the methods and results of Rebelo and co-workers (blue boxes). GENESIS platform = a software platform for genetic analyses and matchmaking; \( \text{H}_2\text{O}_2 \) = hydrogen peroxide; LOF = loss-of-function variant; PRDX3 = peroxiredoxin 3, a mitochondrial protective antioxidant enzyme; Prx3 = Drosophila orthologue of PRDX3; PREPARE Ataxia = ‘Preparing for therapies in autosomal recessive ataxias’, a global
consortium of ataxia researchers; ROS = reactive oxygen species. Illustration created using BioRender.com.

Figure 2 Mitochondrial dysfunction and mechanisms affected by PRDX3 variants. A simplified overview of the mechanism of ROS-induced neurodegeneration (grey boxes), and the mechanism resulting from the PRDX3 variants as described by Rebelo et al. (blue boxes).\(^4\)

mtDNA = mitochondrial DNA; nDNA = nuclear DNA; ROS = reactive oxygen species. Illustration created using BioRender.com.

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Pathogenic biallelic PON1 variants → Mitochondrial dysfunction → ↑ ROS → mtDNA and nDNA damage, lipid peroxidation, protein oxidation, cell apoptosis → ↓ PON1 protein, ↓ PON2 protein, ↓ glutathione peroxidase activity, ↑ H2O2, ↑ ROS-induced apoptosis, ↓ cell viability → Neurodegeneration

Cerebellar ataxia with or without additional neuroanatomical basal ganglia or mitochondrial signs
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