New treatment for phenylketonuria (PKU) clears brain fog

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In the 1959 novella Flowers for Algernon by Daniel Keyes (and the 1968 film Charly), 32-year-old Charlie Gordon, a janitor at a New York City bakery, undergoes experimental surgery that has boosted the intelligence of a laboratory mouse, Algernon. Soon, Charlie is devouring books, asking questions, and even solving problems at work. But then Algernon dies, and in a short while Charlie returns to his normal state of intellectual dullness. But now he becomes distraught, recognizing his limitations in a way that he didn't before the surgery.

Dan Peterson is about Charlie's age, and he, too, has recently experienced a new clarity of thinking thanks to a treatment. In Dan's

case it's an enzyme substitution therapy called Palynziq (pegvaliase-pqpz), which he injects under his skin daily. FDA approved it on May 24 to treat phenylketonuria (PKU). But unlike Charlie's brief experience, Dan's treatment should last.

Awakenings

In PKU, deficiency of an enzyme (phenylalanine hydroxylase) leads to build-up of the amino acid phenylalanine (Phe), which would otherwise break down to the amino acid tyrosine.

Because tyrosine is a precursor of melanin, people with classic PKU tend to have fair hair and light skin, and are prone to eczema. The low levels of tyrosine also block synthesis of several neurotransmitters. Developmental delay, behavioral problems, psychiatric disorders, seizures, and musty body odor are part of the clinical picture too. In the U.S. one in 16,000 newborns is diagnosed with PKU, or about 250 each year.

People with PKU must severely limit their intake of protein to minimize the Phe buildup, but avoiding protein-rich foods isn't enough. They must drink a daily bitter-tasting formula reconstituted from powder, a "medical food" with a balance of the other 19 amino acids.

The dietary regimen is challenging, managing meals and formula. "As a kid I was constantly asked about what I was eating. Family meals or having dinner at a friend's house were hard. I felt like a burden, because they had to make special meals. PKU was a separator," Dan recalls. He ate no meat, dairy, wheat, cheese, or yogurt. "We made our own low protein bread and cookies. But my sister and I – she has PKU too – would always want Chips Ahoy and Oreos; our brother, who didn't have PKU, could have them," he adds.

That feeling of separateness began to lift within months of joining the clinical trial for Palynziq in October 2014. "My friends are filming me on YouTube eating something for the first time. I can enjoy meals with business colleagues. I can even have a turkey dinner at Thanksgiving!" Dan laughs. Here's a look at how hard it is for a person with PKU to eat on Thanksgiving.

Like Charlie's new awareness on the job, Dan's work experience changed. "PKU had limited me. Even though I never strayed off the formula, I can tell now my cognitive abilities were not where they are now. I couldn't stay focused. I was doing mindless manual labor but I couldn't read emails, handle invoices, or think. I went from a grunt laborer to a location manager with the auto detailing company I work for. Now I handle everything with the dealership; before I was stocking shelves in a grocery. I always struggled in school, needed constant help, just to be able to get through with passing grades. Now I'm essentially running a business."

The story of the PKU diet is a classic in life science lore.

It Began With Stinky Diapers

In 1931, in Norway, a mother of two young disabled children noted a musty odor to their urine. The father mentioned this to a friend, who told a friend, Asbjörn Fölling, who was a doctor interested in biochemistry. In a lab at the University of Oslo, Dr. Fölling analyzed the foul urine, which the mother hauled over a bucketful at a time. He identified the metabolic block that lay behind the odor as well as the children's mental sluggishness – the condition was later named PKU – and then found other cases among people languishing in mental institutions.

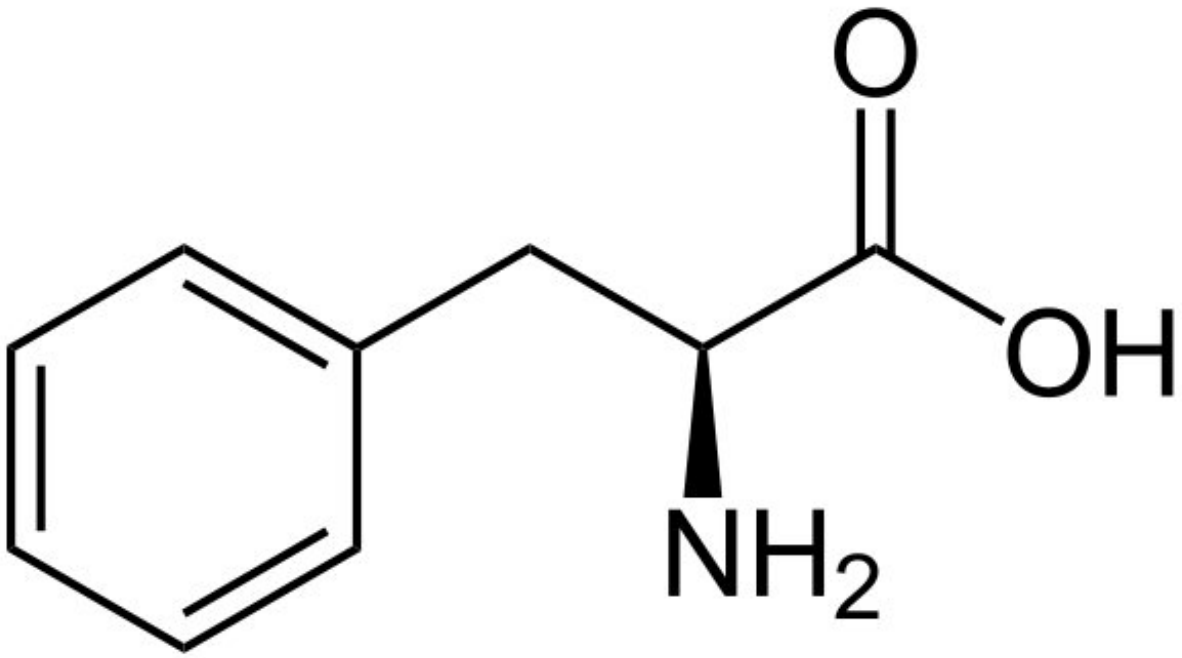
In the early 1960s, physician and microbiologist Robert Guthrie, whose child had PKU, developed what would become the "Guthrie test" to

detect metabolites in blood (using mass spectrometry) that indicate abnormal biochemistry, detected from a sample taken from a baby's heel. The PKU heelstick became the first newborn screening test for a metabolic disorder. PKU is autosomal recessive, inherited from carrier parents.

Limiting dietary protein was a logical way to counter the biochemical buildup. But controlling diet alone wasn't enough; protein restriction needed to continue beyond early childhood, and even during pregnancy, when any fetus, not just ones who'd inherited PKU, would be at risk of brain damage. Here's a story about a young woman with PKU who adopted four boys with the disease from China.

Beyond Medical Food

PKU severity is quantifiable. Average normal blood Phe level is 60-100 micromolar per liter. In a patient off the diet that may soar to 1200-1400, but nutritional intervention brings it down to 600-700.



Phenylalanine

"We recommend people try to get below 360 micromolar, which is still 6 times normal range," explains Cary Harding, MD, of the Oregon Health & Science University. He's first author on the recently published paper in *Molecular Genetics and Metabolism* that reports the phase 3 clinical trial findings. The threshold for Palynziq is above 600.

The new treatment was a long time in coming, perhaps for an unusual reason. Even though the PKU formula couldn't lower Phe levels to a normal range, it's existence may have stifled research into alternative approaches, Dr. Harding speculates. "When I started my career 30 years ago, I couldn't put in an NIH grant 'new therapy for PKU' because the dogma was that someone had solved the problem, so why risk a gene therapy?" He credits BioMarin Pharmaceutical Inc., manufacturer of Palynziq, with pioneering a better treatment.

The PRISM-2 Trial

The clinical trial, PRISM-2, began a decade ago and has treated 335 adults. Phase 3 utilized a clever "randomized discontinuation" design that tested what would happen if patients doing well on the drug went off it for 8 weeks. A conventional double-blinded placebo-controlled trial wasn't feasible because about half the patients experience rash, itching, or swelling of the face, lips, eyes, or tongue that may precede anaphylactic shock, and would therefore know they'd received the drug. (The product package has a warning because anaphylaxis occurred in 9% of people in the trial.)

So instead, all 86 patients in the phase 3 trial reached an optimal dose and then a subset of the group for whom the drug lowered Phe by at least 20%, 28 individuals, switched to placebo for the 8 weeks.

"No one knew who was on what. We didn't analyze blood levels and neurocognition and didn't unblind the study until after it was completed. At the end of 8 weeks everyone was put back on active drug. In those on placebo, blood phenylalanine levels went right back up to where they were pre-treatment," Dr. Harding says. That is, it worked. The studying findings were a powerful confirmation of efficacy.

Perhaps more exciting than the dropping Phe levels were the neurocognitive benefits, although these were challenging to quantify, Dr. Harding says. "Everybody feels the effect of high blood phenylalanine differently. Two individuals with the same blood level can have different experiences. Some people have attention problems, others have anxiety, others depression," he adds.

But assessing cognitive improvement was difficult, because, like Charlie in "Flowers for Algernon," the participants hadn't been aware of previous deficits. "We measured anxiety, depression, and attention. Unfortunately the tests relied on the patients telling us. We learned right at the beginning that they didn't think they had had any problems. Self-reporting didn't work until after the fact and they realized the problems they had had!" Dr. Harding says. Like Dan, other participants mentioned new joy at reading books, being able to fill out complicated forms, and navigate detailed instructions. Patients commonly described the drug's effect as "brain fog that completely lifts," Dr. Harding says.

Enzyme Substitution, Not Replacement

The new drug doesn't provide the missing enzyme, which is unstable and requires a co-factor, but instead delivers a different enzyme,

phenylalanine ammonia lyase (PAL), which degrades phenylalanine. The enzyme is manufactured using recombinant DNA technology, with the PAL gene from a cyanobacterium (Anabaena variabilis) transferred to and cultured in E. coli.

PAL (the enzyme) was tried orally in PKU patients in 1980, but digestive juices destroyed it. The new drug is injected subcutaneously, once daily, by the patient, and physicians must take a training course to learn how to instruct patients. As the drug is metabolized, phenylalanine blood levels drop. The enzyme is "PEGylated," with polyethylene glycol (antifreeze) bonded to it to increase its stability, a common pharmaceutical modification. Hence the generic pegvaliase.

Like enzyme replacement therapy, enzyme substitution therapy is expensive: BioMarin estimates $192,000 annually for Palynziq. But the cost may be worth the ability to return to eating normal foods. Long-term effects of the formula on the oldest patients, who've been on it for more than 50 years, aren't known. "I wonder if some of the sense of wellbeing from the new drug is due to effects of having more normal protein. Maybe there's something in synthetic diets we don't understand, something else patients are missing and we're not giving them," says Dr. Harding, even though there aren't any data on such an effect yet.

Gene Intervention?

Treatment at the gene level would be more lasting than daily injections, and so ultimately would cost less. But gene therapy's track record for PKU has been spotty. Experiments in mice since the 1990s using conventional viral vectors have had poor or transient effects, and the term "gene therapy" doesn't show up at all in the 80 listings for PKU at ClinicalTrials.gov.

But the newer gene editing technologies may outpace gene therapy

efforts. At the recent annual meeting of the American Society of Gene & Cell Therapy, Dr. Harding and colleagues introduced a pig model of PKU bearing the human phenylalanine hydroxylase gene fashioned using TALENs (an older gene editing technique) and a correction of the mutation in mice using CRISPR.

Also at the meeting researchers from Homology Medicines reported on their novel approach. It uses adeno-associated viruses (AAV) from human bone marrow stem cells engineered to direct natural DNA repair to edit a selected DNA sequence without using the cutting enzymes of CRISPR. Their candidate product, called HMI-102 for now, will be "a one-time treatment designed to restore the normal metabolic pathway in PKU," according to the company website. An initial clinical trial on adults with the disease is expected to begin soon.

PKU has held a prominent place in the history of genetics by setting the precedent for the value of newborn screening to detect and counteract a metabolic disease before symptoms start. Let's hope that the new protein substitution therapy and gene-based approaches will provide a lifelong, one-time treatment – a forever fix, rather than an ephemeral Algernon effect.

More information: Cary O. Harding et al. Pegvaliase for the treatment of phenylketonuria: A pivotal, double-blind randomized discontinuation Phase 3 clinical trial, *Molecular Genetics and Metabolism* (2018). DOI: 10.1016/j.ymgme.2018.03.003