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Perhaps a wee bit of sugar would help

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Abstract

A new genetic disorder might be treatable through consumption of a simple sugar, but the relative contributions of endogenous and dietary sources are mostly unknown. It's time to change that.

Fewer than 10% of the 5,500 disorders with a known molecular basis can claim a therapy, but suppose treatment could be as simple as consuming the right sugar. That is the suggestion of a report published in the current issue of *Nature Genetics* (ref. 1). Sugars required for protein glycosylation can be made *de novo*, salvaged from degraded glycans or captured from the diet. The problem is that we don't know how much each pathway contributes to glycosylation in different cells or at different times in development, so we cannot predict whether a sugar supplement could be beneficial.

Brains and bones need glycosylation

Karnebeek, Bonafe, Wen *et al.*¹ combined state-of-the-art genetic and metabolic analysis to define a new congenital disorder of glycosylation (CDG) that selectively impairs skeletal and brain development. Mutations in *NANS* partially block the *de novo* pathway for *N*-acetylneuraminic acid (NeuNAc; also known as sialic acid, Sia), a critical sugar found in glycoproteins and glycolipids of all human cells (Fig. 1).

The team identified eight individuals with intellectual development disorder (IDD) who had similar facial dysmorphisms, skeletal dysplasia, short stature and other bone abnormalities. Next-generation sequencing identified various substitutions, insertions, duplications and splice-altering intronic mutations in *NANS*. *In silico* protein modeling positioned mutations in or near the active site or at a critical dimer-forming interface, while others predicted protein misfolding. Molecular predictions require functional confirmation. *NANS* activity for the reaction *N*-acetylmannoseamine-6-P (ManNAc-6-P) + phosphoenolpyruvate (PEP) → *N*-acetylneuraminate-9-P is required for the *de novo* biosynthesis of sialic acid (Fig. 1), and the fibroblasts of these patients showed a ~75% deficiency in enzymatic activity.

Another patient with IDD and a similar clinical phenotype, but with an unknown mutation, initially underwent high-resolution metabolic profiling that showed accumulation of ManNAc in plasma, urine and cerebrospinal fluid. The team surmised that the ManNAc probably came from intracellular precursor ManNAc-6-P (Fig. 1), strongly suggestive of

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mutations in *NANS*, which they then confirmed. Similar analysis of the other patients demonstrated that they also accumulated ManNAc in their body fluids and ManNAc-6-P in fibroblasts. Metabolic tracers for sialic acid and ManNAc showed that the patients' cells incorporated the former, but not the latter, highlighting the impact of the mutations on the *de novo* pathway. Surprisingly, bulk fibroblast glycoproteins and liver-derived plasma glycosylation biomarkers showed normal sialylation. Therefore, sialic acid seems to be salvaged from either degraded glycoproteins or from the diet.

Could sialic acid supplements benefit these patients? To investigate this, the team knocked down *nansa* (the ortholog of *NANS*) in zebrafish embryos, which displayed abnormal skeletal development when assessed at 6 days post fertilization. The skeletal abnormality could be partially rescued by simply adding 200 μ M sialic acid to the water within 24 h post fertilization. Thus, free sialic acid bypassed the metabolic block. Although it is difficult to extrapolate from the fish model to humans, perhaps supplements might help patients. This is not a novel concept.

A therapeutic confectionary menu

Sugar (monosaccharide) supplements benefit patients with other glycosylation disorders. Patients with MPI-CDG bypass their defect (reducing the conversion of fructose-6-P to mannose-6-P) with a few grams of oral mannose that corrects protein-losing enteropathy, coagulopathy and hypoglycemia². The rare patients with fucose deficiency (SLC35A2-CDG) have frequent infections and highly elevated numbers of neutrophils, but provide them with a few grams of fucose supplements, and their neutrophil levels normalize in a few days³. Fucose restores synthesis of depleted fucosylated glycans found on precursor cells in the bone marrow. Galactose supplements normalize glycosylation of liver-derived glycoproteins in patients with PGM1-CDG (a deficiency of the interconversion of glucose-6-P and glucose-1-P)⁴. ManNAc and sialic acid are in clinical trials now for patients with adult-onset inclusion-body myopathy caused by mutations in *GNE* (affecting UDP-GlcNAc $\rightarrow$ ManNAc-6-P)⁵ (clinicaltrials.gov IDs NCT02346461, NCT02639260 and NCT02377921). GlcNAc has been successful for treating children with severe treatment-resistant inflammatory bowel disease⁶ and is being tested on patients with PGM3-CDG (affecting GlcNAc-6-P $\rightarrow$ GlcNAc-1-P, the latter a precursor of activated UDP-GlcNAc)⁷ (clinicaltrials.gov ID NCT02511041). Other studies in mouse models of multiple sclerosis show that adding modest amounts of GlcNAc to the drinking water improves their clinical outcome⁸. Human therapeutic trials are pending.

Except for extensive studies of mannose^{9,10}, very little is known about the relative or absolute contributions of *de novo*, salvage and dietary sources of these monosaccharides to cellular glycosylation. Incorporation of appropriately tagged stable (heavy) isotope-labeled precursors provides good estimates of different sources¹⁰. Meaningful experiments in cells require physiological concentrations of the relevant monosaccharides, such as glucose, which is often included in standard media at 10–25 mM rather than at a physiological concentration of ~5 mM. Sialic acid salvaged from degraded glycans by some tissues or cells would probably account for selective impact of the *NANS* mutations on glycosylation in selected organs. The current study did not define cellular conditions that deplete sialylated

glycans and titrate sialic acid to restore the normal state. Clearly, 200 μ M sialic acid is sufficient to rescue zebrafish over a defined time frame, but this cannot be generalized to patients with NANS-CDG. The authors correctly advocate extensive studies to determine the contributions of *de novo*, salvage and dietary sources.

Monosaccharide therapy presents an exciting therapeutic opportunity and a compelling argument for addressing fundamental biochemical questions about the origins and contributions of these sugars to glycosylation, how they enter and exit cells, their efficiency of reutilization, their ports of entry and/or the specific transporters required for their uptake. This is an unmapped landscape, and the studies here join the established literature in showing that each cell type will have its own confectionary preferences. Knowing the fundamental biochemistry, employing physiological conditions and carefully measuring the contributions of each will be necessary. So let's get started.

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Figure 1.

Sialic acid metabolism. Sialylated glycans made in the Golgi apparatus (lower left) require sialic acid (NeuNAc) derived from *de novo* pathway, diet or recycled glycans. The relative contribution of each source is unknown. Mutations in *NANS* impair *de novo* biosynthesis, define a new congenital disorder of glycosylation (NANS-CDG) and suggest that dietary NeuNAc might supplement the impaired *de novo* pathway. (This illustration is adapted from Fig. 4 of ref. 1.)