well for eventually describing quantitative genetic variation in terms of complex genetics rather than complex statistics.

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Gangliosides help stabilize the brain

Richard L Proia

Defects in the breakdown of gangliosides are associated with a class of disorders known as lysosomal storage diseases. Now, a defect in the synthesis of gangliosides, glycolipids that contain sialic acid and are abundant in the brain, has been shown to underlie an inherited form of epilepsy.

Epilepsy is a serious neurological disorder affecting as many as 60 million people worldwide[1,2]. Individuals with epilepsy suffer from repeated, spontaneous seizures due to a dysregulation of neuronal excitability. There is a genetic basis for ~40% of cases, which are divided into symptomatic epilepsies (where there is a structural or metabolic abnormality of the brain) and idiopathic epilepsies (where there is no such abnormality). Most genetic forms of epilepsy are complex disorders, in which multiple genes, in combination with environmental factors, contribute to the disease. A small fraction of these disorders are caused by defects in a single gene. These mendelian epilepsies are particularly informative because the genes that underlie them can uncover mechanisms regulating neuronal hyperexcitability and because these genes may also contribute to the much larger group of nonmendelian epilepsies.

Idiopathic epilepsies have been linked to genes controlling ion channel function and receptor signaling on neurons, whereas symptomatic epilepsies have been linked to genetic defects causing neurodegeneration and disordered brain development. Now, on page 1225 of this issue, Simpson *et al.*[3] describe a new gene underlying a severe early-onset symptomatic epilepsy syndrome. The gene, *SIAT9*, encodes a glycosyltransferase involved in the synthesis of gangliosides, glycosphingolipids containing sialic acid that are abundant in the plasma membrane of neurons. Numerous genetic diseases have been linked to defects in the ganglioside degradation pathway[4], but this is the first report of a human disease caused by a defect in ganglioside biosynthesis.

Simpson *et al.*[3] began their work by searching for the genetic defect underlying an autosomal recessive epilepsy syndrome present in an Old Order Amish pedigree. The affected children in this pedigree develop seizure activity, including generalized tonic-clonic seizures, within the first year of life. Coincident with the onset of seizure activity, the affected children show stagnation in the acquisition of developmental milestones and subsequently undergo neurological decline and become blind.

Based on the assumption that the mutated gene derived from a single founder in this genetic isolate, the authors used homozygosity mapping to pinpoint chromosomal regions that might contain the gene of interest. They found a single region of homozygosity on chromosome 2p12–p11.2 and then looked for mutations among the ~50 candidate genes present in this interval. They discovered that *SIAT9*, encoding GM3 synthase, contained a nonsense mutation that was predicted to cause premature termination of the protein product. Analysis of plasma glycosphingolipids in affected individuals confirmed the predicted block in the ganglioside biosynthesis pathway, as marked by substantial changes in the levels of several key gangliosides[3].

Gangliosides are amphipathic molecules composed of a ceramide lipid anchor attached to an externally oriented oligosaccharide chain of variable length and complexity[5]. They are found on the surface of essentially all mammalian cells but are particularly abundant on neuronal cell surfaces. These lipids are the primary glycoconjugates on neurons and carry most of the sialic acid present in the brain[6]. The oligosaccharide chains of the main brain gangliosides are assembled in a combinatorial fashion by a biosynthetic pathway controlled by a few key glycosyltransfereases (**Fig. 1**). GM3 synthase occurs at a pivotal point in this pathway, transferring a sialic acid residue to lactosylceramide to produce a simple ganglioside, GM3, which serves as a precursor to complex gangliosides of the a- and b-series that are common in the brain (**Fig. 1**). Thus, a deficiency of GM3 synthase results not only in the absence of GM3 ganglioside but also in the absence of many key gangliosides normally found in the brain.

Lessons from knockout mice

Because brain samples from the affected individuals described by Simpson *et al.* were not available for analysis of brain gangliosides and pathology, mice deficient in ganglioside glycosyltransferases are important for gaining insight into the potential mechanisms underlying this disorder. As predicted from the ganglioside synthesis pathway, mice with mutations in *Siat9* divert the synthesis of complex brain gangliosides of the a- and b-series to gangliosides of the o-series. Unlike the individuals described by Simpson *et al.*, however, these mice do not show obvious seizure activity or shortened lifespan[7]. The reason for the lack of an obvious seizure phenotype in *Siat9* knockout mice is not known, but it could be due to differences between mice and humans

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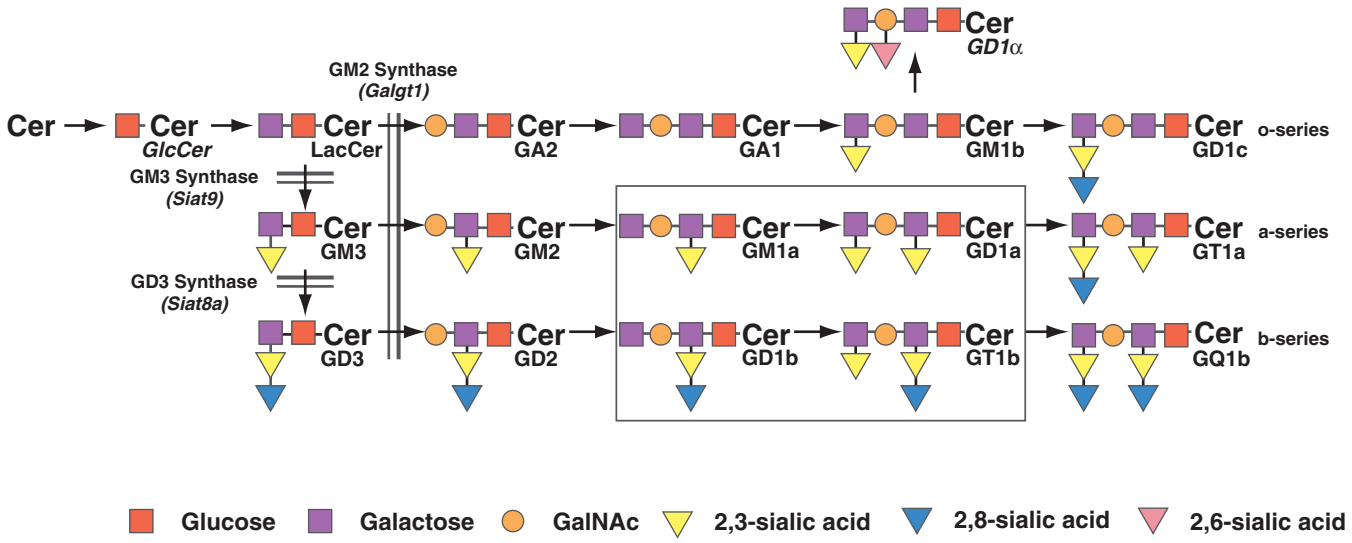

Figure 1 The ganglioside biosynthesis pathway for the ganglio-series of glycosphingolipids. Key gangliosides occurring on neurons in adult mammalian brain are boxed. GM2 synthase (UDP-N-acetyl-D-galactosamine:GM3/GM2/GD2 synthase, EC 2.4.1.92). GM3 synthase (CMP-NeuAc:lactosylceramide a-2,3-sialyltransferase, EC 2.4.99.9). GD3 synthase (CMP-sialic acid:GM3 a-2,8-sialyltransferase, EC 2.4.99.8). Mice with mutations in both *Galgt1* and *Siat8a* have a block after GM3 ganglioside synthesis[10,15]. Mice with mutations in both *Siat9* and *Galgt*1 have a block after lactosylceramide (LacCer) synthesis (R.L.P, unpublished data). Cer, ceramide; GalNAc, N-acetylgalactosamine; GlcCer, glucosylceramide.

in their respective biosynthetic pathways or to the effects of modifier genes, as discussed by Simpson *et al.* Notably, *Siat9* knockout mice show an increased sensitivity to insulin caused by a hyper-responsive insulin receptor, demonstrating a key function of gangliosides as modifiers of signaling pathways[8,9].

Mice carrying mutations in two ganglioside glycosyltransferase genes, *Galgt1* and *Siat8a*, which truncate the synthesis pathway after the production of GM3 ganglioside, are markedly susceptible to sound-induced tonic-clonic seizures[10]. This leaves us with the apparently confounding situation in which individuals that do not synthesize any GM3 ganglioside and mice that synthesize only GM3 ganglioside both suffer seizures. A possible explanation is that in both cases, crucial complex gangliosides in the brain are absent. We recently derived mice carrying mutations in *Siat9* and *Galgt* that have a block after the synthesis of lactosylceramide and are thus unable to produce any of the gangliosides shown in **Figure 1**. These mice have a very short lifespan with severe neurodegeneration, showing that ganglioside synthesis is necessary for a stable central nervous system post-natally (R.L.P., unpublished data).

Possible mechanisms

The diverse functions attributed to gangliosides, when considered with known causes of epilepsy, suggest several possible mechanisms to explain how a block in ganglioside synthesis might cause this disorder. As plasma membrane components, gangliosides are known to modulate ion channel function[11] and receptor signaling, both of which are key for regulating neuronal excitability. Adding to the complexity of potential mechanisms is the severe neurodegeneration in mice with mutations in both *Siat9* and *Galgt*, which are totally deficient in the common brain gangliosides (R.L.P., unpublished data). Though long invoked as a possible function of gangliosides, brain development seems to be largely normal in ganglioside-deficient mice[7,10,12–14], suggesting that developmental defects are unlikely to be the underlying cause of the disorder, though subtle defects have yet to be ruled out.

The discovery by Simpson *et al.*[3] that a human epilepsy syndrome is caused by a defect in ganglioside synthesis is just the beginning of this story. Unraveling the mechanism underlying this disease will not only increase our understanding of epilepsies but also illuminate the crucial functions, now unequivocally established, of gangliosides in the brain.

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