Multiplex analysis of SLC2A1 variants
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Many patients now receive a diagnosis of GLUT1 deficiency syndrome from genetic testing. However, genetic testing interpretation is challenging because many variants in SLC2A1, the gene that causes this disorder, have never been seen before. There are many tools that clinicians can use to help make a diagnosis when variants are not already known to cause disease.

Most importantly, a spinal tap is essential to identify low glucose in the spinal fluid to confirm the diagnosis when GLUT1 deficiency is suspected. Second, testing parents can be helpful to identify variants that are *de novo*, which means that the variant is only present in an affected child and is not present in either unaffected parent. But, not all variants that cause GLUT1 deficiency are *de novo*. Some genetic variants are inherited from parents who may be minimally affected or affected with different or related symptoms.

New technologies are now being developed to study the function of the transporter in cells in a dish. These technologies rely on the requirment of cells in a dish for many genes that are responsible for growth. This growth assay was used previously by other scientists to quantify the effects of all variants in BRCA1, a major gene responsible for familial breast cancer. As might be expected, if cells do not have a functioning SLC2A1 gene, they do not transport glucose into the cell and they do not grow in a dish. We and other investigators, including Elizabeth Radford and Matt Hurles' group at Cambridge, are using this assay to study all possible variants in SLC2A1. CRISPR is used to introduce all possible SLC2A1 variants in a pool of cells in a dish. Cells are collected at different time points and sequenced. Variants that are damaging will be lost over time as cells divide and grow. The ratio of sequenced variants at different time points can be used to calculate a score that correlates with overall function of the glucose transporter.

At the end of the experiment, there will be quantitative data about the degree to which each SLC2A1 variant is damaging to glucose transport and growth of cells. This will provide clinicians a look-up table with which they can identify how a patient's variant functions compared to other variants and the normal protein. We expect that there will be a correlation between the degree of impairment of the protein and the patient's clinical symptoms. Of course, each individual will also have other genetic and environmental modifiers that will impact their outcome, but this is an important first step in developing predictive outcome data.

Overall, this high-throughput functional testing will be immediately useful for clinicians and patients. It will ensure that individuals can be accurately diagnosed with GLUT1 deficiency syndrome. It will also ensure that clinical trials can recruit and appropriately assess outcomes of interventions based on an individual patient's own genetic data.