



Perspectives in Pediatric Neurology

A Needle in a Haystack: Sturge-Weber Syndrome Gene DiscoveryAnne M. Comi MD^{a,*}, Douglas A. Marchuk PhD^b, Jonathan Pevsner PhD^a^aNeurology and Pediatrics, Kennedy Krieger Institute and Johns Hopkins Medicine, Baltimore, Maryland^bDepartment of Molecular Genetics and Microbiology, Duke University, Durham, North Carolina

At the most basic level, the time was finally right; the technology existed to do whole-genome sequencing without it being prohibitively expensive. Computers and servers could handle the massive amount of data, and the software was being developed to analyze the sequencing data. And yet, the journey actually stretches back decades, and right up until the moment that the long-time hypothesis was proven correct, success was far from certain. The facts of the causative somatic mosaic mutation in *GNAQ* have been reported in a recent edition of the *New England Journal of Medicine*¹; here, we tell the story of the discovery. We aim to inspire researchers to take up the challenge of the many syndromes about which the cause is poorly understood. If we can also satisfy both the interest of those who want to know the story and the pleasure that we have in telling it, all the better.

Rudolf Happle applied the hypothesis of somatic mosaic mutation to explain human cutaneous syndromes in a seminal manuscript published in 1987; a mutation occurring early in fetal development affecting a somatic progenitor cell could result in a localized abnormality of structures derived from that progenitor. Sturge-Weber syndrome (SWS) was one of several syndromes used to illustrate this hypothesis: not inherited, occurring in a localized area of the body, and likely to be lethal if the condition were to be present in every cell of the body. Happle's well-articulated hypothesis was the first major step forward toward this discovery because, if true, this genetic mechanism dictated what had to be done to find the gene: compare the DNA from affected tissue to that of unaffected tissue from the same individual with SWS.

Unfortunately, back in 1999 when the National Institutes of Health (NIH) held its first consensus conference on SWS, we were nowhere close to having the ability to do this. Still, the conference was a critical step; it emphasized the need for tissue donation—which the Sturge-Weber Foundation

took on with the help of the NICHD Brain and Tissue Bank for Developmental Disorders—resulting over time in the essential collection of tissue later used in our recent studies. In addition, the NIH consensus conference brought the few clinicians and researchers already familiar with the syndrome, Drs. Roach, Chugani, Maria, and Bodensteiner among them, together with researchers new to its questions; we enter the story here.

In 1999, cutting-edge molecular technology was microarrays and sequencing of candidate genes based on known function or pathways. Given that microarrays compare mRNA levels rather than directly determining differences in DNA and that it was not feasible to sequence every gene known to possibly contribute to abnormal vasculogenesis, none of us was particularly surprised that these efforts, although producing interesting results, brought us no closer to our real goal.

Nevertheless, the clinical and laboratory research efforts persisted. In 2003, a multidisciplinary center under the direction of Dr. Comi, specifically created for the expert care and research of SWS, was created as a collaboration between clinicians and researchers at the Kennedy Krieger Institute and Johns Hopkins School of Medicine. Over the ensuing decade, 30 manuscripts were published on diagnosis, clinical aspects, biomarker development, and approaches to treatment. More than 300 patients were seen at the Hunter Nelson Sturge-Weber Center by a multidisciplinary group of clinicians dedicated to advancing treatment standards. Several foundations (especially Hunters Dream for a Cure Foundation and Celebrate Hope Foundation), families, and donors played critical roles in maintaining this center and its research. These research efforts maintained crucial momentum in the struggle to better understand and treat SWS.

And as each technology became available, we tried it. Eventually single nucleotide polymorphism (SNP) arrays were tested. At least we were now studying DNA. Although unsuccessful, these results served as pilot data for the SWS project on an NIH Rare Disease Consortium grant application. Through this grant, we obtained more funds to do more and better SNP arrays to find the somatic mutation. This Brain Vascular Malformation Consortium was funded

Article History:

Received 28 June 2013; Accepted in final form 11 July 2013

* Communications should be addressed to: Dr. Anne M. Comi; Neurology; Kennedy Krieger Institute and Johns Hopkins Medicine; 801 North Broadway; Baltimore, MD 21205.

E-mail address: comi@kennedykrieger.org

by a Rare Disease Consortium grant to Dr. William Young in 2008. The cost of SNP arrays had decreased. Our initial plan was to do more comparisons of DNA from affected and unaffected tissue from many individuals with SWS. Perhaps we would get lucky.

However, by the time we got our protocols approved, we began to wonder if whole-genome or whole-exome sequencing might be a better approach. The application of these techniques to genetic diseases was being developed in Dr. Jonathan Pevsner's laboratory, which also included the infrastructure to store large amounts of genomic data and the bioinformatics expertise necessary for data analysis. We debated that choice—undertake whole-genome sequencing with more coverage of the genome but less read depth (loosely meaning less confidence in the results) and at higher cost or go with whole-exome sequencing (fewer genes, but greater read depth, less expensive)? In the end, we went with whole-genome sequencing knowing that we only had funds to do three sets of comparisons, and with so few comparisons we might well fail.

Once the three pairs of whole-genome sequencing data from affected and unaffected tissue from three individuals with SWS were obtained, software was used to identify somatic single nucleotide variants and insertions/deletions that differed in the affected DNA samples as compared with the unaffected samples from the same individuals. Matt Shirley, a graduate student in the Pevsner laboratory, found that only 10 variants were consistently seen in all three pairs, and of these only one was in a coding gene: *GNAQ* on chromosome 9, coding for the protein $G\alpha_q$. The mutation in *GNAQ* predicted activation of downstream pathways, some of which were already known to be involved in vascular malformations. We were optimistic that our candidate was authentic.

But still, we were cautious. We replicated the results in many other samples, with two other sequencing technologies, in two laboratories. Most of the tested SWS and port-wine samples had the mutation—and it was present somatically as predicted. The mutation was not found in normal controls or cerebral cavernous malformation samples. We thoroughly replicated and verified the finding in 97 DNA samples from 50 individuals. At this point, the excitement really began to grow as we became confident that the somatic mutation was the cause of both SWS and port-wine birthmarks.

Many poorly understood genes have been identified for diseases, and because of the lack of “drug-ability” of the protein or pathway, identification of the causative gene has done little to further treatment development. This is not expected to be the case for SWS and port-wine birthmarks. The laboratory of Dr. Douglas Marchuk has already shown that the mutation in *GNAQ* is mildly hyperactivating in ERK-phosphorylation and there are clinically available ERK inhibitors. Plus, the same mutation in melanocytes in adults causes uveal melanoma, which is already being studied in clinical trials; the pathways modulated by this mutation are the target of many drugs. After more than a decade of active research, we can now look forward to the development of cell and animal models and rapid progress toward the development of new, targeted, and more effective treatment strategies for SWS and port-wine birthmarks.

Reference

1. Shirley MD, Tang H, Gallione C, et al. A *GNAQ* somatic mutation causes Sturge-Weber syndrome and port-wine stains. *New Engl J Med*. 2013;368:1971–1979.