Gleaning Genetic Insight about Neurodegenerative Disorders

TruSeq™ Neurodegeneration Panel enables researchers to investigate the complex genetic landscape of Alzheimer's, Parkinson's, and other neurodegenerative diseases.

Introduction

In a recent *Annals of Neurology* editorial, leading neurologists reported that the estimated annual cost of common neurological diseases in the United States now exceeds $800 billion. Common aging-related disorders such as Alzheimer's disease and Parkinson's disease take up more than $250 billion of those total expenses.[1] With the aging population expected to nearly double by 2050, those costs are expected to rise. To manage the private and public burden of these disorders, the authors suggest that researchers across the globe expand their translational research into preventive and disease-modifying therapies.

Such a mandate is easier said than done. It has been known for some time that neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis (ALS, better known as Lou Gehrig's Disease) have strong genetic components. However, little is known about the specific genetic variants involved in the development of each disease or the precise mechanisms of neurodegenerative progression over time. That makes it difficult for clinicians to make early, confident diagnoses with prognostic certainty. It also limits the ability of scientists to develop safe, effective treatments to combat these debilitating disorders, before and after patients show symptoms.

Array-based methods, such as genome-wide associations studies (GWAS), can't detect rare risk variants. To discover these variants, researchers such as Alan Pittman, PhD developed their own next-generation sequencing (NGS) panels for their neurodegenerative disease studies. A Senior Research Associate in Molecular Neuroscience at the University College London (UCL) Institute of Neurology, Dr. Pittman has spent his career studying Parkinson's disease and other neurodegenerative disorders, including rare, early-onset neurodegenerative disease. Together with a group of 16 other leading researchers, he helped Illumina design and test a new tool for studying the genetics underlying neurodegenerative diseases, the TruSeq Neurodegeneration Panel.

iCommunity spoke with Dr. Pittman about the challenges of understanding the etiology of neurodegenerative disorders, what he learned while testing the panel, and the potential of the panel to inform future clinical trials and treatment development.

Q: What sparked your interest in neurology and early-onset neurological disease?
Alan Pittman (AP): Most everyone, either directly or indirectly, has been affected by neurological diseases. Many of us have family members and loved ones with conditions like Alzheimer's disease and Parkinson's disease. The cost to society of these diseases is tremendous. That motivates me to find a cure. The hope that I could do something to help is what led me into this line of research.

Q: What is the focus of your research studies at UCL?
AP: My group is interested in finding the causes of neurodegenerative diseases. If we can identify the genetics and how it influences the biology, we can begin unraveling the biological basis of these diseases.

Despite their prevalence, many neurodegenerative diseases are difficult to diagnose. There's significant heterogeneity within and among these disorders. Some have similar symptoms, but different underlying genetic causes. Others have the same genetic cause, but different symptoms.

Focusing on the genetics will enable us to develop better tools for diagnosis. After we have those tools, clinicians will be able to provide people with information about what to expect from these diseases. It will also inform the development of effective treatment options for people living with these disorders.

Q: Will a deeper understanding of neurodegenerative disease genetics also improve the characterization of the neuropathology?
AP: With an understanding of the genetics, we will be able to characterize the different subtypes of a single neurodegenerative disease more effectively. A classic example is frontotemporal dementia. It's an umbrella term for a wide range of different disorders that result from different pathologies. The result is different causes, clinical symptoms, and clinical outcomes for each patient. Genetic studies will improve our clinical understanding of the overall condition and different subtypes.

Alan Pittman, PhD, is a Senior Research Associate in Molecular Neuroscience at the UCL Institute of Neurology.

Q: How will genetic studies help in the development of pharmaceutical agents or other treatments?
AP: Unfortunately, there aren't many treatments available for most neurodegenerative disorders at the moment. The ones that we do have don't always work as well as we'd like them to. It's paramount that we do a better job of developing good, effective treatments. That's where understanding the genetics of neurodegenerative diseases could be valuable. Genetic studies could inform future clinical studies. For example, we could test treatments on specific groups of patients that share a mutation. Identifying individuals who might be at risk or grouping patients with a disorder into particular subgroups, could help in the development of new treatments that actually work. It starts with understanding the genetics and the corresponding biology.

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Q: You've been the NGS Facility Lead at the UCL Institute of Neurology. What are some of the sequencing technologies you use to do this kind of research?
AP: There's no one perfect method for all research studies, so we use various technologies. We use Sanger sequencing if we are sure that a sample has a particular genetic mutation. NGS technologies enable us to screen for a wide range of genes that may be involved with neurodegenerative disease. We might use targeted resequencing panels or whole-exome sequencing (WES), depending on the kind of question we are asking. If those approaches don't give us the answers we're looking for, we then use whole-genome sequencing (WGS) techniques. Usually, we can obtain a clear answer with WES, but not always. We have a mixture of many different technologies at our disposal, including the MiSeq™ and HiSeq™ 3000 Systems. We've been using Illumina NGS systems consistently since 2011, with a few upgrades here and there.

Q: What are the benefits of targeted resequencing?
AP: When we know what we're looking for, we don't want or need a full readout of the exome or genome. That's a significant amount of data to store and analyze and there's also the issue of incidental findings. We'd rather look at a small set of genes. Targeted resequencing is less expensive for labs that want to sequence large numbers of samples very quickly.

Q: How did you become involved with the design and testing of the TruSeq Neurodegeneration Panel?
AP: The TruSeq Neurodegeneration Panel is a product that has been needed for a long time. While there have been targeted resequencing panels available to study different types of cancers, there were no commercial sequencing panels to study neurodegenerative diseases. Neurology researchers had to design their own custom panels. There was a serious gap in the market that needed to be filled. When I was asked to help design and test the TruSeq Neurodegeneration Panel, I was happy to do so.

Q: What genes are covered by the panel?
AP: We designed the panel to include coding and noncoding segments of 118 genes that were previously associated with disorders such as Alzheimer's, Parkinson's, and ALS using GWAS. The non-coding segments include introns, untranslated regions (UTRs), and promoter regions. It provides data on the presence of certain gene variants, as well as additional content to answer research questions. For example, we can use the panel to identify the splicing mutations associated with certain diseases.

Having all these genes on one panel is also good for cross-disease and cross-phenotype situations. For example, frontotemporal dementia and ALS are two very different diseases. Yet, they share a significant amount of genetic overlap. We can use this panel to work on a single disease or on various diseases simultaneously.

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Q: What is the value of including markers on the X and Y chromosomes?
AP: Too often targeted panels lack content on the X and Y chromosomes. If we are performing a large population research study, we want to be able to infer the gender of our samples. It ensures that there are no sample or plate mix-ups, and is a good way to check data integrity.

Q: Why is an intron/exon-based gene panel an advantage over WES?
AP: Using an intron/exon-based gene panel like the TruSeq Neurodegeneration Panel enables us to capture potentially disease-relevant non-coding variants and detect rearrangements located beyond the introns that might otherwise be missed with WES.

Q: How long did it take to test the panel?
AP: We spent the bulk of our time thinking about what to sequence and collecting the samples. We had used an array in the past to perform rare variant analysis of Parkinson's disease.[2] We decided to use the TruSeq Neurodegeneration Panel to take an in-depth look at the penetrants of the *LRRK2* G2019S codes in Parkinson's disease.[3] There is significant variability in *LRRK2*. Our goal was to characterize the G2019S pathogenic haplotype in a new multiethnic cohort and to find genetic modifiers of early age at onset (AAO).

We selected 41 samples with Parkinson's disease and 7 unaffected relatives of North African, Ashkenazi Jewish, European Caucasian origin that were *LRRK2* G2019S mutation carriers. It took us only 2–3 days to create the libraries, sequence them, and perform a quick data analysis.

Q: How did you analyze the data?
AP: We looked at quality metrics, such as coverage, to make sure that the genes we were interested in were covered. I'm a bioinformatician so I use my own analysis methods, including open source software and a genome analysis toolkit that I modified for the panel data.

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Q: How did the TruSeq Neurodegeneration Panel perform?
AP: We were happy with how the TruSeq Neurodegeneration Panel performed in our study. It uses the same principles and workflows of other Illumina systems and panels. It has a standardized, easy-to-use method that was fast and straightforward to use. There weren't any dropouts to worry about. We were pleased with the quality of the data.

Q: What are the advantages of the TruSeq Neurodegeneration Panel?
AP: The main benefit of the TruSeq Neurodegeneration Panel is cost. The panel targets known genes, so researchers don't have to invest as much to sequence the whole genome. It's fast and can be used to sequence many samples simultaneously, which is great when performing large studies. It's also an excellent screening method, enabling researchers to reserve WGS or WES studies for those samples where a mutation is not found.

Researchers also want a method that they can validate and that doesn't change. They want reliability and consistency in their data. The TruSeq Neurodegeneration Panel supports that.

Q: How will the TruSeq Neurodegeneration Panel advance our understanding of neurodegenerative disease?
AP: The TruSeq Neurodegeneration Panel will be an important research tool for a wide spectrum of neurological conditions. There are many research groups that will benefit from its use.

It will enable researchers to look at the genetic architecture of individual disease and neurodegeneration as a whole. I believe that many neurological diseases share some genetic architecture. It may not always seem like certain diseases belong together. However, they do from a genetic standpoint. So, being able to look at individual diseases and groups of diseases at the same time will be very useful.

The panel includes common risk factors and it could offer improved predisposition screening for high-risk populations. It could enable researchers to study non-Mendelian types of risk factors, like those in Alzheimer's disease, as well as lower penetrants and the top hits found in GWAS studies.

In terms of clinical studies, it's advantageous to identify carriers, particularly presymptomatic carriers, for clinical trials. That way we can see if there might be some way to intervene beforehand. We can try new targets for therapeutics that haven't been tried yet. Too often, by the time we diagnose patients with neurodegenerative disease, the damage has been done and we aren't able to make a difference. If we can identify subjects early on, it could be very important in informing clinical trials, and ultimately, beginning therapies to prevent or treat the disease.

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Q: What are the next steps in your research?
AP: Our recent work on oligogenic inheritance patterns in *LRRK2* mutation carriers in Parkinson's disease was just a pilot study. It was successful in characterizing the G2019S pathogenic haplotype in a multiethnic cohort. It was clear that we needed a larger sample size to address the question of genetic modifiers of *LRRK2* G2019S disease. We're in the process of recruiting more patients with that mutation to conduct a much larger research study with the TruSeq Neurodegeneration Panel to boost our statistical power.

We are also beginning a second research study using ALS samples. We'd like to understand why some people have certain ALS-associated mutations, yet never become afflicted with the disease. We'll also be studying why one person gets the disorder at age 30, while another person with the same mutation is never afflicted with ALS. There has to be some modifying genetic factors there and we'll be using TruSeq Neurodegeneration Panel to assist us in identifying them.

Q: What do you hope people understand about the genetics of neurodegenerative disorders?
AP: The genetics of neurodegenerative disorders is complex. We still have so much to learn and a lot more research to do. The TruSeq Neurodegeneration Panel gives us an opportunity to better understand the genetic pathways underlying these disorders. It will enable us to understand the onset of these

diseases and what we might be able to do one day to prevent or treat them.

Learn more about the systems and products discussed in this article:

TruSeq Neurodegeneration Panel Data Sheet, https://science-docs.illumina.com/documents/LibraryPrep/truseq-neuro-panel-data-sheet-1070-2017-002/Content/Source/Library-Prep/TruSeq/truseq-neuro-data-sheet-1070-2017-002/truseq-neuro-data-sheet.html

MiSeq System, www.illumina.com/systems/sequencing-platforms/miseq.html

NextSeq 550 System, www.illumina.com/systems/sequencing-platforms/nextseq.html

HiSeq 3000 System, www.illumina.com/systems/sequencing-platforms/hiseq-3000-4000.html

References

1. Gooch CL, Pracht E, Borenstein AR. The burden of neurological disease in the United States: A summary report and call to action. *Ann Neurol.* 2017; 81:479-484.
2. Lubbe SJ, Escot-Price V, Gibbs JR, et al. Additional rare variant analysis in Parkinson's disease cases with and without known pathogenic mutations: evidence for oligogenic inheritance. *Hum Mol Genet*. 2016; 25:5483-5489.
3. Pittman A, Brown E, Hughes D. Comprehensive analysis *LRRK2* mutation carriers in Parkinson's disease from the Illumina TruSeq Neurodegeneration Panel, www.illumina.com/content/dam/illumina-marketing/documents/products/appnotes/truseq-neuro-dna-panel-app-note-1070-2017-007.pdf

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