

## Review

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# The Identification of Alpha-Synuclein as the First Parkinson Disease Gene

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**Dr. Nussbaum** is the Chief Medical Officer of Invitae, a genetic information company. From 2006–2015, he was the Holly Smith Professor of Medicine at UCSF. He came to UCSF from the National Human Genome Research Institute, NIH, where he and Mihales Polymeropoulos carried out the original research leading to the discovery of mutations in  $\alpha$ -synuclein in hereditary Parkinson disease. He was awarded the Klaus Joachim Zülch-Prize for Neurological Research and the Jay Van Andel Award for Outstanding Achievement in Parkinson's Disease Research. He has co-authored >200 peer-reviewed publications and was elected to the National Academy of Medicine in 2004 and the American Academy of Arts and Sciences in 2015.

**Abstract.** In this Commentary, I describe the events that led from an NINDS-sponsored Workshop on Parkinson Disease Research in 1995, where I was asked to speak about the genetics of Parkinson disease, to the identification a mere two years later of a mutation in alpha-synuclein as the cause of autosomal dominant Parkinson disease in the Contursi kindred. I review the steps we took to first map and then find the mutation in the alpha-synuclein locus and describe the obstacles and the role of serendipity in facilitating the work. Although alpha-synuclein mutations are a rare cause of hereditary PD, the importance of this finding goes far beyond the rare families with hereditary disease because it pinpointed alpha-synuclein as a key contributor to the far more common sporadic form of Parkinson disease. This work confirms William Harvey's observation from 350 years ago that studying rarer forms of a disease is an excellent way to understand the more common forms of that disease. The identification of synuclein's role in hereditary Parkinson disease has opened new avenues of research into the pathogenesis and potential treatments of the common form of Parkinson disease that affects many millions of Americans and tens of millions of human beings worldwide.

**Keywords:** Alpha-synuclein, hereditary Parkinson disease, gene mapping, positional cloning

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I had been a Branch Chief in the newly organized intramural program of the National Center for Human Genome Research (NCHGR) for about a year when I was approached by Zach Hall, Director of the National Institute for Neurological Disorders and Stroke (NINDS), and asked if I would speak at a Parkinson Disease Research Workshop planned for August 1995. Parkinson disease (PD) researchers were concerned and patient support groups disheartened by what they perceived as a lack of progress in understanding the causes of PD and the absence of any major therapeutic advances in the 25 years since the demonstration of the efficacy of L-DOPA [1, 2]. Hall wanted someone who had never worked on PD to provide an outsider's view of whether human genetics in general and, in particular, new approaches such as "positional cloning", might open novel avenues for PD research.

Positional cloning is an approach to finding the gene and mutations responsible for an enigmatic hereditary disease even when we do not know enough about the pathogenesis of the disease to even formulate a hypothesis about which candidate genes might be involved. Instead, positional cloning proceeds by first mapping the disease locus using structural chromosome abnormalities in patients or performing linkage analysis in families, and then sifting through all the genes in the region of a chromosome in which the locus of the disease resides to find which gene is altered in affected patients. As of 1995, the approach had already had a decade of spectacular successes, particularly the discovery of the cystic fibrosis gene through genetic linkage analysis alone [3]. We need to remember that 1995 was still the very early days of the Human Genome Project, and so positional cloning often required the investigator to first find all the genes in the region in which the disease was located and, only then, examine those genes for alterations in people affected with the disease.

I agreed to review the literature on what was known on the genetics of PD to see if any research directions suggested themselves. In discussing my participation in the workshop, Francis Collins, the Director of the NCHGR, told me how, as a medical student, he had challenged an attending physician to name even one disease that had absolutely no genetic contribution to its etiology and was told with utmost confidence that PD was just such a disease. My work was cut out for me!

As I reviewed the literature, I saw that there were twin concordance studies and family studies attempting to answer the question of whether there was a

genetic contribution to PD. Initial reports suggested that low concordance rates for monozygotic twins, with little difference between monozygotic and dizygotic twins, made a genetic contribution unlikely [4, 5]. The tide turned, however, as these older studies were re-interpreted [6] and some twin studies actually showed increased concordance among monozygotic twins [7, 8], especially if imaging were used in the unaffected (presymptomatic?) discordant twin or the twins were stratified by age. The literature also contained two large families with apparent autosomal dominant inheritance of PD, one from the Midwestern United States [9] and the other from New Jersey [10] that was later dubbed the "Contursi kindred" because a branch of the family had emigrated from Contursi Terme, a small town near Salerno, Italy [11]. I observed that a simulated linkage analysis would allow definitive mapping of the gene responsible in the Contursi kindred. The other observation I made was that the same name, Roger Duvoisin, kept coming up in the literature on the genetics of PD; of particular interest to me was that he was the senior author of the papers describing the Contursi kindred. He had been initially quite negative about the role of genetics based on older twin studies but had re-examined his data as well as engaged in family studies and had undergone an epiphany to become one of the strongest advocates for the importance of examining the role of genetics in PD.

I prepared my literature review and gave my talk in August 1995, which was later summarized in the Workshop Report as follows:

*"The proportion of inherited Parkinson's cases is not yet clear. Some people do appear to inherit Parkinson's disease, and several large multi-case families have been carefully studied. Regardless of the percentage of inherited cases, finding genes responsible for familial Parkinson's should be helpful for understanding all forms of the disease. Techniques now available should allow researchers to find the genes responsible for familial Parkinson's disease in a relatively short time."*

([http://www.ninds.nih.gov/news\\_and\\_events/proceedings/parkinson\\_workshop\\_proceeding.htm](http://www.ninds.nih.gov/news_and_events/proceedings/parkinson_workshop_proceeding.htm))

After my talk, Roger Duvoisin sought me out at the coffee urn and introduced himself. I asked him if he had localized the gene responsible for PD in the Contursi kindred and he told me "not yet", that they were having some issues with getting the linkage nailed down. I offered to help, since my colleagues

and I had had a lot of experience with positional cloning and were heavily involved in a number of such efforts. After some lengthy negotiations, facilitated by Zach Hall and then Scientific Director of intramural NINDS, Story Landis (later to become NINDS Director), we obtained the DNA samples from Roger Duvoisin and his collaborators William Johnson, Larry Golbe and Alice Lazzarini, and undertook genome wide mapping. An expanded pedigree and additional DNA samples from Italy were the valuable contributions of Giuseppe Di Iorio, a neurologist from the area of Italy near Contursi.

At this point, the power of serendipity seemed to govern the progress of this work. The first and best piece of luck was having Mihales Polymeropoulos as a colleague. Mihales is a psychiatrist by training and an impressive genomic scientist of striking intelligence, determination and vision. I had hired him into my Branch at NIH from St. Elizabeth's Hospital in Washington DC and I turned to him and asked if he wanted to collaborate - he readily accepted. Mihales went to work immediately and began to map the PD locus. By chance, he began with markers from chromosome 4, because he happened to be using chromosome 4 short tandem repeat marker primer sets for another mapping project, for Wolfram syndrome, which also mapped to chromosome 4. He struck gold 9 days after receiving the DNA samples. Every patient, save one, with PD in the family had the same set of markers for a particular region of chromosome 4, thereby definitively mapping the gene locus in the Contursi kindred to chromosome 4q21-23 by multipoint linkage analysis [12].

As mentioned above, there was one individual with PD in the Contursi kindred who had markers in and around 4q21-23 that were different from those seen in every other affected person in the family. His disease, which was a fairly typical, classic PD, appeared unlinked to the markers we found linked in every other affected person. It turned out that, although he had an affected parent, that parent was *not* connected to the Contursi kindred, and it was his *unaffected* parent through whom he was connected to the rest of the family. Given he did not share the 4q21-23 markers with the rest of the affected individuals in the family, we decided to drop him from our analysis. By declaring this individual to have PD for some other reason irrelevant to what was going on in the Contursi kindred, we were able to show definitive linkage (a maximum LOD score over 6) with no recombination between the marker D4S2380 on the long arm of chromosome 4 and the disease.

Soon after we published the paper showing the map location for the PD gene in the Contursi kindred, we received a call from the White House. President Clinton wanted to make mention of the finding in his 1998 State of the Union Address. Indeed, Clinton spoke about the finding as follows:

*“Think about this, the entire store of human knowledge now doubles every 5 years. In the 1980’s, scientists identified the gene causing cystic fibrosis. It took 9 years. Last year scientists located the gene that causes Parkinson’s disease in only 9 days.”*

In the mid 1990’s, the next obligatory step in a positional cloning project was to build a physical map of the region in overlapping fragments of cloned DNA in order to look for exons and identify all of the genes in the region. Of course, as was often the case in the pre-Human Genome Project era, positional cloning projects could easily get bogged down when faced with the daunting task of searching through millions of basepairs of DNA, containing mostly unknown genes, and systematically studying affected individuals for mutations. After all, 9 years had elapsed between mapping the Huntington disease gene to chromosome 4 [13] and identifying the gene itself [14]. Nonetheless, the physical map was quickly constructed by a team led by Christian Lavedan, a dedicated and resourceful French Research Scientist in the lab [15].

This is when a second bit of good luck occurred. Before taking on the arduous and labor-intensive effort of searching through this large region to find the right gene, we of course had looked in Genbank and the literature to see which known genes had already been mapped to the region of linkage in the Contursi pedigree. There were a few and we named them in our paper describing the linkage mapping in the Contursi pedigree. Among them was the gene encoding alpha-synuclein, a protein of unknown function that got its name because it appeared to be enriched in both synapses and the nuclei of neurons. Alpha-synuclein was originally called NACP, or the non-amyloid component of plaque, because it had been isolated from amyloid plaques derived from Alzheimer disease brains [16]. The alpha-synuclein gene had been previously mapped to chromosome 4q21 by Spillantini and her colleagues [17].

Given the presence of alpha-synuclein in the linked region, Mihales and I looked at each other with typical positional cloning insouciance, and said “alpha-synuclein is in central nervous system neu-

rons, so it's in the right tissue; it's involved somehow in Alzheimer disease, a neurodegenerative disease like PD; the gene maps to the right region; it's as good a candidate as any . . . let's have a look". Christian Lavedan and his colleagues, Susan Ide, Elisabeth Leroy, and the late Anindya Dehejia (a young and promising molecular geneticist who sadly passed away from brain cancer in 2001) sequenced the alpha-synuclein gene in the Contursi family. They found a missense change, alanine to threonine, at position 53 (A53T) of the alpha-synuclein protein in every affected person whose DNA was in our hands. The one exception was the individual in the family that we had dropped from the linkage analysis, consistent with our theory that he was a phenocopy. The A53T mutation was not present in a few hundred controls, including some that were ethnically matched anonymous blood samples.

A missense mutation in a single family does not prove we had either the right gene or the pathogenic mutation in that gene. It could have been a benign variant that had no effect on gene function. Knowing it was not likely to be a common polymorphism was helpful but did not prove it was a pathogenic change - it could be a very rare variant, so rare as to be absent from ethnically matched controls, and yet still be benign and simply linked to the mutation causing PD in the family. One way to assess potential pathogenicity is to ask whether the residue is conserved across animal species, the argument (although admittedly not a definitive one) being that a mutation in a highly conserved residue is less likely to be tolerated over millions of years of evolution. We found that alpha-synuclein was limited to vertebrate species but, in fact, a threonine at position 53 was the *wildtype* allele in all non-primates.

Mihales and I had pinned the Contursi pedigree up on a wall outside our labs in Building 49, right next door to the lab of NIH director Harold Varmus, and were standing in the corridor examining the data. Harold came striding by and asked what we were doing and got very excited until we told him the A53T mutation was, in fact, not a mutation in mice but was, in fact, the wild-type residue in all non-primate vertebrates. He questioned whether we had it right and admonished us to gather more evidence.

That is when we had a third stroke of luck. Mihales was born in Greece and attended medical school at the University of Patras in the northwestern corner of the Peloponnese. He approached two faculty members at his medical school, Thodoros Papapetropoulos, a neurologist, and Aglaia Athanassiadou, a molecular

geneticist, to ask whether they had any patients with autosomal dominant, early onset PD. They replied they had five such families, all too small to allow definitive linkage analysis, but they obtained consent for us to look at the alpha-synuclein gene in these families. We did and three of the five showed the same A53T mutation. These results strengthened the case but still did not eliminate the possibility that we were dealing with a rare variant in a gene that happened to sit within the region of linkage of the PD locus. Nonetheless, we decided to publish the results in order to make them known to the scientific community and allow scientists worldwide to either confirm or reject the finding [18].

Supporting evidence for the involvement of alpha-synuclein mutations in hereditary PD came quickly. First, a different missense mutation, A30P, in alpha-synuclein, was described in a German family with autosomal dominant PD [19]. Next, Andy Singleton at the NIH and his collaborators cracked the positional cloning problem presented by the large Midwestern family with early onset PD when they recognized the mutation was in fact a triplication of approximately 2 million basepairs of DNA in chromosome 4q containing the alpha-synuclein gene (Singleton, Farrer et al. 2003). Amalia Dutra, a gifted cytogeneticist from our group, contributed beautiful FISH studies demonstrating the triplication. Similar copy number changes involving duplications of the alpha-synuclein gene were quickly discovered and reported, but the disease in these families had a somewhat later onset and seemed less aggressive than in the families with a triplication [20]. The implications of finding that overexpression of wild-type protein was capable of causing disease in a dosage dependent manner firmly established a role for alpha-synuclein mutations in hereditary PD. These few key observations confirmed that alpha-synuclein mutations were a cause for autosomal dominant PD but a flurry of papers following our report indicated they were a rare cause [21–24].

The real significance of the discovery really became obvious with the report by Spillantini et al. showing that alpha-synuclein was a major constituent of Lewy bodies, not only in patients with synuclein mutations but, most importantly, in patients with sporadic disease as well (Spillantini, Schmidt et al. 1997). We were in the process of demonstrating the same thing with our NIH colleague Eva Mezey when the Spillantini et al. report was published and so were able to confirm their original finding (Mezey, Dehejia et al. 1998). These results meant that alpha-synuclein

was likely involved in the much more common sporadic disease and was not a rare, genetic “side-show” in the pathogenesis of PD.

There is an additional historically interesting aspect to this story. After the original identification of the A53T mutation in three Greek families, the University of Patras investigators found four additional Greek families with early onset PD and the A53T mutation in alpha-synuclein. The three original and four additional Greek families came from three villages in the northern Peloponnese in Greece. Six families came from two villages that are only 17 km apart and the seventh from a village that was further away, 120 km. Working with Mihales’ lab, the University of Patras investigators performed haplotype analysis of the Contursi pedigree and these seven families and showed there was a shared haplotype across an interval of approximately 600 kb that included the alpha-synuclein gene [25]. The implication was that the Contursi pedigree and all 7 Greek families with the A53T mutation were related through a common ancestor. Although little is known of the origins of the population of Contursi itself prior to 400 AD, the Region of Campania that contains Contursi, Salerno and Naples had been colonized by Bronze Age Greeks over 3000 years ago. The name of the city of Naples is derived from the Greek word Neapolis, meaning “new city”, dating from its re-founding in the 6th century BC. It is likely that the Contursi pedigree is an example of a founder effect resulting from the migration of a Greek settler who carried the A53T mutation from Greece to Italy thousands of years ago, leading eventually to the Contursi pedigree and the discovery of a role for alpha-synuclein in PD.

Over the past 20 years, synuclein has been implicated repeatedly in the pathogenesis of hereditary and sporadic Parkinson disease.

- Additional missense mutations (p.E46K, p.H50Q, p.G51D and p.A53E) and more families with duplications in alpha-synuclein were found to be rare causes of hereditary PD or PD-like syndromes [26–28].
- Genome-wide association studies have repeatedly demonstrated a significant association between non-coding variants in and around the alpha-synuclein gene and sporadic disease [29]. Very recently, exciting work using genetically engineered iPS cell-derived neurons showed that a polymorphic site within an intron of alpha-synuclein is a transcriptional enhancer binding site in which the allele associated with

increased risk for PD causes upregulation of alpha-synuclein expression [30].

- Immunohistochemistry of alpha-synuclein in sporadic PD was used by Heiko Braak and colleagues to show that Lewy bodies containing alpha-synuclein were common in the CNS in regions besides those typically associated with PD, such as the dorsal motor nucleus of the vagus [31, 32]. Lewy bodies containing alpha-synuclein were found to be widespread outside the central nervous system in PD, particularly in regions that correlated with many of the early findings of autonomic and enteric nervous system dysfunction that predate the typical motor signs of striatal dopamine depletion and substantia nigral degeneration responsible for the classical motor signs in PD [33].
- A role for decreased turnover of alpha-synuclein has been demonstrated in the striking increased PD susceptibility in people carrying even a single mutation in the glucocerebrosidase gene, the gene in which mutations are responsible for Gaucher disease, a lysosomal storage disease [34]. Cell culture and animal studies link defects in glucocerebrosidase to enhanced alpha-synuclein accumulation as contributing to this increased risk for PD [35, 36].
- Synuclein has been implicated in other important steps in the pathogenesis of PD in animal models including enteric nervous system dysfunction [37] and cell-to-cell transmission of misaggregated protein [38, 39].

Looking back on this very exciting time in my life as a human genetics researcher, I am continually reminded of the extraordinary role that serendipity played in this discovery. What was the probability that I would be asked to give a talk at a workshop on a topic I knew nothing about, that I would have recruited to my Branch at NIH a geneticist born in Greece who happened to go to medical school at the University of Patras, which turned out to be the geographic focus for a rare form of hereditary PD that was the basis for a founder effect in a rare hereditary PD family that immigrated to the United State, settled in New Jersey, and caught the eye of a PD researcher who had a keen eye for possible genetic causes for the disease and who attended the workshop where I gave my talk.

In 1657, William Harvey wrote in a letter to the physician John Vlackveld

“Nature is nowhere accustomed more openly to display her secret mysteries than in cases where she shows traces of her workings apart from the beaten path; nor is there any better way to advance the proper practice of medicine than to give our minds to the discovery of the usual law of Nature by careful investigation of cases of rarer forms of disease” [40].

Harvey’s prescient observation has no better affirmation than in the story of how the investigation of one of the rarer forms of PD, an early onset, autosomal dominant PD found in the Contursi kindred, led to the discovery of alpha-synuclein as a key protein involved in both rare hereditary and common sporadic PD. Following that discovery, the last 20 years of synuclein research has begun to unlock the “secret mysteries” of how and why PD occurs, as well as opening up avenues for the development of new therapies.

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Michael J. Fox Foundation, the National Institute on Aging and the National Institute of Environmental Health Science, the National Parkinson Foundation, and the Institute for Human Genetics and the Department of Medicine of the University of California.

#### CONFLICT OF INTEREST

Dr. Nussbaum is an employee of and owns stock and stock options in Invitae, a commercial genetic diagnostic laboratory.

In the past two years, he has served as a consultant for Personalis, Inc. and Complete Genomics, Inc., two DNA sequencing companies.

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