MPN RESEARCH FOUNDATION

CALR MUTATION:

10 years of progress propelling MPN research forward

EVOLUTION OF PERSONALIZED MEDICINE

March 2024

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THE TERM "PERSONALIZED MEDICINE" FIRST APPEARED IN 1999, IN A SHORT ARTICLE PUBLISHED IN THE *WALL STREET JOURNAL*. REPRINTED 2 MONTHS LATER IN *THE ONCOLOGIST*,[1] THE ARTICLE DISCUSSED THE EMERGING TREND OF MOVING FROM "ONE-SIZE-FITS-ALL MEDICINES" TO THE PREMISE OF INDIVIDUALIZED MEDICINES TAILORED TO A PERSON'S SPECIFIC GENETIC MAKEUP.

But science was already on the move,

and by the time the article appeared in print, the first treatment based on a monoclonal antibody had been approved by the US Food and Drug Administration (FDA), and a diagnostic test for detecting patients who were most likely to respond to the treatment was approved.

The completion of the Human Genome Project in 2001 also had a profound impact on healthcare and allowed for the emergence of genomic medicine. Genomic information as part of diagnostic or therapeutic decision-making has become increasingly important in fields such as oncology to drive tailored treatment strategies. Continued advances in genomic medicine and technology have allowed for the identification of pathogenic genes and variants, the development of diagnostics to detect them, and therapies targeted to a specific genetic biomarker found in a given cancer.

In the past decade alone, the FDA granted approval for some 40 new targeted therapies for 12 different cancer types.[2] For patients with the blood cancers essential thrombocythemia (ET), polycythemia vera (PV), and myelofibrosis (MF), known collectively as myeloproliferative neoplasms (MPNs), 10 years ago marked an exceptional occasion that paved the way for these patients: discovery of *CALR* mutations.

Drug discovery, however, relies on resources and collaboration beyond the boundary of the laboratory. When MPN Research Foundation (MPNRF) was founded in 1999, meaningful research on MPNs was scarce. Established with a mission to stimulate original research in pursuit of new treatments — and eventually a cure — MPNRF has since played a key role in funding some of the most important work seen over the last 20 years, including foundational research that led to the identification of mutated *CALR* in MPN patients.

RESEARCH HISTORY:

Discovering mutations in *CALR*

One of MPN Research Foundation's first grants (2000) funded laboratory work that investigated genetic drivers of MPNs, a virtually unexplored area of research. This unlocked answers which led to a body of work that subsequently identified Janus 2 kinase *(JAK2)* and thrombopoietin receptor *(MPL)* mutations. But this still left questions for patients with an MPN whose genetics showed neither *JAK* nor *MPL* mutations. So, with two MPNRF-funded grants (2009 and 2011), a team led by Robert Kralovics, PhD, set out to find the missing pieces, looking for other mutations that might be at play. It was this work that directly led to his discovery of the *CALR* mutations a few years later.

These grants are representative of the spirit of MPNRF, with early funding contributing to long-term, practice-changing moments in hematologic cancers, such as the critical role CALR can play.

In 2013, Dr. Kralovics' discovery was one of two seminal papers published back-to-back in the same issue of the *New England Journal of Medicine*,[3,4] both describing mutations that had been identified in *CALR* in a subgroup of patients with ET or MF. These mutations appeared to be mutually exclusive of the *JAK2* and *MPL* mutations, which are found in the majority of ET and MF patients. The *CALR* gene encodes a protein called calreticulin, which is believed to play a role in protein folding, calcium regulation, gene activity, cell proliferation, migration, adhesion, and apoptosis.

Using different strategies, the two teams of researchers demonstrated that in its mutated state, the calreticulin protein is a major driver of MPNs in patients without *JAK2* and *MPL* mutations.

> I remember the squawk of excitement writing that email at 2 a.m. explaining everything that had happened that day and what I had found, recalls Dr. Jyoti Nangalia.

> And very soon, the word on the street in the Cambridge scientific community was that they had found this exciting gene in this blood cancer.

In the Kralovics study, exome sequencing was performed on samples from 6 patients, and acquired insertion or deletion mutations in *CALR* were identified in all of them. Targeted sequencing was then conducted on a large cohort of patients with both MPNs and other myeloid cancers.

Concurrently coming from a different angle, a team led by Jyoti Nangalia, MBBS, PhD, who is now a member of MPNRF's Scientific Advisory Board, performed exome sequencing on samples obtained from a large cohort of 151 patients with MPNs. This provided an unprecedented view of the mutational landscape in MPNs, allowing for *CALR* mutations to be identified in patients with ET or primary MF without *JAK2* or *MPL* mutations.

These two new studies filled a large gap in the existing knowledge of the genetic origins of MPNs, by identifying the most common mutation in patients without *JAK2* or *MPL* mutations. The papers, however, were the culmination of years of investigating the genetic complexity of these disorders.

LONG RESEARCH LIFECYCLE

Jyoti Nangalia, MBBS, PhD

Dr. Nangalia is currently a group leader at the Wellcome Sanger Institute and the Cambridge Stem Cell Institute in Cambridge, UK; a consultant hematologist who cares for patients with chronic myeloid malignancies at Cambridge University Hospitals NHS Foundation Trust; and a member of the Scientific Advisory Board of the MPN Research Foundation.

After working as a medical doctor for some 8 years, Dr. Nangalia was in the middle of her PhD at the University of Cambridge, supervised by Professor Tony Green, when she was delving deep into whole exome sequencing data from patients with MPNs. For her, a major question in the field was, "What is causing disease in patients with MPN who do not have the *JAK2* or *MPL* mutations?"

"When I entered the field, we only had a genetic cause for 50% of our MPN patients," she said. "And for the other 50% of patients, it was really a struggle to diagnose them because you just didn't know if there was some other cause for their abnormal blood counts or if it was driven by some sort of underlying bone marrow disorder or cancer."

Dr. Nangalia recalls sitting in the library in early July 2013 and going through a seemingly endless array of genes that appeared to have mutations in 1-2 patients out of their large MPN patient cohort of 150 in which they had undertaken DNA sequencing. "It's hard to tell whether some of these rarer mutations are genuine or artifacts of sequencing, and I was sifting through these manually, investigating each one." She was looking through the data, one mutation and one gene at time, and asking herself the question, "Are these real?"

She came across mutations in many genes that looked like artifacts of sequencing. "And if they're not artifacts, then you won't find them in other samples," she noted. Many of the mutations were present in a number of samples — they were artifacts of sequencing and they got 'chucked out.' And then on to the next mutations to tidy up the dataset.

As she worked, she noticed 2 mutations in the *CALR* gene in the dataset. They were in 2 different patients and seemed unusual and convincing when looking directly at the DNA sequences. Jokingly, Dr. Nangalia explained she wasn't initially sure how to pronounce the gene — it reminded her of Kal-L from Superman. She screened all other samples at the sequencing facility for such mutations. Much to her surprise, she found similar mutations in several dozen samples.

She suddenly realized as the list of those samples were running down her laptop screen, "But these are all MPN samples too." A *CALR* mutation was present in many of her samples, but in most of them, the mutation had not been reported in the final mutation files. They had been filtered out by the mutation calling algorithms, for a variety of technical reasons to do with the unusual nature of the mutation.

After spending the rest of the day and evening exploring any reason why this may not be a real finding, and curating the final set of patients that had *CALR* mutations, she realized that *CALR* mutations were present in pretty much all patients that did not have the *JAK2* or *MPL* mutation. This was the answer!

That night at 2 a.m., she emailed her supervisor, the esteemed molecular biologist and MPN specialist Dr. Tony Green, and colleague Dr. Peter Campbell. Green is a hematologist who has been involved in seminal MPN research for several decades, and Campbell, a close collaborator, was a senior group leader at Sanger at that time. "I remember the squawk of excitement writing that email to them explaining everything that had happened that day and what I had found," she said. And very soon, the word on the street in the Cambridge scientific community was that we'd found this exciting gene in this blood cancer, she recalls, as they were putting together the study to report the finding.

"There were so many memorable moments — from that special day of discovery, to calling one of our patients in July 2013 to kindly donate another blood sample so we could confirm the mutation. I remember thinking she was likely the first person in the world with a confirmed *CALR* mutation and a cause for her disease. Then to implementing the test in clinic at the hospital within a few months, and testing all our historical patient samples to confirm their diagnosis.

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"I am grateful to all those scientists around the world that have taken this discovery and then worked tirelessly to understand how the mutation causes MPN, and then taken that knowledge towards the development of cutting-edge novel therapies for the benefit of patients."

Robert Kralovics, PhD

Dr. Kralovics was still a graduate student when he entered the world of MPN genetics in 1995, after accepting an offer to join hematologist/researcher Josef Prchal, MD, in Birmingham, Alabama for a 1-year program. Other than a brief trip back home to the Czech Republic to complete his PhD, Dr. Kralovics stayed in the US and worked with Dr. Prchal for about 7 years.

During this time, he came upon one of the first major genetic findings explaining the pathogenesis of MPN — the loss of heterozygosity somewhere on chromosome 9. After years of investigation to rule out a possible deletion, the researchers concluded that myeloproliferation was caused by a mutational defect in the chromosome. This critical discovery was made in part through MPNRF and was the Foundation's very first research grant. He and Dr. Prchal published their findings in *Experimental Hematology* in March 2002.[5]

Relocating to Switzerland, Dr. Kralovics now had access to a larger number of patients through Swiss and Italian data banks. The number of patients in the cohort was expanded to nearly 300, which enabled them to narrow down the specific deficit on chromosome 9 to 15 genes, including the *JAK2 kinase V617F*. These findings were published in 2005, alongside reports from other groups internationally, and it soon became apparent that virtually all PV patients and half of ET and MF patients were positive for the *JAK2* mutation.[6]

"But there was still a major problem — that we didn't have the genetic basis for a third of myelofibrosis and ET patients, and that was one of the projects that we were trying to solve," said Dr. Kralovics, who is now a principal investigator at the Medical University of Vienna, Austria, and co-founder, MyeloPRO Diagnostics and Research GmbH.

Next generation sequencing was just getting started at about this time, and Dr. Kralovics relocated once again, this time to Vienna, where he formed his own group and obtained one of the first high throughput sequencers in Europe. He also applied for funding from MPN Research Foundation as well as the Austrian Academy of Science, and for the next 6 years worked on identifying the underlying pathology of MPN in patients lacking a *JAK2* mutation.

"And then one afternoon my PhD student at that time, Thorsten Klampfl — this was actually his project — calls me and says, 'well I found something that's strange, in this strange gene,' he said. 'CALR is a chaperone protein, it's almost like a housekeeping gene, so how can this actually be a hematopoietic oncogene?'"

"But we eventually knew that we now had a hematopoietic oncogene, and it was probably going to be a cancer-specific antigen," Dr. Kralovics added. "And we were right in the end."

CLINICAL IMPLICATIONS OF THE MUTATED *CALR*

Since the initial discovery of the *CALR* mutation and its relationship to MPNs, research has continued and is ongoing, and detection of *CALR* mutations has been embedded in the World Health Organization and other international diagnostic guidelines. The two most frequent *CALR* mutations, a 52-base pair (bp) deletion and a 5 bp insertion, account for 85% of the mutations and have been termed types I and II.[7]

Distinct clinical and laboratory associations of *CALR* mutations have been identified together with their prognostic significance. As compared to those with *JAK2* mutations, *CALR* mutant patients have a better prognosis and overall increased survival.

CALR also represents a new therapeutic target and raises the possibility of new therapeutic approaches.[8] Rare disease research, however, takes time. Simultaneous presentation of the data at the American Association of Hematology Annual Meeting (ASH) and publication in the *New England Journal of Medicine* heralded the breakthrough in December 2013; 10 years later clinical trials are now beginning.

These breakthroughs have led to two companies investigating CALR-targeted treatments that are currently in clinical trials, with potential in the coming years to be available if approved. At present, patients with this mutation receive standard cytoreductive therapies, such as hydroxyurea, interferon-α and ruxolitinib, which have shown similar improvement in cell counts and symptoms, regardless of mutation status. But the discovery of this mutation has had a major impact, according to Ann Mullally, MD, a physician-scientist at Brigham and Women's Hospital/Dana-Farber Cancer Institute and Associate Professor of Medicine, Harvard Medical School.

"First of all, I think it's very important in definitively making a diagnosis of MPN," she said. "Prior to the discovery of this mutation, we would see patients who looked like they had MPN and then do a biopsy. The biopsy results looked like MPN, but we really didn't have a mutation that was causative for the disease and so there was always some question as to whether they truly had MPN or not."

But that paradigm has now changed with the discovery of the *CALR* mutation, which is the second most common mutation after *JAK2*. "Its presence is very definitive for making a diagnosis, since the mutation is not found in other diseases," said Dr. Mullally. "So, I think that [discovery] was very, very, very important."

Knowing the type of mutation also helps in determining prognosis. "We know that there are differences as to whether you have MPN with this mutation versus MPN with the *JAK2* mutation, for all different types of complications and prognosis," she said. "In and of itself, just being able to identify whether somebody has the mutation can already be very helpful in terms of counseling patients about what you know their risks are."

The discovery of the mutation, and understanding how it causes the disease, has laid the Foundation for the development of new treatments. “We are already within 10 years of discovery of the mutation, and we are moving into clinical trials directly targeting the mutation,” she said.

Activity generated around this mutation during the past 10 years includes a phase 1 trial conducted in Denmark that evaluated a therapeutic cancer vaccination developed with peptide vaccines derived from mutant *CALR*.[9] The results showed that it was safe and tolerable.

Dr. Mullally noted that a clinical trial of a mutant-specific *CALR* antibody, designed to target this mutation was presented at the 2022 Annual American Society of Hematology meeting. It is expected to begin in the United States in early 2024.

“The 10-year anniversary of the discovery of the *CALR* mutation is really a time for everybody to kind of take stock,” said Dr. Mullally. “The MPN Research Foundation has been really instrumental in this and the amount of research that’s been going on all around the world is really impressive, to understand how this mutation causes disease.”

She added that much of the work is highly collaborative. “This involves not only what happens in the laboratory but what happens in the clinic, clinical research, and patient observations.”

PATIENT IMPACT

The discovery of the *CALR* mutation has promising future implications for patients. At this time, it has been proven to be instrumental in making a definitive diagnosis of the disease.

For one patient, the discovery of the *CALR* mutation helped to explain her symptoms and provide answers. Ed Bartholemy explained that his wife, Nancy, tested negative for the *JAK2* mutation in 2005, but skip forward to 2013 and she was positive for the *CALR* type 1 mutation. “That explained it somewhat, and it started to all make sense, now that there was a driver mutation responsible for Nancy’s condition,” he said.

Nancy was diagnosed with myelofibrosis in 1981 when she was 23 years old, and initially misdiagnosed with chronic leukemia and treated for that. When she finally received the correct diagnosis, finding information on the disease proved to be a formidable task.

“We went to a medical library at the University of California, Irvine, and we found one page on MPNs in the whole medical library,” said Bartholemy. “We then went to Mayo Clinic and learned what we could, but there was no original research and no information when new medications would come available.”

Essentially, explained Bartholemy, there were few experts in the field and research was scant, but MPN Research Foundation helped change the landscape. “What they did was draw in talented young researchers and fund them in this field,” he said. “Besides the actual scientific discoveries, they’ve had a huge impact in building the roster of researchers who are out there today.”

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>
> — ED BARTHOLEMY

Fast forward from 1981 to 2023, and there has been a vast improvement in diagnostics as well as recognition of MPNs. “They’re still not on the front page of every medical book, but they are an established disorder with research funding and there are therapeutics,” said Bartholemy.

“The *JAK* mutation was discovered in 2005, which was a huge step forward in treatment and diagnostics. And so here we are with a new mutation — well, not that new, it’s 10-years-old since it was identified — but possibly new therapeutics coming down the road.”

When the *CALR* mutation was discovered, Nancy already had been living with an MPN for 32 years. “Learning about *CALR* helped explain things, because people with *CALR type 1* live longer and she was very young when she was first diagnosed,” he said. “It started to make a little sense that she had lived so long and that was really what brought us to the MPN Research Foundation — now we knew the driver mutation for Nancy’s disease, and we wanted to be involved in pushing for follow-up research that might lead to treatments or a cure.”

Over the course of her illness, Nancy had been treated with most of the therapies that exist for MPNs, even a clinical trial for thalidomide, when that was considered very promising. But none of the treatments were disease modifying. “She had about 15 years of relatively good health and a normal life working as a busy traveling executive,” he explained. “And then for the last 20 years or so, she was pretty disabled from fatigue and bone pain.”

However, Nancy will not be participating in any more clinical trials evaluating potential therapeutics. “She had a stem cell transplant 2 years ago and so we’ve been in that world for the last 2 years,” said Bartholemy. “She relapsed at 1 year and had to undergo more treatment but is currently cancer free and doing very well.”

He added that he hopes that new therapeutics will be able to help future patients. “There’s someone else out there who’s 23 years old, who’s being diagnosed right now, and we want that person to have a lot better life than Nancy was able to have,” he said.

ROLE OF MPN RESEARCH FOUNDATION

Convening the MPN community to advance research

The mission of MPN Research Foundation is straightforward: to fund and actively support original research in pursuit of new treatments — and eventually cures — for the blood cancers essential thrombocythemia (ET), polycythemia vera (PV), and myelofibrosis (MF), known collectively as myeloproliferative neoplasms (MPNs). Founded in late 1999, MPNRF was the first organization in the MPN space and remains the primary organization focused on advancing MPN research.

From its unique position at the intersection of the key stakeholders — patients and caregivers, researchers and clinicians, and the biopharmaceutical industry — MPNRF facilitates a collaborative approach to research that elevates the patient voice in research and drug development, brings disease experts together, and accelerates the industry's understanding of an extremely complex and heterogeneous group of cancers.

The Foundation was started by Chicago businessman Robert Rosen, who was diagnosed with PV in 1997 and was shocked to discover how little research was being conducted on this group of disorders. In addition, there were no advocacy groups working to assist people with these rare blood cancers. Two years later, Rosen established MPN Research Foundation — then known as the MPD Foundation — with a group of other patients, as a means of promoting research for advanced treatments. To date, MPN Research Foundation has awarded more than $18.4 million for MPN cancer research, focusing on projects that accelerate understanding of MPNs and lead to the development of new treatment options.

"MPN Research Foundation is proud to have contributed to Dr. Kralovics work that in part led to the *CALR* discovery in 2013. And we have long-standing support of Dr. Nangalia, Dr. Mullaly and their colleagues that furthered our body of knowledge and the conversation. We are now right at the cusp of that 8-10 year lifecycle it takes from when a target is discovered to a potential therapy going into phase 1 human trials this year," according to Kapila Viges, CEO of MPN Research Foundation.

> MPN Research Foundation made a very clear strategic decision decades ago to focus on the solution by accelerating the research and to pursue the cures, because ultimately that's what patients need.
>
> — KAPILA VIGES, CEO

The goal of developing targeted therapies for MPNs requires not only research but significant funding. "The average medicine takes 10 years and sometimes more than $1 billion to develop. Much of the early-stage discovery happens in academic or research institutions before it attracts the investment of pharmaceutical companies to advance it through development, clinical trials, and regulatory approvals," she explains. "Some of these very early stages of lab work and discovery has to be funded from another sector — from government funding or the spaces in between, like philanthropy, charitable organizations, and research Foundations like ours. We are willing to take the early risk, aiming for a promising outcome to accelerate those discoveries."

Thus, organizations like MPN Research Foundation play an important role in helping to fill early funding gaps, especially for rare diseases, like ET, PV, and MF, with small populations of patients and clinicians who are looking for answers. Although they are serious cancers, MPNs are perceived to be chronic cancers, so they don't always appear to have the urgency and surface-level awareness within the healthcare ecosystem that something needs to be done now. "We try to create that awareness and urgency," says Viges.

When MPN Research Foundation was founded more than 2 decades ago, it was the only organization devoted to these disorders, before they were even classified as cancers. Now there are others in this space, but MPN Research Foundation stands apart because of its focus on research.

"MPN Research Foundation made a very clear strategic decision decades ago to focus on the solution by accelerating the research and to pursue the cures, because ultimately that's what patients need," Viges says. "We are eager to advance research and development of treatments that reduce the burden and worry of patients, while improving quality of life until we achieve sustainable cures for this family of blood cancers."

However, the landscape has changed dramatically over time.

And so must the way in which the Foundation develops a research strategy.

"We usually think of drug discovery and development as a relay race where the baton gets handed off from function to function — from scientists and clinicians to industry leaders developing and manufacturing drugs, to regulatory approval and then finally to patients," notes Viges. "Patient needs and perspectives, however, are often only considered toward the last leg of the race. As a result, we may have more options and new treatments to manage symptoms, but that benefit isn't as meaningful or impactful to patients as it could be. We still aren't modifying, stopping, or slowing MPNs yet."

MPN Research Foundation recognizes it takes a team-based approach and each stakeholder has a role to play. With the goal of bringing all the players together in the earliest stages of discovery and development, to develop a game plan and set of plays to communicate, coordinate, and financially support the science, the Foundation will stay focused on the endgame: cures.

SINCE 2000, MPN RESEARCH FOUNDATION HAS AWARDED MORE THAN $18 MILLION FOR BLOOD CANCER RESEARCH PROJECTS

A significant number of these projects contributed to and advanced major scientific findings, such as the discovery of the *JAK2 V617F* mutation in 2005. The Foundation was convinced it would change everything — and it has.

One of the projects resulted in identifying the *CALR* mutation, which accounts for one-third of MPN cases not associated with *JAK2*.

MPNRF continues to fund work in the area of targeted therapies including *CALR*-driven MPNs, in addition to preclinical research toward the inhibition of *JAK* and *MPL* driver mutations. Today, some of the investigators who received the Foundation's earliest awards are now leaders in the field.

Fundraising is critical to MPNRF's mission, and the Foundation will continue to seek financial support from industry and individuals alike to fund MPN research.

"As a small but mighty Foundation, we've always had a 'high-risk, high-reward' approach to funding research. Our founder instilled that in our mission, and the MPNRF Board of Directors continues to take that same approach, with our eyes set on the long game," says Viges. "Filling funding gaps that others don't, in hopes that it will lead to something promising, may just prove to be worth the wait, as we watch the investigational CALR-mutation targeted treatments unfold."

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