From Genetic Disease to Funded Therapy:

An Analysis of Familial Dysautonomia and Mucolipidosis Type IV

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Introduction

The Jewish Ashkenazic community is affected by many genetic diseases, some of which are devastating to the individual and to the well-functioning of the family. Regrettably, there are many recessive genetic disorders for which Ashkenazic Jews are carriers, thereby creating the possibility of transmitting the disease to their offspring. It is estimated that about 1 in 4 Ashkenazic Jews is a carrier for at least one genetic disorder. Diseases like Tay Sachs and Gaucher disease are recognized as posing significant risk due to their high frequency in the Jewish Ashkenazic community, with the heterozygote frequency of 1:29 for Tay Sachs disease (TSD) and 1:16 for Gaucher disease (GD). Thankfully, today it is much less common to find these disorders in Jewish communities due to genetic testing and progressive biomedical research which enabled the prevention of these diseases from manifesting themselves. Unfortunately, there are yet many other genetic diseases which affect families, sometimes undermining the family structure. Two such diseases, both of which are devastating and life-threatening, are Familial Dysautonomia (FD) and Mucolipidosis Type IV (ML4), with a heterozygote frequency of 1:29 for FD and of 1:67 for ML4. As with TSD, both FD and ML4 are lysosomal storage diseases which interrupt the metabolic processes in neurons of affected individuals. Although these latter two diseases are not as commonly known as TSD and GD, they are extremely significant to those born with these health issues, presenting horrific realities to affected families and loved ones. This paper evaluates FD and ML4 regarding those biochemical pathways adversely affected by the disease-causing mutations. Additionally, current research and treatment options will be discussed to better understand the possibilities of therapy for those afflicted with the diseases. Lastly, to present a more total overview of the health issues, attention will turn to

funding the research needed to develop the appropriate therapies. As grants are the backbone of research, this paper will examine two ways in which researchers secure funding. With proper support, researchers can better grasp the nature of FD and ML4, which in turn will lead to better treatments and therapies for those afflicted by these disorders.

Genetic Disorders

Familial Dysautonomia

Familial Dysautonomia (FD), the first of the genetic diseases to be discussed, is a rare genetic disorder which is transmitted as a recessive mutation. In similar fashion to other recessive genetic diseases, a person must carry both recessive mutations to exhibit symptoms of FD. In 1949, a scientific article was published[1] which compared the similarity of five symptomatic children with overlapping features indicative of a unifying underlying condition. The symptoms present in the subjects included excessive sweating, blotchy skin, and reduced production of tears. The researchers surmised that these symptoms were all indicative of the same disease, which would later become known as Familial Dysautonomia. Since the publication of this article, much more research has been directed to this disease and it is now evident that these are all clear indications of FD.

In 2001, an article published by lead researchers at Fordham University[2] in conjunction with Massachusetts General Hospital successfully identified the point mutation and the specific gene responsible for the disease. *DYS*, the gene, also referred to as the *IKAP* gene and more accurately as the *ELP-1* gene, was discovered to be connected to the disease, and it is located on the long arm of chromosome 9. For many years it was understood that this was a neuropathic disease which caused interruptions in the sensory and sympathetic pathways of affected individuals. Upon identification of the exact gene, researchers studied the specific errors which resulted from the mutation. This research identified that the mutated *ELP-1* gene lacked exon 20. This caused a frameshift mutation which resulted in the IKAP protein being excised from the sequence. Upon further analysis, the authors discovered that IKAP mRNA was found in the cerebellum, thalamus, pituitary glands and the testes. The underdevelopment of the sensory pathways may be attributable to the concentration of IKAP mRNA found in the brain regions. Additionally, perhaps this can explain the extreme temperature swings and dulled sense of pain in individuals affected by FD.

Additional symptoms expressed by individuals with FD included difficulty in chewing and swallowing, respiratory and cardiovascular problems, and extremely elevated blood pressure. Individuals also exhibited extra sensitivity to adrenergic and cholinergic agents which was related to impaired nerve function[3]. The underproduction of tears and elongated pupil cycle time was also a result of the impaired nervous system[4]. Due to the degenerative nature of the disease, pain sensation decreased as patients aged. It was reported[5] that the dorsal root

ganglion in individuals with FD was found to be abnormally maintained, and that neuronal levels were reduced. Dorsal root myelinated axons were also found to be lacking. The findings of this research were consistent with decreased sensation and nerve responses evident in patients with FD. In an additional study published by the same authors[6] similar results were produced. The researchers reported that FD patients had significantly less superior cervical sympathetic ganglia than controls. This report provided insight into the diminished autonomic functions of individuals with FD. The five diagnostic features of an individual with FD, according to Axelrod *et al*.[7], are the inability to produce an axon flare following a histidine injection, the lack of fungiform papillae on the tongue, constriction of the pupil following the addition of methacholine chloride to the eye, lack of responses by deep tendons, and reduced production of tears.

Mucolipidosis type IV

The second disease to be discussed is Mucolipidosis Type IV (ML4), an autosomal recessive genetic disorder which most significantly affects Ashkenazic Jews. Individuals affected by ML4 are typically diagnosed with the devastating disease within the first few months of life. Identifying external symptoms may include cross-eyes as a result of clouding of the cornea and the inability to meet expected milestones. The degenerative nature of the disease results in worsening of the eyesight of affected individuals, as well as other intellectual and motor delays which will be discussed.

The gene mutation for ML4 is found on the short arm of chromosome 19. The *MCOLN-1* gene was identified as the mutated gene connected to the disease. The *MCOLN-1* gene is a protein encoding gene that initiates the production of a transmembrane protein which is a part of the transient receptor potential cation channel gene family[8]. The channel protein is responsible for regulating lysosomal exocytosis, as well as other endocytic pathways. The mutated form of *MCOLN-1* interferes with the assembly of the transport channel receptor proteins which results in the failure of the lysosomes to properly exocytose waste material from the cell. The defective endocytic activity of the mutated channel protein results in food that is not properly ingested into the cell, and therefore causes a buildup of waste material around the cells[9]. This is characteristic of a lysosomal storage disorder, a category of diseases to which ML4 belongs. TRPML1, the protein encoded by *MCOLN-1*, is translocated outside of the nucleus in its mutated form. This impairs the exocytic properties of the lysosomes. The buildup of excess material in the cells leads to a significant decrease in function of the body's systems.[10] Furthermore, a study which analyzed intracellular material in the autopsy of an ML4 patient, suggests that ML4 is not a disorder due to the mutation of one enzyme, but rather it is a disease which affects the packaging and transport of material within the cell.[11]

Another affected pathway in individuals with ML4 interrupts acid production in the stomach. Gastrin levels are typically unusually high, and stomach acid production is significantly lower than normal. This may be caused by the lack of secretion of hydrochloric acid by parietal

cells[12]. Hypergastrinemia was also suggested due to the hyperplasticity of the enterochromaffin-like cells lining the digestive tract. Elevated gastrin levels are common to individuals with typical ML4, as well as those with atypical ML4. The differences in severity of expression of the disease is most noticeable in regard to the patient's motor skills. Individuals with typical ML4 exhibit severely defected motor skills and abilities, though some individuals may be taught to take a few steps with significant assistance. Most, however, remain in wheelchairs for their entire lives. Individuals with atypical ML4 may learn to walk on their own and may be able to speak with limited vocabulary. For both forms of ML4, weak muscles and recurring muscle spasms present challenges regarding eating, swallowing, and controlling limb movements. Individuals with typical and atypical ML4 have significant mental disabilities and their cognitive abilities do not exceed those of a young child. As mentioned earlier, eyesight degenerates with age, rendering many individuals blind in their early teenage years. Individuals with atypical ML4 exhibit slightly milder cognitive deficits and undergo less eyesight degeneration than those with typical ML4.

Genetic Screening

The severity of genetic diseases requires precautionary measures to be put in place to prevent the birth of a child with a genetic disease. Parents must each carry the same genetic mutation in order to pass it on to their child. Therefore, with the development of biotechnology and furthered scientific understanding, genetic testing has become a popular method for couples to ascertain whether they are carriers for the same genetic disorder. For every conception of a couple both carrying a mutated allele, there is a 1 in 4 or 25% chance of transmitting the disorder

to offspring. Genetic testing has become a more widespread and accepted practice for couples prior to having children. Blood sampling and saliva collection are the two most common modes of collecting specimens for genetic analyses. While genetic testing may be offered in a doctor's office at a pregnancy check-up, it is important that couples be tested before starting a family. Organizations, such as JScreen, offer a comprehensive panel of genetic diseases for screening individuals. JScreen's testing is through a saliva sample collection, and the DNA is then analyzed through Next Generation Sequencing.

Next Generation Sequencing is an inclusive term used to refer to various methodologies through which DNA is sequenced, *i.e.,* the order of DNA nucleotides is analyzed. Sanger Sequencing, the initial sequencing method prior to the development of the Next Generation Sequencing technology, utilizes DNA polymerase and produces a complementary DNA strand to the original DNA being studied. Each added nucleotide is tagged with a unique fluorescent dye which enables their identification and localization. The mixture is then analyzed through gel- or capillary-based automated electrophoresis. Next Generation Sequencing is now more widely used due to its ability to sequence thousands of DNA fragments in one run, while Sanger Sequencing is limited to sequencing one DNA fragment at a time. The improved mechanisms utilized in Next Generation Sequencing make it a reliable and cost-efficient system to identify DNA mutations. Identifying the problematic gene paved the pathway for researchers to develop therapies aimed at addressing the specific mutation in the DNA.

Treatments and Therapies

When a child is born with a genetic disease, there are various routes which parents might opt toward regarding how to best provide and care for their child. Unfortunately, there are not many known treatments for genetic diseases such as FD and ML4. Therefore, parents will often turn toward therapies and treatments, in the hopes that one day a cure will be developed. There may be various goals and expectations for the treatments. In the case of FD, parents may attempt to improve the child's sensory motor skills, autonomic functions, and other neurological side effects of the disease. Medications such as benzodiazepines to reduce crisis symptoms and corticosteroids to address metabolic deficiencies and hypotension were suggested for individuals with FD. However, when the gene responsible for the mutation was discovered, treatment options for FD individuals became much more directed and effective.

As it will later be discussed, therapy available for FD focuses on treating the underlying cause of the disease, rather than allaying the external symptoms. Similarly, current research underway for ML4 is focused on the genetic mutation. While the current research and hopeful therapies for each disease are technically classified as genetic therapy, the actualization of the therapies differ. Therapy for FD, which is readily available, corrects the mutated gene by increasing the protein production from the gene, while the gene therapy for ML4 that is currently in progress is reflective of a therapy that is more classically labeled under the category of “gene therapy.” This therapy necessitates a much more exhaustive process for the therapy to be approved and eventually to become available to patients.

This paper will look at two diseases, FD and ML4, the discovery of the genes involved in the diseases and the forms of therapy on which researchers are currently working. Additionally, it

is imperative to note that research is impossible without proper funding. Therefore, lastly, this paper will examine the ways in which researchers receive the finances with which to conduct their research for their life altering work.

Familial Dysautonomia

Gene Discovery

Although researchers in Harvard University spent many years attempting to identify the causative mutation in individuals with FD, Dr. Berish Rubin at Fordham Laboratory, would be noted as the one to discover the FD gene. In the spring of 1999, the founder of Dor Yesharim, Rabbi Ekstein, contacted Dr. Rubin and requested that he identify the FD mutation. Dr. Rubin, along with Dr. Andersen and their team of eight researchers, began their work in the spring of 2000. Within six weeks of their work, they successfully identified the gene. Originally termed the *IKAP* gene, Dr. Rubin noted that the mutation caused a splicing error in mRNA. The team's findings were soon published[13]. The discovery of the gene enabled genetic testing to screen individuals and determine their carrier status of the *IKAP* mutation, and thus ascertain whether a couple had the potential to produce a child with FD (**figure 1**). With the first step to better understanding the nature of FD complete, Dr. Rubin was urged to follow his discovery with the development of therapies for FD patients.

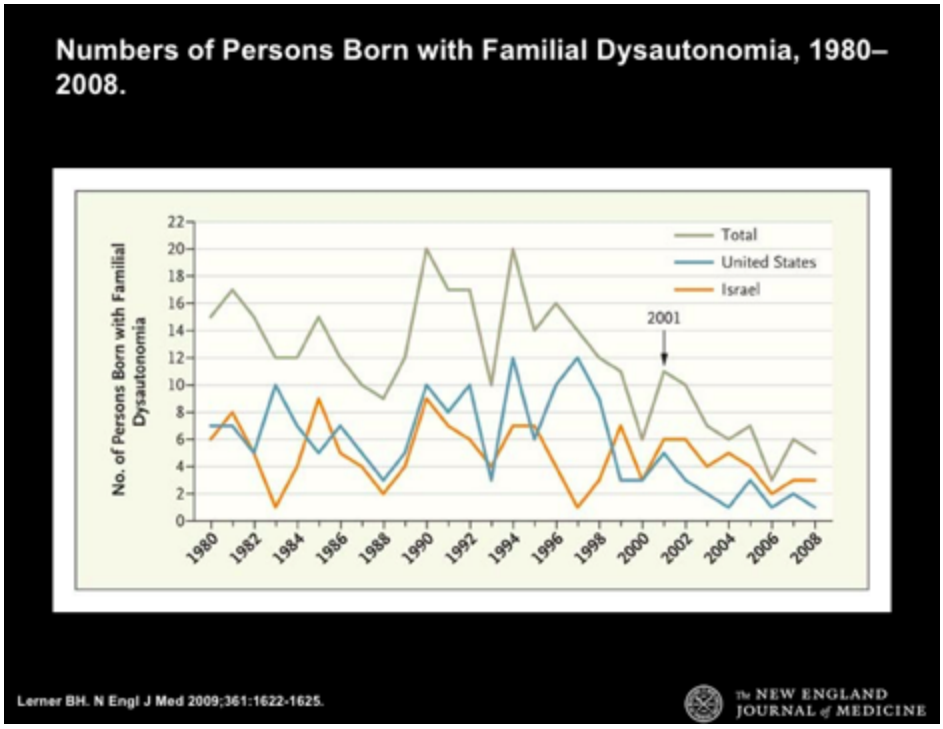


Figure 1 The graph represents the decrease of individuals born with Familial Dysautonomia following the discovery of the mutated gene in 2000. The immediate incorporation of the disease in genetic testing panels made it possible to identify carrier couples, and thereby prevent a child being born with FD.[14]

The discovery of the FD mutation was also crucial because it allowed researchers to analyze the nature of the mutation. Individuals with the most common form of FD, often found in Ashkenazic Jews, produced a limited amount of the encoded protein. Termed a "leaky" gene, the mis-splicing of the gene caused a significant reduction in the synthesis of functional protein. Initially, the mutated gene was termed the *IKAP* gene as it was involved in the synthesis of IKAP protein. Later, as it was discovered that the gene mutation was found within the elongator protein complex, the gene was renamed, *ELP-1*. With this knowledge, Dr. Rubin aimed to provide patients with a therapy that would increase the rate of properly synthesized ELP-1. By increasing

the protein, Dr. Rubin reckoned that FD patients would be able to function in a more typical manner and exhibit less symptoms of FD.

Therapy

While many researchers aim to develop drugs to allay symptoms of the disease being studied, Dr. Rubin was hesitant to follow in this path because receiving FDA approval for new drugs can be an exhausting, time-consuming process. Slowing the degeneration of the FD patients was crucial, and timing was everything. Therefore, Dr. Rubin chose to search within the natural realm for remedies. Drs. Rubin and Andersen founded The FD Research Laboratory at Fordham University, New York, and initiated their mission to discover non-toxic, readily available products which would treat FD patients and enhance the production of ELP-1 protein. In personal discussion with Dr. Rubin, he explained that he began by visiting local grocery stores and health food stores searching for foods which he posited may contain ingredients which would potentiate the production of ELP-1 protein, and thereby increase its level in FD patients. Dr. Rubin's first successful discovery was found in a bottle of rice milk from the brand Rice Dream. When Dr. Rubin and his team treated FD cells grown *in vitro* from patients with drops of the rice milk, the result was an increased production of ELP-1. It was postulated that vitamin E, or tocotrienols, in rice milk contained the necessary elements which created the desired outcome in the cells. After testing this hypothesis repeatedly with the same positive effects, the team at Fordham University quickly published a paper on their findings[15]. Immediately, parents began to incorporate tocotrienols into their FD children's meals. The effects of the vitamin E therapy were instant. Dr. Rubin poignantly remarked with a sigh, "had we come along earlier, we

would've had 10 year olds now that would be marathon runners."[16] In other words, he pointed out that had these ten year olds been taking tocotrienols as infants, they would not have been noticeably affected by FD. He further suggested that there are children with FD who are asymptomatic because they were on this diet therapy since birth. While the typical life expectancy for individuals with FD was not beyond the age of 5, today, Dr. Rubin related that he knows individuals with FD who are living into their thirties[17].

Another drug, discovered by The FD Research Laboratory, that was effective in correcting the mis-splicing of the ELP-1 protein, was the plant-derived molecule, epigallocatechin gallate (EGCG), common in green tea. When added to the FD cells grown *in vitro* in the laboratory, similar results were noted to those produced by amendments with tocotrienol. The laboratory not only focused on identifying effective compounds, but it worked to ascertain which companies produced the highest quality product in order to direct parents to secure the most effective and trustworthy brands for their FD children.

While Dr. Rubin and his research team were analyzing the effectiveness of tocotrienols and EGCG, the Harvard Laboratory was invested in developing a pharmaceutical to reduce symptoms in FD patients. Dr. Sue Slaugenhaupt, a lead researcher in the Harvard laboratory, led her team to create an FD mouse model with the mis-splicing to mimic the defective gene of an individual with FD. In 2016, the laboratory announced the completion of the implantation of the mis-spliced exon in the mouse, with the mouse producing symptoms like those present in FD patients[18]. The symptoms included fungiform papillae on the tongue, kyphosis, and reduced

growth. The intent was to utilize this mouse model to create therapies and drugs to benefit FD patients. The laboratory developed the drug termed kinetin, which targeted and modified the error in splicing. Kinetin increased ELP-1 mRNA which in turn increased the production of the functional protein. The drug, first tested in a small group of FD patients in 2009, increased production of the functional protein[19].

However, the drug caused some adverse side effects, such as nausea. This prompted the researchers to continue investing in the drug to attempt to eliminate side effects. In a subsequent study on mice conducted in 2016 the researchers reported that kintetin increased protein production, improved kyphosis, and increased the volume of dorsal root ganglia and the number of proprioceptive neurons[20]. While this study suggested that kinetin fixed splicing in mice, similar findings were not seen in FD patients. Due to the drug's limited potency in humans, large amounts of the drug were necessary to administer to FD patients in order to achieve the required result. The laboratory therefore developed a modified version of the drug, termed superkinetin. The laboratory formed a partnership with PTC Therapeutics in 2015. The drug has yet to be approved by the FDA, and researchers continue to work on the pharmaceutical.

Funding

Dr. Rubin was first approached by the director of Dor Yesharim, and his research on FD was funded by that organization. However, as he moved on from identifying the gene to identifying therapies for affected individuals, his research needed a sponsor. FD Now, a

non-for-profit organization run by a mother of an FD child, was founded as parents became desperate to help their children. FD Now funds Dr. Rubin's research with donations from families of individuals with FD and family friends of affected FD individuals. Dr. Rubin's goal in his therapies is to design a low cost therapeutic option for parents to give their children a chance at normal life. In discussion with Dr. Rubin, he estimated that the therapies he uncovered have an unbelievable low cost of about a few hundred dollars a year and provide individuals with FD easily accessible and highly effective treatments.

Harvard University's laboratory is funded by the FD Foundation, which also provides funding to the NYU Dysautonomia Center. The Dysautonomia Center focuses on helping individuals with various dysautonomias by running diagnostic tests and screenings to better understand the cause of the dysautonomia and to work to develop and provide treatment plans. The FD Foundation also receives funding from the National Institute of Health (NIH). In 2012, Dr. Slaugenhaupt at Harvard University received funding from the Blueprint Neurotherapeutics Network, a project of the NIH[21]. With this grant, her laboratory tested kinetin for effectiveness in the mouse FD model system. In 2015, Dr. Slaugenhaupt's team formed a partnership with PTC Therapeutics. The funding from this company, as well as from the FD Foundation, enabled the laboratory to further their research and subsequently they designed the drug, PTC258, to treat FD patients. While there is no longer a partnership with PTC Therapeutics, Dr. Slaugenhaupt's laboratory continues to receive funding from the FD Foundation to create a safe drug to treat FD patients.

Mucolipidosis Type IV

Gene Discovery

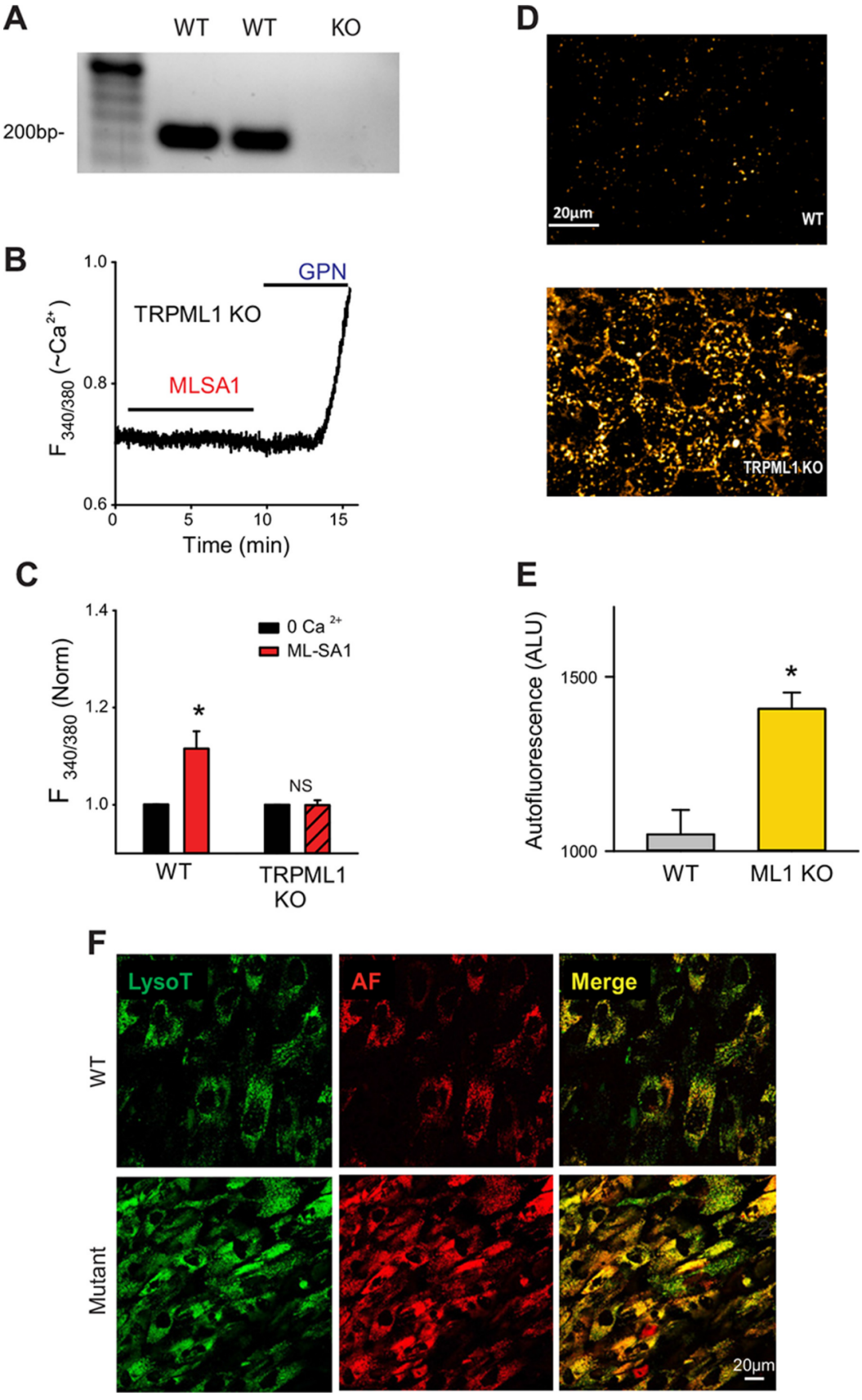
In early 2000, a team of researchers from the Hadassah Medical Organization and the Weizmann Institute of Science, Israel, discovered the gene for the devastating ML4 disease[22]. While the first case of ML4 was identified in 1974, the exact mutation was unknown for almost three decades. The discovery of the gene was crucial to provide genetic testing for the disease, as well as laying the foundation for researchers to begin developing therapies for individuals affected by the disease. Around the same time that the researchers in Israel published their breakthrough, researchers at the Mass General Research Institute, Massachusetts, were making headway in the discovery of that gene, as well[23]. Led by Dr. Sue Slaugenhaupt, her team of researchers reported the identification of the location of the gene responsible for ML4 disease and proceeded to develop a screen to test the carrier status of a person to prevent children born with ML4.

Therapy

To begin researching and developing therapies for individuals with ML4, Dr. Slaugenhaupt and her team of researchers worked on creating a mouse model with the genetic information that reflected that of an individual with ML4. The successful creation of this mouse

model enabled them to begin delving into possible therapy and treatment options for ML4 patients.

To develop the most effective treatments, an extensive understanding of the biochemical pathways affected by the disease is required. ML4 is a lysosomal storage disease. Lysosomal storage bodies accumulate in neurons due to ineffective carrier channels unable to transfer waste from the interior of the cell to the exterior. In a functioning cell, this process occurs through the aid of the mucolipin-1, MCOLN-1, protein, localized to specific compartments within the cell. The protein is responsible for ensuring the proper intracellular concentration of ions, such as Ca^{2+}, Na^{+}, Fe^{3+}, and H^{+} [24]. This protein is a component of a membrane spanning channel which allows the flow of ions into and out of the cell (**figure 2**). With the proper concentration of these ions, lysosomes process the unwanted substances within the cell and transform them into usable material. If the MCOLN-1 protein malfunctions, as is the case of ML4 individuals, the intracellular environment does not allow for the proper lysosome functioning, causing a buildup of waste in the cell's storage bodies.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Time (min)
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0 Ca 2+
ML-SA1
F 340/380 (Norm)
1.4
1.2
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NS
WT
TRPML1 KO
D
20µm
WT
TRPML1 KO
E
Autofluorescence (ALU)
1500
1000
*
WT
ML1 KO
F
LysoT
AF
Merge
WT
Mutant
20µm

Figure 2 This graph is representative of the role of the *MCOLN-1* channel. ML-SA1 is an agonist particular to the *MCOLN* family of channels. This graph indicates that when the ML-SA1 channel opener was introduced to cells which were homozygous for the *MCOLN-1* mutation, cytoplasmic Ca^{2+} levels did not increase as would be expected. This suggests that the mutation is directly involved with the regulation of lysosomal release of Ca^{2+}. A) shows a mouse that is homozygous for the *MCOLN-1* mutation, while B) represents the ineffectiveness of ML-SA1 to increase the levels of cytoplasmic Ca^{2+}. C) is a quantification of the insignificant increase of Ca^{2+} from the lysosomes. D) shows a control and *MCOLN-1*$^{-/-}$ mouse. The autofluorescence is indicative of levels of Ca^{2+} measured. E) is a quantification of the levels of Ca^{2+} in the control and mutated mouse. F) confirms that the *MCOLN1*$^{-/-}$ mouse showed greater amounts of Ca^{2+}.[25]

It is suggested that macroautophagy does not occur in the cells of ML4 patients. Macroautophagy is the process by which waste material forms cellular vesicles which fuses with the lysosomes. According to Dr. Slaugenhaupt's research, the neurons of the mouse models which lacked the MCLON-1 protein did not exhibit macroautophagy[26]. The waste products in the neuronal cells of the mice did not converge on the lysosomes for processing and exportation. This discovery revealed important information regarding the dysfunction of the disease. It also provided the researchers with direction to which biochemical pathways the therapies should target. Understanding the lack of macroautophagy in the neurons led researchers to investigate drugs to reduce the buildup of the storage bodies.

Gene Therapy

Dr. Slaugenhaupt's lab is currently utilizing the science of gene therapy to provide a treatment for individuals with ML4. Gene therapy is a progressive field which targets the specific mutated gene to treat the disease. While certain treatments for various genetic diseases attempt to treat the clinical symptoms, gene therapy aims at correcting the underlying cause of the disorder,

i.e., the genetic mutation. Gene therapy is a relatively new and evolving science which provides a hopeful future to individuals with genetic disorders. The American Society of Gene + Cell Therapy notes, "the concept behind gene and cell therapy is to target the exact ***cause*** of the disease, so that the person should no longer have recurring symptoms, ideally after a single treatment. For example, gene therapy is done by adding working genes within specific cells."[27] Below is an outline of the process by which gene therapy works.

The mechanisms behind gene therapy rely upon vectors, genetically engineered carriers to transport the modified gene to the body's cells. Tiffany Lucas, an FDA reviewer for gene therapy products, described gene therapy as "products that mediate their effects by transcription or translation of transferred genetic material, or by specifically altering host genetic sequences."[28] The improved genetic sequence may be administered into the cell's DNA through *ex vivo* insertion, a technology in which cells are removed from the body, treated, and then returned into the body. Another technology is *in vivo* therapy, in which the target cells remain inside the body during the treatment, is the more common form of gene therapy.

New DNA can be inserted into the cell through a few different pathways. The recent discovery of CRISPR[29], an antiviral defense system found in bacteria, has been utilized in gene therapy. CRISPR includes two strands of RNA to match the sequence of DNA of interest, which will be cleaved. The RNAs form a complex with the endonuclease protein, Cas9, which

recognizes the viral sequence of DNA to be cleaved. This bacterial CRISPR/Cas9 system has been modified and adapted for use in gene therapy in humans to modify and/or DNA sequences.

CRISPR/Cas9 can be programmed to target the mutated DNA, and to modify and/or excise the nucleotides of the mutated gene. This process has allowed scientists to replace new DNA into the cell's genome. The next phase of gene therapy typically utilizes vectors, or systems to deliver the gene therapy into the DNA of the appropriate target cells. Vectors may be programmed with a specific virus whose role is to replace the mutated genome with the normal genome. There are two common forms of viral vectors used in gene therapy. One version utilizes an RNA retrovirus, serving as the viral vector to integrate into the target DNA by converting its single-stranded RNA into double-stranded DNA[30]. Retrovirus vectors are termed integrating vectors, because they insert genes into the nuclear chromosomes of the target cells, and therefore integrate the new DNA into all future daughter cells. The second commonly used vector is a non-integrating vector, a vector which influences only its target cell. The adeno-associated virus (AAV) vector, a commonly used non-integrating vector, carries smaller genes than the retrovirus vector, but it may have a longer lasting effect on the cells because its single-stranded DNA embeds into the new host cell[31]. However, it lacks the ability to effect lasting change on future cells like the integrating retrovirus vector.

Upon selection of the type of vector, the next step is to formulate the vector. The general process for constructing an AAV vector is as follows. Mammalian cells in culture are treated with DNA instructions to create the AAV vector. The viral-laden cells are then grown in a

laboratory bioreactor. After 3-5 days, the AAV-containing cells are harvested and the vector is purified. The treatment and purification processes involve an iodixanol column gradient and density gradient ultracentrifugation to separate the virus from the cellular contents in the supernatant. Once formulated, the vectors must be directed to the proper location within the human body. This may be accomplished by physically inserting the vector into a specific site, such as an arm or eye, or the vector may be inserted intravenously. With *ex vivo* therapy the patient's cells will be extracted, treated with the vector, and then reinserted into the patient.

In part, current gene therapy research for ML4 is closely modeled after the gene therapy designed for individuals with Niemann-Pick Disease Type C (NPC1). In 2016, researchers successfully delivered a functioning gene *in vivo* into the mouse models for NPC1[32]. The researchers used an adeno-associated virus serotype 9 (AAV9) to deliver the gene. Once delivered, researchers identified those symptoms which decreased with a single insertion of the vector. Additionally, through histological sectioning, they discovered that the gene targeted the brain, which was a central and crucial finding which determined the success of the therapy. Once the corrected gene was inside the neuron, it was able to produce the protein lacking in individuals with NPC1.

ML4 researchers are currently invested in developing a gene therapy for patients which will produce results similar to the NPC1 vector study. Because both diseases are lysosomal storage diseases and involve the central nervous system (CNS), ML4 researchers hope to mimic the therapy for NPC1. The goal is that the functional gene will be inserted *in vivo* into the CNS. With the working gene in place, MCOLN-1 protein will be produced and the biochemical

pathways, such as the lysosomal degradation system, will effectively carry out their tasks. Researchers intend that when the ML4 body produces the MCOLN-1 protein, the membrane spanning channel will properly control the flow of ions into and out of the cell. This will then create the proper environment for lysosomes to execute their functions and clear the cell of waste material by transforming it into usable substances and then exporting them from the cell.

The development of an effective vector is exhaustive and thorough. Researchers want to ensure that the vector will deliver the gene with the highest success rate. Before the process of creating a vector begins, researchers must conduct a thorough analysis of all patients with the disease in order to be aware of the varied existing symptoms, concerns, and the various physical processes occurring within the body. With ML4, the leading team of researchers based in Mass General Research Institute met with nearly most of the known individuals with ML4 to document their medical data. This also enabled them to gain a better understanding of the lifestyle of a person with ML4. Dr. Rebecca Oberman, Executive Director of the ML4 Foundation remarked in personal correspondence, "[w]e need to be able to detail what exactly, on every level happens in MLIV, at what age and stage. Many different physical processes take place, and we need to acquire documentation of all sorts to understand them."[33] These meetings took place in the laboratory, at national conferences, or virtually. Researchers then worked with a vector core laboratory to form the vectors and test various forms of insertion into mice. With the functional gene inserted, the mice were analyzed and studied. Mice were subjected to various tests, such as the open field test, balance beams, and motor-rods to assess behavior[34]. After the mouse reached a certain predetermined age, it was euthanized and

histological assessments were conducted to determine expression of the gene in various tissues. The process of developing gene therapy is continuing as the researchers analyze the effectiveness of the vector. The laboratory may choose to reject a specific vector and start anew with a different form of the vector, improve the current vector and modify it, or opt for a different choice.

In a most recent article published by Dr. Slaugenhaupt's lab[35], successful injection of the functioning *MCOLN-1* gene is reported. The authors used an AAV9-based vector, AAV-PHP.b-MCOLN1, to implant the functioning *MCOLN-1* gene into the young mouse prior to the mouse developing symptoms typical of an individual with ML4. The researchers reported that there was significantly less buildup found in the lysosomes of the mice as well as an increase of the presence of the MCOLN-1 protein in the brain. This data suggests that gene therapy may prevent the onset of devastating symptoms typical of ML4. Furthermore, their research addressed the possibility of restoring developmental motor skills which occur in the first few months of life. Using the mice treated to mimic the genetic expression of an ML4 individual, the researchers posit that their results indicate that the cerebrospinal injection of the AAV9 vector restored neurologic activity and motor function. Research in this area is constantly progressing and the development of a successful vector for *MCOLN1*-gene delivery seems promising.

Funding

As with all research, funding is crucial to the success and possibility of the research projects. Dr. Oberman expressed that limited funding from the NIH[36] requires that research laboratories receive funding from other sources. When Dr. Slaugenhaupt discovered the gene for ML4, families of children with ML4 approached her to follow up with the disease and requested that she continue her research to help their children. This was the beginning of the ML4 Foundation. Founded in 1984, the organization's goal is to finance progressive research to discover treatments, and eventually a cure for ML4 while providing a supportive network for families affected by ML4[37]. The foundation hosts a conference biennially to create a forum for researchers to meet with each other and share their developments, for donors to meet with families and individuals with ML4, and to provide opportunities for researchers to interact with the children for whom their research is dedicated. Most of the funding for ML4 research therefore stems from the ML4 Foundation which finances the research by securing funds through national agencies, as well as through garnering support and donations from individuals.

Orphan Diseases and Funding

FD and ML4 are most commonly found in individuals with Ashkenazic Jewish heritage. According to the Familial Dysautonomia Foundation's website, there are less than 400 known cases of FD in the world.[38] The ML4 Foundation notes similar rates of occurence of ML4.[39] The rarity of FD and ML4 places the diseases within a category referred to as orphan diseases. The United States defines an orphan disease "as a condition that affects fewer than 200,000

people in the US."[40] Due to the relatively small number of people which these diseases affect, pharmaceutical companies deem these diseases unworthy of investing in and shy away from producing and marketing drugs and therapies for them. The Orphan Drug Act was therefore created in 1983 to incentivize pharmaceutical companies to invest financial means into these rare diseases through tax cuts and other financial benefits. The National Organization for Rare Diseases (NORD), the coalition vital to the institutionalization of the Orphan Drug Act remains a crucial organization and element in orphan disease drug developments.

The NIH has also created various branches of its organization which are catered toward helping the rare disease community. One such example of this is the Therapeutics for Rare and Neglected Diseases (TRND) program. The NIH states that the mission of this program is to provide resources and assistance to these rare diseases and enable the development of treatments for these diseases. The NIH recognizes that there are, unfortunately, many rare diseases which can now become the beneficiaries of serious research and therapeutic developments. While this branch provides medical and informational assistance, it does not directly provide the much needed funds to the researchers invested in rare diseases.

Conclusion

Some genetic diseases cause tremendous devastation to those afflicted with the disorder and much heartbreak to their families. Research into such diseases has enabled scientists to better grasp the ways in which the human body functions when homeostatic mechanisms are interrupted by genetic mutations. With proper funding and support, researchers have been able to

make breakthroughs leading to the development of therapies and treatments with life-altering transformations to those affected individuals. Individuals with FD have benefitted invaluable measures from the treatments developed by Drs. Rubin and Anderson. Advances in FD outpaced those for ML4, possibly reflecting the greater incidence of FD, than ML4, in the population. Those afflicted with ML4 remain eagerly awaiting for gene therapy to proceed until it will be available for patients. There are various routes through which researchers can navigate the process of drug development, doing a juggling act between solid research, salesmanship, and business. Securing adequate funding, whether through governmental grants or support from private foundations, remains the cornerstone of successful research. The impact of successful treatments and therapies developed through research has a priceless impact on those with the disorder.

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