Clinical Lipidology Roundtable Discussion

JCL Roundtable: Enzyme replacement therapy for lipid storage disorders

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Abstract: There are several inherited disorders that involve abnormal storage of lipids in tissues leading to severe compromise of organs. Sadly, these are often accompanied by lifelong morbidity and early mortality. Disorders such as Gaucher, Fabry, and lysosomal acid lipase deficiencies (Wolman and cholesterol ester storage diseases) have been known for many years, and provide a difficult and frustrating set of problems for patients, their families, and their physicians. With recombinant methods of protein synthesis, it is now possible to literally replace the defective enzymes that underlie the basic pathophysiology of many such disorders. The delivery of these enzymes into the affected cells is possible because of their location in the lysosomes where the natural degradation of their lipid substrates occurs. I have asked 2 well-known investigators to join us for this Roundtable. These are professors who have been involved with the research that has made this type of therapy possible and who have participated in the clinical trials that demonstrated the value of enzyme replacement therapy. They are Dr. Robert Desnick, dean of Genetic and Genomic Medicine and professor and chairman emeritus of the Department of Genetics and Genomic Sciences at the Icahn School of Medicine at Mount Sinai in New York City, and Dr. Gregory Grabowski, professor of Microbiology, Biochemistry, and Pediatrics, at the University of Cincinnati College of Medicine. Dr. Grabowski recently retired from that school to become the chief science officer of Synageva, a company involved in producing enzymes for this type of therapy. © 2014 National Lipid Association. All rights reserved.

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Dr. Brown: I would like to focus our discussion on 3 examples of these lysosomal lipid storage disorders. I suggest we start with one of the best-known, Gaucher disease.

How do we define Gaucher today from a clinical perspective?

Dr. Grabowski: Classically, the literature describes three forms of Gaucher disease, types 1, 2, and 3. Type 1 disease has been classically called a non-neuropathic disease so there’s no primary neurologic involvement, whereas types 2 and 3 involve the brain directly. It results in neurodegeneration in either early childhood or in childhood or adolescence.
Type 1 disease is the most common in the Western world. It affects mostly the visceral tissues, primarily the liver, spleen, and bone marrow, with significant effects leading to hepatosplenomegaly, major effects on blood-formed elements, thrombocytopenia, anemia, and some bleeding disorders. With time, bone disease, destructive bone disease that leads to fractures, and very severe, very painful crisis episodes that are very similar to those in sickle cell disease, and that destroy bone. With time then, it becomes a chronic, very disabling disease.

In the Western world, a clinician would suspect Gaucher disease, first of all, in an Ashkenazi Jewish person. Although not uniquely present, it’s most prevalent in that population. In a person with hepatosplenomegaly and thrombocytopenia or anemia, the thought might initially be leukemia, cancer. As a result, they are referred to a hematologist-oncologist and get a bone marrow examination where characteristic Gaucher cells are found; these are massively infiltrated macrophages with very characteristic cytoplasm that’s been called crumpled tissue paper on hematoxylin and eosin staining.

**Dr. Brown:** Dr. Desnick, are there other clinical findings that would cause one to suspect Gaucher disease in a young adult patient?

**Dr. Desnick:** The first clues are the hepatosplenomegaly, secondary hyperplenism causing pancytopenia, and/or evidence of bone disease. These findings ought to lead one to suspect the diagnosis. One point we should make that’s very important, in all the lysosomal diseases, is there is a spectrum of severity. If the disease-causing mutation results in no or very low enzyme activity, the patients will have an early-onset severe disease phenotype. If there is some residual enzyme activity, the patient will have a less severe form of the disease, typically a later onset presentation. Thus, the severity in each lysosome storage disease depends on the specific disease-causing mutation and its impact on the respective enzyme’s function. In Gaucher disease, we have 3 major phenotypes: infants with neuropathic disease (type 2) have mutations that result in no or very little enzyme activity, children to adults who have no neurologic involvement and primarily reticulon endothelial involvement (type 1) have mutations that result in residual enzymatic activity, and patients with a juvenile-onset phenotype with a slowly progressive neurologic component (type 3) have mutations that result in lower levels of residual activity that lead to an intermediate phenotype.

In fact, one of the key rationales for enzyme replacement therapy was the early discovery that it only took a few percent of normal enzyme activity to correct the metabolic defect and substrate accumulation in cultured cells from patients with various lysosomal diseases, and that the presence of residual enzyme activity modified the phenotype of these diseases from very severe (no enzyme activity) to a disease phenotype with less severe manifestations (because of a few percent of normal enzyme activity).

**Dr. Brown:** What has been the prognosis for patients with untreated type 1 Gaucher?

**Dr. Grabowski:** Historically, it looks as though type 1 Gaucher disease leads to about a decade loss in life span. That’s highly variable because like other lysosomal diseases, there’s a whole spectrum of abnormalities. Some of the kids with type 1, nonneuropathic Gaucher disease can be very severely involved and die in childhood, whereas others have a more indolent progress toward adulthood. It’s a little difficult to get a real life span, but you have to take the presentation—let’s say early onset childhood development of the disease—and watch the progression. That’s going to affect the lifespan more than a more indolent course later on. But both are very debilitating diseases, if not lethal.

**Dr. Brown:** What is the lipid that actually accumulates in the tissues of patients with Gaucher disease?

**Dr. Desnick:** In Gaucher disease, the lipid that accumulates in the lysosomes is a particular glycosphingolipid called glucocerebrosidase. It’s a component of most cell membranes and is normally degraded in the lysosome by a specific lysosomal enzyme, acid beta-glucosidase, which is also called beta-glucocerebrosidase. In Gaucher patients, the glucocerebroside accumulates because of absent or markedly decreased acid beta-glucosidase activity. The clinical manifestations that evolve are all secondary to the degree of the enzyme deficiency and the resultant level of accumulation of its glycolipid substrate.

**Dr. Brown:** Today we are discussing Gaucher because it is the archetypical disorder that represents the group of lysosomal disorders that can be treated with enzyme replacement therapy. What difference has this type of treatment made to patients with various forms of Gaucher disease?

**Dr. Grabowski:** There was a survey conducted last year that polled various specialty groups, one of which was the geneticists. They were asked “what do you think is the most transformational drug that’s been developed in your field in the past 2 decades?” The treatment for Gaucher disease with replacement of beta-glucocerebrosidase came out number 1.

This has been a transformational drug. People that have gone from chronic debilitating disease—and the only analogy would be similar to very severe rheumatoid arthritis—to being healthful people. When we first started with enzyme therapy in 1991 and 1992, people would come in with these massively involved spleens and livers and the secondary effects of massive cytokine production and cachexia as well as broken bones. They looked like people with cancer. Now, we don’t see that anymore.

Two reasons, one of which is we’ve taken the adults with Gaucher disease with the severe and long-term complications, thought to be irreversible, and on treatment stopped...
progression and in some reversed their disease completely. Certainly, the hepatosplenomegaly and blood-formed element problems can go away.

In the kids, with early disease, we’ve prevented the clinical complications from happening. We almost never see bone disease anymore when treated early. It has been truly transformational. We now have many young adults who were children or adolescents when we started treatment who are completely normal people. There’s no other way to say it than “transformational.”

**Dr. Desnick:** When we were performing the first infusions of human enzymes in the early 1970’s and 1980’s, no one thought the disease pathology could be reversed. We assumed that we could stop disease progression. To watch the Gaucher patients’ livers and spleens revert toward normal size with treatment, when the splenomegaly was 10 to 40 times normal, and the liver volume was up to 1.5 times normal, was truly amazing.

This has proven true for all of the diseases that we’re discussing today. If you diagnose them early enough and intervene early enough, you can prevent the manifestations. Gaucher was the prototype and demonstrated how effective early treatment was.

**Dr. Brown:** The very thought that you could infuse a protein into the bloodstream and have it go to the appropriate cellular location in the right tissues and actually do the work for which it evolved would have seemed like an impossible dream only a few years ago. How is it possible to replace glucocerebrosidase in patients genetically deficient in this important protein?

**Dr. Grabowski:** The first thing we have to do in any lysosomal disease is to define what we have called the target site of pathology. What’s the major target site of pathology in Gaucher disease? The macrophage system.

Lucky for us, there’s a very nice receptor on a macrophage called the mannose receptor. We may want to talk about the mannose-6-phosphate receptor a little later, but this is the mannose receptor.

What happened in the initial trials? Enzyme was purified from placenta. When you isolate it from placenta, it has complex carbohydrate modifications on it, which include terminal sialic acids. This form of the enzyme was injected this into people and not much happened.

Then the macrophage mannose receptor was discovered by Sly, Accord, Stahl, and coworkers and it was soon noted that this is the cell that’s involved in Gaucher. Somebody said “Why don’t we modify these carbohydrates on the enzyme, remove the terminal sialic acid and expose mannose?” When infused, this form bound to the mannose receptor and was preferentially taken up into the macrophage. This enzyme degraded the abnormal stores of glucocerebrosides and actually reversed the disease process.

**Dr. Desnick:** That was the key cell biology finding that opened the door for enzyme replacement therapy. Understanding what the receptors were on cell surfaces for enzyme uptake into the cells, and then to the lysosomes, was key. Once you knew that lysosomal enzymes were targeted to the lysosomes both within the cell as well as when you inject the enzymes into the circulation, it provided the rational for receptor-targeted enzyme delivery to the lysosomes where the glycosphingolipid was accumulated. If you could make enough of the human enzyme that was targeted to the sites of pathology, via the mannose or mannose-6-phosphate receptor, you had an effective replacement therapy. Thus, Mother Nature will take an intravenously infused, receptor-targeted enzyme directly to the lysosomes where it can degrade the accumulated substrate.

**Dr. Brown:** What does this teach us about normal macrophage physiology? Do macrophages secrete these enzymes through the Golgi apparatus like most proteins and then reabsorb them?

**Dr. Grabowski:** It depends. The biology of each of these enzymes is a little bit different. The ones, for example, for Fabry disease, alpha-galactosidase A and for lysosomal acid lipase deficiency a little enzyme is secreted from cells. The Gaucher enzyme is usually not secreted from a cell at all. It doesn’t secrete and then correct the neighboring cells. Here, you have to really direct the enzyme into the cell involved with the pathology.

Fortunately, for example, in the liver, if you do a liver biopsy on a person with Gaucher disease, what you will see is Kupffer cell pathology. The Kupffer cells have turned from a cell at all. It doesn’t secrete and then correct the neighboring cells. Here, you have to really direct the enzyme into the cell involved with the pathology.

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All we had to do for Gaucher disease was get the enzyme into the macrophage—the target site of pathology. The mannose receptor is not involved with normal biology of degrading glucocerebrosides in the macrophage. It is believed to be there as part of an innate immune defense system against fungal infections, because many fungi just express mannose-terminated or mannan antigens on their surface, and they would be taken up. Fortunately, we could just use this as a means to deliver the glucocerebrosidase as a Trojan horse and it has worked extraordinarily well.

**Dr. Desnick:** In the beginning, the early experiments of enzyme replacement involved the isolation of the enzyme from human tissues, usually placentae. However, these efforts resulted in relatively small amounts of active, purified enzyme. We now appreciate that you need milligrams per kilogram of enzyme to get to the lysosomes of various organs when you infuse it intravenously.

We’ve learned over time about the biodistribution of an infused lysosomal enzyme, particularly from studies in the mouse knock-out models. Most enzymes go to the liver, then the spleen, so the treatment of type 1 Gaucher disease was quite effective. The more enzyme you administer, the more that is delivered to the “difficult to reach” organs like the kidney and heart. For each disease, there’s a therapeutic dose. The infused enzymes have a dose effect like every other drug, and the more enzymes you give, the more they get to the target sites of pathology, resulting in greater degradation of the accumulated substrates, and ultimately lead to greater clinical benefit.
We’ve learned this principle over the years with Gaucher disease as the prototype. In the very early experiments that Roscoe Brady did at the NIH, his laboratory was focused on purifying sufficient enzyme for clinical trials. Initially, low doses of the normal enzyme were administered, and the effects were highly variable. Later, the enzyme was purified commercially, and the larger amounts of infused enzyme proved clinically effective. Today, the standard dose for Gaucher disease is 1.6 mg/kg every 2 weeks, and probably even more would be better.

**Dr. Grabowski:** To put this in a little perspective, in the original experiments with mannose terminated placental enzyme, it required 20,000 pounds of placenta to provide sufficient enzyme for treatment of a single patient with Gaucher disease for 1 year. Obviously that was a self-limiting issue.

Now, when you heavily overexpress the enzyme in Chinese hamster ovary cells or other types of cells—it overwhelms the system and becomes secreted. It can now be manufactured in massive tissue culture systems and provides us with a very homogenous enzyme system in amounts that allowed dose-response studies in patients. With these recombinant systems, we have sufficient enzyme to treat a large number of patients with effect. This applies to the other diseases that we’re going to talk about as well.

**Dr. Brown:** This must have brought the cost down tremendously. What does it cost to treat a Gaucher patient on an annual basis?

**Dr. Desnick:** It is a complicated process to produce the large amounts of the purified recombinant enzymes needed for replacement therapy. Treating a 70-kg Gaucher patient costs about $250,000 a year.

**Dr. Grabowski:** Yes, it is still very expensive. The only way to modify right now the cost of the drug is to modify the dose. What we’ve learned over the past 20 years is that you have to individualize therapy, just other pharmacotherapy. You can individualize therapy. Some people need less, some people need more. It’s a physician’s acumen and experience that go into that judgment of how much enzyme they use, both in terms of dose size and in the frequency of dosing.

The standard is, let’s say 60 units (1.6 mg) per kilo every 2 weeks. That works well in almost everybody. We’ve had a few patients where it hasn’t. We’ve had to move to weekly dosing, then they respond. Later on, we change their dosing regimen or we change their dose or both. As people get better, you can change doses. You have to monitor them carefully.

That’s where the expertise in these diseases is important because good biomarkers are not available to determine quantitatively the response to treatment. Over the past 20 years, we’ve learned the art of managing this disease. We have a variety of different ways to improve the response of these patients now—personalized medicine.

**Dr. Desnick:** As we already noted, Gaucher disease was the first lipid storage disease that was treated by enzyme therapy. It markedly improved the lives of affected patients. But, it also taught us the principles of enzyme therapy. Those principles include (1) producing an enzyme with the appropriate glycosylation for receptor-mediated uptake, (2) providing an adequate dose for enzyme delivery to the critical organs, (3) initiating early intervention to reverse and even prevent disease manifestations, and (4) appreciating that treatment is lifelong, because if treatment is suspended for a period of weeks, patients experience progression of the clinical disease. These principles hold true for the other lysosomal diseases as well.

**Dr. Brown:** Indeed, I would like to ask specifically about Fabry disease. How does the story of Fabry differ from Gaucher?

**Dr. Desnick:** Fabry disease is an X-linked lysosomal storage disease. Like Gaucher, it also results from the lysosomal accumulation of a glycolipid. The Fabry glycolipid has 3 sugars (galactose-galactose-glucose) attached to a lipid called ceramide. Because the disease is X-linked, males are more severely affected than females. We now know more than 1000 different mutations that can cause this disorder. The specific mutation determines whether it encodes for no enzyme or a small amount of activity. The absent or amount of residual activity will determine if the manifestations begin early in children (the early onset, severe “classic” type 1 phenotype) or in adulthood (the less severe later-onset type 2 phenotype).

The target sites of pathology in Fabry disease are the small vessel endothelial and smooth muscle cells, the kidney (primarily the glomerular capillaries and podocytes), and the heart (cardiomyocytes).

The early manifestation in children with the “classic” type 1 phenotype is the burning, tingling pain in the hands and feet, which begins around 4 to 10 years of age. Affecting children and adolescents experience progression of the disease with age; the renal involvement progresses to renal failure and the left ventricular hypertrophy progresses to hypertrophic cardiomyopathy. Because fibrosis in the heart and in the kidney occurs late in the disease and are irreversible, early treatment in childhood with enzyme replacement is very important. Starting early, you can prevent the fibrosis. The vascular damage can lead to transient ischemic attacks and strokes.

Before there was renal dialysis or transplantation, the average age at death was 41 years in a series of 94 males with the “classic” disease. Life expectancy has been extended with renal replacement therapy, but with enzyme therapy, we’re seeing reduced progression of the disease and an even longer lifespan.

**Dr. Brown:** Macrophages are not involved at all in Fabry disease. It’s only the specific cells you have mentioned. What’s the property of these cells that causes them to be vulnerable to the genetic defect in Fabry?

**Dr. Desnick:** I wish we understood the cell biology, why the glycolipid substrate, globotriaosylceramide, has a proclivity for these particular cells. It probably has a biologic basis. But from a lipid point of view, most of the glycolipid
is synthesized in the liver, and secreted with the very low-density lipoprotein particle to circulate with the low-density lipoprotein (LDL) and the high-density lipoprotein (HDL) particles. The LDL particles containing the accumulated glycolipid are taken up into the vascular endothelium via the LDL receptor–mediated uptake system. They’re delivered to the lysosomes of the microvasculature and smooth muscle. That’s the biology of the vascular endothelial involvement.

In the heart and in the kidney—the target cells are the cardiomyocytes and the podocytes—both lifelong cells, which slowly accumulate the glycolipid over time. In the heart, the accumulation leads to left ventricular hypertrophy and then to hypertrophic cardiomyopathy, which is devastating and really untreatable other than by ablation procedures or transplantation.

**Dr. Brown:** But we know that most LDL is cleared from the blood by the liver. How does the liver handle the lipid in a way that does not cause hepatic damage?

**Dr. Desnick:** It’s interesting because the glycolipid becomes part of the lipoprotein particle. If you measure this glycolipid in the plasma of males with Fabry disease, it’s elevated 5- to 10-fold over the normal level. If you measure it in patients with type II familial hypercholesterolemia, with markedly elevated LDL cholesterol, it’s about 2 to 3 times normal.

**Dr. Brown:** It follows the LDL pathway?

**Dr. Desnick:** It travels with the LDL particle.

**Dr. Brown:** Basically, the liver passes the buck back into the plasma with very low-density lipoprotein and feeds it to the endothelium.

**Dr. Desnick:** Exactly. When you look at the liver, there’s little, if any, accumulation of the glycolipid. The liver’s excreting it.

**Dr. Grabowski:** A fundamental difference between the pathophysiology of Fabry disease and Gaucher disease is that Gaucher disease, it’s really a phagocytic disease, at least in the viscera, where old and aging cells are eaten up by macrophages and the membrane lipids are degraded all the way to glucoceramide and then degradation stops and abnormal storage is the result. Fabry disease is more of a synthetic problem, with delivery through LDL from synthesis in the liver to the podocytes or in the cardiomyocytes where degradation is inadequate. There are really fundamental differences in the pathophysiology of how this works.

A major site of pathology in Fabry, the endothelial cell turns over about every 60 days or so. Preventing delivery of the lipid allows those cells to reform without the stored lipid. However, the renal podocytes, myocardiocytes hang around. They’re lifelong. Therefore, you really have to deliver enzyme to them. You can’t rely on cell turnover. The pathophysiology is very different.

**Dr. Brown:** I have seen case reports of congestive heart failure due to the presence of obstruction in the left ventricular outflow tract because of septal hypertrophy related to the cardiomyopathy of Fabry disease.

**Dr. Grabowski:** In a sense, those kind of things are really important. You’ve got 1000 mutations in Fabry disease, you have 400 or 500 in Gaucher disease. But, the point is, you have several thousand patients now and you get to see the spectrum of the disease, the usual and the unusual manifestations. What is it that really happened to these people over time? When do you give enzyme? What gets treated? What doesn’t get treated? How can we change those things? There are sequestered sites that are not amenable to enzyme therapy, particularly in Gaucher disease.

**Dr. Brown:** I have been told that in some with Fabry disease you may have genetic defects that can completely impair the enzyme but in others, there may be a bit of functionality left. How does that affect the course of the disease?

**Dr. Desnick:** Fabry disease is interesting from that point of view because there are mutations in the alpha-galactosidase A gene that encode no functional enzyme, whereas others result in mutant enzymes with residual activity. Patients with essentially no functional enzyme have the early onset, more severe “classic” type 1 phenotype.

Patients who have missense mutations that encode low levels of activity (>1% of normal) have a somewhat less severe course, the type 2, later-onset phenotype. One of the hallmarks of the type 2 disease is that patients don’t present in childhood with the pain, or other type 1 classic manifestations. Instead, they show up in the emergency room with renal failure or in the cardiac clinic with left ventricular hypertrophy or hypertrophic cardiomyopathy. These later-onset type 2 patients are typically not recognized as having Fabry disease, because they do not have the features of the earlier-onset type 1 patients, who have the very characteristic skin manifestations (angioedema), the hypodermis, and the gastrointestinal problems.

**Dr. Brown:** I was taught in medical school to look for the little black angiomytoma on the skin. But people who have onset in middle life may not have this classical finding?

**Dr. Desnick:** Correct. The later-onset type 2 males do not have the angioedermas. In fact, when they screen cardiac clinics, particularly hypertrophic cardiomyopathy clinics, they find about 1% of patients have unrecognized later-onset type 2 Fabry disease. When they screen hemodialysis clinics, about 0.3% have unrecognized type 2 Fabry disease. Because it’s X-linked, the internist, the nephrologist, or the cardiologist should contact the medical genetics team to investigate the patient’s family to identify the affected younger males and heterozygous females for treatment. Diagnosis can be accurately made by enzyme analyses in plasma or isolated leukocytes in the males and by alpha-galactosidase A gene sequencing in the females.

**Dr. Brown:** They don’t need genetic testing because the biochemical tests are so straightforward with these enzymes?

**Dr. Grabowski:** Yes and no. But there are two real issues. First of all, Fabry is classically an X-linked disease.
Everybody thinks males are affected. Turns out that at least half the females have disease, less in kidney disease, but more often with heart disease or stroke.

At our clinic when I was at Cincinnati Children’s, 60% of the women that were found through family studies had either early stroke or heart disease. The other thing is, some of these women can look like they have multiple sclerosis. There’s a whole spectrum of disease in women that has to be treated. That’s a very important aspect of screening.

In terms of the mutations, in Gaucher disease, we have some very useful genetic tests because there’s predictability, particularly in separating the neuronopathic forms of Gaucher disease from the nonneuronopathic forms. There’s 1 particular mutation that’s termed N370S that either in heterozygous state with another mutant allele, or as homozygotes, leads to nonneuronopathic disease. Whereas there are some other mutations that clearly are heavily predisposed to develop the neuropathic disease. So that changes management.

Also, in Gaucher, depending specific mutations, there is some predictability as to when you’d expect onset and this allows prediction of the need for earlier or later enzyme therapy. Similar situations also exist in Fabry disease.

Dr. Brown: The treatment of Fabry disease with enzyme replacement therapy produces a reversal of some of the tissue damage. What about the cardiomyopathy?

Dr. Desnick: The accumulated glycolipid in the cardiomyocytes is the most difficult for the enzyme to reach. In the Fabry mouse model, less than 0.1% of dose reaches the heart. In the older Fabry patients who have developed the cardiomyopathy, some may already have significant fibrosis as identified by late-enhancement on the magnetic resonance imaging. That is why it is so important to identify the young, classically affected males for early treatment before the irreversible fibrosis has occurred.

Dr. Brown: Does the kidney respond with improved function?

Dr. Desnick: Yes. It’s been shown recently, by analysis of biopsies at baseline and after 5 years of treatment that, if the patient receives an enzyme dose of 1 milligram per kilogram, and the treatment is started in childhood that the enzyme not only reverses the endothelial, interstitial, and mesangial cell accumulations, but also the podocyte accumulation—which is hugely engorged with the undegraded glycolipid—is cleared. Thus, if you start early enough in young classically affected males, say at 4 to 10 years of age, you will achieve clearance of the kidney.

Dr. Brown: Perhaps the least known but one of the more common lipid storage disorders is cholesterol ester storage disease. In young children, it is known as Wolman disease. Could you tell us about Wolman disease? How does that present and how does it differ from cholesteryl ester storage disease?

Dr. Desnick: We currently refer to these two disorders as lysosomal acid lipase deficiency as they represent the spectrum of the disease. Due to different mutations in the acid lipase gene, the more severe mutations result in clinical onset in early infancy with failure to thrive. Affected infants will have feeding problems from the first days of life.

Patients who have mutations with residual activity will have onset as children or adults; they were previously referred to as having cholesterol ester storage disease. This later-onset form often escapes detection and diagnosis. But the unrelenting accumulation of cholesteryl esters and triglycerides will lead to hepatic disease, elevated transaminases, bridging fibrosis, and ultimately cirrhosis. These patients will end up on the liver transplant list. What we’re learning is that many of these patients are not recognized as having this genetic lipid disorder as they are often misdiagnosed or undiagnosed.

Dr. Grabowski: Again, like Fabry and Gaucher, as you go from individual centers that have experience in rare disease to a broad case of larger and larger populations, you actually start to understand the diseases better. When they first found patients—they called it Wolman disease because the presentation early in life is characterized by early onset with a mean lifetime of 3.5 months. Then, there were these later-onset people, but now we’re finding people in between. Indeed, in some of the work that’s been done recently on the natural history, I think it’s astounded many people that 80% of the people that we find with “cholesteryl ester storage disease” are children younger than age 18, many of them younger than 10, who have progressive liver disease and fibrosis and cirrhosis and get transplanted.

There are some patients that are presenting later than that even. That’s to be expected in the whole spectrum of the disease. But, the underlying pathophysiology is essentially identical in all the variants of this disease. So, the spectrum really has to be considered.

Recently, in a presentation, I saw people really astounded. This was at a conference called the World Conference to see that there was a gap between very young patients and patients aged 4 years. But that’s because we haven’t looked, but they’re there. They’re in the literature.

As we get more and more patients and we find more and more, they’re going to spread out and we’re going to find that this whole spectrum of the pathophysiology is going to be expressed. It’s the identical enzyme deficit, and it’s the identical pathophysiology. That’s really what we’re learning about this.

Dr. Brown: How does the acid lipase reenter the lysosome? Does it have a specific receptor as well?

Dr. Grabowski: Yes. Fortunately, like Fabry disease, it uses the mannose-6 phosphate receptor. This is a soluble enzyme. It can be secreted in small amounts from cells and there is reuptake into lysosomes through the mannose-6 phosphate receptor. Or if you manufacture an enzyme with the mannose-6 phosphate tag on it, it’ll be taken up by that receptor.

Also, if you look at the major organ of involvement that presents usually in this disease, it’s the liver. At least 2 cell types of the many in the liver are involved: hepatocytes and
Kupffer cells. Fortunately, when you have mannose-6-phosphate, you can be taken up by hepatocytes. And because there’s some mannose exposed, there is uptake by Kupffer cells. You can get to both cell types effectively. Certainly, some of the preclinical trial studies that I did many years ago in mice show that you could do that. You have to fix both cell types. That’s a very important aspect.

**Dr. Brown:** Does the enzyme preparation that is given therapeutically consist of 2 different forms, with a terminal mannose on the oligosaccharide chance and a portion with mannose-6-phosphate as the terminal moiety in the glycoprotein?

**Dr. Grabowski:** It depends a little bit upon what exactly the enzyme preparation is, but in general, when you have mannose-6-phosphate stuck to the carbohydrate residues, there’s also some mannose there. It can use both, yes.

**Dr. Brown:** With the enzyme treatment, what benefits can accrue to patients with cholesteryl ester storage disease? Do they show shrinkage of the liver? Is there a reversal of this disease process?

**Dr. Grabowski:** Yes. I think for me, as with the other lysosomal enzymes that we’ve talked about, one of the things that’s been very surprising has been the ability to reverse disease manifestations.

In a recent presentation, again at the World Conference in February, data were presented on the early variant of this disease. The amazing results in terms of life expectancy and in terms of a little bit of biopsy data that show actual reversal of liver involvement. From the data that are publicly available, later onset variants from the trials show that you can get rid of the early fibrosis that occurs in the liver. With end-stage liver disease, it becomes irreversible.

I found this very surprising because when I first got into this field, I started reading about the non-alcoholic fatty liver disease and nonalcoholic steatohepatitis. The bariatric surgeons were saying we see reversible fibrosis. I was initially doubtful but now I believe it happens. We’re seeing such effects in reversal of this disease process, in animal models of LAL deficiency and we believe in the human disease.

**Dr. Brown:** Why should the internist or pediatrician suspect the presence of LAL deficiency?

**Dr. Grabowski:** There are several scenarios, which should prompt the pediatrician. For example, a child comes in, is not thriving in the first month or so of life. Maybe the belly’s a little bit big and an X-ray of the abdomen shows calcified adrenal glands. In about 60% of the cases that is pathognomonic of the early-onset variant. An expert radiologist may not see calcification, but notices enlarged adrenal glands; this can be a clue. The plasma cholesterol concentration is bit elevated. When you add that to hepatomegaly, splenomegaly, and malnutrition from malabsorption from the macrophage infiltration of the intestines, you have the clinical diagnosis. Even water absorption can be compromised—severely limiting lifespan. Total parenteral nutrition can only keep you alive for so long. That’s a pretty aggressive presentation.

The later presentation, let’s say children that are between 2 and 10 years of age often have mild hepatomegaly or maybe quite obvious hepatomegaly. The next important clue is the hypercholesterolemia—it can look like familial hypercholesterolemia. This may lead to a referral to a gastroenterologist and a liver biopsy, which shows classic lysosomal storage of cholesterol ester and cholesterol clefts. Bridging fibrosis in the liver biopsy is often found even at this stage. One should immediately suspect LAL. The biochemical diagnosis is then easy. A drop of blood on a piece of filter paper sent to the laboratory to measure lysosomal acid lipase and you have a definitive diagnosis. For confirmation, one can do molecular testing. The older patients, teenagers or young adult patients, may be smoldering along. They may either present with hypercholesterolemia or liver disease—even liver failure.

**Dr. Brown:** Are there usually elevated transaminases?

**Dr. Grabowski:** When you find hypercholesterolemia and elevation of transaminases at the present time, you think immediately of nonalcoholic fatty liver disease. But LAL-deficient patients are usually young and are not obese or even significantly overweight. Hypercholesterolemia in this age group without family history and liver enlargement should cause you to seriously consider a liver biopsy. Furthermore, this disorder usually produces hypercholesterolemia that is difficult to control. That’s a further clue that it may be LAL deficiency.

**Dr. Brown:** The associated plasma lipoprotein elevations do not respond to statins or other treatment?

**Dr. Grabowski:** There is some response, but not as expected.

**Dr. Desnick:** We did a number of studies in the 1980s that showed that statins could reduce the lipids and the apolipoproteins. But even with aggressive statin treatment, those patients often progressed to liver transplantation.

**Dr. Brown:** Is statin therapy now thought to be appropriate in LAL deficiency? Does this slow the disease process or make it worse if we inhibit cholesterol synthesis in the liver?

**Dr. Desnick:** It may not help, and it may even make the disease worse. In those days, we were treating the lipid abnormalities. By giving statins, you may enhance LDL-receptor synthesis and increase cholesteryl ester delivery into the lysosomes. The cholesteryl esters are stored in the lysosomes from the LAL deficiency, so the cholesteryl esters cannot be cleaved to escape as free cholesterol. Today, we’re specifically treating the lipid accumulation and the pathophysiology of the disease by replacing the deficient enzyme activity.

**Dr. Grabowski:** Patients with LAL deficiency and hypercholesterolemia must be monitored like you would for anybody on statins. You start with statins and you measure the transaminases. Some people without LAL deficiency get real elevation of transaminases. But, those with LAL deficiency generally have pretty difficult-to-control hypercholesterolemia. I have 1 patient that’s on 5 different medications. She’s in the middle of her reproductive age. For
her to get pregnant, we have to take her off. That has to be
looked at in terms of the total management.

Then if you treat the fundamental pathophysiology,
these things start to resolve as indicated by reports in the
literature.

**Dr. Brown:** If the cholesterol level does come down,
that’s an indicator that you’re doing the right thing for
the hepatocyte and the macrophage?

**Dr. Grabowski:** There are reports that the first doses of
enzyme in some patients with LAL deficiency, may pro-
duce a spike in serum cholesterol. This is probably due to
mobilization of free cholesterol from those lysosomes
into the endoplasmic reticulum suppressing the LDL recep-
tor synthesis.

It’s very interesting. The lysosome, of course, is the
entrance pathway for cholesteryl esters and triglycerides
into the cell. It sticks in the lysosome. What does the cell
see? The cell sees cholesterol starvation. Its entire meta-
boles pathway for cholesterol synthesis is turned on.

**Dr. Brown:** So 3-hydroxy-3-methylglutaryl-coenzyme
A reductase activity is elevated and LDL receptors can
not do their job because the recipient organ, the lysosomes
are already full of cholesteryl ester?

**Dr. Grabowski:** The cell membrane cholesterol trans-
porter, ABCA-1, seems to be important as well, releasing
cholesterol out of the cell and into the plasma HDL. That
creates a peak potentially, before you cool off the whole
synthetic pathway and unload the lysosomes.

**Dr. Brown:** One of the things reported in terms of the
histologic pathology in the liver was the existence of
cholesterol clefts. Cholesteryl ester doesn’t form a cleft.
That means that cholesterol is being hydrolyzed because
free cholesterol forms crystals but the esters do not. Choles-
sterol is the source of clefts in formalin treated tissue. This
creates a mystery. Of course, there’s another cholesterol
esterase in the cytoplasm of cells, which is there to mobi-
lize cholesteryl ester. That’s stored differently, not in lys-
somes, but in cytoplasmic droplets. Perhaps some
cholesteryl ester escapes from break down of these packed
lysosomes and is hydrolyzed by the cytoplasmic esterase?

**Dr. Grabowski:** If you stain for free cholesterol, it’s
there. How it gets there and where it is located in the cell
is very difficult to say. These cells are very distorted. It is
microvesicular steatosis. But there is some macrovesicular
steatosis also. That’s part of the lipid droplets that could
very well have free cholesterol in them. There could be
cholesteryl esters that sneak out of the lysosome and get
cleaved by the neutral lipase and give you cholesterol
clefts. There could be some broken cell debris and so forth.
We’ve seen in other storage diseases—and, again, in
Gaucher where we’ve looked at some of the macrophages
that are really full, we see cholesterol clefts there, too. I
imagine this is just part of the cell organelles breaking up.

There is some very interesting pathophysiology that
needs to be worked out. One of the problems is that there
are few animal models for the human condition. The mouse
and rat models that we have are not very good for this.

Perhaps this is because they are more HDL-dependent. As
we find more patients with LAL deficiency, perhaps we can
find ways of studying these processes in more detail.

**Dr. Brown:** What do you believe the true incidence of
lyosomal acid lipase deficiency to be in its various de-
grees? How often do you think these genetic abnormalities
occur?

**Dr. Desnick:** I think it’s greatly underrecognized. There
are some common mutations, and we’ve looked at the preva-
ence of these in the general population. There’s’ ethnicity
in their occurrence. All the lysosomal diseases are uncom-
mon to rare, but I believe it’s surprising how frequent they
do occur. A primary care physician can expect to come
across them. I would say that LAL deficiency occurs some-
where around 1 in 50,000 to 150,000 individuals. Unfortu-
nately, many of these patients are not recognized until late
in the disease when they develop cirrhosis. In fact, many
may be misdiagnosed or undiagnosed for years.

**Dr. Grabowski:** Yes. The published literature indicates
that the prevalence varies between 1 in 40,000 (German
studies) and 1 in about 200,000 or so. It seems to have
settled down to about 1 in 150,000 or so. That’s based on
the assumption that a common mutation makes up 50% of
all mutations causing LAL deficiency.

**Dr. Brown:** This is the so-called German mutation?

**Dr. Grabowski:** Yes. The assumption is that it makes up
50% of the whole group. That’s probably not going to be
true. As we find more mutations, missense, nonsense, and
so forth, that number (50%) is probably going to shrink. I
think Dr. Desnick is right that the incidence is going to
be much greater than we thought in the past. It’s still a
rare disorder, but it’s going to be there and it causes
many different manifestations.

**Dr. Desnick:** What we’ve learned over time from the
lyosomal storage diseases is that they’re treatable. If you
start early and you have sufficient enzyme, you’re going
to reverse or prevent the severe and ultimately irreversible
manifestations. That’s what’s so exciting.

In most developed countries, screening is performed on all
newborns for certain genetic and metabolic diseases. How-
ever, with all the technology we have, we can’t really alter
the morbidity of these disorders, particularly because many
are progressive neuropathic diseases. We can only delay the
symptoms. In contrast, the lysosomal diseases such as
Gaucher type 1, Fabry, and LAL deficiency can be effectively
treated. There are more than 50 lysosomal storage diseases.
Of these, there are only seven with Food and Drug
Administration–approved enzyme therapies. If you start
early enough with an effective dose, you get clinical benefit
in virtually all of these 7 lysosomal disorders.

There’s also another half-dozen lysosomal diseases in
which clinical trials are under way, where scientists and
clinicians are working together to develop new treatments. I
think this is a very exciting area. We’re going to see this
continue to be clinically beneficial.

Relevant to our discussion, enzyme replacement therapy
has been in clinical trials for children and adults with LAL.
deficiency, most notably, the primary and secondary clinical end points in the phase 3 trial to determine the safety and efficacy of enzyme therapy were achieved. These results will be reviewed by the Food and Drug Administration and hopefully this treatment will become available for the patients with LAL deficiency.

Enzyme therapy allows us to replace the fundamental defect in these disorders. But there are other approaches that are being investigated in several of the diseases. One is to decrease the amount of the accumulated substrate by inhibiting or decreasing its synthesis. Others use chaperone therapy, the concept that if the enzyme has a residual activity—and maybe it’s only 1% of normal—the other 99% probably misfolds and gets trapped in the endoplasmic reticulum, and then is delivered to the proteosome for degradation. Thus, the misfolded enzyme doesn’t get to the lysosome. Providing a protecting molecule allows it to be transported to the lysosome without destruction. There are small molecules, such as specific competitive reversible inhibitors that will bind to the enzyme, stabilize it, get it out of the endoplasmic reticulum, into the Golgi where it gets glycosylated further, and then is delivered to the lysosome. That’s an encouraging additional approach for certain genetic disorders with residual mutant enzyme activities. Of course, people are thinking about combination or other therapies, for example, combine substrate reduction or a chaperon with enzyme replacement.

Dr. Brown: If you are right about the prevalence of lysosomal acid lipase storage disease in a diverse population, there should be several score individuals with this disorder in a large city. A medical system in a city of 5 million people may have 100 patients in its catchment area.

Dr. Grabowski: That’s true.

Dr. Brown: How do you suggest we find them?

Dr. Grabowski: They will be found in the clinics for hyperlipidemia. You find them in pediatric hepatology groups. The patients with the clues mentioned earlier are easily missed if you are focused entirely in the plasma lipid abnormalities. This is a new area that can provide specific benefits if recognized. I believe it’s important to get the message out there regarding the characteristic presentations.

There’s another important aspect. Some of the newer therapies for hypercholesterolemia may make the lysosomal disorders worse, particularly LAL deficiency. We need to be aware for that reason as well.

These new compounds, as they come out, are potentially really good treatments for hard-to-treat hypercholesterolemia as seen in LAL deficiency. So, it becomes important to screen for LAL deficiency, because you don’t know the consequences, in this condition, of PSCK9 inhibition for example.

Dr. Desnick: The other point is to consider LAL deficiency when the transaminases are increased. If you do a liver biopsy, the pathologist should be asked to consider LAL deficiency in the diagnostic evaluation. But I think many pathologists may not differentiate lysosomal from cytoplasmic lipid accumulation. They should use cathepsin D or one of the other lysosomal membrane stains to identify the lysosomes. Some are commercially available in kits. These stains allow pathologists to appreciate the lysosomal lipid accumulation and recognize LAL deficiency. This may change certain histologic diagnoses from nonalcoholic steatosis to LAL deficiency.

Dr. Brown: That would seem to be a very practical approach and one that is immediately available. Are there any other clinical or histological points we need to consider?

Dr. Grabowski: Just one really interesting major point about LAL deficiency. It doesn’t involve the brain. To me, this is an enormous surprise. It is present in the brain, but it appears to play—from Dietschy’s work and others—LAL does not appear to play a significant role in brain cholesterol metabolism.

Dr. Brown: Maybe it has something to do with the blood–brain barrier and that cholesterol or LDL transport into neuronal cells is different. The liver differs from most other tissues in that the LDL receptor is helped in its function by an “adaptor protein” that links it into clathrin-coated pits, which then fuse with the lysosomes. Other cell types such as fibroblasts do not have this adaptor protein and are less efficient in taking up LDL into lysosomes.

It is wonderful that mental function is not compromised in the children with this disorder, but we should be developing a strategy to identify affected individuals as early as possible and prevent the hepatic damage.

Dr. Grabowski: I certainly agree.

Dr. Brown: There are neonatal screening programs for genetic disorders already active and publicly supported in several states. Perhaps this is a potential starting point?

Dr. Grabowski: Being a geneticist, I have to agree with you completely. We must dispel the idea that genetic testing is expensive. Individual tests may cost $1000 or so. Managing hypercholesterolemia may generate hundreds of tests over years, but be off the mark. A single test, done once for a genetic mutation, can offer predictability of disease outcome and may focus appropriate therapy on a very expensive chronic disorder.

In no state in the union right now is LAL deficiency on the newborn screening panel. There are lots of reasons for that, but eventually it should come. I believe Austria is doing this and there are discussions about doing it in South America.

This is very important in the early onset variant. Without early detection, they develop very severe abnormalities and without enzyme therapy, they die. The earlier, clearly, the better.

The same is going to be true for the variants that come a bit later. You don’t want them to develop severe liver disease, requiring transplantation at the time you’re diagnosing them.

Yes, I think there should be panels and they should have certainly treatable, predictable outcomes. It’s not just LAL deficiency. It’s the other diseases as well.

Dr. Brown: I want to express my appreciation to Drs. Desnick and Grabowski for participating in this Roundtable
discussion on the lysosomal storage diseases. They have made it clear that these are now treatable with enzyme replacement therapy. From our discussion, it is very important for a clinical lipidologist to be aware of the often subtle presentation of these disorders. They can mimic other more common causes of hypercholesterolemia and of primary liver disease. Keeping in mind the characteristic but somewhat variable presentation of Gaucher, Fabry, and LAL deficiency can allow truly effective, even life-saving therapy in such patients.

Recommended reading