Central Symptoms and Comorbidities in Lysosomal Storage Diseases

Ozlem Goker-Alpan:

Hello, everyone. I would like to invite the audience for the last presentation of the CME series on lysosomal storage diseases. For the ones who don't know me, I'm Ozlem Goker-Alpan. I'm the founder and chief medical officer of lysosomal and rare diseases research and treatment center. On today's presentation we are going to focus on central symptoms and comorbidities in lysosomal disorders. But before we start, I would like to wish everyone a happy, healthy, prosper near year.

These are my disclosures. And as most who deal with lysosomal disorders know, lysosomal disorders, the majority of them have some sort of central nervous system or brain involvement. And some of them actually presents with pure CNS involvement with very little or no visceral involvement. And as a general rule, the ones that has a juvenile and infantile presentation, they do also present with the central nervous or brain involvement.

So the disease severity in this slide goes from MPS that can have attenuated forms and also some, the ones with the primary essential nervous system involvement to Fabry disease that doesn't have an infantile presentation, but it is associated with central nervous system involvement is presenting with strokes.

The neurodegeneration can begin in diseases such as in MPS types IVA and VII, type 2 Gaucher disease, GM1 gangliosidosis, Niemann-Pick disease type C, Fabry disease, infantile-free sialic acid storage disease, and mucolipidosis type II (non-immune hydrops fetalis).

So when we teach the medical students, we always remind them to actually test for lysosomal storage diseases as we are evaluating a baby with symptoms in addition to congenital infections and chromosomal disorders.

So to expand on this notion, that brain is an organ that not only regulates the electrical activity and others lively functions, it also regulates the mood, the behaviors, and also the emotions. So lysosomal disorders, in addition to primary nervous degeneration, they can present as a psychiatric disorder.

And one of the differential diagnosis of psychosis or psychotic presentation should be actually lysosomal storage diseases as well. So if you run through the list again, MPS 3 Sanfilippo types A through D can present initially a childhood as ADHD or pure hyperactivity or aggressiveness and follow by cognitive decline.

And these children initially was follow with a diagnosis of ADHD and they start falling off the curve educationally. And then before they present with the prime manifestations of Sanfilippo disease.

The other disease on the list is MPS 2 or Hunter disease. The milder versions they do not present with significant visceral or facial involvement may present with hyperactivity, follow by cognitive decline aggression. This could be part of the whole disease spectrum. And similarly mannosidosis can present with psychosis and hallucinations.

Niemann Pick C patients, types C1 and C2, can have a psychiatric presentation. These were two siblings that presented with bipolar disorders and nobody recognized actually they had that abnormality, and until they got diagnosed with ataxia and the other presentation of Niemann-Pick C in their mid 30s.

Similarly MLD, which is almost a pure CNS disorder can present similarly with ADHD, dementia that may mimic a frontal temporal dementia and psychosis. Similarly Tay-Sachs disease can present with depression, delusion, psychosis, and schizophrenia diagnosis may antedate the actual diagnosis of lysosomal disorder.

In Fabry disease, we do see depression quite often, and depression is we regard that as a part of the disease, rather than situational or secondary depression that is associated with presence of a chronic disorders. Gaucher disease, we often encounter anxiety and almost the patients that I follow are treated for depression or anxiety.

But having said that, the neuronopathic forms of Gaucher disease, we see, especially in Middle East countries, there is a report from Egypt that children exhibit hyperactivity, aggression, but my experience and the US experience is these patients may present with autistic behaviors prior to the diagnosis of Gaucher disease.

So I'm jumping from one subject to another, but now we are going to talk about little bit the comorbidities or the association of neurodegenerative disorders that we encounter in the heterozygous in addition to the homozygotes, that is characterized by the aggregation of proteins. To be exact, this is synuclein.

So we're going to be talking about the synucleinopathies and their association with lysosomal disorders. So this slide depicts the theory that a mutant lysosomal protein may affect the lysosomal functions on lysosomal structure and integrity, and may be a culprit for defibrilization of the aggregate proteins.

So in this case, we're going to be talking about synuclein that leads to ultimate neuronal cell death. So in addition to the mutation, so we are expecting this organelle dysfunction decrease aggregate clearance that is mainly the jobs of the lysosome. So lysosomal clearance is very important in the proteins that are aggregate prone and synuclein.

There is an earlier article that has shown that synuclein is the protein that actually that helps synuclein to shadow in the lysosome and synuclein may need the aggregated synuclein gets cleared through the lysosome.

So this brings us to obviously the association of Gaucher disease or the GBA mutations with Parkinson disease. So initially this was an observation, but through the major work of the NIH, we now know that glucocerebrosidase pathogenic variants are the major or the prime genetic cause or association with Parkinson disease and other associated synucleinopathies.

So this slide shows a publication from 2017, where the researchers looked, this was a multicenter work, and the researchers looked at the association of other lysosomal disorders in the patients with Parkinson disease. So they performed whole exome sequencing from more than 1000 patients with Parkinson disease, and they compare to control subjects.

So in the Parkinson cohort, there was a significant burden of variants that are associated with lysosomal disorders and with Parkinson disease risk. And obviously there was the strong association of glucocerebrosidase, GBA, or the gene that encodes for the Niemann-Pick C disease.

In addition to these known or already established loci, there was also association between CTSD, which associated with ceroid lipofuscinosis, CLC 1785, which sialic acid storage diseases, and ASAH1, which is associated actually that is the mutations cause fibro disease, and also SMA, spinal muscular atrophy, myopathy encephalopathy. And also implicated as candidate Parkinson disease susceptibility genes.

So these obviously expands the notion of lysosomal disorders associated with more common, actually most common neurodegenerative disorders, which is the Parkinson disease, dementia, MSA, and so on. And in this study, the majority of Parkinson disease cases have at least one damaging variant in lysosomal storage diseases, and about one fifth carry two variants.

So from this point on, I would like to give the word to Dr. Swati Sathe, who is a colleague and friend. Dr. Sathe has an extensive experience in the neurological manifestation of rare disorders to start with lysosomal diseases.

She's the current medical director of Cure Huntington's Disease Initiative, CHDI Foundation in New York. After receiving her medical degree from India and then she completed her medical residency at NYU Langone. She works and she achieved a fellowship in lysosomal disorders. And then she moved to research area where she was the director of the Pompe Clinical Development program at Amicus Therapeutics.

So in addition to her position as being the medical director of CHDI, she also is a volunteer neurologist and associate professor at Rutgers New Jersey Medical School. Dr. Sathe has extensive both clinical and basic research. So her primary research interests are enhancing awareness and diagnosis of rare disorders, exploring the natural history as it relates to endpoint development. So it's going to be our delight to listen to Dr. Sathe on today's presentation. Thank you.

Swati Sathe:

Thank you, Dr. Goker-Alpan for the kind introduction. I appreciate this opportunity to present on the central nervous system symptoms of lysosomal storage diseases. So thanks to CheckRare CE, LDRTC, as well as Affinity ME for this educational program.

Just to start off, there are a few disclosures. I have no conflict of interest and I will be presenting non FDA approved products and development for lysosomal storage diseases, and I'll indicate them as we go along. So just a brief slide, not that this particular audience would need an introduction, but the first description of lysosomal storage diseases was independently by Dr. Tay and Dr. Sachs in 1881, which constitutes the Tay-Sachs disease.

At the time, although it was not known that it's a lysosomal storage diseases, because lysosome was eventually discovered in 1955. And the first demonstration of the link between enzyme deficiency and the subsequent storage causing a lysosomal storage disorder was Pompe disease by Hers in 1963.

So in general, the scheme behind lysosomal storage disease is that there is a defective lysosomal acid hydrolysis of endogenous macromolecules, that leads to accumulation of these molecules in the cells with consequent, either impairment of the cellular function or in most cases, especially in the CNS cell death.

So I'm going to speak about the pathophysiology neurologic involvement in LSDs. But before that, I just wanted to give an overview of the sphingolipids pathway. There are other lysosomal storage diseases beyond sphingolipids. However, these constitute a major bulk and are more common than the other neuropathic lysosomal storage diseases

And what's very evident here is that each of these disorders is about one step away in the metabolic or the catabolic pathway in the lysosome. And although these are closely related in terms of their metabolic position, the manifestations of each of these conditions may be very, very different.

Now, not all of these are necessarily enzyme deficiencies. And we'll talk a little bit about those in the future slides. Some of these for example, especially type one form of Gaucher disease, for example, will have minimal to no neurological involvement. And so even though they may be one step away from each other, the manifestation of the disease may be totally different.

And this is generally considered to be because of the utilization of a particular macromolecule by that specific tissue type or cell type, and then subsequent accumulation of it when it is non-degraded leading to cell impairment. Not every cell type in the brain or in the body will use a particular metabolite in a similar fashion, produce it in a similar fashion or degraded for that matter in a similar fashion. And we attribute these differences in manifestations to mostly that aspect of anabolic and metabolic and catabolic pathways.

So just a few slides on the basics of lysosomal physiology..

And so, product A is converted to product B to C to D, bringing it down to the basic metabolic components, which may either be expelled from the cell, or maybe reutilized by the cell for future anabolism.

Now the products that are delivered to the primary lysosomes for degradation may either come from outside the cell by the process of an endocytosis as shown here, and the will fuse with the endosomes to imbibe those exogenous molecules, or there may byproducts of metabolism coming from inside the cell itself in the form of autophagosomes, which will then infuse with the lysosomes forming the secondary lysosomes.

Eventually when the catabolic processes are over, what's left behind is the residual body with this degraded material. Now what happens when there is a enzyme missing, so to say, the normal lysosomal degradation pathway is disrupted. And in this particular instance, the enzyme that converts the product B to C is missing, and that will lead to accumulation of product B being on the catabolic side, absence of C usually doesn't because much of pathology, but the accumulation of product B and the subsequent damage to the cell is what causes pathology.

So as we said, most often it is the missing enzyme or defects in synthesis of folding of the enzyme that lead to lysosomal storage disease. However, there are LSDs caused by other mechanisms, for example, activation defects such as either there is saposin deficiency or GM2 activated deficiency, in which case the phenotype is usually like the primary lysosomal disorder.

However, it is not because of the enzyme itself, but it is because of the deficiency of the activator of the enzyme. And the phenotype does get modified a little bit. There may be membrane protein defects such as Niemann-Pick C usually related to the cholesterol metabolism, or post translational modification defects targeting defects or defects in the fusion of the primary lysosome with the endosomes such as mucolipidosis.

So these are some of the other mechanisms by which lysosomal storage diseases may present itself. In case where there is an enzyme deficiency, a pure enzyme deficiency, the phenotype is largely related to the residual enzyme activity.

So the residual enzyme activity in turn is dependent on the kind of mutation that's present in the gene. So if the mutation is a severe or a deleterious mutation, such as a nonsense mutation, that indicates a stop code on or a frame shift mutation, which will also lead to either non formation of the protein or degradation of the protein.

In that case, the residual enzyme activity is almost nil, and that leads to a severe early onset form of lysosomal storage diseases. However, if it's a missense mutation and maybe away from the catalytic side, that will lead to some amount of residual enzyme activity being present, and the phenotype getting modified to a milder one and may lead to survival for even decades.

Now, the pathophysiology may be dependent just on the primary effect of the storage or secondary effects of the storage, such as macrophage or cytokine activation. And some lysosomal storage diseases do have an inflammatory component such as Krabbe disease.

There is a temporary form of the metabolite, usually called a physocine, and there may be impairment of the function or pathology related to this altered form of the metabolite, as we see in Fabry disease. And of course, once the capacity of the lysosome to accommodate for this accumulation is exceeded, then there's extra lysosomal accumulation of metabolites, and that will lead to further damage, which very commonly seen, say, for example, in Pompe disease.

Now that leads us to what kind of manifestations do we see in lysosomal storage diseases? So these are just listings. And of course, we don't expect that everybody knows this by heart. However, there is an

overwhelming theme. So the most common organ involvement is the eye. And you'll see a number of abnormalities starting from cataracts to retinitis pigmentosa and optic atrophy.

Now, if we see it, look at optic atrophy, a number of lysosomal storage diseases may be responsible for it. However, retinitis pigmentosa is quite specific for neuronal ceroid lipofuscinosis. So in some cases, a particular finding may be diagnostic of a particular lysosomal storage diseases, if the history that the patient presents with is consistent as such.

For example, the corneal walling Fabry disease is diagnostic of Fabry, very useful for picking up patients early in the disease even while undergoing the routine eye examination by an optometrist or an ophthalmologist.

More severe neurological manifestations, of course include myoclonic seizures, cortical atrophy, which maybe cerebellar or cerebral atrophy. Fabry is a unique disease where blood vessels are affected. And so stroke-like events are very common in fibro disease as a neurological involvement.

And then manifestations related to a specific area within the brain. So ataxia may be related to cerebellar atrophy or hyperplasia, extrapyramidal signs related to basal ganglia involvement, peripheral neuropathy, and then dementia or psychosis.

Psychosis or behavioral abnormalities are in general, not that common in lysosomal storage diseases. However, we do see them with later onset, GM2 gangliosidosis or later onset Tay-Sachs disease. In Sanfilippo disease or MPS type 3, especially in children, the behavioral disorders may be more pronounced hyperactivity, difficult to control behavior, aggressive behaviors about the behavioral manifestations of LSDs in very specific disorders.

So neurological involvement in LSDs is very heterogeneous. And as we said, it may be related to the residual enzyme activity, but I would like to highlight the spectrum of neurological involvement and the variation of the phenotype based either within a subtype of a disease. So for example, Gaucher type 3, and all the variations within Gaucher type 3, maybe related to a specific mutation.

Or it may be allelic disorder, however, present in a very different manner or variation within a subtype of a disease. And so, as we were speaking about Gaucher type 3, these are four individuals with Gaucher type 3, these diagnosed Gaucher type 3, each one of them may have a different mutation. The age of onset, the degree of hepatosplenomegaly and the degree of neurological involvement is very different in each of these patients.

Some phenotypes are very mutation specific, and I have highlighted two here. One of them is a homozygous D409H mutation in Gaucher disease, which causes a type three variant. However, it also causes cardiac involvement.

And on the right we see the axial chest CT showing the aortic wall classification as well as the hydrocephalus, which is very specific to this particular mutation in this sub type of Gaucher disease type 3. And we do not have enough explanations as to why this particular mutation should cause an additional involvement of the aorta or the heart. And, there's clearly a genetic modified effect here that we have not elucidated yet.

Now within the same mutation or within the same family, here is an example of siblings with Fabry disease. On the left is the brother who was the oldest sibling, who already at 17 had weakness of the hand. And the overall illness was fairly rapidly progressive, eventually became bed bound with spasticity in all four limbs, and had a much more severe phenotype than his sister, who we happened to see when she was 29 years old and had a very, very localized involvement.

And if you look at the MRI on the side, she had temporoparietal lesions just located on one side of the brain. That's on the right side of the brain, and she had specific weakness on the left side. And what she

really manifested along with the weakness and the spasticity where the arm would do things such as, pull her hair or grab things without she having any control over that activity, which was very unusual for this kind of presentation.

Now, this kind of presentation without the history of the brother may very well have been missed for other demyelinating disorders, such as MS, which is very often what happens in late-onset leukodystrophies, that if it's an unusual presentation, then other demyelinating disorders become, or may seem more likely in the differential diagnosis than say a storage disorder.

And then within a subtype variability. So here I would, as we said, the general scheme is that, more severe the mutation, earlier is the age of onset, more severe is the phenotype, and the survival may be limited.

Now in case of severe classic infantile forms of lysosomal storage diseases, such as Tay-Sachs or Krabbe, they're not very heterogeneous. The onset is fairly homogeneous and the progression is also homogeneous. But as soon as it becomes a little late-onset, and there is a residual enzyme activity, then the age of onset and the progression may really be very, very different, even though they're all, say juvenile Tay-Sachs or later onset Tay-Sachs.

Now, this is very important because in situations where therapy is available, for example, in Pompe disease, even a small change in the enzyme activity will change the survival or will change the outcome of the patient, even when treated with enzyme replacement therapy.

So for example, I put this example of Tay-Sachs disease here. We have three forms, the infantile onset at around six months, and then juvenile onset, we say early to mid childhood, and later onset say beyond teenage. And if you look at the manifestations and the involvement of the organelles of the brain, it is very, very different.

And what is most striking is that cherry red spot, which is the highlight and which is the diagnostic feature of most of the infant-onset is totally absent in late-onset Tay-Sachs.

And spasticity is really what happens in the beginning and eventually there may be hypotonia. cerebellar involvement is not seen in infantile and not even recognized as a say, a pathological feature. However, severe cerebral atrophy and ataxia dysarthria are the prominent features of late-onset Tay-Sachs. So the variability as the residual enzyme activity increases is drastic in some of the lysosomal storage diseases.

And here's just an example of how attenuated can the phenotype be. And here I'll give example of Pompe disease because classically Pompe disease is an infantile presentation onset before the age of six months. Severe failure to thrive cardiomegaly and untreated death usually occurs around age of one year or a little later.

And what we see here is a 78 year old woman who has had muscle weakness for 26 years. And for about 20 years of those, she was diagnosed as limb-girdle muscular dystrophy. She had extensive workup. She had two muscle biopsies, several EMGs, and the way this particular patient was diagnosed was that she was not happy with the diagnosis of limb-girdle muscular dystrophy, because there are several subtypes of limb-girdle muscular dystrophy, and she wanted to know what exact subtype she belonged to.

At the time, genetic testing was very expensive and diagnosing a particular subtype of limb-girdle muscular dystrophy was probably not going to be helpful for her. So we decided to test her for Pompe disease because therapy was available. And we explained to her that if she has Pompe disease, then she might actually be able to be treated and we regard that as more important than ascribing a specific subtype to her muscular dystrophy.

And that's how we send for testing, never, ever expecting that the test will actually be positive. And lo and behold, she did have low levels of alpha-galactosidase and eventually had two mutations in alpha-

galactosidase diagnostic of Pompe disease. I think the one curious part about patient is that then she declined therapy for Pompe disease.

Now, as we said that sometimes we may have a little different phenotype, but overall, the presentation fits a particular gangliosidosis or a particular lysosomal storage diseases. And very how often may happen because of an activated deficiency and not because of deficiency of the primary enzyme.

And here is one that was because of a GM2 activator deficiency. This particular patient presented at 15 months, which is a little later than what classic infantile Tay-Sachs patients would present. And she had failure to thrive. She did have a cherry red spot. She had macrocephaly, and she had easy startle response.

Her brain MRI, this time was unremarkable. She had seizures and a very abnormal EEG. Overall the presentation was like gangliosidosis likely GM1 or GM2. And as we said, this was because of a GM2 activator deficiency and not because of another deficiency.

This is one other patient who really had all hallmarks of a storage disorder. She had progressive involvement of one neurological organelle after another, starting with cognitive decline, indicative of more dementia or a gray matter disorder, seizures. And then myoclonus eventually had a peripheral involvement with muscles and anti EMG showed anterior horn cell involvement, then which led to eventually involvement of the lung function. She didn't have a cherry red spot, and finally extrapyramidal involvement with dystonian Parkinsonism.

And as you can see from age of six to nine, really, there was no diagnosis. This patient underwent extensive testing, and eventually the whole genome sequencing identified mutations and making this a diagnosis of neuropathic Farber's disease. Neuropathic Farber's disease has really been described only in the past, I think, 10 years or so. And cases have been reported, mostly picked up on whole genome sequencing. So this may be one case where a newer modality has been able to identify a newer phenotype of an older disease. And this was very unusual.

So now that we have focused on the spectrum of the lysosomal storage, neurological spectrum of lysosomal storage disease, and the whole idea of pathophysiology and how it might lead to clinical signs and symptoms. We might focus a little bit on therapy. I will not focus on the workup that is required for lysosomal storage diseases.

Most of the time, if a storage disorder is suspected, it is fairly easy these days to get an enzyme estimation for any of the disorders, as well as genetic testing. There is testings available where a single gene need not be tested at a time. And therefore, suspicion of a storage disease is the key, because if it is suspected, then diagnostics are much easier in the past, say 15 to 20 years than they were before.

Again, an infantile form of a storage disease is really not difficult to pick up. In that case, the urgency often is for disorders that have therapy. The likelihood of a therapy working is much higher if the disease is picked up as early as possible in life. And for example, in Pompe disease, the time lag of two months can mean a difference of survival or being on a ventilator or having severe comorbidity.

So in infantile forms of storage disease, the urge is to make early diagnosis, but it is very difficult to miss those. The late-onset forms of lysosomal storage diseases on the other hand are very divergent in presentation. And each of them may overlap with some other more common neurological acquired disorder or either it's an inflammatory disorder or denominating disorder, whatever the case might be.

In that case, the likelihood of misdiagnosis is very high. Delay in diagnosis for storage disorders that are late in onset is sometimes to the extent of a decade or so. Therefore suspicion of a storage disorder is the most important key in making the appropriate diagnosis at the appropriate time.

Very often, it is useful to just list out or enumerate each of the sub organs of the brain that are involved. And if more than three brain systems seem to be involved at the same time, and there is a history of slow progression or neurodegeneration, then a storage disorder should be suspected.

A family history really, really is not relevant most of the time. A sibling might be affected, but not necessarily. And therefore we don't rely so much on the family history for autosomal recessive disorders to make the suspicion stronger.

But there is a role for genetic counseling, especially of the siblings, and for family planning, where even autosomal recessive disorder is diagnosed. And therefore early diagnosis and intervention is still relevant and key for late-onset forms of lysosomal storage diseases.

Now, as we were saying, therapy for neurological forms of lysosomal storage diseases is more difficult than the other disorders where systemic involvement is predominant and neurological involvement may not be there. And mostly it is because of the blood brain barrier issue.

So on the right if you see the picture, lining of the blood vessel is usually porous, meaning that the two cells are really not in contact with each other. And that leaves a room for passage of molecules or medications from the blood vessel out into the tissue, and therefore for systemic treatment, that usually is not a problem.

But when you come to the endothelium or the lining of the blood vessels in the brain, these are really characterized by tight junctions. And therefore there is no room for molecules to pass from inside the blood vessel into the tissue.

These tight junctions are in additional supported by the glial cells that form a second layer around it. And therefore brain is essentially protected from any macromolecule entering the brain by this kind of a design.

So the skeletal meninges have a physical protection around the brain, and the blood brain barrier has a structural or a physiologic protection for the brain to not get affected by a number of toxins or other chemicals. Only small molecules can pass, fat soluble molecules can pass, but we are stuck with this kind of a system for any other therapy that we might want to introduce in the brain.

Now, in cases such as meningitis, when there is inflammation, there is breakdown of this blood brain barrier. And that works to our advantage because in that case, antibiotics can pass through and we can treat illnesses like meningitis by antibiotics because of the breakdown of blood brain barrier.

However, in chronic disorders like lysosomal storage diseases, there's hardly any inflammation and no reason for a breakdown of the blood brain barrier. So all the current therapies, the enzyme replacement therapies are not effective for the neurological forms of the disease. So for Gaucher disease, for example, type one will easily be treated by enzyme replacement therapy, however, type two, and type three, there will be a systemic response. The liver and spleen size will decrease. However, the neurological manifestations will not change. The neurological prognosis will not change with enzyme replacement therapy.

So there are newer strategies that we can apply to circumvent this blood brain barrier issue. Now, one thing that can be done is that the drug can directly be introduced into the CSF and thereby bypassing the blood brain barrier. And this can be done either intrathecally by doing say, spinal tap and introducing the drug into the lumber space, or directly placing electrodes into the brain and introducing the drug into the CSF by that method.

Now as you can see that it is not the easiest thing to do. Repeated lumbar punctures will usually cause chemical arachnoiditis. And we are already seeing some of the long term effects of these repeated intrathecal injection in disorders.

There are also a number of therapies now that are trying to introduce the enzyme replacement therapy by direct placement of catheters in the brain. So that's a possibility.

Second possibility is either decrease the peripheral degradation of lysosomal enzyme replacement therapy that is being given. This is done by either inactivating the terminal sugar chain so that the mannose-6-phosphate receptor is not utilized for degradation, and then allowing higher levels of enzyme in the periphery, and so by sheer concentration gradient, some of the drug can enter the brain. So that's one other possible mechanism.

And we see some places where this is being utilized. Third possibility is using fusion protein. So because there can be a receptor mediated uptake in the brain through the endothelial cells, we provide a side chain or we provide an additional poly peptide, which can be picked up by the receptor that is fused with the enzyme replacement therapy.

And so essentially because of this polypeptide side chain, the entire molecule will be picked up and we'll see that this is being applied in some newer enzyme replacement therapies to allow for ERT to enter the brain. And a similar concept of nanoparticles, where the nanoparticle is conjugated with the enzyme, similar uptake through the endothelium because of the recognition of nanoparticles.

So these are some of the modalities of therapies for lysosomal storage diseases involving CNS. And we will go through some of them. Mostly we are relying either on gene therapy or cell therapy, and then let's see what small molecules can do in this space. So we start with the approved therapy, which is enzyme replacement therapy for neuronal ceroid lipofuscinosis.

As we said, this involves direct catheter replacement into the brain and an enzyme infused every two weeks. The enzyme was approved for late infantile neuronal ceroid lipofuscinosis type two caused by tripeptidyl peptidase 1 deficiency in patients who are three years of age or older.

Now this slowed loss of ambulation in symptomatic subjects. Not much information is available on language or seizure control, which are the two other major manifestations of NCL. The procedure related complications still are major adverse event where placement of the catheter may cause CNS infection. And again, the device needs to be replaced every four months. So it's a very complicated process and we are following the evolution of this therapy over time. And let's see how it works out.

This allogeneic hematopoietic stem cell transplantation was one of the first therapies that was devised for neuropathic lysosomal storage diseases. It did not pan out for several orders, but for MPS 1, it is still the treatment of choice and it's essentially bone marrow replacement. And the idea is that once we replace the bone marrow, the stem cells eventually migrate to the brain and settle there as microglia.

And basically the enzyme that is deficient allowing for receptor mediated uptake of the enzyme secreted by these microglial cells. And in this situation, then the donor of the hematopoietic stem cell has to be a non-carrier.

For MPS 1, this therapy has really panned out to be an excellent therapy, especially if diagnosed early and treated early, the outcomes are fairly good. For other LSDs, this has not really worked very well.

So the other possibility, if we don't want to have a bone marrow donor, stem cells are isolated from the patient themselves, then a gene therapy is introduced into these isolated stem cells, such that now the additional gene expresses the deficient enzyme. Those cells are replaced back into the patient.

So there's not much chance of rejection of the bone marrow because it is essentially the patient's own stem cells and therefore immunological reaction is less likely. And the stem cells that have now been engineered to secrete this enzyme will provide the enzyme in an overexpressed manner. Again, the same idea, these cells will become resident microbial cells in the brain and express the enzyme that required enzyme.

There are some combination therapies like intrathecal laronidase with allergenic bone marrow transplantation that is being tried for say MPS 1. There is also chemically modified variant of sulfamidase being tried for MPS III that allows entry of this modified variant into the brain, and therefore expected to have impact for patients with MPS III. All of these modalities are in development. Not yet approved by the FDA undergoing clinical trial.

As we said before, we will try ERT with fusion protein. And one of the ones that is being used currently, especially for MPS is human insulin receptor. And so human insulin receptor antibody is tagged along with the enzyme replacement therapy. The human insulin receptor will recognize the antibody, allow for endocytosis of the antibody, with the enzyme tagged to it.

Swati Sathe:

And so these additional proteins or the additional peptides attached to the enzyme replacement therapy really behave as trojan horses, allowing for the entry of the enzyme to bypass the blood brain barrier. Some other molecules that are being tried, or some other peptides that are being tried for a similar kind of approach is apolipoprotein, transferrin, and insulin-like growthfactor 2.

And then that brings us to gene therapy. Again, we spoke about ex vivo gene therapy, where we isolate the bone marrow cells, introduce a gene therapy in them and reintroduce the same bone marrow cells into the patient. And that is autologous bone marrow transplantation with engineered cells, and then hoping for the overexpression of the enzyme, and as we said, replacement of the microglia in the brain with these new modified stem cells and replacement of the deficient enzyme.

In vivo gene therapy is directly introducing a viral vector that has been modified and expresses the deficient enzyme. It is introduced either peripherally, but again, the question of whether it is neurotropic and whether it'll reach the brain always remains.

So some of the gene therapies will directly introduce this viral vector into the brain parenchyma. This is a one time therapy hoping that this viral vector will then continue to secrete the enzyme that is deficient and provide the enzyme replacement that is much needed.

Now that brings us to therapies that are under investigation for the ease of administration, which are the small molecules. These can be given orally. They may not have as many adverse events as say gene or autologous bone marrow transplantation. But they do have their own limitations in terms of whether they will work on all forms of the disease and whether they be as effective as say enzyme either ERT or gene therapy.

So a pharmacological chaperone, this is a very opportunistic idea, which has really worked for some of the lysosomal storage diseases. As we said, that when there is missense mutation, that does not lead to a premature termination of the protein chain or degradation of the protein chain, there may be a misfolded mutant enzyme that is actually produced by the endoplasmic reticulum. Now that is actually producing the ribosome.

However, because the cell has a mechanism to recognize a misfolded mutant enzyme and send it for endoplasmic reticulum related degradation of this misfolded enzyme, the residual enzyme activity may actually be very, very low.

So there are some endogenous chaperones that we have, but in this situation, we are giving an exogenous chaperone that will stabilize that misfolded enzyme and allow the enzyme to reach the lysosomal and execute whatever residual enzyme activity it is able to do in this, because even a slight increase in the rest enzyme activity can really mean a big change in the phenotype.

These being small molecules are not expected to have very big side effects. And therefore, when they work, this might be a very effective mode of treatment for the disorders caused by missense mutations or more milder mutations.

Now, the table lists a number of enzymes and there's specific chaperone. So as expected for each enzyme, there'll be a specific chaperone molecule. So far the one that is approved is for alpha-galactosidase and the molecule is migalastat. Whether this will have impact on the neurological manifestations of Fabry remains a question because Fabry is very specific.

The neurological manifestations are not necessarily related to the primary involvement of the brain, but because of the blood vessel involvement and stroke like syndrome related to the blood vessel involvement.

ambroxol is used in Gaucher disease, even in neuropathic Gaucher disease. There have been trials of pyrimethamine and HexA. However, tolerance of pyrimethamine on a chronic or a long term basis is not really the best. So more work needs to be done in that regard.

Now, similar small molecules that are used as chaperones may actually work as inhibitors of the enzyme when they're given in very high or slightly higher doses or slightly hard concentration, because they do bind to the enzyme. And so if the concentration is high enough, they may actually be inhibitors of the enzyme and this is the idea behind substrate reduction therapy.

Small sugars that can attach to enzymes and daily behave as enzyme inhibitors. And therefore for Gaucher disease, there are two substrate reduction therapies approved miglustat and eliglustat. And there are ceramide synthase inhibitors, which lessen the formation of glucosylceramide, the molecule that is accumulating in Gaucher disease. So if we cannot take care of the accumulated molecule, the idea here is to not let the accumulated form be produced in such a high quantity so that we maintain the balance of non accumulation.

A couple of other cyclodextrin was tried for NCP1 intrathecal intravenous, and then genistein for NPS. So these are some of these substrate reduction therapies, either approved or being worked on.

Now for diseases say for Duchenne muscular dystrophy, there has been an approved therapy for read through of the DNA that is a piece of the DNA that is causing pathology. So in this case, for example, in the lysosomal storage diseases situation, if we can read through the premature stop code on and continue the protein chain to be formed, it may actually again, have a residual enzyme activity that may be relatively good depending on where that premature stop codon occurred.

And this is being tried as a methodology of therapy for lysosomal storage diseases, where we allow for read through, through the stop codon. This is already known for drugs such as aminoglycoside and gentamycin. However, the drug itself gentamycin is quite toxic and has side effects of kidney damage and hearing loss, and therefore cannot be used on a long term basis.

So similar drugs are being tried to see if we can bypass the toxicity of aminoglycoside and use it for therapy for MPS. And ataluren, which was originally tried for Duchenne, is maybe tried for Krabbe disease, but we are yet to see any action in that regard.

And then protein homeostasis is affected in lysosomal storage diseases because of the accumulation of the substrate and the downstream effects from there. And therefore use of proteostasis inhibitors as treatment for or as a treatment modality, I would say for NPC has been tried with vorinostat and bortezomib.

Again, we'll see if it eventually becomes an approved therapy and what is the degree of improvement that occurs in symptoms or presentation related to these drugs. And some new modalities of therapy are coming for symptomatic treatment of N-acetyl-L-leucine therapy in Tay-Sachs and NPC.

Now these are all curative therapy as we saw where we are trying to either increase the enzyme activity, or trying to replace the deficient enzyme. However, it is very important to also focus on symptomatic treatment of neurological symptoms in lysosomal storage diseases. Most importantly, seizure control, which can be very, very difficult in a number of gangliosidosis and even other lysosomal storage diseases.

Simple therapies for management of say drooling or management of spasticity, management of extrapyramidal symptoms, such as dystonia or Parkinsonism, whatever therapy could be offered for symptomatic benefit is always balancing it with the side effects of the drug, but always makes the management of the patient easier as well as improves the quality of life for the patient themselves. And so there is no reason to underestimate the use of symptomatic therapy for each of the neurological symptoms in lysosomal storage diseases.

Additional therapies such as physical therapy or use of devices say for prosthesis for spasticity, or even injections for spasticity, all of those could be tried for each of the individual symptoms, neurological symptoms in lysosomal storage diseases.

This particular presentation does not focus on the symptomatic therapies. But again, it's very important to identify and treat or identify and try to ease the discomfort related to these symptoms of lysosomal storage diseases. Hopefully some of the upcoming therapies provides a breakthrough for availability of the enzyme or the pathology modification in any of this lysosomal storage diseases.

And that is what really the field is waiting for, for a breakthrough to allow the neurodegenerative diseases to be ameliorated and not just in lysosomal storage diseases, this is a need or a requirement across the board for neurological or neurodegenerative disorders. And we'll see how the field does over the next 10 years or so. Hopefully this presentation and was helpful and provided an overview of the neurological involvement in LSDs. And now I think it's time for any question.

Q&A

Ozlem Goker-Alpan:

I would to thank Dr. Sathe for this very detailed and almost enlightening presentation. I'm particularly thankful to her because she also covered the therapeutic options, which we did not so far touch base. Before I open the Q and A session, I would like to invite the attendees to do the brief survey. And also I would like thank all our sponsors that helped us to actually put these CME sessions in reality.

So I will read the questions. Please, the ones that have a question, submit through the Q and A that I will present those questions to Dr. Sathe. And also please fill out the survey that will help us to improve the CME on lysosomal diseases. While waiting for the question, I'm just going to ask actually, probably a hypothetical question. So we are now, this is the era of gene therapy, I think, is that right?

We are moving from the 2010s, the small molecules substrate inhibition, the chaperones to gene therapy. So obviously that is, we are having the first in human trials for the primarily neurological disorders, such as GD2.

So what do you think we can achieve by gene therapy? Meaning are we going to be able to successfully treat, I'm not saying cure, the disease, or we are going to see a modified phenotype that is going to be emerging after gene therapy? This, especially true that we were discussing this about 20 years ago or 15 years ago for Pompe disease, infantile Pompe disease, and it evolves to something else with the success of systemic ERT.

Swati Sathe:

So I think the best that we can expect at this time is that we have a modified phenotype. It will also very highly depend on at what time the therapy was initiated. So earlier the treatment, then the possibility of a better outcome is much higher. And that's true for any neurological disease.

And the permanent question of diagnosing a disease before it's onset, and then the possibility of treating it before the onset of symptoms. But then how do you monitor given the variability in the presentation, whether the timing was right. So that will be a question for a long time, I think.

Ozlem Goker-Alpan:

I mean, I just want to make this as a little chat for the listeners or the audience. The reason is, I mean, this brings us to the question, whether some of the presentations, such as GD two, is it already too late to treat these patients? Because I mean, we have some evidence that actually some phenotypes such as in GD II, we know that it's already there is a neural effect.

So it all involved four patients with GD III, and they all had almost close to zero enzymatic activity. And so basically some of them went to college, some of them died. Actually, the baby died of really with myoclonic epilepsy years later, and the middle patient actually that was transplanted years ago and they died of complications.

So, I mean, genotype is helpful, obviously to predict, but is not 100% clear to whether some patients will go to GD II versus GD III. I mean, obviously there is a neuropathic involvement. So how would you comment on the genotype phenotypes and how would you choose the patients for such trials?

Swati Sathe:

So, again, doing a trial before in presymptomatic cases is a very difficult task given that we, unless there is a very, very strong biomarker, or there is a very clear cut estimation of progression and time of onset and such. We barely ever see trials that are initiated prior to onset of symptoms. At least thus far.

That would be a very challenging, and that's the reason for emphasis on the biomarker discovery and so on and so forth. If there's no endpoint, if the patient has no symptoms, then there's no endpoint to demonstrate.

Ozlem Goker-Alpan:

Exactly. Is it by lack or is it a success? Is that right? I have two questions in the Q and A part. So first question is, can we infer that the neurological symptoms like optic involvement all have the same pathophysiology or the involvement of ostracized glial cells, et cetera? That's the first one.

Swati Sathe:

Well, there is always involvement of the ostracized glial cells. But for most part, we would believe that it is a reactive involvement because the primary metabolites in lysosomal storage diseases are actually components of the neurons, either of the gray matter or of the white matter myelin.

Swati Sathe:

And very often we believe that the primary dysfunction is either of the myelin itself or the neuronal cell body itself. And the astrocyte involvement is reactive. I mean, there are leukodystrophies, which are primarily glial in on-set such as Alexander disease, but those, we considered to be rarer that the glial involvement is primary. But with any degenerative disease, we always expect some reactive involvement of the supporting cell.

Ozlem Goker-Alpan:

Well, the second question is, is there any open trial for late-onset disorders? I mean, there are trials for late-onset disorders.

Swati Sathe:

I know that there's one, at least for late-onset Tay-Sachs.

Ozlem Goker-Alpan:

They're asking for Gaucher. I mean, usually there is late-onset from Gaucher, what we mean either GD one, usually the trial start with the adult patients anyway. So they [inaudible 01:04:50]. Can you comment on the particular late-onset Tay-Sachs?

Swati Sathe:

I think it's a chaperone therapy for late-onset Tay-Sachs. That's an therapy. Again, I'm not very sure of what the endpoints are, but given the very heterogeneous presentation that will again be very challenging.

Ozlem Goker-Alpan:

If we're talking about the attenuated forms, actually their gene therapy trials that are currently be available for attenuated forms of MPS II. So in general, it's a proof of concept, we start with the adult presentations in the adult patients, usually as a rule, these patients have the later onset of the attenuated forms.

Swati Sathe:

I would also like to say that usually what happens with the diseases like SMA or lysosomal storage disorders is that the trial is usually initiated in the severe most forms if they're infantile or late infantile. The assumption being that if it worked there, then the demonstration of efficacy in a later onset form will be easier. And sometimes the FDA's considerate and may give approval as such for all forms, if there was a demonstration of benefit in the most severe forms. So sometimes that's a consideration as well.

Ozlem Goker-Alpan:

Okay. All right. I guess these are the questions, and we thanked all the audience and stay tuned. This actually concludes our first year of the CME. So stay tuned for the season two. Thank you.