Zellweger Syndrome: A Genetic Disorder That Alters Lipid Biosynthesis And Metabolism

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Citation

Abstract
Zellweger Syndrome (ZS) is a rare, but lethal, inherited autosomal recessive disorder. Patients with ZS lack functional peroxisomes caused by deficient peroxisomal assembly. The peroxisomal deficiency results in abnormal biochemical pathways including oxidation of very-long-chain fatty acids and synthesis of bile acids, plasmalogens, ether lipids, and docosahexaenoic acid. There is no cure for ZS. Death occurs by the age of 6-12 months. The treatments of ZS are based on palliative and supportive care to optimize quality of life for affected children. In this paper an authentic case is presented and the abnormal biochemical pathways and diagnosis of the disease are discussed. The rationale behind palliative treatment and the role of healthcare providers in supportive care is emphasized for both affected children and their parents.

CASE PRESENTATION
Grace was born on May 12, 1998 and weighed 6 lbs and 10 oz. It was evident at birth that something was wrong. She was very floppy and her muscle tone was so poor that she could not suck much at all. Grace was placed in feeding tube when she was 2 weeks old. One month later, she was diagnosed with Zellweger Syndrome (ZS). She was on breast milk for 3 months and then was put on Pregestimil. She never had any problems putting on weight. She, like most Zellweger babies, had seizures. Grace also had an enlarged liver due partly to the fact that her body could not metabolize long-chain fatty acids. She saw a gastrointerologist that prescribed her Actigal for liver function. It seemed to help cleanse her liver. She also took Phenobarbital and Tegretol for seizures and Zantac to help with stomach problems. She regularly took ADEK vitamins. She had a pulmonologist who took care of her also. In addition, she participated in a study about the effects of giving DHA to babies with Zellweger. Grace was hospitalized 3 times; twice for pneumonia and once for unexplained fever. The first time she went into the hospital her pneumonia was severe. She was about 6 months old and she stayed on continuous oxygen. A suction machine was regularly used to clear her secretions. In addition, she was given regular breathing treatments with Pulmicort Respules. Grace never moved a whole lot. At first she would move her head, feet, and arms a little but gradually, she didn't move at all. Most of the time she was sleeping except when she was having seizures. Grace lived to be 12 ½ months old. She died on May 28, 1999, two weeks after her first birthday party.

Because of ZS is a recessive gene disorder, there was 25% chance that Grace parents could have a second child with ZS. Grace brother, Samuel, was born on July 6, 2000. Zellweger can be detected during pregnancy with an amniocentesis. However, Grace parents chose not to have one done, and as a result, they did not know that Samuel had ZS until he was born. Samuel had a weak cry just like his sister, Grace. From the beginning he moved a lot more than Grace did and his seizures weren't as severe. Samuel started taking Phenobarbital when he was about 2 months old. Samuel also took Actigall, ADEK vitamins, Zantac and Pulmicort Respules. Samuel would kick and smack his lips, even though he did not take anything by mouth. Samuel was just 4 days old when they put in his feeding tube. He also had a Nissen Fundoplication surgery to keep him from refluxing. The Nissen is where they loop his esophagus to keep reflux down. He stayed in the hospital for 2½ weeks before he came home. He was much livelier than Grace; kicking and fussing when it was time to eat. When he was about a month and a half he began to have blood in his stool. He was much livelier than Grace; kicking and fussing when it was time to eat. When he was about a month and a half he began to have blood in his stool. Samuel began to have trouble gaining weight. He originally was taking Nutramigin but switched to Pregestimil because Grace had done so well on it. It made things worse for him. It seemed that everything went straight through him. His gastrointerologist, finally diagnosed him with “Dumping Syndrome”. He wasn't keeping anything in his system long
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enough to gain any weight. The “Dumping Syndrome” was caused by his surgery. He was also put on the formula Neocate. Samuel was hospitalized twice, not counting when he died. Samuel also had the blood clotting factors and had to take vitamin K shots. Grace didn't. He went in both times for pneumonia. Samuel also had to have regular breathing treatments. Samuel died on March 12, 2001 when he was 8 months old.

**The above Case is an authentic medical story. Tanna and Frederick Brumfield are biological parents to Grace and Samuel. Mr. and Mrs. Brumfield have written the above case which is a shortened version of what they wrote at their home page at:

http://www.geocities.com/peroxisomal_disorder/grace.html
and
http://www.geocities.com/peroxisomal_disorder/samuel.htm

INTRODUCTION

One of the unique and characteristic differences between prokaryotes and eukaryotes is that a prokaryotic cell is a “one compartment” organism with no organelles. In contrast, a eukaryotic cell is made of many subcellular organelles. Among the unique organelles within the eukaryotic cell is peroxisome. Although it has been suggested that peroxisomes are autonomous organelles of endosymbiotic origin, the controversy still exits as recent studies show that peroxisomes are originated from endoplasmic reticulum (1, 2).

Peroxisomes were discovered in 1965 by Christian de Duve who later was awarded the shared Nobel Prize for Medicine in 1974. Peroxisomes are organelles of the cytoplasm found in all mammalian cells except in the erythrocytes. Their functions include α and β oxidations of fatty acids, biosynthesis of bile acids, ether phospholipids and docosahexaenoic acid (DHA). Dysfunction of peroxisomes leads to many disorders. Up to date, 17 different disorders are known to be related to peroxisome dysfunction (3), wherein Zellweger Syndrome (ZS), a rare autosomal recessive disorder, is the most severe one. The molecular mechanisms causing ZS is unknown at present. However, the excess of very-long-chain fatty acids (VLCFA) in the brain has often been hypothesized to play a major role in the development of ZS.

PEROXISOME, A MULTI-FUNCTIONAL CELLULAR ORGANELLE

Peroxisomes are single membrane bound cellular organelles present in all mammalian cells except erythrocytes. In mammals, they are abundant in the liver and kidney. These small multi-functional organelles are involved in a series of vital cellular reactions such as free radical detoxification, α and β oxidations of fatty acids, and biosynthesis of bile acids, DHA, and ether lipids. It has been suggested that peroxisomes vary between different organisms in their enzymatic content, size, and number (4). Peroxosomal proteins (called peroxins) are encoded by nuclear genes (called PEX-genes) and are synthesized by cytosolic ribosomes. The synthesized proteins are transported into peroxisomes. This indicates that there must be a transport system that facilitates the transportation of newly synthesized proteins into peroxisomes (3). Up to date, 15 human PEX genes have been identified.

α OXIDATION OF FATTY ACID

Branched-chain fatty acids, such as Phytanoyl-CoA, cannot undergo β oxidation due to the presence of a methyl group at the β carbon of fatty acids (see figure 1). However, peroxisomes have the necessary enzymes to make a new unmethylated β carbon, through α oxidation, to facilitate the subsequent β oxidation process. Milk and animal fats are rich in Phytanoyl-CoA.

Figure 1

Figure 1: α and Oxidations of Fatty Acids

β OXIDATION OF FATTY ACID

Mitochondria of animal cells are the major sites for β oxidation of fatty acids. However, peroxisomes of animal cells, similarly to mitochondria, carry out the four fundamental steps of β oxidations, namely: initial dehydrogenation, hydration, dehydrogenation, and thiolytic cleavage. Despite this similarity, there are three major differences regarding the oxidative mechanism of fatty acids in these two organelles of animal cells:

1. The initial dehydrogenation reaction of the β oxidation is different in mitochondria and peroxisomes. Mitochondrion uses its initial dehydrogenation step to oxidize fatty acids to
generate electron carrier coenzyme, FADH$_2$ (reduced Flavin Adenine Dinucleotide) from FAD (Flavin adenine dinucleotide). FADH$_2$ carries electrons which, in the subsequent steps, are funneled into the unique oxidative phosphorylation pathway to regenerate FAD and to produce ATP and water. Similarly, peroxisomes generate FADH$_2$. However, as a consequence to the lack of the oxidative phosphorylation pathway, peroxisomes synthesize a toxic molecule, hydrogen peroxide (H$_2$O$_2$), see figure 2). Fortunately, due to the presence of peroxisomal catalase enzyme, hydrogen peroxide is immediately broken down into harmless water and oxygen before it exerts any damage to the cell.

Peroxisomes are more active than mitochondria in oxidizing VLCFA (ie., fatty acids with more than 20 carbons) such as hexacosanoic acid and branched-chained fatty acids such as phytic acid and pristanic acid from diary products. Phytanic acid has a methyl group on its β carbon which renders the β oxidation process. However, as it was explained in the introduction, following the α oxidation, the β oxidation facilitates phytanic acid oxidative metabolic pathway. Pristanic acid, which is produced upon α oxidation of phytanic acid, and phytanic acid are used as energy sources and are oxidized to produce ATP.

3. Due to the lack of citric acid cycle enzymes in the peroxisomes, acetyl-CoA, the end product of α oxidation, is not further oxidized in peroxisomes, rather is exported into the cytoplasm. Therefore, many of the VLCFA or branched-chain fatty acids are metabolized to shorter-chain fatty acids, such as hexanoyl-CoA, instead of completely being oxidized to acetyl-CoA. The shortened fatty acids are exported to mitochondria to be completely oxidized to provide energy in the form of ATP.

**Figure 2**
Figure 2: Oxidation of Fatty Acids in the Mitochondria and Peroxisomes of animal cells.

**ETHER LIPIDS BIOSYNTHESIS**
Ether lipids, including plasmalogens and platelet-activating factor, are important phospholipids which differ from other fatty acids by having an ether group (ether- linkage) instead of an ester group (see figure 3). Plasmalogens play important role in maintaining the integrity of cell membranes. They are found in all mammalian cells with high amount in muscle, heart, and brain. A significant fraction (about 50%) of heart tissue's phospholipids in vertebrate is enriched in plasmalogens. Plasmalogens play important role in the neural tissues too. Consequently, when the synthesis of plasmalogens is impaired, muscle, heart and brain tissues are affected. Platelet-activating factor is a potent molecular signal that is released from basophils. This factor is involved in acute inflammation, allergic response, platelet aggregation, and the release of the neurotransmitter serotonin from platelets. Serotonin activates platelet aggregation which plays an important role in the blood clotting process (a).

**Figure 3**
Figure 3: The structure of plasmalogens, a typical ether lipid.

**BILE ACIDS BIOSYNTHESIS**
Liver peroxisomes play an important role in the biosynthesis of bile acids (b). Bile acids are derivatives of cholesterol which are synthesized in the liver. Synthesis of bile acids represents the major route for elimination of cholesterol from the body. Approximately 50% of cholesterol is metabolized into cholic acid and chenodeoxycholic acid (see figure 4). These amphipathic molecules are transported and stored in the gall bladder. After ingestion of fatty meals, bile acids are released into small intestine in order to facilitate lipid digestion.

Humans have a total of four different bile acids with 24 carbon atoms in each. The amphipathic property of these molecules permits them to act as detergents assisting emulsification of ingested lipids into soluble micelles. This in turn facilitates the enzymatic digestion and absorption of fats. Lipids are the major source of energy (ATP). ATP plays a major role in the contraction of muscle which involves both myosin and actin proteins (c). A lack of fat absorption
and ATP formation correlates well with the “floppiness” of muscles tone (see the case).

**Figure 4**

Figure 4: The structure of chenodeoxycholic acid and cholic acid, two primary bile acids.

**BIOSYNTHESIS OF DOCOSAHEXAENOIC ACID (DHA)**

Docosahexaenoic acid (DHA) is a polyunsaturated fatty acid with 22 carbon atoms and six cis double bonds (22:6, see figure 5). DHA is found in diet, particularly fish, or is synthesized in the peroxisomes from linolenic acid. Because DHA’s last double bond (the first double bond from the omega carbon) mimics the last double bond of linolenic acid, it belongs to the omega-3 group fatty acids (in contrast to linolenic acid that refers to short-chain omega-3 fatty acid, DHA refers to long-chain omega-3 fatty acid) ($^8$). DHA is a major fatty acid in the sperm, brain, and retina.

**Figure 5**

Figure 5: Docosahexaenoic acid (DHA)

**ZELLEWGER SYNDROME**

Two classes of disorders related to peroxisomes have been identified: i) peroxisome biogenesis disorders (PBD), ii) single peroxisomal enzyme disorders. While normal peroxisome biogenesis facilitates the import mechanism of peroxisomal matrix proteins into peroxisomes, cells from patients with PBD are not able to fully import one or more classes of peroxisomal matrix proteins into these organelles. Peroxisome biogenesis disorders are further divided into 4 groups: Neonatal Adrenoleukodystrophy (NALD); Infantile Resum's Disease (IRD); Rhizomelic Chondrodysplasia Punctata (RCDP) and Zellweger Syndrome (ZS). NALD, IRD, and ZS are referred to Zellweger spectrum, due to their overlapped clinical manifestations. In Zellweger spectrum, ZS is more severe than NALD and IRD ($^{16}$). Although ZS is fatal with a life expectancy as short as one year old, it is a rare disorder that affects one in every 25,000 to 50,000 births ($^{15}$).

Peroxisome biogenesis disorders cause neuronal migration disorder and demyelination. Neuronal migration is a critical development during the infant's growth. Neuronal migration simply means migration of neurons in the developing brain and nervous system i.e., from the sites where they are formed to the sites where they will function. The exact mechanism(s) for neuronal migration defect is not known yet. However, defects of neuronal migration result in a severely disorganized brain which leads to a combination of epilepsy, i.e., having a lower threshold for seizures, and mental retardation. It has been suggested that impairment of neuronal migration is associated with ZS ($^{16}$). Since these dysfunctions also lead to dysmorphic craniofacial features and hepatic involvement, the ZS is also referred to cerebro-hepato-renal syndrome ($^{16}$).

**PEROXISOMAL MACHINERY, A LINK TO ZELLEWGER SYNDROME**

Many enzymes are unstable in the cellular peroxisomes in patients with ZS ($^{14}$). As a result, many of the peroxisomal metabolic functions are absent. However, it is not yet clear how exactly abnormalities in peroxisomal metabolic pathways lead to ZS. Several hypotheses have been made from different studies to explain a link between peroxisomal deficiencies and ZS. In a developing fetus for a few weeks after birth, peroxisomes are abundant in the oligodendrocytes, the cells which surround the developing central nervous system. The excess of VLCFA, due to the lack of oxidation process in peroxisomes, has been suggested to interfere with the growing axons and results in impairment of neuronal migration ($^{16}$). Plasmalogenes are important molecules in the structure of myelin. Animal model studies have shown that the reduced levels of plasmalogenes and platelet activating factor in ZS patients is associated with abnormality in neuronal migration ($^{19}$). Interestingly, a defect in catalase, an important enzyme of peroxisomes, has not been associated with ZS. This indicates that the toxicity of H$_2$O$_2$ is not a cause for the ZS.

The synthesis of platelet-activating factor is reduced in patients with ZS which results in an impaired blood clotting process. The affected children are given vitamin K and blood
clotting factors to facilitate the blood clotting process.

As it was mentioned earlier, peroxisomes contain enzymes that carry out β oxidation of VLCFA. If VLCFA are not oxidized in peroxisomes, they will accumulate in glial cells including oligodendrocytes. The unprocessed VLCFAs are incorporated into glial cell membranes which results in the cell dysfunction and cell death \((16)\). In addition, the accumulation and incorporation of VLCFA in the myelin causes demolition of myelin resulting in hypomyelination. Myelin's function is to insulate the axon through which the nerve impulses are conducted.

Dietary fats provide two essential fatty acids, linoleic acid and linolenic acid, which humans need for the synthesis of eicosanoids (prostaglandins, thromboxanes, and leukotrienes). Because bile acids facilitate digestion and absorption of lipids in the small intestine, deficiency in the bile acids synthesis results in malabsorption of lipids, including the essential fatty acids, in the ZS patients.

Clinical trials are underway to determine the effectiveness of oral bile acid therapy, including cholic acid, in patients with ZS \((17)\).

It has been reported that deficiency in the synthesis of DHA leads to abnormal development of the brain and retina because, in the experimental studies, low levels of DHA have been associated with severe visual and neurological damage \((18)\).

**DIAGNOSIS**

**CLINICAL SYMPTOMS**

Clinical manifestations in ZS are due to a range of severely affected organs such as liver, eye, cartilage, heart, muscle, and brain. Typical and apparent diagnostic prototypes are: high forehead and flattened face; broad nose and widely spaced eyes, respiratory distress and bell-shaped chest \((12)\); severe hypotonia or “floppiness” of muscles tone and an inability to move, glaucoma, difficulty to suck and/or swallow; hyporeflexia; epileptic seizures, nystagmus, mental retardation, impaired hearing, and enlarged liver. These clinical symptoms are important to know in order to diagnose ZS accurately.

**BIOCHEMICAL ANALYSIS**

There are a few markers that screen functional peroxisomes. Because peroxisomes are the sites for oxidation of VLCFA and branched-chain fatty acids, increased level of these two molecules are often used as diagnostic tools for the manifestation of ZS. An increased plasma level of VLCFA is an indication of ZS. Prenatal analysis for VLCFA and plasmalogen are examples that are used from amniotic tests. Absence of peroxisomes in liver biopsy is another sign of ZS. In addition, a high amount of arachidonic acids metabolites may excrete into the urine of ZS patients \((19)\).

**TREATMENTS**

Today there is no cure for ZS. Therefore, any treatment will be based on palliative and supportive care rather than curative treatment to optimize quality of life for affected children. The palliative treatment includes:

- DHA
- Bile acids (Actigall)
- Formula
- Anticonvulsant, to control seizures

**PALLIATIVE TREATMENT FOR PATIENTS WITH ZS**

**DOCOSAHEXAENOIC ACID (DHA)**

Peroxisomes are the sites where DHA are synthesized and deficient peroxisomes cannot produce DHA. DHA is essential for the functional development and synaptic transmission of the brain in infants. Breast milk is enriched in DHA and its level is higher in women with a diet high in seafood. Food and Drug Administration (FDA) has approved DHA as an ingredient in the infant formula

**ACTIGALL**

Actigall, also called ursodiol, is one of the bile acids extracted from Chinese black bear's gallbladder. However, actigall is synthetically produced by pharmaceutical companies. Because of the ZS, infant patients cannot make their own bile acids in order to digest fats. Actigall helps ZS patients to improve lipid metabolism.

**ZANTAC**

Ranitidine (Zantac) is a histamine-2 antagonist (H\(_2\) blocker). Zantac competitively inhibits the action of histamine on the H\(_2\) receptors of parietal cells which results in reducing gastric acid secretion into the stomach. ZS patients often have gastrointestinal bleeding problem that is aggravated by the gastric acid secretion. Another class of drugs, proton pump inhibitors, is used instead of H\(_2\) blocker to reduce gastric acid secretion. However, lack of IV preparations for proton pump inhibitors, makes Zantac more appropriate for
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ZS patients.

**PULMICORT RESPULES**

Budesonide (Pulmicort) is an inhaled corticosteroid. This drug is used in the treatment of asthmatic attacks and for the improvement of lung function and bronchial hyperresponsiveness. Corticosteroids inhibit synthesis of leukotriene D4. Leukotriene D4 induces contraction of muscles lining the airways to the lung. Budesonide improves ZS patients’ lung function in order to facilitate their breathing process.

**PREGESTIMIL**

Pregestimil is an infant formula for ZS patients with fat malabsorption. It has short chain fatty acids. All three forms: saturated (which the highest amount is caprylic acid with 8 carbons), monosaturated, (which the highest amount is oleic acid with 18 carbons) and polysaturated fatty acids (which the highest amount is linoleic acid with 18 carbons) are present. In addition linolenic acid and arachidonic acid are provided. The β oxidation of the fatty acids provide the necessary energy (ATP) for infants.

**PHENOBARBITAL AND TEGRETOL**

These two drugs are antiepileptic drugs that help ZS patients with their recurring seizures. Phenobarbital belongs to the barbiturates and is one of the most effective drugs for seizure with minimal sedative effects. Phenobarbital increases the effect of GABA, an inhibitory neurotransmitter. Carbamazepine (Tegretol) inhibits sodium channels and thereby blocks neurons from generating repetitive firing of action potentials. It is worth knowing that carbamazepine induces the enzyme that metabolizes other anticonvulsant drugs including phenobarbital. Therefore, dosing adjustment is necessary when these two drugs are prescribed to patients.

**VITAMIN A, D, E, AND K**

These vitamins are lipid soluble vitamins and have different important biological roles for humans. The malabsorption of fats for the ZS patients causes a reduced absorption and consequently inadequate storage of the lipid soluble vitamins. It is important to provide these vitamins as they are involved in many vital biochemical reactions and pathways such as: cell differentiation (vitamin A), Ca2+ and phosphate metabolism (vitamin D), prevention of oxidative damage (vitamin E) and blood clotting process (vitamin K).

**SUPPORTIVE TREATMENTS FOR PARENTS TO CHILDREN WITH ZS**

ZS is an autosomal recessive disorder which means there is always a probability that a single genetic defect can lead to the devastating symptoms mentioned in the case. So far only a few genetic diseases can be treated. Genetic counseling is important to inform parents of the severity of ZS. Genetic tests can enable parents to make more informed decision. It is important for parents to understand the absolute and relative risks that a new born baby will inherit ZS. Of particular concern are families who have (had) at least one child with ZS. Not only it is important to diagnose a child but it is important to diagnose the parents to predict the probability of having a child with ZS.

Pharmacists provide extensive information regarding appropriate medication use. They confirm indication and dosing, provide guidance on potential side effects, cautions or monitoring parameters. In this case, phenobarbital dosing must be adjusted in cases of renal impairment. Additionally, some medications (such as phenobarbital) are available in solution forms, which would make it easier for ZS parents to administer to their children via feeding tubes.

Pharmacists also are the health care professionals patients see on a regular basis, up to 10-12 times per year. As a result, a relationship of trust often develops. It is important for pharmacists to understand how difficult it is for parents knowing their child would not live to see her/his first birthday. When patients are dealing with difficult health concerns, they often seek the pharmacist’s interpretation. In situations of genetic predispositions, patients can discuss information obtained from the genetic counselors with their pharmacist. Pharmacists can offer additional information or perspective.

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A very special note of gratitude to Grace, Samuel, and hundreds of other affected children with Zellweger Syndrome.

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