Diplomarbeit

Phenotypical Variations in Pyridoxine Dependent Epilepsy caused by Mutations in the Antiquitin Gen

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Ort, Datum …….. (Unterschrift)

Ich erkläre ehrenwörtlich, dass ich die vorliegende Arbeit selbstständig und ohne fremde Hilfe verfasst habe, andere als die angegebenen Quellen nicht verwende habe und die den benutzten Quellen wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht habe.

Graz, am …… *Unterschrift*

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.

Zusammenfassung

Die Pyridoxin abhängige Epilepsie (pyridoxine dependent epilepsy, PDE) wurde im Jahr 1954 von *Hunt et al*. erst beschrieben als eine spezifische Form von Epilepsie. Sie ist charakterisiert durch einen neonatalen Beginn von therapieresistenten Anfällen. Ein späterer Anfallsbeginn ist möglich, mit einer wahrscheinlich weniger gravierenden Ausprägungsform. Pyridoxin (Vitamin B_6) Gabe führt zu einem Stop der Anfälle und eine lebenslange Therapie ist nötig, um eine Anfalls- Wiederholung zu vermeiden. Bisher wurden Patienten, je nach ihrem Ansprechen auf Pyridoxingabe bzw. Absetzen desselben klassifiziert, eine definitive, wahrscheinliche oder mögliche PDE zu haben. PDE wird autosomal rezessiv vererbt, wobei der Gendefekt sich auf dem Chromosom 5q31 befindet. Die Prävalenz wird auf 1:100 000 geschätzt. Lange Zeit wurde GAD (glutamic acid decarboxlase) verdächtigt, das defekte Genprodukt darzustellen. 2006 konnte jedoch gezeigt werden, dass PDE durch einen Defekt der alpha-amino adipic semialdehyde (AASA) dehydrogenase (*Antiquitin*) im zerebralen Lysin Abbau verursacht wird.

Die Prognose von PDE Patienten wird durch viele Faktoren bestimmt, allen voran durch das Alter bei Anfallsbeginn, Therapieverzögerung und wahrscheinlich auch durch die grundlegende Mutation im Antiquitin Gen.

Diese Arbeit, in Zusammenarbeit mit 17 verschiedenen internationalen Zentren, konzentriert sich auf phänotypische Merkmale von 30 PDE Patienten, mit einer nachgewiesenen *Antiquitin* Gen-Mutation.

Abstract

Pyridoxine dependent epilepsy (PDE) was first described by *Hunt et al*. in 1954 as a specific form of epilepsy. It is characterized by neonatal onset of therapy resistant seizures. Late onset cases are possibel with a probably less severe form of the condition. Application of pyridoxine (vitamin B_6) leads to cessation of seizures and lifelong treatment is necessary to avoid seizure recurrence. Patients so far have been classified clinically as having definite, probable or possible PDE. PDE follows autosomal- recessive inheritance and maps to chromosome 5q31, with an estimated incidence of 1:100 000. For a long time glutamic acid decarboxylase (GAD), has been claimed to be the abnormal gene product. Studies on glutamate and gamma-aminobutyric acid (GABA) in cerebrospinal fluid (CSF) have brought contradictory results to support this hypothesis. In 2006 PDE has been shown to be caused by a defect of α-amino adipic semialdehyde (AASA) dehydrogenase (*Antiquitin)* in the cerebral lysine degradation pathway.

The long- term outcome of PDE patients is determined by several factors, mainly by age at seizure onset, delay of specific treatment and probably also by the underlying mutations of the *Antiquitin* gene. This work is focussing on the phenotypical characterization of 30 molecularly defined PDE patients, with mutations of the *Antiquitin* gene, collected in the framework of an international cooperation of 17 centers.

Contents

Glossar und Abkürzungen

- **AASA:** α-amino adipic semialdehyde
- **ACTH:** Adrenocorticotropic hormone
- **AED:** Antiepileptic drug
- **ALDH7A1:** *Antiquitin* Gene
- **CBZ:** Carbamazepine
- **CInz:** Clonazepam
- **CNS:** Central nervous system
- **CSF:** Cerebrospinal Fluid
- **CT:** Computer Tomography
- **EEG:** Electroencephalogram
- **DRI:** Dietary Reference Intakes
- **DV:** Daily value
- **GAD:** Glutamic acid decarboxylase
- **GT:** Generalized Tonic
- **GTC:** Generalized Tonic Clonic
- **IBE:** International Bureau for Epilepsy
- **ILAE:** International League Against Epilepsy
- **MR:** Mental Retardation
- **MRI:** Magnetic Resonance Imaging
- **PA:** Pipecolic acid
- **PB:** Phenobarbital
- **PDE:** Pyridoxine dependent epilepsy
- **PHT (DPH):** Phenytoin

- **PLP:** Pyridoxalphosphat
- **P5C:** L-Δ1-pyroline-5- carboxylate
- **P6C:** L-Δ1-Piperideine-6-carboxylate
- **RCT:** Randomized clinical trial
- **SD:** Standard Deviation
- **SW:** Slow Wave
- **TC:** Tonic clonic
- **TNSAP:** Tissue non specific alkaline phosphatase
- **UK:** United Kingdom
- **Vgb:** Vigabatrin
- **VPA (Vps):** Valproic acid

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1. Introduction

1.1. Pyridoxine (Vitamin B_6)

Vitamin B refers to a group of 9 vitamins. This group contains vitamin B_1 (Thiamine), vitamin B_2 (Riboflavin), vitamin B_3 (Niacin), vitamin B_5 (Pantothenic acid), vitamin B_6 (Pyridoxine), vitamin B_7 (Biotin), vitamin B_8 (Myo-inositol), vitamin B_9 (Folic acid) and vitamin B_{12} (Cobalamin) [18]. Vitamin B plays a significant role in cell metabolism. Nutritional supplements containing all or some of the eight B vitamins are referred to as vitamin B complex [18].

There are six forms of vitamin B_6: pyridoxal (PL), pyridoxine (PN), pyridoxamine (PM), and their phosphate derivatives: pyridoxal 5'-phosphate (PLP), pyridoxine 5'-phosphate (PNP), and pyridoxamine 5'-phospate (PNP). In body fluids Vitamin B_6 exists mainly as a mixture of pyridoxal, pyridoxine and pyridoxamine and their phosphates. Phosphorylation is controlled enzymatically [20]. The only active form of vitamin B_6 is pyridoxal5'-phosphate (PLP), which is an important coenzyme in the metabolism of various enzymes in the metabolism of amino-acids, glycogen and sphingoid bases [18]. It is mainly catalyzing reactions of transamination and decarboxylation [19]. PLP is the most abundant form of vitamin B_6 in human tissue and mostly found in muscle, bound to phosporylase.

1.1.1. Absorption and Metabolism

Pyridoxal and pyridoxol absorption take place passively, mainly in the jejuneum. According to *Tsjui et. al.* [20] pyridoxine enters the duodenal cells by passive diffusion Absorption in the gut involves phosphatase-mediated hydrolysis and transport of the non-phosphorylated form to the mucosal cells [19]. Pyridoxal- and pyridoxamin (phosphate) are well absorbed, also in higher quantities, whereas pyridoxine-glucoside is less well absorbed [21]. The majority of the absorbed non-phosphorylated vitamin B_6 goes to the liver where B_6 vitamers undergo rephosphorylation by the ATP-dependent pyridoxal- kinase. (**Figure 1)**

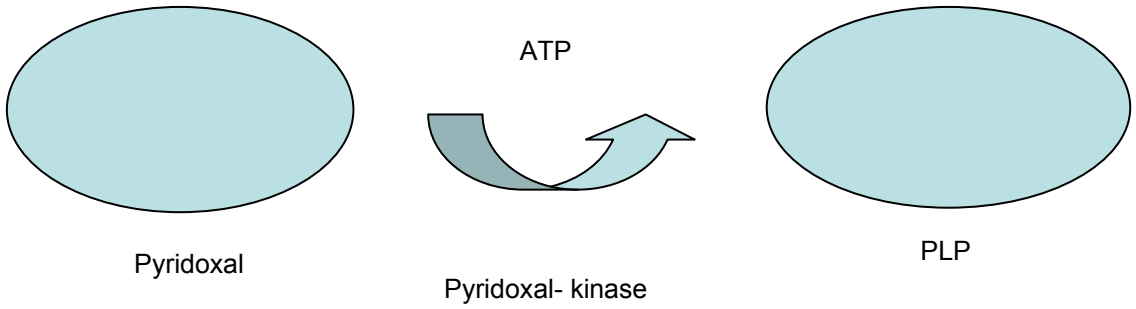

Figure 1 Phosphorylation of pyridoxal to pyridoxalphosphat (PLP) by the ATP-dependent Pyridoxal- Kinase.

1.1.2. Metabolism in Liver Cells

The liver is playing a central role in the metabolism of vitamin B_6. The proper regulation of this pathway in the liver is of importance for the vitamin B_6 status of the whole organism.

In the liver, pyridoxamine- and pyridoxine phosphate are converted into the only active cofactor pyridoxal 5'-phosphate (PLP) by the pyridox(am)ine-phosphate oxidase (Figure 2) [24]. PLP is then released into circulation. Its intracellular uptake is mediated by the tissue non specific alkaline phosphatase (TNSAP) The liver also contains the enzymes that degrade pyridoxal 5'-phosphate to pyridoxic acid (aldehyde oxidase(s) and/or an NAD-dependent dehydrogenase) [23, 25], which is the dead-end catabolite, excreted in urine.

In **Figure 2** the major metabolic transformations by human liver are shown. All three vitamers are phosphorylated by pyridoxol-kinase (K). Pyridox(am)ine 5' phosphate oxidase (O) oxidizes the 4-hydroxymethyl and the 4-aminomethyl groups to pyridoxal 5'-phosphate (PLP) which are rephosphorylated or oxidized to the dead-end catabolite pyridoxic- acid.

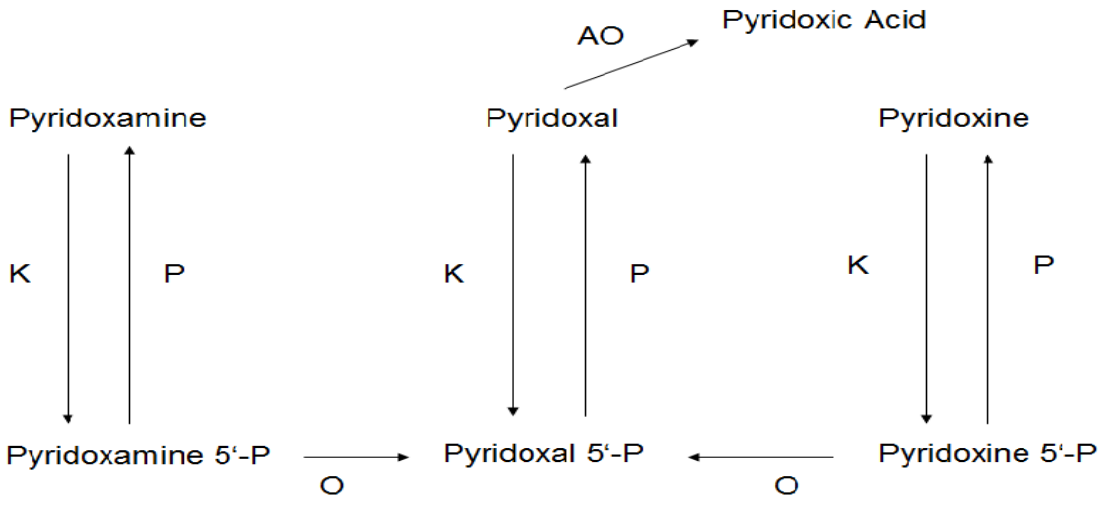

Figure 2 Metabolism of Vitamin B_6 by Human Liver Enzymes.

K: Pyridoxal-kinase; P: Phosphatase; O: Pyridoxine (Pyridoxamine) 5' phosphate oxidase; AO: Aldehyde oxidase.

1.1.3. Dietary Sources

Vitamin B can be found in a wide variety of foods including cereals, beans, meat, poultry, fish, and some fruits and vegetables [27]. The table of selected food sources lists main dietary sources of B_6 [27].

Table 1 Food Sources of Vitamin B_6

Food	**Milligrams (mg) per serving**	**% DV***
Ready-to-eat cereal, 100% fortified, ¾ c	2.00	100
Potato, Baked, flesh and skin, 1 medium	0.70	35
Banana, raw, 1 medium	0.68	34
Garbanzo beans, canned, ½ c	0.57	30
Chicken breast, meat only, cooked, ½ breast	0.52	25
Ready-to-eat cereal, 25% fortified, ¾ c	0.50	25
Oatmeal, instant, fortified, 1 packet	0.42	20
Pork loin, lean only, cooked, 3 oz	0.42	20
Roast beef, eye of round, lean only, cooked, 3 oz	0.32	15
Trout, rainbow, cooked, 3 oz	0.29	15
Sunflower seeds, kernels, dry roasted, 1 oz	0.23	10
Spinach, frozen, cooked, ½ c	0.14	8
Tomato juice, canned, 6 oz	0.20	10

Avocado, raw, sliced, ½ cup	0.20	10
Salmon, Sockeye, cooked, 3 oz	0.19	10
Tuna, canned in water, drained solids, 3 oz	0.18	10
Wheat bran, crude or unprocessed, ¼ c	0.18	10
Peanut butter, smooth, 2 Tbs.	0.15	8
Walnuts, English/Persian, 1 oz	0.15	8
Soybeans, green, boiled, drained, ½ c	0.05	2
Lima beans, frozen, cooked, drained, ½ c	0.10	6

* DV = Daily Value. DVs are reference numbers based on the Recommended Dietary Allowance (RDA). They were developed to help consumers determine, if a food contains a lot or a little of a specific nutrient. The DV for vitamin B_6 is 2.0 milligrams (mg) for adults. The percent DV (%DV) listed on the nutrition facts panel of food labels tells, what percentage of the DV is provided in one serving. Percent DVs are based on a 2,000 calorie diet. Daily Values may be higher or lower depending on calorie needs. Foods that provide lower percentages of the DV also contribute to a healthful diet [27].

Table 2 B vitamins for infants aged 6–8 months: requirements, amount in human milk and amount needed in complementary foods [28]

B vitamins for infants aged 6–8 mo: requirements, amount in human milk and amount needed in complementary foods

					Assuming average human milk intake (0.674 L/d)[3]				
						Amount needed in complementary food			
Nutrient	AI	DRV	AI/100 kcal[1]	Concentration/L milk[2]	Intake from milk	% AI	Amount	Per 100 kcal	Per 40 g[4]
Thiamin, *mg*	0.3	0.2	0.048	0.21	0.14	53	0.16	0.08	0.15
Riboflavin, *mg*	0.4	0.4	0.065	0.35	0.24	40	0.16	0.08	0.15
Niacin, *mg NE*	4.0	4	0.650	1.8	1.21	70	2.79	1.38	2.67
Vitamin B-6, *mg*	0.3	0.3	0.049	0.13	0.09	71	0.21	0.10	0.20
Folate, *μg DFE*	80	50	13.010	85	57.29	28	22.71	11.24	21.76
B-12, *μg*	0.4	0.4	0.065	0.42	0.25	37	0.15	0.07	0.14
Pantothenic, *mg*	1.8	1.7	0.293	2.2	1.48	18	0.32	0.16	0.31
Biotin, *μg*	6	—	0.976	8	5.39	10	0.61	0.30	0.58
Choline, *mg*	150	—	24.390	160	107.84	28	42.16	20.87	40.41

[1] Assuming energy requirement is 615 kcal/d (2).
[2] Concentrations on which the AI is based (3).
[3] From reference 43, using data from developing countries. This volume contains 413 kcal.
[4] Assumed typical intake of infants this age, and 440 kcal/100 g dry product (4). Abbreviations: AI, Adequate Intake; DRV, United Kingdom Dietary Reference Value; NE, niacin equivalents; DFE, dietary folate equivalents.

These values were taken from the report on Dietary Reference Intakes (DRI) [28] for the B vitamins issued by the Institute of Medicine. They were estimated on the basis of all available information about normal milk concentrations. The sources of the data used to make these estimates are referenced in the Institute of Medicine report [28].

1.2. Historical Background

1.2.1. Description by *Hunt et al.*

Pyridoxine dependent epilepsy (PDE) was first described by *Hunt et al* in 1954 [1]. He and co-workers reported about a case in an infant with intractable convulsions, which ceased after the administration of pyridoxine. The seizure-ceasing effect of pyridoxine was discovered „by serendipity“. The child had an early-onset of constant and intractable seizures 3 hours after birth and was hospitalized at the age of 13 days.

During a course of intercurrent pneumonia the child was given a standard multivitamin mixture (Berocca C®, containing 6 mg of pyridoxine) i.v. over 5 days. During this period the child was seizure free, but the correlation to pyridoxine was not appreciated at this time. Fifty hours after the last injection of Berocca C® seizures recurred again [1].

She was then transferred back to the Delaware Hospital, Wilmington, where at the age of 23 days, she again received a daily injection of Berocca C® for other 7 days. The patient again was seizure free and became more alert. On discharge Berocca C® was replaced by 2 cc. multibeta® liquid (containing 0.3 mg of pyridoxine). 50 hours after discharge, the patient developed intractable severe generalized seizures. After receiving one injection of Berocca C® intramuscularly, the convulsions ceased within 15 minutes [1].

For understanding the reason and identifying the effective component of Berocca C® further investigations concerning the contents were done. The contents of a 2cc. ampule of Berocca C®, as stated by the manufacturer, include 6mg pyridoxine HCl, whereas the non effective 2 cc multibeta® liquid contains only 0.3 mg pyridoxine HCl [1].

To confirm this finding, Berocca C® was withheld on repeated occassions, each resulting in the recurrence of seizures. *Hunt* describes that seizures re-occured within 48 to 56 hours following each withdrawal of Berocca C®. Fifteen to thirty minutes after injection of Berocca C® the convulsion ceased and the child became more alert.

Therefore it was ascertained that the patient needed 2mg pyridoxine orally each day for staying seizurefree [1].

Hunt's interest for understanding the vitamin B_6 effect was raised and he carefully studied pregnancy and sibling's history of the affected girl. The patient's mother was 31 years old and in good health. There was no history of convulsions or any other central nervous system disease in the family. The patient had a 6 year old sister, who had no signs of abnormal development and also this pregnancy was normal. The second pregnancy of the mother however resulted in an infant with convulsions, starting 4 hours after birth, resistant to phenobarbitone. The boy died at 30 hours of

age. Hunt describes that spinal fluid and necropsy of the brain were normal, despite a moderate cerebral edema [1].

In the second and third pregnancy the mother suffered from severe nausea and vomiting. Therefore the mother had received regular injections of pyridoxine and thiamine, during the first 4 months of both pregnancies. In the third pregnancy the mother received intramuscular injections consisting of 50 mg pyridoxine HCl and 50 mg thiamine HCl 3 to 4 times weekly [1]. *Hunt* therefore speculated, that an up-regulation of the fetal enzyme system may give rise to an abnormally high pyridoxine requirement and postnatal pyridoxine dependency.

Since the first case report by *Hunt et al* in 1954 [1], more than 100 additional cases have been reported [2, 3] and PDE has soon been understood as an inherited epilepsy. At least half of these cases were diagnosed retrospectively, after the death in siblings with refractory seizures. Since then pyridoxine dependency has been traditionally considered in the differential diagnosis of neonatal seizures, infantile spasms and status epilepticus. In many patients with neonatal seizure onset, additional clinical features as jitteriness, hypothermia, neonatal dystonia, and a prodrome of restlessness, irritability, abdominal discomfort and bile stained vomiting have been described. As PDE can also present in atypical forms beyond infancy, a pyridoxine trial has to be considered in all cases with intractable seizures, at least up to 3 years of age [2, 4, 5].

In contrast to this genetic condition of PDE, a peculiar phenomenon was described in the early 1960's in the United States of America. During 1952 and the beginning of 1953 infants were admitted to different hospitals with a uniform history and clinical course [6]. All of them were having a normal pregnancy- and birth history, normal growth and development, until at the age of 8 to 16 weeks sudden generalized seizures occurred. Preceeding the convulsions there was an apparent gastrointestinal phase of colic with abdominal distress, vomiting and hyperirritability. Parents of the infants recognized alarming signs, like abnormal crying, stiffening of the body, extension of the head and also periods of upward rolling eye movements.

It was discovered that all of these children received the same infant formula, named liquid SMA (SMA is the brand name), which consisted of pasteurized defatted cow's milk, vegetable and animal fats, and vitamins, plus iron [6].

Seizures stopped when children were switched to another milk formula, or when they were fed supplementary food such as cereals, fruits, meats and vegetables. Authors could show, that the liquid SMA contained less than 60 mcg of pyridoxine per liter [7] while the age dependent daily demand would have been 0,3 mg (adequate intake) [28].

1.2.2. Understanding the Pathophysiology of PDE

Long before the description of *Hunt et al*. in 1954 [1] and the case reports of convulsing children fed a liquid formula lacking pyridoxine [6, 7], the relationship of seizures, due to pyridoxine deficiency has been well established in rats [8], chicken [9], and pigs [10].

Since the discovery of PDE several hypotheses have been investigated, to define the pathogenesis of PDE. Studies of pyridoxine turnover [11], tryptophan pathways, transaminases and amino acids [12] have been carried out in serum and urine of patients suffering from this autosomal recessive condition. The results of these examinations showed no significant differences to healthy controls.

PLP is a co-factor of glutamate decarboxylase (GAD), the enzyme converting an excitatory neurotransmitter to GABA, the most important inhibitory neurotransmitter. There are two isoforms of GAD, GAD-67 and GAD-65. They are located at different subcellular compartements and differ in their affinity to pyridoxine [17]. GAD-65 is the smaller isoform, located in synaptic nerve terminals and is involved in controlling and regulating GABA concentrations [18, 19]. GAD-67 is located in neuronal somata and is probably important for the synthesis of the intracellular GABA pool [18, 19].

For long time it was assumed [12, 13, 14], that PDE is caused by diminished activity of its biosynthetic enzyme, GAD and reduced synthesis of the inhibitory neurotransmitter, gamma-aminobutyric acid (GABA), resulting in a lowered seizure threshold.

Plecko et al. [39] first reported about PA elevation in plasma and CSF of two patients with PDE [39]. It could be shown that PA elevation seems to be a specific finding in PDE as plasma concentrations of PA were continuously elevated in both patients with PDE, while they were normal in patients with non–pyridoxine-dependent seizures [39].

High PA concentrations were obviously not related to defective transport of vitamin B6 vitamers across the blood-brain barrier [7], as concentrations of pyridoxalphosphate in CSF were within, or above normal range during the 72-hour withdrawal [39].

The inverse correlation of PA and PLP suggested a variant of a-aminoadipic transaminase, a pyridoxine-dependent enzyme involved in PA degradation. In case of impaired activity this would result in the accumulation of its specific substrates (a-aminoadipic acid) and of their precursors (e.g.: Δ^1-piperideine-6 carboxylic acid) [39]. Findings of normal concentrations of a-aminoadipic acid and of piperidines in CSF of

the two described patients could not confirm this putative enzyme defect by means of specific metabolite patterns [39]. However the authors claim that the significance of normal pipereidine concentrations was restricted by the fact that their method is not suitable to detect Δ^1-piperideine-6 carboxylic acid in particular [39].

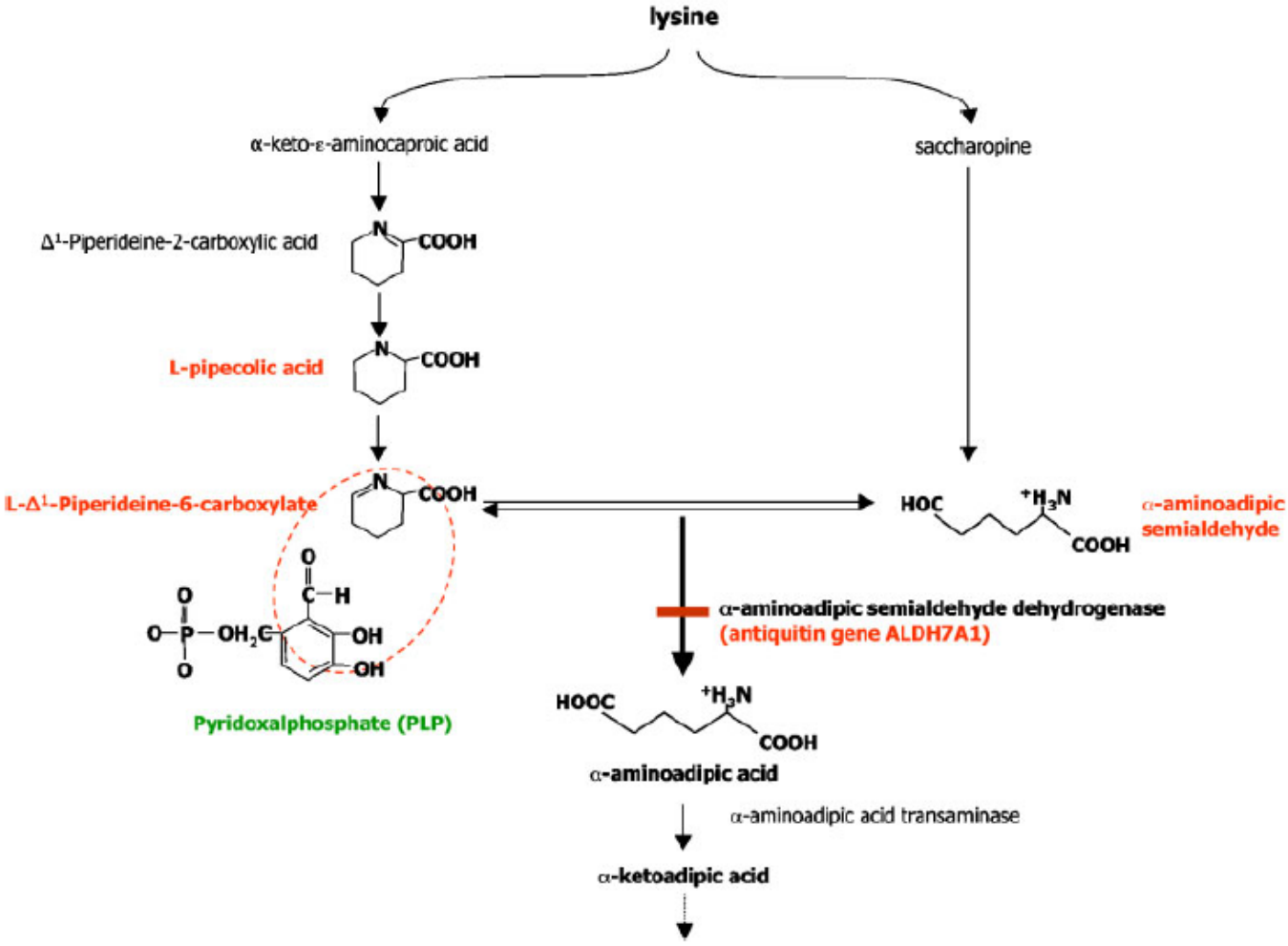

Figure 3: Lysine degradation pathway via saccharopine in liver and via PA in the brain. Mutations of *antiquitin* (ALDH7A1) lead to disturbed function of a-aminoadipic semialdehyde dehydrogenase. The accumulating compound L- Δ^1-Piperideine-6-carboxylate (P6C) inactivates PLP by a Knoevenagel reaction. PA and AASA are elevated and serve as diagnostic markers [50].

Plecko et al reported [39] in the year 2000 that their data suggest that increased plasma and CSF PA- concentrations may represent a diagnostic marker for the disease and could prevent patients from dangerous pyridoxine withdrawals [42] which until this time have been claimed to prove the diagnosis.

In 2005 *Plecko et al* [43] confirmed elevated PA values in plasma in another 6 unrelated PDE patients. Values were 4.3- to 15.3 fold above the upper normal range, even when patients were on pyridoxine at the time of sampling. *Plecko et al.* [43] described, that the degree of PA elevation in plasma seems to be „individual" [43] and is not in correlation to the actual dosage of pyridoxine. In the article it is recommended that, if it is possible, PA should be determined in plasma, urine and/ or CSF, before the first administration of pyridoxine. A higher PA level in CSF than in plasma can be used as a clear discrimination between PDE and peroxisomal diseases, where PA is more markedly elevated in plasma than in CSF.

In parallel to the underlying mechanism of PLP inactivation in PDE, a similar phenomenon has been described in hyperprolinemia type II [44], a defect in L- Δ^1-pyrroline-5- carboxylate (P5C) dehydrogenase. In this inborn error of metabolism the accumulating compound P5C reacts with PLP by a Knoevenagel Condensation [44,] causing secondary PLP deficiency and seizures. Comparison of the structure of P5C, with P6C, suggested that P6C probably also reacts with PLP by a Knoevenagel condensation [38].

The conversion of P6C to a-AASA requires a 'P6C dehydrogenase' and its gene has been sequenced [45]. P6C is the cyclic Schiff base of a-AASA and these two compounds are in equilibrium [46]. The P6C dehydrogenase is most probably identical to a-AASA dehydrogenase (antiquitin).

By homology sequencing it could be found, that the *antiquitin* gene encodes a-AASA dehydrogenase in the pipecolic acid pathway of lysine catabolism. It was concluded correctly that deficiency of *antiquitin* causes seizures because accumulating P6C condenses with PLP by a Knoevenagel reaction and inactivates this cofactor, which is essential for normal metabolism of aminoacids and neurotransmitters [38].

Mills et al. [38] suggested that measurement of a-AASA may facilitate rapid diagnosis of PDE, but it should be pointed out, that neonatal status epilepticus is an emergency.

To avoid diagnostic delay pyridoxine should be given, if clinical and ‚electrophysiological features' are suggestive of PDE [38, 47].

Measurements of a-AASA and if elevated, ALDH7A1 molecular analysis could be used to avoid the potentially dangerous withdrawal of pyridoxine previously recommended for the definitive diagnosis of PDS [50].

Following the administration of pyridoxine it was noticed that there is a lower a-AASA concentration, which suggests elimination pathways of the products formed by the Knoevenagel condensation [43]. That is why the administration of pyridoxine may not only correct secondary PLP deficiency in these patients, but in addition seems to reduce a-AASA (and P6C) as presumably toxic compounds [50]. As a-AASA has highly reactive aldehyde groups with a great tendency to interact with other cellular molecules, accumulation of a-AASA could add to neuronal cytotoxicity in PDE [41].

1.2.3. Molecular Genetic Pathogenesis

As mentioned above, for many years, it was hypothesized that PDE was caused by an abnormality of the enzyme GAD, which needs PLP as a cofactor [12]. GAD converts glutamic acid, an excitatory neurotransmitter, into GABA, an inhibitory neurotransmitter. Both of these neurotransmitters play important roles in the control of epileptic processes. A number of neurochemical studies indirectly supported this hypothesis [33, 34, 35]. However, four different laboratories failed to document linkage to either isoform of GAD [16, 17, 30, 36].

Battaglioli et al [15] performed genotype analysis of three PDE families using polymorphic markers close the two GAD genes (GAD1 and GAD2). The result of this genotype analysis showed that neither mutations of the GAD1, nor of the GAD2 gene were the underlying cause of PDE by haplotype and mutational analysis in PDE pedigrees [15, 30]. The authors found further support of these findings from a study done on Asian families [16]. Also postmortem quantifications of brain GABA and glutamate levels as well as genotype analyses have failed to demonstrate the hypothesis of a consistent underproduction of GABA [20].

Lott et al [33] explored brain tissues of five patients having disorders unrelated to PDE, serving as control for this study. They came to the result that the brain of their PDE patient was weighing 350 gram less than expected. White matter of the centrum semiovale was decreased in overall quantity as compared with the normal state. Also corpus callosum was abnormally thin, especially in the posterior regions. *Lott at al* [33] describe that correspondingly the lateral ventricles were enlarged. Concerning the biochemical results of *Lott et al* [33], they found out that glutamic acid was elevated above all control values in the frontal and occipital cortices, but was found within the normal range within the spinal cord. GABA concentrations were below all control values in the frontal and occipital cortices and were very slightly above control values for the spinal cord. In summary *Lott et al* [33] were able to show that in their patients concentration of glutamic acid were increased and values of GABA were decreased in both brain areas [33].

In the year 2000 *Cormier- Daire et al*.[30] reported about the performance of a genomewide search, using microsatellite markers at an average distance of 10 cM, and the search revealed linkage of the disease-causing gene to markers on chromosome 5q31.2-q31.3 (maximum LOD score [Z_{max}] 8.43 at recombination fraction [θ] 0 and Z_{max}=7.58 at θ=0 at loci D5S2017 and D5S1972, respectively). A recombination event, between loci D5S638 and D5S463 in one family defined the distal boundary, and a second recombination event between loci D5S2011 and D5S2017 in another family defined the proximal boundary of the genetic interval encompassing the PDE gene (5.1 cM) [30].

The homozygosity-mapping strategy in consanguineous families is based on the assumption that affected individuals from the same kindred are homozygous for the disease-causing gene mutation by descent. Microsatellite DNA markers from the entire genome were chosen from the Généthon map [31], and two-point linkage analyses using the MLINK option of the linkage package were performed [30, 32].

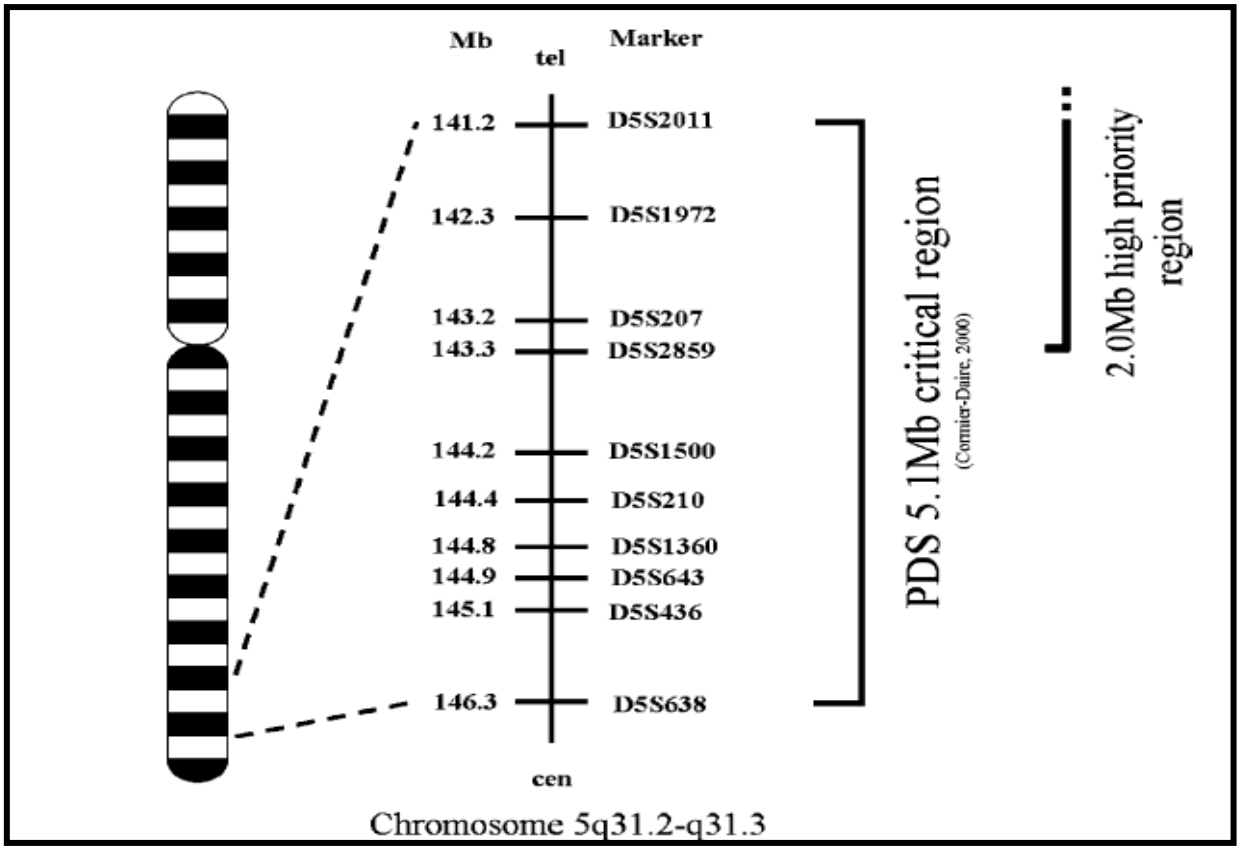

Figure 4: Ideogram of chromosome 5 that shows the map placement of markers in band 5q31.2–q31 and the interval containing the PDE locus. Genetic distances are given in megabases [48].

Bennett et al. [48] in 2005 reported about the findings of a haplotype segregation analysis, using polymorphic DNA markers from 5q31 in six PDE North American families. They concluded, that genetic heterogeneity exists for PDE and that non-penetrance has not yet been reported for PDE. *Bennett et al*. [48] also reported that their results confirm that at least one other PDE locus exists, a finding that has also been reported for UK PDE pedigrees. Nevertheless there was no phenotypical difference in patients with PDE mapping to the 5q31 locus and the PDE patients with a non linked pedigree. In particular, there were also no differences in age of onset, or diagnosis, severity, seizure type, responsiveness, accompanying developmental sequelae, or outcome.

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About Epilepsy

1.3. Definitions

1.3.1. Epilepsy

Epilepsy is the name of a brain disorder characterized predominantly by recurrent and unpredictable interruptions of normal brain function, called epileptic seizures. Epilepsy is not a singular disease entity but a variety of disorders reflecting underlying brain dysfunction that may result from many different causes. Little common agreement exists on the definition of the terms *seizure* and *epilepsy*. At least one seizure is required to establish the diagnosis of epilepsy; a predisposition, for example, by a family history, or by the presence of epileptiform EEG changes, is not sufficient to determine epilepsy [52].

Such definitions are important for communication among medical professionals and also for communication of others involved in legislation, disability pensions, driving regulations, workplace safety, education etc. The current defintions are a result of consensus discussions held by representatives of the International League Against Epilepsy (ILAE) and the International Bureau for Epilepsy (IBE). It presents practical and operational definitions applicable both in medical and non medical settings [52].

2.2.1.1. Elements of a Definition of Epilepsy

Elements of a definition of epilepsy
➢ History of at least one seizure
➢ Enduring alteration in the brain that increases the likelihood of future seizures

➢ Associated neurobiologic, cognitive, psychological and social disturbances

Table 3: Epilepsy can be defined following different criteria [52].

1.3.2. Seizures

S*eizure,* the word itself derives from the Greek meaning '*to take hold'*. Nowadays the word *seizure* is used for any sudden and severe event (for example, "he had a heart seizure"). Different definitions emphasize different features of an epileptic seizure (**Table 4**): nature of onset and termination, clinical manifestations, and enhanced neuronal synchrony.

Some previous definitions also considered issues of etiology, classification, and diagnosis [52].

Elements of a definition of epileptic seizures
➢ Mode of onset and termination
➢ Clinical Manifestation
➢ Abnormal enhanced synchrony

Table 4: Epileptic seizures can be defined following different criteria [52].

2.1.1.1. Classification of Epileptic Seizures

- **Generalized Seizures:**

Generalized seizures are characterized by a hypersynchronic agitation of all nerv cells, taking place in the whole cortex. Patients with generalized epilepsy always experience a loss of consciousness with variable duration.

Generalized seizures can be categorized into:

- Absences
- Myoclonic astatic seizures
- Atonic Seizures
- Tonic/ clonic seizures

- **Focal Seizures:**

In contrast to generalized seizures, the hypersynchronons excitation of the nerv cells is clearly focused on one area of the brain. There can be an aurea preceeding the focal seizures, the symptoms of which can give a hint about the origin of seizures.

Focal seizures can be:

- **simple**: without the loss of consciousness
  - with motor symptoms
  - with somatosensory or sensory symptoms
  - with autonomic symptoms
  - with psychic symptoms

- **complicated**: with loss of consciousness
  - a focal start with a following disturbance of consciousness
  - disturbance of consciousness from the start

- **secundary generalized**: the focal seizure, focused on one area of the brain, spreads all over the brain and becomes a generalized seizure.

2.1.1.2. Antiepileptic drug therapy (AED)

Antiepileptic drugs (AEDs) are the initial treatment modality for the vast majority of patients with epilepsy. Since the beginning of bromide therapy 150 years ago, clinicians have selected initial AED therapy for patients with newly diagnosed epilepsy in large part based on the patient's seizure/epilepsy type, as determined according to the classification scheme of the time [54]. *Glauser T. et al*. [54] claim in their report, from the year 2006, that unfortunately, during the majority of these 150 years, minimal formal scientific assessment of the efficacy, safety, and tolerability of AED's has been done. As an example they mention that a number of older commonly used present-day AED's [e.g.: phenobarbital (PB), phenytoin (PHT)] were registered and marketed in countries around the world without any randomized clinical trial (RCT), evidence of efficacy or tolerability in patients with epilepsy. The clinical development programs of carbamazepine (CBZ) and valproic acid (VPA) in the 1960's and 1970's marked the beginning of more formalized AED efficacy and tolerability assessment [54].

It is clear that the selection of an AED for pediatric epilepsy is not a simple task. Numerous variables should be considered, including:

- AED-specific variables: (seizure- or epilepsy- syndrome- specific efficacy/effectiveness, adverse effects, pharmacokinetics, formulations, and so on),
- Patient-specific variables: (genetic background, sex, age, co- morbidities, socio-economic status) ,
- Nation- specific variables: (AED availability, AED cost) [54].

Typically, in epileptic encephalopathies due to inborn errors of metabolism, there is partial or complete resistance to common anticonvulsants, but specific therapies, as cofactor substitution (PDE) or a ketogenic diet (in GLUT 1 deficiency) lead to quick cessation of seizures.

1.4. Specific Features and Aspects of PDE

After a short introduction into epilepsy, now specific features of PDE are explained and listed. Pyridoxine dependency is a rare cause of seizures. Pyridoxine dependent epilepsy (PDE) occurs despite normal vitamin B_6 levels in plasma. In literature more than one hundred cases have been described.

1.4.1. Clinical Presentation

1.4.1.1. Onset of Seizures

Typically, seizures begin during the first few hours to days of life, whereas there are also few articels reporting about delayed seizure- onset, up to 18 months of age [35, 52, 63, 64, 65]. In addition to the classic neonatal presentation, it has been reported in a substantial number of cases that this disorder may even manifest before birth with convulsive intrauterine movements. Intrauterine seizures are usually reported retrospectively by mothers as a sustained hammering sensation, beginning at 5 months gestation or later [61, 65].

Goutieres and Aicardi [65] suggested, that the diagnosis of PDE should be considered in patients up to 18 months of age with seizures, when one or more of the following situations are present:

- cryptogenic seizures in a previously normal infant without an abnormal gestational or perinatal history,
- a history of a severe convulsive disorder in a sibling, leading to death during status epilepticus or when patients are having consanguineous parents,
- the occurrence of long-lasting focal or unilateral seizures,
- irritability, restlessness, crying and vomiting preceeding seizure onset [65].

1.4.1.3. Type of Seizures

Seizure types and EEG in PDE are non- specific. Various types of seizures have been described even in the individual patient [64]. The more typical seizures in PDE are long lasting and generalized tonic clonic seizures which frequently evolve into status epilepticus. However, *Gupta et al.* [59] report that various other types of seizure disorders have been described, including:

- brief seizures,
- both partial and generalized,
- atonic,
- myoclonic
- visual seizures, and
- infantile spasms

Seizures can also often be provoked by external stimuli [59, 62, 68].

1.4.1.3. Associated Clinical Abnormalities

Beside the characteristic appearance of neonatal seizures, associated features have been described [59, 67] in PDE. *Haenggeli et al*. [62] published a review about clinical and therapeutic aspects in PDE, and listed all abnormalities which have been reported in the following **Table 5** [62]. *Haenggeli et al*. [62] report that the incidence of these abnormalities can not be evaluated from a retrospective review, unfortunately the number of cases analyzed is not named.

Abnormal clinical signs	Number of Patients

Irritablity, agitation, jitteriness, hyperalertness, sleeplessness	22
Startle reaction to touch and sounds	14
Feeding difficulties, vomiting	12
Asphyxia at birth, meconium staining	10
Shrill, high- pitched cry	9
Distended abdomen	8
Hypotonia	7
Decreased visual alertness	5
Hypertonia	3
Changing muscle tone	2
Absent primitive reflexes	2

Table 5: Clinical abnormalities other than seizures [62]

Other reported findings in PDE patients are:

- prolonged seizure-free intervals up to 5 1/2 months that occur after the pyridoxine withdrawal [59, 62]
- initial response to anticonvulsants [35, 66, 67]
- initial failure of pyridoxine to control seizures during the first 8 months of life, followed by the successful treatment of recurrent seizures with pyridoxine [67].

Approximately 10% of the patients may initially show signs resembling those of birth asphyxia or intrauterine infection. Seizures following those signs may be considered as part of hypoxic-ischemic encephalopathy, and lead to misinterpretation of clinical findings, and hence to inappropriate treatment with delay of the correct diagnosis [62]. Several authors come to the conclusion that increasing recognition of atypical forms of PDE indicates that these types of PDE may be more common than the classic presentation [5, 67, 68].

1.4.2. Diagnosis

Concerning the clinical diagnosis, *Gupta et al.* [59] suggested the following criteria which were both simple enough for widespread use and broad enough to recognize typical and atypical cases.

- Cessation of clinical seizures with the administration of pyridoxine, either orally or parenterally.
- Complete seizure control on pyridoxine monotherapy.
- Withdrawal of pyridoxine leads to a recurrence of seizures.

Nowadays pyridoxine dependency is no longer just a clinical diagnosis, as the underlying gene-defect has been discovered and reliable biomarkers are available. Pyridoxine withdrawal is no longer recommended.

Other associated findings, which are supportive of the diagnosis, include:

- for PDE suspect EEG patterns,
- seizures resistant to usual anti- epileptics,
- normalization of the EEG after pyridoxine,
- positive family history,
- intrauterine seizures and
- neonatal onset of seizures [59].

2.2.2.1. EEG Findings in PDE

The EEG features of patients with PDE show both diffuse and focal dysfunction. However, a typical EEG pattern has been reported by *Mikati et al.* [64] consisting of bursts or runs of high-voltage, relatively bilateral synchronous sharp and slow (1–4 Hz) wave activity (either ictal or interictal). This pattern, although highly suggestive of

neonatal PDE, may occur with various other (non-specific) paroxysmal abnormalities [47]. This illustrates, that there is no real specific EEG for PDE, but an identifiable EEG pattern that is highly suggestive of PDE. The sensitivity and specificity of the above mentioned pattern for the diagnosis of PDE has not yet been determined [59].

Gupta et al. [59] stated, that EEG patterns of PDE may also show focal discharges, probably as a result of incomplete myelination of the central nervous system (CNS) with various other EEG abnormalities [64].

Nabbout et al. [47] have done a retrospective study of all clinical records and electroencephalograms of neonates, identified with PDE between 1983 and 1994 at the *Saint Vincent de Paul Hospital*. Follow up ranged from 2 to 10 years. They reported that EEG recordings before pyridoxine administration produce a suggestive pattern. Continuous diffuse high voltage rhythmic delta slow waves, with myoclonic jerks are typical [47]. A similar pattern has been reported in several pyridoxine dependent patients, but it occurs only in bursts and intermingled with spikes [64].
Such continuous delta activity has also been reported in rats with pyridoxine depletion [90].

In the study of *Nabbout et al.* [47] neonates showed various types of seizures. In all cases pyridoxine lead to the resolution of clinical symptoms and normalized the EEG within a few hours. Four neonates were given pyridoxine intravenously at the dose of 100 mg, and immediately after the injection they developed major hypotonia with bradycardia, bradypnoea, and hypothermia. One patient had to be referred to the neonatal intensive care, because of prolonged apnea. Two patients of this study received pyridoxine orally at a dose of 200 mg/ day and did not show these side effects. *Nabbout et al.* [47] reported that the therapeutic benefit was similar to that following intravenous administration [47]. They recommend that a pyridoxine trial should be performed orally with 200 mg as the initial dose, and the intravenous route restricted to patients with status epilepticus [47].

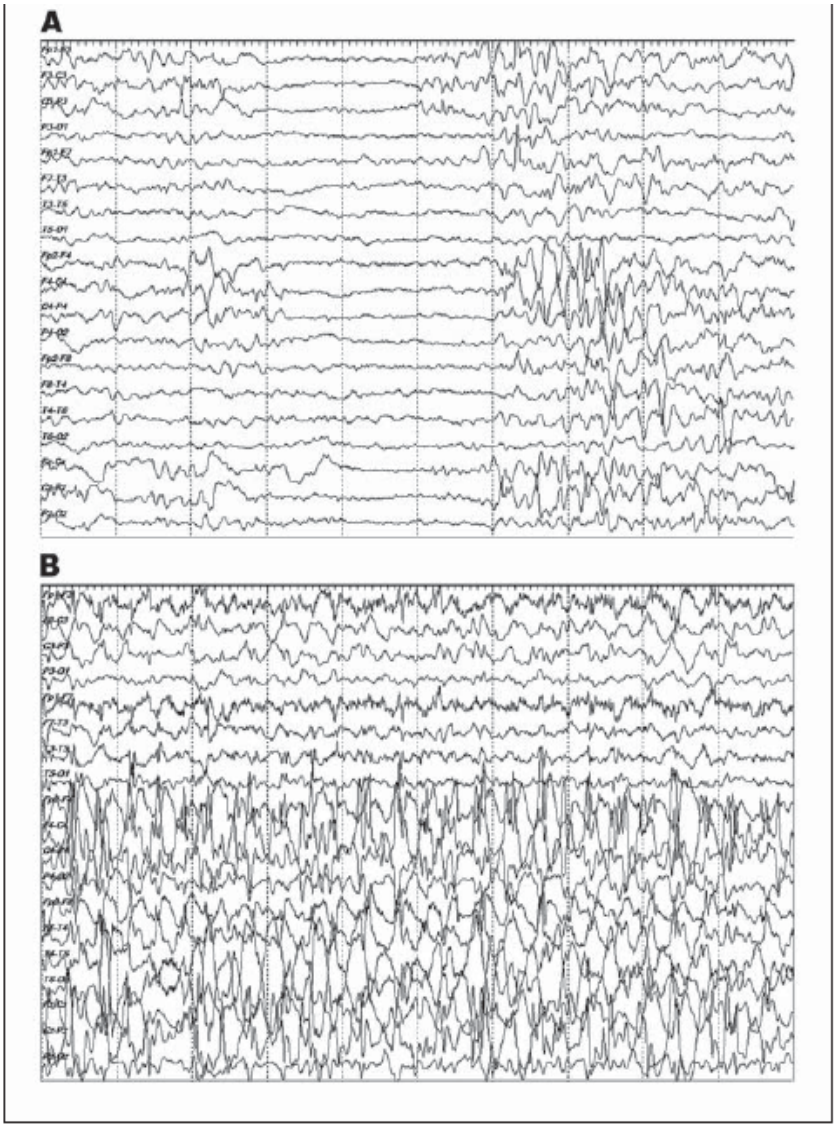

Fig 1. (A) Sleep EEG with burst-suppression pattern. (B) High-voltage eletrographic seizure activity consisting of spike and polyspike-wave complexes lateralized to the right cerebral hemisphere.

Sensibility = 10 μV, time constant = 0.1 s, high frequency filter = 35 Hz

Figure 5: EEG in a 29 days old PDE patient before pyridoxine [66].

1.4.3. Therapy

Diagnosis depends on a demonstration that the seizures cease after pyridoxine has been administered and recurr when it is withdrawn. Till these days it is not clear what dose or route should be used when a trial of pyridoxine has been given [77]. *Baxter et al.* [77] note that over the last 40 years the usual dose has increased from a range of 5 to 50 mg/ kg up to 50 to 100 mg/ kg (a range of approximately 15 to 30 mg/kg) and the intravenous route has become more commonly used. Though not specified, we assume that this recommendation is mainly concerning the neonatal period. They write that the route but not the dose affects the speed of the clinical response which is very rapid if intravenous pyridoxine is given [77]. It is important that patients with suspected PDE should receive pyridoxine at least more than one time, as EEG can take up to 24- 48 hours to normalize [64]. EEG does not always respond immediately: in general, spikes will disappear rapidly, but slow activity can persist for many hours [77].

For the testing of suspected PDE in a neonate an initial intravenous test dose of 100 mg appears to be widely accepted [61]. Additional 100 mg doses of pyridoxine may be given up to 500 mg, if there is no clinical or EEG improvement within 10 min after the first dose [62]. Seizure activity usually stops within 2–4 min of the pyridoxine test dose, although EEG discharges may take slightly longer to normalize. The commonly reported time to resolution of epileptic potentials is approximately 2–6 min, however *Mikati et al*. [64] report that in their patient the EEG was still abnormal after 48 hours [64].

Another interesting fact is that administration of pyridoxine by any route (including oral) not only stops the seizures, but can also lead to marked CNS depression and even apnea, just in early onset seizures [77]. Thus full resuscitation equipment has to be at hand. *Baxter et al*. [77] describe that more typically, a child, following pyridoxine application, may sleep for some hours, with marked associated hypotonia. The risk for this CNS depression seems to be greater when the child is on other anticonvulsants. It is remarkable, that there are no similar central effects for late-onset instances or when pyridoxine is restarted after pyridoxine withdrawal. To our knowledge apnea due to pyridoxine administration has never been reported in non-responders.

The oral maintenance dose of pyridoxine varies between 2 mg and 300 mg/day [62]. *Haenggeli et al.* [62] reported in their review that most patients remained seizure-free on doses between 20 mg and 100 mg/day. Some patients required small doses as low as 2-5 mg/day [69, 70, 71, 72] whereas others needed doses up to 200 mg or even 300 mg/day to remain seizure-free [74, 75]. Some patients had immediate cessation of seizures, while in others it was took 24 to 48 hours to achieve complete seizure control [74].

Some patients suffered from seizure recurrence during an intercurrent illness while on pyridoxine supplementation. For this reason *Haenggeli et al*. [62] and *Baxter et al.* [77] suggested an increase of the oral maintenance dose or an additional parenteral dose during febrile infections.

Therapy resistance to an adequate dose of pyridoxine normally excludes the diagnosis. However, *Baxter at al.* [77] point out that both liquid and tablet preparations have a very unpleasant taste. For this reason it should be considered that compliance, particularly in young children, can be poor, with occurring breakthrough seizures.

2.2.5. Imaging

Although several imaging studies have been done in PDE, no consistent or disease specific abnormality has been found. Nevertheless an unexpectedly high prevalence of variable structural CNS defects has been reported [63, 70, 80].

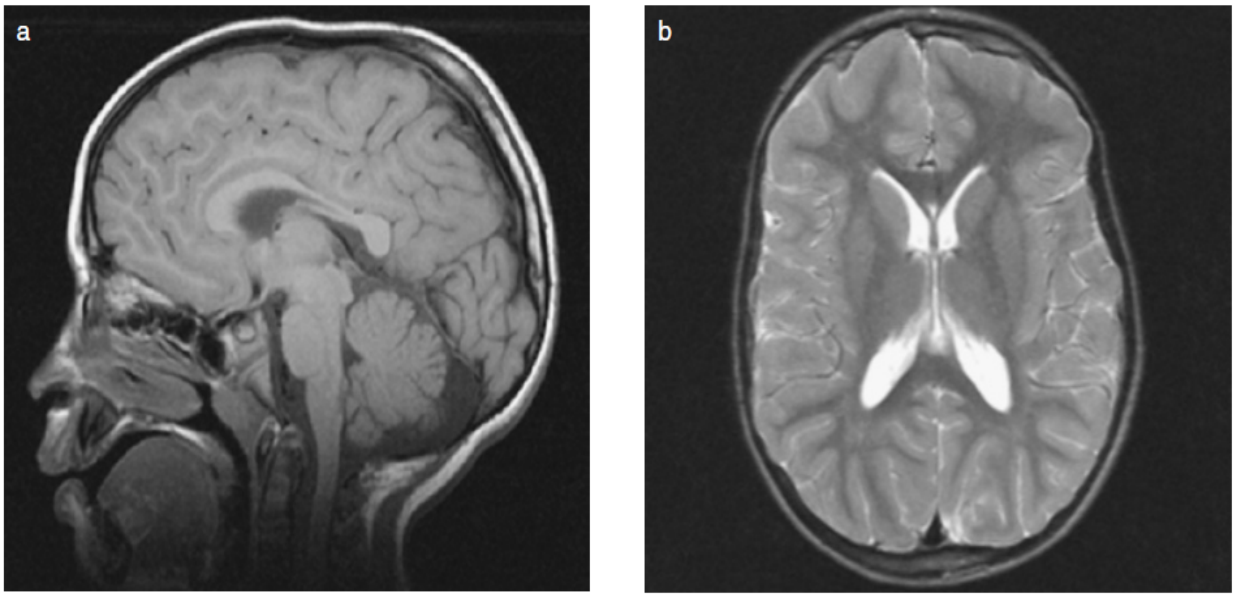

Figure 6: MRI aged 7 years 3 months. Minimal global lack of white matter bulk, with some thinning of the posterior region of corpus callosum [89].

The most typical structural abnormality is hypoplasia of the posterior part of the corpus callosum (**Figure 6**) which was a consistent finding in all six of the children in the Northern Region study of *Baxter et al.* [63] and which has since been confirmed in a study of two other children [63, 80]. In the Annotation of *Baxter et al.* [63] it is noted that another finding in PDE is cerebellar hypoplasia with a megacisterna magna which was seen in two of the six children in the Northern Region study [63]. In addition, abnormalities of the white matter may be present. Some neonatal- ultrasound scans have shown apparent cysts adjacent to the lateral ventricles [81].

Untreated seizures can be associated with intraventricular and/or subarachnoid haemorrhage as well as white matter changes, which may be partially reversible [63, 80, 81] Also hydrocephalus can be present: the reason is not known but both intraventricular haemorrhage and aqueduct stenosis due to gliosis may be contributing factors.

This correlates with neuropathological findings which were performed on siblings, with a retrospective diagnosis of PDE. Most of those neuropathological studies come from siblings who died acutely in the early neonatal period. Their brains largely showed oedematous changes and intracerebral bleeding in some. Later changes are mainly those of widespread gliosis, particularly around the aqueduct, which could explain the association with hydrocephalus [33, 82].

2.2.5. Outcome

Several factors affect the intellectual and physical outcome in PDE patients:

- Age of clinical presentation: It is the most obvious factor. Children presenting after 1 month of age do considerably better than those with early onsets; while infants with early seizures, initially responsive to routine anticonvulsants have a poor prognosis [77],
- Delay before treatment: There is some correlation between delay of treatment and outcome, but *Baxter et al*. [77] write that this is less precise. In early onset seizures, delays of up to 4 days do not seem to cause additional harm, while delays of more than 1 week are associated with an increasing risk of learning difficulties and cerebral palsy. Late- onset patients tolerate much longer delays without apparent harm. *Haenggeli et al.* [62] did not identify a correlation between prognosis and delay of treatment, but they did not consider the age at onset.

- Maintenance dose: As PDE patients need lifelong treatment, it is of crucial importance that they receive pyridoxine continuously. Poor compliance, leading to seizures recurrence is having a negative impact on the longterm outcome [77]. The dosage of pyridoxine may have to be adjusted with the growth and age of the children [77].

Several authors state, that even with early treatment the outcome of PDE patient is poor [33, 62] and many children show learning difficulties. More detailed psychometric assessments show, that on non-verbal measures children who have been treated early are within the normal range. However, verbal scores are poor, and *Baxter et al*. [77] think, that this may give a misleading impression of their overall intellectual ability. More detailed psycholinguistic analysis demonstrate, that receptive verbal skills are normal, but expressive language functions are impaired. *Baxter et al.* [77] report that this appears to be a specific feature of PDE and is therefore part of the clinical picture of early onset forms, whereas it is less marked in children with later onset forms. Some children with early onset forms also show a very marked articulatory dyspraxia to the extent that their spoken language is difficult to understand, necessitating special schooling [77].

Another interesting observation was done in one child with late-onset seizures in the Northern Region [63] study, who presented with school failure at 10 years of age. His dose of pyridoxine had remained unchanged at 50 mg throughout his life. Increasing the dose to 150 mg resulted in a sharp increase in his IQ from below normal to within the average range. *Baxter et al.* [77] studied this prospectively in five other children in the region. They came to the conclusion that IQ appears to improve with a pyridoxine dose up to, but not above, 15 to 18 mg/kg/day [63, 77]. Dosage should not exceed 500 mg per day. Such therapy is required for life, affected individuals are metabolically dependent on the vitamin, rather than pyridoxine deficient. Parents think that their children's improvement appears to be linked to a better and higher attention [77]. These results suggest that the optimum dose of pyridoxine is maybe not the minimum for seizure control, but the one which leads to best IQ [77] without side effects.

The over-zealous use of pyridoxine must be avoided, as a reversible sensory neuropathy (ganglionopathy) caused by pyridoxine- neurotoxicity can develop. Primarily this ganglionopathy was reported in adults who have received "megavitamin therapy" with pyridoxine [85]. After the description of a non-disabling sensory neuropathy in one adolescent with possible PDE, who received 2 grams of pyridoxine per day [84], recent concerns about possible side effects, particularly in non-medical uses of pyridoxine emerged.

For this reason affected individuals should be followed for the development of clinical signs of a sensory neuropathy, including regular assessments of sensory nerve conduction velocity, ankle jerks, gait, and station [84]. Also regular assessments of intellectual function should be offered as it is important to follow the intellectual development [84].

Patients, Methods, Results

3.1. Patients and Methods

Demographic data of 30 patients were collected in the form of a standardized questionnaire from 17 different centers, from Austria, Belgium, Brussel, Germany, Hungary, Italy, Swiss and Turkey. Of the 30 patients, 12 were female and 18 were male. Parents of patient 21 and 22 were consanguineous. *Patient 19* had an older brother who died undiagnosed with neonatal seizures, and who was not included in this study.

The questionnaire, received by all centers, included 16 main topics, which had to be filled out by the treating physician:

- Somatic data at birth (Gestation age, birth weight, length, head circumference)
- Onset and type of seizures (Onset and types of seizures)
- EEG findings (EEG before -, after - and the last EEG while on B_6)
- Dosage of B_6 (age at first B_6 administration, initial dosage response, actual dosage of B_6, form of B_6)
- Breakthrough seizures while on B_6 (age, fever, B_6 form and dosage, other anticonvulsants)
- Withdrawal (age at withdrawal, time period to seizure recurrence, type of seizures, additional symptoms, response to B_6 dosage)
- MRI Findings (ventriculomegaly, posterior corpus callosum agenesis, intraventricular bleeding, age at MRI)
- Neurologic outcome (age of assessment, head circumference, IQ, mental retardation, spasticity, hypotonia)
- Pipecolic acid in plasma before B_6
- Pipecolic acid in plasma while on B_6
- Pipecolic acid in cerebrospinal fluid before (or while off) B_6

- Pipecolic acid in cerebrospinal fluid while on B_6
- Pipecolic acid in urine before B_6
- Pipecolic acid in urine while on B_6
- Anticonvulsants before B_6 (substance, dosage, effect on seizures)
- Anticonvulsants before B_6 (substance, dosage, reasons for co-medication)

3.2. Results

3.2.1. Birth Percentile

All patients were born beyond the 37th week of gestation, birth weight of children varied from 2825g to 4000 gram, whereas the length varied from 49 cm to 55 cm and the head circumference was determined in 10 children and ranged from 32.7 cm to 37.5 cm at birth (±SD= 1.46 cm). Out of these ten, 4 patients were macrocephalic (>97 percentile) (*patients 7,11, 27, 29*), one patient was at 90 percentile (*patient 20*), one patient was > 75 percentile (*patient 21*) and 4 patients were > 50 percentile (*patients 24, 26, 28 and 30*).

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In 18 patients Apgar- Score was determined, and just in two patients (*patient 8 and 20*) fetal distress was present, while in the other 16 Apgar Score was normal (**Table 6**).

Table 6: Apgar Scores of patients

Patients Number	Apgar Score
1	Not available
2	No fetal distress
3	Not available
4	Not available

5	Not available
6	No fetal distress
7	No fetal distress
8	Fetal distress
9	No fetal distress
10	Not available
11	No fetal distress
12	Not available
13	Not available
14	Not available
15	No fetal distress
16	Not available
17	Not available
18	Not available
19	10/10
20	5/7/9
21	10/10
22	Not available
23	10/10
24	8/9/9
25	10/10
26	9/9/10
27	9/8/8
28	No fetal distress
29	No fetal distress
30	9/10/10

3.2.2. Seizure Onset and Type of Seizure

Out of the 30 patients all had seizure onset in their neonatal period from minutes after birth up to 3 weeks of age.

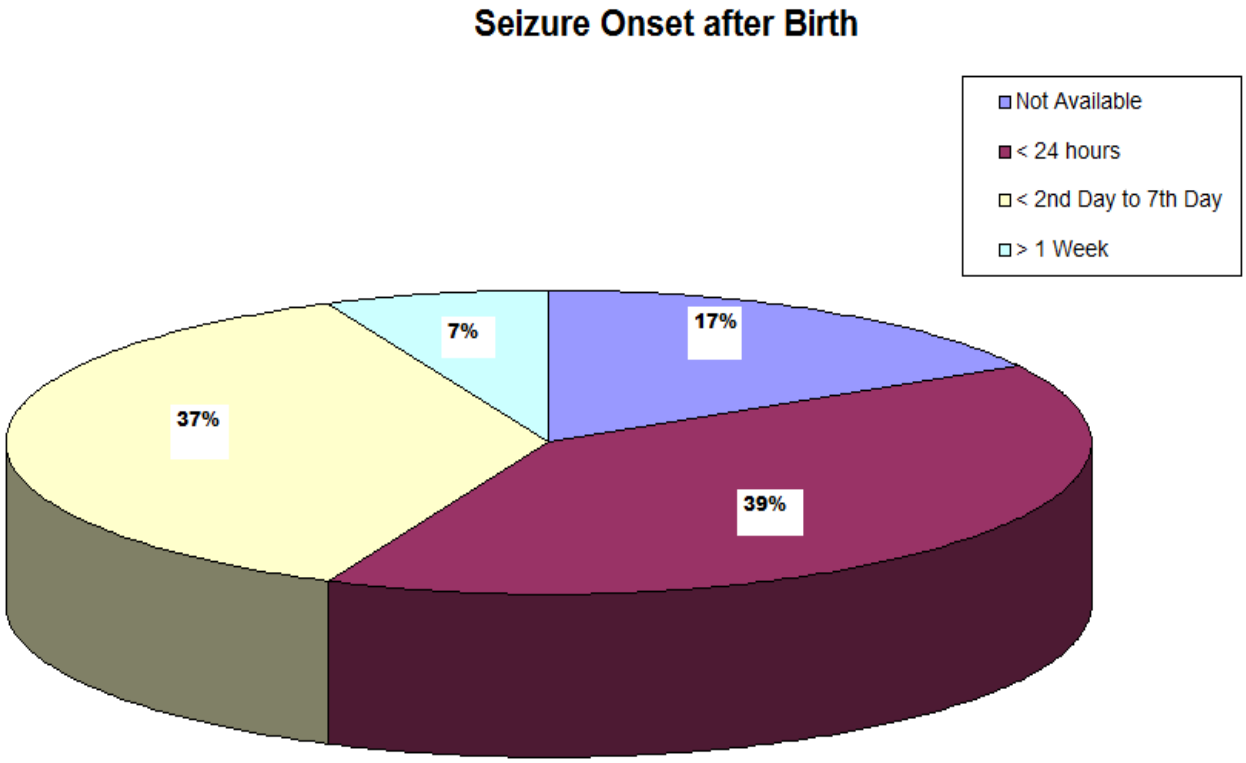

Figure 7: Seizure onset after birth. N=30

Figure 7 shows that 39% of cases had a seizure onset within the first 24 hours of life, while 37% had seizure onset from day 2nd to day 7th and only 7% experienced seizures onset beyond the first week of life.

Seizure types at onset were various and included the following types, which were specified in 22 patients:

- Generalized tonic clonic (GTC) n= 10
- Myoclonic, n= 6
- Infantile spasms n=1

- Eye deviations n= 1
- In 4 cases seizures were mixed and specification to one seizure type was not possibel.

Additional features were present in 15 neonates. Also in this category signs and symptoms were various and included the following.

- Poor sucking n= 2
- Restlessness n= 2
- ARDS (Adult respiratory distress syndrome) n= 1
- Abnormal crying n= 5
- Apnea n= 2
- DIC (disseminated intravascular coagulation) n= 1
- Hypoglycemia n= 1
- Hypotonia n= 7
- Irritability n= 6
- Sleeplessness n= 3
- Abdominal signs (e.g.: abdominal distension, vomiting) n= 3
- Macrocephalus n= 1
- Tremor n= 1
- Infection like symptoms (e.g.: fever, paleness) n= 1

Most patients had a combination of irritability, abnormal crying, abdominal signs and sleeplessness.

3.2.3. EEG Findings

In 11 patients the EEG before B_6 administration was not performed or not available. The EEG's done before treatment with Vitamin B_6 showed different abnormal patterns:

- Focal epileptic discharges n= 2
- Burst suppression n= 7

- Multifocal spike waves n= 3
- Focal slowing n= 1
- Hypsarrhythmia n= 4
- Bilateral frontal-central slow wave n= 1
- EEG with lack of normal background activity n= 1

EEG data were available from 24 patients while on vitamin B_6 treatment. From the 25 EEG's performed, 15 were normal (*patients 1, 5, 6, 7, 9, 10, 13, 15, 17, 20, 21, 22, 28, 29, 30*), while the other 9 EEG's showed:

- Slight focal slowing (*patient 18*)
- Moderate slowing (*patient 14*)
- Mild slowing (*patient 4, 16*)
- Diffuse slowing, sharp- and sharp slow waves (*patient 2*)
- Diffuse slowing (*patient 8*)
- Bilateral frontoparietal slow waves (*patient 11 and 12*)
- Moderate general slow activity without paroxysmal pattern (*patient 19*)

Patient age of last EEG varied from 6 months up to an age of 19 3/12 y.

3.2.4. Dosage of Vitamin B_6

Only 4 patients had their first administration of pyridoxine within the first 24 hours. 9 patients received their first pyridoxine from the 2nd day of life until 6 weeks of age, while in 10 patients treatment was markedly delayed (6 weeks to 5 years). The initial dosage to test B_6 response varied from 50 mg up to 300 mg daily. The actual dosage of pyridoxine treatment ranged from 1.7 mg/kg (*patient 19*) up to 65 mg/kg daily (*patient 20*). In most cases vitamin B_6 was applied in form of Pyridoxine HCl, while other forms of pyridoxine application were not specified.

3.2.5. Breakthrough Seizures while on B_6

Breakthrough seizures while on B_6 treatment were observed in 4 patients *(patients 6, 7, 8, 11)*. Out of the 4 patients, *patient 11* experienced 5 breakthrough seizures during pyridoxine therapy, but just during one of these episodes he was suffering from fever (>38.5). *Patient 11* had all break through seizures in the 4th year of life (4, 4 1/12, 4 3/12, 4 10/12 and 4 11/12 years). He was treated with dosages between 300 up to 500 mg Pyridoxin HCl daily. During the first three breakthrough seizures no additional anticonvulsants were administered, while the last two seizures occured while on CBZ comedication.

The other three patients were not suffering from fever when they developed the breakthrough seizures and just one patient (*patient 7*) was receiving another anticonvulsant than pyridoxine. Add on therapy in *patient 7* was performed with PHB (Phenobarbital) The only measurement taken in *patient 8*, was increasing the pyridoxine dosage. He suffered from two breakthrough seizures at the age of 3 and 8 years.

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3.2.6. Withdrawal of Vitamin B_6

Before genetic testing became available the diagnosis of PDE was confirmed by a vitamin B_6 withdrawal. From the 30 patients vitamin B_6 withdrawal was not performed in 11 patients. They probably did not need to undergo withdrawal, because of selective biochemical or genetic testings. The youngest patients experienced their withdrawal in the newborn period (*patients 6, 9 and 11*), while the latest withdrawal was performed at the age of 16 years (*patient 12*). It has to be mentioned that all patients where withdrawal was performed showed seizure recurrence. Time to recurrence of seizures ranged from 24 hours up to 6 weeks.

Following types of seizures could be seen:

- Status epilepticus n= 2
- Myoclonic n= 3
- Generalized tonic-clonic n= 4
- Complex partial n= 1
- Focal n= 1
- No specification of seizure type n= 8

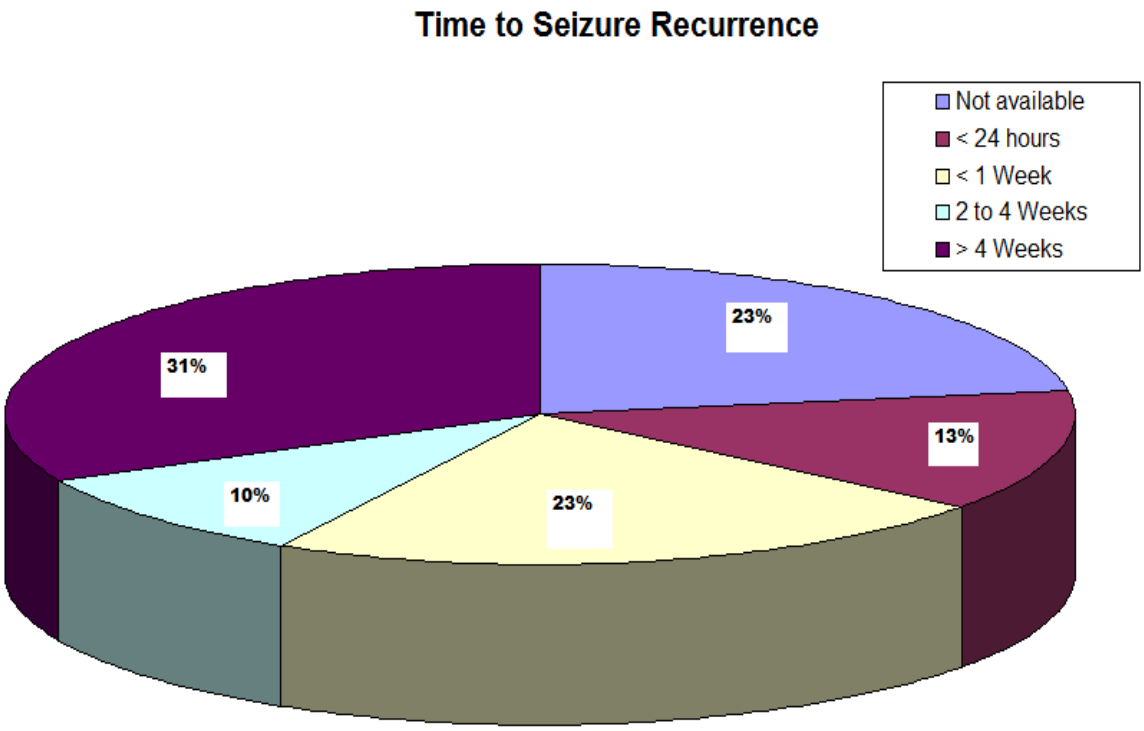
Beside seizure recurrence 6 patients presented additional symptoms:

- Vomiting n= 1
- Sleeplessness n= 2
- Irritability n= 2
- Arrousels n= 1
- Abnormal Crying n= 1
- Opisthotonus n= 1
- Somnolence n= 1
- Tachykardia n= 1
- Hypotonia n= 1

After vitamin B_6 withdrawal, all patients showed a favourable pyridoxine response. Dosages to assess the pyridoxine response after withdrawal varied from 40 mg to around 300 mg/day.

In our cohort pyridoxine was stopped (**Figure 8**) as a planned withdrawal to establish a diagnosis in 16 of the 30 cases. In around a third of these patients, seizures reocurred after more than four weeks (31%). 23% of the patients were seizure free for one week only while 13% of the patients, experienced seizure recurrence within the first 24 hours. 10% were seizure free for two to four weeks,

Figure 8: Recurrence of Seizures after Pyridoxine Withdrawal.



3.2.7. MRI Findings

All but 5 patients underwent MRI examination. Out of these 5 patients, *patient 2* and *9* were examined by CT at the age of 11 months and 4 ¾ years (*patient 2*) and 6 years (*patient 9*). *Both patients* showed ventriculomegaly, but did not need ventriculoperitoneal shunting. *Patients 21, 24* and *30* were examined by US (Ultrasound), where no pathologic changes could be detected.

The youngest patient to undergo MRI was 21 days old (*patient 26*) while the oldest was 17 years old (*patient 19*). MRI showed *ventriculomegaly* in 7 patients but only two patients (patient 2 and14) had to be shunted.

Three patients (*patient 6, 7* and *29*) had posterior corpus callosum agenesis and two patients had signs of intraventricular bleeding (*patient 6 and 7*)

In addition the following pathologies were visible on MRI:

- Delayed myelination (*patient 6, 8*)
- Bilateral subcortical and periventricular white matter alterations with nodular aspect in the frontal and occipital areas. Probably white matter lesions in the cerebellum (*patient 20*)
- Slight generalized atrophy (*patient 19*)
- Not specified severe damage (*patient 23*)
- Subependymal leukencephalopathy (*patient 26*)

3.2.8. Neurologic Outcome

Data on neurologic outcome were available for all patients except one case (*patient 3*). Age of assessment was between 7 months and 19 3/12 years. Unfortunately precise IQ, DQ or SON-R assessments were only determined in 6 cases, with the age of assessment:

- *Patient 15:* IQ 90 (14.5 years)
- *Patient 19*: IQ 61, VQ 72, PQ 58 (17 years)
- *Patient 20*: 85 SON-R* (3.5 years)
- *Patient 23*: IQ< 24 age (16 years)
- *Patient 26*: DQ* 75; mild motor delay, no behavioural abnormalities (16 months)
- *Patient 27*: IQ 75 (7 years)

* SON-R: The **SON-tests** are individual intelligence tests for general application which do not require the use of spoken or written language. The **SON-tests** are especially suited for children with problems in the area of language and verbal communication and for immigrant children.

* DQ: The developmental quotient is a norm used to express aspects of a child's development as measured by the Gesell Development Schedules

Mental outcome varied from normal, mild, moderate to severe mental retardation (7 normal, 10 mild, 4 moderate and 6 severe mental retardation, while in the other cases some degree of mental outcome was present but not specified).
In addition 7 patients suffered from hypotonia and 3 patients showed spasticity. *Patient 23* is suffering from severe quadriplegia, microcephaly, and severe mental retardation, most probably as sequelea after prolonged resuscitation due to general collaps on the 2nd of life.

Figure 9 shows that in 87% of our patients, the degree of mental outcome was assessed. 20% showed a normal performance and neurologic outcome. 34% were mildly retarded, while 13% suffered from moderate and 20% from severe mental retardation. In our series two children (*patient 2 and 14*) needed to be shunted because of hydrocephalus and both are severely mentally retarded.

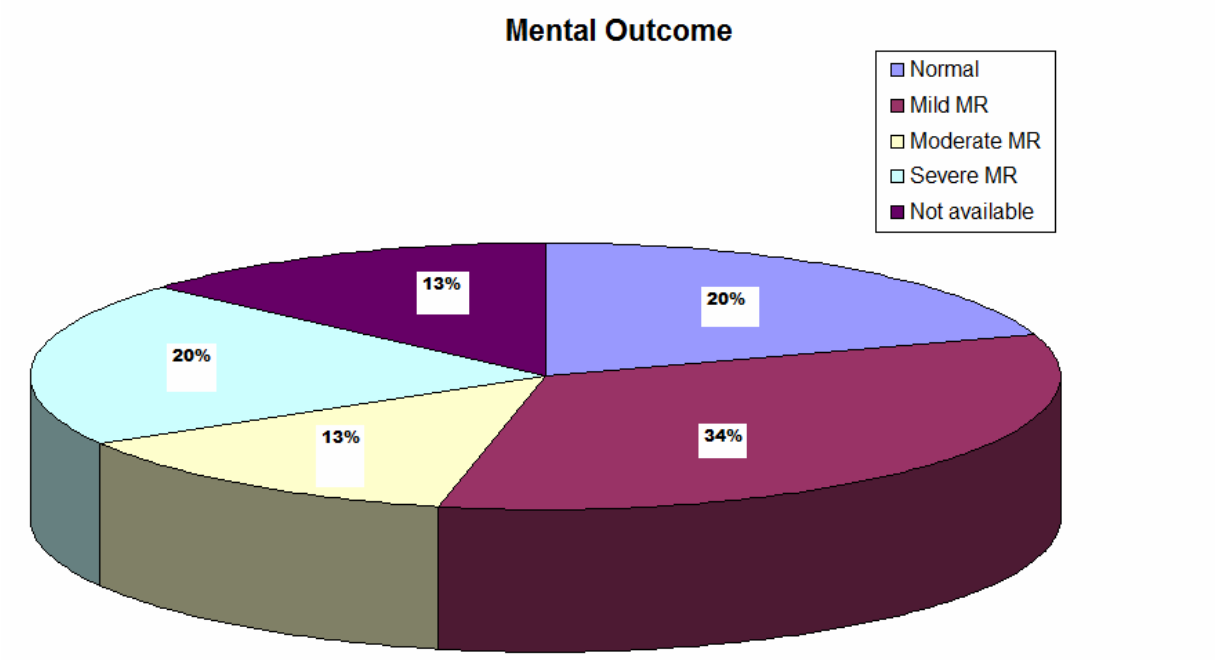


Figure 9: Degree of Mental Outcome in PDE Patients

In the following **Table 7**, the patient's mental outcome assessments are compared to the delay of specific pyridoxine therapy. The results are sorted according to the degree of mental outcome (normal to severe) and the delay of specific pyridoxine treatment. In our series, there is no clear correlation between the start of the treatment and outcome. For example *patient 13* had a normal outcome, although specific treatment was started when he was already 2 weeks old. *Patient 17* show a mild retardation, although pyridoxine was applied with a delay of 5 month. *Patient 23* is the most severe case, as described above, although he received pyridoxine 5 days after birth.

Patient Number	Age at first Pyridoxine Trial	Mental Outcome
4	1st day	Normal
9	1st day	Normal
10	2nd day, 4 weeks, 14 months	Normal
24	7 days	Normal
13	2 weeks	Normal
5	Not available	Normal
6	1st day of life	Mild Mental Retardation
11	1st day of life	Mild Mental Retardation
1	1st week	Mild Mental Retardation
26	6 days	Mild Mental Retardation
16	4 weeks	Mild Mental Retardation
15	14 weeks	Mild Mental Retardation
17	5 months	Mild Mental Retardation
20	Not available	Mild Mental Retardation
25	Not available	Mild Mental Retardation
12	Not available	Mild Mental Retardation
21	2 days	Moderate Mental Retardation
19	3 days	Moderate Mental Retardation
7	3.5 months	Moderate Mental Retardation
27	Not available	Moderate Mental Retardation
23	5 days	Severe Mental Retardation
22	2 weeks	Severe Mental Retardation
2	~6 weeks	Severe Mental Retardation
18	12 months	Severe Mental Retardation

14	17 months	Severe Mental Retardation
8	18 months	Severe Mental Retardation
3	Not available	Not Available
28	Not available	Not Available
29	3 months	Not Available
30	Not available	Not Available

Table 7: Delay of Treatment in Comparison to Mental Outcome

3.2.9. Pipecolic Acid (PA) in Plasma while on Vitamin B_6

In 14 patients plasma pipecolic acid was available while on vitamin B_6 therapy. In all PDE patients PA was elevated from 1 to 5.6 times (*patient 6*) over the normal age range In four patients (*patient 7, 11, 15, 30*) plasma pipecolic acid was measured several times.

- Pipecolic acid of *patient 7, treated at the Medical University Graz* was determined 15 times **(Table 8)**. The values of pipecolic acid from *patient 7*, were collected over a period of 10 9/12 years, and are therefore illustrating that plasma pipecolic acid values are decreasing during Vitamin B_6 treatment. While pipecolic acid at the age of 7 month was 9.87 µmol/ l, it reached the lowest level with 2.6 µmol/ l at the age of 9 6/12 years. *Patient 7* received 200 mg pyridoxine daily, which was increased up to 300 mg daily. Last measurement of pipecolic acid was showing a result of 4.9 µmol/ l. Compared to age-related control ranges the elevation of pipecolic acid while on pyridoxine ranged between 1 to more than 2.3 times of normal.

- Pipecolic acid of *patient 11, also treated at the Medical University Graz,* was determined 15 times **(Table 9).** The values of plasma pipecolic acid were collected over a time period of 5 5/12 years. Again plasma pipecolic acid decreases over time along with vitamin B6 therapy. While plasma pipecolic acid at the age of 5 months was 14.3 µmol/ l, it markedly decreased to 4.9 µmol/ l at the age of 5 10/12 years. At the beginning *patient 11* received 70 mg pyridoxine HCl/day, the dosage was raised up to 500 mg daily (*patient 11* was 4 5/12 years old) and then decreased again to 300 mg/ day. Elevation of pipecolic acid was between 1.2 to more than 3.5 times above the normal age dependent range.

Table 8 Plasma pipecolic acid values of *patient 7*, over a time period of 10 years and 5 months.

PA in Plasma µmol/l	Normal Age Range µmol/l zitat	X- elevation	Age at Sampling	B6 dosage/ day
9.87	0.8-5.3	1.9	7m	200mg
9.51	0.4-4.9	1.9	14m	200mg
5.7	0.4-4.9	1.2	3 6/12y	300mg
6	0.7-2.6	2.3	6 4/12y	300mg
5.8	0.7-2.6	2.2	7 1/12y	300mg
4.07	0.7-2.6	1.5	7.2y	300mg
5.3	0.7-2.6	2	7.4y	300mg
4.0 (after 48 hours withdrawal)	0.7-2.6	1.5	7.4y	300mg
3.6 (after 72	0.7-2.6	1.4	7.4y	300mg

hours withdrawal)				
4.6	0.7-2.6	1.8	7 11/12y	300mg
4	0.7-2.6	1.53	9 1/12y	300mg
2.6	0.7-2.6	1	9 6/12y	300mg
5.15	0.7-2.6	2	10y	300mg
4.1	0.7-2.6	1.6	10 6/12y	300mg
4.9	0.7-2.6	1.9	11 4/12y	300mg

The following diagram shows all plasma PA values in µmol/l of *patient 7.*

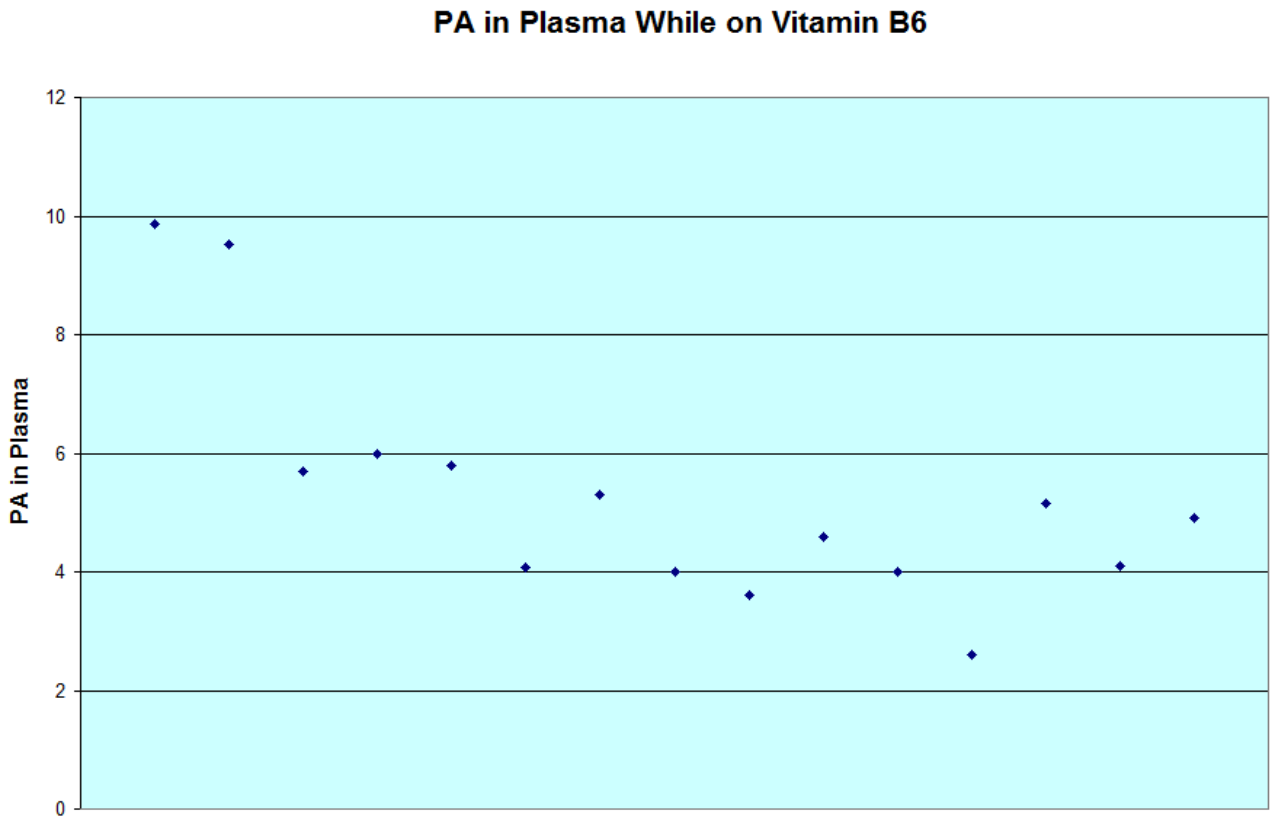


Figure 10: All measured plasmatic values of PA (µmol/l) in *patient 7*

Table 9: Plasma pipecolic acid values of *patient 12*, over a time period of 5 years and 10 months.

PA in Plasma µmol/l	Normal Age Range µmol/l	X- elevation	Age at Sampling	B6 dosage/ kg BW
14.3	0.6-4.1	3.5	5m	70mg PHCl
10.1	0.8-5.3	1.9	9m	100mg
8.2	0.4-4.9	1.7	22m	120mg PHCl
8.0	0.4-4.9	1.6	23m	150mg
7.4	0.4-4.9	1.5	35m	200mg
6.4	0.4-4.9	1.3	3 11/12y	300mg
6.3	0.4-4.9	1.3	4 5/12y	500mg PLP
6.1	0.4-4.9	1.2	4 10/12y	300mg
5.6	0.7-2.6	2.2	5 1/12y	300mg
5.8	0.7-2.6	2.2	5 4/12y	300mg
4.9	0.7-2.6	1.9	5 10/12y	300mg

3.2.10. Anticonvulsants before Vitamin B_6

4 patients were not receiving any anticonvulsants therapy before vitamin B_6 treatment (*patients 22 and 23*, who are brothers and patients *21 and 30*). In 12 patients it is not known if they received prior anticonvulsant treatment.

Following substances were given, before specific pyridoxine treatment was started:

- Phenobarbital
- Carbamazepine
- Chloralhydrate
- Clonazepam
- Valproat
- Vigabatrin
- ACTH
- Phenytoin
- Diazepam (Benzodiazepam)
- Brom

In the following **Table 10** all patients, who received anticonvulsant therapy prior to pyridoxine, are listed.

Table 10: Anticonvulsants Before Vitamin B_6 Therapy .

Patient Numbers	Substances	Dosage mg/ kg	Effect on seizures
2	Phenobarbital Phenytoin	-	-
6	Phenobarbital	10 mg/kg	Temporary 03-04/02
7	Phenobarbital, Carbamazepin	Up to 7.5 mg/kg	No effect
8	Phenobarbital		Phenobarbital: partial

	Diazepam Clonazepam, Valproat, Vigabatrin, ACTH, Chloralhydrate		responsive ACTH: improvement
9	Phenobarbital	25 mg/kg	No effect
11	Phenobarbital Chloralhydrate	-	-
14	Phenobarbital Diazepam ACTH	-	Transient seizure reduction
18	Phenobarbital Diazepam (i.v. in neonatal period)	30 mg/kg Phenobarbitol+0.3 ml/kg Diazepam	No effect
22	Phenobarbital	5 mg/kg	No effect
23	Benzodiazepine	One dose	No effect
24	Benzodiazepine, Brom, Phenobarbital	Brom Phenobarbital	Brom: No effect Phenobarbital: seizure free for a short period
25	Diazepam, Phenytoin, Phenobarbital	Diazepam: 0.3mg/kg BW Phenytoin: 18mg/kg BW, Phenobarbital: 10 mg/kg BW	No effect
26	Phenobarbital	8 mg/kg	Seizure free for a short period

27	Benzodiazepine, ACTH, Phenytoin, Phenobarbital, Carbamazepine	-	No effect
29	Phenobarbital	5-10 mg/kg	No effect

3.2.11. Anticonvulsants while on Vitamin B_6

Although patients were already on Vitamin B_6 treatment, 8 were continuing other anticonvulsants as shown in **Table 11**. There were several reasons for co- medication during vitamin B_6 therapy:

- Prolonged status epilepticus at the age of 6 years which lead to the final diagnosis of PDE (*patient 7)*
- Breakthrough seizures on Vitamin B_6 (*patient 11*)
- Infantile spasms (*patient 14*)
- Headache (*patient 18*)
- Unclear diagnosis (*patient 26*)

In the following **Table 11** all substances are listed, with the reasons for co-mediaction while on Vitamin B_6.

Table 11: Anticonvulsants While on Vitamin B_6Therapy

Patient initials	Substance	Dosage/ kg	From to	Reason for co-medication
2	Phenobarbital	1.8 mg/kg	-	-
7	Phenobarbital	12.5mg/kg	7-7 10/12y	Unrecognized diagnosis before prolonged status at age of 6 years. Could be terminated without seizure recurrence
11	Carbamazepine	-	-	Afebrile breakthrough seizures on B_6
14	ACTH Phenobarbital Clonazepam	 3.7mg/kg/d 0.1mg/kg/d	14 to 18w 3w to 5m 5m to 16m	Infantile spasms
18	Carbamazepine	4mg/kg (low not-antiepileptic dose)	Only from 16y	Head ache
19	Phenobarbital DPH	5mg/kg/d		Stop at 08.12.03
24	Illegible	400 mg/d	-	-
26	Phenobarbital	8 mg/kg	1st day to 2 months	Unclear diagnose

This work is focussing on phenotypical variations in pyridoxine dependent epilepsy (PDE) with proven mutations in the *antiquitin* gene. **Table 12 and 13** summarize the most important information on clinical and EEG findings recorded in the questionnaires,

- Seizure Semiology- EEG findings and the initial pyridoxine trial (**Table 12**)
- Clinical and EEG findings on pyridoxine maintenance (**Table 13**)

Table 12: Seizure Semiology- EEG findings and Pyridoxine Trial

Pat. Nr.	First Seizures	Seizure type at onset	EEG prior to B6	Age at first B6 dosage	1st dosage of B6	Age at B6 withdrawal	Intervall to seizure recurrence
1	5th day	Tonic and infantile spasms	Burst suppression pattern	1st week	100 mg i.v.	Not performed	-
2	5 hours	GT, apnea	Burst suppression pattern	6 weeks	150 mg i.v.	7 weeks, 11 months	6 weeks, 24 ho
3	Not available	Not available	Not available	Not available	Not available	Not performed	-
4	1st hour	GTC	Not performed	1st day	100 mg i.v.	Not performed	-
5	Hours	Not available	Not available	Not available	Not available	2 months	Few days
6	1st minute	GTC	Multifocal SW discharges	1st day of life	50 mg i.v.	Newborn, Newborn	11 days, 9 day
7	2nd day	GTC	Focal epileptic discharges	3.5 months	300 mg i.v.	7 years	2 days
8	1st hour	GTC	Burst suppression pattern	18 months	Not available	7 years	2 days
9	14 hours	GTC and myoclonic	Not available	1st day of life	50 mg i.v.	Newborn, 6 months, 6 y	17 days, 6 day days

10	3 hours	GTC, TC, myoclonic	Burst suppression pattern	2nd day, 4 weeks, 14 months	20 mg i.v. no effect, 400 mg i.v. "uncertain effect", 500 mg i.v. stop seizures/24h	Not performed	-
11	1st hour	GTC	Burst suppression pattern	1st day of life	100 mg i.v.	18 days	18 days
12	6-8 hours	Not available	Not available	Unknown	Unknown	16 years	24 hours
13	3rd day	Multifocal, myoclonic	Burst suppression pattern	2 weeks	100 mg i.v.	Not performed	-
14	1st hour	Generalized myoclonic	Bilateral frontal-central SW	17 months	40 mg p.o.	Not performed	-
15	3 weeks	GTC, partial motor	Focal discharges	14 weeks	100 mg p.o.	5 months, 19 months, 32 months	10 days, 7 days, 14 days
16	4-6 hours	Myoclonic	Not available	4 weeks	120 mg i.v.	2.5 years	Few days
17	5th day	GTC	Focal slowing right and S	5 months	Unknown	Not performed	-
18	3rd day	Clonic	Not available	12 months	200 mg i.v.	9 years	3 days
19	1st day	Myoclonus	Like hypsarrythmia with multifocal spikes	3 days	100 mg i.v.	3 years	3 days

20	5th day	Eye deviation, myoclonic	Burst suppression	Not available	50 mg i.v.	Stop B6 at 27thdec 2003	2-3 days
21	2nd day	Generalized clonic	Depressed amplitude	2 days	100 mg	1 week pp	2 days
22	3rd day	Generalized clonic	Not available	2 weeks	100 mg	Not performed	-
23	3rd day	Myoclonic/Clonic	Not available	5 years	Not available	Not performed	-
24	6-7th day	Not available	Not available	7 days	100 mg i.v. fractured by 2 per 40 mg/day	Not performed	-
25	8th day	Myoclonic	Not available	10.7 months	Not available	Not performed	-
26	2nd day	Myoclonic than clonic	Not available	6 days	Not available	2nd day	-
27	Not available	Not available	Normal Ictal: generalized sharp wave	Not available	20 mg/kg	Not performed	3 weeks
28	Not Available	Not available	Hypsarrhythmia	Not available	80 mg/kg	Not performed	5 days
29	Not	Not available	Slowly right	3 months	80 mg/kg	Not performed	-

	Available		hypsarrythmia				
30	Not available	Not available	Lack of normal background activity. Right frontotemporal sharp waves with frequent generalisation during myoclonic seizures	Not available	200 mg fractional as single dose	After first dose	3 days

Table 13: Clinical and EEG Findings on Pyridoxine Maintenance

Patients Number	Present Age at 7.7.2006	Actual B6 Dosage/day	Total daily B6 dose	Last EEG while on B6	Hydrocephalus/ shunt	Neurologic Outcome
1	2 5/12 years	29 mg/kg	-	Normal	Yes/no	Mild MR, Hypotonia
2	14 3/12 years	18 mg/kg	-	Diffuse slowing, sharp waves and sharp slow aves	Yes/yes	Severe MR, Spasticity
3	-	-	-	-	-	-
4	2 1/2 months	10 mg/kg	50 mg	Mild slowing	Yes/no	Normal
5	3 9/12 years	15 mg/kg	-	Normal	No/no	Normal
6	2 11/12 years	30 mg/kg	-	Normal	Yes/no	Mild MR, Hypotonia
7	15 8/12 years	4.2 mg/kg	300 mg	Normal	Yes/no	Moderate MR, Spasticity, Hypotonia
8	13 7/12 years	30 mg/kg	-	Diffuse slowing	No/no	Severe MR, Hypotonia

9	19 3/12 years	11 mg/kg	-	Normal	Yes/no	Normal
10	2 3/12 years	28 mg/kg	-	Normal	No/no	Normal, Hypotonia
11	7 8/12 years	5 mg/kg ??	300 mg	Bilateral frontoparietal slow waves	Yes/no	Mild MR
12	21 5/12 years	5 mg/kg	-	Bilateral frontoparietal slow waves	No/no	Mild MR
13	4 months	28 mg/kg	-	Normal	No/no	Normal
14	10 years	Not available	120 mg	Moderate slowing	Yes/yes	Severe MR, Spasticity
15	17 4/12 years	20 mg/kg	-	Normal	No/no	Mild MR
16	3 6/12 years	Not available	120 mg	Mild slowing	Yes/no	Mild MR, Ataxia
17	6 2/12 years	2 mg/kg	-	Normal	No	Mild MR, Speech delay
18	26 years	5.6 mg/kg	-	Focal slowing	No	Severe MR, Hypotonia

19	-	100 mg/day-1.7 mg/kg	-	Moderate general slow activity without paroxysmal pattern	No	Moderate MR
20	-	65 mg/day	-	Normal	No	Mild MR
21	-	250 mg/4 kg BW	-	Normal	No	Moderate MR
22	-	200-0-200 mg/37 kg BW/d	-	Normal	No	Severe MR
23	-	100 mg	-	Not available	No	Severe MR, quadriplegia with
24	-	16 mg/kg	-	Not available	Non symetrical ventricels	Normal

25	-	13 mg/kg	-	Not available	Normal	Mild MR
26		Not available		Not available	Ventricular assymetry, no shunt	Mild MR
27	-	10 mg/kg	-	5/07 sharp wave focus from 3. to 6. year of life	No	Mild MR
28		60 mg/kg		Normal	No	Not available
29		60 mg/kg		Normal	No	Not available
30		20 mg/kg		Normal	No	Not available

4. Discussion

PDE is an orphan disease (estimated prevalence of 1:100 000) caused by mutations of the human *antiquitin* gene (ALDH7A1). The *antiquitin* gene is involved in the cerebral pathway of lysine catabolism. Antiquitin deficiency results in elevation of a-AASA, and PA in plasma, CSF and/or urine. Accumulating P6C condenses with PLP by Knoevenagel reaction and inactivates this enzyme cofactor, which is essential for normal metabolism of aminoacids and neurotransmitters [38, 50.] That is why measurements of a-AASA, PA and genetic analysis can be used to establish the diagnosis and to avoid the potentially dangerous withdrawal of pyridoxine [50]. Values of PA in PDE infants were 4.3- to 15.3 [43] fold above the upper normal range. Also in our cohort PA in plasma before pyridoxine treatment was markedly higher ranging from 3.31 up to 9.25 times above the normal range. The degree of PA elevation is not in correlation with the actual dosage of pyridoxine, but may be correlated to the dietary intake of lysine and the individual residual activity of the a-AASA dehydrogenase. A higher PA level in CSF than in plasma can be used as clear discrimination between PDE and peroxisomal diseases [50], which are also associated with elevated levels of PA in plasma. A-AASA is the disease specific marker and helps to select patients for molecular analysis.

ALDH7A1 is the only gene identified so far to be associated with PDE, but there is at least one other gene locus encoding another inherited PDE, as has been shown by linkage studies [48].

Prenatal diagnosis for pregnancies at risk of PDE is possible by molecular analysis. This genetic test is usually performed by amniocentesis at about 10 to 12 weeks' gestation [86] or chorionic villus sampling (CVS) at about 15-18 weeks' gestation [86]. Both disease-causing alleles/mutations must have been identified in the index patient before prenatal testing can be offered [86]. Parents asking for genetic testing should be informed that there is a higher risk of abortion, because of intra-uterine infections due to amniocentesis or CVS [86]. The higher risk of miscarriage after amniocentesis has been reported to be between 0.06 to 1.8% [91] while miscarriage after CVS is 1% higher [92].

Classically, children with PDE are presenting with neonatal or even with intrauterine seizure onset. However, there are rare cases with seizure onset in the second up to third year of life [5, 59, 77]. In this study, the majority of the children experienced seizure onset during the first 24 hours of age (39%). Only 6% developed seizures after one week of life. An important aspect which has to be considered by pediatricians, is that neonates with PDE may initially show signs resembling those of birth asphyxia or intrauterine infection. Seizures following those signs may be considered as part of hypoxic-ischemic encephalopathy, which leads to misinterpretation of the clinical findings, and hence to inappropriate treatment. Generally children can show signs of encephalopathy with “atypcal” forms of PDE, like restlessness, poor sucking, hypotonia, and may have abdominal distension or vomiting [65]. These signs have been reported in at least 16 newborns of our study and should be considered in the diagnosis of PDE. Due to the high proportion of atypical cases all children with early onset (younger than 3 years) intractable seizures or status epilepticus should receive a trial of pyridoxine whatever be the suspected cause [59, 65, 77].

Results of this study and several other studies show, that the morphology of seizures seen in PDE, does not help to suspect the diagnosis [47].

Seizure semiology in PDE varies, from generalized tonic and clonic to myoclonic seizures, infantile spasms and brief partial seizures [47, 59, 77]. In our study seizure types at onset were various and ranged from generalized tonic clonic (GTC), myoclonic, infantile spasms, to eye deviations only.

Electroencephalographic patterns in PDE patients are also diverse, with normal or slowed high voltage background activity, usually abnormal sleep patterns [77], generalized rhythmic slow wave activity, paroxysmal features including sharp elements, or bursts of runs of high voltage sharp and slow wave activity [59].
Our patients showed various EEG patterns ranging from burst suppression pattern, focal epileptic discharges, multifocal spike waves, hypsarrhythmia, up to bilateral frontal- central slow waves.

Although several imaging studies have been done in PDE, no consistent or disease specific abnormality has been found. Nevertheless an unexpectedly high prevalence of variable structural CNS defects has been reported [63, 70, 80]. The most typical structural abnormality is hypoplasia of the posterior part of the corpus

callosum, which was a consistent finding in all six of the children in the Northern Region study of *Baxter et al.* [63] and which has since been confirmed in a study of two other children [63, 80].

In the MRI examinations which were done in our study, 7 patients showed ventriculomegaly, but just two patients needed a ventriculo-peritoneal shunt system. Another 2 patients showed ventriculomegaly revealed by CT examination. 3 patients (*patient 6, 7* and *29*) had posterior corpus callosum agenesis and two patients had signs of intraventricular bleeding (*patient 6 and 7*)

As PDE is quite a rare disease, delay of the correct diagnosis and initiation of specific treatment is common, at least in the index case. Especially in cases with partial response to conventional anti-epileptic drugs diagnosis is often delayed [66]. In the 76 patients, including isolated and familial cases, reviewed by *Haeggenli et al*. [62], the mean age at the time of diagnosis was 3.5 months, ranging from 1 day to 22 months [62]. Also in our study there was significant treatment delay in several cases. The time till specific treatment was performed ranged from 1 day to 18 months. The mean age at the time of first pyridoxine administration was 3.18 months (±SD=161.69 days).

PDE is an illness needing lifelong treatment. There are no clear recommendations for dosage adjustment of pyridoxine over time, as the need may vary from patient to patient. The recommended daily allowance for pyridoxine in healthy subjects is 0.5 mg for infants and 2.0 mg for adults [62]. In general, individuals with PDE have excellent seizure control when treated with 50-100 mg of pyridoxine per day [62, 76]. In our cohort the initial dosage to test B_6 response varied from 50 mg up to 300 mg daily, while the actual dosage of pyridoxine treatment ranged from 1.7 mg/kg (*patient 19*) up to 65 mg/kg daily (*patient 20*). In most cases vitamin B_6 was applied in form of Pyridoxine HCl, while other forms of pyridoxine application were not specified. For the risk of sensory neuropathy total daily dose should not exceed 300 to 500 mg.

The majority of PDE patients remain seizure-free on pyridoxine monotherapy Some children with breakthrough seizures may need higher doses during intercurrent illness [62, 77]. PDE patients should be monitored for their psychomotor development, regular assessments of intellectual function and clinical signs of a sensory neuropathy [84, 85].

A majority of PDE patients show some degree of mental retardation. There are rare descriptions about patients with normal development. Our study could confirm these findings. Just 20% of our patient had a normal outcome, while 34% were mildly retarded. 13% were assessed to be moderately retarded and 20% had a severe degree of mental retardation. Therapeutic delay may of course contribute to an unfavorable outcome [59]. Therefore it is important to, once again, stress out the role of early specific treatment to avoid additional brain damage due to recurrent seizures or even status epilepticus. Affected children are showing normal receptive verbal skills, but impaired expressive language functions which seems to be a specific feature of PDE [77]. *Baxter et al*. [77] reported that this is much more pronounced in PDE patients with early onset are showing a much more severe impairment of expressive language than late onset children.

It is not yet clear, which factors have the highest impact on neurologic outcome of PDE patients. There are children who a poor outcome despite early treatment, while others have a favourable outcome despite significant treatment delay.. This underlines PDE being an epileptic encephalopathy and supports the hypothesis of potential a- AASA toxicity [50]. Future studies will show, if lysine restriction will enable normalization of a- AASA and lead to better outcome.

Regarding the issues of compliance the diagnosis of PDE means life long treatment and follow up. Therefore counselling of parents is of high. Well informed decision making is an essential part of management of any chronic illness. Non-compliance is reported to be quite common in children on chronic treatment [87]. The parents therefore need to be informed about the genetic nature of the disease, its differences from other seizure disorders, the requirement for life-long therapy and follow-up, and the risks of non-compliance [88].

5. Conclusio

As conclusion it can be noted that most of the PDE patients experience seizure onset in the neonatal period. According to the data generated during this study, it can be stated that a big part of neonatal PDE patients present with apparent birth asphyxia and/or suspected hypoxic ischaemic encephalopathy or additional symptoms, just as vomiting, hypotonia, poor sucking and more.

All children with early onset (younger than 3 years old) intractable seizures or status epilepticus should receive a trial of pyridoxine, whatever the suspected cause might be.

First administration of pyridoxine, if intravenous or oral has to be done under controlled conditions, due to the risk of apnea and unresponsiveness

It is of utmost importance, that diagnostic algorithms are well established on neonatal wards and pyridoxine, 30 mg/kg/day is administered over 3 consecutive days to test for PDE. Urine, plasma and, if possible, CSF samples should be saved before the first administration of pyridoxine. In the presence of a positive response, pyridoxine should be continued until results of the selective screening are available.

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