Psychosocial impact and residual resource needs following institution of enzyme substitution therapy for PKU

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Abstract

Background: Phenylketonuria (PKU) is one of the most common inherited metabolic disorders and the first to be included on newborn screening. Even treated, it can impact quality of life (QoL) due to strict dietary management and potential for neuropsychiatric symptoms including anxiety, depression, and impaired executive functioning and attention. This study aims to learn about the impact the newest treatment for PKU – pegvaliase, an enzyme substitution therapy – has on QoL and to assess the residual support and resource needs of patients taking it. In clinical trials, pegvaliase was shown to improve certain neuropsychiatric symptoms. No research is currently published on the impact of pegvaliase on overall QoL or residual support and resource needs.

Methods: Adults who have PKU were surveyed, including adults who are and are not taking pegvaliase. Survey questions assessed current management, overall QoL, QoL PKU-related, and currently utilized resources and supports and remaining needs. Standardized questions assessing depression and cognition were also included. Responses were compared by whether the respondent was taking pegvaliase and whether their phe levels were above or below a set threshold.

Results: Significant differences were found in satisfaction with management, impact of PKU, general satisfaction with life, and cognition when comparing by phe level. When comparing by pegvaliase status, a significant difference was only found in satisfaction with management.

Residual resource and support needs predominantly involved social, financial, and adult-specific needs, although many respondents reported utilizing supports or resources in these domains.

Conclusions: The knowledge gained by this study is relevant to public health and genetic counseling by adding to the existing literature on QoL related to PKU, specifically related to use of pegvaliase and phe levels. Phe level seemed to have the most robust impact on QoL, although pegvaliase use was shown to improve QoL to a degree – likely related to its ability to lower phe levels. PKU-related worries seem to have a similar impact on QoL regardless of management, and should be a focus for future care. Ensuring that adult patients are aware of available supports and resources is also important in filling residual needs reported in this study.

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Preface

      I would like to acknowledge my committee for all of their invaluable guidance throughout this project. Their diligent editing, advice, and encouraging words made this project possible. My chair, Cate Walsh Vockley, and her expertise in inborn errors of metabolism and related resources and ongoing issues was especially integral. Not to mention her prompt response times to my many emails and impromptu thesis check-ins during clinic-related meetings, all of which helped keep me to continue progressing smoothly throughout the project.

      There are also many "behind the scenes" people that I could not have completed this project, or even started it, without. First, there are the individuals from UPMC Children's Hospital of Pittsburgh who provided key insights during my survey development and later helped with recruitment. Their perspectives as people who are fully immersed in the nuances of being an adult who has PKU, from both a clinical and personal standpoint, were extremely helpful in fine-tuning my survey. Adding to recruitment, there is also the director of the NPKUA Patient Registry for all of her communication and updates during the process of getting my project approved for distribution to the registry and for coordinating the eBlasts and newsletter for recruitment. It was truly amazing to watch the number of respondents grow with the final recruiting push!

      And to my roommate and classmate, Julia Gerow, thank you for letting me camp out on your floor on days where I needed a bit more motivation during our endless days at home this last year, bouncing ideas off of you, helping me de-stress leading up to my defense, and putting up with my babbling about the exciting (at least to me) details I was learning during my literature review and the findings of my project.

1.0 Introduction

Phenylketonuria (PKU) is one of the most common inherited metabolic disorders, with an estimated incidence of 1 in 15,000[1]. It was the first condition to be included in newborn screening programs, following development of an assay by Guthrie and Susi in 1963 that could be conducted on a dried blood spot[2]. PKU is part of a group of conditions titled hyperphenylalaninemia, all of which are characterized by elevated levels of the essential amino acid phenylalanine (phe). Severity of hyperphenylalaninemia in the context of PKU can be separated into three categories: classical PKU (phe concentrations greater than 1,200 µmol/L), mild PKU (phe concentrations between 600 to 1,200 µmol/L) and non-PKU hyperphenylalaninemia (phe concentrations between 120 to 600 µmol/L)[1]. PKU is inherited in an autosomal recessive pattern due to pathogenic variants in *PAH,* which encodes phenylalanine hydroxylase (PAH) – the enzyme responsible for converting phe to tyrosine in humans[1]. Over 1,100 different pathogenic variants have been reported to date[3]. with some correlation between variant type and severity of PAH deficiency[4–6].

When untreated, classic PKU causes a range of symptoms including intellectual disabilities, seizures, and impaired motor skills[7,8]. With the advent of newborn screening, early treatment can be initiated to prevent or mitigate these symptoms, however certain symptoms such as anxiety and depression, attention and executive functioning deficits, and lower IQ (but not usually to the extent of intellectual disability) may continue even with appropriate treatment[9–14]. Treatment for PKU originally relied solely on dietary restriction of protein, and therefore of phe, supplemented by medical formulas that contain all amino acids except phe, and other essential nutrients[15]. These dietary restrictions were once only routinely followed during childhood[16,17], but treatment for life has been the standard recommendation in the United States since 2000[16].

Management options for PKU have expanded in recent years. In 2008 an oral therapy, sapropterin dihydrochloride (Kuvan, BioMarin Pharmaceuticals), a synthetic form of tetrahydrobiopterin (BH$_4$) – a cofactor of PAH, was approved by the FDA[18]. This therapy successfully lowers phe levels in many people who have mild PKU, but it typically does not provide a clinical benefit to people who have more severe, classical PKU[6,18,19]. Several studies have been conducted to measure the impact this therapy had on the quality of life of patients who responded to the medication. These studies largely found that there was not a significant increase in quality of life subsequent to treatment with sapropterin and the associated relaxation of dietary restrictions, with only one study showing an improvement in multiple domains of quality of life[11,20–22]. Notably, the study that showed improvements in quality of life was also the only one to use a quality of life questionnaire specific to PKU; this questionnaire was adapted for use in the current study. It has also been postulated that improvements in quality of life due to dietary relaxation for some patients following treatment with sapropterin may be limited, since these patients typically have a less stringent diet at baseline compared with people who have more severe or classic PKU[21–24]. It therefore can be postulated that patients who have more severe PKU may experience a greater improvement in quality of life following an effective treatment that allows for dietary relaxation.

In 2018, the newest management option for PKU, and the first to be effective for a substantial portion of adults who have classical PKU, was approved by the FDA. Pegvaliase (Palynziq, BioMarin Pharmaceuticals) is an injectable enzyme substitution therapy using recombinant phenylalanine ammonia lyase, a bacteria-derived enzyme that converts phe to cinnamic acid and ammonia[25]. It has been shown to be useful in lowering phe levels for adult patients with phe levels over 600μmol/L[25–29]. As this is a more recently approved therapy for PKU,

many studies have been published on the effectiveness and safety of pegvaliase from a clinical standpoint, including improvement of neuropsychiatric symptoms[26,30,31]. However, no studies on the impact it has had on quality of life for individuals taking it have been published to date.

Research into treatments for PKU, including gene therapies, is currently ongoing. If these therapies are proved to be safe and effective and receive FDA-approval, their impact on quality of life and residual needs will be an interesting area for future research projects. However, as these therapies are still in development, they will not be discussed in detail here.

The specific aims for this study were to use an online questionnaire to:

1. Assess residual effects of PKU in adult patients taking pegvaliase, focusing on psychosocial impact, including neuropsychiatric symptoms, and related quality of life.
2. Evaluate how dietary decision-making has been impacted for PKU patients taking pegvaliase and how this has affected their quality of life.
3. Identify potential residual resource and support needs for adult patients with PKU taking pegvaliase, stratified by geographic area based on the reported zip code of the clinic they attend.

This questionnaire was open to all adults who have PKU, including adults who are taking pegvaliase as well as adults who are not taking it. Forty-three eligible adults responded to the survey, contributing to current knowledge surrounding the impact of PKU and pegvaliase on quality of life. By learning more about how a treatment like pegvaliase can impact a patient's life beyond lowering their phe levels, providers can continue to improve overall care for these individuals. These continued improvements may include providing anticipatory guidance on the

benefits and limtations of such treatments and linking patients to additional resources and supports

as needed.

2.0 Literature Review

2.1 Background on PKU

Phenylketonuria (PKU) is an inborn error of metabolism that is inherited in an autosomal recessive pattern. With an estimated prevalence of 1 in 15,000 in the United States, it is one of the most common inherited metabolic disorders. PKU is caused by mutations in phenylalanine hydroxylase (*PAH*). PAH is responsible for converting phenylalanine (phe) to tyrosine. When PAH function is impaired, as in PKU, phe accumulates to toxic levels in both the blood and the brain[1]. Untreated, this leads to an array of clinical presentations including intellectual disability, seizures, and impaired motor skills[7,8].

PKU is part of a larger catergory of inborn errors of metabolism – hyperphenylalaninemias (HPA) – which are all characterized by increased levels of phe in the blood. While most of these conditions are caused by deleterious mutations of varying severity in *PAH*, a small subset (approximately 1-2%) of cases of hyperphenylalaninemia are caused by deficiencies in other enzymes responsible for either the synthesis or regeneration of tetrahydrobiopterin (BH_4), a key cofactor of PAH[1]. GTP cyclohydrase I (GTPCH) and 6-pyruvoyl-tetrahydropterin synthase (PTPS) are the enzymes involved in synthesis of BH_4, and pterin-4a-carbinolamine dehydratase (PCD) and dihydropteridine reductase (DHPR) are the enzymes involved in BH_4 regeneration[32].

Even within PKU due to *PAH* mutations, there are multiple sub-types defined by the severity of the phenotype that are delineated by blood phe concentration. Normal reference blood phe concentrations are between 50 and 110 μmol/L[1]. Classical PKU is the most common of these sub-types and is the most severe with phe concentrations greater than 1,200 μmol/L[1,33]. It is

followed by mild PKU with concentrations ranging from 600 to 1,200 μmol/L, and non-PKU mild hyperphenylalaninemia (mHPA) with concentrations ranging from 120 to 600 μmol/L[1]. These differences among sub-types are largely due to differences in PAH activity, as influenced by the *PAH* mutations present. Specifically, the average PAH activity in each of these sub-types has been estimated to be 51.7% (compared to wild type levels) in mHPA, 40.4% in mild PKU and 3.8% in classic PKU[34].

2.1.1 Genotype-Phenotype Correlations

To date, over 1,100 mutations in *PAH* have been reported[3]. This large number of mutations is associated with the phenotypic heterogeneity seen within PKU, as different mutations within *PAH* lead to the wide range in severity of PAH dysfunction and deficiency and thereby affect clinical presentation[4,5].

Although there is an established correlation between genotype relative to *PAH* and PKU phenotype, it is not a perfect correlation. Studies focused on genotype-phenotype predictions have consistently predicted a majority of participants' phenotypes based on their genotypes, but there are always discrepancies in the remaining individuals[4–6]. Most people who have the same genotype will have the same phenotype. However, several cases have been reported where this is not true. Certain mutations have been reported in all three phenotypes or only in the highly dissimilar classical PKU and mHPA[5,6]. Although such discrepancies are more common in patients with identical heteroallelic genotypes, patients with identical homoallelic genotypes have also been clinically classified with different sub-types of PKU[5]. While a small number of these discrepancies may be explained by possible clinical misclassification, it is more likely that a combinations of other factors such as activity level of BH_4 or epigenetics are influencing phenotype along with

PAH mutations[35,36]. Interallelic complementation – a phenomenon where compound heterozygous genotypes produce a more or less severe phenotype than would be expected in the homozygous form of either mutation – must also be considered due to PAH being a tetramer. This phenomenon could play a role in the approximately 76% of people who have PKU who have a heteroallelic *PAH* genotype[37].

Even though there is not a perfect correlation between genotype and severity of PKU, a person's genotype can still be informative in estimating PAH activity[4–6]. It is therefore important to establish a patient's genotype because level of PAH activity can influence treatment approach. This is because the severity of *PAH* mutations influences dietary management due to differences in phe tolerance, as well as the types of pharmaceutical treatments available to a patient. People who have mild or moderate PKU are more likely to respond to an oral treatment, sapropterin, while people who have classic PKU may instead be candidates for an injectable treatment, pegvaliase[36]. Both of these treatment approaches will be discussed in more detail in later sections.

2.1.2 Neuropsychological Presentation

An increased prevalence of a wide range of neuropsychiatric disorders has been reported in adults who have PKU when compared with the general population and when compared to people who have diabetes mellitus[10]. In this study, people who have diabetes mellitus were included to capture differences in mental health that may come with managing a chronic metabolic disease. The results of this study found that even among adults 20 to 39 years old who would have received recommendations to follow treatment for life, there were higher rates of intellectual disability and autism spectrum disorder, as well as eating disorders, OCD, and ADHD[10]. In contrast, other studies examining the intellectual abilities of people who have early-treated PKU do not report higher

rates of intellectual disabilities among these individuals, suggesting that people who have early-treated PKU typically have an IQ within normal range. However, studies do often report that people who have PKU have lower IQs when compared to controls[11,13,14]. Reports of increased rates of intellectual disability in people who were diagnosed when lifetime treatment was recommended may be explained by variation in treatment history of these individuals[9]. A separate publication reported increased rates of anxiety disorders among people who have PKU when compared to both the general population and people who have diabetes, and increased rates of depression when compared to the general population, but not the diabetes cohort[10]. Although there are increased rates of depression and anxiety among adults who have PKU and follow treatment-for-life guidelines, these rates are further increased among adults who have PKU but have discontinued treatment since childhood[12].

People who have PKU also often have impaired executive functioning. Executive function involves several cognitive tasks: 1) working, or short-term, memory, 2) inhibitory control, the ability to prevent unnecessary or irrelevant information from preventing one from reaching a specific goal, 3) cognitive flexibility, the ability to effectively switch between tasks with different goals, and 4) organization and planning. Of these, cognitive flexibility and organization and planning tend to be more complex to measure[38]. Bilder et al (2016) reported deficits in attention, inhibitory control, and cognitive flexibility, but not working memory, when people who have early-treated PKU were compared to members of the general population[9]. Feldmann et al (2019), also found a significant difference between healthy controls and people who have early-treated PKU in regard to attention, but only when comparing older adults who have PKU (over age 42), not younger adults who have PKU, to healthy controls[11]. Similarly, they reported deficits in information processing speeds among older adults who have PKU[11]. Differences between older

and younger early treated individuals is potentially due to the length of time that has passed since discontinuing or relaxing treatment in adulthood, if this was done[11]. Regarding working memory, there is mixed evidence of working memory being largely comparable between children who have early-treated PKU and healthy controls, with the treated individuals becoming impaired to varying degrees as they age[38].

Similar findings were identified by a meta-analysis of studies examining previously published papers comparing people who have PKU and controls regarding executive functioning and other neuropsychological tasks such as attention maintenance. The meta-analysis found that people who have PKU tend to perform poorly compared to controls on tests of information processing speed, attention, inhibitory control and motor control, but reported no significant impairement of working memory[13].

Social functioning is another important aspect of cognitive function that can be affected by increased phe levels through its detrimental impact on serotonin and dopamine levels. Jahja et al (2016) measured the ability of people who have PKU and healthy controls to exhibit necessary social skills such as facial and emotional recognition and Theory of Mind (identifying and understanding the feelings and thoughts of other people). They found that people who have PKU begin to have less developed social skills compared to their peers in the general population starting in adolescence (age 12), with these social impairments increasing over time. Higher lifetime phe levels, but not current levels, were similarly associated with poorer social skills beginning in adolescence[39].

2.1.2.1 Biological Mechanism of Neuropsychological Presentation

There are multiple ways that PAH deficiency can lead to an impact on neuropsychological functioning. In addition to increased phe levels being potentially toxic to the brain, PAH deficiency

limits the amount of tyrosine that crosses the blood-brain barrier (BBB). This happens in two ways: 1) lower tyrosine levels due to PAH having limited ability or inability or convert phe to tyrosine, and 2) phe competing with tyrosine to cross the BBB and crossing more successfully than tyrosine and other large neutral amino acids (LNAAs), such as tryptophan, especially when phe levels are increased[1,40,41]. LNAAs are key precursors for neurotransmitters, with tyrosine and tryptophan being precursors for dopamine and serotonin, respectively[30,40]. By inhibiting LNAAs from crossing the BBB, cerebral protein synthesis – a process that is crucial to cognitive development during childhood and early adulthood as well as cognitive functioning throughout a person's lifetime – is also impaired[41]. Elevated phe can also interfere with cholesterol production which can in turn impact neurotransmitter production[40].

These neurochemical changes can be linked to the neuropsychiatric presentation of PKU. Executive function impairments and ADHD can be related to the impact of increased phe levels on dopamine production. These functions are primarily carried out by the prefontal cortex, an area of the brain that relies heavily on the presence of dopamine[30,38]. Increased phe levels can also impact levels of glutamate, another important neurotransmitter that has been linked to memory, depression and anxiety, as well as norepinephrine, which has been linked to inattention and ADHD[30,40]. Decreased serotonin can be related to sleep disorders and depression, both of which can be seen in people with PKU[30].

There are also structural differences that can be linked to the neuropsychiatric presentation in PKU. White matter abnormalities, detected as hyperintensities on MRI, have been extensively studied in people who have PKU. These abnormalitites are present in the majority of both early and late treated individuals who have PKU, including those who currently have well-controlled phe levels[11,14,42–44]. Subcortical and periventricular areas, especially the frontal and occipital-

parietal lobes, are the most commonly affected[14,42–44]. Early studies correlated the degree of abnormality with recent phe levels, with some studies reporting the best correlation with current phe levels[42,44,45] and others reporting the best correlation with cumulative phe over the past few years[14]. Length of time on a relaxed or unrestricted diet may also play a role in these white matter abnormalities independent of recent phe levels[43,44].

In contrast, more recent studies that use diffuse tensor imaging (DTI), a newer MRI scanning technique, often find correlations between degree of microstructural white matter abnormality and historical phe levels or lifetime phe exposure. One such study reported the strongest correlation with white matter abnormality was phe level at diagnosis, not recent phe levels, although current phe levels were also correlated[43]. Another study using DTI with children who have PKU found correlations between microstructural white matter abnormalities and lifetime phe exposure and mean phe levels over time. In this study, children who had more severe microstructural abnormalities were more likely to have experienced high or variable phe levels throughout their lives[46]. Similarly, a different study involving early treated children who had phe levels under 600μmol/L found that children with higher past and current phe levels typically had greater microstructural abnormalities and reported that this trend increased with time[47].

There are few established correlations between degree of microstructueral abnormality and neurological function, with most studies, including a study using DTI[47], being unable to find correlations with IQ or other neurological symptoms of PKU[14,42,44]. One study that used DTI with people who had early-treated PKU did find a correlation between microstructural white matter abnormalities and IQ, as well as executive function tasks, including working memory[45].

One proposed theory regarding these white matter abnormalities is that they are related to reversible altered myelination because of their close relationship to recent phe levels, compared to

historical phe levels[14,42,44,45]. Data from DTI studies can also been interpreted as suggestive of abnormal myelination[47]. The altered myelination theory is supported by studies that show abnormal myelination in patients who have untreated PKU. This impaired myelination may be due to impaired cholesterol synthesis and can lead to impaired dopamine synthesis, further decreasing dopamine levels in people who have PKU[40]. Alternative theories include accumulation of water inside cells due to excess of hydrophilic metabolites or cellular debris[43,45].

2.2 Newborn Screening & PKU

In 1963, Robert Guthrie and Ada Susi published a method to test newborns for PKU using a bacterial inhibition assay with dried blood spot on a filter paper card[2]. This assay began newborn screening. Since then, numerous other screening methods have been developed for a variety of diseases[48].

Newborn screening (NBS) practices are determined on a state-by-state basis, leading to variability in the number of conditions for which screening is conducted and the methodologies used. Further, states vary in their ability to support patients diagnosed following NBS, including the availability of treatment and management services due to a lack of specialists knowledgeable about these rare conditions in certain areas of the country. This in turn creates the potential for health disparities among these patients[49].

In 2006, a list of conditions recommended to be included on NBS nationwide was assembled and published by the American College of Medical Genetics (ACMG) at the request of the Maternal and Child Health Bureau within the federal Health Resources and Services Administration (HRSA). This request was in response to a statement by the American Academy

of Pediatrics Newborn Screening Task Force providing evidence that having a nationally standardized screening process would be beneficial to both patients and the public health system as a whole[49]. The list produced by ACMG was labled the Recommended Uniform Screening Panel (RUSP) and included 29 conditions which should, at minimum, be part of each state's NBS program[49]. This list has now been expanded to 36 conditions[50]. The criteria for inclusion in the RUSP are: 1) availability of an appropriately sensitive and specific test for the condition that can be used within 24 to 48 hours of birth, 2) established benefits for early detection and early intervention or treatment, 3) availability of treatment and care by appropriate specialists, 4) burden of the condition, including severity of the phenotype and incidence, and 5) understanding of the natural history of the condition, especially in regard to how it affects newborns. The cost of adding a condition to NBS was also considered, but was not used as a primary criterion due to its subjective nature[49].

PKU was one of the highest scoring conditions included on this panel. This is because 1) testing has been available since the 1960s, 2) there is substantial evidence for the benefits of early treatment to minimize the effects of what would othersise be a devastating disorder, 3) treatment is readily available in the form of dietary management, and 4) the condition has a relatively high incidence. By beginning dietary management for PKU in the first two weeks of life, overall cognitive outcomes for the patient are significantly improved[51], providing a prime example of the beneftis of NBS. Even in the early years following the development of a newborn screening method for PKU by Guthrie and Susi, significant improvements in detection rate and age of diagnosis were noted. This was especially true in states like California which adopted mandatory screening during the 1960s[7].

2.3 Management of PKU

Current guidelines for management of PKU, as established by the American College of Medical Genetics and Genomics (ACMG), state that the goal for blood phe concentration in patients who have PKU is 120 to 360μmol/L. However, levels below this range may not be considered too low as long as they occur in the presence of relatively high phe intake and do not fall below 30μmol/L. To achieve this, dietary management beginning in the first week of life is recommended for infants who have a blood phe level of over 600μmol/L, with the intent to have phe levels in the target range by the second week of life. Treatment should then be continued for life[15].

Management recommendations for women who have PKU before and during pregnancy are slightly different. This is because of the teratogenic effect of elevated phe, referred to as maternal PKU syndrome. Maternal PKU syndrome has been shown to cause microcephaly, intrauterine growth restriction, congenital heart defects, and low IQ. With the exception of congenital heart defects, which are only related to elevated phe levels prior to eight to ten weeks of gestation due to the timing of fetal heart development, the likelihood and severity of these symptoms have a direct relationship to both the degree of phe elevation and the length of time the fetus was exposed to elevated phe levels. Guidelines in the United States recommend a stricter goal for blood phe levels of 60 to 360μmol/L, with the best outcomes associated with achieving a phe level below 360μmol/L prior to pregnancy. International guidelines are more strict and recommend phe levels under 240μmol/L during pregnancy[15].

2.3.1 Historical Management Approaches

The benefits of a low-phe diet in people who have PKU were first reported in 1953[52]. Initiation of a low-phe diet as soon as possible following a diagnosis of PKU has been recommended since this discovery. A 1978 survey assessing the practices and recommendations of 72 PKU clinics throughout the United States found that about half of children were discontinuing diet by age 7, with 8 clinics recommending discontinuation at or before age 6, one of which recommended discontinuation at age 3. Only 16 of the 72 clinics were recommending treatment for life, although about a quarter of clinics surveys indicated that they were considering recommended discontinuing diet at a later age than they were currently[17]. This inconsistency among clinics at the time of the survey was likely due to the conflicting evidence of the impact of dietary discontinuation. Many of the clinics with either a later recommended age of discontinuation or recommendation of treatment for life had experience with patients who had been negatively impacted by discontinuing diet, while many of the clinics who recommended earlier discontinuation did not report having similar experiences[17]. This set the stage for an increasing number of clinics recommending treatment for life throughout the 1980s. By the 1990s, treatment for life was a commonplace recommendation made by PKU clinics in the United States[16]. This shift was supported by a paper published in 1991 with evidence to support a negative impact of dietary discontinuation as late as age 10, and further suggested that dietary discontinuation in adulthood after full brain maturation would have a similar, although attenuated, impact[53].

However, the first nationwide recommendation for treatment for life was not released until the early 2000s[16,54]. The implications of maternal PKU syndrome were factored into this recommendation due to the increased difficulty of regaining metabolic control prior to conception and maintaining metabolic control throughout pregnancy when a low-phe diet had been previously

discontinued[16]. These original guidelines included more lenient blood phe levels than those recommended in the current guidelines as follows: 120 to 360µmol/L for infants and children under the age of 12, 120 to 600µmol/L from age 12 through adolescence, and 120 to 900µmol/L for adults[16]. Previously, it was not uncommon for clinics to allow relaxation of diet after age 6, as the brain was thought to be essentially fully developed by this time[55].

2.3.2 Dietary Management for PKU

Many people who have PKU have to follow a strict low-phe diet in order to reach the target range of 120 to 360 µmol/L for blood phe levels. This involves a diet that is low in natural protein yet contains an adequate amount of phe, an essential amino acid, for normal growth and development. The amount of natural protein and overall amount of phe a person can consume and still maintain the target range varies from patient to patient depending on their residual PAH activity and their current stage of life. Careful monitoring of protein and phe intake to consume only what is needed can be achieved by use of specialized low-phe and phe-free medical foods and beverages and by tracking phe exhanges throughout the day[15]. A single phe exchange consists of 15mg of phe, which equates to approximately 0.3 grams of protein when using an average of 50mg of phe per 1g of protein[56]. Patients may also use foods that are modified to have lower protein levels while maintaining a similar taste and appearance to their natural, high-protein equivalents[15].

Consistent monitoring of blood phe levels is critical to fine-tuning the recommended dietary management for each individual patient. The recommended frequency of blood phe monitoring varies over a persons lifetime, being most frequent in infancy (weekly) and decreasing

in frequency over time until adulthood (monthly). More frequent monitoring may be necessary if a patient is not well-controlled or during certain life events, such as growth spurts or pregnancy[15].

2.3.3 Adherence to Management Recommendations

Adherence to management recommendations over time is critical for the overall health of a person who has PKU. As described above, uncontrolled PKU after childhood can lead to various neuropsychiatric impacts, including impaired executive functioning skills. These skills, such as the ability to plan for the future and self-monitor, are necessary for a patient to follow a regular dietary schedule. Therefore, when a patient stops following management guidelines, the associated decline in executive functioning skills can make it more difficult for the patient to go back on diet and adhere to management recommendations in the future[15]. Treatment for life is also beneficial in this sense as the continuous metabolic control is entails can mitigate these neuropsychiatric impacts[15]. Similarly, it has been shown that it is difficult for women who have discontinued diet to achieve the recommended phe level prior to conception and maintain it throughout pregnancy[15,16]. This makes treatment for life a way to limit the effects of maternal PKU by encouraging women who have PKU to have good metabolic control throughout the lifespan, as is recommended for all people who have PKU, allowing metabolic control during pregnancy to be more achievable.

Measurement of blood phe levels is the standard method to monitor and assess metabolic control and, in turn, dietary adherence[51,57,58]. Using phe level measurements, it has been repeatedly shown that adherence to diet and control of blood phe levels tend to decrease after childhood[51,58,59]. A meta-analysis of publications comparing people who have different sub-types of PKU (mHPA, mild PKU, or classical PKU) studying blood phe levels in relation to clinical outcomes reported

27% of young children (ages 0 to 6) having phe levels that exceed the recommended threshold, compared to 78% of patients over the age of 18[58]. Similar findings were reported by a 2015 survey distributed by the National PKU Alliance (NPKUA), with only a quarter of patients under 18 exceeding the recommended range, compared to almost two-thirds of adult patients, even though 68.4% of patients (all ages) reported that they wanted to have phe levels within the recommended range[60]. This trend is also present in regard to patients becoming increasingly lost to follow up as they age, with adults being more likely to be lost to follow up than children. Over half of adults over 30 years old were lost to follow up compared to approximately 10 percent of children ages 0-4[51]. However, compared to historical levels, blood phe levels in patients on diet are on average lower now than they were in the past[51,59].

Supporting the linkage between increasing phe levels and changes in compliance with age, a study focusing only on adolescents and adults who have PKU found that 75% of their participants reported following their dietitian's recommendations about using their protein substitute. However, only about 50% of their participants reported being "on diet"[61]. Further, 56% of their participants reported measuring their blood phe levels as often as their doctor recommended and only 41% of participants reported weighing or measuring their phe exchanges all the time or most of the time[61]. The majority of their participants had a strong understanding of each of these recommendations, yet this knowledge did not lead to reliable adherence to these recommendations[61]. Their findings regarding continuation of diet into adulthood were similar to the results of a study in the United Kingdom which showed that 46% of their participants were continuting to follow the recommended diet into adulthood. This study also found the majority of adults who discontinued the diet never resumed dietary management. This was especially true for males, possibly because of strict dietary management for women before and during pregnancy[62].

The results also revealed that 89% of their participants who reported being on diet were receiving less than the recommended amount of amino acid supplement, with over half (55%) receiving less than 25% of their recommended amino acid supplements. This is in contrast to the substantially higher numbers reported by Durham-Shearer et al[61,62].

There are several nuances to using phe level statistics to estimate overall adherence. These statistics typically come from clinical data, leaving the possibility that patients who are lost to follow up, and therefore potentially not following management recommendations, may have significantly higher phe levels than those reported in published studies[51]. This is supported by data from the 2015 NPKUA survey, which found that 58% of patients who have phe levels within recommended range had been seen in a clinic within the last 12 months, compared to only 38.6% of patients who had not been seen in a clinic for at least 12 months[60]. A subset of patients who follow with clinics and know their phe levels are higher than recommended may manipulate when they test their phe levels to make them appear consistently lower. An example of this would be waiting to do their test until they know their phe intake is lower instead of measuring at regular intervals[51,57].

2.3.3.1 Barriers to Adherence

There are a number of barriers that can make it difficult for a person who has PKU to adhere to management recommendations. Some of these barriers are related to clinical services, as patients, especially adult patients, may have difficulty accessing a clinic with providers who are knowledgeable about PKU, especially in rural areas[59], although recent increases in telemedicine usage may help diminish the impact of distance. A recent survey based in Michigan reported that, on average, patients had to spend over 4 hours in travel time to get to and from their metabolic clinic appointments[63]. Additionally, a survey based in Colorado reported patients travelling

anywhere from 5 to 584 miles to their clinic, with an average of 136 miles[59]. This barrier is exacerbated in some cases because not every clinic sees adult patients. This can be compounded by the fact that some patients would rather continue to follow at the clinic where they were treated as a child and are not comfortable seeking care elsewhere. Alternatively, not all people who have PKU will feel comfortable seeking care at a clinic based at a pediatric center, even when the clinic sees both pediatric and adult patients[55].

There is also the issue of access to medical foods, which can be costly especially when following a low-phe diet for life. The average annual cost of medical foods for people who have PKU in the United States is estimated at $6,400 for children and $9,000 for adults. These cost numbers become even more substantial when considering that they are limited to medical foods alone and do not include other PKU-related costs such as pharmaceutical treatments or co-pays and travel costs of attending clinic visits. The cost of these medical foods makes it difficult for people who have PKU to obtain them. Sixty-percent of parents and 68% of adults reported that it was difficult to obtain the necessary medical foods, with the cost of purchasing them being a major factor, especially among parents[63]. Ideally, people who have PKU would have consistent insurance coverage for these low-protein modified foods and low-phe medical formulas that are crucial to adhering to dietary management guidelines. However, over 20% of states do not require insurance coverage for any medical foods, low-protein foods or formulas, for patients of any age. Several other states mandate coverage, but only for children, not adults, despite treatment-for-life guidelines. There are also several states that only mandate coverage for either medical formulas or low-protein foods, not both[55]. Even when insurance coverage of one or both categories is available, there are often stipulations that is has to be prescribed to the patient or that the patient must have a feeding tube to use with the medical formula, which is not necessary for people consuming

medical formula for PKU[15]. Currently, the only consistency among states is that Medicaid provides coverage for medical foods. It is a complex process to apply for Medicaid though, which poses another barrier, especially since Medicaid is typically reserved for children, those who have significant disabilities, or people who have low-income, which is not the case for all people who have PKU[55].

The Medical Nutrition Equity Act (S.3657), a bill that was introduced to the Senate in 2020, may help address some barriers related to insurance coverage if it is passed into law. In its current form, it would do so by expanding coverage of medically necessary foods under Medicare, Medicaid, and private insurance, as well as redefining medically necessary foods to include both low-protein modified foods and medical foods or formulas not requiring a prescription[64]. Companies who produce medical foods also recognize the difficulty of gaining insurance coverage for these medically necessary products. Many such companies offer free assistance in navigating insurance to help patients and families obtain coverage for this reason[65–67].

Cost barriers may also be partially alleviated by private assistance programs. BioMarin, the company that produces both of the pharmaceutical treatments for PKU, offers a co-pay assistance program for eligible patients to help minimize the costs of these treatments. However, this is only available to patients who have commercial insurance, leaving some people who have PKU ineligible for this assistance regardless of income [68,69]. The National Organization of Rare Disorders (NORD) also provides financial assistance to patients and families with qualifying income levels to help with the cost of managing PKU including clinic visits and medical foods, as well as pharmaceuticals[70].

Time commitment required to prepare low phe foods, including weighing and calculating phe exchanges throughout the day, is also a barrier for some individuals[63,71]. This time

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commitment has been estimated to be 300 hours a year (0.9 hours a day)[63]. This can be especially problematic when factoring in demanding work schedules for adult patients[71]. The social burden of adhering to a PKU diet, including preparing and packing separate low-phe meals to eat at social events and potential harassment by other attendees for doing so, is also a key factor that has been reported as making it difficult to follow diet, mainly among adult patients[63,71]. Adult patients have also reported feeling like they are posing a burden on their peers to meet their dietary needs when planning social events[71]. For adults specifically, it has also been reported that it is even more difficult to return to dietary management following a lapse in adherence. This may be due to a combination of the unpleasant taste of medical foods for PKU compared to foods containing high levels of natural protein and the symptoms of PKU associated with an increase in phe levels that make it more difficult to plan ahead and stick to a diet, such as a "mental fog" or difficulty planning[71].

2.3.4 Health-Related Quality of Life in PKU

Health-related quality of life is a complex concept. It can be defined as a patient's perspective on the impact their health has on their physical, emotional, social, and psychological well-being[72]. People who have PKU have reported feeling they are not reaching their full potential in work and in social relationships. They have also struggled with feelings of isolation due to stigma surrounding PKU or feeling that they could not explain their PKU to others in a way that would be understood[71]. While these statements were not explicitly tied to a decrease in quality of life, self-esteem and social relationships are key parts of quality of life, along with the neuropsychological impact of PKU.

There is evidence to suggest that people who have PKU experience an overall quality of life comparable to people in the general population[23,24,73,74]. However, some studies have found that quality of life is only comparable between people who have PKU and the general population for certain age groups. Huijbregts et al. found a significant difference in adults but not children or adolescents[21], while Cotugno et al. found a significant difference in children and adolescents but not adults[72]. These two studies used different questionnaires to assess quality of life, neither of which was specific to PKU, and had different cut offs for when they used adult versus child questionnaires, with Huijbregts et al. using 16 as the minimum age for the adult questionnaires and Cotugno et al. using 18. They were also conducted in different parts of Europe (the Netherlands and Spain, respectively)[21,72], which may also have played a role in their differing results.

2.3.5 Management with Sapropterin

Tetrahydrobiopterin (BH4), a cofactor of PAH, (and its synthetic form, sapropterin dihydrochloride) has been shown to be useful in managing mild cases of PKU, helping these patients have fewer, if any, dietary restrictions[6,18,19]. Sapropterin has been approved for use in people who have mild PKU in the United States since 2008[18]. This treatment works by using sapropterin's ability to promote PAH activity by stabilizing the enzyme and preventing it from being degraded prematurely[36]. A few studies have also shown a small proportion of patients who have classical PKU to benefit from sapropterin[6,18]. Sapropterin responsiveness is closely related to genotype. Quirk et al. found that the majority of patients who experience a definitive response to sapropterin had at least one, if not both, of their *PAH* mutations known to be mild or moderate in severity, while non-responders were more likely to have two severe mutations. There is also an intermediate group – provisional responders. These individuals show an initial decrease in phe

following initiation of sapropterin therapy, but cannot maintain phe levels of less than 360 μmol/L after increasing dietary phe intake[75]. However, genotype is not always predictive of responsiveness. The same study by Quirk et al. found that the majority of provisional sapropterin responders had two severe *PAH* mutations, suggesting that they were more likely non-responders. They also reported multiple instances where two patients who have the same genotype had different responses to sapropterin, with one being a provisional responder and the other being a non-responder[75].

In order to determine if a patient will be a responder to sapropterin, typically their genotype is first assessed to see if they have a mutation associated with mild or moderate reduction in PAH activity, allowing for residual PAH activity needed for sapropterin to be beneficial[36]. A trial of sapropterin may be considered for all patients, except those with two null *PAH* mutations[15]. When a potentially responsive genotype is established and sapropterin is administered, regular blood phe monitoring must then occur to determine if there has been a decrease in the patient's phe levels. This monitoring often involves comparing the baseline phe level to phe levels at 24 hours, 1 week, and 2 weeks after initiating the sapropterin trial, but may continue to 3 or 4 weeks in some cases to capture a delayed response to sapropterin[15]. Typically, a patient will be considered a saptropterin responder if they have a 30% or greater decrease in their phe levels over this time, as long as their diet has remained stable or even increased in protein content during the trial[15,36]. Continued use of sapropterin can also be useful for patients who do not experience a decrease in phe levels, but do experience an increase in phe tolerance during the trial. While patients are typically told to maintain constant protein intake during the sapropterin trial, systematically adding protein to a patient's diet during a sapropterin trial may be necessary for patients who have milder or better controlled PKU in order to see a substantial response[15].

2.3.5.1 Effect of Sapropterin on Quality of Life

There have been mixed findings on whether treatment with sapropterin leads to an improvement in quality of life. Studies that found improvement in quality of life typically only saw improvements in specific domains of quality of life compared to an overall improvement. Douglas et al. found that treatment with sapropterin, regardless of level of response, did significantly improve quality of life specific to the impact of PKU, but did not improve other areas of quality of life defined in their study (PKU-related life worries, satisfaction with life and medical managment, support from social network and medical community, and general well-being). They also noted significant increases in satisfaction only for definitive responders and in total quality of life score for both definitive and provisional responders, but not for non-responders[20]. Huijbregts et al. reported no significant differences in overall quality of life, but did find improvements in quality of life for adults taking sapropterin in the domains of pain, happiness, anger, and social functioning. They reported no similar improvements in children or adolescents[21]. Conversely, multiple studies have found no improvement in quality of life in any domain[11,22]. However, two studies reported participants who mentioned anecdotally that they enjoyed the increased dietary freedom provided by taking sapropterin, as well as the savings that came with a reduced need to purchase medical foods[22,24].

These discrepant findings have been explained in part by the fact that the questionnaires used in many of these studies were not disease-specific, suggesting that a questionnaire specific to PKU, or at least a chronic condition involving dietary restriction in general, would have been more informative[21–24]. This is especially relevant as Douglas et al., the study reporting the most significant improvements in quality of life, did use a questionnaire designed specifically for individuals who have PKU[20]. Further, the one significant improvement in quality of life seen by

Ziesch et al. was in patients who ultimately were not sapropterin responders, but who were on a less restricted diet for three months while determining their response status[22]. Although this was a temporary improvement that ended following a return to restricted diet after three months, this difference may be explained by non-responders tending to have lower PAH activity, and therefore lower phe tolerance and more restrictive diets, compared to responders[33]. The higher baseline phe tolerance of responders, and its associated less restrictive diet, may have limited the influence of initiating sapropterin on quality of life. This suggests that people who have classical PKU would have a higher chance of an improved quality of life with a less restricted diet, where such a benefit was not seen in patients who have milder forms of PKU[20,73]. This may be relevant as changes in quality of life are explored for newer therapies, such as pegvaliase, that are efficacious for patients who have classic PKU.

A significant proportion of patients taking sapropterin eventually discontinued it. Insurance concerns, difficulty taking the required number of sapropterin tablets, undesirable side effects, and the treatment not working as well as expected were all reported reasons for discontinuation. While patients who discontinued sapropterin were more likely to have been less responsive to the drug, which is consistent with the complaint that it did not work as well as expected, the distribution of side effects was equivalent in patients who discontinued sapropterin and patients who chose to continue it. On the other hand, patients who chose to continue treatment with sapropterin focused on the dietary benefits of sapropterin, which allow for a lower phe level while maintaining a less restrictive diet involving fewer medical foods[76].

2.4 Background on Palynziq/Pegvaliase

Pegvaliase is an enzyme substitution therapy using recombinant phenylalanine ammonia lyase (PAL) from the bacterium *Anabaena variabilis* that is conjugated to polyethylene glycol (PEG), giving it it's original name – PEG-PAL[27,29]. It is important to conjugate PAL to PEG in order to both mitigate an immune response against pegvaliase and maintain enzyme activity and stability because PAL is a bacteria-derived protein that does not naturally occur in humans[27,77].

PAL metabolizes phenylalanine into cinnamic acid and ammonia, which are both able to be metabolized safely by people who have PKU[25]. This allows PAL to be used to reduce phe levels to therapeutic range even in the absence of functional PAH[26,27,29,31]. PAL is an enzyme *substitution* therapy, as opposed to an enzyme *replacement* therapy, because it provides an alternate pathway for phe metabolism. Enzyme replacement therapies have been previously used in certain lysosomal storage disorders, another category of inborn errors of metabolism, and work by directly replacing the defective enzyme, instead of providing an alternative pathway[78].

Pegvaliase has been FDA-approved since 2018 for use in adults who have PKU and phe levels over 600μmol/L in the presence of past or current incomplete management with diet, sapropterin, or a combination of these management approaches[25,28]. It is not currently approved for use during pregnancy[28]. For this reason, some clinics may recommend women who are taking pegvaliase to continue incorporating specialized medical formula into their diet, but to a lesser degree than they might have needed prior to pegvaliase initiation. This is in order to ease the transition to dietary-based management during pregnancy (R. Mohring, personal communication, August 24, 2020).

2.4.1 Use of Pegvaliase

Pegvaliase is self-administered by the patient via sub-cutaneous injection. Patients taking pegvaliase are informed about the potential adverse effects and administer pegvaliase in the presence of a trained observer, with both individuals being educated on how to use an epinephrine autoinjector in the event of a severe allergic reaction[28,79]. If support in the form of a trained observer is not available for a patient, then the risks and benefits of management with pegvaliase should be weighed[28]. All doses should be administered in a clinical setting until the patient and designated observer exhibit understanding of pegvaliase dosage and administration and ability to recognize signs of anaphylaxis, including what to do in the event of anaphylaxis. Use of antihistamines, specifically H1-receptor antagonists, prior to daily administration of pegvaliase is also recommended to help mitigate hypersensititivy reactions[79].

Initiation of pegvaliase therapy involves gradual titration of the drug up to 20mg/day over the course of 9 weeks or more to help minimize the risk of severe hypersensitivity reactions. Patients who experience adverse events may require a longer timeline due to the need to reduce pegvaliase dosage following a hypersensitivity reaction[28,79]. Complete discontinuation of pegvaliase following a hypersensitivity reaction is not recommended as this will delay desensitization to pegvaliase, and therefore put the patient at greater risk for future hypersensitivity reactions[79]. The dosage may then be titrated up to 40mg/day if the patient does not experience at least a 20 percent decrease in phe levels while on the 20mg/day dosing. When a patient does not experience this 20 percent decrease in phe levels within a year of pegvaliase initiation, treatment discontinuation is recommended. In contrast, if a patient experiences hypophenylalaninemia (phe levels under 30μmol/L) for three months or more, then dietary protein intake must be increased or pegvaliase dose decreased when dietary changes are not sufficient to raise phe levels[28]. Patients

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who need to cease taking pegvaliase due to pregnancy or for other reasons may resume treatement with pegvaliase once they are able. However, they should start at a lower dose than they were taking when they stopped and gradually increase their dosage to prior levels as recommended by their clinic[79].

The goal of treatment with pegvaliase is to lower blood phe levels to 31-120µmol/L in the presence of a liberalized diet. Patients should also be educated on appropriate portion sizes and cooking methods for protein rich foods such at meat, eggs and fish, as they can be expected to have little prior experience with these foods if they were following the recommended low-protein diet. For some patients, it may also be important to address eating behaviors, as there is an increased risk of disordered eating patterns among people who have early-treated PKU due to the strict dietary management involved[10,28,71]. Although there is limited literature on this topic, it has been reported that women who have PKU scored higher on the oral control section of an assessment of eating attitudes and behaviors. This finding alone does not indicate a higher incidence of eating disorders, but does suggest a difference in the attitudes women who have PKU may have towards food. Specifically, questions in this section assess self-control regarding food intake and whether a person feels pressured by others to gain weight, as may be the case for someone who is underweight due to an eating disorder, thereby possibly linking these higher scores to the recommended decreased protein intake of the typical PKU diet, which can require a significant level of self-control related to food[80].

2.4.2 Clinical Trials for Pegvaliase

Clinical trials for pegvaliase were initiated by BioMarin in 2008[25]. Approximately half of the 24 patients enrolled in a phase 2 clinical trial of pegvaliase were able to reach phe levels below

120μmol/L within 24 weeks without decreasing their dietary protein intake. The majority of the remaining half of the patients were able to achieve phe levels under 600μmol/L within 48 weeks, with many of these patients achieving levels below 120μmol/L by this time. The dosing regimen used in this clinical trial involved starting each patient at 2.5mg/week (0.5mg/day, 5 days a week) for the first 4 to 8 weeks. Their dose was then gradually increased over the course of at least 4 weeks until their phe levels decreased to below 600μmol/L and remained beneath 600μmol/L for an additional 4 weeks without any additional dosing changes. The maximum dose used was 375mg/week (75mg/day, 5 days a week)[29]. This was the first clinical trial of pegvaliase to show both decreased phe levels over time and an overall manageable safety profile, suggesting this was an appropriate dosing schedule[27]. The extension of this clinical trial reported that by week 48, 57.4% of patients were able to reach phe levels below 600μmol/L, with 45.9% able to reach levels below 360μmol/L. Overall, by the end of the extension study, 82.5% of patients had experienced phe levels below 600μmol/L. This was accomplished while many of these individuals were on unrestricted diet[27].

The phase 3 randomized control trial for pegvaliase confirmed the efficacy and safety of pegvaliase in reducing blood phe levels. This 8-week trial recruited patients who were enrolled in previous clinical trials for pegvaliase and were therefore already taking pegvaliase. These patients were then randomized to continue taking their current dose of pegvaliase (either 20 or 40mg/day) or an equivalent dose of placebo. Patients who were switched to a placebo had significant increases in phe levels, while patients who continued on pegvaliase maintained their previously achieved decrease in phe levels. Patients in both groups were instructed not to change their dietary intake during the trial[26].

The 20mg/day versus 40mg/day distinction for dosage was established during an earlier phase 3 clinical trial where pegvaliase-naïve patients were enrolled and randomized to either a 20mg/day or 40mg/day dosage plan. These dosages were informed by the aforementioned phase 2 studies and followed a similar titration plan – starting at 2.5mg/day and gradually increasing up to the dosage to which the person had been randomized. Overall, 78.3% of participants during phase 3 trials achieved a 20% or greater decrease in phe levels after taking pegvaliase for one year, while 60.7% reached levels below 360µmol/L and 51.2% reaching levels below 120µmol/L during this time[31].

2.4.2.1 Neuropsychiatric Improvements with Pegvaliase

In a PKU mouse model, pegvaliase has been shown to reduce phe levels both in the blood and the brain. Along with this, tyrosine levels in the brain increased, even though blood tyrosine levels remained low. Accordingly, dopamine and serotonin levels increased as well. Norepinephrine levels were also noted to increase as phe levels decreased due to pegvaliase[30]. In humans, similar patterns were seen in patients who had a successful response to pegvaliase, including improvement of attention scores related to norepinephrine and dopamine levels being increased back to normal following a decrease in phe levels[30].

During the randomized clinical trial, patients who were switched to the placebo, and consequently experienced increased phe levels, showed worsening in multiple executive functioning domains (working memory, cognitive flexibility, and inhibitory control), as well as attention. In contrast, patients who continued on pegvaliase remained stable or improved in each of these abilities from week 1 to week 8 of the trial[26]. The earlier phase 3 clinical trial also showed that attention improved with lower phe levels induced by pegvaliase, demonstrating that patients

who experienced the greatest decrease in phe levels also had the most significant improvement on attention tests[31].

Significant improvements in mood were also reported during the earlier phase 3 trial, but not the randomized control trial. This may be because the initial phase 3 trial lasted for 2 years, while the randomized control trial only lasted for 8 weeks, and a longer period of time is likely required to see improvements in mood[26,31].

2.4.2.2 Immune Responses to Pegvaliase

Enyzme replacement therapies and similar therapies that use an enzyme foreign to the patient's body, such as enzyme substitution therapies like pegvalise, are known to induce an antibody response in some patients, which can lead to adverse reactions[81]. All patients in the phase 2 clinical trial developed antibody responses against PAL and many developed a temporary response against PEG, both of which decreased overtime with the exception of PAL IgG, which remained consistently increased. No patients were positive for a drug-specific IgE, including patients who experienced anaphalaxis[27,29,31,81,82]. For PAL, over 75% of patients had PAL IgM at week 24 compared to under 50% at week 120. For PEG, PEG IgG and IgM were elevated in most patients at week 8, but had returned to normal by week 36[27,31]. Many participants had low-level titers for anti-PEG antibodies prior to starting pegvaliase due to the use of PEG in other medications and cosmetics, which did not seem to impact the safety of pegvaliase for these individuals[82].

The extent of a person's immune reaction against pegvaliase is also important because it likely influences the effectiveness of the treatment. Phase 2 studies suggested that if there is a strong immune response, then the immune system will clear pegvaliase from the person's system more rapidly, requiring a higher dosage[29]. This was further supported by phase 3 trial data showing

that patients who had lower antibody titers early in treatment with pegvaliase were more likely to have a greater and more rapid decrease in phe levels. However, even participants who had a strong immune reaction were shown to have a significant reduction in phe levels[81]. It was also noted in several of the clinical trials that adverse events, described below, were most common in the early months following initiation of pegvaliase when antibody levels were highest, especially anti-PEG antibodies[28,29,31]. This is not unexpected because PEG is on the surface of PAL, making it easier for anti-PEG antibodies to bind to pegvaliase and initiate an immune response compared to anti-PAL antibodies[82]. However, presence or level of a specific antibody was not predictive of any particular adverse event[31,81].

2.4.2.2.1 Safety Profile of Pegvaliase

Anaphylaxis occurred in the clinical trials but was not common. It happened only in two patients out of the 80 who enrolled in phase 2 trials. Both of these patients had multiple events of anaphylaxis, with anaphylaxis occurring both during the initial 24 weeks of the trial and during long-term treatment [27,29]. Other hypersensitivity adverse events were more common, occurring in most (91.2%), but not all patients in phase 2 trials. These reactions included rash, arthralgia (joint pain), urticaria (hives), pruritis (itching), and pyrexia (fever)[27]. Overall, during the first 24 weeks, the most common reactions were arthralgia and injection-site reactions. After the first 24 weeks, when many patients had reached their maintenance dose associated with decreased phe levels, the most common reaction was urticaria. In general, adverse events decreased in frequency over time once an effective dose for an individual had been established and their dosage was no longer being systematically increased[27,29]. Most adverse events were also mild or moderate in nature and did not lead to discontinuation or changes in dosing[26,27,29,31].

A similar adverse event profile was reported by phase 3 clincal trials[26,31,81]. Importantly, there were similar rates of adverse effects reported by both the placebo and pegvaliase groups during the randomized control trial[26]. This could not be investigated during the phase 2 trials as there were no control groups for comparison.

2.4.3 Impact of Pegvaliase on Patients

A survey distributed to people who have PKU by NPKUA in 2015 showed that over 90% of respondents stated it was important to find new, improved treatments for PKU. This was true

for patients both within the recommended phe range of 120 to 360µmol/L and patients above 360µmol/L. Similarly, 90% of respondents, both with phe levels within and exceeding the recommended range, reported desiring a new treatment to help lower blood phe levels. Fifty-seven-percent of respondents stated that they were content with their current treatment, but were hopeful for better treatments to be developed in the future, while 35% stated that they have difficulty with their current treatment[60]. Pegvaliase has the promise to begin to address these concerns for a substantial portion of people who have PKU, with the exception of those who have a preference for oral medications over injections[60].

A more recent study compared data on blood phe levels and natural protein intake from participants in the phase 2 and 3 pegvaliase clinical trials to those enrolled in a PKU patient registry. The study was designed to investigate the effectiveness of pegvaliase versus sapropterin plus diet versus diet alone management approaches in adults with PKU. They found that patients who were taking pegvaliase had both the lowest phe levels and highest natural protein intake on average, suggesting that pegvaliase is the most effective treatment for patients who have been previously unable to keep their phe levels below 600µmol/L[83]. This creates the potential for pegvalise to improve quality of life for people who have PKU on the basis of reducing neuropsychiatric and cognitive symptoms by lowering phe levels and allowing for liberalization of diet.

These anticipated impacts on quality of life are highlighted by a study on patient perspectives of pegvaliase and how they weight the risks versus benefits of the treatment. Using a survey, this study was able to show that, on average, adults who have PKU and currently have a blood phe level of over 600µmol/L are willing to use pegvaliase as long as there is at least a 22.7 to 34.4% chance of achieving phe levels below 600µmol/L. These patients were aware of the

potential adverse effects and the frequency of these events among patients enrolled in clincal trials. They were also aware that pegvaliase is administered via injection, which contributed to a sub-set of respondents (4 out of 45) being less willing to use pegvaliase. Notably, the percent of patients who reach and maintain phe levels below 600μmol/L by taking pegvaliase is much higher than 34.4%, as discussed previously[84]. This is similar to the results of a survey distributed by the NPKUA, which showed that most respondents were willing to experience mild reactions such as injection site reactions or skin irritation, but less willing to risk severe reactions such as anaphylaxis[60]. As part of the risk-benefit survey, respondents also reported on the burdens created by managing and living with their PKU. The reported burdens impacted multiple areas of life, including social activities, cognitive ability, and overall lifestyle. These burdens possibly explain why many respondents were willing to try pegvaliase even if there was an over 70% chance that it would not reduce their phe levels below 600μmol/L[84].

A smaller study involving 26 patients in Germany who had phe levels above 600μmol/L reported that most of the patients (73%) refused treatment with pegvaliase due to fear of the adverse effects, administration via injection, or not feeling a need for additional treatment at this time; there was a 4-week gap between the initial appointment providing information on pegvaliase and the follow-up appointment where the patient was asked for a decision regarding pegvaliase, during which time several of the patients who refused pegvaliase were able to reduce their phe levels closer to or below 600μmol/L. These differences in patient preference may reflect the differences in guidelines in the United States compared to Europe. As Europe has a higher target phe level for adults (600μmol/L), patients in Europe may feel less pressured to achieve lower phe levels with new treatments like pegvaliase, compared to patients in the United States where the target phe level is lower[85,86].

2.5 Future Directions for PKU Management: Gene Therapy

Gene therapy is actively under investigation for PKU. It has typically targeted the liver, the site of PAH activity. As mentioned previously, PKU is inherited in an autosomal recessive pattern, with carriers having normal phe levels despite having decreased PAH activity. This will likely help with the gene therapy approach because it will only be necessary to restore PAH activity to the level of a healthy carrier, not 100% activity. In addition, mouse models have shown that having at least 10% of liver cells with functional PAH is sufficient to lower phe levels to normal range, even if this subset of cells are heterozygous for *PAH* mutations[86].

The initial approach to gene therapy for PKU in mouse models involved a recombinant retrovirus. This approach caused a strong immune response against the virus, necessitating a different approach, a recombinant adeno-associated virus. Multiple pharmaceutical companies have stated that they plan to conduct clinical trials for gene therapies that would use a viral vector to introduce functional *PAH* in liver cells. However, the benefits may only be temporary, especially in pediatric cases where liver cells are still rapidly dividing, because these approaches do not incorporate the vector carrying functional *PAH* into the cell's genome[86].

Non-viral approaches, such as RNA, are also under investigation, along with gene editing using CRISPR/Cas9[86,87]. While previous approaches with CRISPR/Cas9 have been prone to error and therefore not suitable for use in humans, a new method was recently developed that allows for precise editing of single base pairs. This newer method has since been tested in a mouse model and shows potential for use in future gene therapy. Such an approach would be expected to have a more permanent impact than one using viral vectors, as this would permanently repair the mutation within cells, allowing it to be passed on when the cell divides[86,88]. One study on gene editing as a treatment for PKU in mice used a recombinant adneo-associated virus vector to deliver the Cas9

enzyme, guide RNA, and repair template to liver cells of the mice in order to correct a missense mutation in *Pah* for which the mice were homozygous. While the rate of successful gene editing by homology directed repair was only 13.06%, they did report a decrease in blood phe levels and a complete reversal of associated physical and behavioral symptoms. This improvement persisted for the remaining lifetime of the mice that had successful gene editing. A separate study on gene editing in *Pah*-deficient mice used base editors that do not require homology directed repair to edit the gene. This study reported up to 25.1% correction of *Pah*[89]. However, as the mice in each of these studies all had the same mutation, the same guide RNAs were used, which would not be the case in humans considering there are over 1,000 known *PAH* mutations, further complicating the translation of this approach to humans[88,89].

It is also important to consider the high costs that will be associated with gene therapies, if they are successfully developed. This must then be weighed against potential benefits, such as requiring fewer injections – possibly only one in a lifetime – compared to pegvaliase which requires daily injections[87,88]. There is an apparent interest in a one-time management approach, like gene therapy, among people who have PKU. A survey distributed to people who have PKU by the National PKU Alliance found that 85% of respondents would be interested in a single-dose gene therapy for PKU[60]. Overall, future comparisons will be possible between the ability of pegvaliase and of gene therapy to impact quality of life for people who have PKU due to their potential similarities and differences in cost, administration method and frequency, efficacy, and side effects.

3.0 Manuscript

3.1 Background

Phenylketonuria (PKU) is one of the most common inherited metabolic disorders, with an estimated incidence of 1 in 15,000[1]. It was the first condition to be included on newborn screening, following development of an assay by Guthrie and Susi in 1963 that could be conducted on a dried blood spot[2]. PKU is part of a group of conditions titled hyperphenylalaninemia, all of which are characterized by elevated levels of the essential amino acid phenylalanine (phe). Severity of hyperphenylalaninemia in the context of PKU can be separated into three categories: classical PKU (phe concentrations greater than 1,200 μmol/L), mild PKU (phe concentrations between 600 to 1,200 μmol/L) and non-PKU hyperphenylalaninemia (phe concentrations between 120 to 600 μmol/L)[1]. PKU is inherited in an autosomal recessive pattern due to pathogenic variants in *PAH,* which encodes phenylalanine hydroxylase (PAH) – the enzyme responsible for converting phe to tyrosine in humans[1]. Over 1,100 different pathogenic variants have been reported to date[3], with some correlation between variant type and severity of PAH deficiency[4–6].

When untreated, classic PKU causes a range of symptoms including intellectual disabilities, seizures, and impaired motor skills[7,8]. With the advent of newborn screening, early treatment can be initiated to prevent or mitigate these symptoms, however certain symptoms such as anxiety and depression, attention and executive functioning deficits, and lower IQ (but not usually to the extent of intellectual disability) may continue even with appropriate treatment[9–14]. Treatment for PKU originally relied solely on dietary restriction of protein, and therefore of phe, supplemented by medical formulas that contain all amino acids except phe, and other essential

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nutrients[15]. These dietary restrictions were once only routinely followed during childhood[16,17], but treatment for life has been the standard recommendation in the United States since 2000[16].

In 2008 an oral therapy, sapropterin dihydrochloride (Kuvan, BioMarin Pharmaceuticals), a synthetic form of tetrahydrobiopterin (BH4) – a cofactor of PAH, was approved by the FDA[18]. This therapy successfully lowers phe levels in many people who have mild PKU but typically does not provide a clinical benefit to people who have more severe, classical PKU[6,18,19]. Several studies have been conducted to measure the impact this therapy had on the quality of life of patients who responded to the medication. These studies largely found that there was not a significant increase in quality of life subsequent to treatment with sapropterin and the associated relaxation of dietary restrictions, with only one study showing an improvement in multiple domains of quality of life[11,20–22]. Notably, the study that showed improvements in quality of life was the only one to use a quality of life questionnaire specific to PKU; this questionnaire was adapted for use in the current study. It has also been postulated that improvements in quality of life due to dietary relaxation for some patients following treatment with sapropterin may be limited, since these patients typically have a less stringent diet at baseline compared with people who have more severe or classic PKU[21–24]. It therefore can be postulated that patients who have more severe PKU may experience a greater improvement in quality of life following an effective treatment that allows for dietary relaxation.

In 2018, a treatment received FDA approval for adults who have classical PKU. Pegvaliase (Palynziq, BioMarin Pharmaceuticals) is an injectable enzyme substitution therapy using recombinant phenylalanine ammonia lyase, a bacteria-derived enzyme that converts phe to cinnamic acid and ammonia[25]. It has been shown to be effective in lowering phe levels for adult patients with phe levels over $600\mu mol/L$[25–29]. As this is a more recently approved therapy for PKU, many studies have been published on the effectiveness and safety of pegvaliase from a clinical

standpoint, including improvement of neuropsychiatric symptoms[26,30,31]. However, no studies on impact of pegvaliase on quality of life for individuals taking it have been published to date. The goal of this study was use an online questionnaire to investigate quality of life impacts, focusing specifically on 1) assessing psychosocial and neuropsychiatric impacts of pegvaliase, 2) determining how pegvaliase has changed the dietary decision making of patients using it and how this could translate to an impact on quality of life, and 3) identifying residual resource and support needs for patients taking pegvaliase. By learning more about how a treatment, like pegvaliase, can impact a patient's life beyond lowering their phe levels, providers can continue to improve overall care for these individuals. These continued improvements may include providing anticipatory guidance on the benefits and limtations of such treatments and linking patients to additional resources and supports as needed.

3.2 Methods

3.2.1 Study Participants

The target population for this study was adults 18 and older who have PKU, including individuals who are currently taking pegvaliase, have never taken pegvaliase, and have taken pegvaliase previously but are no longer taking it. Children were excluded from this study as pegvaliase is currently only FDA approved for use in adults. This study and its corresponding survey have been approved by the University of Pittsburgh (Appendix A) and National PKU Alliance (NPKUA) IRBs.

3.2.2 Survey Development

The survey constructed for this study (Appendix B) included the following topics: 1) introductory paragraph, 2) eligibility questions and demographics, including phe levels, management of PKU and employment and/or student status, 3) general quality of life, 4) quality of life in relation to management of PKU, 5) quality of life in relation to impact of PKU on daily life, 6) worries surrounding PKU, 7) assessment of current resources and supports and residual needs, 8) cognitive symptoms, and 9) depressive symptoms. The quality of life portions of the survey were based in large part on a previously developed survey of PKU-specific impacts on quality of life, used with permission from Dr. Rani Singh, the corresponding author for the study which originally constructed the survey (Appendix C.1). Additional questions were added to assess residual resource needs, and certain questions determined to be redundant were removed to help keep the survey at a reasonable length. Internal consistency was calculated with Cronbach's alpha values for each construct in the present survey to assess whether items within the construct were correlated with each other, as intended. This was done to ensure that this addition and removal of questions did not compromise the survey, although they were previously calculated for each construct in the questionnaire on which the quality of life portion of the present survey was based.

Questions assessing cognitive and depressive symptoms were taken verbatim from Neuro-QOL short forms (Neuro-QOL Item Bank v2.0 – Cognition Functions – Short Form, Neuro-QOL Item Bank v1.0 -Depression – Short Form) available from the NIH Toolbox HealthMeasures and were adapted for use in an online survey with permission from the HealthMeasures staff (Appendix C.2). Overall, the process of determining which questions to use, add, or exclude was conducted with the input of multiple people, including providers in the PKU Clinic and a neuropsychologist

at UPMC Children's Hospital of Pittsburgh and an individual who has PKU and is who is pegvaliase.

The survey implemented skip logic at multiple points to ensure that participants were only asked relevant questions. Examples of this included only asking follow-up questions about pegvaliase for individuals who reported using pegvaliase either currently or previously and only inquiring about the impact of PKU on a person's work or schooling if they reported being employed or having student status. The survey was accessible through an anonymous Qualtrics link, in compliance with the University of Pittsburgh's University Data Security standards.

Written informed consent was deemed unnecessary due to the nature of the study as an anonymous online survey, and an introductory paragraph detailing the study was provided to potential participants upon accessing the survey link. This included information on the purpose of the study, eligibility, potential benefits and risks, and contact information for the primary investigators (Appendix B).

3.2.3 Survey Recruitment and Distribution

Recruitment for this study was conducted in two ways, 1) through coordination with NPKUA on emailing an informational paragraph on the study, including a link to the survey, to their patient registry twice – once in December to initiate data collection and once in February as a final reminder to complete the survey, and 2) through the PKU clinic at UPMC Children's Hospital of Pittsburgh, with help from the clinic's nurse. Both of these methods distributed the same informational paragraph (Appendix D). Recruitment at UPMC Children's Hospital of Pittsburgh did not begin until after the survey had been distributed to the NPKUA patient registry.

NPKUA further helped with recruitment by including a section on the study in their January newsletter which was similar to the original informational email. Recruitment from both methods lasted from December 2020 through February 2021. The informational paragraph used by both recruitment sites consisted of a condensed version of the introductory script of the survey (Appendix E).

3.2.4 Data Analysis

The anonymous responses to the Neuro-QOL short-forms on cognition and depression were transferred to a Microsoft Excel spreadsheet where they were scored based on the standardized scoring system previously available from the NIH Toolbox Health Measures. For the cognition short-form, responses scoring 16 or less (2 SD below the mean) were identified to allow two separate data analyses – one including these respondents' answers and one excluding them. Demographics (degree of phe control, management approaches, and student/employment status) were noted for these respondents as well. This was done in order to determine if there were any respondents who may not have been able to fully understand the questions asked in the survey by assessing different response patterns, and the impact this may have had on the data. These individuals were included in some parts of the analysis in order to better understand how PKU can impact cognition in adults, even ones who are using various management approaches.

Responses to quality of life questions were also scored in accordance with the scoring method for the questionnaire on which the present survey was based, with each response being assigned a value of 1 to 5 and higher scores indicating a lower quality of life. In cases where a question was not applicable to a respondent or the respondent chose to skip a question, leaving the question unanswered, the missing responses were imputed based on present responses. Scoring

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protocols for the quality of life questions, by sub-section, and the Neuro-QOL Short forms are available in Appendix E.

These scores were then compared between respondents who reported taking pegvaliase and those who reported not taking pegvaliase, as well as between respondents who reported their most recent phe level being below 600μmol/L and those who reported their most recent level as 600μmol/L or higher. These comparisons were made using a Wilcoxon rank-sum test. Specific questions from the quality of life assessment were analyzed using a Fisher's exact test in order to further investigate the relationship between elevated phe levels and the emotional costs of dietary management and guilt related to phe levels, to enable comparison to previously published literature on this topic[90–92]. Comparisions regarding the distribution of reported phe levels, reported frequency of phe measurements, and presence of residual resources needs, each related to pegvaliase use, as well as comparisions of degree of dietary change by reported phe level and length of time taking pegvaliase, were done using a Fisher's exact test. The statistical software used was StataSE, version 16.1.872.

For the three open-ended questions (Table 1), thematic analysis was conducted as described by Braun and Clark (2006)[93]. The codes and themes and overarching patterns were first identified by the primary investigator, then reviewed by two additional study team members. Modifications in the coding scheme and themes were made based on this review process.

Table 1 Open-Ended Questions Included in Survey

Question was visible to	Question Content
Only respondents taking pegvaliase	What caused you to make this change in your diet (or to not make a change) [following initiation of pegvaliase]?
All respondents	Are there any other areas of your life where you wish you had more support?
All respondents who reported they need additional resources	Please describe these additional resources you need.

3.3 Results

3.3.1 Demographics

Forty-three eligible individuals began the survey, with 36 individuals completing the entire survey. Ten of the 43 (23.2%) respondents reported being a current student and 31 (72.1%) reported being currently employed, with 4 (0.9%) individuals being both a student and employed. Of these, 13 reported taking pegvaliase currently and one reported taking pegvaliase previously. All 14 of these individuals completed the survey in its entirety. Details about the number of respondents taking and not pegvaliase relative to the number of participants with phe levels at or below 600μmol/L appear in Table 2. Although a higher percent of respondents who were taking pegvaliase had phe levels below 600μmol/L compared to the group of respondents who were not taking pegvaliase, this did not reach statistical significance (p-value: 0.49)

Table 2 Number of respondents by pegvaliase status and phe level range

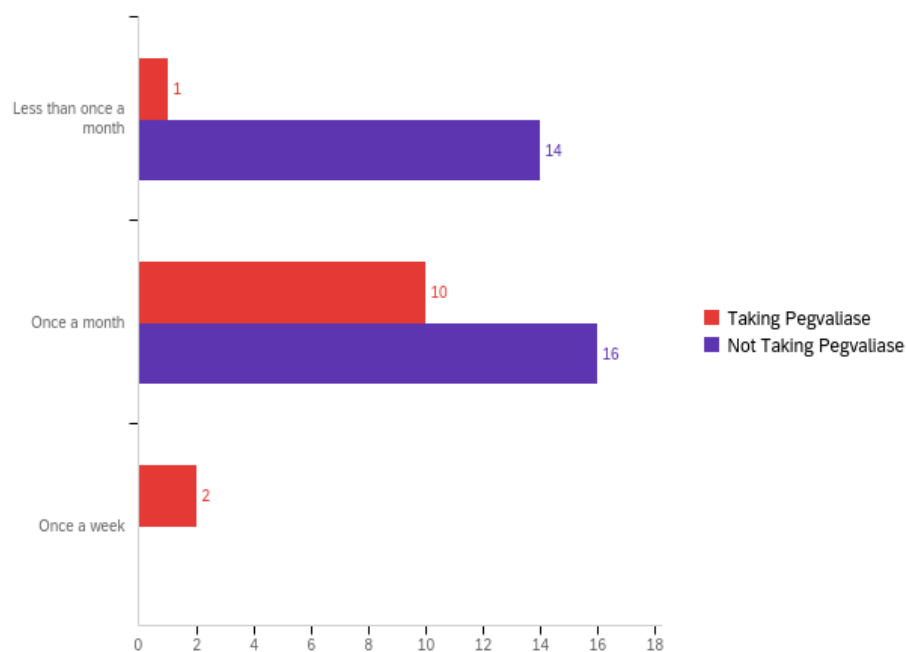|	Taking pegvaliase (%)	Not taking pegvaliase (%)	Total
Phe levels at/below 600μmol/L	10 (77%)	18 (60%)	28
Phe levels above 600μmol/L	3 (23%)	12 (40%)	15
Total	13 (100%)	30 (100%)	43

With regards to other management approaches, 17 respondents reported taking sapropterin and 35 reported using dietary management. A more detailed combination of management approaches, including combinatory approaches, is available in Table 3.

Table 3 Number of respondents reporting utilization of different management approaches, including those involving multiple different types of management

Management Approach(es)	Number of Respondents Reporting Use
Pegvaliase only*	4
Pegvaliase + Sapropterin	1
Pegvaliase + Diet	3
Pegvaliase + Sapropterin + Diet	4
Sapropterin only	2
Sapropterin + Diet	10
Diet only	18

*One respondent who reported taking pegvaliase did not answer the question regarding additional management approaches and is not included in this table.

Reported frequency of phe measurements ranged from once a week to less than once a month. Phe measurements were taken significantly more frequently in respondents who were taking pegvaliase than respondents who were not (p-value: 0.02), and both of the respondents who reported measuring their phe levels weekly were taking pegvaliase (Figure 1). One of these respondents had been taking pegvaliase for less than six months and the other had been taking it for a year or more.

Figure 1 Number of respondents measuring their phe levels at specified frequencies, by pevaliase status

Phe levels were also lower on average among respondents taking pegvaliase than participants who were not, and all three respondents who reported phe levels below 120μmol/L, were taking pegvaliase (Figure 2). However, as stated above, this trend did not reach statistical significance
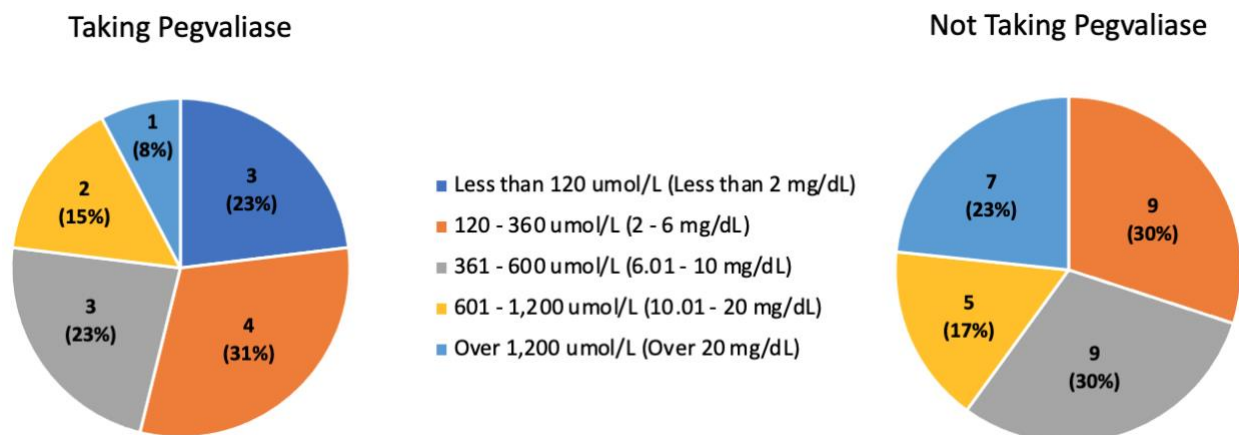
Figure 2 Blood phe levels, by pegvaliase status

3.3.2 General Impact of Pegvaliase

Of the participants who reported taking pegvaliase, four had been taking it for less than six months, two had been taking it for six to nine months, and seven had been taking it for a year or more. All of the respondents who reported that their diet had changed appreciably had been taking pegvaliase for at least a year, although there was variation in the degree of dietary protein change among respondents who reported taking pegvaliase for this length of time (Table 4). There was no

significant difference when comparing reported degree of dietary change by length of time taking pegvaliase (p-value: 0.29) or recent reported phe levels (p-value: 0.15).

Table 4 Degree of dietary protein change since initiation of pegvaliase, by length of time taking pegvaliase

	No change in diet	Diet changed a little	Appreciable change
<6 months	2	2	0
6 months to <9 months	2	0	0
1 year or more	2	1	4
TOTAL	6	3	4

*No respondents reported taking pegvaliase for 9 months to less than a year.

Reasons for change in dietary protein intake, or lack thereof, were elucidated with the open-ended question, "What caused you to make this change in your diet (or to not make a change)?" Seven of the 13 respondents taking pegvaliase answered this question. One respondent may have misunderstood the question and answered "My levels started getting harder to manage in my teens," which is not directly applicable to the question asked. Otherwise, each respondent mentioned either the time delay associated with lowered phe levels after starting pegvaliase (n=1), positive changes related to pegvaliase, including decreased phe levels, increased protein intake, and improvements in day to day life (n=4), or both a time delay and positive changes (n=1). Time delays were cited as a reason for why a respondent's diet had not yet changed since initiation of pegvaliase, and were potentially a source of frustration for the two respondents who were still waiting for the potential positive effects of pegvaliase to be realized. Notably, the respondent who mentioned both future positive impacts of pegvaliase, and the expected time needed for those to take effect, placed particular emphasis on expectations for cognitive improvements, saying:

I have a greater desire to feel more normal. Mostly when it comes to

cognition and brain clarity. I need to be able to function better, especially with

my memory. The food option changes (once they come) will just be an added

bonus. I'm more concerned with my mental clarity and how that will translate

to my mental capabilities when I'm in my 80s and on…

Another respondent shared his/her positive experiences in relation to participation in clinical trials and beyond, and positive feelings towards the process and resulting outcome:

Palynziq. I went from 5 grams of protein a day to 55. I have been on it

for over 4 years as I was a patient in the FDA trial and I do not regret that
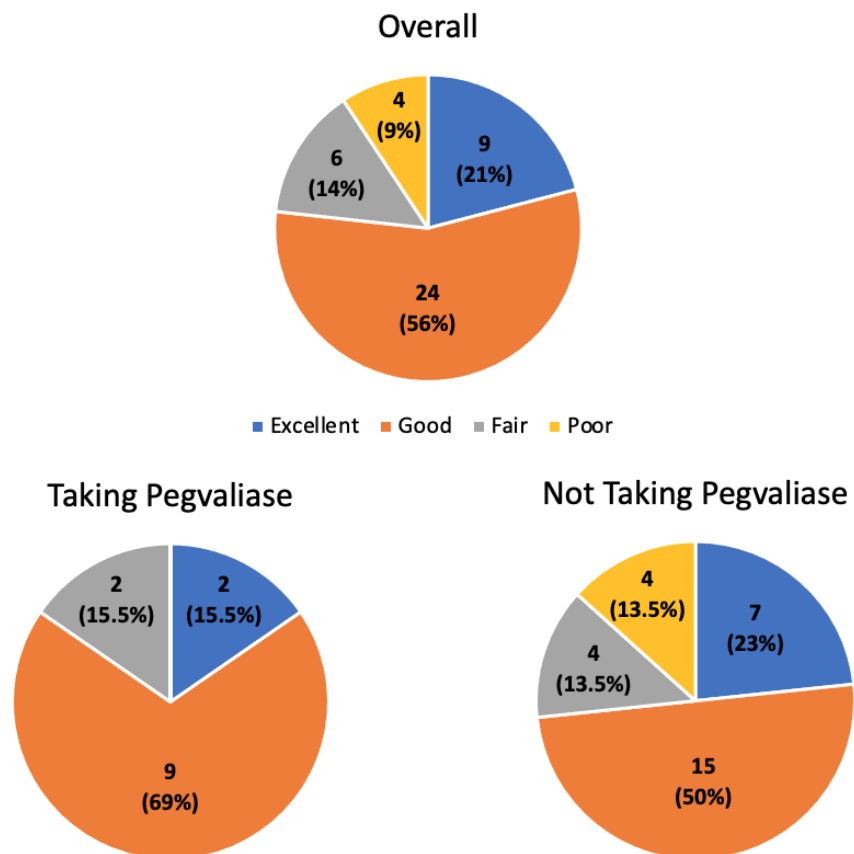
decision at all.

Taking pegvaliase came with some degree of side effects for the majority of respondents (85.7%), with six of these reporting that these side effects did not impact their quality of life and

the other six reporting that their side effects impacted their quality of life. No respondents reported

that the side effects of pegvaliase impacted their life significantly (Table 5).

Table 5 Impact of pegvaliase side effects on quality of life, by length of time a respondent has been taking

pegvaliase

	IMPACT OF SIDE EFFECTS		
	Experiencing side effects		Not experiencing side effects
TIME ON PEGVALIASE	Moderately impacting quality of life	Not impacting quality of life	
<6 months	3	1	0
6 months to <9 months	0	2	0
1 year or more	2	3	2
TOTAL	5	6	2

3.3.3 Quality of Life

Figure 3 Self-reported overall health: All respondents (top), by pegvaliase status (bottom)

Overall, the majority of respondents reported they felt their health is good or excellent compared to other people their age, with only four (9.3%) of all respondents stating they felt their health was poor compared to others (Figure 3). Each of the individuals who selected "Poor" also reported not taking pegvaliase, and three of the four had phe levels above 600µmol/L

The scores for the quality of life portions of the survey are summarized in Table 6, sorted by sub-section and sub-group within the study sample. When comparing respondents taking pegvaliase to those who do not, a significant difference was found for satisfaction with

management (p=0.03). This was the only significant difference identified when comparing these two groups but general satisfaction with life approached statistical significance (p=0.05). A number of differences were noted when comparing respondents who had phe levels below 600μmol/L to those who had phe levels of 600μmol/L or higher. These differences were seen in general life satisfaction (p=0.02), satisfaction with management (p=0.01), impact of PKU (p=0.04) and overall quality of life score (p=0.02). No significant differences in PKU-related worries or satisfaction with supports were seen when comparing by pegvaliase status or phe level. Across all sub-sections, regardless of whether a statistically significant difference was seen, respondents who had lower phe levels had better scores on average than those who had higher levels and respondents who were taking pegvaliase had better scores on average than those who were not taking it. This pattern was also present for total scores.

Internal consistency of each sub-section, as calculated with Cronbach's alpha were: 0.85 for general satisfaction with life (11 questions), 0.88 for satisfaction with management (14 questions), 0.88 for impact of PKU (15 questions), 0.91 for PKU-related worries (11 questions), and 0.78 for satisfaction with support (four questions). These scores indicate acceptable to excellent internal consistency among the questions in each of the sub-sections, making it appropriate to analyze differences between the average scores of different groups on these sub-sections, as was done for the above analysis.

Table 6 Quality of life section(s) scores and comparisons by pegvaliase status and phe level

	Sub-Group	n	Mean ± SD	Min-Max (Range)	p-value
General Life Satisfaction Possible scores: 11-55 (median: 33)	Pegvaliase	13	22.17 ± 4.95	13.75-30 (16.25)	0.05
	No Pegvaliase	30	28.11 ± 9.37	13-46.75 (33.75)	
	Phe <600µmol/L	28	23.9 ±7.19	13-37.125 (24.125)	0.02*
	Phe ≥600µmol/L	15	30.8 ± 9.64	16-46.75 (30.75)	
Satisfaction with Management Possible scores: 14-70 (median: 42)	Pegvaliase	13	33.15 ± 13.38	14-60 (46)	0.03*
	No Pegvaliase	30	41.27 ± 10.3	25-63 (38)	
	Phe <600µmol/L	28	35.61 ± 11.23	14-55 (41)	0.01**
	Phe ≥600µmol/L	15	44.8 ± 10.66	25-63 (38)	
Impact of PKU Possible scores: 15-75 (median: 45)	Pegvaliase	13	37.92 ± 9.51	22-54 (32)	0.38
	No Pegvaliase	29	41.65 ± 11.33	23-62 (39)	
	Phe <600µmol/L	27	37.95 ± 10.97	22-62 (40)	0.04*
	Phe ≥600µmol/L	15	45.07 ± 9.22	31-60 (29)	

Lower score = higher quality of life (for any sub-score and overall score), *p-value less than 0.05, **p-value less than or equal to 0.01

Table 6 Quality of life section(s) scores and comparisons by pegvaliase status and phe level

PKU-Related Worries Possible scores: 11-55 (median: 33)	Pegvaliase	13	29.27 ± 10.84	14-47.3 (33.3)	0.66
	No Pegvaliase	28	30.75 ± 12.33	11-52.8 (41.8)	
	Phe <600µmol/L	27	28.41 ± 11.32	11-49.5 (38.5)	0.16
	Phe ≥600µmol/L	14	33.88 ± 12.17	15.4-52.8 (37.4)	
Satisfaction with Support Possible scores: 4-20 (median: 12)	Pegvaliase	13	7.31 ± 2.86	4-12 (8)	0.32
	No Pegvaliase	28	8.68 ± 3.88	4-17 (13)	
	Phe <600µmol/L	27	7.81 ± 3.39	4-15 (11)	0.36
	Phe ≥600µmol/L	14	9.07 ± 4.01	4-17 (13)	
TOTAL Possible scores: 55-275 (median: 165)	Pegvaliase	13	129.82 ± 34.02	72.86-189.3 (116.44)	0.25
	No Pegvaliase	28	139.2 ± 41.09	84.3-223.5 (139.2)	
	Phe <600µmol/L	27	133.117 ± 37.77	72.86-209.5 (136.64)	0.02*
	Phe ≥600µmol/L	14	162.69 +36.95	118.9-223.5 (104.6)	

Lower score = higher quality of life (for any sub-score and overall score), *p-value less than 0.05, **p-value less than or equal to 0.01

Analysis by phe level of responses to the questions that were specifically analyzed due to their indirect relationship to the emootional costs of dietary restrictions and the potential for guilt surrounding elevated phe levels is included in Table 7. There was a clear, statistical difference in satisfaction with phe levels ($p = 0.01$), as people who reported lower phe levels reported higher satisfaction with their levels. For the possible emotional costs of dietary restriction, the only significant difference was found in the impact of PKU on leisure activities, where people who reported lower phe levels reported less of an impact ($p = 0.01$). In contrast, there was not a significant difference by phe level for the impact of PKU on going out to eat ($p = 0.22$). However, only people who reported phe levels under 120 μmol/L consistently reported having no negative impacts for both the general-leisure and going-out-to-eat questions. There was no significant difference found regarding perceived restrictiveness of diet in either question assessing this ($p = 0.08$ and $p = 0.15$).

Table 7 Analysis of responses to specific QOL questions related to the potential for guilt associated with elevated phe levels and the emotional costs that can be associated with a strict low-phe diet Responses were grouped by reported phe level allowing comparisions to be made between the group of people who reported phe levels ≥600µmol/L and the group who reported phe levels <600µmol/L.

| Sub-Section	Question	p-value
Satisfaction with Management (n=43)	How satisfied are you with your current phe level?	0.01**
	How satisfied are you with the flexibility you have in your diet?	0.08
Impact of PKU (n = 42)	How often do you feel restricted by your diet?	0.15
	How often do you think that your PKU interrupts your leisure-time activities?	0.01**
	How often do you find that your PKU prevents you from going out to eat with your friends?	0.22

*p-value less than or equal to 0.01

3.3.3.1 Resources and Supports, Present Use and Residual Need

Satisfaction with support from family and friends, as well as doctors and dieticians, was assessed as part of the quality of life portion of the survey, summarized in Table 6. Additional supports were identified by 16 repondents. Identified support persons included mental health professionals and significant others, with pets being mentioned, as well. Support organizations, such as churches or university-based disability services were also mentioned. One respondent reported that they do not receive any support. The types of support provided are shown in Figure 4. Two respondents provided more detailed information about the types of support received: 1) "Housing accommodations to prevent my food needs from interfering with my school schedule"

in relation to support from University-based disability services and 2) "[My boyfriend] helps me make low protein meals often."
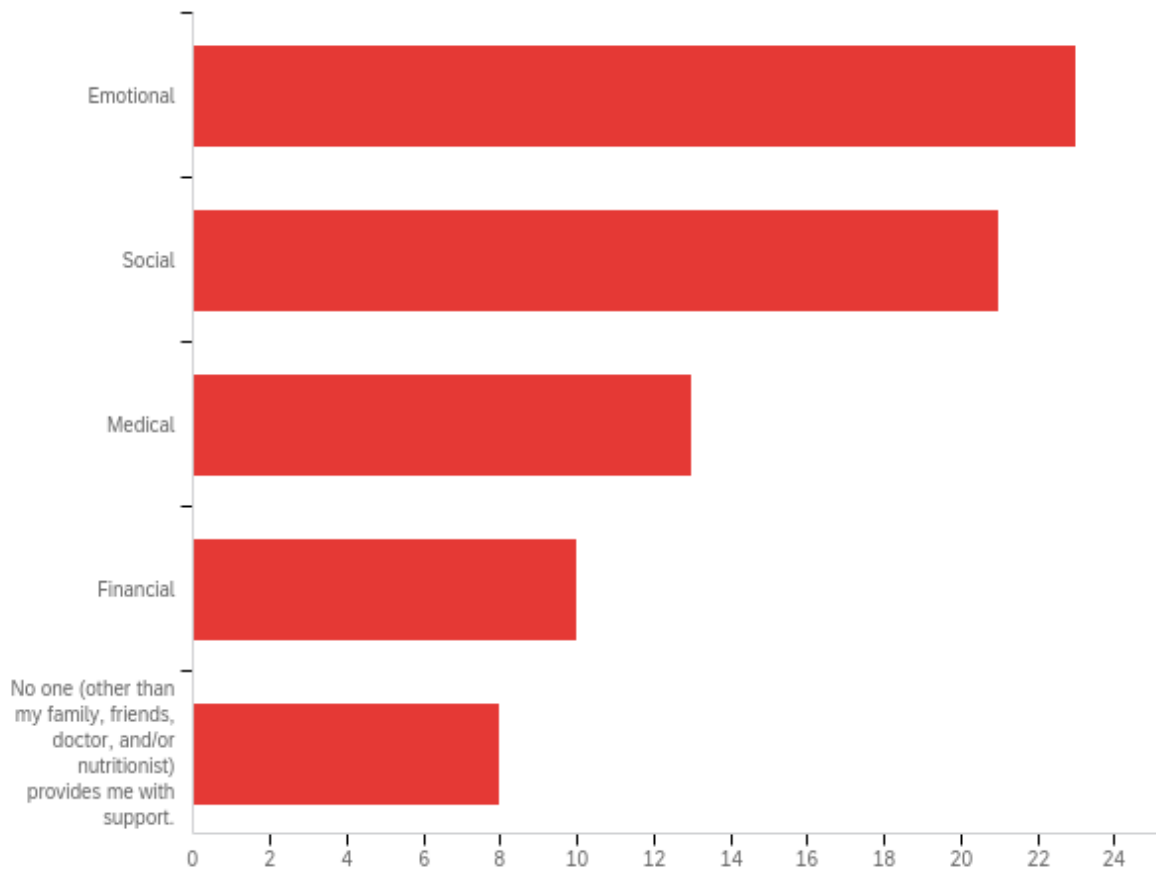
Figure 4 Number of respondents reporting types of support provided by support persons and organizations

*Total exceeds the number of participants as respondents were able to select multiple options.

Eighteen respondents answered the open-ended question, "Are there any other areas of your life where you wish you had more support?" Four stated "No" or "None," including one respondent taking pegvaliase. Of the other fourteen responses which indicated a residual support need, only one of these was from a respondent taking pegvaliase. This individual focused on support from insurance companies and the government. Three respondents who were not taking

pegvaliase also reported needing financial supports or better support from insurance, although supports related to social interactions and health care were more common.

Responses regarding social support ranged from general statements of isolation such as "I wish I had more people in my life with PKU" and the "social effects of lack of friends" to more complex social situations. As one respondent stated:

I wish I had more food support, as in I wish my significant other/s

didn't eat things I can't eat around them because that is when I break my diet

(even though its an unrealistic expectation).

The difficulty of navigating such social situations was also mentioned by another respondent in the later open-ended question regarding resource needs.

From a health care perspective, several of the responses revolved around support in managing PKU or accessing different management approaches such as sapropterin. One respondent also mentioned a need for support regarding "weight management/weight loss with PKU."

Other areas of residual support needs included support in employment and education, including identification of unspecified educational leaders by one respondent.

There were a variety of utilized resources reported, with the most common being: NPKUA and online support groups. A full summary is included in Figure 5. For the two respondents who selected "Other", one reported using their state's PKU organization and the other reported using pharmaceutical companies, although that individual did not select either of the pre-populated BioMarin resources.
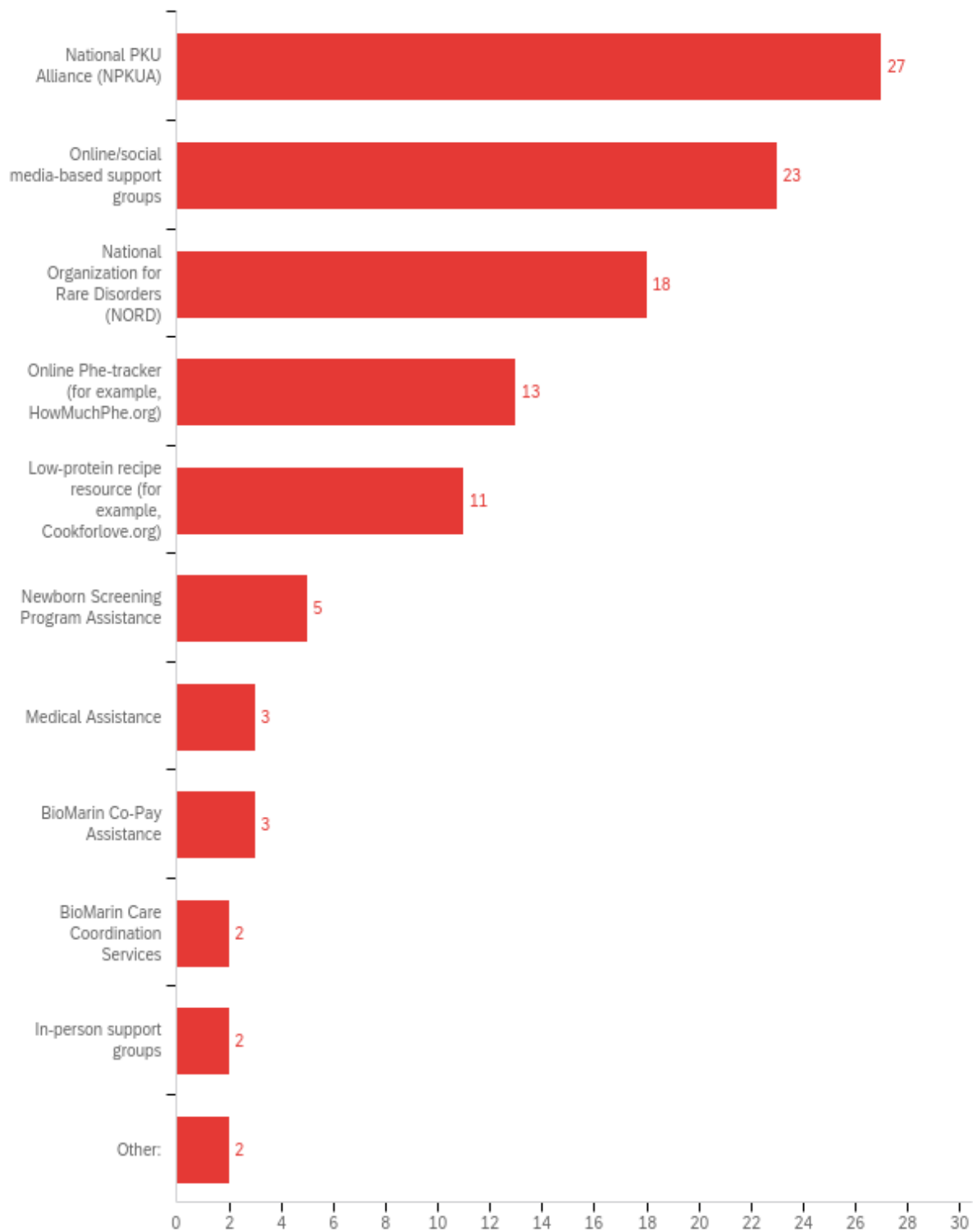
Figure 5 Number of respondents utilizing various resources (n=38); The total reflected in this figure exceed the number of respondents because respondents were able to select more than one option.

There was a trend among respondents who were taking pegvaliase toward not reporting additional unmet resource needs, although there were a few respondents who were taking pegvaliase who indicated they were missing key resources (Figure 6). However, this trend did not reach statistical significance (p-value: 0.19).
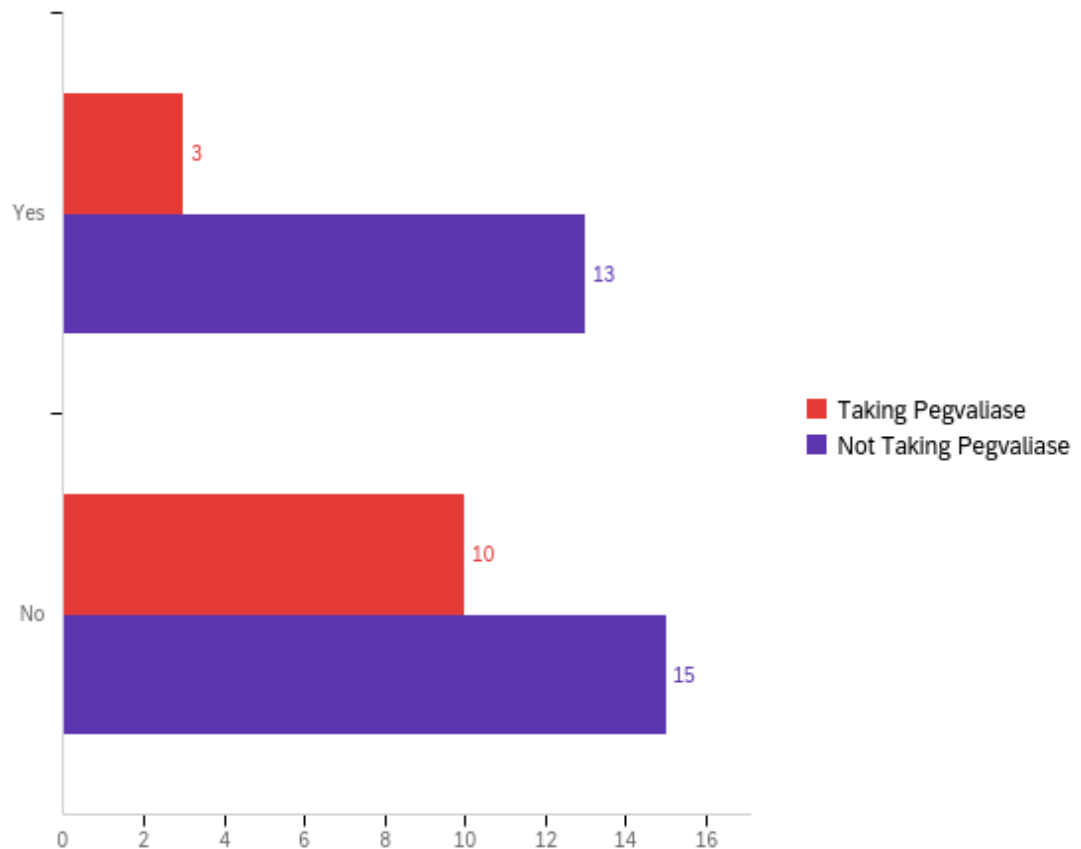
Figure 6 Number of respondents reporting residual resource needs, by pegvaliase status (n=41)

Of the 25 respondents who selected "Yes" on the multiple choice question – "Are there any other areas of your life where you wish you had more support?" (Answer Options: Yes/No), 15 provided additional details as to what kinds of additional supports they desire. Two of these 15 respondents were taking pegvaliase, and both reported residual financial support needs alone.

Among respondents not taking pegvaliase, financial resources, including "tools to make financial management easier with PKU," were mentioned as well, with one respondent specifically mentioning the financial burden of paying for certain resources like Howmuchphe. This respondent stated:

Howmuchphe is 45 dollars a year which is expensive, I'm a student and

don't work and that's a lot of money to spend on an app.

The other primary concerns related to resources for these individuals included health care, encompassing access to doctors and management approaches like sapropterin and pegvaliase (including one respondent based in Canada who was waiting for pegvaliase to become available there) and adult-specific resources. These adult-specific resources included both adult-focused PKU clinics and social resources. As one respondent mentioned:

I wish I knew about more support groups for adults with PKU and

especially women of childbearing age, I have no idea who to talk to about

personal maternal PKU experiences.

Another respondent mentioned:

Age specific peer support. Everyone else my age already has kids and

*support is always geared toward kids or people who have kids.*

Three respondents were also seeking additional information as a resource. Two of these respondents were seeking information for themselves in the form of either "information about the simplified diet" or "peer reviewed journal articles; more scientific knowledge of PKU at the biochemistry level in the brain" and the other wanted their providers outside of the PKU clinic to

be more informed on the actual impact of PKU on health, stating a need for "education for general practitioners that not all medical issues are PKU related."

There were no apparent trends when analyzing resource and support needs by geography, although only 29 out of the total 43 respondents provided the zipcode for their clinic, which was required for them to be included in this specific analysis (Figure 7). Even within California, which had the highest number of zipcodes provided (n=7), variations were noted between respondents. Two respondents (who both provided zipcodes for the same city) reported no additional support or resource needs, while the other five (two of whom also provided zipcodes for the same city noted above) did report either residual resource needs only (n=3) or both residual resource and support needs (n=2). One of these respondents said "Low protein food, drink has been good in CA, but moving," suggesting no residual resource need at this time, while others in California commented on insurance needs. One respondent expressed challenges accessing resources, saying:

My state has resources but requires information from doctors that I

cannot get until I have the resources to see the doctor.

As California was the only state with more than two known respondents, resource needs mentioned by California respondents were compared to responses from the rest of the United States. This comparison showed that the majority of respondents outside of California did not report residual needs (59-percent), compared to only 29-percent of respondents in California. The individuals outside of California who reported no residual needs included five taking pegvaliase and eight who were not, while the respondents in California who reported no residual needs were both taking pevalisase. Financial concerns were also more prominent outside of California, with three respondents explicitly stating financial concerns and three more stating residual needs regarding insurance coverage.
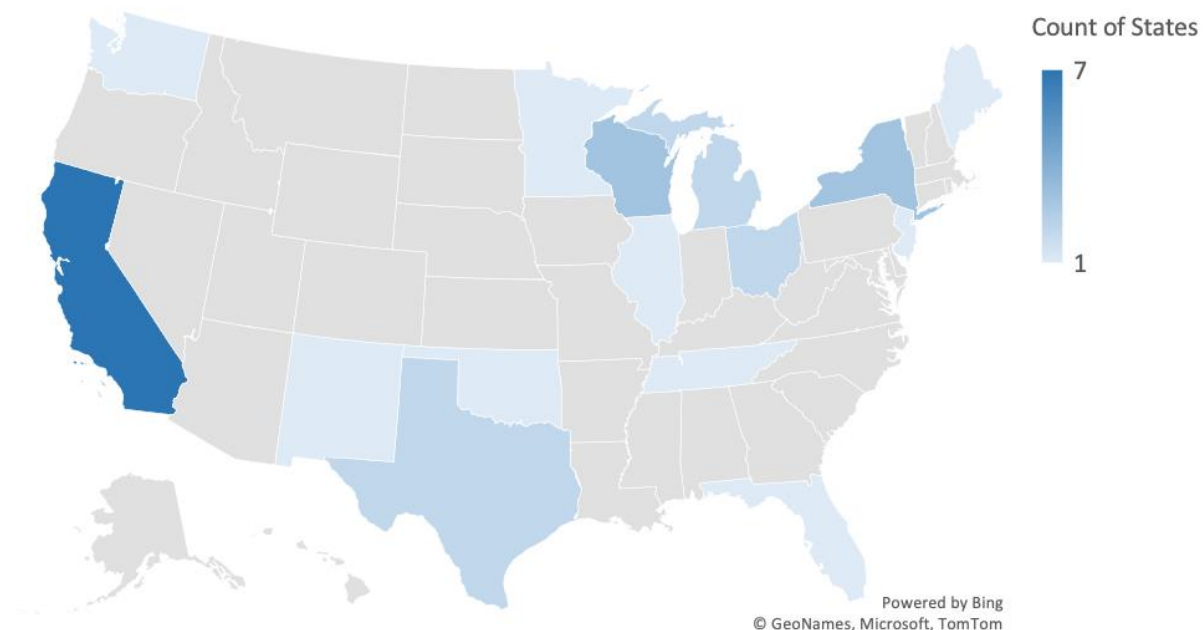
Figure 7 Distribution of reported zipcodes, grouped by state

3.3.3.2 Neuropsychiatric Symptoms

Three individuals were found to score 16 or lower (2 or more SD below the mean) on the cognition section. None of these individuals was taking pegvaliase, and they were all employed, non-students. Their phe levels varied, with one individual reporting phe levels in the 120-360μmol/L range, another reporting levels of 361-600μmol/L, and the final individual reporting levels of 601-1,200μmol/.

There was no significant difference between cognition or depression scores when comparing all respondents by pegvaliase status (taking versus not taking pegvaliase) or phe levels (below versus at or above 600μmol/L). However, when the three respondents who scored 16 or lower on the cognition section were excluded from the analysis, a significant difference became apparent for cognition by phe level (p=0.04). No other comparisons of neuropsychiatric symptoms

or quality of life by either grouping were changed from significant to non-significant, or vice versa, following exclusion of these three individuals. Scores are summarized in Table 8.

Table 8 Summary of scores on Neuro-QOL short forms and comparisons by pegvaliase status and phe levels

	Sub-Group	n	Mean ± SD	Min-Max (Range)	p-values (excluding ≤16 on cognition)
Neuro-QOL: Cognition Short Form Possible scores: 8-40 (median: 24)^	Pegvaliase	13	29.77 ± 4.92	20-39 (19)	0.34
	No Pegvaliase	28	27.64 ± 7.61	12-40 (28)	(0.65)
	Phe <600µmol/L	27	29.56 ± 6.99	12-40 (28)	0.067
	Phe ≥600µmol/L	14	25.93 ± 6.21	14-38 (24)	(0.04*)
Neuro-QOL: Depression Short Form Possible scores: 14-70 (median: 24)^^	Pegvaliase	13	14.62 ± 7.92	8-26 (18)	0.09
	No Pegvaliase	27	18.26 ± 8.36	8-31 (23)	(0.20)
	Phe <600µmol/L	27	16.48 ± 7.83	8-31 (23)	0.47
	Phe ≥600µmol/L	13	18.31 ± 8.79	8-31 (23)	(0.45)

^Higher score on Neuro-QOL Cognition Short Form = better cognitive functioning; ^^Lower score on Neuro-QOL Depression Short Form = less severe signs of depression; *p-value less than 0.05

3.4 Discussion

The aim of this study was to begin to assess the impact of pegvaliase on several domains of life in individuals who take it, including changes in dietary management, impact on health-related quality of life, improvements in neuropsychiatric functioning, and support and resource needs. The following sections discuss the results of this study, and how the findings may help to inform care for people who have PKU and future research needs in these areas.

3.4.1 Impact of Pegvalise on Dietary Management and Phe Levels

Across all respondents to the survey, the only individuals who had phe levels below 120μmol/L were respondents who were taking pegvaliase, although one respondent who reported taking pegvaliase also reported phe levels of over 1,200μmol/L. The overall trend towards lower phe levels in respondents who are taking pegvaliase compared with other management approaches, although not significant, is consistent with another study which compared the effectiveness of pegvaliase to management involving low-phe diet alone or low-phe diet with sapropterin[83]. However, the majority of respondents taking pegvaliase reported that they had not experienced a significant change in their dietary protein intake (6/13 reporting no change; 3/13 reporting minimal change), which is interesting considering that most of the respondents taking pegvaliase had been taking it for at least a year, by which point dietary benefits would be anticipated. This, along with the finding that both length of time taking pegvaliase and reported phe levels were not associated with a statistically significant change in diet, suggests that decreases in phe levels did not

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consistently translate to a significant change in diet. It is possible that some respondents had higher phe levels prior to starting pegvaliase due to consuming more protein than recommended. Additionally, some respondents may have experienced a substantial decrease in phe levels after initiating pegvaliase, but they started from a phe level high enough that even with a substantial decrease in phe levels, it was not possible to increase dietary protein appreciably.

Regardless of these trends, there was an overwhelmingly positive response among respondents who shared the reasons for their change in diet following initiation of pegvaliase. A number of them commented on how their phe levels had improved, allowing for positive changes in the amount of protein they could consume. One respondent even stated directly that he/she had no regrets starting pegvaliase when it was still in clinical trials, emphasizing the positive changes that pegvaliase can offer many people who have classical PKU. Even among respondents who had not yet seen an improvement in phe levels, due to recently initiating pegvaliase, there seemed to be a significant amount of hope regarding the promise of pegvaliase. One respondent provided greater context to how increases in dietary protein can be beneficial, sharing a desire to feel more normal. By increasing the amount of dietary protein, respondents could be more like their peers in social settings, which was an important theme in later open-ended questions about support and resource needs as well.

There were also improvements associated with pegvaliase that were not directly related to dietary changes but rather to other benefits related to decreased phe levels. One respondent detailed how they wanted the improved cognition, including stronger memory and greater mental clarity, that would come with improved control thorugh pegvaliase. Notably, this same respondent was forward-thinking, also sharing how important it would be to maintain this neurological function throughout life and well into older ages.

3.4.2 Factors in Quality of Life

There appeared to be potential for improvement in overall self-perceived health among individuals taking pegvaliase compared to those who were not. However, the majority of respondents reported that their health was "good" or "excellent," which is consistent with the literature[90,91]. Only four respondents of the total 43 reported their health was "poor" compared to others their age, none of whom were taking pegvaliase. However, phe level may play a role in this as well, as three of these four individuals had a phe level above 600μmol/L. These four individuals also consistently scored at or above the median possible score on each sub-section of the quality of life questionnaire that they completed (one respondent only completed the first three sub-sections), suggesting a poorer quality of life among these four individuals in addition to perceiving their health to be poor. The only exception to this was the one respondent who had a phe level below 600μmol/L and scored just under the median possible score on the satisfaction with management sub-section.

Specific significant differences were noted in the domains of satisfaction with management, general satisfaction with life and impact of PKU, when comparing by phe level. In contrast, only satisfaction with management was significant when comparing by pegvaliase status. Although this finding of improved satisfaction with management among respondents taking pegvaliase was expected due to the improvements in phe levels and dietary flexibity that can come with pegvaliase use, it is interesting in the context of the finding that respondents who were taking pegvaliase measured their phe levels significantly more often than respondents who were not. One of the questions in the management sub-section assessed satisfaction with the time it takes to manage PKU, and measuring phe levels regularly does require time. It may be that the improved

dietary flexibility, which minimizes the time-consuming planning and preparing of low-protein meals, outweighs the time that it takes to measure phe levels more regularly for these individuals.

A significant difference in total quality of life score was only seen when comparing by phe level, not when comparing by pegvaliase status. This difference suggests that phe level may be a better predictor of quality of life than pegvaliase status alone, although the finding that pegvaliase status was significantly related to satisfaction with management may reflect the interplay between pegvaliase and lowering phe levels as co-dependent predictors of satisfaction. This suggests that pegvaliase may still be a defining factor in improving at least some aspects of quality of life for people who have classic PKU. This was difficult to assess thoroughly in the present study due to the limited sample size, especially because of the known interrelationship between pegvaliase use and phe levels[26,27,29]. It is also possible that there were greater differences when analyzing based on phe level compared to pegvaliase status because there were 18 respondents who had phe levels at or below 600μmol/L and were not taking pegvaliase, whereas only nine of the 13 respondents taking pegvaliase had phe levels at or below 600μmol/L, possibly lending more weight to the "low" phe group.

Overall trends in quality of life scores between groups, although not statistically significant, showed respondents who were taking pegvaliase having better quality of life scores than those who were not, and respondents who have phe levels at or under 600μmol/L having better scores with those who have phe above 600μmol/L. This trend was seen in each sub-section individually, as well as in overall quality of life scores. This may suggest that both pegvaliase status and phe level are related to multiple domains of quality of life, although further research with a larger sample size is needed to determine to what extent this may be true. Further, the group of respondents who reported a phe above 600μmol/L, consistently averaged poorer quality of life

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scores than any other grouping, again across all sub-sections and as compared to the total score. This sub-grouping was also the only one to have an average score on any sub-section that exceeded the median possible score. This occurred for three sub-sections: satisfaction with management, impact of PKU, and PKU-related worries, suggesting that these may be quality of life domains where people who have significantly elevated phe levels struggle the most.

These findings are consistent with reports in the literature regarding the emotional costs of following a strict low-phe diet[92], as well as the potential for feelings of anxiety or guilt surrounding having high phe levels and the financial costs of having a stricter diet[90,91]. Regarding the potential emotional costs of maintaining dietary restrictions, the management and impact sub-sections indirectly assessed this through questions about satisfaction with dietary flexibility, for which people who reported higher phe levels were not statistically more likely to report being less satisfied. However, responses to the question assessing this within the management sub-section did approach statistical significance. The impact sub-section also indirectly assessed the potential for emotional costs associated with dietary restriction through questions regarding a respondent's ability to participate in general leisure activities and go out to eat, with the finding that people who reported lower phe levels were significantly more likely to report less impact on leisure activities. Further, only people who reported phe levels under 120 μmol/L consistently reported having no negative impacts. Regarding anxiety or guilt related to phe levels, a respondent's satisfaction with their phe levels was assessed by the management sub-section, which showed that most people who reported phe levels above 600μmol/L were less satisfied with their phe levels. Finally, the impact of finances was assessed by the management and worries sub-sections, where again people who reported phe levels above 600μmol/L reported more issues with finances. This suggests that an inability to afford the cost of low-protein foods and medications necessary to decrease phe levels

is associated with increased worries. Notably, the only instance of another sub-group's average score coming close to the median possible score was regarding satisfaction with management among the group of respondents not taking pegvaliase. This is consistent with the finding that the only significant difference seen in the quality of life assessment when comparing those taking pegvaliase to those not was in the sub-section assessing satisfaction with management.

Trends were also noted in visually comparing average scores of respondents grouped by having phe levels at or under 600μmol/L and by their status as current users of pegvaliase, acknowledging that there is a high degree of overlap within these two groupings and that their average scores were often fairly similar. Even though the greatest number of significant differences in domains of quality of life were seen when comparing respondents reporting relatively low phe levels to respondents reporting relatively high phe levels, respondents taking pegvaliase typically had the best average score on each sub-section. The exception to this was the sub-section assessing worries related to PKU, in which respondents grouped by having phe levels at or under 600μmol/L scored slightly better than those grouped based on currently taking pegvaliase. It is also worth noting that the average scores for these two groups on the sub-section assessing the impact of PKU were essentially identical, with a difference of only 0.03 points, especially when standard deviation is taken into account. However, statistically speaking, phe level still seems to be the most significant factor impacting quality of life for adults who have PKU based on the finding of this study. This is interesting as it has been previously reported that increased protein intake is associated with improved quality of life in the context of diet-based management for PKU[72]. This does not align with having low phe levels unless a person is using pharmaceutical management approaches that allow for increased natural protein intake such as sapropterin or pegvaliase. However, the improvements seen in quality of life related to lower phe levels reported in this study

may be explained in part by the benefits of improved cognitive functioning in the presence of lower phe levels[1,21].

Non-significant differences were seen in the PKU-related worries and support sub-sections. In both of these sub-sections, the average scores across all four groups (taking pegvaliase, not taking pegvaliase, phe at or under 600μmol/L, phe above 600μmol/L) were highly similar. This is consistent with the study by Douglas et al, on which the quality of life questionnaire used in the present study was based, as Douglas et al also did not find a significant difference in the worries or supports sub-sections.

In the case of PKU-related worries, the scores all averaged close to the median possible score, indicating that there may be a greater negative impact on quality of life relating to worries surrounding PKU than other domains of quality of life, regardless of management approach or degree of phe control. Some of the worries respondents most commonly reported worrying about either "Often" or "All the Time" included worries about body image, finances, deciding whether to have children or being behind on having them, and potential for complications related to PKU. The diversity of these most common worries emphasizes how much PKU can impact those who are affected by it and affects many areas of their lives. Certain ones – namely financial concerns and uncertainty surrounding having children – were mentioned in several open-ended responses on resource and support needs as well.

Regarding satistfaction with support, each of the averages was close to eight points, suggesting that respondents were overall satisfied on average with the supports they received from their family, friends, doctor, and dietician, again regardless of management approach used or level of phe control. There was variation among respondents on whether each respondent was more satisfied with social supports (family or friends) compared to clinical supports (doctor or dietician).

This suggests that although a desire for adult-focused clincal care was reported by respondents and also previously reported in the literature[55,59], it does not detract from satisfaction with their current clinical care team. Rather, it suggests adult-based clinical care may be a preferred care setting.

Quantitative assessment of quality of life in the present study is partially consistent with the results from the study by Douglas et al which developed the quality of life questionnaire on which the present study's survey was based. As was seen in the present study with respondents taking pegvaliase, Douglas et al saw a significant improvement regarding satisfaction with management among sapropterin-responders[20]. However, they also saw improvements among sapropterin-responders regarding the impact of PKU and overall quality of life[20]. Significant differences in these domains were only seen in the comparisons by phe level in the present study, not in comparisions by pegvaliase status. The significantly better scores among respondents with phe levels at or below 600μmol/L compared to those with phe levels above 600μmol/L on sub-sections about satisfaction with management and impact of PKU are consistent with the study by Douglas et al, though, as that study also saw improvements on management scores and impact scores related to lowered phe levels and increased phe tolerance[20]. Interestingly, the study by Douglas et al did not find any significant differences in quality of life by phe level prior to initiation of sapropterin. This does not directly contradict the findings of the present study regarding the impact of phe level on quality of life because most of the respondents in this study were using pegvaliase or sapropterin at the time of survey and quality of life was assessed at a single point in time, instead of correlating changes in phe level with improvements in quality of life over time. However, this finding by Douglas et al is interesting to note as it supports the changes caused by a pharmaceutical approach – in this case, sapropterin – being more impactful than baseline phe

level on quality of life, warranting further research into the impacts of phe levels compared to pegvaliase use.

These differences in results are likely due the differences in study structure, as essentially the same questionnaire was used for quantitative assessment of quality of life. The study by Douglas et al utilized a longitudinal approach, tracking changes in quality of life from baseline to a year after initiation of sapropterin[20]. In contrast, the present study was cross-sectional and compared people who are taking pegvaliase and those who are not, without excluding people who use other approaches for managing their PKU, including a variety of combinatory approaches used by respondents both taking and not taking pegvaliase. Sample size was similar in both studies.

Another important consideration in comparing the study by Douglas et al to the present study is how the side effects of pegvaliase impact quality of life. Although the majority of respondents taking pegvaliase reported that they were either not experiencing side effects of pegvaliase or were experiencing side effects that had no impact on their quality of life, the potential side effect profile of pegvaliase is not negligible, especially in the first several months following initiation. These can include hypersensitivity reactions such as injection site reactions, rashes, or joint point, as well as a risk for anaphylaxis, although most adverse reactions reported were still mild to moderate[27,81]. In contrast, sapropterin has a relatively benign side effect profile. In clinical trials, the majority of adverse events were mild to moderate, with the only significant adverse event being deemed unrelated to sapropterin, and the most common effects were headache, diarrhea, and abdominal pain[94]. These contrasting side effect profiles are important to consider when examining the potential impacts different management approaches can have on quality of life.

3.4.3 Neuropsychiatric Impact

No significant differences were found when comparing cognitive functioning or depressive symptoms among respondents who are and are not taking pegvaliase, even though there is evidence in the literature to support pegvaliase being associated with improved neuropsychiatric outcomes after initiation of pegvaliase. During clinical trials, it was found that patients who were taking pegvaliase had better executive functioning skills, including working memory, cognitive flexibility, and inhibitory control, as well as better attention compared to other patients who were taking a placebo or compared to themselves at baseline prior to initation of pegvaliase[26,31]. In addition to the small sample size and cross-sectional nature of this study, it is possible that the present study did not see evidence of improved cognition among respondents who were taking pegvaliase compared to those who were not because attention was only assessed by a sub-set of the questions asked on the Neuro-QOL cognition short form. As improved attention is a previously established benefit following initiation of pegvaliase, a questionnaire more focused on attention may have been better able to identify a difference in cognition. Executive function, the other area of cognition shown to improve with pegvaliase in clinical trials, also could not be assessed with the Neuro-QOL cognition short form due to the limited question content of this standardized assessment. The sampling approach used in this study may also have limited the ability to detect a difference, as people with higher cognitive functioning may have been more likely to complete the survey.

Interestingly, a significant difference was noted when comparing by phe level with exclusion of the three respondents who scored 16 or lower on the Neuro-QOL cognition short form. This may have been because each of these three individuals reported different ranges of phe levels (120-360μmol/L, 361-600μmol/L, and 601-1,200μmol/L), potentially confounding the

comparison by phe levels, and none of them was taking pegvaliase, which could have minimized the impact on the comparison by pegvaliase status. Additionally, the self-report nature of this study may also have skewed the data and contributed to this unexpected result. However, the finding that lower phe level, is associated with better cognitive functioning is consistent with findings from the clinical trials on pegvaliase as well, as these trials found improved attention as a result of the improved phe levels that resulted from pegvaliase treatment[26,31].

The lack of a significant difference regarding depressive symptoms in any comparison, with or without the three individuals with low scores on the cognition section, may be explained by the length of time it takes for improvements in mood to occur following initiation of pegvaliase. Specifically, no improvements in mood were seen during the randomized control trial, which lasted only eight weeks, but improvements were seen in the initial phase three trials, which lasted two years. Approximately half of respondents taking pegvaliase in this study had initiated it less than a year prior to taking the survey, suggesting that enough time may not have passed to allow these particular benefits of pegvaliase to take effect, and making it even more challenging to detect a significant improvement in the few respondents who had been taking pegvaliase for a year or more.

3.4.4 Supports and Resources

There were no significant differences reported in the satisfaction with support sub-section of the quality of life questionnaire. This was consistent with the non-significant differences in the number of respondents reporting residual resource needs between the pegvaliase and no pegvaliase sub-groups. However, there did seem to be a pattern for people taking pegvaliase reporting fewer residual resource and support needs.

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Several of the residual needs that were reported by respondents have previously been reported in the literature as well. One example is the need for adult-specific clinical settings for PKU identified by a study respondent on both the question assessing residual support needs and the question assessing residual resource needs. Barriers to accessing care for PKU as an adult have been reported in the literature, with some adults encountering difficulty finding care at all and others preferring to be seen in an adult-focused setting as opposed to the pediatric clinics where they were seen as children[55,59]. The financial impact of PKU, which was prevalent in responses to both the question about residual support needs and residual resource needs among respondents taking and not taking pegvaliase, has also been reported in the literature, especially for adults, due to the tendency of states to not provide financial support for medical formulas and foods for adult patients[55,63]. This dearth of financial support for adults who have PKU who are trying to purchase medical foods and specialized formulas in many states likely explains why finances and financial management, along with insurance, were a prevalent topic in the survey responses.

This study also identified unique residual resource needs regarding social supports intended for adults who have PKU. This was the first time social supports intended for adults, including support groups, opportunities to discuss maternal PKU experiences, and the difficulties navigating certain social settings given the dietary restrictions that are traditionally a key part of PKU management, are reported. Previous studies on residual needs for adults, as mentioned above, have primarily found these needs to be related to adult-focused clinics[55,59], not adult-focused social supports and resources. However, need for social supports in general, and feelings of isolation which can be addressed through improved social supports, have been reported[71]. These previously reported feelings of isolation seem to stem from the stigma surrounding PKU, related to the difficulty many respondents experienced in navigating complex social situations, and feeling

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unable to explain PKU to others in a way that is understandable[71]. This latter point seems especially pertinent to the respondent who mentioned not knowing who to talk to about her maternal PKU experiences. Overall, filling these social support and resource needs is likely a critical part of improving quality of life for people, especially adults, who have PKU, as social relationships are a key component of quality of life[72]. The importance of social social supports was further reinforced by responses to the select-all-that-apply question about types of support provided by individuals other than the respondent's doctor, dietician, family, and friends, which predominantly focused on emotional support (n=23) and was closely followed by social support (n=21).

It was not possible to identify geographic trends regarding these residual resource and support needs due to the small number of respondents who provided a zipcode to be included in this analysis. However, there was preliminary evidence for a trend involving more financial needs outside of California. As mentioned above, it is well-known that financial supports, at minimum, vary by state, although this variation is less prevalent for adult patients due to almost universal lack of state-based support for adults who need specialized medical foods or formulas[55]. Upon closer look at California, as the state with the largest number of zipcodes reported, respondents seemed to have differing experiences. This is to be expected to an extent as each respondent is a unique adult who has a unique set of needs. One respondent in California explicitly said that the medical food and formula supports in California were good, while another respondent stated a need for better resources regarding insurance, suggesting that not all Californians may be equally able to access these resources. Also, there was the respondent who was trapped in a resource loop – unable to see the doctor without resources, but unable to access resources without first seeing a doctor – further emphasizing potential accessibility issues, even in states, like California, known to have relatively good supports and resources in place for people who have PKU.

For resources utilized by respondents, the most common resource reported was NPKUA (n=27), which is to be expected as the NPKUA patient registry was the main source of recruitment for this study. Support groups via social media or other online services were the next most commonly reported (n=23), with in-person support groups only being reported by two respondents. It is not clear from the survey responses if online support groups are more commonly used than in-person options in general, which could be the case due to PKU being a rare disease, or if these responses were skewed due to the survey being conducted in the midst of a pandemic where fewer in-person options would have been available. Also notable was the fact that NORD, which does offer patient assistance programs, was the third most commonly used resource (n=18), while co-pay assistance programs (n=3) and care coordination services (n=2) through BioMarin – the pharmaceutical company that produces sapropterin and pegvaliase – were far less commonly used. Medical assistance (n=3) and assistance from the state newborn screening program (n=5) were also among the less commonly used resources, although the latter is expected based on respondents being adults, many of whom would have aged out of such services[55]. The resources commonly utilized – and those not as commonly utilized – do suggest an important disconnect between exisiting resources and patients being aware of them and able to access them. For example, social supports and resources were commonly mentioned in the open-ended questions assessing residual needs, even though many respondents reported participating in support groups in the select-all-apply-question about utilized resources. Perhaps, as mentioned by a few respondents, there are not as many support groups that meet their needs as adults, as compared to support groups for children who have PKU or families. There is also the issue of finances, which was mentioned by many respondents, even though financial assistance programs through NORD and BioMarin exist. While it is possible that the individuals reporting needs related to finances or financial management are

not eligible for these programs based on income-level and/or management approach, it is also possible that these respondents are not aware that these programs exist or are aware of them but are unsure how to access them. These potential social and financial resources suggest that adults who have PKU could benefit from additional conversations with their providers or other members of the PKU community to help them learn about such resources that could benefit them by meeting their residual needs.

3.4.5 Limitations & Future Directions

The ability of the present study to identify significant aspects of quality of life, dietary management, neuropsychiatric functioning, and resource and support needs was limited by the sample size. This is exacerbated by the small number of people taking pegvaliase in the sample, especially those who have been taking it long enough to see a positive impact on phe levels and protein tolerance, relative to the total sample size. In addition, not all respondents completed the survey in it's entirely, although all did complete the majority of the survey. Qualitative analysis was also limited due to the majority of respondents offering brief responses to the open-ended questions and many more respondents skipping those questions entirely. Also, due to the small sample size, respondents who were using multiple management approaches, including pegvaliase in conjunction with sapropterin and dietary management, were not excluded, which may have made distinctions in quality of life between the sub-group taking pegvaliase and the sub-group not taking pegvaliase less apparent. It was also not possible to determine causation due to the cross-sectional nature of this study, as well as the self-report nature of the questionnaire and the potential for sampling bias due to the relatively passive recruitment methods used. It must also be considered that the self-reported phe levels, which were a critical aspect of the quality of life and

neuropsychiatric data analyses, may have been affected by response bias if respondents reported lower phe levels than their actual average levels, which would have affected the overall statistical analysis.

With these limitations in mind, this study is the first to investigate the impact of pegvaliase on quality of life and support and resource needs. As such, the preliminary evidence presented here can be used to aid in the development of future studies collecting more direct data by tracking any improvements in quality of life and residual resource and support needs over time in the same participants following initiation of pegvaliase. This could be done using a structure similar to the study on the impact of sapropterin on quality of life by Douglas et al[20], or limiting participants to those managing their PKU with pegvaliase, with or without dietary management, sapropterin with dietary management, or dietary management alone, as was done by Zori et al in their investigation of the effectiveness of each of these approaches in lowering phe levels.[83]

3.5 Conclusions

The preliminary data collected by the present study provide insight into potential factors that influence quality of life for people who have PKU and which domains of quality of life may be most likely to be impacted for affected individuals. Specifically, these domains seem to be satisfaction with management, which is likely modifiable by management approach and phe level, and worries related to PKU. Phe level is also likely to be the strongest influence on quality of life, regardless of management approach used to achieve low phe levels, as phe level was shown to have a significant impact on satisfaction with life in general, the impact of PKU, and total quality of life score, in addition to satisfaction with management. This is consistent with the well-

established relationship between low phe levels and quality of life related to alleviation of symptoms associated with increased phe levels in adulthood, especially in regard to promoting mental clarity and function[1,21], as noted by one respondent taking pegvaliase in an open-ended response. Further research is indicated to determine how the effects of PKU-related worries can be addressed, as worries were high among all comparison groups.

This study also provides insights into reasons why people who have PKU change their dietary protein intake in response to increased phe tolerance and what residual support and resource needs still exist, even when phe tolerance is greatly improved. Most of the respondents taking pegvalise who shared their reason for changing their diet cited a positive change or benefit, including improved phe control, a more normal life, improved day-to-day life, and better mental clarity and memory. Respondents taking pegvaliase also appeared to have fewer residual resource and support needs. Even when residual needs were reported by respondents taking pegvaliase, these needs were focused on finances, compared to the constellation of residual needs – including primarily financial, social, and adult-focused resources and supports – reported by respondents who were not taking pegvaliase. Special attention should be paid to adult-focused social supports, as many respondents cited a need for either social supports in general or social supports for situations unique to adults who have PKU. This included adult-focused social support needs of adults who have PKU being reported for the first time. In comparing the residual needs that were reported to the utilized resources that were reported, it appears there is a disconnect between supports and resources that exist and patients' ability to consistently access them. This was seen in both social and financial support areas. This could be happening because many adults who have PKU are unaware of these resources or are unsure of how to access them. Alternatively, it may be that not all adults who could benefit from these resources and supports are eligible for them.

Regardless, it will be important to work towards improving access to these key resources and supports for adults who have PKU.

Evidence presented here supports worries surrounding PKU as a crucial factor in quality of life for people who have PKU which may not be ameliorated by existing treatments or lowered phe levels alone. Several of these worries were also noted through responses to open-ended questions regarding resource and support needs, thus, more clearly defining and addressing these worries may be a better way to improve quality of life. The impact of satisfaction with management is also noteworthy, as this seems to be an area of quality of life that can be addressed with improved treatments that help to decrease phe levels and improve phe tolerance, especially as this area was seen to have a significant difference when comparing scores by both pegvaliase status and phe levels. This was reinforced through analysis of the open-ended questions inquring about reasons for changes in dietary protein and the overall positive feedback from respondents who were already experiencing positive changes due to pegvaliase and the hopes other respondents had for the promise of positive changes to come now that they had access to pegvaliase.

Although there are limitations, the study provides preliminary evidence regarding key areas of quality of life in adults who have PKU, including general domains of quality of life, as well as assessments of neuropsychiatric symptoms and existing gaps in resources and supports. It was the first to do so with a focus on the impact of pegvaliase and found evidence of unique residual needs that had not been previously reported in the literature. The nuances gleaned from the quality of life assessment and thematic analysis can contribute to providers' awareness of the interests, worries, and needs of the patients who have PKU for whom they provide care, which may in turn help to further promote quality of life for these individuals.

4.0 Relevance to Genetic Counseling and Public Health

The overall goal of this study was to explore the impact of pegvaliase, compared to other management approaches, on the quality of life and resource needs of individuals who have PKU. This has the potential to improve care for people who have PKU by identifying gaps in support and ways to promote their quality of life. The knowledge gained by this study can inform all providers in a PKU clinic, including nurses, dietitians, physicians, and genetic counselors, about how they interact with and care for their patients.

As PKU is one of the most common inherited metabolic disorders,[1] the information learned from this study has the potential to benefit a significant number of people. This study is further relevant to public health as it aligns with two of the 10 Essential Public Health Services: 1) assess and monitor population health status, factors that influence health, and community needs and assets, 2) communicate effectively to inform and educate people about health, factors that influence it, and how to improve it[95]. The survey addresses the first essential service by assessing factors that influence health and community needs and assets among people who have PKU by inquiring about their PKU management, quality of life, mental health, current supports, and residual resouces needs. This then relates to the latter service as information can inform both health care providers and people who have PKU, providing these individuals with the knowledge to influence and improve patients' health, related to having PKU.

Information gained from this study includes the benefits of pegvaliase in improving satisfaction with management and potentially other domains of quality of life. As pegvaliase use and phe control are often closely related, the benefits in quality of life related to general satisfaction with life and the impact of PKU seen by respondents who have lower phe levels, may also be

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applicable to people who utilize pegvaliase. It will also be important for providers to be aware of the potential impacts of PKU-related worries on quality of life, as this is a survey section in which all comparison groups scored relatively poorly. This may involve spending time in an appointment assessing what a patient's worries might be related to their PKU and working to address those in a collaborative manner. Key gaps in resources and supports, such as financial needs and resources tailored to adult patients, as well as medical and social needs are also important for providers, or in the case of social supports – the PKU community and organizations at large – to spend time addressing, as is often already done by members of the PKU health care team..

In relation to genetic counseling specifically, the knowledge gained by this study will be useful in providing patients and families with anticipatory guidance and supportive counseling to address their psychosocial needs. As the first study on quality of life among people who have PKU who are taking pegvaliase, this information will help provide preliminary answers as to how new treatments for PKU can improve quality of life. This relates to a number of the Practice-Based Competenies for Genetic Counselors established by the Accreditation Council for Genetic Counselors (ACGC): "Effectively educate clients about a wide range of genetics and genomics information based on their needs, their characteristics and the circumstances of the encounter," "Integrate knowledge of psychosocial aspects of conditions with a genetic component to promote client well-being," and "Demonstrate the skills necessary to successfully manage a genetic counseling case"[96]. This is important because a genetic counselor's role extends beyond solely explaining the genetic basis and inheritance of PKU; it encompasses addressing psychosocial concerns, such as questions regarding quality of life, mental health, and resources, for patients and their families, as well. In short, the results of this study can begin to help genetic counselors accurately and effectively educate patients who have PKU on the impact pegvaliase can have on

quality of life, available resources that patients have reported to be helpful, and areas where additional support or resources may still be needed.

Appendix A IRB Approval

University of Pittsburgh | Institutional Review Board
Office of Research Protections

EXEMPT DETERMINATION

Date:	November 5, 2020
IRB:	STUDY20060241
PI:	Trinity Sprague
Title:	Psychosocial impact and residual resource needs following institution of enzyme substitution therapy for PKU

The Institutional Review Board reviewed and determined the above referenced study meets the regulatory requirements for exempt research under 45 CFR 46.104.

Determination Documentation

Determination Date:	11/5/2020
Exempt Category:	(2)(i) Tests, surveys, interviews, or observation (non-identifiable)

Approved Documents:	• Survey, Category: Data Collection; • Exempt Application Form, Category: IRB Protocol; • Flyer, Category: Recruitment Materials; • Introductory Paragraph, Category: Recruitment Materials; • NPKUA Final Eblast, Category: Recruitment Materials; • NPKUA letter of support.docx, Category: Other; • NPKUA letter of support.docx, Category: External Site Permission Letter; • Permission for NeuroQOL Short Forms.pdf, Category: Other; • Permission for PKU-QOLQ.pdf, Category: Other;

If you have any questions, please contact the University of Pittsburgh IRB Coordinator, Carolyn Ivanusic.

Please take a moment to complete our Satisfaction Survey as we appreciate your feedback.

PKU QoL Thesis

If you are an adult (18 years or older) with phenylketonuria (PKU)…   I am inviting you to take part in a research study that is part of my training to become a genetic counselor. My name is Trinity Sprague, and I am a student at the University of Pittsburgh Graduate School of Public Health. I am interested in learning more about 1) how living with PKU affects your "quality of life" (your health, comfort, and happiness), and 2) if you have unmet support or resource needs. To do this, I am working with the UPMC Children's Hospital of Pittsburgh and the National PKU Alliance to share a survey. It will take you about 15 to 20 minutes to complete the survey.

This survey will ask about:
- If you are happy with how you are managing your PKU
- The impact of PKU on your life
- Your satisfaction with the resources and support you use and receive
- How happy you are with your health and your quality of life
- Your thinking skills and if you have any feelings related to depression.

We will compare answers from people who are taking pegvaliase (brand name: Palynziq), a medication that has been approved for people with PKU who have not responded to previous phe-reducing management options, to people who are not taking it.

We hope to share the results of this study with medical providers who care for people who have PKU. Risks of participating in this study are limited but may include negative feelings due to answering some of the questions. If you have negative feelings or other distress while taking the survey, you should contact your primary care provider or other health professional. There are no direct benefits to you for participating in this study. If you choose to participate, your responses will be confidential and anonymous. This means we will store your responses securely and your responses will not be linked to you in any way. Your participation is optional, and you may

withdraw from this study at any time. Any responses you provide prior to withdrawing will be used by the study team. Your decision on whether to participate will have no effect on future care you might need at UPMC Children's Hospital of Pittsburgh.

This study is being conducted by myself, Trinity Sprague, and you can reach me at spraguet@upmc.edu. You can also contact my mentor, Cate Walsh Vockley, MS, LCGC, a genetic counselor at Children's Hospital of Pittsburgh, at catherine.walshvockley@chp.edu if you have any questions or concerns about this study. Thank you for considering participating in this study. If you are interested in taking the survey, please click the arrow below to begin.

Page Break ──

Before you begin the survey, please answer the next two questions to confirm that you are eligible for this study.

Are you 18 years of age or older?

○ Yes (1)

○ No (2)

Do you have phenylketonuria (PKU)?

○ Yes (1)

○ No (2)

Page Break ─────────────────────────

Thank you. You are eligible to complete the remainder of this survey. The next set of questions will focus on your PKU management.

What was your average Phe level over the last month (the last 30 days)?

○ Less than 120 umol/L (Less than 2 mg/dL) (1)

○ 120 - 360 umol/L (2 - 6 mg/dL) (2)

○ 361 - 600 umol/L (6.01 - 10 mg/dL) (3)

○ 601 - 1,200 umol/L (10.01 - 20 mg/dL) (4)

○ Over 1,200 umol/L (Over 20 mg/dL) (5)

How often do you typically measure your Phe level?

○ Less than once a month (1)

○ Once a month (2)

○ Once a week (3)

○ More than once a week (4)

Are you **currently taking** pegvaliase (Palynziq) for your PKU?

○ Yes (1)

○ No (2)

How long have you been taking pegvaliase (Palynziq) for your PKU?

○ Less than 6 months (1)

○ 6 months - Less than 9 months (2)

○ 9 months - Less than 1 year (3)

○ 1 year or more (4)

Have you **ever taken** pegvaliase (Palynziq) for your PKU?

○ Yes (1)

○ No (2)

How long did you take pegvaliase (Palynziq) for your PKU?

◯ Less than 6 months (1)

◯ 6 months - Less than 9 months (2)

◯ 9 months - Less than 1 year (3)

◯ 1 year or more (4)

✱

When did you stop taking pegvaliase (Palynziq) for your PKU? Please estimate if you are not certain. (mm/dd/yyyy)

_____

Please select any other ways you manage your PKU. You may select more than one.

☐ Kuvan (also known as sapropterin or BH4) (1)

☐ Diet/formula (2)

☐ Other: (3) _____

☐ I do not manage my PKU with anything other than pegvaliase (Palynziq). (4)

The following section will focus on your quality of life (your health, comfort, and happiness). It will start with questions that ask you to think more generally.

Compared with others your age, would you say your health is:

○ Excellent (1)

○ Good (2)

○ Fair (3)

○ Poor (4)

Are you currently a student?

○ Yes (1)

○ No (2)

Are you currently employed?

○ Yes (1)

○ No (2)

This set of questions will focus on your satisfaction with your life in general. Please rate your answer to each question on a scale of 1 (very satisfied) to 5 (very unsatisfied) using the table below.

Display This Choice:

    If Are you currently a student? = Yes

    Or Are you currently employed? = Yes

Display This Choice:

    If Are you currently a student? = Yes

    Or Are you currently employed? = Yes

Display This Choice:

    If Are you currently a student? = Yes

    Or Are you currently employed? = Yes

	Very Satisfied (1)	Somewhat Satisfied (2)	Neutral (3)	Somewhat Unsatisfied (4)	Very Unsatisfied (5)
How satisfied are you with your sleep? (1)	◯	◯	◯	◯	◯
How satisfied are you with your social relationships and friendships? (2)	◯	◯	◯	◯	◯
How satisfied are you with your romantic relationships? (11)	◯	◯	◯	◯	◯
Display This Choice: *If Are you currently a student? = Yes* *Or Are you currently employed? = Yes* How satisfied are you with your performance in school or at work? (3)	◯	◯	◯	◯	◯

How satisfied are you with your attendance in school or at work? (4)

○   ○   ○   ○   ○

How satisfied are you with how your classmates or co-workers treat you? (5)

○   ○   ○   ○   ○

How satisfied are you with your ability to complete household activities? (6)

○   ○   ○   ○   ○

How satisfied are you with the appearance of your body? (7)

○   ○   ○   ○   ○

How satisfied are you with the time you spend exercising? (8)	○	○	○	○	○
How satisfied are you with your leisure time? (9)	○	○	○	○	○
How satisfied are you with life in general? (10)	○	○	○	○	○

Page Break ─────────────────────────────────

This set of questions will focus on your satisfaction with how you manage your PKU. Please rate your answer to each question on a scale of 1 (very satisfied) to 5 (very unsatisfied) using the table below.

	Very Satisfied (1)	Somewhat Satisfied (2)	Neutral (3)	Somewhat Unsatisfied (4)	Very Unsatisfied (5)
How satisfied are you with the amount of time it takes to manage your PKU? (1)	○	○	○	○	○
How satisfied are you with the number of checkups you have each year? (2)	○	○	○	○	○
How satisfied are you with the length of time your check-up visits take? (3)	○	○	○	○	○
How satisfied are you with the time it takes to determine your Phe level? (4)	○	○	○	○	○
How satisfied are you with how often you measure your Phe level? (5)	○	○	○	○	○

How satisfied are you with the process for getting the materials you need to measure your Phe levels (filter paper, etc.)? (6)	○	○	○	○	○
How satisfied are you with the financial costs associated with managing your PKU? (7)	○	○	○	○	○
How satisfied are you with insurance coverage associated with managing your PKU? (8)	○	○	○	○	○
How satisfied are you with the assistance you get from your state for managing your PKU? (9)	○	○	○	○	○
How satisfied are you with the effect managing your PKU has on your family? (10)	○	○	○	○	○

How satisfied are you with your knowledge about your PKU? (11)	○	○	○	○	○
How satisfied are you with your current treatment for your PKU? (12)	○	○	○	○	○
How satisfied are you with your current Phe level? (13)	○	○	○	○	○
How satisfied are you with the flexibility you have in your diet? (14)	○	○	○	○	○

How has your diet changed since you started taking pegvaliase (Palynziq)?

○ My diet hasn't changed (1)

○ My protein intake has changed a little (2)

○ My protein intake has changed a lot (3)

What caused you to make this change in your diet (or to not make a change)?

_____

How much have the side effect(s) of pegvaliase (Palynziq) impacted your quality of life (your health, comfort, and happiness)?

○ Significantly (1)

○ Moderately (2)

○ Slightly (3)

○ I am experiencing side effect(s) of pegvaliase (Palynziq), but they are not affecting my quality of life at all. (4)

○ I am not experiencing side effect(s) of pegvaliase (Palynziq). (5)

Page Break

This set of questions will focus on the impact of your PKU on your daily life. Please rate your answer to each question on a scale of 1 (never) to 5 (all the time) using the table below.

	Never (1)	Very Seldom (2)	Sometimes (3)	Often (4)	All the Time (5)
How often do you have a bad night's sleep? (3)	○	○	○	○	○
How often does your PKU interfere with your family life? (2)	○	○	○	○	○
How often do you find your PKU limiting your social relationships and friendships? (4)	○	○	○	○	○
How often do you feel restricted by your diet? (5)	○	○	○	○	○
How often do you miss work, school, or household duties because of your PKU? (6)	○	○	○	○	○
How often do you find yourself explaining what it means to have PKU? (7)	○	○	○	○	○
How often do you think that your PKU interrupts your leisure-time activities? (8)	○	○	○	○	○
How often are you teased because you have PKU? (9)	○	○	○	○	○
How often do you find you eat something you shouldn't rather than tell someone that you have PKU? (10)	○	○	○	○	○

How often do you find that your PKU prevents you from going out to eat with your friends? (11)	○	○	○	○	○
How often are you embarrassed by having to deal with your PKU in public? (1)	○	○	○	○	○
How often do you feel that your PKU limits your ability to do other things that interest you (for example: playing a sport, volunteering in your community)? (12)	○	○	○	○	○
How often do you find that your parents/spouse/friend/relative are too protective of you? (13)	○	○	○	○	○
How often do you feel that your parents/spouse/friend/relative worry too much about your PKU? (14)	○	○	○	○	○
How often do you find that your parents/spouse/friend/relative act like PKU is their disease, not yours? (15)	○	○	○	○	○

Page Break

This set of questions will focus on the worries you might have surrounding your PKU. Please rate your answer to each question on a scale of 1 (never) to 5 (all the time) using the table below.

	Never (1)	Very Seldom (2)	Sometimes (3)	Often (4)	All the Time (5)
How often do you worry that PKU will affect whether you will get married, or affect the status of your current marriage? (1)	◯	◯	◯	◯	◯
How often do you worry that PKU will affect whether you will have any children, or whether you will have more children? (2)	◯	◯	◯	◯	◯
How often do you worry about whether you will not get a job you want due to PKU? (3)	◯	◯	◯	◯	◯
Display This Choice: *If Are you currently a student? = Yes* How often do you worry that PKU will affect whether you will be able to complete your education? (4)	◯	◯	◯	◯	◯

How often do you worry that your body looks different because you have PKU? (5)	○	○	○	○	○
How often do you worry that you will get complications from your PKU? (6)	○	○	○	○	○
How often do you worry about whether someone will not go out with you because you have PKU? (7)	○	○	○	○	○
 How often do you worry that your instructors or your work supervisor treat you differently because of your PKU? (8)	○	○	○	○	○

How often do you worry that because of your PKU you are behind in terms of dating, marriage, making friends, or going to parties? (9)	○	○	○	○	○
How often do you worry that because of your PKU you are behind in terms of having children? (11)	○	○	○	○	○
How often do you worry about the financial costs associated with managing your PKU? (10)	○	○	○	○	○

Page Break

The following questions will ask about your current resources and supports, as well as areas where you feel you would benefit from additional resources and/or supports.

This set of questions will focus on your satisfaction with the supports you receive for your PKU. Please rate your answer to each question on a scale of 1 (very satisfied) to 5 (very unsatisfied) using the table below.

	Very Satisfied (1)	Somewhat Satisfied (2)	Neutral (3)	Somewhat Unsatisfied (4)	Very Unsatisfied (5)
How satisfied are you with support you receive from your family? (1)	○	○	○	○	○
How satisfied are you with support you receive from your friends? (2)	○	○	○	○	○
How satisfied are you with support you receive from your doctor? (3)	○	○	○	○	○
How satisfied are you with support you receive from your nutritionist? (4)	○	○	○	○	○

Who else provides important supports for you?

_____

What kind of support do they provide? You may select multiple options.

☐ Emotional (1)

☐ Social (2)

☐ Medical (3)

☐ Financial (4)

☐ Other: (5) _____

☐ No one (other than my family, friends, doctor, and/or nutritionist) provides me with

support. (6)

Are there any other areas of your life where you wish you had more support?

_____

Which of the following resources do you use? You may select multiple options.

☐ National Organization for Rare Disorders (NORD) (1)

☐ National PKU Alliance (NPKUA) (2)

☐ Medical Assistance (3)

☐ Newborn Screening Program Assistance (4)

☐ BioMarin Care Coordination Services (5)

☐ BioMarin Co-Pay Assistance (6)

☐ Low-protein recipe resource (for example, Cookforlove.org) (7)

☐ Online Phe-tracker (for example, HowMuchPhe.org) (8)

☐ In-person support groups (9)

☐ Online/social media-based support groups (10)

☐ Other: (11) _____

Are there any other resources (information, assistance, materials, other) that you need but have not been able to get?

○ Yes (1)

○ No (2)

Please describe these additional resources you need.

_____

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PKU often affects people's ability to think and can impact their emotions. The following questions explore how pegvaliase (Palynziq) might impact these areas of health compared to other types of PKU management.

This set of questions will focus on your ability to think. Please respond to each question or statement by marking one box per row.

In the **past 7 days**...

	Never (1)	Rarely (once) (2)	Sometimes (2-3 times) (3)	Often (once a day) (4)	Very Often (several times a day) (6)
I had to read something several times to understand it. (1)	○	○	○	○	○
My thinking was slow. (2)	○	○	○	○	○
I had to work really hard to pay attention or I would make a mistake. (3)	○	○	○	○	○
I had trouble concentrating. (4)	○	○	○	○	○

How much DIFFICULTY do you **currently** have...

	None (1)	A little (2)	Somewhat (3)	A lot (4)	Cannot do (5)
reading and following complex instructions (e.g. directions for a new medication)? (1)	○	○	○	○	○
planning for and keeping appointments that are not part of your weekly routine (e.g. a therapy or doctor appointment, or a social gathering with friends and family)? (2)	○	○	○	○	○
managing your time to do most of your daily activities? (3)	○	○	○	○	○
learning new tasks or instructions? (4)	○	○	○	○	○

Page Break

This set of questions will focus on any feelings you might have related to depression. Please respond to each question or statement by marking one box per row.

In the past **7 days**...

	Never (1)	Rarely (2)	Sometimes (3)	Often (4)	Always (5)
I felt depressed. (1)	○	○	○	○	○
I felt hopeless. (2)	○	○	○	○	○
I felt that nothing could cheer me up. (3)	○	○	○	○	○
I felt that my life was empty. (4)	○	○	○	○	○
I felt worthless. (5)	○	○	○	○	○
I felt unhappy. (6)	○	○	○	○	○
I felt I had no reason for living. (7)	○	○	○	○	○
I felt that nothing was interesting. (8)	○	○	○	○	○

✳

Thank you for taking the time to complete our survey. To finish, please enter the zip code for the **PKU clinic where you receive care (NOT your home zip code)**. This is so that we can compare unmet resource and support needs across the country.

_____

Appendix C Permissions

Appendix C.1 PKU-QOL Permission

Department of Human Genetics
Division of Medical Genetics
Metabolic Nutrition Program
http://genetics.emory.edu

September 30, 2020

Dear Trinity,

This letter states that you have permission to use and adapt the PKU-Quality of Life Questionnaire (PKU-QOLQ) previously developed by myself and my team for the purpose of your thesis project.

Please let me know if you have any additional questions or need to use the PKU-QOLQ for a purpose other than your thesis project in the future.

Sincerely,

Rani H. Singh, PhD, RD, LD
Professor
Department of Human Genetics
Division of Medical Genetics

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Tel 404.778.8521 • Fax 404.778.8562
An equal opportunity, affirmative action university

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Appendix C.2 NeuroQOL Permission

Subject: Online Use of NeuroQOL - Case 00027164
Date: Monday, August 17, 2020 at 5:07:10 PM Eastern Daylight Time
From: HealthMeasures
To: Sprague, Trinity

Hello Trinity,

Thank you for this additional information. You have permission to use the PROMIS measure without fee for this specific purpose you have described . You may find additional information regarding scoring here: http://www.healthmeasures.net/score-and-interpret/calculate-scores. Additionally, please review and adhere to our Terms of Use and Conditions: : http://www.healthmeasures.net/images/PROMIS/Terms_of_Use_HM_approved_1-12-17_-_Updated_Copyright_Notices.pdf

Please let us know if you have additional questions or a need to use PROMIS for other purposes.

Best Regards,

David Ortiz
HealthMeasures Support Team

--------------- Original Message ---------------
From: HealthMeasures [help@healthmeasures.net]
Sent: 8/17/2020 1:20 PM
To: trs106@pitt.edu
Subject: Online Use of NeuroQOL - Case 00027164

Hello Trinity,

Thank you for contacting HealthMeasures Support and your interest in Neuro-QoL measures.

We are forwarding your inquiry to our colleague, David Ortiz, who will follow up with you directly on this case. In the meantime, please see our Terms and Conditions of Use for your reference. http://www.healthmeasures.net/images/neuro_qol/Terms_of_Use_HM_approved_1-12-17_-_Updated_Copyright_Notices.pdf

Best Regards,
Customer Service Support

Appendix D Informational Paragraph/Flyer

Quality of Life and Resource Needs Following Treatment of PKU with Pegvaliase (Palynziq)

** Are you an adult (18 years or older) with phenylketonuria (PKU)? **

My name is Trinity Sprague, and I am a graduate student working on my master's degree in Genetic Counseling at the University of Pittsburgh.

I am interested in learning more about how taking pegvaliase (Palynziq) can impact the lives of people who have PKU. To do this, I have developed a survey to ask people who have PKU about their satisfaction with their PKU management and their access to supports and resources, as well as the impact of their PKU on their quality of life. A person's quality of life includes their health, comfort, and happiness.

If you are an adult who has PKU, you may be eligible for this study, even if you have never taken pegvaliase.

To find out more about the questionnaire and to participate, please follow the link below:
https://pitt.co1.qualtrics.com/jfe/form/SV_cPkvZeZgxA8GXFH

If you have questions or concerns about this research study, please contact me at: spraguet@upmc.edu

Appendix E Scoring Protocols

Appendix E.1 Quality of Life Sub-Sections

Appendix Table 1 Scoring Scheme for General Satisfaction with Life, Satisfaction with Management, and

Satisfaction with Support

Response Option	Score
Very Satisfied	1
Somewhat Satisfied	2
Neutral	3
Somewhat Unsatisfied	4
Very Unsatisfied	5

Appendix Table 2 Scoring Scheme for Impact of PKU and PKU-Related Worries

Response Option	Score
Never	1
Seldom	2
Sometimes	3
Often	4
All the Time	5

Appendix E.2 Neuro-QOL Short Forms

Appendix Table 3 Scoring Scheme for Cognition Short Form

	Response Choice	**Score**
"In the past 7 days…" Questions	Never	5
	Rarely (once)	4
	Sometimes (2-3 times)	3
	Often (once a day)	2
	Very often (several times a day)	1
"How much difficulty do you currently have…" Questions	None	5
	A little	4
	Somewhat	3
	A lot	2
	Cannot do	1

Appendix Table 4 Scoring Scheme for Depression Short Form

Response Choice	**Score**
Never	1
Rarely	2
Sometimes	3
Often	4
Always	5

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