THE ROLE OF ANXIETY IN THE RELATIONSHIP BETWEEN PHENYLKETONURIA AND WORKING MEMORY

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by

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The undersigned, appointed by the dean of the Graduate School, have examined the dissertation entitled

THE ROLE OF ANXIETY IN THE RELATIONSHIP BETWEEN PHENYLKETONURIA AND WORKING MEMORY

presented by Kelly Boland,

a candidate for the degree of doctor of philosophy of clinical psychology,

and hereby certify that, in their opinion, it is worthy of acceptance.

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DEDICATION

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ABSTRACT

Phenylketonuria (PKU) is a rare genetic condition associated with disruption in the synthesis of tyrosine, a precursor for dopamine. Although early diagnosis and treatment largely prevent the severe impairments associated with untreated PKU, individuals with early-treated PKU (ETPKU) nonetheless experience significant neurocognitive sequelae, including difficulties in working memory (WM). ETPKU is also associated with an increased risk of psychiatric problems, most notably anxiety. Given the well-documented relationship between anxiety and impaired WM in the general population, it remains unclear to what extent WM difficulties and comorbid anxiety may be inter-related in the ETPKU population. To examine this issue, we recruited a sample of 40 adults with ETPKU and a demographically-matched sample of 40 healthy adults without PKU. WM performance and anxiety symptomatology were assessed using a variety of remote assessment methods (e.g., web-based neurocognitive tests, semi-structured interviews, report-based measures). As expected, the ETPKU group demonstrated poorer WM performance and higher anxiety as compared to the non-PKU group. In addition, anxiety moderated the relationship between metabolic control (as reflected by blood phe levels) and WM performance in the ETPKU group. The presence of elevated anxiety was associated with poor WM performance regardless of phe levels. For individuals with lower anxiety, there was a negative relationship between phe level and WM performance. This study highlights the importance of considering how metabolic control and comorbid conditions such as anxiety may contribute to neurocognitive difficulties experienced by individuals with ETPKU.

Chapter 1: Overview of Phenylketonuria

Phenylketonuria (PKU) is a rare autosomal recessive condition associated with disruption in tyrosine synthesis. PKU is caused by mutations in the gene that codes for phenylalanine hydroxylase, an enzyme which is essential for the metabolism of phenylalanine (phe) into tyrosine. Tyrosine is a precursor for dopamine and other catecholaminergic neurotransmitters. Phe is a large amino acid present in many food products (e.g., dairy and meat). If not properly metabolized, excess phe can have disruptive/toxic effects on the brain (Schuck et al., 2015).

PKU was discovered in 1934 by a Norwegian biochemist and physician, Asbjørn Følling (Følling, 1934). Følling was introduced to two young siblings whom had both been diagnosed with retardation (nowadays referred to as intellectual disability) and whom curiously omitted an atypical odor. The children's parents had taken them to many other specialists, but no cause of their condition was identified. Følling agreed to examine the children and during a routine urine test of ketones, he discovered an abnormal result which eventually led to his discovery that these children were excreting phenylpyruvic acid. He eventually sought out urine samples from over 400 patients identified with retardation at local institutions and found eight more individuals with phenylpyruvic acid present in their urine. These individuals all shared a number of symptoms, including severe intellectual disability, fair complexions, eczema, broad shoulders, stooping figure, and spastic gait (Christ, 2003). A later study by Paine (1957) found that PKU was associated with significant developmental delays for both motor and speech milestones, with a vast majority of individuals demonstrating intelligence quotients (IQs) under 50.

Individuals with PKU were also found to frequently experience epilepsy and microcephaly (for review, see Brenton & Pietz, 2000).

There are a number of pathways by which PKU may negatively affect neurological and cognitive development. The two immediate effects of a deficient phenylalanine hydroxylase enzyme are decreased tyrosine and excessive phe (Williams et al., 2008). The shortage of tyrosine is problematic for neurotransmitter production because the hydroxylation of tyrosine to L-dihydroxyphenylalalnine (L-dopa) allows for the synthesis of dopamine and other catecholamines (i.e., epinephrine and norepinephrine). Therefore, a shortage of tyrosine may also lead to depletions in these important neurotransmitters in the brain.

Excess phe may also contribute to decreased central levels of other neurotransmitters. The main active transport channel across the blood brain barrier (BBB) for phe and other LNAAs is the LAT1 transporter. The LAT1 has a greater affinity for phe over other types of LNAAs (Hargreaves & Pardridge, 1988). As such, excess phe may compete with tyrosine and other available LNAAs to cross the BBB. The result can be lower-than-normal central levels of neurotransmitter precursors such as tryptophan, a precursor of the neurotransmitter serotonin. Consistent with this, lower-than-normal levels of serotonin have been found in studies of PKU mouse brains (Yuwiler et al., 1965). Serotonin has been linked to brain functions such as social behaviors, sleep and memory, and mood (Bacqué-Cazenave et al., 2020).

Fortunately, a neonatal screening method was developed in the 1960's that allowed for the early identification of PKU (Guthrie & Susi, 1963). Subsequent research revealed that dietary restriction of phe was associated with improved outcomes in patients

(Bickel et al., 1953, 1954). Of note, the diet was initially stopped after age 6-12 years old due to the belief that the brain was largely developed by mid-childhood and therefore not as susceptible to hyperphenylalaninemia-related insults by adolescence. Later evidence suggested that the late-adolescent and even adult brain is still negatively impacted by high phe levels and that the best cognitive and psychosocial outcomes were associated with dietary adherence across the entire lifespan. According to current United States guidelines, individuals with PKU should aim to achieve phe levels in a range of 120-360 μmol/L at all stages of life (Vockley et al., 2014).

Even with early treatment, individuals with PKU remain at risk for significant neurologic and cognitive impairment albeit not as severe as that associated with untreated PKU. Neurologically, the most consistent finding in individuals with early-treated PKU (ETPKU) is abnormalities of the white matter of the brain. White matter represents the myelin sheath around neuron axons, a sheathing which allows for more efficient signaling between neurons. The typical focus of white matter lesions is in posterior periventricular regions of the brain. With increased severity, the abnormality extends more anteriorly, typically along the lateral ventricles. More recent studies utilizing diffusion tensor imaging (DTI), an advanced MRI technique, have revealed that the magnitude of actual damage is much more extensive than originally thought based on visual inspection alone (e.g., Peng et al., 2014; Vermathen et al., 2007; White et al., 2010). Furthermore, the extent of white matter disruption is associated with increased age and poorer metabolic control, as reflected by higher blood phe levels (Anderson et al., 2007; Antenor-Dorsey et al., 2013; Cleary et al., 1994).

The precise cause of the white matter lesions remains unknown, but several theories have been put forth to explain their etiology. One researcher found evidence suggesting that high phe disrupts cholesterol metabolism in oligodendrocytes, the cells producing myelin in the brain (Dyer, 1999), therefore impairing myelin synthesis. Another theory is that high phe levels cause a reduction in $Na^{+}K^{+}$-ATPase activity (Kono et al., 2005) leading to the accumulation of fluid inside oligodendrocytes. This process results in status spongiosis (Phillips et al., 2001), which is associated with the swelling and separation of myelin sheaths from axons, creating spaces for vacuoles to form. This causes white matter lesions known as intramyelinic edema (for review see Anderson & Leuzzi, 2010; Vermathen et al., 2007). In addition to white matter lesions, growing evidence suggests that gray matter structures in the brain may also be affected, including cortical gray matter (Christ et al., 2016), basal ganglia (Bodner et al., 2012), and cerebellum (Aldridge et al., 2020).

Overall, ETPKU is associated with a slight decrease in overall intellectual functioning (Waisbren et al., 2007) coupled with impairments in specific domains/abilities including processing speed, attention, motor control, and emotional regulation (Antshel & Waisbren, 2003; Janzen & Nguyen, 2010). Working memory and related aspects of executive function represent another domain that is particularly affected (Christ et al., 2010, 2020). One meta-analysis (DeRoche & Welsh, 2008) of the PKU literature found moderate-to-large effect sizes for a number of executive functioning domains, including planning (0.51), working memory (0.59), inhibition (0.78), and flexibility (1.15). In contrast, they found only small-to-moderate effect sizes for measures of intelligence (overall, verbal, and non-verbal IQ; 0.29-0.42). There is

additional evidence suggesting that better metabolic control (as reflected by lower phe levels) in individuals with ETPKU is associated with better performance across several cognitive domains including working memory (for review, see Burlina, 2019). Within this context, a focus of the present study was on working memory in individuals with ETPKU.

Chapter 2: Working Memory in ETPKU

Working memory (WM) can be conceptualized as compromising two component processes (Baddeley & Hitch, 1974; Engle et al., 1999). The first process is storage and maintenance of information. Representations of information are held in an easily accessible state by using strategies such as phonemic rehearsal and chunking. In traditional neuropsychological literature, this process is often referred to as short-term memory. The second process in WM is the executive component. The executive component involves manipulating and updating information that is being held in the storage component. Aspects of this component may include suppression of irrelevant information, balancing concurrent processing demands, and updating information based on provided rules for data transformation (Postle & Oberauer, 2020). Postle and Oberauer (2020) refer to the relationship between the storage and executive processes as the stability-flexibility dilemma. The dilemma arises from WM needing to protect the integrity of relevant information from the effects of extraneous information (stability) while also allowing for the rapid updating of WM contents with the newest relevant information, based on changing goals or rules (flexibility). Almost all WM tasks involve both the storage and executive processes to at least some degree.

A related conceptualization of WM is based on Baddeley and Hitch's (1974) tripartite working memory model, which divides WM into three main components. This model effectively takes the storage component from the aforementioned model and further subdivides processing into domain-specific verbal and visual components called the phonological loop and visuospatial sketchpad, respectively. Baddeley and Hitch's third component is called the central executive and is a domain-general component

similar to the aforementioned executive component. The central executive encompasses overall information processing and data manipulation, as well as controlling the flow of information between the other two domain-specific stores.

Brain areas implicated in WM include fronto-parietal brain regions, most notably the dorsolateral prefrontal cortex (DLPFC), cingulate regions, and the posterior superior parietal cortex (Chein et al., 2011; C. Kim et al., 2015; Osaka et al., 2003). Neuroimaging studies have also implicated posterior cingulate regions (Moore et al., 2013) as being more involved in the maintenance function of WM while the DLPFC appears to be sensitive to manipulation of information (C. Kim et al., 2015). In addition, there is research suggesting that the ventrolateral prefrontal cortex is involved in the suppression or inhibition of task-irrelevant information during WM tasks (for review, see Dillon & Pizzagalli, 2007). Recent studies have also found a positive association between WM and white matter integrity, particularly in fronto-parietal brain tracts (Chung et al., 2018; Schulze et al., 2011).

In addition to identifying brain regions involved in WM, another line of research has linked the neurotransmitter dopamine to WM processes. For example, dopamine agonists have been shown to improve executive functioning in patients with frontal lobe injuries (McDowell, Whyte, & D'Esposito, 1998). In addition, dopamine depletion, accomplished by providing participants with an amino acid drink selectively lacking tyrosine and phenylalanine over a prolonged period of time, is associated with decreased WM functioning in healthy individuals (Harmer, McTavish, Clark, Goodwin, & Cowen, 2001). Regions of the prefrontal cortex are believed to be especially sensitive to

decreased dopamine levels due to dopaminergic neurons in these regions having higher firing and turnover rates (Diamond, 1994; Ford, 2014).

As noted earlier, WM represents one of the neurocognitive domains that is particularly affected in ETPKU. Evidence for this comes from a number of studies and meta-analyses (e.g., Bartus et al., 2018; Brumm et al., 2004; Channon et al., 2004; DeRoche & Welsh, 2008; White et al., 2002). Furthermore, WM appears to be impaired across both storage and executive domains and for both the phonological loop and visuospatial sketchpad. As previously mentioned, in DeRoche and Welsh's (2008) meta-analysis, they found a moderate effect size for WM (g = 0.59). The studies used in this WM analysis (e.g., Anderson et al., 2004; Antshel & Waisbren, 2003; Stemerdink et al., 1999) utilized tasks tapping into various components of WM, including the Tower of Hanoi (storage and executive components), Digit Span (storage and executive components; phonological loop), California Verbal Learning Test (storage component; phonological loop), and Rey-Osterrieth complex figure (storage component; visuospatial sketchpad).

Additional evidence implicating WM storage impairments in ETPKU include tasks tapping into both the phonological loop and visuospatial sketchpad. For example, a study by White et al (2002) examined short-term recall of verbal (letters) and non-verbal (novel shapes and locations) stimuli in children with and without ETPKU. Participants were shown a series of 2-9 stimuli one-at-a-time on a computer screen. After a brief delay, an array appeared which included all of the stimuli from the series, and children were asked to point to the stimuli in the order in which they were presented in the memory series. Results revealed impaired WM performance across verbal, visual, and

spatial domains for the ETPKU group as compared to age-matched peers. Interestingly, they also found that these deficits were more pronounced in older children as compared to younger children, suggesting an emerging deficit in WM.

Evidence suggests that PKU-related impairments in WM storage may persist into adulthood. Brumm et al (2004) conducted neuropsychological testing on a group of adults with ETPKU. WM tasks included the California Verbal Learning Task (CVLT) which requires remembering a list of verbally presented items, the Rey-Osterich Complex Figure (ROCF) in which participants are asked to redraw a visuospatial picture from short-term memory, and a digit span test that requires participants to recall an auditorily-presented list of numbers in forward and reverse order. Brumm et al found that the ETPKU group demonstrated marked impairments on all three of these tests of WM, as compared to normative data.

In addition to WM storage impairments, Bik-Multanowski et al (2011) also found impairments in central executive domains. In this study, young adults with ETPKU were given a neuropsychological battery that included tests of processing speed, sustained attention, and spatial WM. The spatial WM task involves a series of squares presented in pseudorandom locations on the computer, one of which reveals a target stimulus (i.e., smaller yellow square) when chosen for that trial. The participant must use the process of elimination to reveal the target square from trial to trial, without revisiting boxes that previously held a stimulus. This task assesses a person's ability to retain and manipulate visuospatial information. They reported that the ETPKU group performed significantly poorer than the general population based on normative data. Within the ETPKU group, they also found that those individuals with better metabolic control (as defined by having

phe levels below 720 µmol/L over past month) performed significantly better than those with poorer metabolic control. These results implicate hyperphenylanine (i.e., high phe levels) as a mechanism by which ETPKU negatively affects WM.

Other research groups have also found evidence for a relationship between phe level and WM. For example, Channon et al (2007) recruited adults with ETPKU who discontinued their diets in adolescence (off-diet group) with adults with ETPKU who stayed on diet during their whole lifetime (on-diet group), which was confirmed by significantly higher lifetime phe levels in the off-diet versus on-diet groups. Both groups were given a series of neuropsychological tests including a common test of WM, the n-back task. In the n-back task, participants are presented a series of letters and asked to press a button when the current letter matches the letter that was presented *n* items before (e.g., DXD or FDF; 2-back). Their design included three conditions with increasing WM load (0-back, 1-back, 2-back). Channon and colleagues found that the off-diet group had significantly worse accuracy on this task across all three conditions than the on-diet group, implying a relationship between higher phe and poorer WM outcomes. Of note, other studies have failed to find a significant relationship between phe levels and WM (e.g., Bartus et al., 2018; Brumm et al., 2004), and so this relationship warrants further investigation before definite conclusions can be drawn.

Findings from recent functional neuroimaging studies (Christ et al., 2010, 2013) provide insight into the potential nature of the WM impairments associated with ETPKU. Christ et al (2010) found atypical activation in individuals with ETPKU during performance of an n-back task in a number of WM-related brain regions including bilateral superior frontal gyrus, anterior cingulate cortex, and left parietal area. They also

found decreased functional connectivity both among prefrontal regions as well as between PFC regions and more distal parietal regions during performance of the WM task. Similarly, a later study by Christ et al (2013) also found atypical activation during performance of WM task in WM-related brain regions including the left intraparietal sulcus, bilateral dorsolateral prefrontal cortex, and anterior cingulate cortex. Taken together, these findings support the notion that both dysfunction in PFC and parietal regions as well as disruptions in functional connectivity may contribute to WM problems in PKU. This is consistent with notions that ETPKU and elevated phe levels are associated with depletion in dopamine (a neurotransmitter that is important for PFC function) and abnormalities of the white matter (Anderson et al., 2007; Antenor-Dorsey et al., 2013; A. Hood et al., 2015), respectively.

Chapter 3: Anxiety and Other Mental Health Concerns in ETPKU

Given the extent of neurodevelopmental insults in ETPKU, it is not surprising that PKU is also associated with increased psychiatric concerns. Before widespread newborn screening, untreated PKU patients were susceptible to a range of emotional and behavioral concerns. While some patients displayed only mild symptoms, such as shyness and anxiety, others presented with more severe psychotic symptoms, such as catatonia, aggression, psychosis, and self-mutilation (Følling, 1967; Penrose, 1963). With early identification and treatment, such extreme symptoms are no longer a staple of this disease.

Unfortunately, growing evidence suggests that ETPKU is still associated with an increased risk of psychiatric problems. One study found that 54% of adult patients screened positive for clinically significant psychiatric distress symptoms based on self-reports, a figure higher than that of normative data (vs 10% of general adult population; Burton et al., 2013). Another study used a structured interview of psychiatric diagnoses and found that 25.7% of the individuals with ETPKU met criteria for an internalizing disorder while only 8% of controls met criteria (Pietz et al., 1997). However, not all ETPKU studies have found evidence of increased psychological symptomatology (Brumm et al., 2004; Channon et al., 2005, 2007; Clacy et al., 2014; Palermo et al., 2020). In fact, one study examining children with ETPKU found that parents reported better psychological adjustment in their children with ETPKU than expected based on normative data (Landolt et al., 2002). These results raise questions about what could be driving the differences seen across various PKU studies.

One factor that may vary across studies is the disease severity or level of metabolic control of the participant samples. A handful of studies have demonstrated a relationship between phe levels and psychiatric distress. For example, concurrent phe levels have been demonstrated to be related to reports of anxiety (Waisbren et al., 2017) and thinking problems (Jahja et al., 2013). Lifetime phe levels have been related to psychiatric distress (Burton et al., 2013), anxiety (Clacy et al., 2014; Didycz & Bik-Multanowski, 2018), and depression (Jahja et al., 2017). Evidence for a relationship between concurrent phe levels and psychiatric symptoms has also been explored by directly manipulating phenylalanine intake. One randomized, double-blind study gave participants with ETPKU either a placebo or a phe supplement to examine the influence of short-term phe loading on mood and cognition (ten Hoedt et al., 2011). They found that after just 4 weeks of daily phe supplementation, individuals experienced a marked decrement to their self-reported mood and that these symptoms were so apparent that close friends also reported seeing heightened psychological distress in the participants with phe supplementation. This study provides more causal support for the acute effects of phenylalanine on one's psychological state.

However, several other studies have failed to find a significant relationship between measures of metabolic control (i.e., phe levels) and psychological functioning (Bilder et al., 2013; Brumm et al., 2004; Palermo et al., 2020; Pietz et al., 1997; Ris et al., 1997; Weglage et al., 2000) or have found the opposite relationship (Manti et al., 2016). Although still debated, if the relationship between phe levels and psychiatric problems does exist, this could pose a problem for generalizing psychiatric outcomes across studies that have different levels of metabolic control in their sample. However, in comparing the

range of phe levels across previous studies, there does not seem to be a pattern between the group of studies that did versus did not find a significant relationship (i.e., mean phe levels of studies that did find a significant relationship ranged from 471-918 μmol/L while for studies finding no evidence of this relationship the range was 528-1332 μmol/L).

Another difference across studies related to metabolic control was the use of phe level as a continuous variable versus assigning a cut-off to group individuals into low and high phe groups. Among the values previously chosen as cutoffs were 360 μmol/L (Jahja et al., 2017), 500 μmol/L (Manti et al., 2016), and 1,000 μmol/L (Brumm et al., 2004). Brumm's study was the only study of these three to not find a significant difference between groups on psychiatric distress, which may be attributable to having the highest cutoff value to distinguish groups. Future research should be weary of dichotomizing phe values, or at the least provide justification for dividing up their groups in this way.

In addition to differences in phe levels, which mental health disorders researchers decide to investigate may also contribute to differences across studies in prevalence rates in this population. For example, using a structured interview, Pietz and colleagues (1997) found that, compared to healthy controls, their ETPKU group endorsed significantly more emotional symptoms overall. However, when broken into specific concerns, they found that the ETPKU group had higher rates of depressed mood, phobias, generalized anxiety, hypochondriac worries, and anxiety in the workplace but did not differ from controls on the presence of panic disorders, school phobia, somaticizing symptoms, or suicidal tendencies. Other research groups have found increased risk for internalizing disorders but not externalizing disorders (Bilder et al., 2013; Jahja et al., 2013). These

results highlight that there may be risks for particular mental health concerns, rather than an overall risk for all types of psychiatric disorders.

When studies do specify types of psychiatric concerns, anxiety (Bilder et al., 2013; Koch et al., 2002; Manti et al., 2016; Pietz et al., 1997; Smith et al., 1988; Waisbren et al., 2017; Weglage et al., 2000) and depression (Bilder et al., 2013; Jahja et al., 2017; Koch et al., 2002; Manti et al., 2016; Pietz et al., 1997; Trefz et al., 2019; Waisbren et al., 2017; Weglage et al., 2000; Wu et al., 2011) emerge as the two most widely reported comorbid conditions with ETPKU (see Table 1 for brief description of past anxiety studies). However, there are a handful of studies suggesting no evidence of heightened anxiety and/or depression in this population (Brumm et al., 2004; Channon et al., 2005, 2007; Clacy et al., 2014; Palermo et al., 2020). One possible explanation may be differences in methodology, including choice of psychiatric symptomatology measure (Brumm et al., 2004; Channon et al., 2005, 2007; Palermo et al., 2020), exclusion criteria (Channon et al., 2005, 2007), and/or insufficient statistical power related to small sample sizes (only had 8 participants with ETPKU and compared to norms; Clacy et al., 2014).

Table 1

Summary of Previous Research Studies Examining Anxiety in ETPKU

Study	**Ages**	**# of participants**	**ETPKU vs Control/Norms**	**Measure of Anxiety**	**Relationship with Phe**
Smith et al., 1988	8 y.o.	544 ETPKU; 1,088 controls	ETPKU > controls	Teacher-report Rutter Behavior Questionnaire	N/A

Pietz et al., 1997	17-33 y.o.	35 ETPKU; 181 controls	ETPKU > controls (for most anxiety categories)	Structured interview based on ICD-10 diagnoses	N/A
Weglage et al., 2000	10-18 y.o.	42 ETPKU; 42 diabetics; 2,900 controls	ETPKU > controls; No difference between ETPKU & diabetics	Parent-report CBCL	Anxiety not related to lifetime or concurrent phe
Koch et al., 2002	~26-31 y.o.	73 ETPKU	41% of ETPKU who discontinued diet after age 6-10 y.o. had mental health problems (most notably phobias and depression)	Unspecified 'questionnaire'	N/A
Brumm et al., 2004	21-32 y.o.	24 ETPKU	No difference found	BAI	Anxiety not related to concurrent phe when dichotomized into high vs

					low (> vs < 1000mol/L)
Channon et al., 2005	18-33 y.o.	25 ETPKU; 25 controls	No difference found	BAI	N/A
Channon et al., 2007	18-38 y.o.	50 ETPKU; 45 controls	No difference found	BAI	N/A
Bilder et al., 2013	17-48 y.o.	64 ETPKU	ETPKU > norms	Brief Symptom Inventory	Anxiety not related to phe
Jahja et al., 2013	7-40 y.o.	53 ETPKU (30 adults; 23 children); 21 controls (14 adults; 7 children)	ETPKU (adults only) > controls for internalizing problems	ASEBA (adults), CBCL (children)	Thinking problems and somatic problems (children only) positively correlated with concurrent phe
Clacy et al., 2014	15-25 y.o.	8 ETPKU	No difference found	Depression Anxiety Stress Scale	Anxiety positively correlated with lifetime phe and phe-to-tyr, but not concurrent phe, tyr, or phe-to-tyr

Manti et al., 2016	12-44 y.o.	46 ETPKU; 30 controls	ETPKU > controls	STAI, ASEBA, psychiatric interview	Better phe in childhood (<12 y.o.) when dichotomized into high vs low (> vs < 500mol/L) predicted worse psychiatric outcomes
Didycz & Bik-Multanowski, 2017	13-17 y.o.	25 ETPKU	N/A	STAI	Anxiety positively correlated with mean lifetime phe and most recent phe
Jahja et al., 2017	18-40 y.o.	57 ETPKU; 57 controls	No difference found	ASEBA	Anxiety not related to concurrent or lifetime phe
Waisbren et al., 2017	22-38 y.o.	9 ETPKU	7/9 of participants had anxiety or	BAI and supplemented by self-reported	Anxiety positively correlated with

			depression as determined by BAI/BDI cutoffs or self-report treatment for these conditions	treatment for anxiety or depression	concurrent phe, as measured in both the bloodstream and the brain
Trefz et al., 2019	18-96 y.o.	377 ETPKU; 3,770 controls	No difference found, however reaction to severe stress and adjustment disorders was elevated in ETPKU	Healthcare claims with diagnoses	N/A

Note. ASEBA = Achenbach System of Empirically Based Assessment; BAI = Beck Anxiety Inventory; CBCL = Child Behavior Checklist; STAI = State-Trait Anxiety Inventory

Among the mental health measurements previously utilized for this type of research are questionnaires, structured interviews, and pre-existing mental health diagnoses from a mental health professional. In regards to questionnaires, some researchers have used scales specific to anxiety and depression, such as the State-Trait Anxiety Inventory or the Beck Anxiety and Depression Scales (Channon et al., 2005, 2007; Manti et al., 2016), while others have used anxiety-related subscales of more

comprehensive mental health measures, such as the Achenbach System of Empirically Based Assessments (ASEBA; Jahja et al., 2017; Manti et al., 2016). In addition, while the ASEBA was used as an adult self-report, studies examining youth with ETPKU have utilized parent-report questionnaires, such as the Child Behavior Checklist (Weglage et al., 2000), and even a teacher-report, the Rutter Behavior Questionnaire (Smith et al., 1988; for comprehensive list of measurements, see Table 1).

A study by Manti and colleagues (2016) provides additional insight into the issue of different types of measurement of psychological functioning in ETPKU. The researchers utilized questionnaires specific to anxiety (STAI) and depression (BDI), a general mental health questionnaire (ASEBA), as well as a structured interview of diagnostic criteria. All modes of measurement yielded higher psychiatric symptoms/diagnoses in their ETPKU group as compared to the non-PKU comparison group. However, they found that while overall agreement between these tools was 65-93% for the ETPKU participants, the self-report STAI always showed less than 50% concordance with the other measures, calling the validity of the STAI in this population into question. In addition, the majority of studies finding no group differences in anxiety or depression used anxiety/depression-focused self-reports similar to the STAI, most notably, the Beck Anxiety and Depression Inventories (Brumm et al., 2004; Channon et al., 2005, 2007; Palermo et al., 2020). Taken together, these findings suggest that certain diagnosis-specific self-reports may be less sensitive to unique anxious symptomatology in this medical population.

One reason that self-reports may be insufficient in this population is the comprehensiveness of the questions. For example, a study examining the appropriateness

of anxiety and depression measures for cancer populations recently criticized the STAI for failing to differentiate between cancer patients with or without anxiety disorders (Shunmugasundaram et al., 2020). In addition, they criticized the BAI for only covering 50% of the criteria for an anxiety disorder (as defined by The Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition; DSM-5; American Psychiatric Association, 2013), missing items including sleep disturbance, irritability, concentration, and fatigue. The poor specificity of symptoms on these measures may create issues for clinicians examining anxiety in non-typical populations.

In addition to type of measures used, cohort differences may also contribute to differences in results. For example, Channon et al's studies (2005, 2007) have been cited in several recent papers (Burlina, 2019; Palermo et al., 2020) as lack of evidence for elevated psychiatric risk in ETPKU. At first glance, this could be attributable to again using the BAI and BDI as measures. However, when examining these studies' methodologies, Channon and colleagues also excluded individuals with a previous psychiatric diagnosis. Therefore, they may have screened out people with anxiety and/or depression diagnoses, leaving their remaining sample to be under-representative of these symptoms in this population. Another source of variability is that Channon's studies had slightly overlapping samples which makes it more difficult to generalize the findings to the broader ETPKU population. Therefore, the evidence against increased psychiatric risk in patients with ETPKU may not be as robust as previously thought and may be attributable to factors such as measurement type and study inclusion/exclusion criteria.

The precise neurophysiological mechanisms underlying the increased mental health risk in ETPKU remains unclear; however, it has been hypothesized that

dysregulation in serotonin and dopamine, two neurotransmitters that are important in mood regulation (Morrissette & Stahl, 2014; Radwan et al., 2019), may contribute to the elevated risk. As described earlier, phe competes with tryptophan and tyrosine (precursors for serotonin & dopamine, respectively) to cross the BBB. As such, elevated phe levels may lead to lower-than-normal central levels of these NT precursors and their associated NTs. In mouse models of PKU, it has been demonstrated that both serotonin and dopamine content in brain tissue is significantly reduced (Puglisi-Allegra et al., 2000).

Importantly, the psychosocial burden of maintaining treatment for a chronic disease may also contribute to the increased risk of mental health concerns (Stanton et al., 2007). The hypervigilance needed for controlling one's diet may substantially increase stress and contribute to medical care fatigue and burnout which may, in turn, then develop into anxiety or depression. Periods of poor metabolic control and psychosocial stressors likely co-occur. Of note, both of these processes can become cyclic with increased depression and anxiety in turn making it more difficult to adhere to medical management of the disease. This cycle of poor adherence and increased psychological concerns has already been identified in other chronic illness groups, including type 1 diabetes (e.g., Hilliard et al., 2011; Hood et al., 2006). However, as mentioned previously, the relationship between metabolic control and psychiatric functioning remains a debated issue in the ETPKU field.

In summary, although somewhat mixed, previous findings generally point towards an elevated risk for psychiatric distress, specifically anxiety and depression, in individuals with ETPKU as compared to the general population. In addition, a review of

past work suggests that the optimal choice of measurement instrument is vital in detecting mental health symptoms in ETPKU, and research groups would benefit from using additional methods (e.g., structured interviews, DSM-5 criteria for clinical diagnoses) beyond just self-report questionnaires.

Chapter 4: Theoretical Relationship between Anxiety and Working Memory

The high prevalence of anxiety within the ETPKU population is particularly interesting in light of evidence of a relationship between anxiety and aspects of executive function, most notably WM (Moran, 2016). Although there are several theories regarding the nature of the relationship, most researchers agree that anxiety-related and WM-related processes compete with each other for limited cognitive resources (for review, see Moran, 2016). In addition, most theories mention at least two main components of anxiety: worrisome thoughts and arousal. Worries are cognitive ruminations that at times can be intrusive. Arousal refers to the physiological heightened state of anxiety, which may include physical symptoms such as racing heart, sweating, and shakiness. One of the main distinctions between theories is whether anxiety has a negative influence on general- or specific- domains of WM.

One example of how anxiety may compete for limited cognitive resources comes from Eysenck's and colleagues' (2007) attentional control theory. Attentional control is the ability to allocate attention to desired stimuli and suppress irrelevant information. According to attentional control theory, there are two attentional systems that interact for optimal cognitive functioning: a goal-directed system and stimulus-driven system. The goal-directed system is a top-down process by which one's goal for a task determines where attention will be focused. In contrast, the stimulus-driven system is a bottom-up, exogenous process whereby stimuli themselves automatically capture attention. Importantly, there are limited attentional resources in WM that are divided amongst the stimulus-driven and goal-directed systems. According to attentional control theory, anxiety disrupts the balance between these two systems, defaulting more resources to the

stimulus-driven system. This is believed to occur because when a person is anxious and 'under threat,' the optimal survival strategy would be to reduce selective focus on any one WM task and instead allocate attentional resources widely to increase the likelihood that the presence of a new threat can be processed more quickly to determine risk. Therefore, performance on a WM task that does not involve threat-stimuli will suffer from these limited attention resources being less focused on the task at hand and more likely to shift away to external or internal task-irrelevant stimuli. For individuals with anxiety, these task-irrelevant distractors are often worrisome, intruding thoughts.

The idea that anxious thoughts may be occupying limited attentional resources is grounded in the assumptions of another theory by Eysenck and colleagues called processing efficiency theory (Eysenck & Calvo, 1992). The two major assumptions of processing efficiency theory are (1) worry is largely responsible for the negative cognitive effects of anxiety, and (2) the main effects of worry on WM are domain-general. Regarding the first assumption, worrisome thoughts are considered internal distractors and are thought to take up limited WM resources, leading to less resources available for efficient cognitive processing. This view is largely supported by other theories of the anxiety/WM relationship (e.g., Sarason, 1988; Shackman et al., 2006).

In contrast, the second assumption of processing efficiency theory regarding domain-general effects continues to be debated. The idea of domain-specific versus domain-general components of WM is based on Baddeley's and Hitch's (1974) tripartite working memory model. The phonological loop and visuospatial sketchpad are considered domain-specific while the central executive is considered domain-general. As previously mentioned, processing efficiency theory states that the domain-general, central

executive is most susceptible to the negative effects of anxiety due to the important role attention plays in many of the processing and storage functions that characterize this component (e.g., reducing interference from distractors, maintaining task goals; Eysenck & Calvo, 1992). With regards to the phonological loop and visuospatial sketchpad components, processing efficiency theory suggests that the former is more susceptible than the latter to anxiety-related disruption given that worries are often verbal in nature. In opposition, Shackman et al (2006) argued that anxiety competes with the visuospatial sketchpad to a greater extent than the other two components of working memory based on neuroimaging findings implicating both spatial WM and anxious arousal activity in the right prefrontal cortex. More recent research suggests that task difficulty may serve as an important moderator of the relationship between anxiety and these two WM components. For example, Vytal et al (2012) proposed that worrisome thoughts interfere with the phonological loop at low task difficulty, but high task difficulty often triggers compensatory mechanisms that minimize anxiety's negative effects. In contrast, anxious arousal disrupts spatial WM across all levels of task difficulty because arousal, which is more associated with the visuospatial sketchpad, is a basic primitive function protecting organisms from threat and so is less amenable to top-down regulation.

Evidence for the detrimental effects of anxiety disorders on WM have typically been explored in experiments with threat- and emotionally- salient stimuli. A growing number of studies have also found evidence for negative effects of anxiety with emotionally neutral stimuli, such as those found in classic neuropsychological tests (e.g., Cupul-García et al., 2018; see Derakshan & Eysenck, 2009 for a review; K. L. Kim et al., 2019; Tempesta et al., 2013). However, results are somewhat mixed, with other studies

showing no detrimental effects of anxiety on aspects of cognition (e.g., Kurt et al., 2017; Leonard & Abramovitch, 2019). Several factors may contribute to these mixed findings.

One explanation for discrepant findings is how these studies define their high anxiety group, with some researchers examining anxious symptomatology in a general population while others use clinical populations of individuals who met diagnostic criteria for current or lifetime anxiety disorders. One study that highlights this distinction characterized their participants according to whether they met criteria for a current anxiety diagnosis or an anxiety disorder any point during their lifetime (Castaneda et al., 2011). They also had participants complete neuropsychological tests, including several WM tasks. They found that when looking at individuals with an anxiety disorder during their lifetime, there was no relationship between anxiety and WM. However, when the researchers excluded individuals whose anxiety disorders were in remission (i.e., included only individuals currently meeting criteria for an anxiety diagnosis), a significant negative relationship between anxiety and visual WM emerged (as measured by a simple visual span task).

In addition, evidence has surfaced indicating that neuropsychological difficulties may depend on what type of anxiety disorder is under investigation. Airaksinen et al (2005) examined neuropsychological test performance across a number of specific anxiety disorders, including social anxiety, generalized anxiety, panic, obsessive-compulsive, and specific phobia. When combining all these anxiety disorders into one group, they found significant impairments in episodic memory and WM in the anxiety group as compared to non-anxious controls. However, a more focused analysis revealed that this relationship did not hold when examining just those with generalized anxiety

disorder (GAD) or specific phobia separately. The findings of WM impairment seemed to be mainly driven by those with panic and obsessive-compulsive disorders. Of note, Airaksinen et al's sample size for GAD was small (n = 7) and may not have had sufficient power to detect a relationship, which seems to also be a limitation for many studies that have attempted to parse out different types of disorders post hoc.

A recent meta-analysis on the anxiety/WM relationship consolidated results from 177 studies for a total of 22,061 individuals (Moran, 2016). Moran included studies whose populations were diagnosed with anxiety disorders, as well as studies employing general populations with subclinical anxious traits. Moran found that overall, anxiety was negatively associated with WM performance with a hedge's g of -.334, suggesting a moderate negative relationship. In addition, Moran categorized WM assessments into three main categories of tasks: simple, complex, and dynamic spans, to see if there were differences in the WM/anxiety relationship based on type of WM task. Simple span tasks are most commonly digit or visual span tasks that require participants to hold a series of digits/numbers in memory as that span gets increasingly larger and recall them in order. Tasks such as this require simple maintenance and rehearsal functions of WM. Complex span tasks include operation span tasks (OSPAN). In these tasks, participants try to remember sequentially presented words while simultaneously solving simple math equations. This type of task requires participants to hold the target stimuli outside of explicit awareness by controlled attention while attending to the secondary task. Finally, dynamic span tasks require more of the updating function of WM. One of the most well-known dynamic span task is the n-back task, which was described previously. Dynamic span tasks only modestly correlate with simple and complex spans ($r \approx .25$), suggesting

they may be tapping into a separate component of WM from these other tasks. Moran found that across all three different types of spans, the negative relationship between WM performance and anxiety held consistent (hedge's g range: -.318 to -.437).

In addition, the WM/anxiety relationship held true regardless of age and population (i.e., clinical vs general population), although effect sizes for clinical populations were significantly larger than nonclinical samples (-.424 vs -.235, respectively). In addition, this relationship held for both measures of state and trait anxiety. Of note, Moran pointed out that the relationship between state and trait anxiety has not been previously explored by WM/anxiety theories and this may be an important avenue of future study. For example, Moran hypothesized that trait anxiety may predispose a person to worry whereas state anxiety may more directly influence arousal, perhaps influencing different aspects of WM (i.e., central executive vs visuospatial sketchpad). Finally, the anxiety/WM relationship was present for both self-reported anxiety symptoms and experimentally-induced anxiety. Taken together, work by Moran and others provide strong evidence for a relationship between anxiety and WM performance.

Chapter 5: The Present Study

The present study examines the potential relationship between WM performance and comorbid anxiety in individuals with ETPKU. Based on the limited cognitive resources theory of WM, one might expect that if two conditions restricting resources are present, there would be more profound impacts on working memory performance than one of those conditions by itself. Therefore, in ETPKU patients with co-morbid anxiety, the concern would be that not only do high phe levels decrease one's WM resources, but the processing of anxiety-related worries would also consume many of those limited resources, leaving less resources available to devote to performing cognitive tasks. Preliminary evidence for this theory comes from Burton and colleagues' study (2013) which found that the majority of individuals with ETPKU who screened positive for psychiatric distress also screened positive for self-reported executive functioning concerns. The relationship between cognition and psychiatric distress has been explored in other clinical populations, including multiple sclerosis (Morrow et al., 2015; Vissicchio et al., 2019), heart disease requiring surgery (Stroobant & Vingerhoets, 2008), and sickle cell disease (Prussien et al., 2018). For example, Morrow et al (2015) found poorer performance on measures of processing speed, working memory, and visual-spatial memory for participants with multiple sclerosis *and* anxiety as compared to participants with multiple sclerosis but no anxiety. Studies such as this highlight the importance of considering co-morbid psychiatric conditions when conceptualizing the neuropsychological profile of individuals with chronic illnesses. Of note, a limitation of many of these studies is the absence of a comparison group of healthy individuals, which would allow for elucidation of how the relationship between mental health and cognitive

performance might differ across clinical and non-clinical populations. The present study will include a healthy comparison group to better characterize this relationship.

There are three main aims of the present study:

Aim #1. The first aim is to test the hypothesis that ETPKU is associated with both (a) impaired WM performance and (b) increased anxiety symptomatology. As described earlier, previous studies have documented WM difficulties and elevated anxiety in individuals with ETPKU. A strength of the present study is the use of a comprehensive approach to evaluating both WM and anxiety. Several methods of assessing both anxiety symptomatology (i.e., self-report, other-report, diagnostic questionnaire) and WM (i.e., tasks tapping into different components of WM) are included in the study.

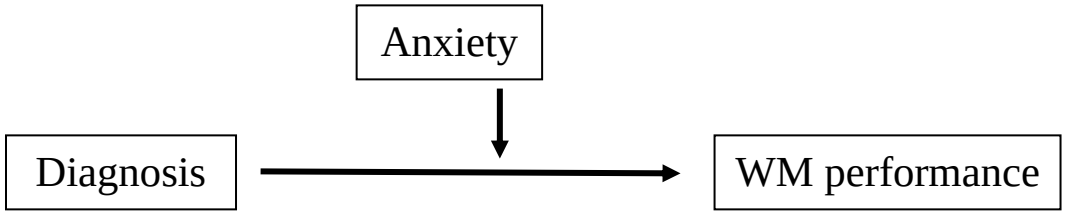
Aim #2. The second aim is to test the hypothesis that anxiety will moderate the relationship between diagnosis (ETPKU vs non-PKU) and WM performance (see Figure 1). As described above, past research suggests that the magnitude and integrity of WM resources available to individuals with ETPKU are decreased as compared to healthy individuals without PKU. Given that ETPKU is associated with a smaller-than-normal pool of available (and intact) WM resources, we hypothesize that devotion of WM resources to anxiety-related processing will have an even more detrimental effect on WM task performance in ETPKU as compared to non-PKU.

Aim #3. The third aim is to test the hypothesis that anxiety will moderate the relationship between metabolic control (as reflected by blood phe levels) and WM performance within the ETPKU group (see Figure 2). Similar to the rationale for Aim #2, to the extent that higher phe levels are associated with more compromised WM

resources, we hypothesize that anxiety will exacerbate the relationship between phe levels and WM performance within our ETPKU group.

Figure 1

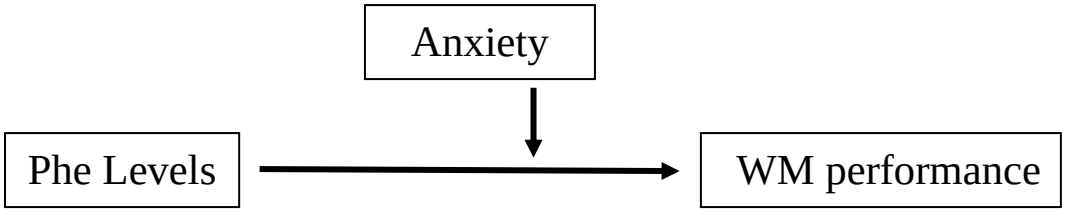
Hypothesized Moderation Effect for Aim 2



Note. Proposed mechanism for Aim 2, by which anxiety moderates the relationship between diagnosis (ETPKU vs non-PKU) and WM performance.

Figure 2

Hypothesized Moderation Effect for Aim 3



Note. Proposed mechanism for Aim 3, by which anxiety moderates the relationship between disease severity, or phe levels, and WM performance within just an ETPKU population.

Methods

Participants

A sample of 42 adults (18-40 years old) with ETPKU and a demographically-matched comparison group of 41 healthy adults without PKU were enrolled in the study. Three participants (2 ETPKU, 1 non-PKU) were consented but did not complete participation due to scheduling conflicts. Demographic and diagnostic information for the final sample of 80 participants (40 with ETPKU) is included in Table 2. The groups were matched for age, sex, and years of education.

Table 2

Sample Characteristics

Variable	ETPKU (*n*=40)		non-PKU (*n*=40)	
	M (SD)	*Range*	*M (SD)*	*Range*
Age (years)	26 (6.0)	18-39	26 (6.0)	18-39
Sex (M/F)	11/29		11/29	
Education (years)	15 (2.2)	11-20	16 (1.8)	12-19
Most Recent Phe Level (µmol/L)	438 (251.9)	64-1114	N/A	N/A
ASR DSM Anxiety T-Score	58 (8.5)	50-75	55 (6.6)	50-75
ABCL DSM Anxiety T-Score	56 (6.9)	50-73	54 (6.1)	50-75

Note. ASR = Adult Self-Report, ABCL = Adult Behavior Checklist (other-report), DSM = The Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition.

GPower3 software was used to conduct statistical power analysis (Faul et al., 2007). Given the proposed sample size (N = 80 total) and an anticipated medium-to-large

effect size (Cohen, 1988) based on data from a recent study in our laboratory (Boland et al., in prep), the power to detect a main effect of diagnosis (ETPKU vs non-PKU; 0.80-0.84), anxiety (0.89), and the interaction between diagnosis and anxiety (0.80) are all high.

Participants with ETPKU were recruited through (1) the Metabolic Genetics Clinic at Emory School of Medicine, (2) a database of research volunteers with ETPKU that is maintained by Dr. Christ's laboratory, and (3) recruitment flyers disseminated to potential participants through various other metabolic clinics and patient advocacy organizations. Participants with PKU were included if they were diagnosed as newborns and immediately started on treatment. Individuals with a history of neurologic compromise, major medical condition unrelated to PKU (e.g., closed head injury, multiple sclerosis), and/or were currently prescribed pegvaliase-pqpz (Palynziq^{TM}) were excluded. Of the final sample, 22 participants with ETPKU were currently taking sapropterin (Kuvan^{TM}). Participants without PKU were recruited through (1) advertisements placed in the University of Missouri's campus-wide weekly email announcements, (2) the aforementioned database of research volunteers maintained by Dr. Christ's laboratory, and (3) participants with ETPKU were encouraged to pass along the study recruitment flyer to friends without PKU of a similar age and background.

For participants with ETPKU, most recent phe level and lifetime phe levels were calculated. Phe level data were collected on the day of testing via self-collection of blood samples on filter paper, which were then sent for analysis to a leading laboratory in the field (PerkinElmer Laboratories). Day-of-testing phe measurements were unavailable for two participants with ETPKU. The most recent phe levels for these individuals were

collected 1 and 17 days prior to the study date. Data on historic phe levels was gathered from available medical records. Lifetime phe level data was unavailable for five participants. Mean phe level for the year preceding study participation was calculated. As described previously (Christ et al., 2021), the index of dietary control (IDC; the mean of all half-year median phe levels) was computed for early childhood (0-5 yrs), middle childhood (6-11 yrs), adolescence (12-17 yrs), adulthood (18+ yrs), and lifetime (0-present). In case of small gaps (e.g., 1-2 yrs) in records, values were extrapolated using linear regression and phe levels of adjacent years for purposes of IDC calculation. In rare cases of larger gaps, the participant was excluded from analysis of the relevant developmental epoch. For example, if a participant was missing phe levels for 7-9 yrs of age, then they would not be included in analysis of middle childhood (6-11 yrs) or lifetime IDC values.

Procedure

The present study was approved by the University of Missouri Internal Review Board (Review ID 281010) and was carried out in accordance with the provisions of the World Medical Association Declaration of Helsinki. Informed consent was obtained for all individuals prior to participation.

After potential participants expressed interest in participating in the study, study coordinators called to go over the informed consent and other relevant information. Study personnel gave a verbal summary of inclusion/exclusion criteria and answered any questions. Participants were then sent a secure link in their email to sign the study's informed consent and HIPAA documentation through RedCap, a web-based tool for administering surveys on the Web. RedCap is considered a secure medium for gathering

PHI. The study visit took place on a HIPAA-compliant cloud-based video and phone conferencing system. The link to the teleconferencing meeting was sent via email to the participant along with instructions for using the platform.

During the meeting, participants completed the cognitive tests, mental health questionnaires, and structured interview. ETPKU participants also completed self-collection of the blood spots on filter paper under the supervision of the investigator. Participants were asked to find a quiet, distraction-free location to complete the tasks. Participants were also instructed to report on any outside distractions that occurred during the study visit that may have impacted performance.

Overall Level of Intellectual Functioning. The Matrix Reasoning subtest from the Wechsler Adult Intelligence Scale 4th edition (WAIS-IV; Wechsler, 2008) was administered (via secure video conference software) in order to estimate overall intellectual ability.

Working Memory Assessment. The web-based version of the Cambridge Neuropsychological Test Automated Battery's (CANTAB) was used to evaluate WM and other executive functioning skills. Originally developed at the University of Cambridge over 30 years ago, CANTAB has since been extensively validated (Lowe & Rabbitt, 1998) and published in over 2000 peer-reviewed papers (for some reviews, see Levaux et al., 2007; Luciana, 2003; Zygouris & Tsolaki, 2015). Recently, a web-based version of the CANTAB was developed, and initial research suggests no significant differences in performance across the platforms (supervised in-person vs remote web-based; Cormack et al., 2017, 2018).

The CANTAB has already shown promise in revealing neuropsychological deficits within ETPKU populations (e.g., Bik-Multanowski et al., 2011; Harding et al., 2018; Luciana et al., 2001; Schindeler et al., 2007; Stroup et al., 2017). In addition, CANTAB's web-based version has the advantage of being available to remotely administer over participant's own computers or tablets from the comfort of their own homes. One of the challenges in studying ETPKU is the disorder's low prevalence in the population, approximately 1 in every 13,500-19,000 births in the USA (National Institutes of Health Consensus Development Panel, 2001). Remote assessment is an innovative solution for recruiting more individuals with rare disorders in a cost-efficient way.

Four CANTAB subtests were administered, each of which involved elements of WM and related executive functioning skills:

The *Spatial Span* (SSP) subtest is a visuospatial WM task in which participants are shown nine white boxes (i.e., small square stimuli) positioned at semi-random locations on the display. On the first trial, two of the boxes are cued (i.e., briefly change color) one at a time. Participants must then recall the identity and order of the boxes that were cued. On subsequent trials, the number of cued boxes is systematically increased. The task is then repeated with participants needing to recall the cued boxes in reverse order. Previous studies have found that individuals with ETPKU performed worse than individuals without PKU on this task (Bik-Multanowski et al., 2011). Studies of non-PKU individuals have also found higher anxiety is related to poorer performance (K. L. Kim et al., 2019; Murphy et al., 2018).

The *Paired Associates Learning* (PAL) subtest is a visuospatial WM task in which participants are shown six white boxes (i.e., small square stimuli) positioned at semi-random locations on the display. The boxes are "opened" (i.e., briefly changes from a white-filled box to an outline of a box) one at a time, with some of the boxes containing a pattern image within the box outline. After the contents of all six boxes are shown, the patterns are then displayed one at a time in the middle of the screen, and the participant must select the box in which the pattern was originally located. If the participant's response is incorrect, the trial is repeated. If they are correct, the task proceeds with a larger memory set (i.e., there are always 6 boxes, but on any given trial 2-6 of them may contain images).

The RVP subtest is a WM task in which participants are shown a continuous stream of digit stimuli (i.e., numbers 2-9) at a rate of 100 stimuli per minute. Participants are asked to indicate when certain 3-digit sequences appear by pressing the spacebar. For example, participants may be given a target sequence of '2-4-6' on the display. The level of difficulty varies according to how many target sequences (1, 2, or 3) must be watched for at the same time (e.g., just looking for '2-4-6' vs looking for both '2-4-6' *and* '3-5-7' simultaneously). This task involves both updating and shifting functions. A previous study found that individuals with ETPKU performed worse than individuals without PKU on this task (Bik-Multanowski et al., 2011).

In the *Spatial Working Memory* (SWM) subtest, participants are shown 4-12 colored boxes (i.e., small square stimuli) positioned at semi-random locations on the display. When the participant clicks on a box, it changes from a filled-in colored box to an outline of a box that is either empty or that contains a "token" (i.e., smaller yellow

square inside the outlined box). The goal of the task is to find all of the tokens. Each block consists of several trials in which only one of the colored squares on the display will reveal a token, with each box revealing only one token per block. Therefore, participants must use a process of elimination on each trial to select boxes that have not previously contained a token for that block. The level of difficulty varies according to how many boxes (4-12) are presented on the display for that block. The color and position of the boxes used are changed from trial to trial to discourage the use of stereotyped search strategies. The primary variable of interests are error rate (i.e., re-selecting boxes that have already been found to be empty or previously contained a token) and strategy use. This task relies on rapidly updating WM and strategy use. Several studies have found impaired performance on this task for individuals with ETPKU (Bartus et al., 2018; Bik-Multanowski et al., 2011) and with higher phe levels (Harding et al., 2018; Schindeler et al., 2007).

The aforementioned CANTAB subtests assess primarily visuospatial WM. In order to also capture aspects of verbal WM, the Digit Span subtest from the WAIS-IV was also administered via secure video conference software. In the Digit Span task, participants are orally presented with a series of digits in pseudo-random order. Participants are then asked to verbally recall the sequence as presented (i.e., forward condition). The number of digits presented is systematically increased over the course of the task. Participants also complete versions of the task in which they must recall the digits in reverse order (i.e., backward condition) and in order from smallest to largest number (i.e., sequencing condition). As previously mentioned, performance on the Digit

Span subtest has been shown to be impaired in ETPKU populations versus normative data (Brumm et al., 2004).

Anxiety Assessment. In order to provide a comprehensive evaluation of anxiety-related symptomatology, several methodological approaches were used, including a self-report, other-report, and diagnostic interview. Note that gathering a secondary 'other' report may be particularly important for an ETPKU population, as insight into one's own general mood state requires the use of cognitive resources (e.g., memory) that may be impaired in individuals with poor dietary control (i.e., high phe levels), and so a close relative or friend may have more accurate information on anxiety-related behaviors that they have observed.

Achenbach System of Empirically Based Assessments (ASEBA; Achenbach, 2003). In order to assess for anxiety, the ASEBA's Adult Self Report (ASR) and Adult Behavior Checklist (ABCL; other-report) were used. These assessments were administered online via a secure website (https://www.aseba-web.org/). The ASR assesses mental health across a range of syndromes, including anxiety. The ABCL is a checklist that parallels the self-report and is given to either a close relative or friend to provide additional data on the participant.

The ASEBA has been used in over 10,000 published studies (https://aseba.org/research-updates-from-around-the-world/) including some past studies of ETPKU (e.g., Jahja et al., 2013; Manti et al., 2016). It has also been shown to have good test-retest reliability and internal consistency (Achenbach et al., 2004).

Per the standardized guidelines for ASEBA administration, the 'other-report' measure may be completed by a variety of individuals associated with the participant

(e.g., spouses, parents, roommates, children, friends, and other relatives). The majority of participants in the present study nominated significant others (N = 48; i.e., boyfriend/girlfriend, spouse), followed by parents (N = 18), close friends (N = 11), and siblings (N = 3).

MINI International Neuropsychiatric Interview (Sheehan et al., 1998). In addition to questionnaires, the MINI was administered. The MINI is a short, structured interview based on DSM-5 criteria and was given to participants over secure video conference software by a trained, doctoral-level clinical psychology graduate student to determine if participants met clinical threshold for any DSM-5 anxiety disorders. The MINI is well-established in the literature and psychometrically sound (Sheehan et al., 1997). For the purposes of the present study, only the modules pertaining to anxiety were administered to participants: Generalized Anxiety Disorder, Social Anxiety Disorder, Specific Phobia, Panic Disorder, Agoraphobia, and Obsessive-Compulsive Disorder.

Data Processing and Analysis

Working Memory (WM) Composite Score. Each of the four CANTAB subtests yielded age-, sex-, and education level-normed standard z scores ($M = 0$, $SD = 1$). The Digit Span subtest from the WAIS-IV yielded age-normed standard scaled scores ($M = 10$, $SD = 3$). Scores on this latter subtest were converted to z-scores so as to allow for easy comparison, as well as calculation of a composite WM score. For the SSP, the outcome variable was the average of z-scores for the longest forward span sequence recalled (SSPFSL) and the longest backward span sequence (SSPRSL). For the PAL, the outcome variable was calculated by averaging the z-scores for total errors (PALTEA) and number of times the participant chose the correct stimulus on their first attempt for a trial

(PALFAMS). The outcome variable for RVP was the average of z-scores for A' (A prime; a signal detection measure of a participant's sensitivity to the target sequence, regardless of response tendency) and probability of false alarms (RVPPFA). The SWM's outcome variable was the average of z-scores for total errors (SWMBE) and strategy use (i.e., number of times the participant begins a new trial with a different box; SWMS). The outcome variable used for the Digit Span subtest was the overall score, which is based on performance across the forward, backward, and sequencing trials. These five WM outcome z-scores were then averaged to generate a composite WM score.

In cases where either (a) a participant's score on an individual subtest was an outlier (>2.5 SD above sample mean) or (b) a participant reported distractions in the testing environment that may have affected performance, the composite score was based on the remaining four subtests. This resulted in a single subtest being discarded for 6 participants (5 PKU, 1 non-PKU; no participants had more than one problematic subtest).

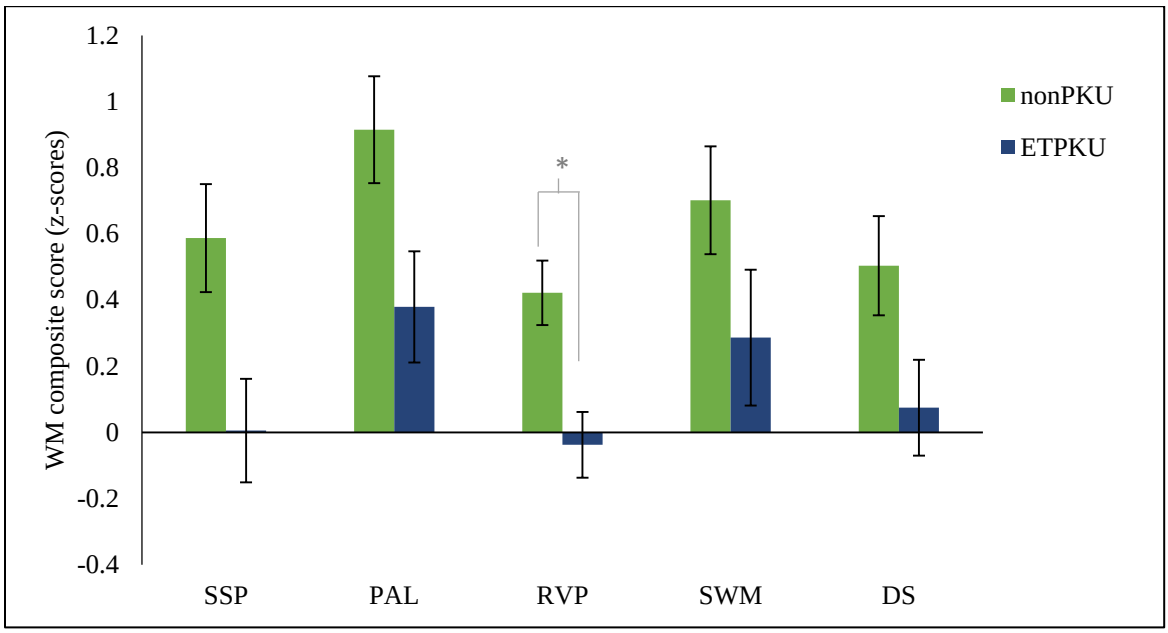
Anxiety Composite Score. Due to the dichotomous nature of the MINI output, an anxiety composite was created that separated participants into two groups ("low anxiety" and "high anxiety"). Participants in the high anxiety group (1) met criteria for an anxiety disorder on the MINI, (2) scored "at-risk" or above on the Anxiety Problems scale of the ASR (overall T-score $\geq$ 65), and/or (3) scored "at-risk" or above on the Anxiety Problems scale of the ABCL (overall T-score $\geq$ 65).

Results

Hypothesis #1a: ETPKU is associated with impaired WM performance. To test this hypothesis, a hierarchical linear regression model was used, with the WM composite score serving as the dependent variable. Matrix Reasoning scores were entered into the first step of the model in order to account for the contribution of overall intellectual ability, and diagnosis (ETPKU and non-PKU) was entered in the second step. A main effect of diagnosis was apparent [$t(77) = 2.48$, $p = .015$, $pr^2 = .07$], with the ETPKU group performing significantly worse than the non-PKU group on overall WM performance ($M_{PKU} = 0.12$; $M_{Non\text{-}PKU} = 0.63$). As can be seen in Figure 3, post hoc analysis revealed a general trend of poorer performance by the PKU group as compared to the non-PKU group across all five subtests; however, the effect reached statistical significance for only the RVP subtest [$t(76) = 2.54$, $p = .013$, $pr^2 = .08$].

Figure 3

Group Differences on Individual Working Memory Tasks



Note: SSP = Spatial Span; PAL = Paired Associates Learning; RVP = Rapid Visual Information Processing; SWM = Spatial Working Memory; DS = Digit Span

*$p < .05$.

To examine the relationship between metabolic control (as reflected by phe levels) and WM performance within the ETPKU group, additional regression analyses were conducted. Matrix Reasoning scores were entered into the first step of the model followed by most recent phe level in the second step. This analysis was repeated with phe level metrics for each of the remaining developmental epochs (i.e., early childhood, middle childhood, etc) serving as an independent variable, in turn. WM performance was not significantly associated with any of the phe level metrics [$t < 1.69$, $p > .10$, $pr^2 < .10$ in all instances]. The resulting partial correlations are included in Table 3. Exploratory

analyses examining the relationship between phe levels and individual WM tasks yielded similar results and are detailed in Supplementary Table S1.

Table 3

Relationship Between Metabolic Markers, WM performance, and Anxiety

Phenylalanine Levels (μmol/L)	WM composite (*pr*)
IDC for early childhood (0-5 yrs)	-.17
IDC for middle childhood (6-11 yrs)	-.31
IDC for adolescence (12-17 yrs)	.01
IDC for adulthood (18+ yrs)	-.25
IDC for lifetime (0-present)	-.22
Mean level for previous year	-.08
Most recent level	-.04

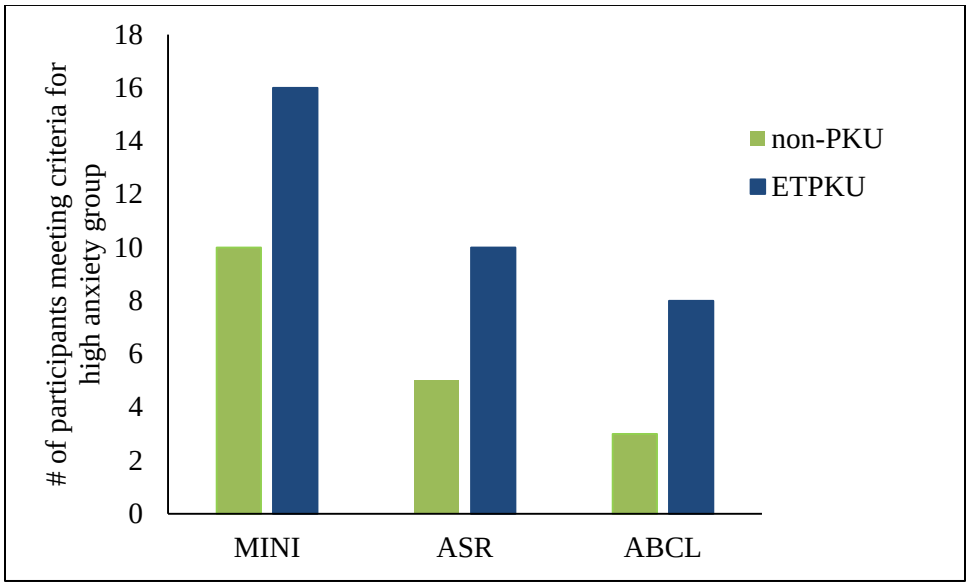
Note. *pr* = partial correlation; IDC = Index of Dietary Control (mean of all half-year median phe levels)

Hypothesis #1b: ETPKU is associated with increased anxiety symptomatology. A chi-square test comparing the ETPKU and non-PKU groups on the anxiety composite was performed. Results indicated significantly more ETPKU participants than non-PKU participants met criteria for high anxiety [X^2 (1, 80) = 4.18, p = .041; 21 ETPKU vs 12 non-PKU]. As can be seen in Figure 4, there was a general trend of more participants with ETPKU meeting criteria for the high anxiety group as compared to participants without PKU, although no significant group

differences were observed on any single assessment measures. Exploratory analyses examining potential group differences on individual ASEBA subscales (including non-anxiety subscales) are illustrated in Supplementary Figures S1 and S2.

Figure 4

Group Differences on Individual Anxiety Assessments



Note. MINI = MINI International Neuropsychiatric Interview; ASR = ASEBA Adult Self-Report; ABCL = ASEBA Adult Behavior Checklist (other-report)

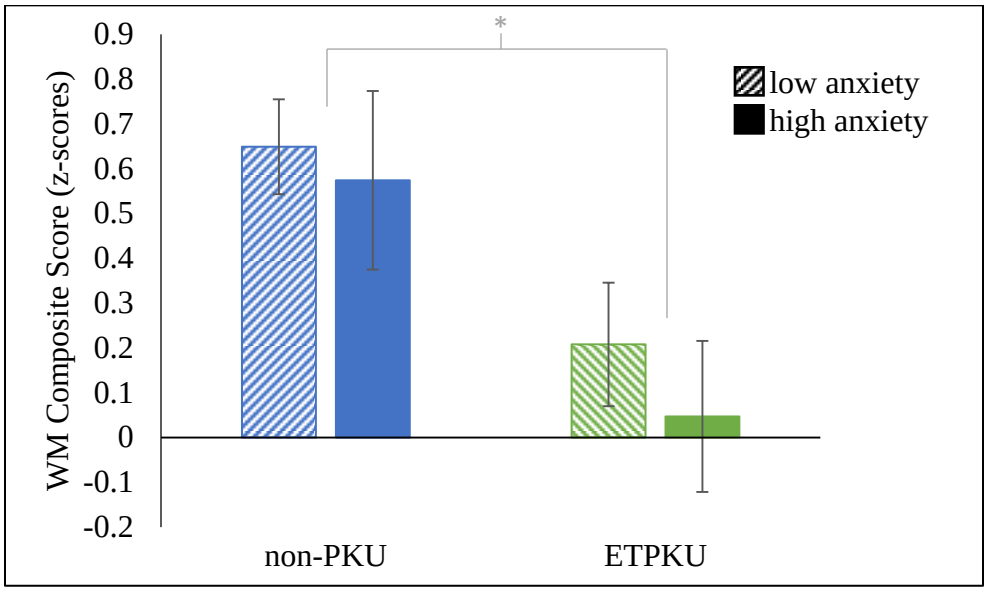
To explore the relationship between phe levels and anxiety symptomatology within the ETPKU group, a series of t-tests was performed comparing the low anxiety and high anxiety groups on phe levels for each developmental epoch. Results indicated that there were no significant differences between anxiety groups on phe levels ($p < .14$ in all instances).

Hypothesis #2: Anxiety will moderate the relationship between diagnosis (ETPKU vs non-PKU) and WM performance. For Aim 2's primary data analysis, a

hierarchical regression analysis was performed with WM task performance serving as the dependent variable. Matrix Reasoning score was entered into the first step of the model, followed by diagnosis (ETPKU and non-PKU) and anxiety group (low and high anxiety) in the second step, and the interaction term (diagnosis x anxiety group) in the third and final step. Results revealed a main effect of diagnosis [$t(76) = 2.20$, $p = .031$, $pr^2 = .06$]. Neither the main effect of anxiety group [$t(76) < 1$, $p = .343$, $pr^2 = .01$] nor the interaction term were significant [$t(75) < 1$, $p = .785$, $pr^2 < .01$; see Figure 5].

Figure 5

WM performance Shown Separately by Diagnosis and Anxiety Group



Note: There was a significant main effect of diagnosis on WM performance. There was no significant effects of anxiety or diagnosis x anxiety interaction. Error bars represent the standard error of the mean.

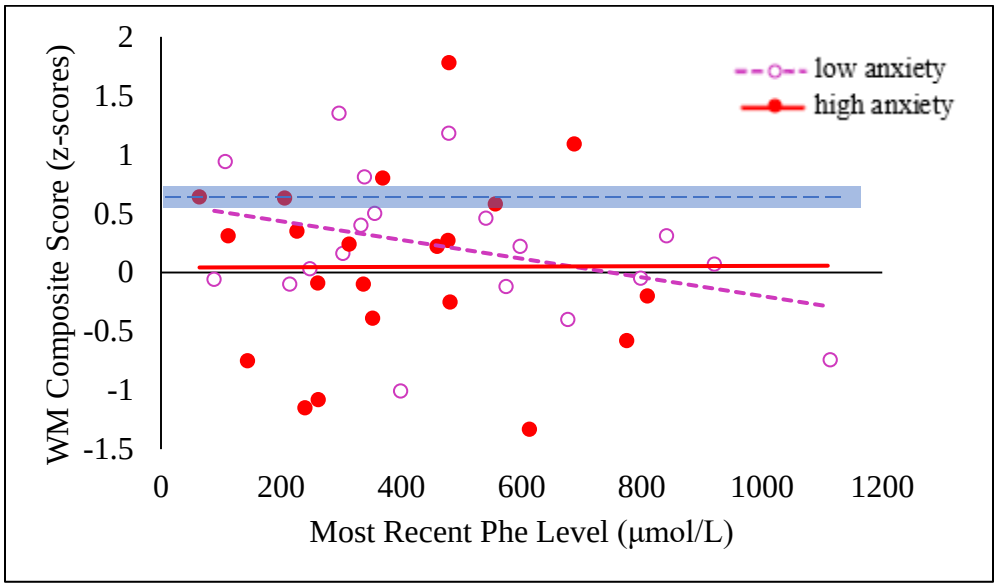
*$p < .05$.

Hypothesis #3: Anxiety will moderate the relationship between metabolic control (as reflected by blood phe levels) and WM performance within the ETPKU group. A hierarchical regression analysis was performed with Matrix Reasoning entered in the first step, followed by most recent phe level and anxiety group (low and high anxiety) entered in the second step, and the moderator factor (phe level x anxiety group) in the final step. This analysis was repeated with phe level data for each of the remaining developmental epochs (i.e., early childhood, middle childhood, etc) serving as an independent variable, in turn.

Results revealed a significant interaction between most recent phe level and anxiety group [$t(35) = 2.859$, $p = .007$, $pr^2 = .19$] and between mean phe level for previous year and anxiety group [$t(35) = 2.674$, $p = .011$, $pr^2 = .17$]. As illustrated in Figures 6 and 7, in both cases the interaction appears to be driven by the presence of a negative relationship between phe levels and WM scores for individuals with low anxiety (i.e., higher phe is related to poorer WM performance) whereas no such relationship was observed for the high anxiety group. There were no significant main effects ($p > .11$; $pr^2 < .01$, in all instances) or significant interactions for the other phe metrics ($p > .10$, $pr^2 < .13$, in all instances). The effect size (i.e., partial correlation) associated with the interaction term for each phe metric are included in Table 4.

Figure 6

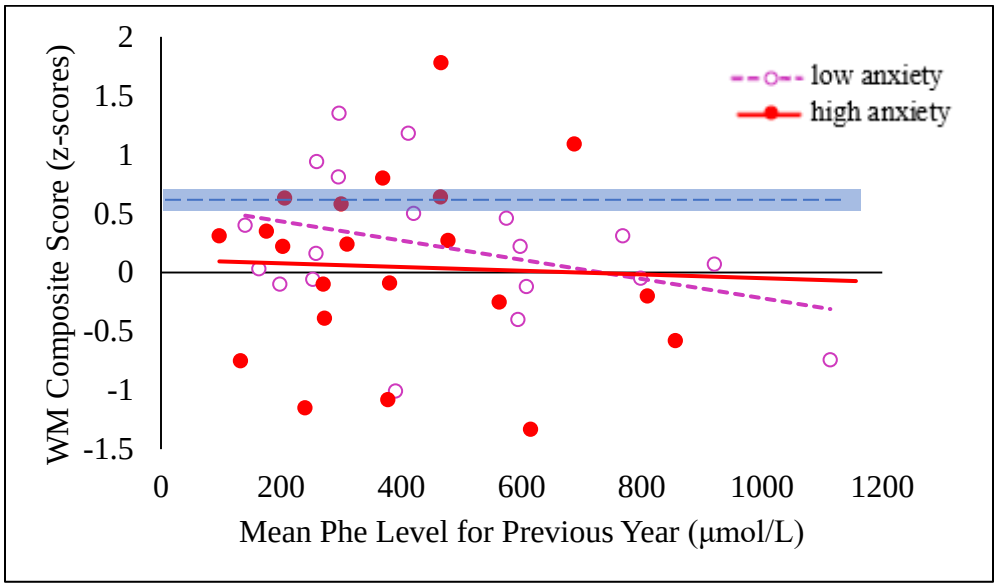
Scatterplot of Most Recent Phe Levels and WM Composite Scores Shown Separately by Anxiety Group



Note. There was a significant interaction effect of phe level x anxiety on WM performance at $p = .007$. The blue dashed line represents the mean of the non-PKU group's WM performance with the light blue shade representing the standard error of the mean.

Figure 7

Scatterplot of Mean Phe Level for the Previous Year and WM composite Scores Shown Separately by Anxiety Group



Note. There was a significant interaction effect of phe level x anxiety on WM performance (p = .011). The blue dotted line represents the mean for the non-PKU group, and the light blue shade representing the standard error of the mean.

Table 4

Interaction Between Metabolic Markers and Anxiety on WM Task Performance

Phenylalanine Levels (μmol/L)	Partial correlation (*pr*) of interaction term
IDC for early childhood (0-5 yrs)	.35
IDC for middle childhood (6-11 yrs)	.09
IDC for adolescence (12-17 yrs)	.09
IDC for adulthood (18+ yrs)	.25
IDC for lifetime (0-present)	<.01
Mean level for previous year	.41*
Most recent level	.44*

Note. IDC = Index of Dietary Control (mean of all half-year median phe levels)

*$p < .05$.

Of note, the aforementioned analyses were rerun excluding participants currently on anti-anxiety medications (10 ETPKU, 6 non-PKU participants). The overall pattern of WM results remained unchanged, with a main effect of diagnosis as well as a significant interaction between phe levels and anxiety.

Discussion

The present study examined WM, anxiety, and the relationship between these two factors in individuals with and without ETPKU. We predicted that the ETPKU group would demonstrate poorer WM performance and increased anxiety symptoms relative to the non-PKU group. Furthermore, we hypothesized that anxiety would moderate the relationship between diagnosis (ETPKU vs non-PKU) and WM performance. Within the ETPKU group, we anticipated that poorer metabolic control would similarly be associated with worse WM performance and elevated anxiety. In addition, it was predicted that anxiety would moderate the relationship between metabolic control and WM performance in this group.

As anticipated, the present ETPKU group performed more poorly than the non-PKU group on the WM composite. These findings align with a growing number of studies demonstrating deficits in WM performance in both children and adults with ETPKU (e.g., Bartus et al., 2018; Brumm et al., 2004; Channon et al., 2004; DeRoche & Welsh, 2008; White et al., 2002). In addition to performance-based studies, a recent study examining executive functioning symptomatology in everyday life through self- and caregiver- reports found that WM was the single most impaired executive functioning domain in ETPKU (Christ et al., 2020).

The present study's WM composite was comprised of several different WM tasks, tapping into various components of WM. As previously mentioned, WM is comprised of multiple component processes including storage and maintenance of information, as well as executive processes involved in information transformation and manipulation. Additionally, WM has separate but inter-related domains for processing phonological and

visuospatial stimuli. Tasks in the present study were chosen so that all components of WM were represented: SSP (storage and executive components; visuospatial sketchpad), PAL (storage; visuospatial sketchpad), RVP (storage and executive components; visuospatial sketchpad), SWM (storage and executive components; visuospatial sketchpad), and Digit Span (storage and executive components; phonological loop). Taken together, these results support previous findings of impairments across WM domains.

In addition to worse performance overall, the ETPKU group also demonstrated a trend of poorer performance on each of the individual WM tasks, with the RVP task reaching statistical significance. These trends support previous findings of ETPKU-related deficits on specific WM tasks. For example, the Digit Span task has revealed impaired WM performance in adults with ETPKU (Brumm et al., 2004) while the child-version of the Digit Span task has trended towards significance (p = .066; Anderson et al., 2004). In addition, Bik-Multanowski and colleagues (2011) utilized several of the CANTAB tasks used in the present study (SSP, RVP, SWM) and found deficits on all 3 of these tasks for their ETPKU participants as compared to normative data. The SWM has also been utilized in other past ETPKU studies, with one study finding evidence of ETPKU impairment (Bartus et al., 2018) and another study finding no evidence of impairment (Luciana et al., 2001). To the best of our knowledge, the present study represents the first to administer the PAL subtest to individuals with ETPKU. Our results suggest that participants with ETPKU demonstrate impaired WM and that impairment is most sensitive to a pooled measure of WM rather than individual tasks.

Presently we also found a higher prevalence of anxiety symptomatology in our ETPKU group as compared to the non-PKU group. As previously mentioned, the pattern of past findings in ETPKU is somewhat mixed, but generally points towards an elevated risk for anxiety (Smith et al., 1988; Pietz et al., 1997; Weglage et al., 2000; Bilder et al., 2013; Jahja et al., 2013; Manti et al., 2016; Waisbren et al., 2017). In the present study, we took a comprehensive threshold-based approach to quantifying anxiety symptomatology in our sample. The cumulative data from three different assessment approaches (self-report, other-report, and diagnostic interview) were utilized to identify individuals who were at clinical risk for high anxiety. Similar to the present study, Waisbren and colleagues (2017) categorized participants as low versus high anxiety based on either meeting cut-off criteria on a self-report anxiety measure and/or receiving treatment for an anxiety disorder and found evidence of elevated anxiety risk in their ETPKU population. The present study's results highlight the importance of examining clinically meaningful cut-offs for anxiety symptoms. Furthermore, comparable to the WM pattern of results, the overall anxiety composite demonstrated a significant group difference while the individual anxiety measures demonstrated non-significant trends. These findings suggest that significant group differences may be overlooked when relying on a single assessment approach (i.e., self-report vs other-report vs diagnostic interview).

Relationship with Metabolic Control

The present study did not find any evidence of a relationship between WM performance and phe levels (most recent or historic). Past research has produced inconsistent findings, with some studies finding a significant relationship between WM

performance and phe levels (e.g., Bik-Multanowski et al., 2011; Channon et al., 2004), and other studies finding no such relationship (e.g., Brumm et al., 2014; Bartus et al., 2018). The present pattern of findings mirror those of a few recent studies (Christ et al, 2020; Romani et al., 2017 and Palermo et al., 2017 combined) which found that WM difficulties were generally elevated in individuals with ETPKU (as compared to non-PKU individuals or normative data); however, the magnitude of the difficulties was not strongly correlated with phe levels.

The aforementioned study by Romani et al (2017) also examined the impact of phe on multiple neurocognitive domains (not only WM). Overall, a pattern emerged such that some cognitive domains (e.g., inhibitory control, verbal memory and learning) were relatively more sensitive to metabolic control (i.e., phe levels) within the ETPKU group, but did not demonstrate strong group-related (ETPKU vs non-PKU) differences. Conversely, other domains (e.g., complex executive functions, orthographic processing) yielded group effects but not phe-related effects. This data suggests that there may be particular neurocognitive tasks or domains that are more sensitive to group versus metabolic control differences and vice versa. As discussed earlier, the neurological impact of PKU is lessened by maintaining good metabolic control (i.e., low phe levels). However, the neurochemical imbalances associated with ETPKU (e.g., depleted levels of dopamine and other catecholamines) cannot be entirely avoided. Although speculative, different neurocognitive domains may be more sensitive to each of these pathways of disruption (higher phe levels vs catecholaminergic metabolism disruption) thus manifesting as phe-related vs group-related effects, respectively. Additional research is needed to better evaluate this hypothesis.

In addition, the present study found no relationship between phe levels and anxiety symptomatology. Whereas some past studies have reported a relationship between anxiety and phe levels (Clacy et al., 2014; Waisbren et al., 2017), others have failed to find any relationship (Weglage et al., 2000; Brumm et al., 2004; Bilder et al., 2013; Jahja et al., 2017). One possible explanation is that, similar to varied sensitivities of neurocognitive tasks to phe level, anxiety symptomatology may be differentially sensitive to the effects of phe level. Based on the hypothesis that anxiety in this population is driven by decreased serotonin (due to competition with phe), a phe-related effect would be expected. Our results suggest the opposite; that, like WM, anxiety may be sensitive to the general, inherent effects of having PKU, rather than a specific neurochemical change associated with phe levels.

Therefore, our results seem to support a group-level mechanism for anxiety. One explanation may be that there is a substantial psychosocial burden of living with chronic illness, and this stress contributes to an increased risk of psychiatric concerns among all individuals with ETPKU regardless of phe levels (Stanton et al., 2007). A survey by the National PKU Alliance (NPKUA) completed by 625 individuals with ETPKU highlights this issue, with 51.7% of respondents indicating difficulty in managing their PKU, including maintaining a phe-restricted diet (Brown & Lichter-Konecki, 2016). Future research studies should employ the use of assessments that address this psychosocial burden hypothesis, such as the Phenylketonuria – Quality of Life Questionnaire (PKU-QOL; Regnault et al., 2015) which has been validated for use in this population.

Interaction with Anxiety and Working Memory

Whereas we did not observe an interaction between diagnosis and anxiety on WM performance, anxiety did moderate the relationship between phe levels and WM within the ETPKU group. The nature of the moderating relationship was slightly different than that predicted. Our original hypothesis was that we would see a detrimental effect of phe level on WM performance for all individuals with ETPKU, but that the effect would be magnified for individuals with anxiety. We found that the high anxiety group appears to be performing generally below their non-PKU peers, regardless of phe level (as can be seen in Figures 6 and 7). In contrast, it appears that individuals with low anxiety perform similarly to their non-PKU peers if they had low phe levels, with worsening performance as phe levels increased. These findings may indicate that for individuals with ETPKU, high phe levels and/or high anxiety are risk factors for worse cognitive performance while low phe levels may only be protective in the absence of clinically high anxiety. High anxiety appears to substantially deplete WM resources to the point that the addition of a second risk factor to WM (i.e., high phe) has no influence. These findings may help explain discrepant findings in the literature between metabolic control and WM. For example, individuals with ETPKU in good metabolic control but who have high anxiety are likely to exhibit WM impairments comparable to those seen in individuals with ETPKU in poorer metabolic control. Failure to account for this relationship may have contributed to mixed findings in past studies of WM in ETPKU. Future neuropsychological research on ETPKU would greatly benefit from the inclusion of measures of anxiety and examination of its impact on neurocognitive results.

The Use of Multiple Measures

A strength of the present study was the utilization of multiple metrics of both WM and anxiety. With regards to WM, the present study examined the composite score of both visual and verbal WM tasks. Interestingly, although performance on the overall WM composite was lower in the ETPKU group than the non-PKU group, there was only one individual task (Rapid Visual Information Processing) that was significantly different between groups. This implies that if we had chosen only one WM task to administer, we likely would have missed a significant group difference. By utilizing multiple measures and a composite score, we were able to capture a broader sample of WM performance, thereby decreasing heterogeneity and variability in performance associated with relying on a single task. This approach may be especially beneficial when dealing with rare diseases, such as PKU, which are often associated with relatively small sample sizes.

In a similar vein, the present study utilized three assessments, a self-report, other-report, and clinical interview, to examine anxiety in this population. Similar to the WM results, although the diagnostic groups differed significantly on the composite anxiety score, no individual anxiety assessment demonstrated significant group differences. Again, this highlights the importance of measuring constructs of interest using multiple tools and may help explain why the literature has been so mixed on the prevalence of anxiety in this population if most studies have only used one anxiety assessment. The majority of previous studies have chosen self-report questionnaires. This is potentially problematic, as an argument could be made that cognitive skills impacted in PKU, such as memory are critical for accurate completion of self-report measures. As such, this

population may benefit from additional input on psychological functioning by an outside observer, such as a close friend, family member, and/or clinical psychologist.

Limitations & Future Directions

The present study illustrated the value and feasibility of remote assessment for studying neurocognitive and psychological functioning in individuals with ETPKU. Potential advantages of this approach include overcoming geographical limitations typically associated with in-person studies and access to larger sample sizes for rare disease research. However, challenges associated with this line of research include issues related to having less control over the testing environment and thus more need to monitor performance (quality control). In addition, the CANTAB has been validated for use as a remote assessment (Cormack et al., 2017, 2018), and there is some preliminary support for administration of the Digit Span subtest for remote administration (Hamner et al., 2021; Wright, 2020). However, there is a need for additional validated remote neuropsychological tests/batteries that work across multiple platforms.

A limitation of the present study was the availability (or lack thereof) of full lifetime phe level data for all of the participants with ETPKU. All efforts were made to acquire/review all available metabolic records for study participants. However, a number of participants had been seen at multiple clinics throughout their lifetime, and older records were sometimes unavailable due to a variety of reasons (e.g., destruction of old records, clinics closing). This will likely continue to be a challenge for studies of adults with PKU moving forward. The use of longitudinal research studies with robust retention efforts may help to fill in these gaps.

Findings from this study have implications for clinical practice. The present study highlights the prevalence of clinically significant levels of anxiety in the ETPKU population, and the potential impact of that anxiety on cognitive performance. In light of these findings, metabolic clinics would benefit from screening their patients for not only neurocognitive difficulties but also psychiatric symptoms and connecting them with appropriate referrals if warranted. In fact, in a survey put out by the National Phenylketonuria Alliance (NPKUA), over half of respondents with ETPKU indicated that reducing anxiety and depression symptoms would be a top priority for them when considering new treatments (Brown & Lichter-Konecki, 2016). In addition to targeting diet and medication adherence rates, professionals working with this population should be aware of the high risk of mental health disorders and include psychiatric or psychological intervention in their treatment plans. In light of findings of the combined impact of anxiety and recent high phe levels on WM performance, patients with ETPKU should also be encouraged to maintain good metabolic control throughout the lifespan, not just during childhood years.

Summary and Conclusions

In summary, the present results indicate higher rates of WM impairments and anxiety symptoms in ETPKU than the general population. Furthermore, in the ETPKU group, anxiety moderated the relationship between recent phe levels and WM performance. In the absence of anxiety, higher phe levels were associated with poorer WM performance. In the presence of anxiety, WM performance was impaired relative to individuals without ETPKU, regardless of phe levels. In addition to advancing our understanding of the inter-relationship between metabolic control, anxiety, and WM in

individuals with ETPKU, the present study demonstrates the feasibility and potential value of remote assessment for studying ETPKU and other rare genetic disorders. In addition, this study highlights the importance of monitoring both cognitive skills and mental health symptoms in this population.

REFERENCES

Airaksinen, E., Larsson, M., & Forsell, Y. (2005). Neuropsychological functions in anxiety disorders in population-based samples: Evidence of episodic memory dysfunction. *Journal of Psychiatric Research*, *39*(2), 207–214.

Aldridge, K., Cole, K. K., Moffitt, A. J., Peck, D., & Christ, S. E. (2020). The Effects of Early-Treated Phenylketonuria on Volumetric Measures of the Cerebellum. *Molecular Genetics and Metabolism Reports*.

American Psychiatric Association. (2013). *Diagnostic and Statistical Manual of Mental Disorders: DSM-5* (Fifth Edition). American Psychiatric Association.

Anderson, P. J., & Leuzzi, V. (2010). White matter pathology in phenylketonuria. *Molecular Genetics and Metabolism*, *99,* S3–S9.

Anderson, P. J., Wood, S. J., Francis, D. E., Coleman, L., Anderson, V., & Boneh, A. (2007). Are neuropsychological impairments in children with early-treated phenylketonuria (PKU) related to white matter abnormalities or elevated phenylalanine levels? *Developmental Neuropsychology*, *32*(2), 645–668.

Anderson, P. J., Wood, S. J., Francis, D. E., Coleman, L., Warwick, L., Casanelia, S., Anderson, V. A., & Boneh, A. (2004). Neuropsychological functioning in children with early-treated phenylketonuria: Impact of white matter abnormalities. *Developmental Medicine and Child Neurology*, *46*(4), 230–238.

Antenor-Dorsey, J. A. V., Hershey, T., Rutlin, J., Shimony, J. S., McKinstry, R. C., Grange, D. K., Christ, S. E., & White, D. A. (2013). White matter integrity and executive abilities in individuals with phenylketonuria. *Molecular Genetics and Metabolism*, *109*(2), 125–131.

Antshel, K. M., & Waisbren, S. E. (2003). Developmental timing of exposure to elevated levels of phenylalanine is associated with ADHD symptom expression. *Journal of Abnormal Child Psychology*, *31*(6), 565–574.

Bacqué-Cazenave, J., Bharatiya, R., Barrière, G., Delbecque, J.-P., Bouguiyoud, N., Di Giovanni, G., Cattaert, D., & De Deurwaerdère, P. (2020). Serotonin in Animal Cognition and Behavior. *International Journal of Molecular Sciences*, *21*(5).

Baddeley, A. D., & Hitch, G. (1974). Working Memory. In G. H. Bower (Ed.), *Psychology of Learning and Motivation* (Vol. 8, pp. 47–89). Academic Press.

Bartus, A., Palasti, F., Juhasz, E., Kiss, E., Simonova, E., Sumanszki, C., & Reismann, P. (2018). The influence of blood phenylalanine levels on neurocognitive function in adult PKU patients. *Metabolic Brain Disease*, *33*(5), 1609–1615.

Bickel, H., Gerrard, J., & Hickmans, E. M. (1953). Influence of phenylalanine intake on phenylketonuria. *Lancet (London, England)*, *265*(6790), 812–813.

Bickel, H., Gerrard, J., & Hickmans, E. M. (1954). The influence of phenylalanine intake on the chemistry and behaviour of a phenyl-ketonuric child. *Acta Paediatrica*, *43*(1), 64–77.

Bik-Multanowski, M., Pietrzyk, J. J., & Mozrzymas, R. (2011). Routine use of CANTAB system for detection of neuropsychological deficits in patients with PKU. *Molecular Genetics and Metabolism*, *102*(2), 210–213.

Bilder, D. A., Burton, B. K., Coon, H., Leviton, L., Ashworth, J., Lundy, B. D., Vespa, H., Bakian, A. V., & Longo, N. (2013). Psychiatric symptoms in adults with phenylketonuria. *Molecular Genetics and Metabolism*, *108*(3), 155–160.

Bodner, K. E., Aldridge, K., Moffitt, A. J., Peck, D., White, D. A., & Christ, S. E. (2012). A volumetric study of basal ganglia structures in individuals with early-treated phenylketonuria. *Molecular Genetics and Metabolism*, *107*(3), 302–307.

Boland, K. M., Christ, S. E., Abbene, E., & Clocksin, H. E. (in prep). *Relationship between Mental Health Concerns and Cognition in Phenylketonuria*.

Brenton, D. P., & Pietz, J. (2000). Adult care in phenylketonuria and hyperphenylalaninaemia: The relevance of neurological abnormalities. *European Journal of Pediatrics*, *159*(S2), S114–S120.

Brown, C. S., & Lichter-Konecki, U. (2016). Phenylketonuria (PKU): A problem solved? *Molecular Genetics and Metabolism Reports*, *6*, 8–12.

Brumm, V. L., Azen, C., Moats, R. A., Stern, A. M., Broomand, C., Nelson, M. D., & Koch, R. (2004). Neuropsychological outcome of subjects participating in the PKU Adult Collaborative Study: A preliminary review. *Journal of Inherited Metabolic Disease*, *27*(5), 549–566.

Burlina, A. (2019). The neurological and psychological phenotype of adult patients with early-treated phenylketonuria:A systematic review. *Journal of Inherited Metabolic Disease*, *42*(2), 209–219.

Burton, B. K., Leviton, L., Vespa, H., Coon, H., Longo, N., Lundy, B. D., Johnson, M., Angelino, A., Hamosh, A., & Bilder, D. (2013). A diversified approach for PKU treatment: Routine screening yields high incidence of psychiatric distress in phenylketonuria clinics. *Molecular Genetics and Metabolism*, *108*(1), 8–12.

Castaneda, A. E., Suvisaari, J., Marttunen, M., Perälä, J., Saarni, S. I., Aalto-Setälä, T., Lönnqvist, J., & Tuulio-Henriksson, A. (2011). Cognitive functioning in a

population-based sample of young adults with anxiety disorders. *European Psychiatry*, *26*(6), 346–353.

Channon, S., German, E., Cassina, C., & Lee, P. (2004). Executive functioning, memory, and learning in phenylketonuria. *Neuropsychology*, *18*(4), 613–620.

Channon, S., Goodman, G., Zlotowitz, S., Mockler, C., & Lee, P. J. (2007). Effects of dietary management of phenylketonuria on long-term cognitive outcome. *Archives of Disease in Childhood*, *92*(3), 213–218.

Channon, S., Mockler, C., & Lee, P. (2005). Executive Functioning and Speed of Processing in Phenylketonuria. *Neuropsychology*, *19*(5), 679–686.

Chein, J. M., Moore, A. B., & Conway, A. R. A. (2011). Domain-general mechanisms of complex working memory span. *NeuroImage*, *54*(1), 550–559.

Christ, S. E. (2003). Asbjørn Følling and the discovery of phenylketonuria. *Journal of the History of the Neurosciences*, *12*(1), 44–54.

Christ, S. E., Clocksin, H. E., Burton, B. K., Grant, M. L., Waisbren, S., Paulin, M.-C., Bilder, D. A., White, D. A., & Saville, C. (2020). Executive function in phenylketonuria (PKU): Insights from the Behavior Rating Inventory of Executive Function (BRIEF) and a large sample of individuals with PKU. *Neuropsychology*, *34*(4), 456–466.

Christ, S. E., Huijbregts, S. C. J., de Sonneville, L. M. J., & White, D. A. (2010). Executive function in early-treated phenylketonuria: Profile and underlying mechanisms. *Molecular Genetics and Metabolism, 99 Suppl 1*, S22-32.

Christ, S. E., Moffitt, A. J., Peck, D., & White, D. A. (2013). The effects of tetrahydrobiopterin (BH4) treatment on brain function in individuals with phenylketonuria. *NeuroImage: Clinical*, *3*, 539–547.

Christ, S. E., Price, M. H., Bodner, K. E., Saville, C., Moffitt, A. J., & Peck, D. (2016). Morphometric analysis of gray matter integrity in individuals with early-treated phenylketonuria. *Molecular Genetics and Metabolism*, *118*(1), 3–8.

Chung, S., Fieremans, E., Kucukboyaci, N. E., Wang, X., Morton, C. J., Novikov, D. S., Rath, J. F., & Lui, Y. W. (2018). Working Memory And Brain Tissue Microstructure: White Matter Tract Integrity Based On Multi-Shell Diffusion MRI. *Scientific Reports*, *8*.

Clacy, A., Sharman, R., & McGill, J. (2014). Depression, Anxiety, and Stress in Young Adults with Phenylketonuria: Associations with Biochemistry. *Behavioral Pediatrics*, *35*(6), 4.

Cleary, M. A., Walter, J. H., Wraith, J. E., Alani, S. M., Whittle, D., Jenkins, J. P. R., & Tyler, K. (1994). Magnetic resonance imaging of the brain in phenylketonuria. *The Lancet*, *344*(8915), 87–90.

Cormack, F., Backx, R., Cotter, J., Taptiklis, N., de Cock, L., Zavitz, K., & Barnett, J. H. (2017). *A comparison of in person and web based computerized cognitive testing using CANTAB*. Clinical Trials on Alzheimer's Disease, Boston, MA.

Cormack, F., Backx, R., Cotter, J., Taptiklis, N., de Cock, L., Zavitz, K., & Barnett, J. H. (2018). *A comparison of in person and web based computerized cognitive testing using CANTAB*. International Society for CNS Clinical Trials and Methodology, Washington, D.C.

Cupul-García, J. C., Hinojosa-Calvo, E., Villa-Rodríguez, M. Á., Herrera-Guzmán, I., & Padrós-Blázquez, F. (2018). Basic neuropsychological evaluation for adults in patients with generalized anxiety disorders. *Revista Chilena de Neuro-Psiquiatria*, *56*(3), 151–160.

Derakshan, N., & Eysenck, M. W. (2009). Anxiety, processing efficiency, and cognitive performance: New developments from attentional control theory. *European Psychologist*, *14*(2), 168–176.

DeRoche, K., & Welsh, M. (2008). Twenty-five years of research on neurocognitive outcomes in early-treated phenylketonuria: Intelligence and executive function. *Developmental Neuropsychology*, *33*(4), 474–504.

Didycz, B., & Bik-Multanowski, M. (2018). Blood phenylalanine instability strongly correlates with anxiety in phenylketonuria. *Molecular Genetics and Metabolism Reports*, *14*, 80–82.

Dillon, D. G., & Pizzagalli, D. A. (2007). Inhibition of Action, Thought, and Emotion: A Selective Neurobiological Review. *Applied & Preventive Psychology: Journal of the American Association of Applied and Preventive Psychology*, *12*(3), 99–114.

Dyer, C. A. (1999). Pathophysiology of phenylketonuria. *Mental Retardation and Developmental Disabilities Research Reviews*, *5*(2), 104–112.

Engle, R. W., Laughlin, J. E., Tuholski, S. W., & Conway, A. R. A. (1999). Working memory, short-term memory, and general fluid intelligence: A latent-variable approach. *Journal of Experimental Psychology: General*, *128*(3), 309–331.

Eysenck, M. W., & Calvo, M. G. (1992). Anxiety and performance: The processing efficiency theory. *Cognition and Emotion*, *6*(6), 409–434.

Eysenck, M. W., Derakshan, N., Santos, R., & Calvo, M. G. (2007). Anxiety and cognitive performance: Attentional control theory. *Emotion*, *7*(2), 336–353.

Følling, A. (1934). Über Ausscheidung von Phenylbrenztraubensäure in dem Harn als Stoffwechselanomalie in Verbindung mit Imbezillität. *Hoppe-Seyler's Zeitschrift Fuer Physiologische Chemie*, *227*, 169–176.

Følling, A. (1967). [Phenylketonuria]. *Tidsskr Nor Laegeforen*, *87*(5), 451–454.

Guthrie, R., & Susi, A. (1963). A Simple Phenylalanine Method for Detecting Phenylketonuria in Large Populations of Newborn Infants. *Pediatrics*, *32*, 338–343.

Hamner, T., Salorio, C. F., Kalb, L., & Jacobson, L. A. (2021). Equivalency of In-Person Versus Remote Assessment: WISC-V and KTEA-3 Performance in Clinically Referred Children and Adolescents. *Journal of the International Neuropsychological Society: JINS*, 1–10.

Harding, C. O., Amato, R. S., Stuy, M., Longo, N., Burton, B. K., Posner, J., Weng, H. H., Merilainen, M., Gu, Z., Jiang, J., & Vockley, J. (2018). Pegvaliase for the treatment of phenylketonuria: A pivotal, double-blind randomized discontinuation Phase 3 clinical trial. *Molecular Genetics and Metabolism*, *124*(1), 20–26.

Hargreaves, K. M., & Pardridge, W. M. (1988). Neutral amino acid transport at the human blood-brain barrier. *Journal of Biological Chemistry*, *263*(36), 19392–19397.

Hilliard, M. E., Herzer, M., Dolan, L. M., & Hood, K. K. (2011). Psychological screening in adolescents with type 1 diabetes predicts outcomes one year later. *Diabetes Research and Clinical Practice*, *94*(1), 39–44.

Hood, A., Antenor-Dorsey, J. A. V., Rutlin, J., Hershey, T., Shimony, J. S., McKinstry, R. C., Grange, D. K., Christ, S. E., Steiner, R., & White, D. A. (2015). Prolonged exposure to high and variable phenylalanine levels over the lifetime predicts brain white matter integrity in children with phenylketonuria. *Molecular Genetics and Metabolism*, *114*(1), 19–24.

Hood, K. K., Huestis, S., Maher, A., Butler, D., Volkening, L., & Laffel, L. M. B. (2006). Depressive Symptoms in Children and Adolescents With Type 1 Diabetes: Association with diabetes-specific characteristics. *Diabetes Care*, *29*(6), 1389–1389.

Jahja, R., Huijbregts, S. C. J., de Sonneville, L. M. J., van der Meere, J. J., Bosch, A. M., Hollak, C. E. M., Rubio-Gozalbo, M. E., Brouwers, M. C. G. J., Hofstede, F. C., de Vries, M. C., Janssen, M. C. H., van der Ploeg, A. T., Langendonk, J. G., & van Spronsen, F. J. (2013). Mental health and social functioning in early treated Phenylketonuria: The PKU-COBESO study. *Molecular Genetics and Metabolism*, *110*, S57–S61.

Jahja, R., Huijbregts, S. C. J., de Sonneville, L. M. J., van der Meere, J. J., Legemaat, A. M., Bosch, A. M., Hollak, C. E. M., Rubio-Gozalbo, M. E., Brouwers, M. C. G. J., Hofstede, F. C., de Vries, M. C., Janssen, M. C. H., van der Ploeg, A. T., Langendonk, J. G., & van Spronsen, F. J. (2017). Cognitive profile and mental health in adult phenylketonuria: A PKU-COBESO study. *Neuropsychology*, *31*(4), 437–447.

Janzen, D., & Nguyen, M. (2010). Beyond executive function: Non-executive cognitive abilities in individuals with PKU. *Molecular Genetics and Metabolism*, *99*, S47–S51.

Kim, C., Kroger, J. K., Calhoun, V. D., & Clark, V. P. (2015). The role of the frontopolar cortex in manipulation of integrated information in working memory. *Neuroscience Letters*, *595*, 25–29.

Kim, K. L., Christensen, R. E., Ruggieri, A., Schettini, E., Freeman, J. B., Garcia, A. M., Flessner, C., Stewart, E., Conelea, C., & Dickstein, D. P. (2019). Cognitive performance of youth with primary generalized anxiety disorder versus primary obsessive–compulsive disorder. *Depression and Anxiety*, *36*(2), 130–140.

Koch, R., Burton, B., Hoganson, G., Peterson, R., Rhead, W., Rouse, B., Scott, R., Wolff, J., Stern, A. M., Guttler, F., Nelson, M., De la Cruz, F., Coldwell, J., Erbe, R., Geraghty, M. T., Shear, C., Thomas, J., & Azen, C. (2002). Phenylketonuria in adulthood: A collaborative study. *Journal of Inherited Metabolic Disease*, *25*(5), 333–346.

Kono, K., Okano, Y., Nakayama, K., Hase, Y., Minamikawa, S., Ozawa, N., Yokote, H., & Inoue, Y. (2005). Diffusion-weighted MR imaging in patients with phenylketonuria: Relationship between serum phenylalanine levels and ADC values in cerebral white matter. *Radiology*, *236*(2), 630–636.

Kurt, E., Yıldırım, E., & Topçuoğlu, V. (2017). Executive functions of obsessive compulsive disorder and panic disorder patients in comparison to healty controls. *Noropsikiyatri Arsivi*, *54*(4), 312–317.

Landolt, M. A., Nuoffer, J.-M., Steinmann, B., & Superti-Furga, A. (2002). Quality of life and psychologic adjustment in children and adolescents with early treated phenylketonuria can be normal. *The Journal of Pediatrics*, *140*(5), 516–521.

Leonard, K., & Abramovitch, A. (2019). Cognitive functions in young adults with generalized anxiety disorder. *European Psychiatry*, *56*, 1–7.

Levaux, M.-N., Potvin, S., Sepehry, A. A., Sablier, J., Mendrek, A., & Stip, E. (2007). Computerized assessment of cognition in schizophrenia: Promises and pitfalls of CANTAB. *European Psychiatry*, *22*(2), 104–115.

Lowe, C., & Rabbitt, P. (1998). Test/re-test reliability of the CANTAB and ISPOCD neuropsychological batteries: Theoretical and practical issues. Cambridge Neuropsychological Test Automated Battery. International Study of Post-Operative Cognitive Dysfunction. *Neuropsychologia*, *36*(9), 915–923.

Luciana, M. (2003). Practitioner review: Computerized assessment of neuropsychological function in children: clinical and research applications of the Cambridge Neuropsychological Testing Automated Battery (CANTAB). *Journal of Child Psychology and Psychiatry, and Allied Disciplines*, *44*(5), 649–663.

Luciana, M., Sullivan, J., & Nelson, C. A. (2001). Associations between Phenylalanine-to-Tyrosine Ratios and Performance on Tests of Neuropsychological Function in Adolescents Treated Early and Continuously for Phenylketonuria. *Child Development*, *72*(6), 1637–1652.

Manti, F., Nardecchia, F., Chiarotti, F., Carducci, C., Carducci, C., & Leuzzi, V. (2016). Psychiatric disorders in adolescent and young adult patients with phenylketonuria. *Molecular Genetics and Metabolism*, *117*(1), 12–18.

Moore, A. B., Li, Z., Tyner, C. E., Hu, X., & Crosson, B. (2013). Bilateral basal ganglia activity in verbal working memory. *Brain and Language*, *125*(3), 316–323.

Moran, T. P. (2016). Anxiety and Working Memory Capacity: A Meta-Analysis and Narrative Review. *Psychological Bulletin*, *142*(8), 831–864.

Morrissette, D. A., & Stahl, S. M. (2014). Modulating the serotonin system in the treatment of major depressive disorder. *CNS Spectrums*, *19 Suppl 1*, 57–67; quiz 54–57, 68.

Morrow, S. A., Rosehart, H., & Pantazopoulos, K. (2015). Anxiety and Depressive Symptoms Are Associated With Worse Performance on Objective Cognitive Tests in MS. *The Journal of Neuropsychiatry and Clinical Neurosciences*, *28*(2), 118–123.

Murphy, Y. E., Luke, A., Brennan, E., Francazio, S., Christopher, I., & Flessner, C. A. (2018). An Investigation of Executive Functioning in Pediatric Anxiety. *Behavior Modification*, *42*(6), 885–913. https://doi.org/10.1177/0145445517749448

National Institutes of Health Consensus Development Panel. (2001). National Institutes of Health Consensus Development Conference Statement: Phenylketonuria: Screening and Management, October 16–18, 2000. *Pediatrics*, *108*(4), 972–982.

Osaka, M., Osaka, N., Kondo, H., Morishita, M., Fukuyama, H., Aso, T., & Shibasaki, H. (2003). The neural basis of individual differences in working memory capacity: An fMRI study. *NeuroImage*, *18*(3), 789–797.

Palermo, L., MacDonald, A., Limback, E., Robertson, L., Howe, S., Geberhiwot, T., & Romani, C. (2020). Emotional health in early-treated adults with phenylketonuria

(PKU): Relationship with cognitive abilities and blood phenylalanine. *Journal of Clinical and Experimental Neuropsychology*, *42*(2), 142–159.

Peng, H., Peck, D., White, D. A., & Christ, S. E. (2014). Tract-based evaluation of white matter damage in individuals with early-treated phenylketonuria. *Journal of Inherited Metabolic Disease*, *37*(2), 237–243.

Penrose, L. S. (1963). *The biology of mental defect* (3rd ed., Vol. xxiv). Sidgwick and Jackson.

Phillips, M. D., McGraw, P., Lowe, M. J., Mathews, V. P., & Hainline, B. E. (2001). Diffusion-weighted imaging of white matter abnormalities in patients with phenylketonuria. *AJNR. American Journal of Neuroradiology*, *22*(8), 1583–1586.

Pietz, J., Fätkenheuer, B., PhD, P. B., Armbruster, M., Esser, G., & Schmidt, H. (1997). Psychiatric Disorders in Adult Patients With Early-treated Phenylketonuria. *Pediatrics*, *99*(3), 345–350.

Postle, B. R., & Oberauer, K. (2020). Bradley R. Postle and Klaus Oberauer. In M. J. Kahana & A. D. Wagner (Eds.), *The Oxford Handbook of Human Memory*. Oxford University Press.

Prussien, K. V., DeBaun, M. R., Yarboi, J., Bemis, H., McNally, C., Williams, E., & Compas, B. E. (2018). Cognitive Function, Coping, and Depressive Symptoms in Children and Adolescents with Sickle Cell Disease. *Journal of Pediatric Psychology*, *43*(5), 543–551.

Puglisi-Allegra, S., Cabib, S., Pascucci, T., Ventura, R., Cali, F., & Romano, V. (2000). Dramatic brain aminergic deficit in a genetic mouse model of phenylketonuria. *Neuroreport*, *11*(6), 1361–1364.

Radwan, B., Liu, H., & Chaudhury, D. (2019). The role of dopamine in mood disorders and the associated changes in circadian rhythms and sleep-wake cycle. *Brain Research, 1713*, 42–51.

Regnault, A., Burlina, A., Cunningham, A., Bettiol, E., Moreau-Stucker, F., Benmedjahed, K., & Bosch, A. M. (2015). Development and psychometric validation of measures to assess the impact of phenylketonuria and its dietary treatment on patients' and parents' quality of life: The phenylketonuria – quality of life (PKU-QOL) questionnaires. *Orphanet Journal of Rare Diseases, 10*, 59.

Ris, M. D., Weber, A. M., Hunt, M. M., Berry, H. K., Williams, S. E., & Leslie, N. (1997). Adult psychosocial outcome in early-treated phenylketonuria. *Journal of Inherited Metabolic Disease, 20*(4), 499–508.

Sarason, I. G. (1988). Anxiety, self-preoccupation and attention. *Anxiety Research*, *1*(1), 3–7.

Schindeler, S., Ghosh-Jerath, S., Thompson, S., Rocca, A., Joy, P., Kemp, A., Rae, C., Green, K., Wilcken, B., & Christodoulou, J. (2007). The effects of large neutral amino acid supplements in PKU: An MRS and neuropsychological study. *Molecular Genetics and Metabolism*, *91*(1), 48–54.

Schuck, P. F., Malgarin, F., Cararo, J. H., Cardoso, F., Streck, E. L., & Ferreira, G. C. (2015). Phenylketonuria Pathophysiology: On the Role of Metabolic Alterations. *Aging and Disease, 6*(5), 390–399.

Schulze, E. T., Geary, E. K., Susmaras, T. M., Paliga, J. T., Maki, P. M., & Little, D. M. (2011). Anatomical Correlates of Age-Related Working Memory Declines. *Journal of Aging Research*, *2011*.

Shackman, A. J., Sarinopoulos, I., Maxwell, J. S., Pizzagalli, D. A., Lavric, A., & Davidson, R. J. (2006). Anxiety selectively disrupts visuospatial working memory. *Emotion*, *6*(1), 40–61.

Shunmugasundaram, C., Rutherford, C., Butow, P. N., Sundaresan, P., & Dhillon, H. M. (2020). What are the optimal measures to identify anxiety and depression in people diagnosed with head and neck cancer (HNC): A systematic review. *Journal of Patient-Reported Outcomes*, *4*.

Smith, I., Beasley, M. G., Wolff, O. H., & Ades, A. E. (1988). Behavior disturbance in 8-year-old children with early treated phenylketonuria: Report from the MRC/DHSS phenylketonuria register. *The Journal of Pediatrics*, *112*(3), 403–408.

Stanton, A. L., Revenson, T. A., & Tennen, H. (2007). Health Psychology: Psychological Adjustment to Chronic Disease. *Annual Review of Psychology*, *58*(1), 565–592.

Stemerdink, N. B. A., van der Molen, M. W., Kalverboer, A. F., van der Meere, J. J., Huisman, J., de Jong, L. W., Slijper, F. M. E., Verkerk, P. H., & van Spronsen, F. J. (1999). Prefrontal Dysfunction in Early and Continuously Treated Phenylketonuria. *Developmental Neuropsychology*, *16*(1), 29–57.

Stroobant, N., & Vingerhoets, G. (2008). Depression, Anxiety, and Neuropsychological Performance in Coronary Artery Bypass Graft Patients: A Follow-Up Study. *Psychosomatics*, *49*(4), 326–331.

Stroup, B. M., Murali, S. G., Nair, N., Sawin, E. A., Rohr, F., Levy, H. L., & Ney, D. M. (2017). Dietary amino acid intakes associated with a low-phenylalanine diet combined with amino acid medical foods and glycomacropeptide medical foods

and neuropsychological outcomes in subjects with phenylketonuria. *Data in Brief, 13*, 377–384.

Tempesta, D., Mazza, M., Serroni, N., Moschetta, F. S., Di Giannantonio, M., Ferrara, M., & De Berardis, D. (2013). Neuropsychological functioning in young subjects with generalized anxiety disorder with and without pharmacotherapy. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 45*, 236–241.

ten Hoedt, A. E., de Sonneville, L. M. J., Francois, B., ter Horst, N. M., Janssen, M. C. H., Rubio-Gozalbo, M. E., Wijburg, F. A., Hollak, C. E. M., & Bosch, A. M. (2011). High phenylalanine levels directly affect mood and sustained attention in adults with phenylketonuria: A randomised, double-blind, placebo-controlled, crossover trial. *Journal of Inherited Metabolic Disease, 34*(1), 165–171.

Trefz, K. F., Muntau, A. C., Kohlscheen, K. M., Altevers, J., Jacob, C., Braun, S., Greiner, W., Jha, A., Jain, M., Alvarez, I., Lane, P., Schröder, C., & Rutsch, F. (2019). Clinical burden of illness in patients with phenylketonuria (PKU) and associated comorbidities—A retrospective study of German health insurance claims data. *Orphanet Journal of Rare Diseases, 14*(1).

Vermathen, P., Robert-Tissot, L., Pietz, J., Lutz, T., Boesch, C., & Kreis, R. (2007). Characterization of white matter alterations in phenylketonuria by magnetic resonance relaxometry and diffusion tensor imaging. *Magnetic Resonance in Medicine*, *58*(6), 1145–1156.

Vissicchio, N. A., Altaras, C., Parker, A., Schneider, S., Portnoy, J. G., Archetti, R., Stimmel, M., & Foley, F. W. (2019). Relationship Between Anxiety and

Cognition in Multiple Sclerosis. *International Journal of MS Care*, *21*(4), 151–156.

Vockley, J., Andersson, H. C., Antshel, K. M., Braverman, N. E., Burton, B. K., Frazier, D. M., Mitchell, J., Smith, W. E., Thompson, B. H., Berry, S. A., & American College of Medical Genetics and Genomics Therapeutics Committee. (2014). Phenylalanine hydroxylase deficiency: Diagnosis and management guideline. *Genetics in Medicine: Official Journal of the American College of Medical Genetics*, *16*(2), 188–200.

Vytal, K., Cornwell, B., Arkin, N., & Grillon, C. (2012). Describing the interplay between anxiety and cognition: From impaired performance under low cognitive load to reduced anxiety under high load. *Psychophysiology*, *49*(6), 842–852.

Waisbren, S. E., Noel, K., Fahrbach, K., Cella, C., Frame, D., Dorenbaum, A., & Levy, H. (2007). Phenylalanine blood levels and clinical outcomes in phenylketonuria: A systematic literature review and meta-analysis. *Molecular Genetics and Metabolism*, *92*(1), 63–70.

Waisbren, S. E., Prabhu, S. P., Greenstein, P., Petty, C., Schomer, D., Anastasoaie, V., Charette, K., Rodriguez, D., Merugumala, S., & Lin, A. P. (2017). Improved measurement of brain phenylalanine and tyrosine related to neuropsychological functioning in phenylketonuria. *JIMD Reports*, *34*, 77–86.

Wechsler, D. (2008). *Wechsler Adult Intelligence Scale—Fourth Edition (WAIS-IV)*. APAPsycTests. https://psycnet.apa.org/doiLanding?doi=10.1037%2Ft15169-000

Weglage, J., Grenzebach, M., Pietsch, M., Feldmann, R., Linnenbank, R., Denecke, J., & Koch, H. G. (2000). Behavioural and emotional problems in early-treated

adolescents with phenylketonuria in comparison with diabetic patients and healthy controls. *Journal of Inherited Metabolic Disease*, *23*(5), 487–496.

White, D. A., Connor, L. T., Nardos, B., Shimony, J. S., Archer, R., Snyder, A. Z., Moinuddin, A., Grange, D. K., Steiner, R. D., & McKinstry, R. C. (2010). Age-related decline in the microstructural integrity of white matter in children with early- and continuously-treated PKU: A DTI study of the corpus callosum. *Molecular Genetics and Metabolism*, *99 Suppl 1*, S41-46.

White, D. A., Nortz, M. J., Mandernach, T., Huntington, K., & Steiner, R. D. (2002). Age-related working memory impairments in children with prefrontal dysfunction associated with phenylketonuria. *Journal of the International Neuropsychological Society*, *8*(1), 1–11.

Williams, R. A., Mamotte, C. D. S., & Burnett, J. R. (2008). Phenylketonuria: An inborn error of phenylalanine metabolism. *The Clinical Biochemist. Reviews*, *29*(1), 31–41.

Wright, A. J. (2020). Equivalence of remote, digital administration and traditional, in-person administration of the Wechsler Intelligence Scale for Children, Fifth Edition (WISC-V). *Psychological Assessment*, *32*(9), 809–817.

Wu, W., Sheng, D., Shao, J., & Zhao, Z. (2011). Mental and motor development and psychosocial adjustment of Chinese children with phenylketonuria: Development and psychosocial adjustment. *Journal of Paediatrics and Child Health*, *47*(7), 441–447.

Yuwiler, A., Geller, E., & Slater, G. G. (1965). On the mechanism of the brain serotonin depletion in experimental phenylketonuria. *The Journal of Biological Chemistry*, *240*, 1170–1174.

Zygouris, S., & Tsolaki, M. (2015). Computerized cognitive testing for older adults: A review. *American Journal of Alzheimer's Disease and Other Dementias*, *30*(1), 13–28.

SUPPLEMENTARY ANALYSES

Table S1

Relationship Between Metabolic Markers and Performance on Individual WM Tasks

	Working Memory Tasks (*pr*)				
Phenylalanine Levels (μmol/L)	DS	RVP	SSP	SWM	PAL
IDC for early childhood (0-5 yrs)	.005	-.091	-.128	-.231	.043
IDC for middle childhood (6-11 yrs)	-.175	-.088	-.135	-.265	-.139
IDC for adolescence (12-17 yrs)	-.204	.056	.182	-.001	.019
IDC for adulthood (18+ yrs)	-.001	-.133	-.008	-.432*	-.130
IDC for lifetime (0-present)	.049	-.351	.387	-.361	-.401
Mean level for previous year	.148	-.114	-.083	-.135	-.022
Most recent level	.093	-.160	.070	-.012	-.118

Note. *pr* = partial correlations; IDC = Index of Dietary Control (mean of all half-year median phe levels)

*$p < .05$

Table S2

Multi-trait, multi-method correlation table for all continuous anxiety and WM subscales

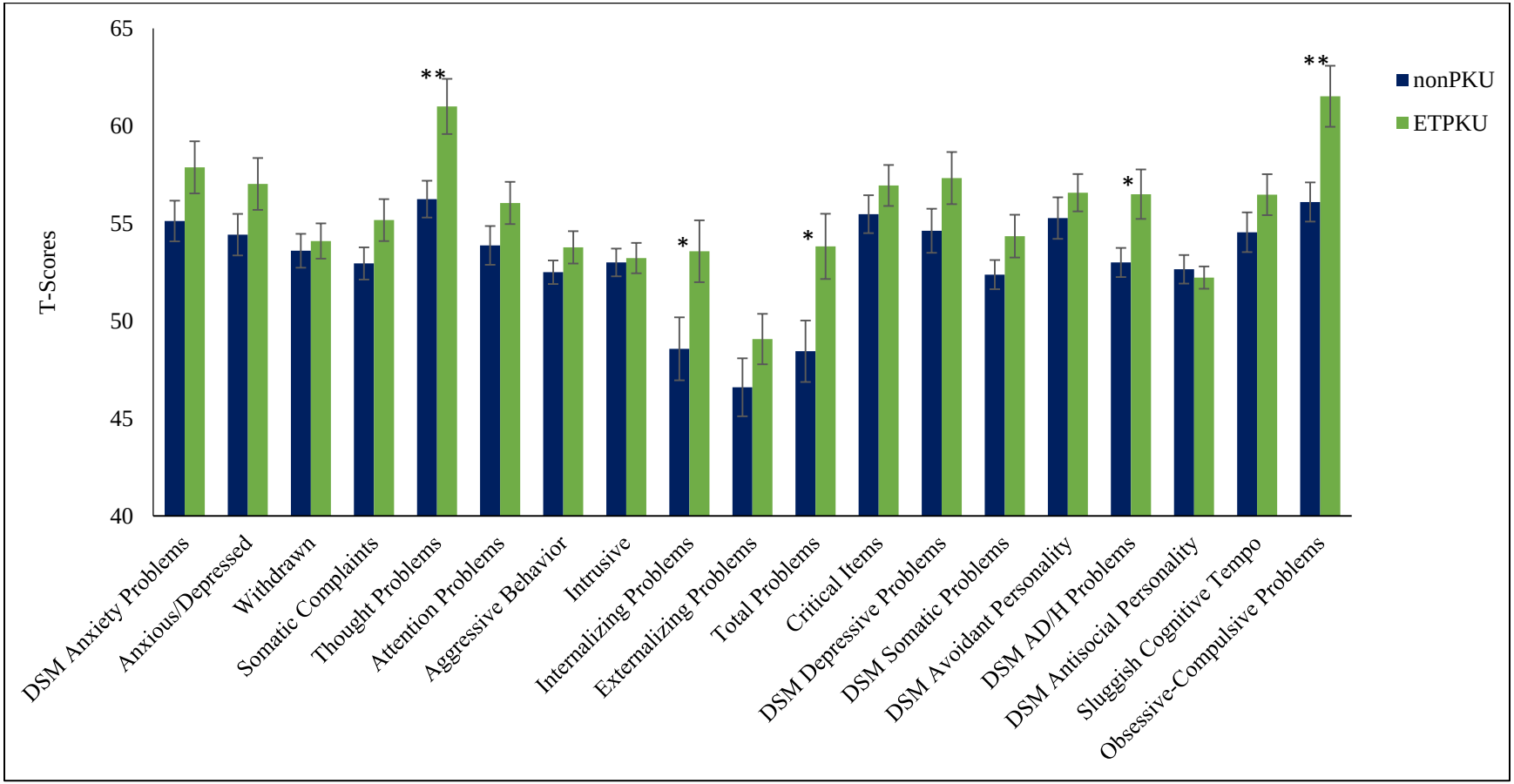
		Anxiety Measures		Working Memory Measures				
		ASR	ABCL	SSP	PAL	RVP	SWM	DS
Anxiety Measures	ASR	1						
	ABCL	.483**	1					
WM Measures	SSP	.001	.083	1				
	PAL	.134	.043	.301**	1			
	RVP	.073	.084	.308**	.286*	1		
	SWM	.049	.034	.441**	.347**	.338**	1	
	DS	.180	.024	.436**	.296**	.389**	.263*	1

Note. ASR = Adult Self-Report, ABCL = Adult Behavior Checklist (Other-report), DS = Digit Span, RVP = Rapid Visual Information Processing, SSP = Spatial Span, SWM = Spatial Working Memory, PAL = Paired Associates Learning.

*$p < .05$, **$p < .01$

Figure S1

Group Differences on Adult Self-Report Subscales

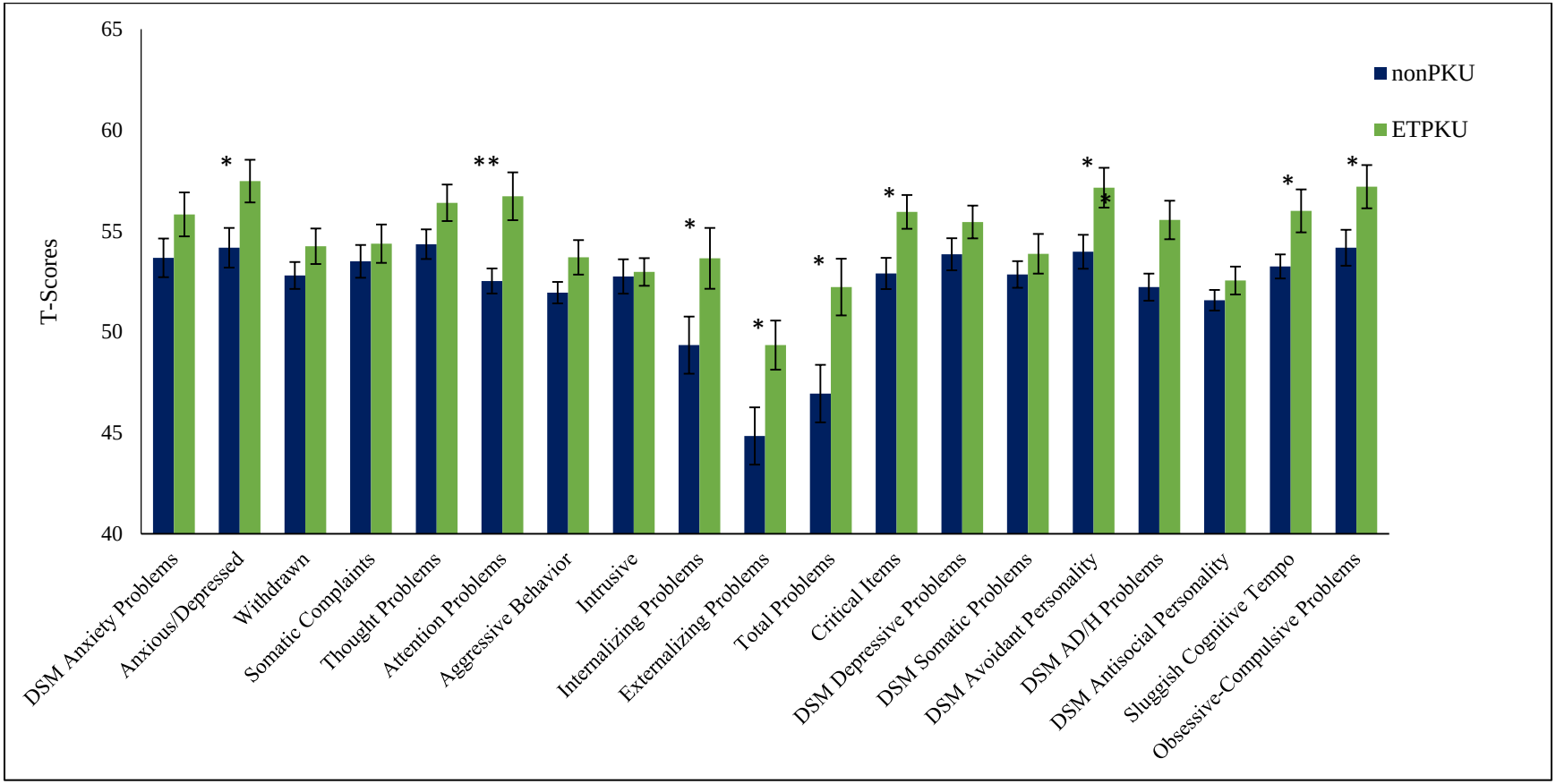


Note. DSM Anxiety Problems was the only subscale from this figure included in the overall anxiety composite.

*p < .05, **p < .005

Figure S2

Group Differences on Adult Behavior Checklist (Other-Report) Subscales

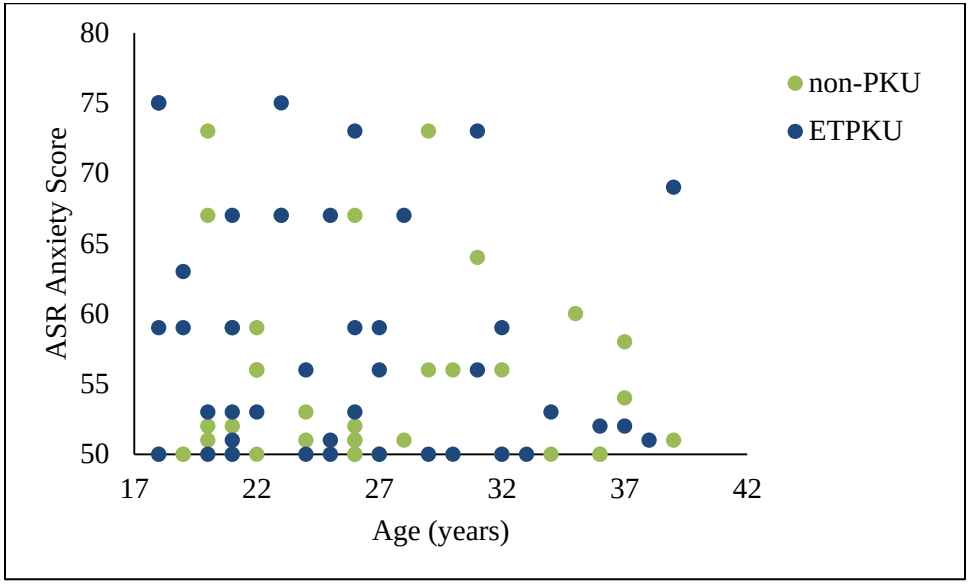


Note. DSM Anxiety Problems was the only subscale from this figure included in the overall anxiety composite.

*p < .05, **p < .005

Figure S3

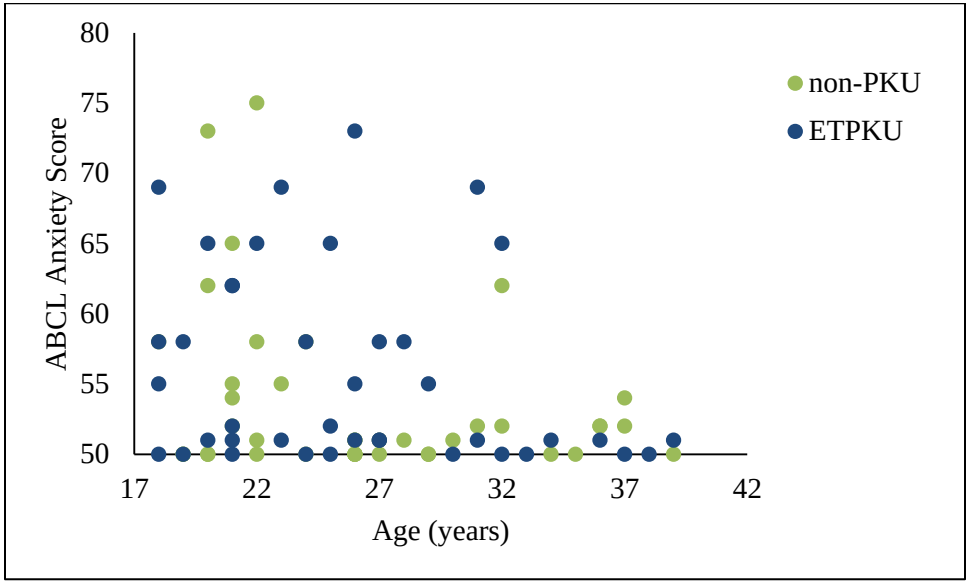
Group Differences on ASR Anxiety Scores



Note. Age was used on X-axis for visual purposes only.

Figure S4

Group Differences on ACBL Anxiety Scores



Note. Age was used on X-axis for visual purposes only.

VITA

Kelly Boland was born in St. Louis, Missouri where she attended school in the Ladue School District. For undergraduate study, she attended Kenyon College in Gambier, Ohio and graduated with her bachelors in Neuroscience. During her time at Kenyon College, she was active in Dr. Tabitha Payne's Cognitive Neuroscience Laboratory. Following graduation, she worked as a research assistant to Dr. Rebecca Treiman in her Reading & Language Laboratory at Washington University in St. Louis. Kelly has been an active member of Dr. Shawn Christ's Clinical Neuropsychology Laboratory at The University of Missouri since her admittance to the Psychology Department's Doctoral Program in 2016. During graduate school, she was also an active student clinician in the University of Missouri's Psychological Services Clinic, The Thompson Center for Autism & Neurodevelopmental Disorders, and the Division of Pediatric Psychology at Women and Children's Hospital. Kelly is scheduled to complete her clinical internship at the Children's Hospital of Michigan in August 2022 and has accepted a postdoctoral fellowship as a pediatric psychologist at St. Louis Children's Hospital.