UNIVERSITAT DE BARCELONA

Avances en el estudio de la fenilcetonuria

María Julieta González

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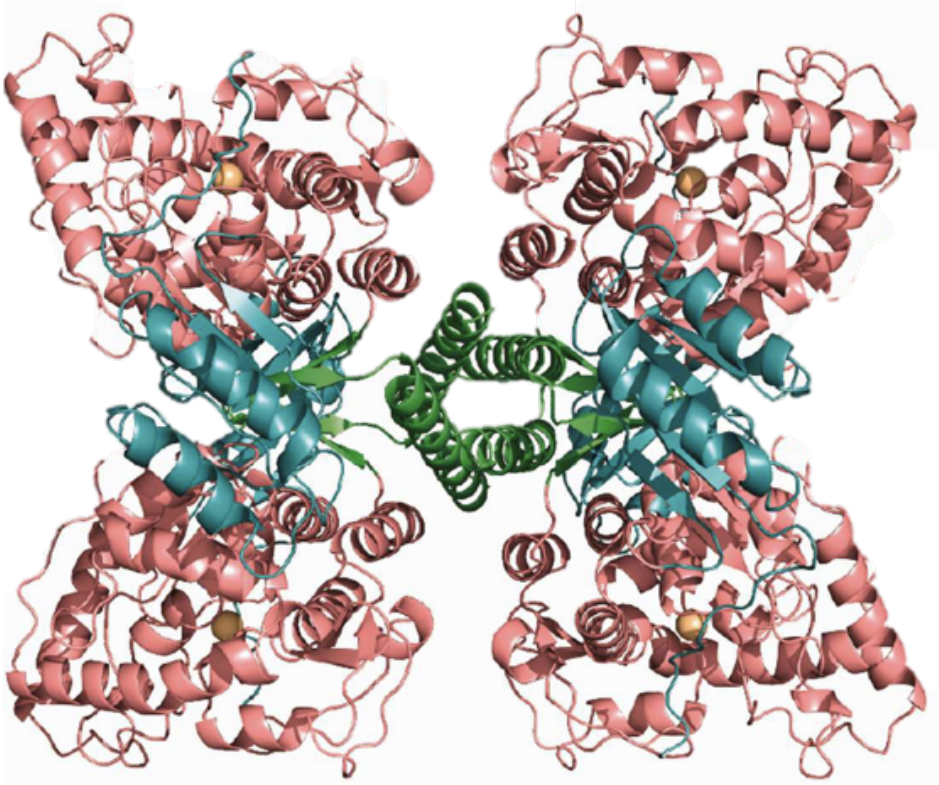

Doctoranda: María Julieta González
Director - Tutor: Dr. Jaume Campistol MD, PhD
Co-Director: Dr. Rafael Artuch MD, PhD

Universidad de Barcelona
Barcelona-2020

Diseño de la página: María Julieta González

Gráfico de la portada: Flydal MI, Martinez A. Phenylalanine hydroxylase: function, structure, and regulation. IUBMB Life. 2013 Apr;65(4):341-9. doi: 10.1002/iub.1150. Epub 2013 Mar 4. PMID: 23457044.

Maquetación: Rodrigo Suarez Ledesma, desarrollo@irodrigosl.com

Encuadernación: Gráficas Cosialls: graficascosialls@gmail.com; graficascosialls@hotmail.com

Facultad de Medicina

Departamento de Obstetricia y Ginecología, Pediatría, Radiología y Medicina Física

Programa de Doctorado en Medicina

Avances en el estudio de la fenilcetonuria

Memoria para optar al título de Doctora en Medicina por la

Universidad de Barcelona

María Julieta González, MD

Realizada bajo la dirección de:

Director - tutor: Dr. Jaume Campistol MD, PhD

Co-Director: Dr. Rafael Artuch MD, PhD

Barcelona - 2020

Departamento de Obstetricia y Ginecología, Pediatría, Radiología y Medicina Física.

Programa de Doctorado e Facultad de Medicina

Avances en el estudio de la fenilcetonuria

Trabajo realizado en el Hospital Sant Joan de Dèu,

Esplugues de Llobregat, Barcelona.

La interesada

María Julieta González

Visto bueno de los directores:

Dr. Jaume Campistol MD, PhD
Servicio de Neuropediatría
Catedrático de la UB
Hospital Sant Joan de Dèu (HSJD)
Director –Tutor de la Tesis

Dr. Rafael Artuch MD, PhD
Servicio de Bioquímica Clínica
Institut de Recerca del HSJD
CIBER-Enfermedades Raras
Co-Director de la Tesis

Barcelona septiembre 2020.

Informe Directores y Tutor de Tesis

El director – tutor de la tesis, Dr. Jaume Campistol (DNI 40270437J) y el co-director de la tesis Dr. Rafael Artuch (DNI 29139314Q),

Certifican que la tesis doctoral titulada: "Avances en el estudio de la fenilcetonuria", presentada por María Julieta González Pasaporte AAA888216, para la obtención del grado de Doctora en Medicina de la Universidad de Barcelona cumple con todos los requisitos administrativos y académicos para ser sometida a su defensa ante la comisión correspondiente y pertenecen a una misma unidad temática.

Así lo certifican,

Dr. Jaume Campistol

Dr. Rafael Artuch

Barcelona noviembre, 2020.

Los artículos científicos de esta tesis se han realizado mientras la doctoranda se encontraba trabajando en el centro Hospital Sant Joan de Dèu, Esplugues de Llobregat, Barcelona, en el servicio de neuropediatría.

Parte de los estudios de esta tesis han sido financiados por:

Instituto de Salud Carlos III, Ministerio de Sanidad. Fondo de Investigación Sanitaria (FIS PI12/01469).

Dedicado a mis queridos padres, los que me han dado todo lo que soy, gracias por nuestra familia.

Papá estoy segura que te dará mucha alegría leerla.

Comienza haciendo lo necesario,

luego haciendo lo posible y

terminarás haciendo lo imposible

San Francisco de Asis

Agradecimientos:

A Jaume gracias por tu confianza y ayuda para trabajar con este grupo de pacientes, gracias por la oportunidad de formarme con los grandes maestros y gracias por el apoyo para trabajar en este tesis.

A Rafa, por tu paciencia, confianza y generosidad para brindarme todo lo necesario para completar esta tesis.

A Rosa por los consejos, tu trabajo y tu tiempo dedicado a esta tesis.

A María Antonia, has sido un gran apoyo y libro de sabiduría para introducirme en el mundo de estos niños con PKU y sus familias.

A Mireia, por tu bienvenida al laboratorio de metabólicas y tu valiosa amistad.

A todos los compañeros del equipo del laboratorio de bioquímica de enfermedades metabólicas que han trabajado en la determinación de metabolitos para completar esta tesis (Aída, Mercedes, Cristina, Raquel y Marta).

A Mónica y Pablo, por vuestra colaboración para realizar parte de esta tesis.

A mis compañeros de neurología, todos los que han pasado en mi vida enseñándome siempre algo más dentro de estas enfermedades (Mercedes, Mar, Ángels) y con todos los que he compartido muchos momentos de trabajo y aprendizaje (Alba, María, Raquel, Delia y Fede), además de viajes y experiencias dentro de este servicio.

A la Unidad de Seguimiento de la fenilcetonuria por el trabajo que realizan por estos pacientes.

A los padres y pacientes con fenilcetonuria por estar siempre dispuestos a colaborar en todo momento con el trabajo de esta tesis.

A la beca FIS PI12/01469, del Instituto Carlos III, CIBER de Enfermedades Raras que ha financiado parte de los estudios de esta tesis.

A Maricarmen y Mercedes por vuestro apoyo incondicional.

A mi gran familia, mis padres y mis hermanos (Diego, Ana, Santi, Tere, Flor y Lulú) que siempre me han acompañado y guiado a la distancia.

Huguito, gracias por estar presente desde el inicio gracias por enseñarnos a vivir la vida, te extraño primo.

A mis queridas tías, Magda, Marina y tía Rosita desde allá, ustedes han sido una gran pilar para mí en mi formación humana y académica.

A José María y a mi bella Julia, por el tiempo que juntas hemos compartido, mientras mamá intentaba completar esta tesis.

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Abreviaturas

- 5-HIAA: ácido 5-hidroxiindolacético
- AF: anisotropía fraccional
- BH4: tetrahidrobiopterina
- BHE: barrera hematoencefálica
- CC: cuerpo calloso
- Coenzima Q10: CoQ10
- CPF: cortex prefrontal
- DM: difusividad media
- DR: difusividad radial
- GPX: glutation peroxidasa
- HVA: ácido homovanílico
- JHU-ICBM DTI 81: atlas de SB del Johns Hopkins University - International Consortium of Brain Mapping, diffusion tensor imaging
- LAT1: transportador de aminoácidos
- LANN: aminoácidos neutros de cadena larga
- NT: neurotransmisores
- PAH: fenilalanina hidroxilasa
- Phe: fenilalanina
- PKU: fenilcetonuria
- PKUDP: fenilcetonuria de diagnóstico precoz
- PKUDT: fenilcetonuria de diagnóstico tardío
- RM: resonancia magnética
- RMC: resonancia magnética craneal
- RMC-TD: resonancia magnética craneal con tensor de difusión
- SB: sustancia blanca

- SNC: sistema nervioso central
- TD: tensor de difusión
- Trp: triptófano
- Tyr: tirosina

RESUMEN

El objetivo general de esta tesis ha sido, investigar las complicaciones neurológicas y neuropsicológicas en el seguimiento de los pacientes PKUDP, a pesar de un adecuado control de la enfermedad a través de investigaciones clínicas, bioquímicas y de neuroimagen.

En el primer trabajo se estudió la relación entre las complicaciones neurológicas, los hallazgos neurorradiológicos y problemas de conducta, edad en el momento del diagnóstico y control dietético a lo largo del seguimiento de los pacientes con PKU en nuestra unidad metabólica.

Se realizó un estudio retrospectivo de los pacientes con PKU diagnosticados y controlados en nuestra unidad desde 1985 hasta 2010. A través del registro de pacientes en una base de datos con 50 ítems completados por medio de la revisión de las historias clínicas.

En la segunda investigación nos planteamos estudiar la integridad de la microestructura de la sustancia blanca en todo el cerebro de pacientes pediátricos PKU de diagnóstico precoz comparados con una población control, mediante la resonancia magnética con tensor de difusión e índices como la difusividad media, la difusividad radial y la anisotropía fraccional. Existen pocos estudios que evalúen todos los tractos de la SB cerebral con éste método en grupos de pacientes pediátricos PKU de diagnóstico precoz. Además se correlacionaron estos índices con los niveles de Phe, el ICD, biomarcadores de neurotransmisores y parámetros neuropsicológicos como la velocidad de procesamiento.

Los pacientes PKU son una población en riesgo de desarrollar trastornos del sueño debidos a déficits en la síntesis de neurotransmisores. En la tercera investigación se estudió la prevalencia de los trastornos del sueño en niños y adolescentes PKUDP y su correlación con marcadores de síntesis de dopamina y serotonina en relación a un grupo control. Además se correlacionó el estudio del sueño con los niveles de Phe concurrente, ICD, variabilidad de Phe en el último año, tirosina, triptófano, prolactina, ferritina en plasma, concentración de serotonina plaquetaria, y la excreción de melatonina, ácido homovanílico y ácido 5-hidroxi-

indolacético en orina. El sueño se evaluó mediante la escala de Bruni para los trastornos del sueño en niños.

En el último estudio de esta tesis se evaluó de forma restrospectiva las concentraciones plasmáticas de CoQ10 en una gran cohorte de pacientes pediátricos y adultos jóvenes durante un período de 12 años. Se estudiaron 597 pacientes , los cuales se dividieron en 6 diferentes grupos (un grupo control de pacientes sanos, pacientes con PKU, con mucopolisacaridosis (MPS), con otros errores congénitos del metabolismo (EIM), con enfermedades neurogenéticas y otros con enfermedades neurológicas sin diagnóstico genético). Se focalizó la atención en aquellos pacientes que presentaban valores bajos de CoQ10 plasmática, evaluando factores genéticos y ambientales que podrían influir en el estado de CoQ10 plasmática.

Luego de cada resumen de resultados se adjuntan las publicaciones realizadas en la tesis y en la parte final, en anexos, se adjuntan las presentaciones realizadas en congresos y reuniones de pacientes y familiares de los afectos de esta enfermedad, muchos de los cuales han participado en en las investigaciones, a fin de dar difusión de los resultados.

INTRODUCCIÓN

1. Introducción

1.1. Definición de fenilcetonuria

La fenilcetonuria (PKU; OMIM 261600) es un defecto genético recesivo del metabolismo de la fenilalanina (Phe), producido por mutaciones del gen *PAH*, el cual codifica para la enzima fenilalanina hidroxilasa (EC 1.14.16.1). Esta enzima cataliza la hidroxilación de Phe a tirosina, utilizando la tetrahidrobiopterina (BH_4) como cofactor.

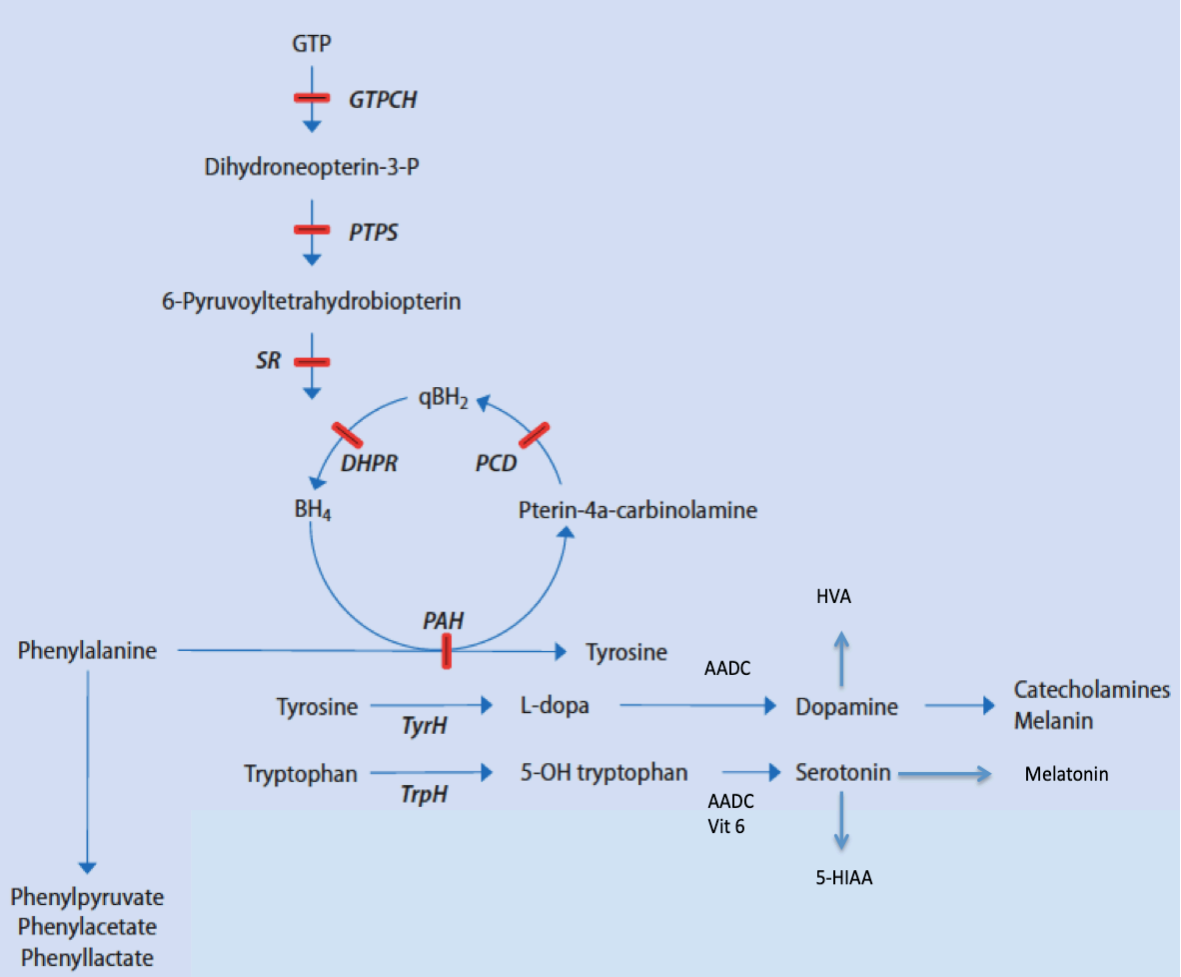


Figura 1: Sistema de hidroxilación de la fenilalanina, incluye síntesis y regeneración de pterinas, síntesis de aminas biógenas. AADC: aminoácido decarboxilasa; BH2: Dihidrobiopterina; BH4: tetrahidrobiopterina; DHPR: Dihidropterina reductasa; GTP: guanosina trifosfato; GTPH: Guanosina trifosfato ciclo hidrolasa; L-DOPA: L-3,4 dihidroxifenilalanina; 5-HIAA: ácido 5-hidroxi-indolacético; HVA: ácido homovanílico; PAH: fenilalanina hidroxilasa; PCD, pterina-4a-carbinolamina dehidratasa ; PTPS, piruvoil-tetrahidrobiopterin sintetasa ; SR, sepiapterina reductasa ; TrpH, triptófano hidroxilasa ; TyrH, tirosina hidroxilasa.
Adaptado en Burgard et al. en Saudubray 6ed, 2016.

Las mutaciones en *PAH* dan lugar a una proteína cuya actividad enzimática está total o parcialmente disminuida, lo que provoca la acumulación de Phe en tejidos y fluidos biológicos (Scriver et al. 2001).

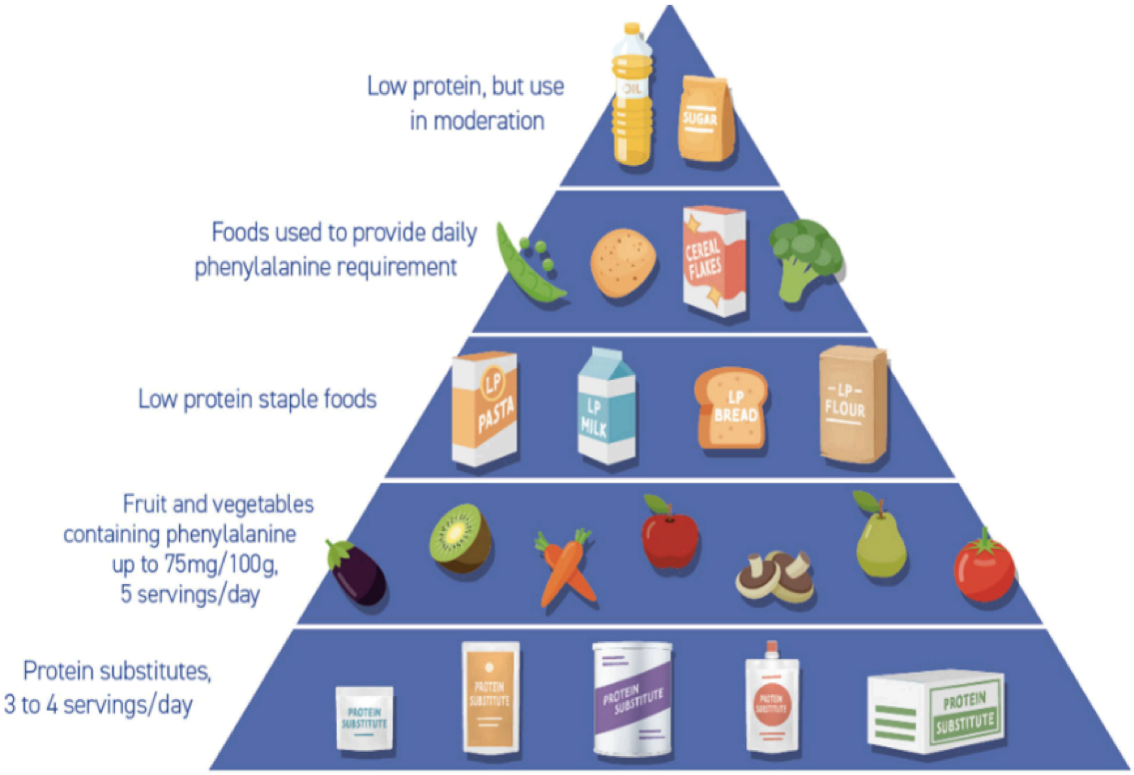


Figura 2 : Esquema de pirámide alimentaria en la PKU. MacDonald et al. 2020.

1.2 Tratamiento de la fenilcetonuria

Si no se trata, la PKU puede producir discapacidad intelectual y otros trastornos neurológicos graves, pero con el diagnóstico en las primeras semanas de vida a través del cribado metabólico neonatal y el inicio de un tratamiento adecuado, permite un desarrollo físico e intelectual prácticamente normal (Scriver et al. 2001). El tratamiento clásico de la PKU

consiste en la restricción de Phe de la dieta por medio de alimentación con bajo contenido en proteínas naturales, suplementada con fórmula especial enriquecida en tirosina y con aminoácidos y micronutrientes (vitaminas, minerales, oligoelementos y ácidos grasos esenciales) necesarios para evitar deficiencias nutricionales (Scriver et al. 2001).

El tratamiento alternativo con BH_4 (comercializado como Kuvan) también logra disminuir las concentraciones de Phe en los pacientes que responden al mismo, permitiendo la liberalización total o parcial de la dieta (Lambruschini et al. 2005). El objetivo con ambos tratamientos, es mantener las concentraciones de Phe en sangre en un rango seguro mediante el cálculo del índice del control dietético (ICD) (inferior a 360 µmol/L hasta los 12 años y a 600 µmol/L a partir de los 12 años). Con ello se alcanza tanto un nivel cognitivo y desarrollo psicosocial adecuados, como una buena calidad de vida a largo plazo (Scriver et al. 2001; Pietz et al. 1998; van Spronsen et al. 2017). No obstante, a pesar de instaurar el tratamiento precoz o lograr un adecuado control metabólico de los pacientes PKU, estos pueden presentar alteraciones neurológicas y neuropsicológicas, aspectos en los que hemos centrado nuestras investigaciones (Pérez-Dueñas et al. 2005; Waisbren et al.2007; DeRoche et al. 2008).

1.3 Fisiopatología de la fenilcetonuria

Desde un punto de vista fisiopatológico, existen diferentes alteraciones bioquímicas que podrían explicar las disfunciones neuropsicológicas en la PKU. Por un lado, los pacientes PKU tratados precozmente y bien controlados, mantienen una elevación de la concentración de Phe en plasma, entre 3-5 veces superior a los valores fisiológicos de la población sana. Se desconoce si esta elevación crónica pudiera contribuir a la fisiopatología de la enfermedad, pero si se sabe que el efecto directo de las concentraciones elevadas de Phe en el cerebro altera, por un lado la producción y mantenimiento de la mielina (Dyer et al. 1999) y, por otro lado interfiere en la síntesis de los neurotransmisores dopamina y serotonina (Dyer et al. 1999; Surtees et al. 2000; van Spronsen et al. 2009; 2017; Feillet et al. 2010).

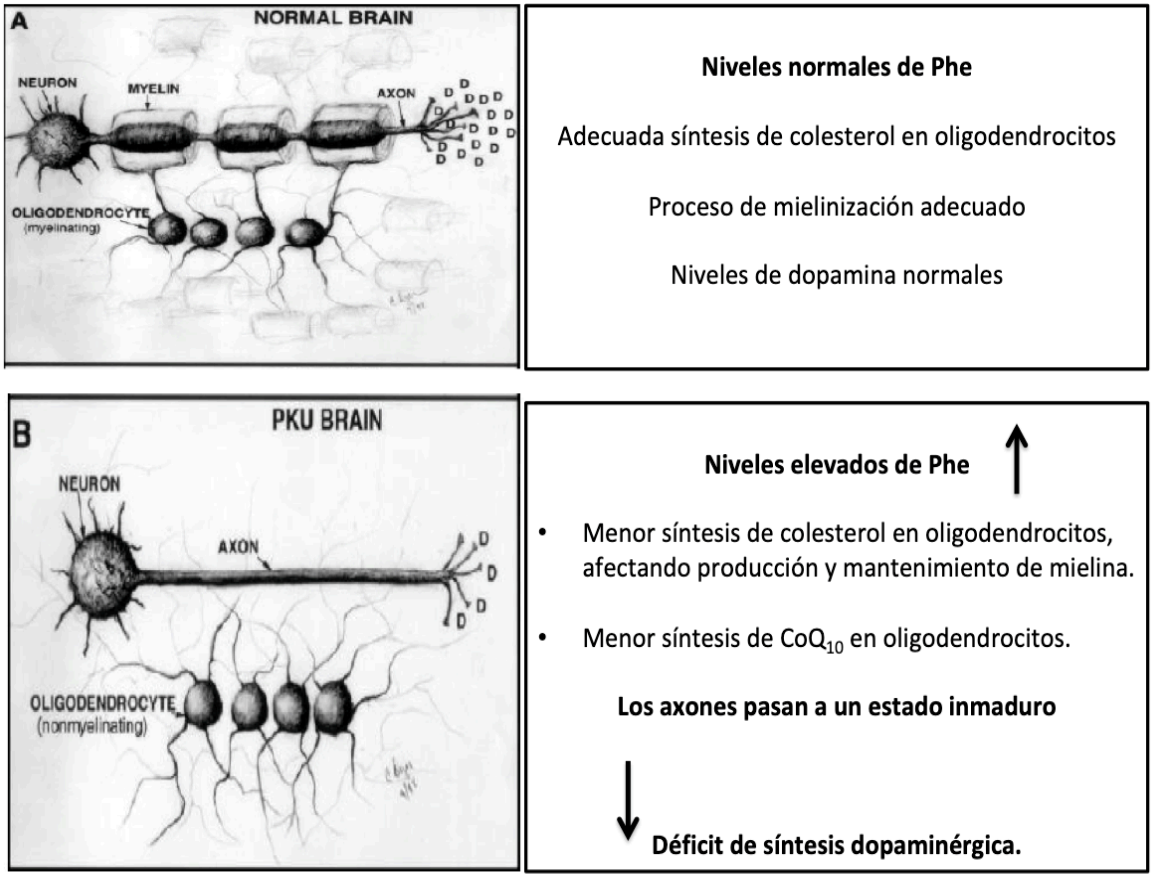


Figura 3: Hipótesis de Dyer, 1999

Las concentraciones elevadas de Phe en el cerebro producen alteración en la mielinización. La prevalencia de estas anomalías es alta, especialmente en los pacientes mayores y en aquellos que no cumplen el tratamiento (Anderson et al. 2007). Estas anomalías se identifican a través de los estudios de resonancia magnética cerebral (RMC) como áreas de aumento de señal en secuencias T2 predominantemente en la sustancia blanca (SB) periventricular parieto-occipital (Bick et al. 1991; Pietz et al. 1996). Las alteraciones en la SB parece que dependen más de las concentraciones de Phe en sangre recientes, de forma que un buen control dietético puede reducirlas (Thompson et al. 1990; Ullrich et al. 1994; Cleary et al. 1995). Estas anomalías en la mielinización cerebral junto con el defecto de los neurotransmisores dopamina y serotonina parecen estar relacionados con los problemas neuropsicológicos descritos en los pacientes PKU, especialmente si el control metabólico no es el adecuado (Feillet et al. 2010).

1.4 Funciones cognitivas y fenilcetonuria

Los pacientes PKU tratados de forma precoz presentan alteraciones en algunas funciones cognitivas, que aunque leves, inciden de forma negativa en su rendimiento escolar (Gassió et al. 2005). Su capacidad intelectual es normal pero menor que la de los grupos control de su mismo entorno social, y muestran déficits principalmente en funciones ejecutivas (control atencional, planificación estratégica, inhibición de respuestas, memoria de trabajo y flexibilidad de pensamiento y acción) (DeRoche et al. 2008; Gassió et al. 2005; Christ et al. 2010). En estudios más recientes también se ha reportado un enlentecimiento de la velocidad de procesamiento y alteraciones en motricidad fina (Anderson et al. 2007; Gassió et al. 2005).

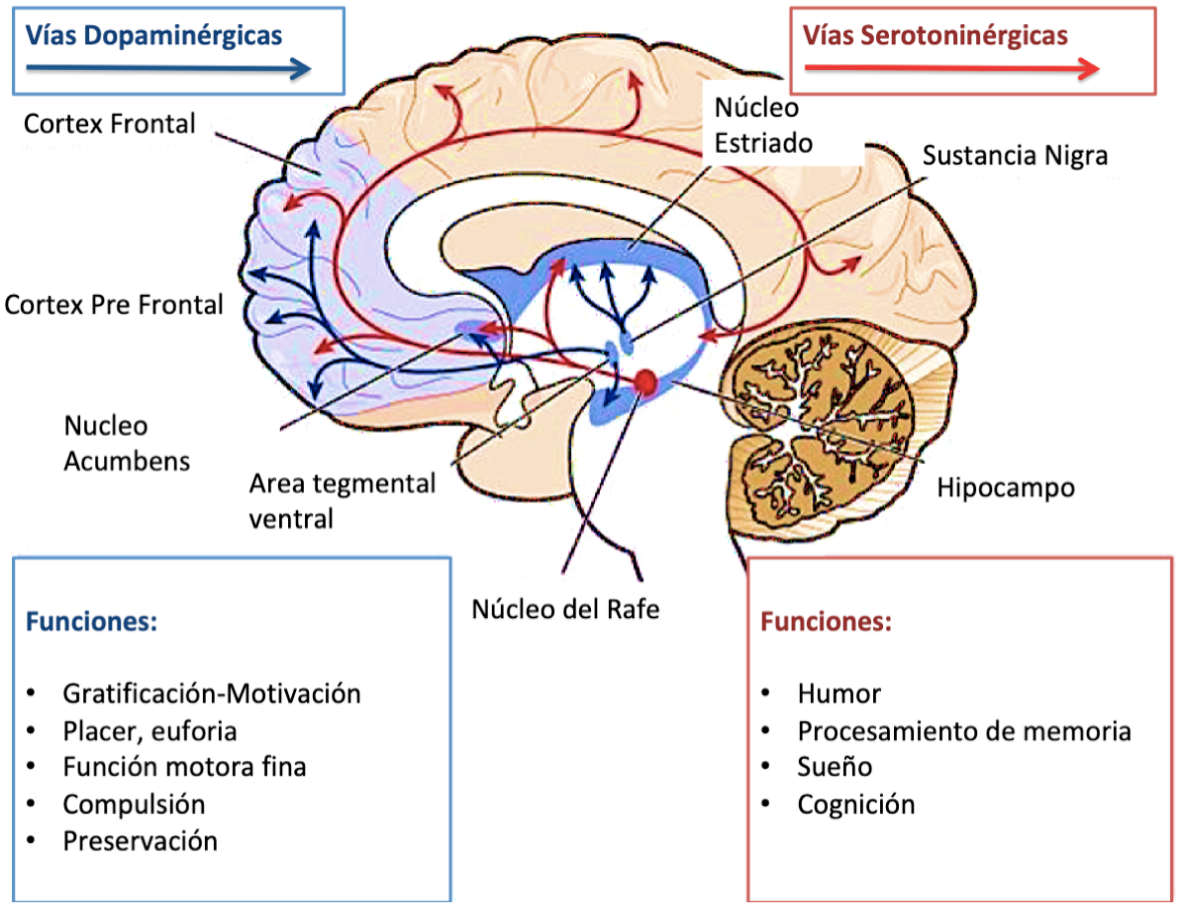


Figura 4: Esquema de funciones dopaminérgicas y serotoninérgica.
Adaptado en https://antroporama.net/nucleo-accumbens-aprendizaje-y-motivación/

Se ha descrito una elevada incidencia de ansiedad y depresión en los pacientes PKU, más relacionada con el déficit de serotonina (Feillet et al. 2010), y la presencia de trastor-

nos del sueño probablemente debida a una menor síntesis de melatonina (Campistol et al. 2010). Actualmente se baraja una hipótesis principal como causa de estas alteraciones neuropsicológicas potencialmente asociadas con la disfunción en la neurotransmisión. Se ha demostrado una disfunción del córtex prefrontal (CPF) debido a una disminución de la síntesis de dopamina, y secundariamente de catecolaminas (van Spronsen et al. 2009). Evolutivamente se objetiva que la adecuada adquisición de las funciones ejecutivas y el control motor están estrechamente vinculados al desarrollo anatómico y funcional del CPF y de las áreas cerebrales asociadas (ganglios basales, córtex cingulado anterior, córtex parietal posterior, y cerebelo).

Por tanto, estas funciones pueden verse afectadas ante cualquier disminución en la disponibilidad de dopamina en el CPF. En la PKU, esta disminución en la síntesis de dopamina se debe a que hay una menor síntesis de tirosina y a una menor disponibilidad de tirosina debido a que las concentraciones elevadas de Phe inhiben el transporte por los transportadores de aminoácidos (LAT1) al cerebro por un mecanismo competitivo (van Spronsen et al. 2009; 2017).

Esto afectaría al transporte de otros aminoácidos neutros (metionina, esencial para la estabilidad de la mielina y triptófano, éste último precursor de serotonina). Además las concentraciones elevadas de Phe en las neuronas incide negativamente sobre la actividad de las enzimas tiroxina hidroxilasa y triptófano hidroxilasa (Surtees et al. 2000), que son las enzimas que intervienen en la síntesis de dopamina y serotonina respectivamente (Figura 1). La pérdida de mielina en pacientes PKU afectaría secundariamente a la producción de neurotransmisores (DeRoche et al. 2008), y causaría disrupciones en la interconectividad entre diferentes regiones cerebrales (Christ et al. 2010).

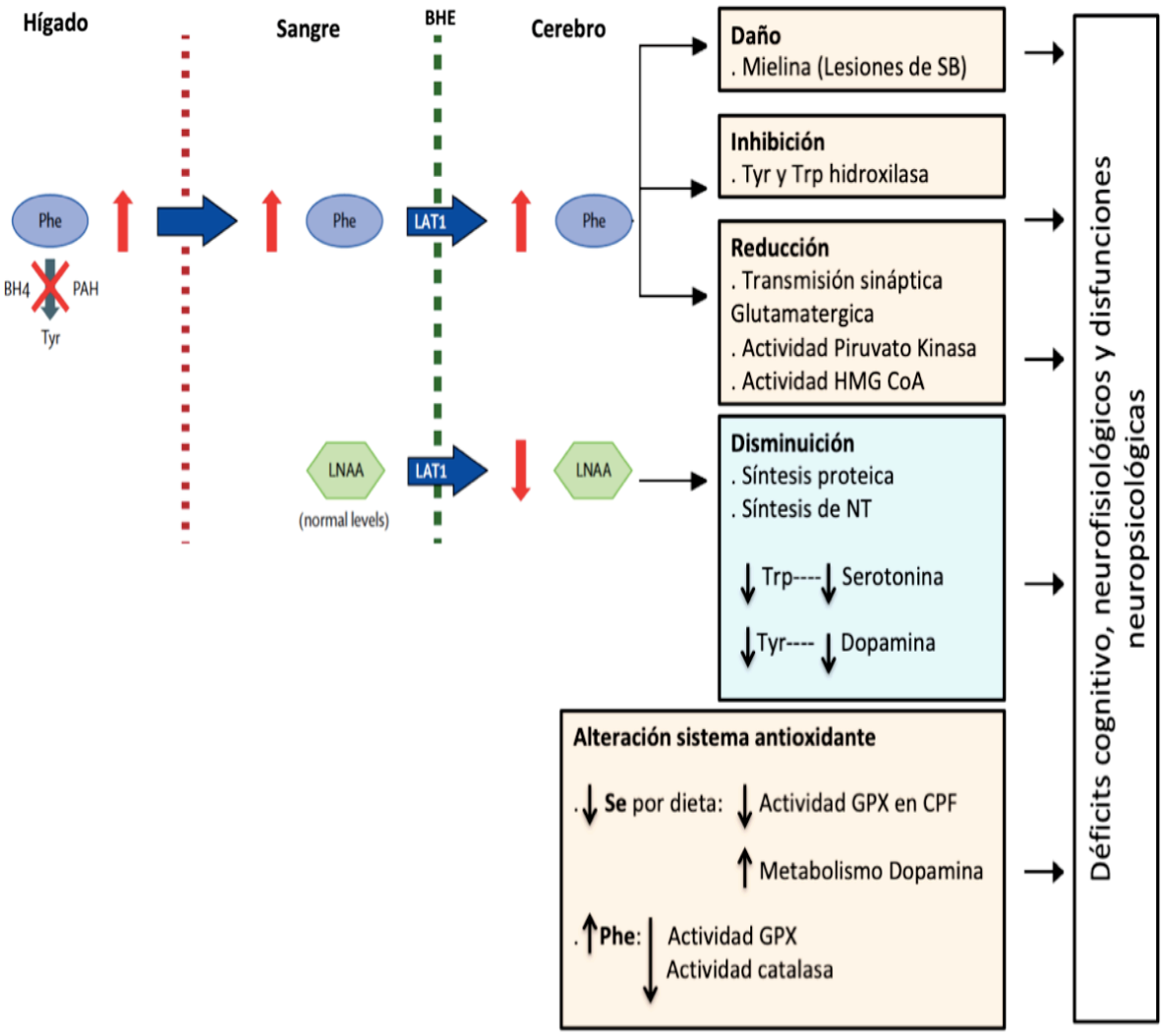


Figura 5: Fisiopatología en la disfunción cerebral de la PKU. Adaptado de Van Spronsen 2017.

1.5 Biomarcadores de la neurotransmisión en la fenilcetonuria

Desde un punto de vista bioquímico, es difícil estudiar la neurotransmisión en la PKU ya que la forma más adecuada sería la práctica de una punción lumbar para analizar directamente los neurotransmisores y sus precursores (Ormazábal et al. 2005). Obviamente, esta práctica no se realiza por motivos éticos. Por otro lado, las nuevas técnicas de neuroimagen para estudiar el metabolismo cerebral no tienen sensibilidad suficiente para analizar determinados metabolitos. Por ello, se ha realizado un estudio bioquímico de la neurotransmisión en fluídos biológicos periféricos para tratar de correlacionarlo con los trastornos neuropsicológicos en los pacientes PKU. Si bien la sangre y orina no serían los especímenes más

adecuados para este fin, nuestra experiencia previa en la PKU sugiere que podrían aportar información valiosa (Ormazábal et al. 2005). Se analizaron tanto precursores necesarios para la síntesis de los neurotransmisores (tirosina, triptófano), metabolitos que reflejan su síntesis y recambio (aminas biógenas: ácidos 5-hidroxiindoleacético y homovanílico) y marcadores de síntesis y función (prolactina como marcador del déficit de dopamina, y serotonina en plaquetas como marcador de síntesis de este neurotransmisor) (Ormazábal et al. 2005).

1.6 Histopatología en la fenilcetonuria

Los cerebros de pacientes con PKU no tratados generalmente muestran alteraciones en la mielinización, que reflejan la palidez de la SB en las tinciones de la mielina (Huttenlocher 2000). Además según investigaciones de Malamud, estas alteraciones se asocian a gliosis astroquística en los tractos de la SB afecta, (Malamud 1966). En modelos animales de PKU, los estudios han demostrado que los oligodendrocitos no forman mielina en respuesta a un alto contenido de fenilalanina (Phe) (Dyer et al. 1999), debido a que la hiperfenilalaninemia produce una inhibición de la síntesis de colesterol a nivel de los oligodendrocitos (Dyer et al. 1999; Shefer et al. 2000) (Figura 3).

Dado que la colesterogénesis es una vía común de síntesis de CoQ_{10}, las elevaciones de Phe podrían asimismo causar una disminución de la síntesis endógena de CoQ_{10}. Esta hipótesis es interesante, teniendo en cuenta que el CoQ_{10} es una sustancia fundamental en el metabolismo neuronal, pues participa en la síntesis de energía a nivel mitocondrial y previene el daño causado por la peroxidación lipídica.

En la etapa intraútero se cree que el desarrollo del cerebro continúa normalmente hasta el nacimiento. Después del nacimiento, los niveles de Phe aumentan rápidamente y desde ese momento se consideran neurotóxicos para el desarrollo posterior del cerebro. Esto denota que las lesiones de la SB en la PKU no tratada ocurren generalmente en regiones en las que la mielinización se produce en etapa post-natal (Dyer et al 1999; Dyer et al 1996). Como consecuencia, se supone que los oligodendrocitos en las regiones que mielinizan después del nacimiento (por elemplo: el tracto óptico, cuerpo calloso, SB subcortical

y sustancia blanca periventricular) son vulnerables a niveles altos de Phe después del nacimiento, mientras que los oligodendrocitos asociados con los tractos de SB que mielinizar antes del nacimiento (por ejemplo, cápsula interna y tronco encefálico) son resistentes a niveles elevados de Phe después del nacimiento (Dyer et al 1999). Dyer en esa misma publicación, sostiene que la patología de la SB en la PKU no tratada es un proceso de desarrollo por el cual los niveles elevados de Phe detienen el proceso de la mielinización, resultando en hipomielinización severa.

El compromiso leve y transitorio de los tractos de SB que se mielinizan después del nacimiento, se cree que es lo que sucede en los pacientes PKU tratados en forma temprana. Es probable que las lesiones de SB en los pacientes PKU no tratados se deba a una formación reducida de mielina, mientras que las lesiones en pacientes bien controlados se deba a una pérdida de la función o deterioro de mielina previamente ensamblada (Dyer et al. 1999).

En conclusión, la PKU se asocia con patología difusa de la SB en pacientes tratados y no tratados. En pacientes no tratados refleja un proceso de hipomielinización (falta de formación de mielina) mientras que en pacientes tratados temprano, esta patología probablemente refleje un edema intramielínico. Las investigaciones demuestran que estas alteraciones se asocian con el control metabólico y, como tal, puede revertirse con la adherencia a una dieta estricta baja en Phe durante al menos 2 meses (Cleary et al. 1995; Leuzzi et al. 1997).

1.7 Resonancia magnética cerebral y la secuencia con tensor de difusión.

La prevalencia de las anormalidades de la SB es alta (Cleary et al. 1994; Thompson et al. 1993), y la mayoría de los pacientes tratados de forma precoz presentan al menos un aumento leve de la intensidad en la secuencia T2 de la RMC en la SB periventricular (Anderson et al. 2004; Cleary et al. 1994; Shaw et al. 1991). Otros estudios también han demostrado que en casos más graves, las anomalías de la SB pueden extenderse a regiones subcorticales (Anderson et al. 2004; Bick et al. 1991; Bick et al. 1993; Thompson et al. 1993).

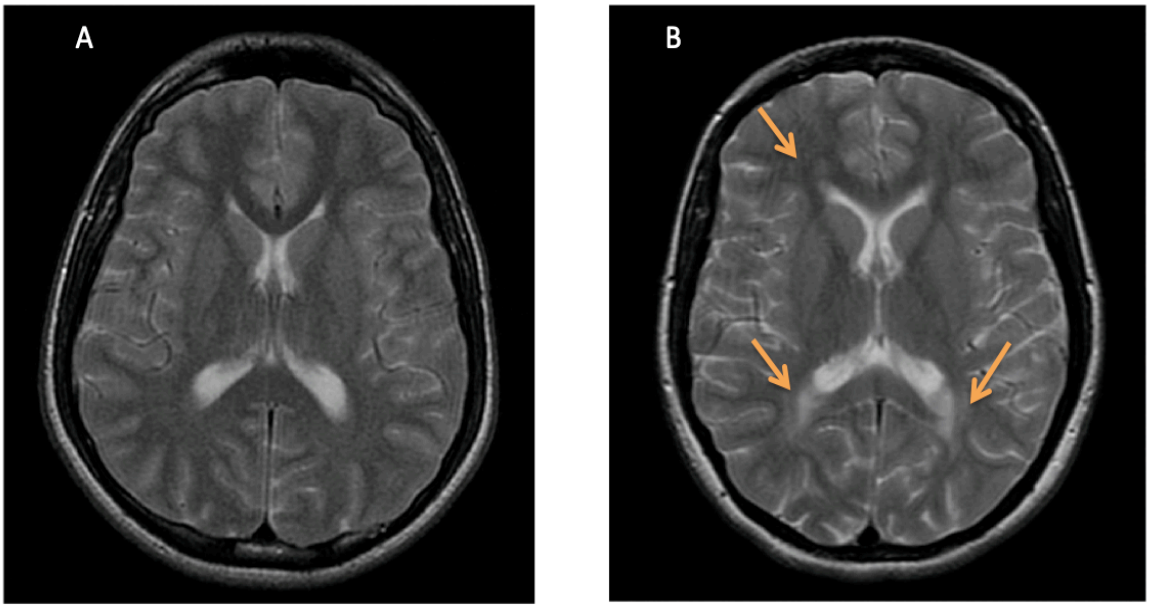


Figura 6: Secuencia T2, corte axial de resonancia magnética de pacientes PKU.

A. Buen control metabólico.
B. Pobre control metabólico, las flechas indican las lesiones de SB periventricular

Más recientemente, se han utilizado imágenes con tensor de difusión (TD) (adquiridas a partir de la RM) para estudiar la integridad de la microestructura de la SB en la PKU.

La técnica de TD es una aplicación cuantitativa de la RM que permite una evaluación no invasiva, in vivo de la estructura axonal y del estado de la mielina, a través de la medición de la difusión de las moléculas de agua en las estructuras cerebrales (Basser et al. 1994; Basser &Pierpaoli 1996).

La RMC-TD mide la extensión y direccionalidad de la difusión del agua, la cual en una SB integra, se mueve de manera paralela a los tractos de la fibra nerviosa, de manera asimétrica. A esta asimetría se la denomina anisotropía, estableciéndose una relación directa entre el nivel de anisotropía y el número de fibras (por lo que a una menor anisotropía menor número de fibras). Es decir, la anisotropía fraccional (AF) cuantifica la preferencia por la difusión en una dirección, y se correlaciona con diámetro axonal, densidad y orientación de las fibras.

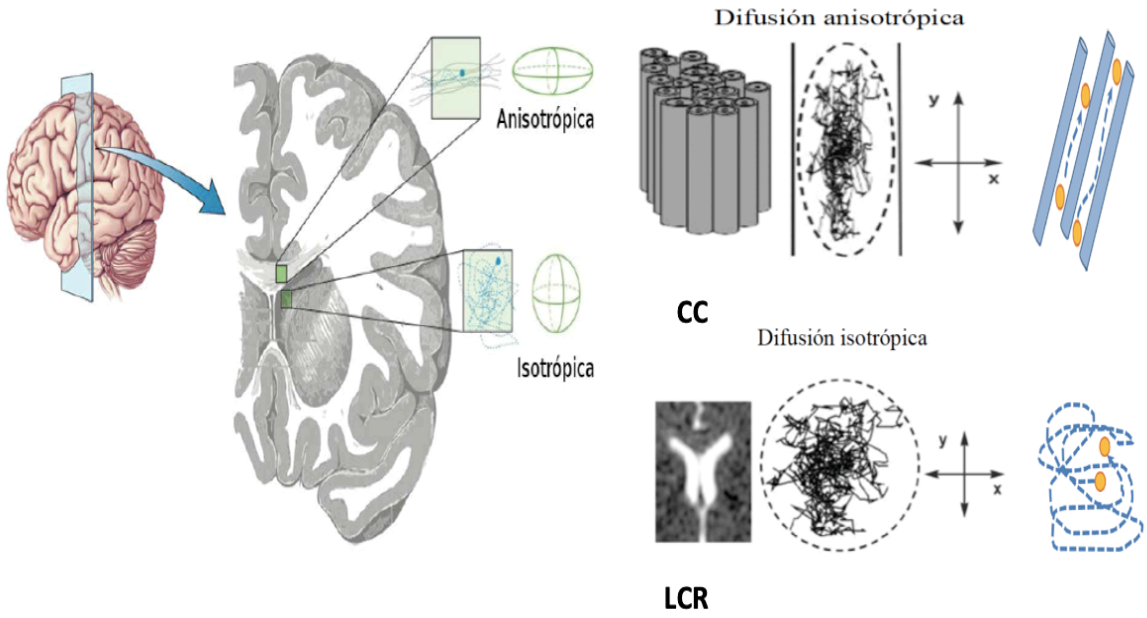


Figura 7: RMC-TD: Resonancia magnética con Tensor de difusión: difusión en los medios biológicos. Difusión anisotrópica: restringida en una o más direcciones, (i.e cc: cuerpo calloso). Difusión isotrópica o libre: igual en todas las direcciones, (i.e. LCR: líquido cefalorraquídeo). Adaptado en Brain´s Cortex and White Matter. Harvard.

Además de la AF se pueden determinar otros parámetros, como la difusividad media y la difusividad radial. La difusividad media mide el movimiento molecular medio del agua, representa la media del movimiento molecular, independientemente de la direccionalidad y proporciona una medida general de integridad del tejido (Sen & Basser, 2005), la difusividad radial (DR) es la difusividad transversa, perpendicular a la fibra axonal, y se relaciona con la integridad de mielina.

Los estudios de TD han identificado una difusividad restringida en individuos con PKU en varias regiones del cerebro, incluido el centro semioval central (CSO), la corteza occipital parietal posterior, la corteza prefrontal, la radiación óptica, el putamen y el cuerpo calloso anterior (Antenor-Dorsey et al. 2013; Christ et al. 2010; Ding et al. 2008; Kono et al. 2005; Peng et al. 2014; Vermathen et al. 2007; White et al. 2013; 2010).

La diferencia se observa en investigaciones en poblaciones sanas que demuestran que los valores de DM disminuyen con la edad durante la niñez, (por el proceso de mielinización, la SB está mas compacta), mientras todavía está madurando (Scantlebury et al. 2014). Mientras que, durante la edad adulta, los valores de DM tienden a aumentar por los

procesos que afectan la integridad de la SB (Madden et al. 2009; Benitez et al. 2018). En cambio la AF disminuye a lo largo de la edad adulta. La disminución de valores de DM y DR con el incremento de la edad se asocian a un mejor estado de la microestructura de la SB (Basser et al. 2014; 2015; Song et al. 2002; 2005). Sin embargo, en poblaciones PKU, los valores reducidos de DM y DR parecen estar más relacionados con el aumento de los niveles de Phe (Peng et al. 2014; Hood et al. 2015). Es decir, que en los pacientes PKU la reducción de los valores de DM y DR, no refleja que la SB está más preservada, sino que, representa el daño de SB ya que los niveles elevados de Phe inhiben la biosíntesis y estabilidad de la mielina en los oligodendrocitos. Por lo tanto, se postula que en poblaciones sanas, la correlación negativa entre los valores de DM y DR con la edad refleja una mejor preservación del proceso de mielinización (Scantlebury et al. 2014), mientras que en la PKU, los resultados refuerzan la hipótesis que los niveles elevados de Phe producen un daño acumulativo en la microestructura de la SB y por eso disminuyen los valores de DM.

El análisis estadístico a través del estudio estadístico de los tractos (TBSS) proporcionan evidencia del compromiso generalizado de la SB en la PKU (Antenor-Dorsey et al.2013; Hawks et al.2017; Hood et al. 2015).

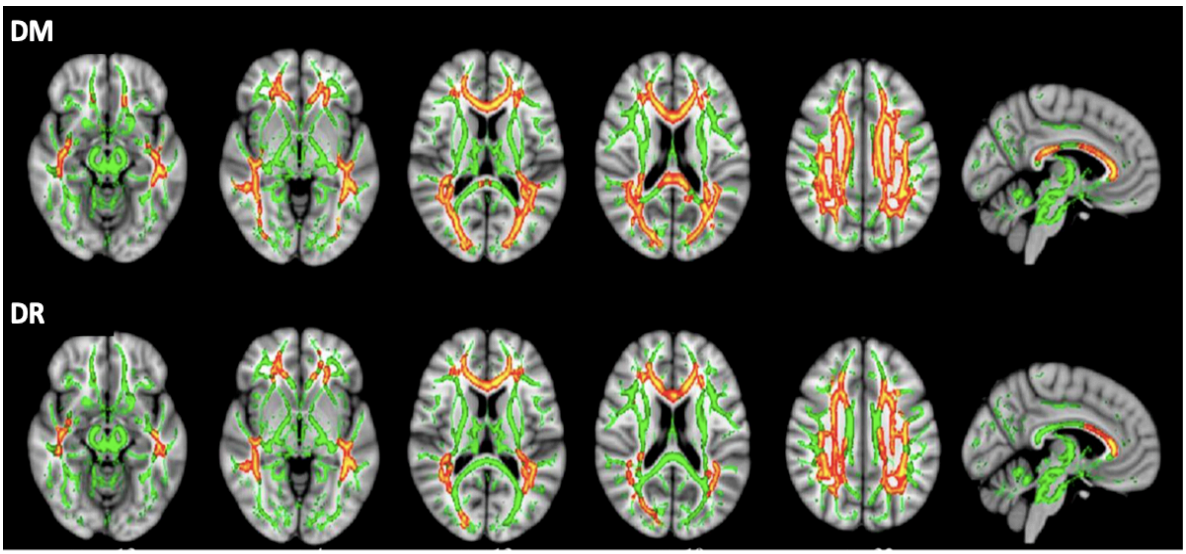


Figura 8: Gráficos de TBSS: muestran regiones de reducción DM y DR en individuos con ETPKU en comparación con el grupo control. El color rojo-amarillo representa vóxeles significativos en p <0.05. El esqueleto medio de FA de todos los participantes se superponen en verde en la imagen del cerebro ponderada en T1 de MNI152. Adaptado en Peng et al. 2014.

1.8 Seguimiento dietético y alteraciones de micronutrientes:

Otro aspecto muy relevante en el seguimiento de los pacientes PKU es el estudio de los efectos del tratamiento dietético restringido en proteínas naturales, que hace que los pacientes PKU sean un grupo susceptible de padecer deficiencias dietéticas de sustancias esenciales, como vitaminas y oligoelementos (Ormazábal et al. 2005; Artuch et al. 2004). De hecho, nuestra propia experiencia y la de otros grupos han revelado cuáles son los nutrientes que pueden estar deficientes en la PKU, y que además potencialmente podrían estar asociados a ciertos trastornos neurológicos como en el caso de la deficiencia de selenio (Gassió et al. 2008). Se ha demostrado en ratones con restricción dietética de selenio una disminución de la actividad del enzima glutatión peroxidasa (GPX) en el CPF y un aumento del catabolismo de la dopamina (Castaño et al. 1997). En un estudio de nuestro grupo (Gassió et al. 2008) encontramos una relación entre los valores bajos de selenio en plasma y un peor rendimiento en las pruebas de atención, función cognitiva dependiente del CPF. Otro nutriente deficiente en pacientes PKU es el CoQ_{10}, deficiencia debida principalmente a una inhibición en su síntesis endógena causada por los valores elevados de Phe (Artuch et al. 2004). La relación de la deficiencia de CoQ_{10} diferentes trastornos neurológicos hace muy pertinente su inclusión en el estudio. De hecho, sería probable que ocasionara trastornos sutiles en el metabolismo energético mitocondrial que podrían estar relacionados con alteraciones en el metabolismo y función de los neurotransmisores, que son procesos demandantes de energía en forma de ATP. Finalmente será de especial interés el seguimiento de los pacientes en tratamiento con BH_4. Este cofactor de la enzima PAH, además de conseguir reducir los valores de Phe, actuaría también mejorando la síntesis de dopamina y serotonina, ya que actúa también como cofactor de las enzimas tirosina hidroxilasa y triptófano hidroxilasa (Surtees et al. 2000). Este tratamiento ha emergido en la pasada década, pero todavía la experiencia en su seguimiento es muy corta y hay pocos datos en la literatura de sus potenciales efectos sobre la neurotransmisión dopaminérgica y serotoninérgica en los pacientes PKU tratados (Lambruschini et al. 2005) y además la experiencia en nuestro país es muy escasa (Campistol et al. 2012). A pesar de instaurar el tratamiento precoz o lograr

un adecuado control metabólico de los pacientes PKU, estos pueden presentar alteraciones neurológicas y neuropsicológicas, aspectos en los que se han centrado nuestras investigaciones.

JUSTIFICACIÓN DE LA UNIDAD TEMÁTICA

2. Justificación de la Unidad Temática

En los estudios realizados en esta tesis se ha intentado caracterizar los determinantes de las alteraciones neurológicas y del comportamiento, son los primeros estudios en conjunto de una cohorte relativamente importante de pacientes PKU seguidos a largo plazo.

También se han investigado las alteraciones neurológicas-neuropsicológicas, y su relación con biomarcadores de la neurotransmisión, y alteraciones de la microestructura de la sustancia blanca en relación a un grupo control.

Hay pocos estudios en pacientes PKU pediátricos y adolescentes de diagnóstico precoz en los que se hayan evaluado la calidad del sueño, y estos se han centrado en evaluar los patrones electroencefalográficos de sueño REM y no REM. En nuestro estudio analizamos la calidad de sueño con cuestionarios y las correlaciones con precursores de melatonina comparándolos con una población control.

Finalmente, se sabe que el déficit de la CoQ10 es una alteración metabólica secundaria de origen multifactorial en la PKU que puede condicionarles un aumento del estrés oxidativo. Se ha evaluado las concentraciones de CoQ10 y el índice coenzima Q10/colesterol en pacientes con PKU, otros errores congénitos del metabolismo, y otros con enfermedades neurológicas genéticas y no determinadas genéticamente, en una cohorte de pacientes pediátricos y adultos jóvenes menores de 22 años. Asimismo se evaluaron factores genéticos y ambientales que pueden influir en las concentraciones plasmáticas de la CoQ10 para intentar determinar el origen de la deficiencia.

Por tanto, hemos desarrollado en este trabajo una investigación orientada al paciente PKU, que ha nacido desde la experiencia clínica apoyándose en datos complementarios tanto de laboratorio como de neuroimagen.

HIPÓTESIS

3. Hipótesis

La hipótesis de esta tesis doctoral postula que a pesar de instaurar el tratamiento precoz o lograr un adecuado control metabólico de los pacientes PKU, estos pueden presentar alteraciones neurológicas-neuropsicológicas, nutricionales y de neuroimagen a medio- largo plazo.

OBJETIVOS

4. Objetivos

4.1. Objetivo general:

Investigar las complicaciones neurológicas y neuropsicológicas en el seguimiento de los pacientes PKUDP, a pesar de un adecuado control de la enfermedad a través de investigaciones clínicas, bioquímicas y de neuroimagen.

4.2. Objetivos concretos:

1. Caracterizar los factores determinantes de las complicaciones neurológicas y los trastornos del comportamiento en una cohorte de pacientes PKUDP seguidos a largo plazo.
2. Estudiar las complicaciones neurológicas y neuropsicológicas en el seguimiento de los pacientes PKUDP a través de investigaciones clínicas, bioquímicas y de neuroimagen.
3. Estudiar las alteraciones del sueño en pacientes PKUDP y su relación con las alteraciones de melatonina y serotonina.
4. Elucidar las posibles causas de la deficiencia de la coenzima Q en pacientes PKU.

PACIENTES, MATERIAL Y MÉTODOS

5.1 Pacientes del estudio

En el conjunto de trabajos que se incluyen en esta tesis, han participado 281 pacientes PKU controlados periódicamente en nuestro hospital (Centro de Referencia para la PKU en Cataluña). Se estudiaron pacientes de diagnóstico precoz y solo la primera investigación incluye pacientes de diagnóstico tardío.

5.1.1. Pacientes del estudio del Objetivo 1:

En este estudio retrospectivo descriptivo se estudiaron 121 pacientes PKU diagnosticados y tratados en la unidad de seguimiento PKU del Hospital Sant Joan de Dèu de Esplugues (Centro de referencia de Fenilcetonuria en Cataluña) desde su creación en 1985 hasta 2010. Del total, 14/121 siguieron tratamiento con BH4, y el resto fueron tratados con dieta restrictiva en Phe.

Criterios de inclusión: A) pacientes PKU diagnosticados y seguidos en este centro, confirmados por diagnóstico genético de la deficiencia de PAH. B) pacientes PKU con concentraciones de Phe superiores a 360 µmol/L.

Registro de pacientes: Se incluyeron 121 pacientes en los que se recogieron datos clínicos (edad, sexo, edad al diagnóstico y tratamiento), complicaciones neurológicas, (epilepsia, temblor, torpeza, espasticidad, discapacidad intelectual), hallazgos neurorradiológicos, y trastornos del comportamiento (déficit de atención, impulsividad, hiperactividad, síntomas de ansiedad y depresión, fobias, agresividad y baja autoestima), datos metabólicos (control dietético) y mutaciones en el gen *PAH*. Los datos fueron extraídos de las historias clínicas de cada paciente.

5.1.2. Pacientes del estudio del Objetivo 2:

5.1.2. A. Se estudiaron 15 pacientes con PKUDP (mediana de edad 12 años, rango 8-18 años) de la unidad de seguimiento del hospital Sant Joan de D**è**u. Todos los pacientes fueron diagnosticados con el programa de cribado neonatal y fueron tratados continuamente desde las primeras semanas de vida. El tratamiento fue el siguiente: 11 pacientes con tratamiento dietético (restricción de Phe), de los cuales 5 fueron clasificados como de buen control metabólico (mediana del último año o Índice de control de dieta) (Vilaseca et al. 1995) fue Phe < 360 µmol/L para pacientes menores de 12 años de edad o Phe < 600 µmol/L para los mayores de 12 años, y el resto mostraron un cumplimiento de la dieta subóptimo (van Spronsen et al. 2017). Los 4/15 pacientes restantes fueron tratados con BH4 y todos presentaban un buen control metabólico. Ningún paciente manifestó historia o clínica de deterioro neurológico.

5.1.2. B. Grupo control de objetivo 2:

El grupo control del estudio de neuroimagen "White matter microstructural damage in early treated phenylketonuric patients" estaba formado por 11 participantes (media de edad: 11 años, rango de edad 9-18 años; 5 varones y 6 mujeres), que fueron referidos para realizar la resonancia magnética craneal (RMC) por cefalea. Ninguno de los controles presentaba historia de discapacidad intelectual, problemas de aprendizajes, trastornos psiquiátricos o problemas médicos relacionados con la PKU. La edad, sexo y las características socio-culturales fueron comparadas con el grupo PKU de diagnóstico precoz.

5.1.3. A. Pacientes del estudio del Objetivo 3:

Se estudiaron 32 pacientes PKU tratados de forma precoz con una edad media de 12 años (DE ± 3.36) (16 mujeres y 16 varones). El grupo de pacientes PKU se dividió en tres subgrupos: 1) 12 pacientes bajo tratamiento dietético y un buen control metabólico (índice de control dietético (IDC) del último año < 360 µmol/L para pacientes menores de 12 años o < 600 µmol/L después de 12 años), edad media 10 años (DE ± 2,21); 2) 7 pacientes bajo

tratamiento dietético y control metabólico deficiente (ICD del último año > 360 µmol/L para pacientes menores de 12 años o > 600 µmol/L después de 12 años), edad media 11,7 años (DE ± 3,09); y 3) 13/32 pacientes en tratamiento prolongado (entre 4 y 12.5 años de tratamiento) con BH4 y buen control metabólico, edad media 13.9 años (DE ± 3.48). Los criterios de inclusión para pacientes con PKU fueron: inicio temprano del tratamiento (durante las primeras semanas de vida), tratamiento continuado con dieta restringida o BH4, cociente intelectual superior a 80, ausencia de medicación el día del análisis y ausencia de patología médica asociada o trastornos del estado de ánimo que podría condicionar el resultado de los estudios psicológicos y de sueño. Tres pacientes en el grupo de PKU (uno en cada subgrupo) fueron diagnosticados de TDAH y recibieron tratamiento farmacológico con metilfenidato de liberación prolongada; tenían buena tolerabilidad y, por lo tanto, se incluyeron en el estudio. Los criterios de inclusión para el grupo control fueron: rendimiento escolar normal, ausencia de medicación crónica, ausencia de patología médica o trastornos del estado de ánimo que podrían conducir a un trastorno del sueño, y no ser hermanos sanos de pacientes PKU (para evitar hábitos familiares o factores ambientales que podrían tener un impacto en la calidad del sueño).

5.1.3. B. Grupo control del Objetivo 3:

En el estudio de sueño participaron 32 sujetos sanos con una media de 11.9 años de edad (DE ± 3.29) (16 mujeres y 16 varones), con las mismas condiciones socio-culturales y demográficas.

5.1.4. A. Pacientes del estudio del Objetivo 4:

Se estudiaron retrospectivamente la base de datos de 597 individuos (rango de edad: 1 mes a 43 años, media 11 años), seguidos durante el período 2005-2016 en el Hospital Sant Joan de Dèu y en el Hospital Great Ormond Street (Londres). 113 con fenilcetonuria (PKU), 44 con mucopolisacaridosis (MPS), 61 con otros errores innatos del metabolismo (EIM), 99 con trastornos neurogenéticos, 197 con otros trastornos neurológicos no determinados genéticamente.

5.1.4. B. Grupo control del objetivo 4:

Los controles fueron niños sanos sin tratamientos farmacológicos crónicos enviados al Hospital Sant Joan de D**è**u para intervenciones quirúrgicas menores (principalmente fimosis, adenoides y drenaje timpánico).

5.2. Aspectos éticos

Los protocolos de estudio y las enmiendas fueron aprobados por el Comité de ética (CE) del Hospital Sant Joan de Dèu. Se obtuvo el consentimiento informado por escrito de todos los padres (cuando el paciente era menor de 18 años de edad o presentaba discapacidad intelectual) y para los participantes del grupo de control en el momento de la visita. Los estudios se realizaron de acuerdo con los principios de la Declaración de Helsinki, revisada y actualizada en 2013.

5.3 Material y métodos

5.3.1. Evaluación neurológica y alteraciones del comportamiento

En el caso del estudio inicial de las complicaciones neurológicas, estas fueron registradas a través de la revisión de historias clínicas en el estudio del objetivo 1, pero en el resto la exploración fue directa. En el exámen neurológico se tuvieron en cuenta las alteraciones neurológicas como temblor, torpeza motora y signos piramidales (hiperreflexia, espasticidad y signo de Babinski).

El registro de problemas de comportamiento se realizó a través de cuestionarios a los padres, pacientes y profesores. Los pacientes fueron controlados por un neurólogo cada 6 meses durante el primer año de vida, anualmente a partir del primer año de vida hasta los dos años de vida y cada 2 años a partir del tercer año de edad. El desarrollo psicomotor fue evaluado al año, y a los tres años y la capacidad intelectual a los 6, 12 y 18 años en los controles de psicología.

Dentro de las alteraciones de comportamiento se incluyeron síntomas internalizantes como ansiedad, síntomas de depresión y baja autoestima, y síntomas externalizantes como hiperactividad, impulsividad y agresividad.

5.3.2. Evaluación neuropsicológica

El coeficiente de desarrollo fue calculado con la escala de Brunet Lezine para valoración del desarrollo en la infancia precoz en pacientes menores de 4 años de edad. El coeficiente intelectual (CI) fue evaluado con Kaufman Bit intelligence Test (K Bit) (pacientes de 4-6 años), por la escala intelectual de niños de Wechsler (WISC-IV) (Weschsler 2007) y por la escala de inteligencia de adultos de Wechsler (WAIS-III) (Wechsler 1999). Dado que las anomalías de la SB se han asociado con un rendimiento más lento en la velocidad de procesamiento, se aplicaron los siguientes test neuropsicológicos para evaluarla: índice de velocidad de procesamiento de Escalas Wechsler, tiempo requerido para copiar la figura compleja de Rey (Rey 2003), velocidad motriz y de búsqueda visual con Trail Making Test Part A (prueba del trazo) (Strauss et al. 2006), velocidad con velocidad de denominación con el NEPSY II -subtest (Korkman et al. 2014), velocidad de respuesta y tiempo de reacción del golpe, Prueba de rendimiento continuo de Conners II (CPT-II) (Conners 2004) y tiempo total de iniciación en la ejecución de la prueba de la Torre de Londres (Culberston et al. 2005). La puntuación típica (PT) de referencia para el coeficiente intelectual (CI) fue de 100 ± 15 para todos las pruebas, salvo la referencia típica de puntuación en la evaluación del tiempo de reacción del golpe en CPT-II, fue de 50 ± 10, ya que se consideró una ejecución más rápida la asociada a una puntuación menor que 40 y una ejecución más lenta a una puntuación mayor de 60.

5.3.3. Material y métodos de laboratorio:

5.3.3. A. Determinación de fenilalanina y tirosina.

La determinación de Phe y tirosina de las muestras de plasma y sangre seca, se realizó por cromatografía líquida de intercambio iónico y derivatización con ninhidrina usando

el equipo Biochrom 30 (Pharmacia-Biotech). La frecuencia de las mediciones varía entre semanalmente (< 2 meses de vida), quincenalmente (entre 2 meses y 4 años de edad) ó mensualmente (> 4 años de edad). Se utilizó el índice de control de dieta de los primeros 6 años de vida, (valor de Phe medio en µmol/L de las medianas anuales) y valores de Phe de toda la vida (calculada como la medición de la media de las medianas de toda la vida de Phe). También evaluamos la variabilidad de Phe (desviación estándar de los niveles de Phe) e ICD (mediana de los niveles de Phe en sangre) durante el último año. En el estudio de neuroimagen se determinó la Phe plasmática concurrente (medición de Phe el día de la realización de la resonancia). Los análisis de sangre se realizaron en condiciones de ayuno, a primera hora de la mañana.

5.3.3. B. Indice de control de la dieta

Se consideró un ICD bueno cuando la Phe < 360 µmol/L para los pacientes menores de 12 años de edad, y < 600 µmol/L en los mayores de 12 años de edad y regular o malo cuando estaban por encima de estos parámetros, (van Spronsen et al. 2017). Previo a la publicación de esta guía, se utilizó el protocolo de la Unidad de Seguimiento de la PKU del Hospital Sant Joan de Dèu, Centro de Referencia en Cataluña de la PKU, según el cual, el ICD del último año dependía de la edad del paciente: se consideró como bueno cuando en los pacientes menores de 10 años el ICD < 360 µmol/L y para los pacientes con edades entre los 10 a 18 años < 480 µmol/L. Se consideró un control regular para los pacientes menores de 10 años si el ICD era entre 360 – 480 µmol/L y para los mayores de 10 años si el ICD era entre 480- 600 µmol/L. El ICD fue considerado malo si los valores se encontraban por encima de estos rangos.

5.3.3. C. Determinación de marcadores de neurotransmisores

Se analizaron biomarcadores bioquímicos relacionados con la neurotransmisión dopaminérgica y serotoninérgica, además de la determinación en plasma de Tyr y Phe (comentados previamente), se analizó la excreción de metabolitos de aminas biógenas en orina como ácido homovanílico (HVA) para dopamina y 5-hidroxiindolacético (5HIAA) para la se-

rotonina, por medio de cromatografía de gases acoplada a detección por espectrometría de masas (Agilent Technologies).

La determinación de triptófano se realizó por cromatografía líquida de alta presión (HPLC) con detección de fluorescencia (Waters, Milford, MA, EE.UU.), según protocolos y procedimientos ya descritos (Ormazábal et al 2005).

La secreción de prolactina es inhibida por dopamina, y por lo tanto una elevación de este biomarcador puede usarse como marcador de déficit dopaminérgico cerebral. La deficiencia de hierro está involucrada en algunos trastornos del sueño y, por lo tanto, se determinaron los niveles de ferritina en sangre (Trotti et al. 2012). La prolactina y la ferritina en suero se midieron mediante inmunoensayo de micropartículas quimioluminiscentes utilizando el analizador automatizado Architect (Abbott). La concentración de serotonina plaquetaria se ha utilizado como un marcador indirecto de la función serotoninérgica central. Las concentraciones de serotonina plaquetaria fueron analizadas por HPLC con detección de fluorescencia (Waters, Milford, MA, EE. UU.), siguiendo un procedimiento publicado por nuestro grupo (Ormazábal et al. 2005). La 6-sulfatoximelatonina en orina (el metabolito final de la melatonina) se analizó por duplicado usando un kit competitivo de ELISA (IBL: Ref. RE54021) (Batllori et al. 2017). Las muestras de orina fueron recolectados en la primera micción de la mañana. Todos los resultados bioquímicos se compararon con los valores de referencia establecidos por nuestro laboratorio.

5.3.3. D. Determinación de coenzima Q_{10}

Los valores totales de colesterol (Col) en suero se analizaron mediante el procedimiento automatizado de colesterol oxidasa en un analizador automatizado Architect (Abbot). La CoQ_{10} total en plasma, la suma de la forma reducida y la oxidada del CoQ_{10} se midió por cromatografía líquida de alto rendimiento en fase reversa con detección electroquímica y con detección ultravioleta a 275nm, según procedimientos publicados previamente (Montero et al. 2008; Duncan et al. 2005). En la figura 9, se representan cromatogramas típicos del material de control de calidad interno y de las muestras de plasma humano. Como podemos observar, la separación de CoQ_{10} es óptima y los sistemas de detección electro-

química son específicos para el análisis de CoQ_{10} en matrices complejas como plasma. La detección electroquímica por HPLC tiene mayor sensibilidad en comparación con la detección ultravioleta.

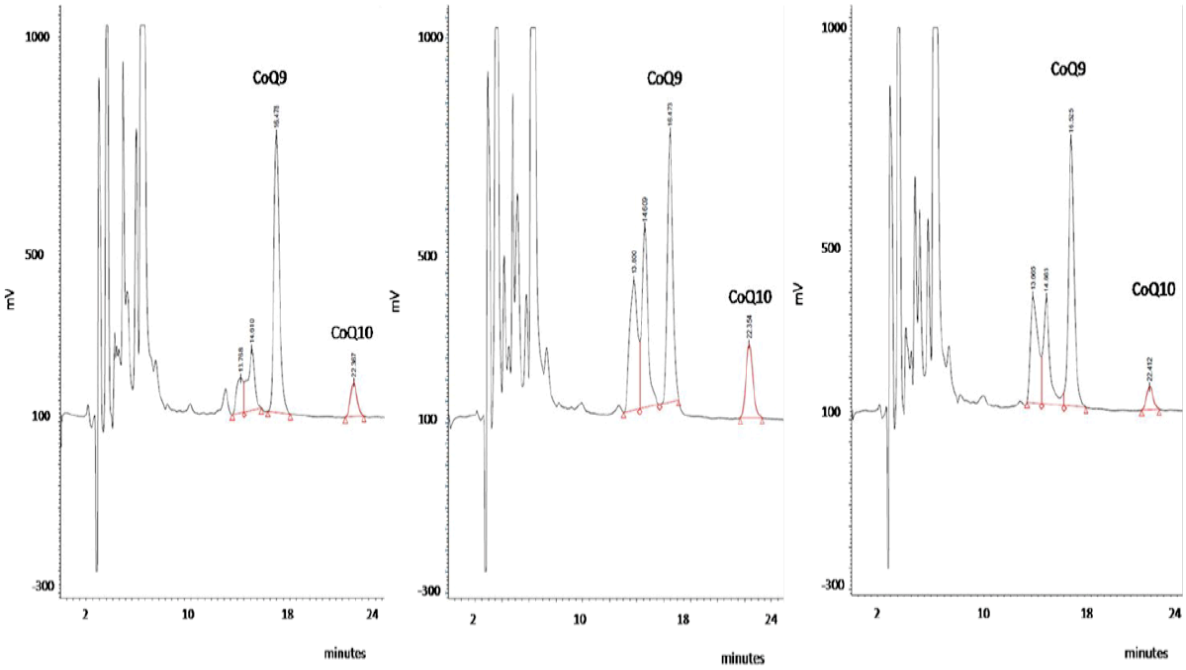


Figura 9. Cromatograma típico (detección electroquímica con HPLC): Panel izquierdo. Control comercial (Coenzima Q_{10} Chromsystens, nivel 1 (Ref.0092): CoQ10 = 0.56 µmol / L), basado en matriz de suero. Panel medio. Muestra de plasma humano con CoQ10 = 1,18 µmol / L. Panel derecho. Muestra de plasma humano con CoQ10 = 0,38 µmol / L. Las muestras, calibradores y controles se enriquecen con estándar interno (coenzima Q9 (CoQ9)).

5.3.3. E. Análisis genético

El estudio mutacional de los errores congénitos del metabolismo y otros trastornos neurológicos determinados genéticamente fueron realizados mediante el estudio de secuenciación génica por SANGER y con tecnología NGS, previamente descrita (Yubero et al. 2016).

5.3.4. Neuroimagen

5.3.4.A. Parámetros de resonancia magnética craneal

Todos los estudios de resonancia magnética craneal (RMC) se realizaron en un escáner de RM de 1.5 Tesla (General Electric Signa HD). Los parámetros de adquisición fueron los siguientes: secuencia EPI spin eco (53 cortes axiales, tiempo de repetición (TR): 15000ms, Tiempo de eco (TE): 104ms, tamaño de matriz de adquisición: 256x256 pixeles, tamaño del voxel: 0.94x0.94x2.5mm3).

Las imágenes se adquirieron con un volumen ponderado sin difusión (usando una secuencia EPI spin eco con cobertura de todo el volumen cerebral) y 25 volúmenes ponderados en difusión (direcciones de gradiente de difusión no colineal, valores-b de 1500 s/mm2).

5.3.4.B. Glosario de herramientas utilizadas para el análisis

FSL: es una biblioteca completa de herramientas de análisis para datos de imágenes cerebrales de resonancia funcional, resonancia magnética y con tensor de difusión.

FDT-FMRIB Diffusion Toolbox (FMRIB Software Library): es parte del software FSL y con una biblioteca completa de herramientas de análisis de imágenes neurológicas de diverso tipo: RM estructural, funcional y de difusión. FDT incluye herramientas para pre-procesamiento de datos, modelado de difusión local y tractografía. Cada etapa de FDT se ejecuta por separado.

TBSS: (de las siglas en inglés “estadística espacial basada en el tracto”) . Es una técnica de análisis que se usa en la normalización, requieren un registro de imágenes de los sujetos mediante el uso de una plantilla. Esto se hace con el objetivo de garantizar que se estén comparando regiones homólogas entre los diferentes grupos de pacientes. Para llevar a cabo el registro, lo más eficaz es utilizar la información del tensor de difusión, que proporciona una mejor normalización de la morfología de los tractos de sustancia blanca. El TBSS se presenta como un método que tiene como objetivo cubrir las falencias de los algoritmos

basados en comparación de vóxeles, mejorando la sensibilidad, objetividad e interpretabilidad del análisis multisujeto de las imágenes de difusión. A grandes rasgos realiza un registro nolinear seguido de proyecciones sobre una representación de tractos invariantes entre sujetos (llamado "media de esqueleto de Anisotropía Fraccional").

Tractografía: es una técnica de modelado que representa una reconstrucción de los tractos neurales a partir de la información contenida en las imágenes de tensor (DTI). Esta modalidad saca provecho de la direccionalidad de los tensores de la DTI y, a partir de diversos algoritmos, reconstruye los tractos de sustancia blanca cerebral.

5.3.4. C. Pre-procesamiento de resonancia magnética con tensor de difusión

Previo al análisis, se realizaron varios procedimientos automatizados de las imágenes:

1. Corregir distorsiones causadas por las corrientes eléctricas inducidas por el campo magnético y por artefactos del movimiento de la cabeza del sujeto con el software FDT-FMRIB Diffusion Toolbox (Leemans et al 2009).
2. Rotación de la matriz de gradientes ortogonales para proporcionar una estimación más precisa de las orientaciones del tensor de difusión, garantizando que tengan la misma orientación que las imágenes de TD (con la rotación del software FDT del comando "rotate_bvecs "(Smith. 2002).
3. Extracción del volumen cerebral usando la herramienta de extracción de cerebro (Wang et al. 2015).
4. Análisis y reconstrucción en 3D de los tensores de difusión utilizando el algoritmo lineal de mínimos cuadrados incluido en difusión con la aplicación DiffusionToolkit0.6.2.2 (http://www.trackvis.org/dtk/).
5. Cálculo de los mapas de AF, DM, DR para cada paciente y control utilizando los valores propios extraídos de los tensores de difusión.

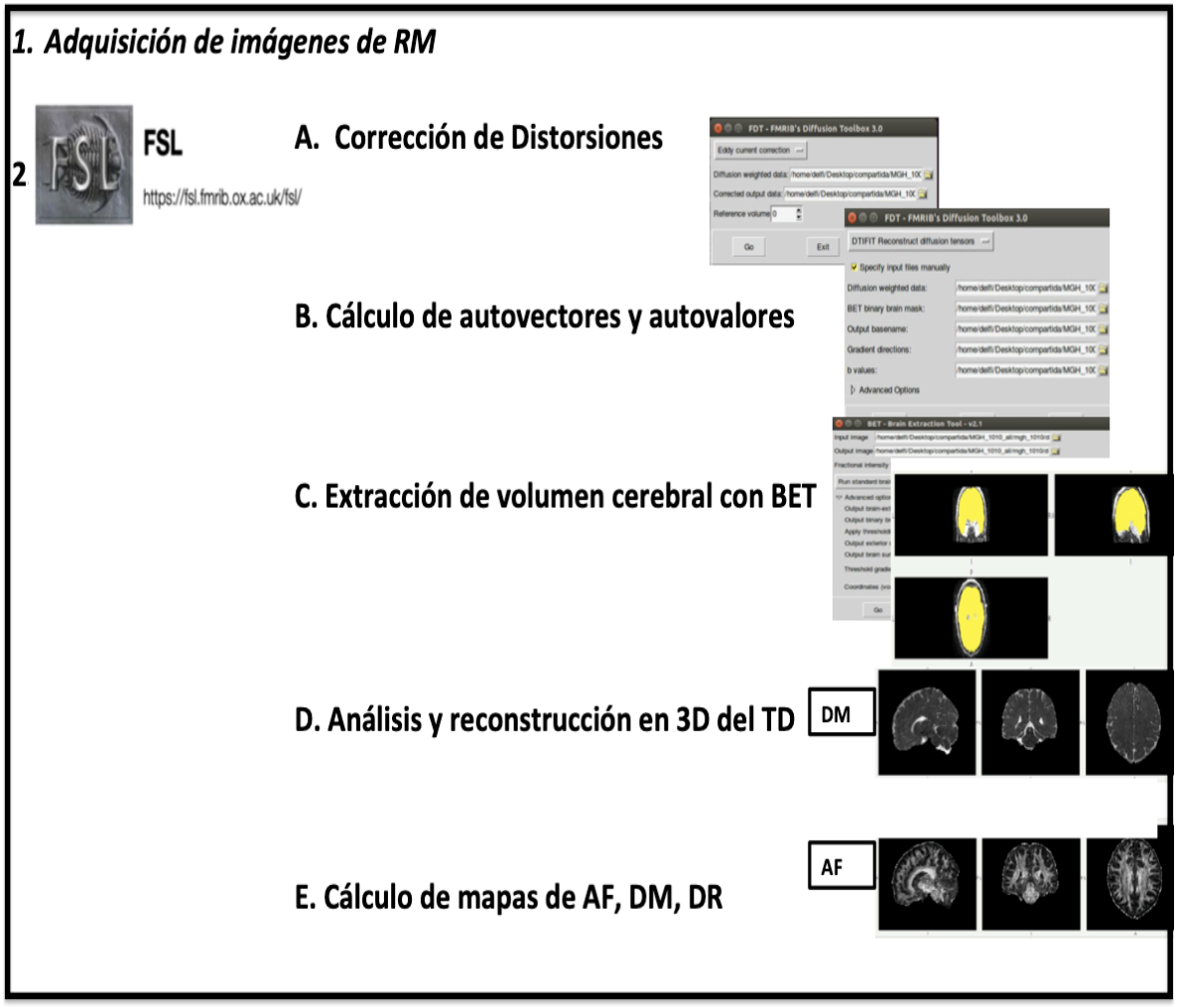


Figura 10: Esquema del Pre-Procesamiento de la imágen de RMC-TD

5.3.5. Revisión sistemática

En la revisión sistemática realizada en el estudio de neuroimagen se revisó la literatura publicada entre 2001 a 2016: en PubMed: http: //www.ncbi.nlm.nih. gobierno / pubmed. Para evitar cualquier sesgo, en la búsqueda general se eligieron los términos: estudios clínicos, PKU / fenilcetonuria, imágenes de tensor de difusión, resonancia magnética, tratamiento precoz, tratamiento tardío, pacientes pediátricos y adultos.

Criterios de inclusión: estudios en humanos con grupo control, estudios de neuroimagen con tensor de difusión realizados en pacientes pediátricos o adultos con PKU, de diagnóstico precoz o tardío. Criterios de exclusión: casos aislados, estudios en animales/o modelos celulares y pacientes con otras enfermedades metabólicas como deficiencias de

BH4 y artículos no publicados en inglés. Se revisaron un total de 12 artículos que cumplieron con los criterios de inclusión, 9 de ellos estudiaron pacientes PKU de diagnóstico precoz (Vermathen et al. 2007; White et al. 2010; White et al. 2013; Antenor-Dorsey et al. 2013; Peng et al. 2014; Wesonga et al. 2016; Hood et al. 2015; Hood et al. 2016; Ding et al. 2008), mientras que los otros artículos describen pacientes PKU de diagnóstico tardío y precoz comparados con un grupo control (Leuzzi et al. 2007; Kono et al. 2005; Scarabino et al. 2009).

5.3.6. Evaluación de los trastornos del sueño

Todos los pacientes del estudio de sueño fueron evaluados con el cuestionario de Bruni: Escala de trastornos del sueño para niños (siglas en inglés: SDSC: Sleep disorders scale classification in children) (Bruni et al. 1996). Para detectar la presencia de trastornos del sueño, se siguieron las recomendaciones de la guía de práctica clínica sobre los trastornos del sueño en la infancia y adolescencia para la atención primaria de España del Sistema Nacional de Salud (Clinical Practice Guideline 2009). La escala consta de 26 ítems y fue desarrollada para detectar trastornos del sueño en los últimos 6 meses en niños y adolescentes basado en los informes de los padres. Seis factores representaron las áreas más comunes de trastornos del sueño en la infancia y adolescencia: trastornos de inicio y mantenimiento, trastornos respiratorios del sueño, trastornos del despertar, trastornos de la transición sueño-vigilia, somnolencia excesiva y trastorno de hiperhidrosis del sueño. Estas sub-escalas fueron consideradas como alteradas cuando la puntuación típica (PT) fue > 70 (PT: 50 ± 10).

5.3.7. Métodos estadísticos

Todas las variables clínicas, neurorradiológicas y bioquímicas fueron recogidas en una base de datos de Microsoft Excel.

Los estudios estadísticos se realizaron con el programa de SPSS (versión 18, 19.0, 23, ver en los diferentes artículos), aplicando las siguientes pruebas:

A. Para la comparación de variables cualitativas: se utilizó la prueba de Chi-cuadrado.

B. Para la comparación de variables cuantitativas:

-Variables paramétricas:

. Prueba T de Student para comparación de medias y Kruskal-Walllis se utilizó para comparar las medias de variables numéricas entre grupos.

. ANOVA para el análisis de varianzas, con corrección de Bonferroni en comparaciones múltiples.

C. Pruebas de correlación simple: Variable paramétrica: Prueba de Pearson.

D. Análisis estadístico: Mapas estadísticos paramétricos:

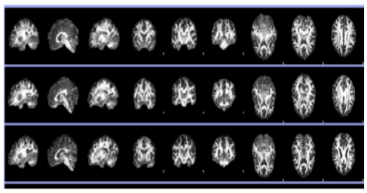
Se realiza el análisis de vóxel de tractos basándonos en mapas de AF, DM, DR utilizando estadísticas espaciales basadas en tractografía (TBSS) (Anderson et al. 2007). Utiliza algoritmos basados en comparación de vóxeles, mejorando la sensibilidad, objetividad e interpretación del análisis de las imágenes de difusión. Se realizan los siguientes pasos:

1. Escala los volúmenes de las imágenes y erosiona ligeramente los mapas de AF.
2. Calcula el registro no lineal, alineando todos los mapas de AF de todos los participantes en una plantilla FMRIB58 AF en espacio MNI (Montreal Neurological Institute, 152 1x1x1mm3).
3. Los mapas registrados de AF se promedian para crear un volumen medio de AF. Se obtiene la AF media del esqueleto de AF, lo cual representa el centro de todos los tractos comunes a todos los participantes del estudio. Los datos alineados de AF de cada paciente se proyectaron en este esqueleto buscando el valor de AF más alto dentro de una búsqueda del espacio perpendicular a cada vóxel del esqueleto medio.
4. Este procedimiento se repitió para los mapas de DM y DR, aplicando las transformaciones previamente calculadas con los mapas de AF.

Finalmente para evaluar las diferencias en la SB entre controles y pacientes PKU, se aplicó la prueba de T student para grupos independientes para los esqueletos AF, DM, DR. Los resultados se informaron con un valor $p<0.05$ corregido por FWE usando una algoritmo con 5000 permutaciones (Nichols et al. 2005). Los tractos de SB se identificaron utilizando el atlas de SB de JHU-ICBM DTI 81 (Hua et al. 2008; Klawiter et al. 2011).

Se calculó el valor medio de todos los vóxeles que mostraron diferencias significativas entre controles y pacientes. Se aplicó la prueba de Pearson entre estos valores (que representaba daño individual SB) y edad, Phe concurrente, mediana del último año, mediana de toda la vida y valores medios de Phe, y concentraciones de HVA y 5HIIA. También se calcularon correlaciones de Pearson entre los valores de la difusividad y las puntuaciones de las pruebas neuropsicológicas. Las correlaciones se calcularon con MATLAB R2012a (The MathWorks, Natick, MA, EE. UU.). Una correlación se consideró significativa si sobrevivía a una $p < 0.05$ con una corrección por múltiples comparaciones con FDR. Dado que los 15 pacientes con PKUDP pueden subdividirse en 2 grupos diferentes (buen control metabólico y pobre control metabólico) completamos un último análisis. Nuevamente, usando el promedio DM, DR (sin que resultados significativos se obtuvieron para AF), para los valores de todos vóxeles que mostraron diferencias entre pacientes y controles (mismos valores utilizados para los análisis correlacionales descritos anteriormente), probamos si los diferentes subgrupos de ETPKU mostraron diferentes porcentajes de reducción en los valores de difusividad (es decir, daño SB). Teniendo en cuenta el número reducido de pacientes por grupo utilizamos pruebas no paramétricas bajo SPSS (versión18.0.0) para realizar estos cálculos.

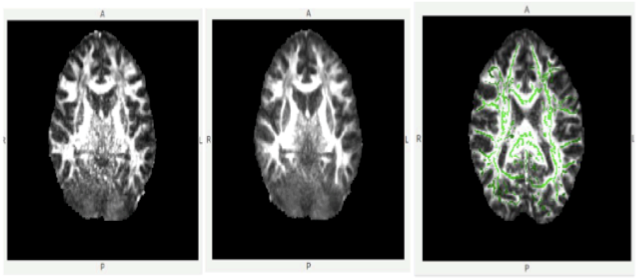
Análisis del voxel de tractos basados en los mapas de AF, DM, DR.

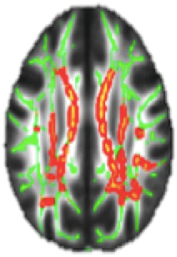


1. Escala los volúmenes de las imágenes y se erosiona ligeramente los mapas de AF

2. Cálculo de registro no lineal, alinea mapas de todos los participantes en una plantilla (MNI, 152 1x1x1 mm3)

3. Cálculo promedio de los mapas para crear volumen medio de AF





4. Se repite el procedimiento para mapas de DM y DR

5. Cálculo de diferencias entre los pacientes PKU y GC para mapas FA, DM, DR

6. Identificación de tractos con atlas de SB del JHU-ICBM DTI 81

7. Correlación de valores de difusividad y variables (Phe, NT, neuropsicológicas)

Figura 11: Esquema de Procesamiento de RMC-TD con TBSS

RESULTADOS

6.1. RESULTADOS 1.

OBJETIVO 1:

Caracterizar los factores determinantes de las complicaciones neurológicas y los trastornos del comportamiento en una cohorte de pacientes PKUDP seguidos a largo plazo.

Neurological complications and behavioral problems in patients with phenylketonuria in a follow-up unit.

María Julieta González, Alfonso Pablo Gutiérrez, Rosa Gassió, María Eugenia Fusté, María Antonia Vilaseca, Jaume Campistol.

Mol Genet Metab 2011;104 Suppl:S73– S79.

En este trabajo se estudió la relación entre las complicaciones neurológicas, los hallazgos neurorradiológicos y problemas de conducta, edad en el momento del diagnóstico y control dietético a lo largo del seguimiento de los pacientes con PKU en nuestra unidad metabólica.

Se realizó un estudio retrospectivo de los pacientes con PKU diagnosticados y controlados en nuestra unidad desde 1985 hasta 2010. A través del registro de pacientes en una base de datos con 50 ítems completados por medio de la revisión de las historias clínicas.

Molecular Genetics and Metabolism 104 (2011) S73–S79

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism

journal homepage: www.elsevier.com/locate/ymgme

Neurological complications and behavioral problems in patients with phenylketonuria in a Follow-up Unit

María J. González [a,b], Alfonso P. Gutiérrez [a,b], Rosa Gassió [a,b], María E. Fusté [b,c], María A. Vilaseca [b], Jaume Campistol [a,b,d,*]

[a] Neuropediatrics Department, Hospital Universitari Sant Joan de Déu, Barcelona, Spain
[b] PKU Follow-up Unit, Hospital Universitari Sant Joan de Déu, Barcelona, Spain
[c] Psychology Department, Hospital Universitari Sant Joan de Déu, Barcelona, Spain
[d] Centre for Biomedical Research on Rare Diseases (CIBERER), Institute of Health Carlos III, Spain

ARTICLE INFO

Article history:
Received 30 May 2011
Received in revised form 14 July 2011
Accepted 14 July 2011
Available online 23 July 2011

Keywords:
Phenylketonuria
PKU
Dietary control
Neurological complications
Behavioral problems
Brain magnetic resonance

ABSTRACT

Objective: To investigate the relationship between neurological complications, neuroradiological findings, and behavioral problems, age at diagnosis and dietary control along the follow-up of the PKU patients in our metabolic unit.
Design: Retrospective study of the PKU patients diagnosed and controlled in our unit from 1985 to 2010.
Methods: Registry of patients in a database with 50 items filled in by review of the clinical histories. Statistical study of the data (SPSS, 19.0 version).
Results: 121 patients were included (median age: 16.0, range 1 month–46 years). 76% of them were diagnosed through neonatal screening. 12.4% had mild-PKU, 19% moderate-PKU and 68.6% classic-PKU. 88.4% of patients were treated with a protein-restricted diet, and 11.6% with BH4. 97.7% of the early diagnosed patients had normal IQ, while 46.3% of late diagnosed patients had mental retardation, 28.5% were borderline and 25% had normal IQ. In early diagnosed patients, there was a significantly negative correlation between IQ [mean (SD) 100 (11.1)] and the index of dietary control during the first six years of life [median (range) 310 (105–992)] and that of the immediately past year [348 (106–1127)] ($p<0.0001$). The proportion of patients with late diagnosis and neurological and behavioral problems was significantly higher than that of the early diagnosed ones ($p<0.001$). The proportion of early diagnosed patients with neurological and behavioral problems who had good, intermediate or poor dietary control during the first 6 years of life and the immediately past year was significantly different ($p<0.001$).
Conclusions: The results show the impact of early diagnosis and good dietary treatment on the IQ and on the percentage of neurological complications and behavioral problems in PKU patients.

1. Introduction

Phenylketonuria (PKU) is an inborn error of phenylalanine (Phe) metabolism caused by a deficiency of phenylalanine hydroxylase (PAH), the hepatic enzyme which synthesizes tyrosine from Phe, using tetrahydrobiopterin (BH4) as a cofactor. Phe accumulation in plasma and tissues and a decrease in tyrosine synthesis seem to be involved in the pathogenesis of the disease [1,2]. Classical therapy of PKU consists of Phe intake restriction, which means a diet with low natural protein content supplemented with a special Phe-free formula, enriched with tyrosine and the other amino acids and micronutrients (vitamins, minerals, oligoelements and essential fatty acids in some of them) to prevent nutritional deficiencies [3,4]. An alternative treatment with BH4 also succeeds in decreasing high Phe concentrations in patients who respond to this therapy [5]. Early treatment of PKU prevents severe neurological damage [1]. However, despite appropriate treatment, some patients may present a slight decrease in intelligence in comparison with the general population [6,7] as well as specific deficits of executive functions, especially when metabolic control is poor [8,9]. Behavioral problems have also been described in patients with early treatment [10–12]. Quality of dietary control is determinant in the disease prognosis [7,13].

The neurological and metabolic evaluation of wide series of patients may allow better understanding of the relationship between dietary control and the neurological outcome. The recently created registry of Spanish PKU patients [14] led us to do a retrospective neurological, behavioral and metabolic study of the whole series of patients diagnosed and followed-up in our metabolic unit from its creation in 1985 until 2010.

Our objective was to investigate the relationship between neurological complications and behavioral problems, age at diagnosis

* Corresponding author at: Neuropediatrics Department, Hospital Sant Joan de Déu, Passeig Sant Joan de Déu 2, 08095-Esplugues (Barcelona), Spain. Fax: +34 93 280 36 26.
E-mail address: campistol@hsjdbcn.org (J. Campistol).

1096-7192/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.ymgme.2011.07.015

and dietary control along the follow-up of the PKU patients in our metabolic unit.

2. Material and methods

2.1. Patients

A retrospective, descriptive study of the PKU patients diagnosed and controlled in the Follow-up Unit of the Hospital Sant Joan de Déu (Reference Center for PKU in Catalonia) from its creation in 1985 until 2010 was performed. The inclusion criteria were: a) PKU patients diagnosed and/or followed-up in our center, with PAH deficiency confirmed by differential diagnosis and/or genetic analysis of the PAH gene, and b) PKU patients with plasma Phe concentrations before treatment above 360 μmol/L. The exclusion criteria were: a) PKU patients with late diagnosis who refused treatment and follow-up; b) foreign patients diagnosed in our center who returned to their country and are not controlled at present in our unit; and c) patients who died owing to causes unrelated with PKU. PKU patients were classified according to plasma Phe concentrations before treatment (mild PKU: 360–600 μmol/L, moderate PKU: 600–1200 μmol/L, and classical PKU>1200 μmol/L) [15].

2.2. Patient's registry

An Excel database was created with 50 patient issues including clinical data (age, sex, age at diagnosis and treatment), neurological complications (epilepsy, tremor, clumsiness, spasticity, and mental retardation), neuroradiological findings, and behavioral problems (attention deficit, impulsiveness, hyperactivity, anxious and depressive mood, phobias, aggressiveness and low self-esteem), metabolic data (dietary control) and mutations. The data were recorded following a review of the patient clinical histories.

2.3. Ethical issues

Adult patients or the parents (when patients were younger than 18 years of age or had mental retardation) signed an informed consent agreement in accord with the Helsinki Declaration of 1964, revised in Edinburgh in 2000. Our hospital ethics committee approved the study.

2.4. Methods

2.4.1. General intelligence measurement

Developmental quotient (DQ) was calculated with the Brunet–Lezine Scale for measuring Psychomotor Development in Early Infancy in patients younger than 4 years of age. Intelligence quotient (IQ) was evaluated with the Kaufman Bit Intelligence Test (K-Bit) (patients from 4 to 6 years), Wechsler Intelligence Scale for Children-Revised (WISC-R) or IV (WISC-IV) (patients from 6 to 16 years 11 months), and Wechsler Adult Intelligence Scale Third Edition (WAIS-III) (patients older than 17 years).

Level of intelligence was classified as normal range (DQ/IQ>80), borderline (DQ/IQ of 70–79) and mental retardation (DQ/IQ<69), according to the rules of the test manuals [16–20]. Mental retardation level was classified according to DSM-IV-TR criteria [21].

2.4.2. Neurological complications, neuroradiological study, and behavioral problems

Neurological complications were registered by review of the patients' clinical histories, and behavioral problems were recorded based on information from patients, parents and teachers.

Patients were controlled by the neuropediatrician every 6 months during the first year of life, annually for the first two years of life, and every 2 years after the age of three. In the neurological examination altered signs such as tremor, clumsiness and pyramidal signs (hiperreflexia, spasticity, and Babinski's sign) were especially focused. Tremor was evaluated with the WHIGET Tremor Rating Scale.

Development evaluation was performed at one, and three years of age, and intelligence quotient was evaluated at the age of 6, 12 and 18 years. Patients were evaluated annually by the unit psychologist until the age of 6 and at 12 and 18 years of age.

The brain magnetic resonance imaging (MRI) had not routinely been performed, but only in selected groups of patients for research or medical reasons.

Behavioral problems include internalizing symptoms such as anxiety, depressed mood, and low self-esteem, and externalizing symptoms such as hyperactivity, impulsivity and aggressiveness.

2.4.3. Biochemical methods

Plasma and dried blood spot Phe concentrations were analyzed by ion exchange chromatography (Biochrom 30, Pharmacia Biotech). The frequency of Phe measurements varied from weekly (<2 month of age) or biweekly (2 months–4 years old) to monthly (>4 years of age).

2.4.4. Index of dietary control (IDC)

We calculated the IDC as the Phe data reduction in half-year medians and the mean of all medians throughout the patient's life [5]. We examined the registry for the IDC of the first 6 years of life and the IDC of the immediately past year. We considered the IDC of the first 6 years of life to be good with Phe <360 μmol/L. The IDC of the immediately past year depended on patient age: in patients below 10 years of age <360 μmol/L was considered to be good and from 10 to 18 years <480 μmol/L was considered also good. In patients younger than 10 years of age an IDC from 360 to 480 μmol/L was intermediate, and it also was in patients older than 10 years of age an IDC<600 μmol/L. Control was deemed poor when the IDC was higher than these values.

2.5. Statistical study

The statistical study was performed with the SPSS program (version 19.0). Pearson chi-square test was applied to search for association between categorical variables. ANOVA with Bonferroni correction was used to compare quantitative variables when the number of data was higher than 30, the distribution was normal and the variances were not significantly different (Levene test). Otherwise, Kruskal–Wallis test was used. Pearson test was used for correlations between quantitative data. Statistical significance was accepted for $p<0.05$.

3. Results

3.1. Patients

3.1.1. Characteristics of the patients

We evaluated data from 121 patients who fulfilled selection criteria. Twelve patients were excluded (3 dead patients; 7 late diagnosed adults who refused treatment and follow-up, and 2 foreigners who had returned to their countries). Of the included patients, 46% (56) were males and 54% (65) females. Ages ranged from 1 month to 46 years (median 16.0 years). The age distribution was as follows: below 6 years: 32/121 (26.4%); from 6 to 11 years: 16/121 (13.2%); from 12 to 18 years: 19/121 (15.7%); and adults: 54/121 (44.6%).

3.1.2. Diagnosis

The newborn screening program started in Catalonia in 1970 but had only a universal coverture in 1985. Of the whole series, 76% (92) of patients were early diagnosed (during the first two months of life) and 24% (29) were late diagnosed. The present age of early diagnosed patients was a median 11.0 years (range: 1 month to 39 years), and that of late diagnosed patients was 34.0 (15 to 46 years).

3.1.3. PKU classification

12.4% (15) were mild PKU, 19% (23) were moderate PKU and 68.6% (83) were classic PKU.

3.1.4. Genetic study

From 242 alleles, 177 mutations were found. Those with higher allelic frequency were summarized in Table 1.

3.1.5. Treatment

All patients were treated with Phe-restricted diet at diagnosis. Fifteen patients (controlled elsewhere at that moment) abandoned the diet after the age of 6–10 years and all of them resumed the diet in 1985, when they started to be controlled in our unit. Seven of them were early diagnosed.

In 2003 a BH4-loading test was performed on 64 patients, and 13 of them who responded to BH4 were subsequently treated with this cofactor. Afterwards one more patient was treated at diagnosis. Therefore, 14 patients were treated at the moment of the registry with doses ranging from 6 to 18 mg/kg per day of BH4, while 107 patients were treated with diet and formula.

3.1.6. Dietary control

In patients with early diagnosis, the IDC for the first 6 years of life was: median (range) 310 (105–992) μmol/L and that for the immediately past year was 348 (104–1127) μmol/L. Fifty six patients (61%) had good dietary control, 22 patients (24%) had intermediate dietary control, and 14 patients (15%) had poor dietary control either during the first 6 years of life or during the last year.

In patients with late diagnosis the IDC for the immediately past year was 433 (260–1247) μmol/L. Seventeen patients (59%) has good dietary control, 4 patients (14%) intermediate, and 8 patients (27%) poor dietary control.

No significant differences were observed regarding sex in any age group.

3.2. Cognitive evaluation

3.2.1. Development and intelligence quotient related to the age at diagnosis

The DQ/IQ of the early diagnosed patients was mean 100 (SD 11.1). Of these, 97.7% had normal DQ/IQ, one patient was borderline, and only one patient with Turner syndrome had slight mental retardation. Three patients could not be evaluated.

The IQ of the late diagnosed patients was mean 62 (SD 21.6). Of these, 25% had severe mental retardation, 3.5% moderate mental retardation, 17.8% slight mental retardation, 28.5% were borderline and 25% had normal IQ. One patient could not be evaluated.

Table 1
Spectrum of the most common mutations in the PAH gene (in bold are those of major allelic frequency).

Mutations in the PAH gene	Detected alleles	Allelic frequency
IVS10-11 G>A	**26**	**14.6%**
V388M	**13**	**7.3%**
IVS4 + 5 G>T	**12**	**6.7%**
S349P	**11**	**6.2%**
I65T	**9**	**5.0%**
R158Q	**9**	**5.0%**
R261Q	6	3.3%
R243Q	6	3.3%
R261X	6	3.3%
R408W	5	2.8%
L48S	5	2.8%
R243X	5	2.8%
Y414C	4	2.2%

When we considered the DQ/IQ related to the age at diagnosis, a significantly different distribution was observed among patients with early diagnosis (before one month and from one to two months of age) and late diagnosis (from two months to one year and older than one year of age) (Kruskal–Wallis; $P<0.0001$) (Fig. 1).

3.2.2. Relationship between DQ/IQ and dietary control in early diagnosed patients

A significantly negative correlation was observed between DQ/IQ and the IDC of the immediately past year and that of the first 6 years of life (Pearson correlation test: $r=-0.478$, and $r=-0.435$, respectively, $p<0.0001$).

The DQ/IQ was significantly different in patients with good, intermediate and poor IDC in the first 6 years of life (Fig. 2) and in the immediately past year (ANOVA, Bonferroni correction $p<0.0001$).

3.3. Neurological complications, neuroradiological findings, and behavioral problems

3.3.1. Characteristics of the neurological complications, neuroradiological findings, and behavioral problems

Characteristics of the neurological complications, such as epilepsy, tremor and clumsiness were examined. The proportion of patients with these problems was summarized in Table 2.

Generalized or focal epilepsy was present in 9 of the 29 late diagnosed patients (31%), with favorable response to antiepileptic drugs, except for two cases. In the 30% of late diagnosed patients epilepsy started with flexion spasms. Only one early diagnosed patient with epilepsy family history had seizures.

Tremor was predominantly postural and kinetic, and was limited to the hands.

Clumsiness was present in 11% of the early diagnosed PKU patients who showed difficulties in gross motor functions, in throwing or catching a ball, in coordinating different limbs or in motor imitation skills. Four of the 29 late diagnosed patients (13.8%) did not walk and were wheelchair-bound. Spasticity was present in 8/29 late diagnosed patients (27.6%).

Brain magnetic resonance imaging (MR) was only performed in a limited number of early diagnosed patients ($N=28$), for special research studies or owing to clinical suspicion of abnormalities because of poor metabolic control. An abnormal image was detected in 17 patients (60.7%) consisting of increased signal in periventricular posterior white matter in T2 sequences. In the case of late diagnosed patients, MR was performed in 17 patients, and an abnormal white matter with cerebral (5 patients) and cerebellar (2 patients) atrophy was found in 12 of them (70.6%) (Table 2).

Behavioral problems were found in 28.3% of early diagnosed patients and in the 86.2% of late diagnosed patients (Table 2).

3.3.2. Neurological complications, neuroradiological findings, and behavioral problems in relation with the age at diagnosis

The proportion of patients with late diagnosis and neurological and behavioral problems, and abnormal neuroradiological findings was significantly higher than that of early diagnosed patients (Pearson chi-square test, $p<0.0001$) (Tables 2 and 3). We could not consider individual complications because of the low number of affected patients with early diagnosis.

3.3.3. Neurological complications, neuroradiological findings, and behavioral problems in patients with early diagnosis in relation to sex

No significant differences were observed in the proportion of patients with early diagnosis and neurological complications or behavioral problems in terms of sex (44 males/48 females) (Pearson chi-square test).

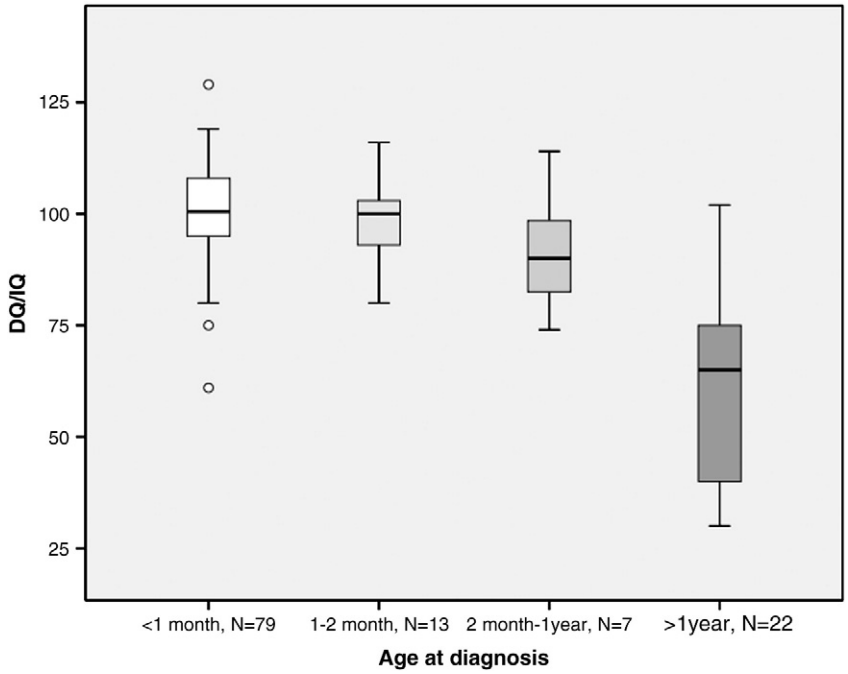


Fig.1. Development or intelligence quotient (DQ/IQ) related to the age at diagnosis of the PKU patients (Kruskal–Wallis test, $p>0.0001$). Figure legend: The length of the boxes indicates the interquartile space (P25–P75); the horizontal line in the box represents the median (P50) and the whiskers indicate the adjacent values, i.e. the maximum and minimum values of the distribution, which may not be considered abnormal. The circles indicate the outliers.

3.3.4. Neurological complications, neuroradiological findings, and behavioral problems in patients with early diagnosis in relation to dietary control

The proportion of patients with neurological complications was significantly different in patients with good, intermediate and poor IDC in the first 6 years of life (Pearson chi-square test $p=0.007$), and those in the immediately past year ($p=0.001$).

The proportion of patients with behavioral problems was also significantly different in patients with good, intermediate and poor IDC in the first 6 years of life (Pearson chi-square test: $p=0.007$) and those in the immediately past year ($p<0.0001$). Neurological and behavioral alterations could not be individually considered because of the low number of affected patients.

Considering early diagnosed patients who abandoned the diet at the age of 8–10 years ($N=7$) and resumed after a period free of dietary control, the proportion of patients with neurological complications and behavioral problems was not significantly different compared with those from a group of early diagnosed patients with similar ages ($N=15$) who continued treatment through their lives.

3.4. Schooling

In the group of patients with early diagnosis and of school age (35 patients from 6 to 18 years), 82.8% (29/35) attended ordinary school and 14.3% (5/35) ordinary school with support. Only one patient attended a special school. Among the 25 early diagnosed patients older than 18 years 36% (9/25) had completed schooling.

Among late diagnosed patients, 7% (2/29) attended ordinary school, 24% (7/29) ordinary school with support, 48% (14/29) attend or attended a special school, and 21% (6/29) had completed schooling.

Among the 54 patients older than 18 years (with early or late diagnosis), 15% (8/54) attend or attended university. At present 2 of them are dieticians (one with late diagnosis), one is an engineer, one studied political science, one is a business manager, one is a chemist and one studied Catalan philology. A further late diagnosed patient studied and is now working as a social worker.

4. Discussion

We previously performed several neurological and neuropsychological studies involving selected groups of PKU patients controlled in our unit [22–26], but we had never evaluated the neurological and behavioral outcome of the whole series of patients either with early or late diagnosis. The recent register of the Spanish PKU patients [14] led us to include all patients diagnosed and controlled in our unit in a database, and to specifically analyze the neurological complications and behavioral problems of the whole series. Moreover, we completed the general information with new data regarding the dietary control during the first 6 years of life, the patients who abandoned the diet, and specific information about the complications found in individual patients and their basic schooling and higher education.

There is a general consensus in the literature on the decrease in the intellectual capacity of early treated PKU patients [6,27–29]. In spite of having an intelligence quotient within the normal limits, these patients show a slight decrease in intellectual capacity compared with identical control groups, including their healthy brothers and sisters. Moreover, they show some deficits, especially in executive functions [8,9,30,31]. All these deficits are related with the early start of treatment and the quality and the duration of the dietary control [32–34]. Our previous results, involving 37 early diagnosed patients and 29 healthy, age and sex-matched subjects [8], and the present study with 92 patients, both found a mean IQ of 100 in PKU patients, which is significantly lower than that found in our control group ($IQ=111$) [8]. Only one patient with combined PKU and Turner syndrome showed slight mental retardation, and a further one had borderline intelligence.

Conversely, in our study late diagnosed patients showed a mean IQ of 62, with 46.3% of patients with severe to slight mental retardation, 28.5% with borderline intelligence and 25% with normal IQ. Two of these late diagnosed patients even achieved university studies (PAH genotype P279fsdelC-V388M) [35]. This proportion of mentally retarded patients is rather low compared with what is found in the literature (96–98% of untreated PKU patients with IQ lower than 50 [36], and 84% in another

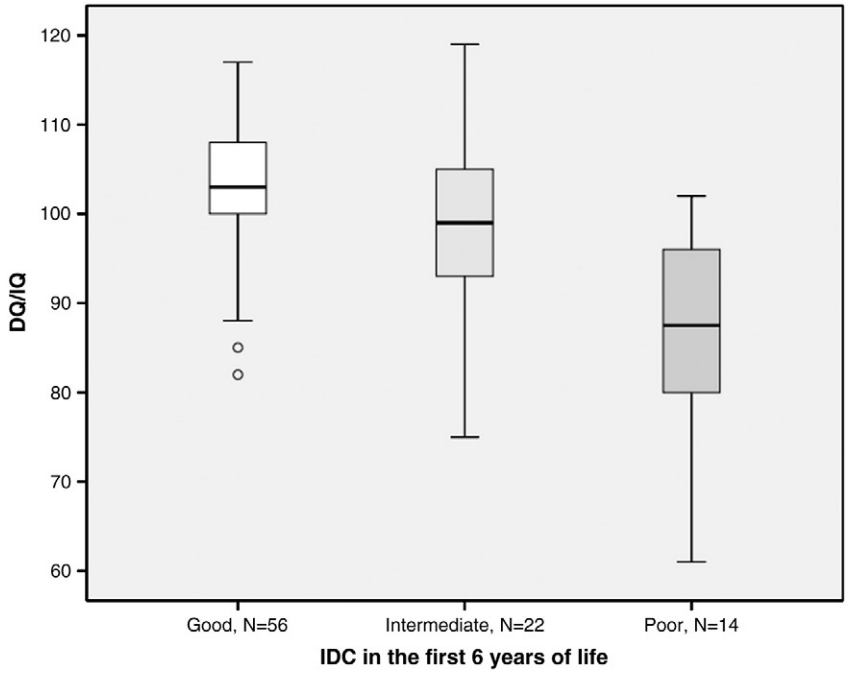


Fig. 2. Development or intelligence quotient in early diagnosed PKU patients with good, intermediate and poor IDC in the first 6 years of life.

series [37]). A possible explanation for this may be the higher proportion of mild mutations in the Mediterranean countries [38,39], which allows a better outcome in some untreated patients. A further reason is that most of our late diagnosed patients (65%) assumed the Phe-restricted diet with rather good control, and this might have had a beneficial effect on their intellectual capacity even in late diagnosed patients [40].

Another relevant question in our study was the similar IQ between patients diagnosed and treated during the first month and those diagnosed and treated during the second month of life. The IQ sharply descended after the second month and kept on decreasing after the first year of life (Fig. 1).

The quality of dietary control, especially during the first 6 years of life, further determined the DQ/IQ of early treated patients, as has already been observed in previous studies with smaller series [8,13]. Similar results were found by other authors [33].

Regarding neurological complications, we focused on epilepsy, tremor, clumsiness and mental retardation. The proportion of late diagnosed patients with epilepsy (26%) was similar than that of other authors (25%) [36,41,42], and this was also the case with tremor and clumsiness [42,25]. However, even a few patients with early diagnosis and acceptable dietary treatment showed tremor and clumsiness. The only early diagnosed patient with seizures had familial antecedents of epilepsy, and the only one with slight mental retardation also suffered Turner syndrome.

A high prevalence of abnormal white matter MR imaging has already been described in the literature, especially in patients with poor metabolic control [43]. These lesions have been associated with high concentrations of Phe in plasma and central nervous system by MR with spectroscopy [44], and they seem to be quite reversible by optimization of the metabolic control. We found these abnormal patterns in a high percentage of early and late diagnosed patients in the present series (Table 2), although most of these patients are asymptomatic. These results should be interpreted with caution in the case of early diagnosed patients because of the limited patients sample (N = 28). Moreover, the brain MR had been performed for special research studies or owing to the clinical suspicion of abnormalities because of poor metabolic control. Nevertheless we found abnormal volumetric studies in this population [26].

Behavioral problems, extensively described in PKU patients, include internalizing symptoms such as anxiety, depressed mood, and low self-

Table 2
Neurological complications and neuroradiological findings in early and late diagnosed PKU patients.

Neurological complications	Early diagnosis	Late diagnosis
	Number of patients (%)	Number of patients (%)
	Total patients = 92	Total patients = 29
Epilepsy	1 (1.1)	9 (31.0)
Tremor	11(12.0)	27 (93.1)
Clumsiness	10 (10.9)	26 (89.7)
Spasticity	–	8 (27.6)
Mental retardation	1 (1.1)	13 (46.3)
MR findings		
Altered brain MR[a]	17/28(60.7)	12/17(70.6)
Total % of patients with neurological complications and altered MR	23 (25)	28 (96.6)

[a] Brain MR was only performed in a reduced number of PKU patients with early diagnosis (N = 28) and late diagnosis (N = 17).

Table 3
Behavioral alterations in early and late diagnosed PKU patients.

Behavioral alterations	Early diagnosis	Late diagnosis
	Number of patients (%)	Number of patients (%)
	Total patients = 92	Total patients = 29
Attention deficit	21 (22.8)	20 (69.0)
Impulsiveness	16 (17.4)	10 (34.5)
Hyperactivity	19 (20.7)	13 (44.8)
Anxious mood	7 (7.6)	14 (48.3)
Depressed mood	7 (7.6)	14 (48.3)
Phobias	3 (3.3)	7 (24.1)
Low self-esteem	11(12.0)	17 (58.6)
Aggressiveness	–	8 (27.6)
Total % of patients with behavioral problems	26 (28.3)	25 (86.2)

esteem [7,45,46], and externalizing symptoms such as hyperactivity and impulsivity [11,47,48,12]. Although half of our late diagnosed patients presented one or more of these symptoms, only a very few of the early diagnosed patients showed them. The externalizing symptoms, including attention deficit, impulsivity and hyperactivity, are the most prevalent ones in our series. We did not find a different proportion of males and females with behavioral symptoms in our series, in contrast to the results of other authors [11].

The early diagnosed patients who abandoned dietary treatment at the age of 8–10 years and resumed it after a diet-free period did not show a higher proportion of neurological complications or behavioral troubles compared with patients who continued treatment throughout life.

As regards schooling, even early diagnosed patients showed a higher incidence of school problems compared with control groups [7,11,49,50]. We had previously performed a study in a limited group of 26 PKU patients [22] and demonstrated that they presented with more learning difficulties than controls, probably related to the disturbed cognitive functions observed in PKU patients. The index of dietary control of the immediately past 6 months showed a close relationship with school performance [18], which points to the importance of good dietary control in PKU. In the present study, 82.8% of early diagnosed patients attended ordinary school, 14.3% attended to ordinary school with support, and only one patient needed a special school. Moreover, of the total group, 15% of adult patients had attended university and are now working in jobs related with their studies.

4.1. Conclusions

Our results demonstrate the impact of early diagnosis and good dietary treatment on IQ and on the incidence of neurological complications and behavioral problems in PKU patients.

Acknowledgments

We very much appreciate the close collaboration of the Metabolic Nutrition staff, the skillful technical assistance of Juan Moreno, and the collaboration of the PKU patients and their families in the study.

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6.1. Síntesis de resultados 1

- La correlación entre el CD/CI y la edad al momento del diagnóstico mostró diferencias significativas entre los pacientes PKUDP (diagnosticados en los primeros 60 días de vida) y los pacientes PKUDT (diagnosticados > 60 días de vida) ($p< 0.0001$).
- Se observó una correlación negativa significativa entre el CD/CI y el ICD del último año y el de los primeros 6 años de vida ($p<0.0001$).
- El CD/CI fue significativamente diferente entre los pacientes con ICD bueno, intermedio y pobre en los primeros 6 años de vida y en el último año ($p<0.0001$).
- La proporción de pacientes de diagnóstico tardío con alteraciones neurológicas, alteraciones del comportamiento y anomalías en la neuroimagen fue significativamente más elevada que en los pacientes de diagnóstico precoz ($p<0.0001$).
- La proporción de complicaciones neurológicas y del comportamiento fue significativamente diferente en pacientes con buen, intermedio y pobre ICD en los primeros 6 años de vida ($p=0.007$) y en el último año ($p<0.001$).

6.2. RESULTADOS 2

OBJETIVO 2

Estudiar las complicaciones neurológicas y neuropsicológicas en el seguimiento de los pacientes PKUDP a través de investigaciones clínicas, bioquímicas y de neuroimagen.

White matter microstructural damage in early treated phenylketonuric patients.

María Julieta González, Mónica Rebollo Polo, Pablo Ripolles, Rosa Gassió, Aída Ormazabal, Cristina Sierra, Roser Colomé Roura, Rafael Artuch, Jaume Campistol.

Orphanet J Rare Dis. 2018;13(1):188.

En esta segunda investigación nos planteamos estudiar la integridad de la microestructura de la sustancia blanca en todo el cerebro de pacientes pediátricos PKU de diagnóstico precoz comparados con una población control, mediante la resonancia magnética con tensor de difusión e índices como la difusividad media, la difusividad radial y la anisotropía fraccional. Existen pocos estudios que evalúen todos los tractos de la SB cerebral con éste método en grupos de pacientes pediátricos PKU de diagnóstico precoz. Además se correlacionaron estos índices con los niveles de Phe, el ICD, biomarcadores de neurotransmisores y parámetros neuropsicológicos como la velocidad de procesamiento.

González et al. Orphanet Journal of Rare Diseases (2018) 13:188
https://doi.org/10.1186/s13023-018-0912-5

Orphanet Journal of Rare Diseases

RESEARCH Open Access

White matter microstructural damage in early treated phenylketonuric patients

María Julieta González[1*], Mónica Rebollo Polo[2], Pablo Ripollés[2,3], Rosa Gassió[1], Aída Ormazabal[4], Cristina Sierra[4], Roser Colomé Roura[1], Rafael Artuch[4] and Jaume Campistol[1]

Abstract

Background: Despite dietary intervention, individuals with early treated phenylketonuria (ETPKU) could present neurocognitive deficits and white matter (WM) abnormalities. The aim of the present study was to evaluate the microstructural integrity of WM pathways across the whole brain in a cohort of paediatric ETPKU patients compared with healthy controls (HCs), by collecting DTI-MRI (diffusion tensor magnetic resonance imaging) data and diffusion values (mean diffusivity (MD), radial diffusivity (RD) and fractional anisotropy (FA)).

Methods: DTI-MRI data and diffusion values (MD, RD, FA) from WM tracts across the whole brain were analized using Tract Based Spatial Statistics (TBSS), in 15 paediatrics TPKU patients (median age: 12 years) and compared with 11 HCs. Areas showing abnormal values in the patient group were correlated (Pearson) with age, lifetime Phe values, last year median and mean Phe, concurrent Phe values in plasma, urine neurotransmitters status biomarkers, and with a processing speed task.

Results: ETPKU showed bilaterally decreased MD values compared with HCs in the body and splenium of the corpus callosum, superior longitudinal fasciculus, corona radiata and in the posterior limb of the internal capsule. RD values followed a similar pattern, although decreased RD values in PKU patients were also found in the anterior limb of the internal capsule and in the cerebral peduncle. Decreased MD and RD values within the aforementioned regions had significant negative correlations with age, last year median and mean Phe and concurrent Phe values. No correlations were found with monoamines in urine or processing speed task.

Conclusions: ETPKU patients showed MD and RD values significantly decreased across the whole brain when compared with HCs, and this damage was associated with high Phe values and the age of patients. Despite this microstructural damage, no affectation in processing speed was observed in patients with good metabolic control. DTI-MRI sequences could be used as a technique to quantify WM damage that is difficult to be detect in T1 or T2-weighted images, but also to quantify damage of WM through the follow up of patients with poor metabolic control in prospective studies.

Keywords: Neuroimaging, Phenylketonuria, Paediatric, Early treatment, Diffusion tensor imaging, Urine monoamines

Background

Despite early and continuous dietary intervention, individuals with early treated phenylketonuria (ETPKU) could have neurocognitive deficits and white matter (WM) abnormalities [1]. The aetiology of this process is not entirely understood.

In the last years, diffusion tensor magnetic resonance imaging (DTI-MRI) has emerged as a crucial neuroimaging technique that allows non-invasive assessment of axonal structure and myelin status [2–4]. In particular, by measuring the diffusion of water molecules in the brain, different diffusion indexes can be calculated. Among them, fractional anisotropy (FA), quantifies the preference for diffusion of water molecules in one direction and correlates with axonal diameter, density, and fibre orientation; while mean diffusivity (MD) is related to cellularity, oedema, and necrosis and measures mean water molecular motion [5]. Radial diffusivity (RD) is also very sensitive for the detection of microstructural

* Correspondence: yuligonza@yahoo.com.ar
[1]Neuropediatric Department, PKU Follow Up Unit, Hospital Sant Joan de Déu (HSJD), Institut de Recerca Sant Joan de Deu (IRSJD), Passeig Sant Joan de Deu 2, Postal code, 08950 Barcelona, Spain
Full list of author information is available at the end of the article

changes and is usually associated with myelination and axonal diameter [6].

Recent studies have reported abnormal diffusion values (as measured by MD) in the WM of individuals with ETPKU as compared with healthy controls (HCs) [7–15]. Strikingly, the differences in diffusivity extend to regions that showed normal signal intensity when visualised on regular T2-weighted images [7, 15]. Findings regarding the direction of diffusion (as reflected by FA measures) are controversial, with some studies reporting decreased values of diffusion anisotropy in ETPKU [7] while others not finding such differences when compared with control groups [10, 16].

Hallmark neuropathological biomarkers in the brain of both treated and untreated PKU patients are hypomyelination, demyelination or both. Cognitive deficits are present in both treated and untreated PKU individuals, although the link between the neuropathological findings and cognitive deficits is poorly understood. Dyer et al. [17] postulate in this sense two interesting hypothesis. The first is based on the fact that cognitive deficits in individuals with PKU result from a deficiency of the dopamine neurotransmitter (NT). Decreased levels of tyrosine in the PKU brain are thought to be the cause of the low dopamine levels. The second is that elevated phenylalanine (Phe) values inhibit biosynthesis and myelin stability in oligodendrocytes. Myelin influences the maturity of axons, suggesting that axonal maturation may be involved in NT production. Also, abnormalities in the WM matter could be involved in the slower processing speed observed in PKU patients [18].

It has been hypothesized also that the hyperphenylalaninaemia-related neurotoxicity could be caused by a deficiency of large neutral amino acids (LNAA), mainly tyrosine and tryptophan, due to transport competition through the blood-brain barrier (BBB) [19]. Tyrosine and tryptophan are precursors of dopamine and serotonin, respectively; and its relative brain deficit may contribute to a reduced synthesis of these NT but also a disruption in protein synthesis [20–22]. Moreover, high brain Phe values may produce an inhibition of tyrosine and tryptophan hydroxylase activity, causing a further reduction of dopamine and serotonin biosynthesis [23, 24]. Dopamine is essential for proper functioning of the prefrontal cortex (PFC), which governs executive functions. In addition, serotonin is involved both in the cognitive processes mediated by the orbitofrontal cortex and in the regulation of mood, emotions, and behaviour [25].

The aim of the present study was to evaluate the microstructural integrity of WM pathways in a cohort of paediatric ETPKU patients, by collecting DTI-MRI data and extracting FA, RD and MD diffusion values from WM tracts across the whole brain. We first compared ETPKU patient data with a group of HCs and then we correlated the areas showing abnormal values in the patient group with age, last year median and mean Phe, and concurrent Phe values in plasma, lifetime Phe values, urine NT status biomarkers and processing speed task.

Methods

Participants

Children with ETPKU ($n = 15$, median age 12 years, range 8–18 years) were recruited through the PKU-Follow-up Unit of Sant Joan de Deu Hospital in Barcelona. All patients were diagnosed by the newborn screening program and were treated continuously from the first weeks of life. Treatment was as follows: eleven with classic dietary control (Phe restriction), five classified with good metabolic control (last year median of Phe values or index dietary control (IDC) [26], was < 360 μmol/L for patients under 12 years old or < 600 μmol/ L after 12 years old), and six with poor metabolic control (IDC was > 360 μmol/L for patients under 12 years old or > 600 μmol/L after 12 years old), according to the European Guidelines [27]. and six with poor metabolic control, IDC was > 360 μmol/L for patients under 12 years old or > 600 μmol/L after 12 years old, according to the European Guidelines [27]. The remaining four patients were treated with tetrahydrobiopterin (BH_4) and all had good metabolic control. No patients had a history or clinical evidence of neurological deterioration.

Control group

The control group formed by 11 healthy participants (mean age 11 years old, range 9–18 years; 5 males and 6 females) that were referred for MRI (magnetic resonance imaging) examination for headache. None of the controls had a history of intellectual disability, learning or psychiatric disorders or major medical disorders unrelated to PKU. They had similar age, sex and sociocultural class when compared with the ETPKU group. Controls were included in a consecutive fashion after the consent for the acquisition of the DTI-MRI data was signed.

Metabolic profile

Concurrent blood Phe (taken the day of the scanning session), lifetime Phe values (calculated as the mean of each median year Phe value across the lifetime), last year median and mean Phe values were measured by ion-exchange chromatography with ninhydrin detection using a Biochrom 30 analyser (Pharmacia-Biotech). Urine excretion of biogenic amine metabolites (homovanillic acid (HVA) for dopamine and 5 hydroxyindoleacetic acid (5HIAA) for serotonin) was analyzed using gas chromatography mass spectrometry detection (Agilent Technologies).

Neuroimaging

Scanning parameters:

A diffusion-weighted MRI (DW-MRI) scanning session was run on a 1.5 T scanner (General Electric Signa HD). Images were acquired with a spin-echo EPI sequence (53 axial slices, TR: 15000 ms, TE: 104 ms, acquisition matrix: 256×256, voxel size: $0.94 \times 0.94 \times 2.5$ mm^3). A run with one non-diffusion weighted volume (using a spin-echo EPI sequence coverage of the whole head) and 25 diffusion-weighted volumes (non-collinear diffusion gradient directions, b-values of 1500 s/mm^2) was acquired.

DTI-MRI preprocessing and statistical analysis:

Diffusion data processing started by correcting for eddy current distortions and head motion using FMRIB's (functional MRI of the brain) Diffusion Toolbox (FDT), which is part of the FMRIB Software Library [28]. Subsequently, the gradient matrix was rotated to provide a more accurate estimate of diffusion tensor orientations, using FSL's (FMRIB Software Library) FDT rotating bvecs [29]. Following this, brain extraction was performed using the Brain Extraction Tool [30], which is also part of the FSL distribution. Analysis continued with the reconstruction of the diffusion tensors using the linear least-squares algorithm included in Diffusion Toolkit 0.6.2.2 [31]. Finally, FA, RD and MD maps for each patient and control were calculated using the eigenvalues extracted from the diffusion tensors. Voxel based analyses of FA, RD and MD maps were performed using Tract Based Spatial Statistics (TBSS) [32]. Briefly, FA maps from all individuals were registered to the FMRIB58_FA template (MNI152 space and $1 \times 1 \times 1$ mm^3) using the nonlinear registration tool (FNIRT) [33]. These registered FA maps were first averaged to create a mean FA volume. Then a mean FA skeleton was derived, which represents the centers of all tracts common to all participants in the study. Each participant's aligned FA data were then projected onto this skeleton

Table 1 Neuroimaging studies with revision samples only ETPKU

Authors	Studied population	Studied regions	Parameters of DTI	Conclusions
Vermathen et al. 2007 [7]	ETPKU adult patients (mean age 32.5 years) ($n = 9$). Control group (mean age 29.4 years) ($n = 7$).	Grey and white matter tracts. Include corpus callosum (CC)	MD, FA	Decreased MD and FA values in lesions and CC. Decreased MD and FA values correlated negatively with Phe values.
White et al. 2010 [8]	ETPKU paediatric patients (mean age 12.2 years) ($n = 34$). Control group (mean age 12.4 years) ($n = 61$).	6 ROI (region of interest) of CC (genu, rostral body, anterior midbody, posterior midbody, isthmus and splenium)	MD, RA = FA	Decreased MD values in anterior part of CC. Non significant differences in FA compared to control group. Age related decrement of anterior WM of CC. Non-significant correlations with MD and Phe values.
White et al. 2013 [9]	ETPKU adults and paediatric patients BH4 responders (mean age 18.2 years) ($n = 12$). Control group (mean age 17.8 years) ($n = 9$).	10 ROI. Include CC (genu, body and splenium)	MD	Basal MD values improve after 6 months with BH4 treatment. MD values correlate negatively with Phe levels.
Atenor-Dorsey et al. 2013 [10]	ETPKU adults and paediatric patients (mean age 18 years) ($n = 29$). Control group (mean age 17.8 years) ($n = 12$)	10 ROI. Include CC (genu, body and splenium)	MD, FA	Decreased MD values correlated with poor executive functions. Decreased MD values compared to control group. Non significant differences in FA values compared to control group.
Peng et al. 2014 [11]	ETPKU adult and paediatric patients (mean age 23.3 years) ($n = 10$). Control group (mean age 23.5 years) ($n = 12$).	12 ROI. Include CC (genu, body and splenium)	MD, RD, AD, FA	Decreased MD, RD and AD values in WM tracts and CC compared to control group. Decreased MD, RD and AD values correlated with older ETPKU. Non-significant differences in FA compared to control group.
Wesonga et al. 2016 [12]	ETPKU paediatric patients (mean age 12.2 years) ($n = 31$). Control group (mean age = 12 years) ($n = 51$).	10 ROI. Include CC (genu, body and splenium)	MD	Age correlated with decreased MD values in 4 out of 10 ROI.
Hood et al. 2015 [13]	ETPKU paediatric patients (mean age 12.2 years) ($n = 36$). Control group ($n = 24$).	Over 10 ROI. Include CC (genu, body and splenium)	MD	Decreased MD values were correlated with high exposure of Phe levels.
Hood et al. 2016 [14]	ETPKU paediatric patients (mean age 12.2 years) ($n = 36$). Control group ($n = 62$).	2 ROI: PPO (posterior parietal-occipital) CSO (centrum semiovale).	MD, RD, FA	Decreased MD and RD values compared to control group. Non significant differences in FA compared to control group.
Ding et al. 2008 [15]	Adult patients (range 17–32 years): ETPKU ($n = 4$). Control group ($n = 4$).	22 ROI. Include CC (corpus and splenium).	MD, FA	Decreased MD values in WM and GM (grey matter) than control group. FA non significant differences than control group.

by searching for the highest FA value within a search space perpendicular to each voxel of the mean skeleton. This process was repeated for the RD and MD maps by applying the transformations previously calculated with the FA maps. Finally, in order to assess WM differences between controls and PKU patients, independent sample t-tests were calculated for the RD, MD and FA skeletons, with age and gender as covariates of nuisance. Results were reported with an FWE corrected $p < 0.05$ value using threshold-free cluster enhancement [34] and a nonparametric permutation test with 5000 permutations [35]. Significant voxels within the skeleton were filled to make the presentation of results easier to follow. WM tracts were identified using the JHU-ICBM DTI-81 white matter atlas [36, 37]. RD describes microscopic water movements perpendicular to the axon [38]. It has been proposed to reflect myelin quality along the axon with demyelination being associated with increased RD [39, 40], while MD is more related to tissue density [41]. Finally, for each patient, diffusivity values within the voxels showing significance between group effects were averaged and a mean value was obtained. Pearson's correlations were computed between these values (which represented individual WM damage) and age, concurrent Phe, last year median, lifetime and mean Phe values, and with HVA and 5HIIA concentrations. Pearson's correlations were also computed between the diffusivity values and the scores of the neuropsychological tests.

Correlations were computed with MATLAB version R2012a (The MathWorks, Natick, MA, USA). A correlation was considered significant if it survived a $p < 0.05$ false discovery rate (FDR) corrected threshold.

Given that the 15 ETPKU patients can be subdivided into 2 different groups (good metabolic control and poor metabolic control) we completed one last analysis. Again, using the average RD/MD (no significant results were obtained for FA, see next section) values from all voxels showing differences between patients and controls (same values used for the correlational analyses described above), we tested whether the different ETPKU subgroups showed different percentages of reduction in diffusivity values (i.e., WM damage). Taking into account the reduced number of patients per group, we used nonparametric tests under SPSS (version 18.0.0) to perform these calculations.

Neuropsychological evaluation

The Wechsler Intellectual Scale of Children (WISC-IV) [43] and Wechsler Adults Intelligence Scale (WAIS-III) [44] were administered to assessment general intellectual ability (intellectual quotient (IQ)). Given that WM abnormalities have been associated with a slower performance in processing speed, the following neuropsychological battery was used to evaluate it: Processing Speed Index of Wechsler Scales, time required to copy the Rey Complex Figure Task [45], motor and visual search speed with Trail Making Test Part A [46], speed naming with Speeded Naming NEPSY (NEuroPSYchological Assessment) II subtest [47], response speed with hit reaction time of Conners' Continuous Performance Test-II (CPT-II) [48] and Total initiation time (sum of time taken to begin each item) in execution of Tower of London test [49].

The reference typical punctuation for IQ, processing speed in Index Scales Wechsler, time required to copy

Table 2 Neuroimaging studies with early (ETPKU) and late treated PKU (LTPKU)

Authors	Studied population	Studied regions	Parameters of DTI	Conclusions
Leuzzi et al. 2007 [16]	Adult and paediatric patients ($n = 32$): ETPKU ($n = 21$) (mean age 17.1 years), LTPKU ($n = 11$) (mean age 22.4 years. Control group ($n = 30$) (mean age 32.9 years) (range: 12–58 years).	4 ROI: Parietal (P), Occipital (O), Frontal (F), Temporal (T).	MD, FA.	Abnormal signal T2-W and FLAIR scans: Parietal periventricular WM abnormalities > O > F > T. WM severity score correlated with age patient at time of the study. MD values and WM scores were closely and inversely correlated ($p < .001$). Blood and brain Phe levels were closely correlated ($p < .001$). Brain Phe levels was unrelated with FA values.
Kono et al. 2005 [50]	Adult and paediatric patients ($n = 21$) (mean age 19.4 years) (age range 3–44 years): ETPKU ($n = 14$), LTPKU ($n = 7$). Control group ($n = 21$) (mean age 20.6 years) (age range 3–33 years).	6–10 ROI (anterior and posterior deep WM).	MD	MD values in posterior deep WM significantly lower than in frontal deep WM ($P < .001$). MD values in the posterior WM tended to be lower with increased concurrent serum Phe levels ($p < .005$) and average serum Phe last year of examination ($p < .001$).
Scarabino et al. 2009 [51]	Adult and paediatric patients ($n = 32$) (mean age 18.9 years): ETPKU ($n = 21$), LTPKU ($n = 11$). Control group ($n = 30$) (mean age 32.9 years).	4 ROI: (P, O, F, T).	MD, FA	Supratentorial (periventricular and subcortical) abnormal T2: P > O > F > T. Decreased 30–50% of MD compared to control group. FA non correlated with Phe values.

Table 3 Clinical characteristics of patients

Patient code	Sex	Age	Mutation	IDC	Concurrent Phe µmol/L	Last year median Phe umol/L	Last year mean Phe umol/L	Lifetime mean Phe umol/L
P1	Female	8 years	R158G/L48S	Good	258	256.5	263	268
P2	Female	8.9 years	Y206X/L348 V	Good	435	330.5	298	434
P3	Male	13.6 years	R261Q-R176X	Good	503	558.5	597.7	319
P11	Female	8 years	I65T/IVS12 + 1A > G	Good	126	134	186.8	151
P12	Female	8 years	R261Q-I65T	Good	443	285	288.5	297
P6	Male	12 years	delF39/F55 L	Good (BH4)	667	434	398	316
P7	Female	13.1 years	R241Q- Not found	Good (BH4)	330	369.5	383	309
P8	Female	13.7 years	Y414C/K396 M	Good (BH4)	376	384	407	334
P9	Female	17.1 years	V388 M-P362T	Good (BH4)	585	410.5	522	322
P4	Male	14.6 years	IVS8nt-7a > g-/IVS8nt + 1g > a	Poor	1162	801.5	789	560
P5	Male	17.8 years	I65T-R261X	Poor	1016	852	837.4	406
P10	Female	8 years	IVS4 + 5G > T / IVS4 + 5G>	Poor	417	442.5	436	351
P13	Male	9.1years	IVS10-IVS10	Poor	567	372	440.5	320
P14	Male	9.3 years	IVS4 + 5G > T/ IVS10	Poor	102	426	366.5	285
P15	Male	10.7 years	R158Q/P281S	Poor	198	427	390.7	367

the Rey Complex Figure Task (RCFT), speed naming in NEPSY II, Trail Making Test A and total initiation time in Tower of London, were 100 ± 15. The reference typical punctuation in the evaluation of hit reaction time in CPT-II: Conners' Continuous Performance Test-II, was 50 ± 10, as it was considered faster execution less than 40 and slower execution more than 60.

Systematic review

The literature published from 2001 to 2016 were systematically searched: in PubMed: http://www.ncbi.nlm.nih.gov/pubmed. To avoid any risk of bias, general search terms were chosen: clinical studies, PKU/phenylketonuria, DTI/diffusion tensor imaging, magnetic resonance imaging, early treated, late treated, paediatric and adults.

Table 4 Results of neuropsychological evaluation

Patient code	Index dietary control (IDC)	IQ[a]	Processing speed Index[a]	RCFT[a] (Time required to copy)	CPT-II[b] (Hit reaction time)	NEPSY II[a] (Naming speed)	Trail Making Test A[a]	Tower of London[a] (Initiation time)
P1	Good	105	110	92	40	95	106	98
P2	Good	95	88	96	58	75	88	116
P3	Good	114	93	94	45	95	103	130
P11	Good	114	115	100	65	80	83	104
P12	Good	113	117	106	61	84	108	120
P6	Good (BH4)	109	99	69	59	95	93	98
P7	Good (BH4)	115	112	90	40	100	113	106
P8	Good (BH4)	115	121	90	51	95	96	122
P9	Good (BH4)	97	102	102	57	–	103	94
P4	Poor	95	85	58	68	84	115	96
P5	Poor	116	107	81	52	–	94	100
P10	Poor	101	115	126	55	95	97	112
P13	Poor	102	99	106	62	84	104	102
P14	Poor	114	91	119	69	75	96	112
P15	Poor	113	91	73	46	80	73	102

IQ intellectual quotient, *RCFT* The Rey Complex Figure Task, *CPT-II* Conners' Continuous Performance Test- II
[a]Typical Punctuation (TP): 100 ± 15
[b]TP: 50 ± 10. Fast: < 40; Slow: > 60

Inclusion criteria: studies in humans with control group, neuroimaging studies of DTI done in paediatric or adult PKU patients, early or late treated. Exclusion criteria: isolated case reports, studies in animal/cellular models, and patients with other metabolic disease such as BH4 deficiencies, articles not published in English. A total of 12 articles met criteria, 9 of them studied only early treated PKU (described in Table 1) [7–15], while the other articles described late and early treated PKU patients compared with a healthy control group (described in Table 2) [16, 50, 51].

Results

The 15 ETPKU evaluated patients had a concurrent Phe mean value of 426 μmol/L (range: 102–1162), lifetime Phe values of 319.5 μmol/L (range: 151–560), a last year Phe median value of 397 μmol/L (range: 134–852), a last year mean value of 440 μmol/L (range: 186–837). The scores for the intellectual quotient (IQ; WISC-IV and WAIS-III) ranged from 95 to 116 (mean: 111). The individual clinical, biochemical and molecular features of the PKU patients were described in Table 3. The results of neuropsychological evaluation were described in Table 4. Only 3 patients had a slower processing speed (more than one altered task), and all were patients with poor metabolic control.

Parameters of DTI: RD, MD and FA

In spite of the diffusion data coming from normal appearing white matter, eleven patients had subtle periventricular abnormalities in a T2 sequence, with only one being more evident and extended. However, only three had normal T2 sequences in MRI. Accordingly, the whole ETPKU group showed decreased MD values when compared with controls bilaterally in the body and splenium of the corpus callosum (CC), superior longitudinal fasciculus, corona radiata and in the posterior limb of the internal capsule (Fig. 1). RD values followed a very similar pattern, although decreased RD values in ETPKU patients were also found

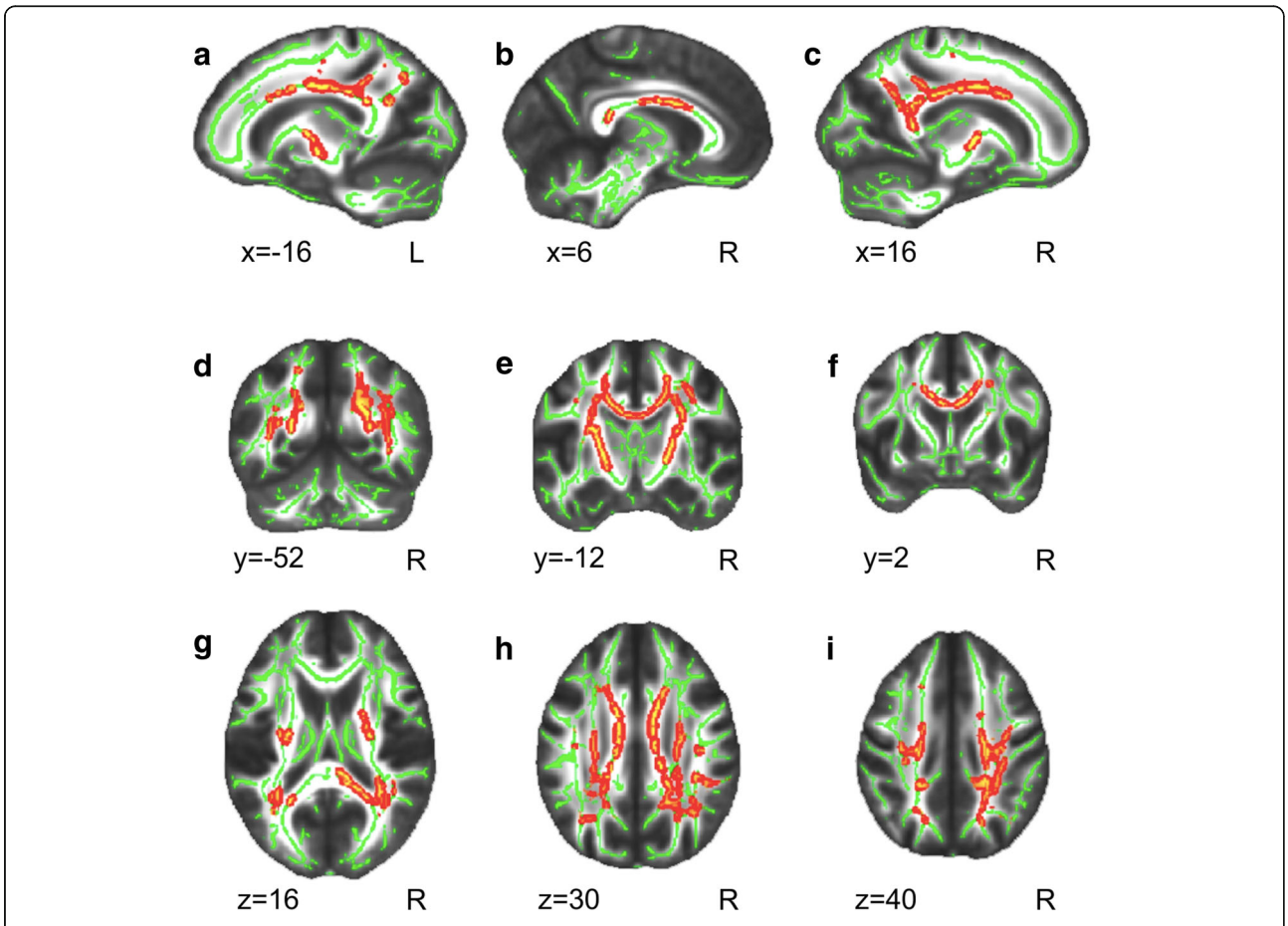

Fig. 1 Decreased Mean Diffusivity values of WM tracts across the whole brain in PKU as compared with controls. **a-i**: Results are shown over the mean group skeleton (in green), which represents the centers of all WM tracts common to all participants in the study (see Materials and Methods). In red-yellow, the WM regions showing decreased MD in patients as compared with controls are shown ($p < 0.05$ FWE corrected). Neurological convention is used with MNI (Montreal Neurological Institute) coordinates at the left bottom of each slice

in the anterior limb of the internal capsule and in the cerebral peduncle (Fig. 2). FA values showed no significant differences between groups.

Associations among age, full scale IQ, processing speed task, blood Phe values and urinary neurotransmitter biomarkers

Average MD values for all voxels showing WM damage, significantly and negatively correlated with age ($r = -0.80$, $p < 0.001$), last year median Phe, last year mean Phe and concurrent Phe values ($r = -0.65$, $p < 0.008$, $r = -0.72$, $p < 0.003$ and $r = -0.71$, $p < 0.004$, respectively; all correlations survived a $p < 0.05$ FDR corrected threshold; Fig. 3). RD values followed the same pattern as MD values, also significantly and negatively correlating with age ($r = -0.82$, $p < 0.001$), last year median Phe, last year mean Phe and concurrent Phe values ($r = -0.60$, $p < 0.02$, $r = -0.68$, $p < 0.005$, $r = -0.65$, $p < 0.008$, respectively; all correlations survived a $p < 0.05$ FDR corrected threshold; Fig. 4).

Thus, the greater the age or the higher the median, mean and concurrent Phe values, the greater the reduction in MD and RD values. This suggests that increased WM damage (reduced MD and RD values) is related to higher concentrations of Phe and that this damage increases with age.

All patients had lifetime Phe values in normal range in recomended levels for age: Lifetime Phe values were less than 600 μmol/l in patients older than 12 years old and less than 360 umol/L in the younger group (< 12 years old). These values fall within the recomended normal ranges for each age reported in the European PKU guidelines [42]. No significant correlations were observed between lifetime Phe values and MD or RD variables.

While correlations were not significant between diffusion values and HVA (MD: $r = 0.28$, $p = 0.30$; RD: $r = 0.30$, $p = 0.27$), the relationship between 5HIAA and MD ($r = 0.47$, $p = 0.073$), and also RD ($r = 0.49$, $p = 0.068$), approached significance. There were no significant correlations between IQ, and processing speed scores and

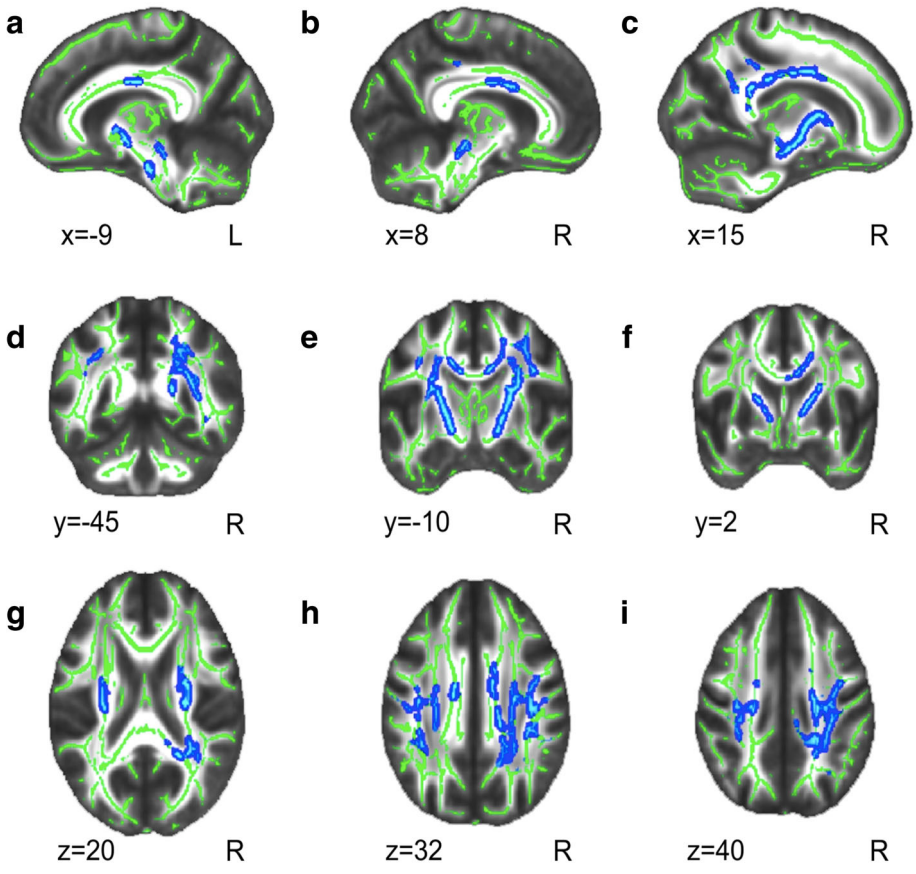

Fig. 2 Decreased Radial Diffusivity values of WM tracts acress the whole brain in PKU as compared with controls. **a-i**: Results are shown over the mean group skeleton (in green), which represents the centers of all WM tracts common to all participants in the study (see Materials and Methods). In blue, the WM regions showing decreased RD in patients as compared with controls are shown ($p < 0.05$ FWE corrected). Neurological convention is used with MNI (Montreal Neurological Institute) coordinates at the left bottom of each slice

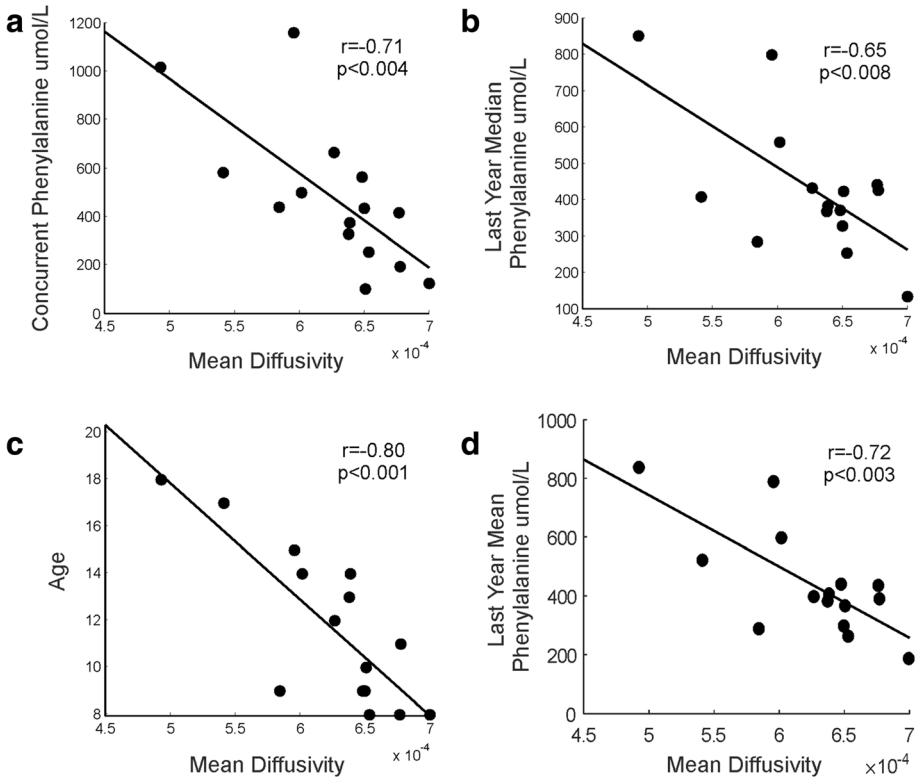

Fig. 3 Correlations between the average Mean Diffusivity value of the WM pathways showing between-group differences and biochemical parameters and age. The scatter plots display: **a** The correlation between the mean MD value of the voxels and concurrent Phe. **b** The correlation between the mean MD value of the voxels and last year median. **c** The correlation between the mean MD value of the voxels and age. **d** The correlation between the mean MD value of the voxels and last year mean. The scatter plots display the correlation between the mean MD value of the voxels showing significant differences and concurrent Phe, last year median and mean Phe values and age (the greater the age/phenylalanine values the greater the reduction in MD)

DTI-MRI variables. No significant differences were found between the PKU-sub-groups regarding the diffusivity values.

Discussion

This work evaluated the microstructural integrity of WM pathways across the whole brain in a sample of paediatric ETPKU patients. By means of DTI-MRI parameters such as FA, MD and RD and using TBSS, we compared WM tracts across the whole brain of ETPKU patients as compared to controls. We found that ETPKU patients showed bilaterally decreased MD values compared with HCs in the body and splenium of the CC, superior longitudinal fasciculus, corona radiata and in the posterior limb of the internal capsule. These findings were consistent with previous observations [7–15]. RD values also followed a similar pattern as previous studies [11, 14].

The most frequent findings in neuroimaging studies in these patients are the presence of WM abnormalities, evidenced as an increased signal intensity in T2-weighted sequences [52–54], with the periventricular WM being the most commonly affected region [55–58]. Only 3 (20%) patients showed normal T2 in our sample. The extent and severity of the WM abnormalities appears to be modulated by patient age, dietary adherence and metabolic control profile of Phe levels [54, 57, 59]. More recent studies demonstrate that DTI-MRI can provide additional insight into the microstructure of WM integrity of ETPKU patients. Indeed, previous findings have shown decreased MD values in ETPKU patients [8–11, 50].

Reviewing previous studies, only two of them studied MD, RD and FA in a paediatric population in more than one brain region. Peng and colleagues [11] studied a smaller series of patients than the present work (but mixed both paediatric and adult patients), and its results agree with those of this study. Hood and colleagues [14] studied a larger series, comparable to our study, which also showed a decrease in MD and RD values in paediatric ETPKU patients, but their analyses were restricted only to the posterior parietal occipital (PPO) and centrum semiovale (CSO; i.e., they did not study WM

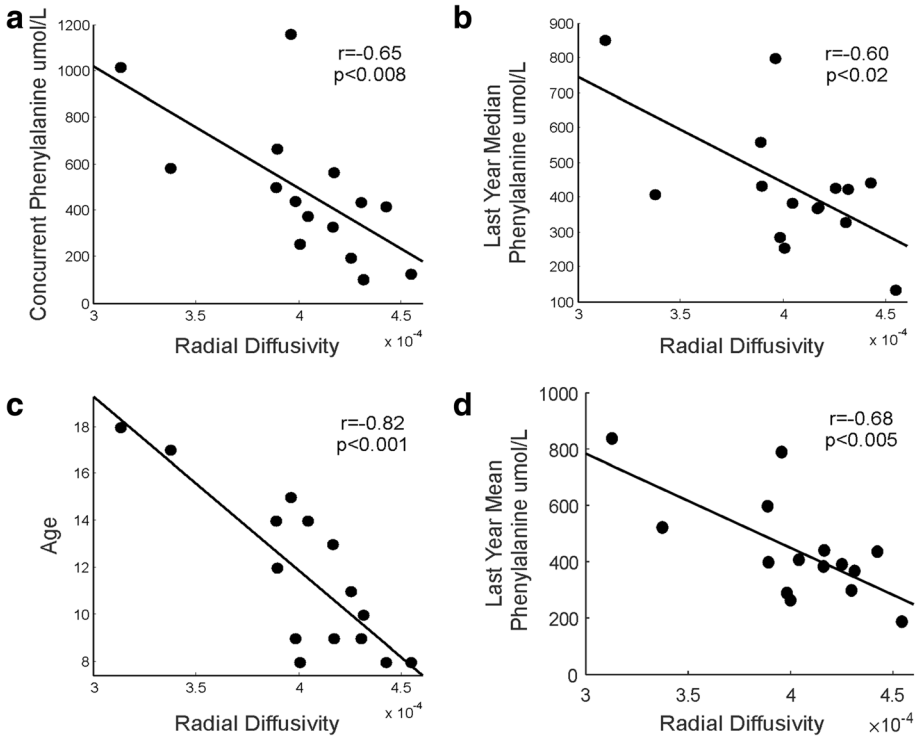

Fig. 4 Correlations between the average Radial Diffusivity value of the WM pathways showing between-group differences and biochemical parameters and age. The scatter plots display: **a** The correlation between the mean RD value of the voxels and concurrent Phe. **b** The correlation between the mean RD value of the voxels and last year median. **c** The correlation between the mean RD value of the voxels and age. **d** The correlation between the mean RD value of the voxels and last year mean. The scatter plots display the correlation between the mean RD value of the voxels showing significant differences and concurrent Phe, last year median and mean Phe values and age (the greater the age/phenylalanine values the greater the reduction in RD)

tracts across the whole brain). Both studies showed non-significant differences in FA values when compared with the control group. A brief revision among the different PKU neuroimaging studies is summarized in Table 1 [7–15] and Table 2 [16, 50, 51].

Associations between decreased MD and Phe values have previously been reported [7, 11–13]. In our study, we demonstrate that last year median and mean Phe and concurrent Phe values were significantly correlated with decreased MD and RD values.

In addition, decreased MD within the CC has also been shown to be related to Phe levels [11, 13] . White et al. [8] showed MD restriction values in the anterior part of the CC (genu and rostral body). Wesonga et al. [12] demonstrated significant abnormal diffusion values in the genu and splenium of the CC. In contrast, we found decreased of MD values but also RD decreased values in the body and splenium of the CC.

Finally, decreased MD values have been previously shown to correlate with increased age [8, 11, 12] in PKU children, which further supports the results shown in this work. MD and RD are thought to be a good marker of myelin structure and, in healthy populations, decreased RD and MD values are usually associated to a better WM microstructure [2, 40, 60, 61]. Moreover, research shows that in healthy populations MD values decrease with age during childhood (increased myelination) while WM is still maturating [62], whereas during adulthood, MD increases with age (decreased myelination) as WM degenerates [63, 64]. However, PKU populations including the one studied in this work usually display reduced MD and RD values that are related to increased Phe levels [11, 13]. That is, in PKU patients reduced RD and MD values, rather than reflecting improved WM structure, are a proxy of WM damage (note that elevated Phe values inhibit biosynthesis and myelin stability in oligodendrocytes). Thus, it could be that in healthy populations the negative association between MD and RD values and age reflects increased myelination [62], while in PKU, the same relationship could suggest quite the opposite: we hypothesize that high levels of Phe produce an accumulative damage in the microstructure of WM.

For future prospective studies, it is important to note that to identify the damaged white matter pathways affected in PKU patients, it is paramount to create and use

age specific DTI templates from healthy participants. While these templates could be obtained from existing atlases, we suggest that, to avoid inter-scanner effects, it would be useful to have a specific template made for each scanner and center. In addition, these templates could be used to compare the microstructural white matter lesions of each individual patient against the control group of the hospital, by means of, for example, Crawford-Howell t-tests [65, 66]; for an example of this test in single-patient DTI data, see Tuomiranta et al. [67].

While we did not find a significant correlation between diffusivity values and HVA, the relationship between 5HIAA and WM damage approached significance. Although it is known that dopamine and serotonin status may be affected in PKU patients, urinary HVA and 5-HIAA are, apparently, not good biomarkers for evaluation. Our results further support that blood Phe level is still the most reliable biomarker in the follow-up of these patients.

In addition, previous studies suggest that slower processing speed is related to the WM damage usually found in PKU, as a result of disruptions in the speed with which neural signals are transmitted [18, 68]. Nevertheless, despite the microstructural damage, not affectation in processing speed was observed in this group of patients. An explanation could be that the PKU population recruited for this study had in general a good metabolic control. Possibly higher Phe values would have led to more extensive WM abnormalities than the ones showed by this group of ETPKU patients, and this, in turn, could have been manifested in a slower performance in processing speed.

Conclusions

When we evaluated the WM tracts across the whole brain using TBSS in paediatric ETPKU patients, we found MD and RD values significantly decreased compared with HCs, and this damage was associated with high Phe values and with the age of patients. No correlations were found with processing speed scores.

Given current and previous research, we recommend the use of DTI-MRI sequences for the neuroimaging study of PKU patients. This technique could be used not only to quantify WM damage that is difficult to detect in T1 or T2-weighted images (MRI sequences routinely used), but also to quantify damage of WM through the follow up of poor metabolic control patients in prospective studies.

In addition, we suggest that neuropsychological evaluation should be performed routinely in patients with good metabolic control after 7 years old, in particular in individuals with WM abnormalities.

Abbreviations

5-HIAA: 5 - hydroxyindolacetic acid; BBB: Blood brain barrier; BH_4: Tetrahydrobiopterin; CC: Corpus callosum; CSO: Centrum semiovale; DTI-MRI: Diffusion tensor magnetic resonance imaging; DW-MRI: Diffusion-weighted MRI; ETPKU: Early treated phenylketonuria; FA: Fractional anisotropy; FDR: False discovery rate; FDT: Diffusion Toolbox; FMRIB's: Functional MRI of the brain; FNLRT: Functional Non Linear Registration Tool; FSL's: Functional MRI of the brain Software Library; HCs: Healthy controls; HVA: Homovanillic acid; IDC: Index dietary control; IEC: Independent ethics committees; IQ: Intellectual quotient; LTPKU: Late treated phenylketonuria; LNAAs: Large neutral amino acids; MATLAB: Matrix laboratory; MD: Mean diffusivity; MRI: Magnetic resonance imaging; NEPSY II: Developmental NEuroPSYchological Assessment version II; NT: Neurotransmitter; PFC: Prefrontal cortex; Phe: Phenylalanine; PKU: Phenylketonuria; PPO: Posterior parietal occipital; RD: Radial difusivity; ROI: Region of interest; TBSS: Tract Based Spatial Statistics; WAIS-III: Wechsler Adults Intelligence Scale version –III; WISC-IV: Wechsler Intellectual Scale of Children version IV; WM: White matter

Acknowledgements

We thank the patients and their parents and the PKU Follow-Up Unit staff who participated in this study. This work was supported by grants from the Spanish Ministerio de Economía y Competividad, FIS PI12/01469 and FEDER Funding Program from the European Union. The Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER) is an initiative of the Instituto de Salud Carlos III.

Funding

This work was supported by grants from the Spanish Ministerio de Economía y Competividad, FIS PI12/01469 and FEDER Funding Program from the European Union. The Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER) is an initiative of the Instituto de Salud Carlos III.

Availability of data and materials

Please contact author for data requests.

Authors' contributions

MJG: contributed in recruiting patients, processing and interpreting data, and wrote manuscript, MRP and PR: they participated in processing and interpreting neuroimaging data, and wrote manuscript, RG and RCR: they participated in processing and interpreting neuropsychological data and wrote manuscript, AO and CS: they participated in processing and interpreting biochemical data, RA and JC: they were the mentors of this work and contributed to guide the writing of the manuscript. All authors read and approved the final manuscript. We confirm that all authors details on the revised version are correct, all authors have agreed to authorship and order of authorship for this manuscript and that all authors have the appropriate permissions and rights to the reported data.

Ethics approval and consent to participate

The study protocols and amendments were approved by local independent ethics committees (IECs). Written informed consent/assent was obtained from all parents/participants of the control group at the time of their visit. The studies were conducted in accordance with the principles of the Declaration of Helsinki, amended in 2013. Our hospital ethics committee approved the study.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Publisher's Note

Author details

[1]Neuropediatric Department, PKU Follow Up Unit, Hospital Sant Joan de Déu (HSJD), Institut de Recerca Sant Joan de Deu (IRSJD), Passeig Sant Joan de Deu 2, Postal code, 08950 Barcelona, Spain. [2]Neuroimaging Section, HSJD, IRSJD, Passeig Sant Joan de Deu 2, Postal code, 08950 Barcelona, Spain. [3]Department of Psychology, New York University, 6 Washington Place, 10003 New York, USA. [4]Clinical Biochemistry Department, HSJD, IRSJD, UB, (CIBERER-ISCIII), Passeig Sant Joan de Deu 2, 08950 Barcelona, Spain.

Received: 18 June 2018 Accepted: 12 September 2018
Published online: 26 October 2018

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6.2. Síntesis de resultados 2

- De los 15 pacientes PKUDP evaluados, el valor de la mediana del último año fue 397 µmol/L (rango: 134–852) y la media del último año fue 440 µmol/L (rango: 186–837).
- Los valores de CI mostraron un rango entre 95- 116 (media: 111). Sólo 3 pacientes del grupo de pobre ICD mostraron ser más lentos en tareas de velocidad de procesamiento (VP).
- En la valoración cualitativa de la RMC, 11 pacientes presentaron alteraciones en regiones periventriculares (PV) (hiperintensidad de señal en la secuencia T2), y sólo en uno se mostraba una asociación entre las lesiones PV y en otras áreas subcorticales. El resto normal.
- En la valoración cuantitativa a través de los índices de la RMC-TD, se observó que todo el grupo PKUDP mostró una disminución de los valores de DM en comparación con el GC (afectación bilateral en el cuerpo y esplenio del CC, fascículo longitudinal superior, corona radiata y en la porción posterior de la cápsula interna). Los valores de DR siguieron un patrón de localización muy similar, aunque también se encontraron valores de DR disminuidos en pacientes con PKUDP en la porción anterior de la cápsula interna y en el pedúnculo cerebral. Los valores de AF no mostraron diferencias significativas entre los grupos.
- Los valores de DM correlacionaron negativa y significativamente con la edad ($p< 0.001$), mediana de Phe del último año, media de Phe del último año y valores de Phe concurrente ($p< 0.008$, $p< 0.003$ y $p<0.004$, respectivamente).
- Los valores de DR también muestran correlaciones negativas significativas con la edad ($p < 0.001$), mediana de Phe del último año, media de Phe del último año y valores concurrentes de Phe ($p < 0.02$, $p < 0.005$, $p < 0.008$, respectivamente).

- No se encontraron correlaciones significativas entre los valores de los índices de difusión (DM, DR, AF) y los de monoaminas en orina (HVA – 5HIAA), CI ni VP.

6.3. RESULTADOS 3

OBJETIVO 3

Estudiar las alteraciones del sueño en pacientes PKUDP y su relación con las alteraciones de melatonina y serotonina.

Prevalence of sleep disorders in early-treated phenylketonuric children and adolescents. Correlation with dopamine and serotonin status.

Rosa Gassió, María Julieta González , Oscar Sans, Rafael Artuch, Cristina Sierra, Aida Ormazabal, Daniel Cuadras, Jaume Campistol

Eur J Paediatr Neurol. 2019;23(5):685–691.

Los pacientes PKU son una población en riesgo de desarrollar trastornos del sueño debidos a déficits en la síntesis de neurotransmisores. En esta investigación se estudió la prevalencia de los trastornos del sueño en pacientes niños y adolescentes PKUDP y su correlación con marcadores de síntesis de dopamina y serotonina en relación a un grupo control.

Además se correlacionó el estudio del sueño con los niveles de Phe concurrente, índice de control dietético y variabilidad de Phe en el último año, tirosina, triptófano, prolactina y ferritina en plasma, concentración de serotonina plaquetaria, y la excreción de melatonina, ácido homovanílico y ácido 5-hidroxiindolacético en orina. El sueño se evaluó mediante la Escala de Bruni para los trastornos del sueño en niños.

EUROPEAN JOURNAL OF PAEDIATRIC NEUROLOGY 23 (2019) 685–691

Official Journal of the European Paediatric Neurology Society

Original article

Prevalence of sleep disorders in early-treated phenylketonuric children and adolescents. Correlation with dopamine and serotonin status

Rosa Gassió [a,b], María Julieta González [a,b,*], Oscar Sans [b,c], Rafael Artuch [a,d,e], Cristina Sierra [a,d], Aida Ormazabal [a,d,e], Daniel Cuadras [f], Jaume Campistol [a,b,e]

[a] Institut de Recerca Sant Joan de Déu, Esplugues de Llobregat, Spain
[b] Department of Neurology, Hospital Sant Joan de Déu, Esplugues de Llobregat, Spain
[c] Pediatric Sleep Unit, Neurophysiology Division, Department of Neurology, Hospital Sant Joan de Déu, Esplugues de Llobregat, Spain
[d] Department of Clinical Biochemistry, Hospital Sant Joan de Déu, Esplugues de Llobregat, Spain
[e] Biomedical Network Research Center for Rare Diseases (CIBER-ER), Institute of Health Carlos III (ISCIII), Madrid, Spain
[f] Methodological and Statistical Advice Service for Research, Parc Sanitari Sant Joan de Déu, Sant Boi de Llobregat, Spain

ARTICLE INFO

Article history:
Received 12 May 2019
Received in revised form
31 July 2019
Accepted 14 August 2019

Keywords:
Phenylketonuria
Sleep disorders
Dopamine
Serotonin
Melatonin

ABSTRACT

Phenylketonuric (PKU) patients are a population at risk for sleep disorders due to deficits in neurotransmitter synthesis. We aimed to study the prevalence of sleep disorders in early-treated PKU children and adolescents and assessed correlations with dopamine and serotonin status. We compared 32 PKU patients (16 females, 16 males; mean age 12 years), with a healthy control group of 32 subjects (16 females, 16 males; mean age 11.9 years). 19 PKU patients were under dietary treatment and 13 on tetrahydrobiopterin therapy. Concurrent phenylalanine (Phe), index of dietary control and variability in Phe in the last year, tyrosine, tryptophan, prolactin, and ferritin in plasma, platelet serotonin concentration, and melatonin, homovanillic and 5-hydroxyindoleacetic acid excretion in urine were analyzed. Sleep was assessed using Bruni's Sleep Disturbance Scale for Children. Sleep disorders were similar in both groups, 15.6% in control group and 12.5% in PKU group. In PKU patients, no correlations were found with peripheral biomarkers of neurotransmitter synthesis nor different Phe parameters, 43.3% had low melatonin excretion and 43.8% low platelet serotonin concentrations. Despite melatonin and serotonin deficits in early-treated PKU patients, the prevalence of sleep disorders is similar to that of the general population.

* *Corresponding author*. Department of Neurology, Hospital Sant Joan de Déu, Passeig Sant Joan de Déu 2, planta 4, 08950, Esplugues de Llobregat, Spain. Fax: +34 93 2033959.
E-mail address: yuligonza@yahoo.com.ar (M.J. González).
https://doi.org/10.1016/j.ejpn.2019.08.005

1. Introduction

Phenylketonuria (PKU; OMIM 261600) is an inborn error of metabolism caused by mutations in the phenylalanine hydroxylase (*PAH*) gene (EC 1.14.16.1). These mutations lead to a total or partial decrease of PAH activity, which catalyzes the hydroxylation of Phe into tyrosine (Tyr), using tetrahydrobiopterin (BH4) as a cofactor. The biochemical consequences are Phe accumulation in biological tissues and fluids, as well as a deficit in the availability of Tyr.[1]

It is well-known that untreated PKU causes severe neurological disorders such as intellectual disability, epilepsy, progressive motor disorder, and severe behavioral disorders. Treatment of PKU should begin in the first weeks of life as early as possible and consists of a Phe restricted diet combined with the administration of a special formula, or alternatively with BH4 and total or partial diet liberalization in patients who respond to it.[2–4] Due to the protein requirements for humans it is not feasible to completely remove Phe from the diet and, therefore, early-treated PKU patients maintain blood Phe levels above normal values (40–70 μmol/L), between 120 and 600 μmol/L according to age. Because of this, even early-treated patients may show slight cognitive alterations (a normal intellectual quotient but lower than in control groups, along with executive dysfunction), altered myelination of the cerebral white matter, and a high incidence of anxiety, depression and attention deficit hyperactivity disorder (ADHD).[5–8]

There are several factors involved in the pathophysiology of PKU, including changes in the synthesis of the neurotransmitters dopamine and serotonin[9] (Fig. 1). These deficits are of particular relevance given that dopamine is essential for executive functions and serotonin is necessary for the regulation of mood. Also, both neurotransmitters play an important role in regulating sleep and wakefulness.[10–13] The alterations in dopamine and serotonin synthesis in PKU are the result of several mechanisms: a) lower Tyr synthesis and decreased Tyr and tryptophan (Trp) transport through the blood-brain barrier, since Phe shares the same L-type amino acid carrier (LAT1, SLC7A5) and competes with them, and b) inhibition of tyrosine hydroxylase and tryptophan hydroxylase activity due to elevated Phe levels.[1,9]

One of the compounds derived from serotonin is melatonin, a hormone synthesized mainly in the pineal gland, whose main function is the control of the sleep cycle.[14] Many studies have been published on the consequences of dopamine deficiency in early-treated PKU patients, and ultimately on a higher incidence of mood problems that may be related to serotonin deficiency.[6,15] Regarding the serotoninergic deficit, references to its repercussions on sleep quality have been described in the literature but are scarce,[16] even studies in which sleep is evaluated. In that sense, our group has performed a previous study in early-treated PKU young adult patients in which alterations in sleep quality were not found[17]; however, recent studies have been published in PKU adults[18,19] reporting more sleep disorders than in controls.

In the present work, we study the prevalence of sleep disorders in early-treated PKU children and adolescents and assessed correlations with different biomarkers related to dopamine and serotonin status.

2. Material and methods

2.1. Participants

We recruited 32 early-treated PKU patients with a mean age of 12 years (SD ± 3.36) (16 females and 16 males), and a control group of 32 healthy subjects with a mean age of 11.9 years (SD ± 3.29) (16 females and 16 males) with the same sociodemographic characteristics. The PKU patient group was divided in to three subgroups: 1) 12 patients under dietary

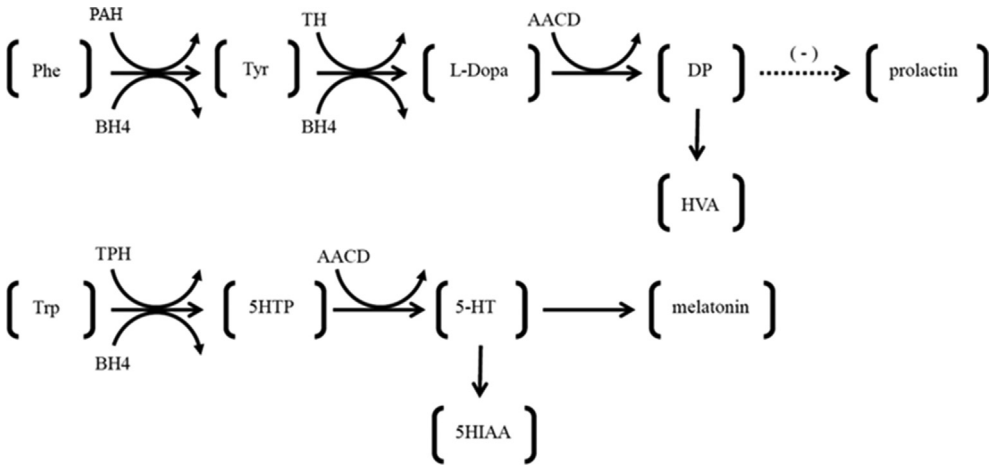

Fig. 1 – Neurotransmitter synthesis. TH: tyrosine hydroxylase. AACD: Aromatic L-amino acid decarboxylase. DP: dopamine. HVA: homovanillic acid. TPH: tryptophan hydroxylase. 5HTP: 5-hydroxytryptophan. 5-HT: serotonin. 5HIAA: 5-hydroxyindoleacetic acid.

treatment and a good metabolic control (last year index of dietary control (IDC) < 360 μmol/L for patients under 12 years old or < 600 μmol/L after 12 years), mean age 10 years (SD ± 2.21); 2) 7 patients under dietary treatment and poor metabolic control (last year IDC > 360 μmol/L for patients under 12 years old or > 600 μmol/L after 12 years), mean age 11.7 years (SD ± 3.09); and 3) 13 patients on prolonged treatment (between 4 and 12.5 years of treatment) with BH4 and good metabolic control, mean age 13.9 years (SD ± 3.48).

Inclusion criteria for PKU patients were: early (started during the first weeks of life) and continuous treatment with restricted diet or BH4, intellectual quotient above 80, absence of medication on the day of the analyses, and absence of associated medical pathology or mood disorders that could condition a sleep disorder. Three patients in the PKU group (one in each subgroup) were diagnosed with ADHD and received pharmacological treatment with prolonged-release methylphenidate; they had good tolerability and therefore were included in the study. Inclusion criteria for the control group were: normal school performance, absence of chronic medication, absence of medical pathology or mood disorders that could lead to a sleep disorder, and not being healthy siblings of PKU patients (to avoid family habits or environmental factors that could have an impact on the quality of sleep).

2.2. *Ethical approval*

Parents and participants older than 12 years of age signed an informed consent agreement in accord with the World Medical Association Declaration of Helsinki adopted in 1964 and amended in 2013. Our hospital ethics committee (CEI - Comité de Ética en Investigación Clínica) approved the study, code PI-47-12.

2.3. *Sleep disorder evaluation*

All participants in the study were assessed according to Bruni's Sleep Disturbance Scale for Children (SDSC)[20] for the presence of sleep disorders following the recommendations of the Clinical Practice Guidelines on Sleep Disorders in Childhood and Adolescence in Primary Care of the Spanish National Health System.[21]

The scale consists of 26 items and was developed to detect sleep disorders within the last 6 months in children and adolescents based on parental reports. Six factors representing the most common areas of sleep disorders in childhood and adolescence were evaluated: initiating and maintaining sleep disorders, sleep breathing disorder, arousal disorders, sleep wake transition disorders, excessive somnolence and sleep hyperhidrosis disorder. These subscales were considered as abnormal when T-score was >70 (T-score: 50 ± 10).

2.4. *Biochemical analysis*

In the PKU patient group, biochemical markers related to dopaminergic and serotonergic neurotransmission were analyzed. As precursors of synthesis: plasma Tyr and Phe were measured by ion-exchange chromatography with ninhydrin detection using a Biochrom 30 analyzer (Pharmacia-Biotech). Plasma Trp was measured by reversed-phase high-performance liquid chromatography (HPLC) with fluorescence detection (Waters, Milford, MA, USA), according to previously reported procedures.[22] Pituitary prolactin secretion is inhibited by dopamine, and therefore can be used as a parameter of brain dopamine availability. Iron deficiency is involved in some sleep disorders and therefore ferritin blood levels were measured.[23] Serum prolactin and ferritin were measured by a chemiluminescent microparticle immunoassay using an Architect analyzer (Abbott). Due to the presence of similar amine storage granules and identical high-affinity serotonin transporter and receptors, the platelet serotonin concentration has been used as an indirect index of central serotoninergic function. Platelet serotonin concentrations were analyzed by HPLC with fluorescence detection (Waters, Milford, MA, USA) following a modified procedure.[22] Urine excretion of biogenic amine metabolites (homovanillic acid (HVA) for dopamine and 5-hydroxyindoleacetic acid (5HIAA) for serotonin) was analyzed using gas chromatography mass spectrometry. Urinary 6-sulphatoxymelatonin (the final metabolite of melatonin) was analyzed by duplicate using a competitive ELISA kit (IBL: Ref. RE54021).[24] We also evaluated concurrent Phe levels, and variability in Phe (standard deviation of Phe levels) and IDC (median Phe levels in blood) for the last year. The blood tests were performed after fasting in the morning and urine tests were collected in the first sample in the morning. All the biochemical results were compared to the reference values established by our laboratory.

2.5. *Statistical analysis*

The categorical variables were described by their frequency table with percentages, and the numerical ones by means of descriptive statistics (mean, standard deviation). The frequencies of categorical variables were compared between groups using the Chi-squared test, whereas Kruskal–Walllis test was used to compare the means of numerical variables between groups.

SPSS software 19 (Armonk, NY: IBM Corp.) was used to perform the statistical analyses. Statistical tests with a p-value less than 0.05 were considered significant.

3. Results

Biochemical values of metabolic control of PKU and dopamine − serotonin status are shown in Table 1.

The poor metabolic control group is the one that presented significantly higher values of concurrent Phe ($p = 0.021$) and worse metabolic control during the last year (higher IDC) ($p = 0.002$). There are significant differences ($p = 0.023$) in variability in Phe levels for the last year between poor control group (higher SD) and BH4 group.

Mean Trp values were lower in the poor metabolic control group however the differences were not significant ($p = 0.252$).

In the PKU group, 43,3% of patients had low melatonin excretion (Table 2). The group with good metabolic control presented highest melatonin values (Fig. 2), although we did not find significant differences ($p = 0.249$) when comparing the three PKU subgroups (Table 1). Additionally, 43.8% of the PKU group presented low serotonin levels (Table 2), with lower levels in poor metabolic control group (Fig. 3), and we found

Table 1 – Biochemical values of metabolic control of PKU and dopamine – serotonin status.

Group		N	Mean	SD	p-values[a]
Concurrent Phe	BH4	13	387	±173	0.021
μmol/L n.v.: 40–70	Poor control	7	662	±353	
	Good control	12	284	±190	
IDC last year	BH4	13	332	±112	0.002
μmol/L	Poor control	7	534	±169	
	Good control	12	264	±110	
Variability in Phe last year (SD)	BH4	13	98.8	±46.8	0.023
	Poor control	7	183	±50.4	
	Good control	12	135	±64.7	
Tyr	BH4	13	57.2	±13.1	0.231
μmol/L n.v.: 40–87	Poor control	7	60.3	±37.0	
	Good control	12	47.6	±8.23	
Trp	BH4	13	47.2	±7.85	0.252
μmol/L n.v: 30–85	Poor control	6	41.0	±4.69	
	Good control	12	46.9	±10.2	
Platelet Serotonin nmol/10^9 platelets	BH4	13	2.41	±0.75	0.040
n.v.: 1.77–4.46	Poor control	7	1.58	±0.30	
	Good control	12	2.03	±0.74	
Prolactin mU/L	BH4	13	209	±43.3	0.186
n.v.: 108-466	Poor control	7	260	±132	
	Good control	12	201	±160	
Ferritin	BH4	13	52.7	±39.5	0.736
μg/L n.v.: 10–120	Poor control	7	46.2	±26.9	
	Good control	12	37.6	±21.4	
6-sulphatoxymelatonin	BH4	12	13.4	±9.30	0.249
μmol/mol creatinine n.v.[b]	Poor control	6	8.33	±6.03	
	Good control	12	22.8	±19.5	
HVA mmol/mol creatinine	BH4	13	3.25	±1.24	0.416
n.v.: 0.1–9.8	Poor control	7	3.16	±0.95	
	Good control	12	5.34	±5.26	
5HIAA mmol/mol creatinine	BH4	13	3.36	±2.54	0.502
n.v.: 0.3–8.9	Poor control	7	2.27	±1.40	
	Good control	12	3.88	±4.03	

Abbreviations: Phe: phenylalanine; BH4: tetrahydrobiopterin; IDC: index of dietary control; Tyr: tyrosine; Trp: tryptophan; HVA: homovanillic acid; 5HIAA: 5-hydroxyindoleacetic acid.
[a] Kruskal–Walllis test.
[b] 7–14 y: 11.9–66.2; >15 y: 6.3–37.9 μmol/mol creatinine.

significant differences (p = 0.040) between BH4 group and poor metabolic control group (Table 1). Only one patient presented with low levels of Trp and another presented with low excretion of 5HIAA. No correlation was found between the levels of Phe, Trp, serotonin and the excretion of melatonin or 5HIAA.

Table 2 – Melatonin and serotonin status.

Melatonin			
Group	normal	low	*p-value*[a]
BH4	6 (50%)	6 (50%)	0.665
Poor control	3 (50%)	3 (50%)	
Good control	8 (66.7%)	4 (33.3%)	
TOTAL	17 (56.7%)	13 (43.3%)	
Serotonin			
Group	normal	low	*p-value*[a]
BH4	9 (69.2%)	4 (30.8%)	0.213
Poor control	2 (28.6%)	5 (71.4%)	
Good control	7 (58.3%)	5 (41.7%)	
TOTAL	18 (56.2%)	14 (43.8%)	

Abbreviation: BH4: tetrahydrobiopterin.
[a] Chi-squared test.

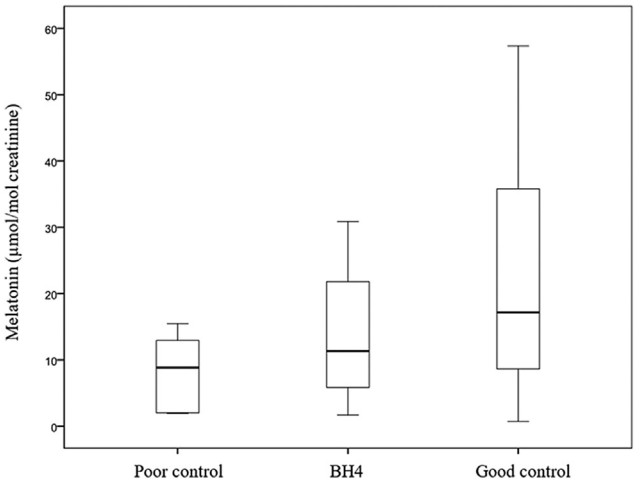

Fig. 2 – Melatonin levels.

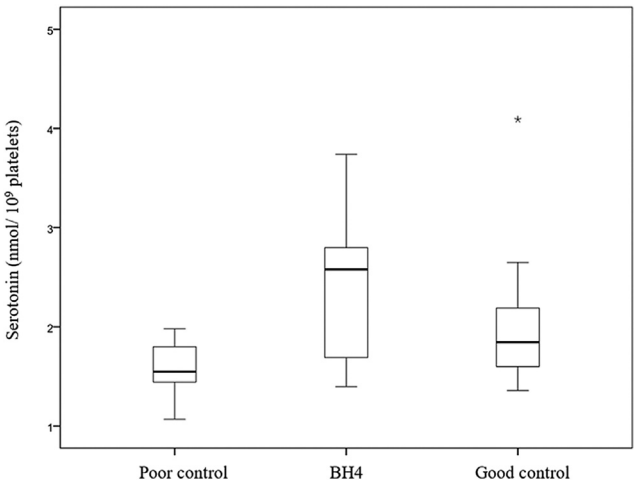

Fig. 3 – Serotonin levels.

We did not find statistically significant differences when comparing the presence of sleep disorders in the whole group of PKU patients and the control group (Table 3). Sleep disorders were slightly more frequent in the control group than in the PKU group (15.6% vs 12.5%), but the difference was not significant ($p = 0.719$). Only 4 subjects in PKU group and 5 in control group presented sleep disorders (one or more than one SDSC subscale abnormal). We did not find significant differences between the different SDSC subscales, or specific questions related to the alteration in the synthesis of dopamine/serotonin and melatonin such as: "How long after going to bed does your child usually fall asleep", "The child has difficulty getting to sleep at night", "The child has frequent twitching or jerking of legs while asleep or often changes position during the night or kicks the covers off the bed".

No differences were found when comparing the four groups for the presence of sleep disorder, the subscales or specific questions. Regarding the nine subjects with sleep disorders, five were in the control group, one in the poor metabolic control group and three in the good metabolic control group. No subjects in the group receiving BH_4 presented sleep disorders.

Biochemically, no correlation was found between the presence of a sleep disorder and the rest of the studied parameters (concurrent Phe, last year IDC and variability in Phe, Tyr, Trp, prolactin, serotonin and ferritin, excretion of melatonin, HVA and 5HIAA).

We did not find any correlations between low melatonin or serotonin levels and the presence of a sleep disorder.

4. Discussion

The prevalence of sleep disorders reported in different countries is between 13% and 27% in children aged 4–12 years.[21] According to the International Classification of Sleep Disorders, seven major categories can be described: insomnia disorders, sleep-related breathing disorders, central disorders of hypersomnolence, circadian rhythm sleep-wake disorders, sleep-related movement disorders, parasomnias and other sleep disorders.[25]

Our study is the first that analyze sleep disorders in early-treated PKU children and adolescents; 12.5% had sleep disorders, similar than in the control group (15.6%) and prevalence reported in normal children.[21] Therefore, our PKU patients presented sleep disorders as expected.

Cognitive functions have been extensively evaluated in PKU patients, but there are hardly any references as to how PKU patients sleep, even though they are theoretically a population at risk of presenting sleep disorders given the alterations in the synthesis of neurotransmitters and melatonin.

Initially, published studies address questions related to EEG sleep patterns rather than sleep quality. Behbehani[26] studied EEG sleep patterns in 22 early- and late-treated PKU patients (8–10 years), detecting minor EEG changes in early-treated children and pathological EEG changes in late-treated PKU patients; however, no significant changes were found during diet therapy and after diet termination. De Giorgis et al.,[27] found a delay in the maturation of patterns of

Table 3 – Bruni's Sleep Disturbance Scale for Children (SDSC) in PKU patients compared control group.

	Initiating and maintaining sleep disorders	Sleep breathing disorders	Arousal disorders	Sleep wake transition disorders	Excessive somnolence disorders	Sleep hyperhidrosis
PKU patients						
BH4 (0/13)[a]	normal	normal	normal	normal	normal	normal
Poor control (1/7)[b]	**abnormal (P_1)**	**abnormal (P_1)**	normal	**abnormal (P_1)**	**abnormal (P_1)**	normal
Good control (3/12)[b]	**abnormal (P_2)**	normal	**abnormal (P_2)**	**abnormal (P_2)**	**abnormal (P_2)**	normal
	normal	**abnormal (P_3)**	normal	normal	normal	normal
	normal	normal	normal	**abnormal (P_4)**	normal	normal
Control group (5/32)[b]	normal	normal	**abnormal (C_1)**	normal	**abnormal (C_1)**	**abnormal (C_1)**
	normal	normal	normal	normal	normal	**abnormal (C_2)**
	normal	normal	normal	normal	**abnormal (C_3)**	normal
	normal	**abnormal (C_4)**	normal	normal	normal	**abnormal (C_4)**
	normal	normal	normal	**abnormal (C_5)**	normal	normal

P: patient; C: control.
[a] In brackets altered cases versus total subgroup.
[b] The same case may have more than one abnormal subscale.

"tracé alternant" and sleep spindles in 16 early-treated PKU infants during their first months of life, during plasma Phe normalization. In 1973, Schulte et al.,[16] hypothesized that PKU patients may present sleep behavior alterations due to Trp metabolism disturbances. They studied 22 untreated PKU infants and young children (16 days and 3.75 years of age) and found no differences in the distribution between rapid eye movement or active, non-REM or quiet, and undifferentiated sleep compared to the control groups (healthy normal subjects and early-treated PKU patients). They concluded that, under chronic reduced conditions and not abruptly decreased, a normal or near normal sequence of quiet and active sleep could be maintained despite a severe lack of blood and cerebrospinal fluid serotonin as it occurs in PKU.

In recent years, interest in evaluating sleep in PKU patients has increased, but studies are scarce and focused on the adult population. In a previous study by our group,[17] 25 early-treated PKU young adults (range 18–31 years, mean age: 23.66 years; 12 females, 13 males) were assessed regarding sleep quality using the Pittsburg Sleep Quality Index. Thirteen out of 25 patients presented with good metabolic control (IDC < 600 μmol/L). All patients showed subjectively good sleep quality, 15 very good and 10 fairly good. By contrast, Bruinenberg et al.,[19] found more sleep disorders (specially insomnia and circadian rhythm sleep disorders) and reduced sleep quality in 25 treated adult PKU patients (mean age: 30 ± 9) compared to controls, but they did not assess metabolic control or provide the age when treatment was started in these patients. Bilder et al.,[18] reported a sleep disorder prevalence of 14.4% in 3714 adult PKU patients (aged 20 to >80 years, mean age: 38.5 years) versus 6.9% in general population controls, which fell to 9% in the age range of 20–39 years (n = 2247) (the authors supposed that this adult cohort were most likely to have had early and continuous treatment). Regard metabolic control, Huijbregts et al.,[28] in a study of health-related quality of life in early-treated PKU patients (7–40.8 years) found, only in adult patients, association between lifetime Phe levels and poorer functioning in the domain sleep (problems/limitations concerning sleeping). We did not find correlation between metabolic control and the presence of a sleep disorder, but in our case, we evaluated more recent Phe levels (concurrent Phe, last year IDC and variability in Phe) and only 21.8% of the sample had poor metabolic control.

In adult PKU, mood disorders are more common than in children, and should be taken into account that could affect sleep independent of neurotransmitter status.[6,18]

Platelet serotonin concentrations and urinary melatonin excretion have been shown to be good long-term indicators of the amount of circulating plasma serotonin and melatonin secretion, respectively.[22,29] In our sample, 43.3% of PKU patients had low melatonin levels and 43.8% had low serotonin concentrations, but these values were not correlated with sleep disorders. These patients have a chronic serotonin and melatonin deficits, and the hypothesis proposed by Schulte,[16] could explain that there is no correlation with sleep disorders, or perhaps, more exposure over time or a greater deficit of neurotransmitter synthesis is necessary to induce a sleep disorder. Other possibility is that urinary melatonin is an indirect estimation of pineal melatonin excretion, and differences in hepatic metabolism of melatonin would explain interindividual differences.

5. Conclusions

In conclusion, we highlight a prevalence of sleep disorders in early-treated children and adolescent PKU patients similar than in the control group, despite having a deficit in the synthesis of serotonin and melatonin. However, we should not completely rule out the higher incidence of sleep disorders given the small sample size we studied, and the fact that we only used questionnaires as a tool to assess sleep disorders. We think it is important to follow up sleep characteristics in PKU patients, especially those who are older or have poor metabolic control.

Conflict of interest

The authors have stated that they had no interest which might be perceived as posing a conflict or bias.

Acknowledgements

This work was supported by a grant from the Instituto de Salud Carlos III of Spanish Ministry of Economy and Competitiveness, European Regional Development Fund (FIS PI12/01469).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejpn.2019.08.005.

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6.3. Síntesis de resultados 3

- Los trastornos de sueño fueron ligeramente más frecuentes en el grupo control que en el grupo PKU (15.6% vs 12.5%), (p = 0.719). No se encontraron diferencias estadísticamente significativas cuando se comparó la presencia de trastornos del sueño en el grupo de pacientes PKU respecto al grupo control.
- Sólo 4 pacientes del grupo PKU (1 del grupo de pobre ICD y 3 del grupo de buen ICD) y 5 del grupo control presentaron desórdenes del sueño. No se encontraron diferencias significativas entre las diferentes sub-escalas y preguntas específicas relacionadas con la alteración de la síntesis de dopamina, serotonina o melatonina. Tampoco cuando se compararon los cuatro grupos y la presencia de trastornos del sueño.
- En el grupo total de pacientes PKU, el 43.3% (13 pacientes) tiene valores bajos de excreción de melatonina. En el grupo PKU con buen ICD, los valores de melatonina tienden a ser más altos, sin diferencias significativas entre los tres sub-grupos PKU.
- Los valores medios de Trp fueron más bajos en el grupo PKU de pobre ICD, (p=0.252).
- El 43.8% (14 pacientes) del total los pacientes PKU muestran niveles más bajos de serotonina. Los valores más bajos aparecen en el grupo de pobre ICD, y se observaron diferencias significativas (p=0.04) entre el grupo BH4.

6.4. RESULTADOS 4

OBJETIVO 4

Elucidar las posibles causas de la deficiencia de la coenzima Q en pacientes PKU.

Plasma coenzyme Q_{10} status is impaired in selected genetic conditions.

Raquel Montero, Delia Yubero, María C. Salgado, María Julieta González, Jaume Campistol, Maria del Mar O'Callaghan, Mercè Pineda, Verónica Delgadillo, Joan Maynou, Guerau Fernandez, Julio Montoya, Eduardo Ruiz-Pesini, Silvia Meavilla, Viruna Neergheen, Angels García-Cazorla, Plácido Navas, Iain Hargreaves, Rafael Artuch.

Sci Rep. 2019;9(1):793. Published 2019 Jan 28.

En este estudio se evaluó de forma restrospectiva las concentraciones plasmáticas de CoQ10 en una gran cohorte de pacientes pediátricos y adultos jóvenes durante un período de 12 años. Se estudiaron 597 pacientes , los cuales se dividieron en 6 diferentes grupos (un grupo control de pacientes sanos, pacientes con PKU, con mucopolisacaridosis (MPS), con otros errores congénitos del metabolismo (EIM), con enfermedades neurogenéticas y otros con enfermedades neurológicas sin diagnóstico genético). Se focalizó la atención en aquellos pacientes que presentaban valores bajos de CoQ10 plasmática, evaluando factores genéticos y ambientales que podrían influir en el estado de CoQ10 plasmática.

SCIENTIFIC REPORTS

OPEN

Plasma coenzyme Q_{10} status is impaired in selected genetic conditions

Received: 26 June 2018
Accepted: 4 December 2018
Published online: 28 January 2019

Raquel Montero[1,2], Delia Yubero[1], Maria C. Salgado[1], María Julieta González[1], Jaume Campistol[1,2], Maria del Mar O'Callaghan[1,2], Mercè Pineda[1], Verónica Delgadillo[1], Joan Maynou[3], Guerau Fernandez[3], Julio Montoya[2,4], Eduardo Ruiz-Pesini[2,4], Silvia Meavilla[1], Viruna Neergheen[5], Angels García-Cazorla[1,2], Placido Navas[2,6], Iain Hargreaves[5,7] & Rafael Artuch[1,2]

Identifying diseases displaying chronic low plasma Coenzyme Q_{10} (CoQ) values may be important to prevent possible cardiovascular dysfunction. The aim of this study was to retrospectively evaluate plasma CoQ concentrations in a large cohort of pediatric and young adult patients. We evaluated plasma CoQ values in 597 individuals (age range 1 month to 43 years, average 11 years), studied during the period 2005–2016. Patients were classified into 6 different groups: control group of healthy participants, phenylketonuric patients (PKU), patients with mucopolysaccharidoses (MPS), patients with other inborn errors of metabolism (IEM), patients with neurogenetic diseases, and individuals with neurological diseases with no genetic diagnosis. Plasma total CoQ was measured by reverse-phase high-performance liquid chromatography with electrochemical detection and ultraviolet detection at 275 nm. ANOVA with Bonferroni correction showed that plasma CoQ values were significantly lower in the PKU and MPS groups than in controls and neurological patients. The IEM group showed intermediate values that were not significantly different from those of the controls. In PKU patients, the Chi-Square test showed a significant association between having low plasma CoQ values and being classic PKU patients. The percentage of neurogenetic and other neurological patients with low CoQ values was low (below 8%). In conclusión, plasma CoQ monitoring in selected groups of patients with different IEM (especially in PKU and MPS patients, but also in IEM under protein-restricted diets) seems advisable to prevent the possibility of a chronic blood CoQ suboptimal status in such groups of patients.

Coenzyme Q_{10} (CoQ) is a lipid that acts in the mitochondrial respiratory chain as an electron transporter essential for ATP synthesis and serves as a lipophilic antioxidant, among other functions[1]. The benzoquinone ring of CoQ is derived from tyrosine, while the polyprenyl side-chain comes from acetyl-CoA, through the mevalonate pathway, which is common to the synthesis of other lipids such as dolichol and cholesterol, in a tightly regulated process[2]. Blood CoQ status depends on liver biosynthesis and is also the result of dietary sources that can influence plasma CoQ concentrations, contributing up to 25% of the total amount[3]. For CoQ, all tissues and cells are able to synthesize the sufficient amounts necessary for their different biological functions; therefore, no noticeable degree of uptake of CoQ seems to occur between the blood and tissues[4].

In blood, CoQ is transported by the lipoprotein cholesterol (Chol) transporters[5]. CoQ has been shown to be very efficient in preventing low-density lipoprotein (LDL) oxidation[6]. Since oxidized LDL is considered to have a key function in the development of the atherosclerotic process leading to cardiovascular diseases, treatment with CoQ to prevent this oxidation may have therapeutic value. Accordingly, clinical trials of CoQ supplementation to patients suffering from cardiovascular diseases have reported a reduction in the level of biochemical markers

[1]Inborn errors of metabolism Unit, Institut de Recerca Sant Joan de Déu, Barcelona, Spain. [2]CIBER de Enfermedades Raras (CIBERER), Instituto de Salud Carlos III Spain, Madrid, Spain. [3]Department of Genetic and Molecular Medicine, Institut de Recerca Sant Joan de Déu, Barcelona, Spain. [4]Departamento de Bioquimica y Biologia Molecular y Celular, Universidad Zaragoza-Instituto de Investigación Sanitaria de Aragón (IISAragon), Zaragoza, Spain. [5]Neurometabolic Unit, National Hospital, Queen Square, London, UK. [6]Centro Andaluz de Biología del Desarrollo, Universidad Pablo de Olavide, Sevilla, Spain. [7]School of Pharmacy, Liverpool John Moores University, Liverpool, UK. Correspondence and requests for materials should be addressed to R.A. (email: rartuch@hsjdbcn.org)

associated with pathology and major adverse cardiovascular events[7,8]. Therefore, identifying diseases displaying chronic low plasma CoQ values may be important to prevent possible cardiovascular dysfunction. The relationship between total blood Chol and CoQ is based on the rationale that both of these molecules share a common biosynthetic pathway, the mevalonate pathway[9]. Thus, the simultaneous measurement of plasma CoQ and Chol levels is of interest to assess the relationship between the presence of these two lipids in the blood[10] and may therefore predict the potential for the oxidative damage of the cholesterol transporter lipoproteins[11].

Some genetic and environmental conditions have been associated with a decreased level of plasma CoQ values in pediatric patients[12–18]. Thus, plasma CoQ status may be a valuable biomarker for certain diseases, both for diagnosis and treatment monitoring. Moreover, the key role of CoQ in the protection of Chol lipoprotein against free-radical damage strongly advocates the identification of patients presenting with chronic low plasma CoQ values.

In view of the diagnostic potential of plasma CoQ status, the aim of this study was to retrospectively evaluate plasma CoQ concentrations and CoQ/Chol ratios in a cohort of pediatric and young adult patients from a period of 12 years. We focused on those patients presenting with low plasma CoQ values, assessing genetic and environmental factors that could influence plasma CoQ status.

Methods

Subjects. We retrospectively evaluated our database containing 597 individuals (age range 1 month to 43 years, average 11 years), studied during the period 2005–2016 in Hospital Sant Joan de Déu (Barcelona) and in the Great Ormond Street Hospital (London). Patients were classified into 6 different groups: control group of healthy participants, phenylketonuric (PKU) patients due to mutations at the *PAH* gene encoding phenylalanine hydroxylase, patients with mucopolysaccharidoses (MPS), patients with other inborn errors of metabolism (IEM), patients with neurogenetic diseases, and lastly individuals with neurological diseases with no genetic diagnosis. Details of these cohorts of patients are provided in Table 1 and in the Supplementary Material 1. From the latter group, we selected 9 cases who were found to have low plasma CoQ values associated with a neurological syndrome for genetic diagnosis through next-generation sequencing (NGS). Total Chol values were also analyzed from the PKU, MPS, and IEM groups (Table 1). The criteria for the group classification were: i) Patients under restricted dietary treatment and at the risk of a suboptimal CoQ status: PKU and IEM patients. ii) Patients with MPS, since these patients may present with low CoQ values, but they are not under dietary restriction. iii) Patients with neurogenetic conditions just to identify diseases that may present with low plasma CoQ levels. iv) Neuropediatric patients with no diagnosis, to assess whether CoQ status would be a surrogate biomarker for the diagnosis of genetic related primary or secondary CoQ deficiencies.

Controls were healthy children with no chronic pharmacological treatments submitted to our Hospital for minor surgical interventions (mainly phimosis, adenoids and tympanic drainage). The different groups of diseases studied here were excluded based on: (1) biochemical data: most of them underwent the expanded newborn screening programs that include PKU and other IEM groups. (2) Clinical data: controls were healthy children with no neurological complications, while the MPS, neurogenetic and other neuropediatric patients included in the present study were severely handicapped.

From the 113 PKU patients, the type of PAH gene mutation was determined in 89 patients, as reported[19] (Supplementary Data Set). The assigned value study classifies the mutations as 4 categories (1 classic PKU, 2 moderate PKU, 4 Mild PKU, and 8 mild HPA) for every mutant *PAH* allele. Thus, the sum of the scores obtained for every allele led to the final classification of patients. In 65 out of 113 PKU patients, the predicted residual PAH activity was also calculated as previously reported[20]. Both variables (assigned value and residual PAH activity) were studied as predictors of the risk of developing low plasma CoQ values. All PKU patients were under dietary treatment at the time of the study, as previously reported[21].

For the 44 MPS patients (Table 1), we increased the number of patients studied in our previous work[15] from Sanfilipo to include patients with other MPS disorders (Supplementary Material 1). For the 61 IEM patients, we compared CoQ values in a subgroup on a carbohydrate-restricted diet (galactosemic and fructosemia) with those of a group on protein-restricted diets (homocystinurias, organic acidurias, urea cycle defects, and other aminoacidopathies) (Supplementary Material 1).

Exclusion criteria included patients taking CoQ. Blood samples were taken in the morning after an 8–12 h fasting period. Blood samples were collected into evacuated glass tubes containing heparin. Blood was immediately centrifuged at 4 °C (1500xg), and the plasma samples were stored at −80 °C until analysis.

Ethical issues. All patient samples were obtained in accordance with the 2013 revised Helsinki Declaration of 1964. For biochemical and genetic investigations, informed consent was collected from patients or their guardians. The Ethical Committee of Sant Joan de Déu Hospital approved the study.

Biochemical methods. Serum total Chol values were analyzed by the automated cholesterol oxidase procedure in an Architect autoanalyzer (Abbot). Plasma total CoQ, the sum of the reduced form ubiquinol plus the oxidized ubiquinone, was measured by reverse-phase high-performance liquid chromatography with electrochemical detection and with ultraviolet detection at 275 nm, as previously reported[22,23]. Plasma CoQ determination was accredited by the norm ISO15189 (ENAC). Reports regarding this accreditation are available upon request. In Fig. 1, typical chtomatograms from internal quality control material and human plasma samples are depicted. As we can see in the Fig. 1, the separation of CoQ and internal standard is optimal and both electrochemical and ultraviolet detection systems are specific for CoQ analysis in complex matrixes such as plasma, HPLC-electrochemical detection having a greater sensitivity when compared with ultraviolet detection or other approaches.

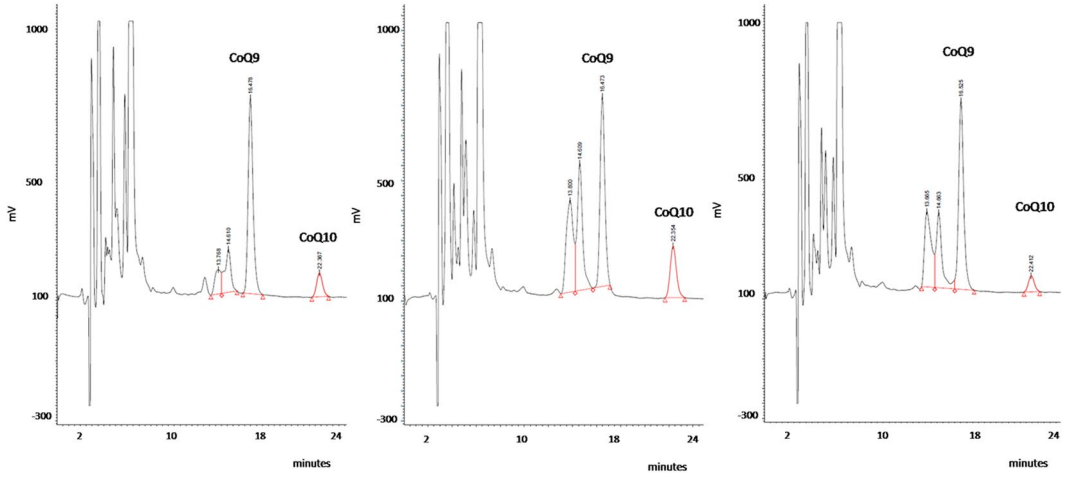

Figure 1. Typical chromatograms (HPLC with electrochemical detection) from: Left panel. Commercial control (Coenzyme Q_{10} Chromsystens, level 1 (Ref. 0092): $CoQ_{10} = 0.56$ µmol/L), based on serum matrix. Middle panel. Human plasma sample with $CoQ_{10} = 1.18$ µmol/L. Right panel. Human plasma sample with $CoQ_{10} = 0.38$ µmol/L. Samples, calibrators and controls are spiked with internal standard (Coenzyme Q_9 (CoQ_9)).

Subject groups: age range (average)	Plasma CoQ (µmol/L)	Plasma Chol (mmol/L)	Plasma CoQ/Chol (µmol/mol)	Number of low CoQ values cases
PKU (n = 113) 9m-43y (16.8)	0.20–1–18 (0.49) SD = 0.20	2.46–8.36 (3.74) SD = 0–74	51–324 (132) SD = 49.7	CoQ: 37/113 CoQ/Chol: 33/113
MPS (n = 44) 3–25 y (11.4)	0.16–0.93 (0.40) SD = 0.17	3.14–6.35 (4.40) SD = 0.85	29–201 (94) SD = 40.3	CoQ 24/44 CoQ/Chol 17/30
IEM (n = 61) 6m-40y (10.4)	0.18–1.21 (0.55) SD = 0.19	2.27–5.89 (4.04) SD = 0.85	69–291 (139) SD = 46.2	CoQ 11/61 CoQ/Chol 13/61
Neurogenetic conditions (n = 99) 1m-27y (10.3)	0.25–1–30 (0.67) SD = 0.21	n.a	n.a	CoQ 5/99
Other neurological disorders (n = 197) 1m-35y (8.2)	0.20–1.67 (0.68) SD = 0.27	n.a	n.a	CoQ 19/197
Control group (n = 83) 8m-22y (10.6)	0.38–1.34 (0.65) SD = 0.24	2.46–5.88 (4.01) SD = 0.77	101–283 (163) SD = 51	

Table 1. Plasma CoQ and Chol concentrations in controls and 5 groups of patients. Reference values are stated as range (defined as 2.5 and 97.5 percentiles), average (in brackets) and SD. For patient groups, data are represented as range (average and standard deviation). Age is expressed as range (average). Only the PKU group showed a significantly higher average age when compared with the other groups. In PKU cases elder than 22 years of age, the % of low plasma CoQ values was slightly higher when compared with to those younger than 22 years of age (8 out of 22). *m: months of age. y: years of age.

Genetic analysis. A PKU and MPS mutation study was performed by Sanger sequencing and NGS technology, as previously reported[24]. For the 9 selected patients with unknown neurological conditions and plasma CoQ deficiency, we applied a commercial panel (TruSight One Sequencing Panel, Illumina) and a NextSeq. 500 sequencer (Illumina) to screen a maximum number of candidate genes.

Statistical methods. The Pearson correlation test was used to determine the correlations among plasma CoQ and Chol values and the patient age and to correlate PAH residual activity with plasma CoQ concentration. The ANOVA with Bonferroni correction test was applied to compare plasma CoQ, Chol, and CoQ/Chol values among the different cohorts of patients and controls. Student T test was used to compare CoQ status in IEM patients with different dietary treatment (carbohydrate and protein-restricted). Chi-Square test was used to search for an association between the type of mutations (classified according to 25) and the CoQ status (deficient or not) in the PKU cohort. Statistical calculations were performed using SPSS 23.0 software.

Results

In the control group, a highly positive correlation was observed between plasma CoQ and total Chol values (Pearson test; $r = 0.523$; $p < 0.0001$) (Supplementary Figure 1). No correlation was observed between the age and plasma CoQ and CoQ/Chol values in controls under 22 years of age. Thus, a unique reference interval was established for this group (Table 1). We defined low plasma CoQ values as those below the lowest limit of the reference interval established in our laboratory, which was 0.38 µmol/L corresponding to the 2.5 percentile (Table 1).

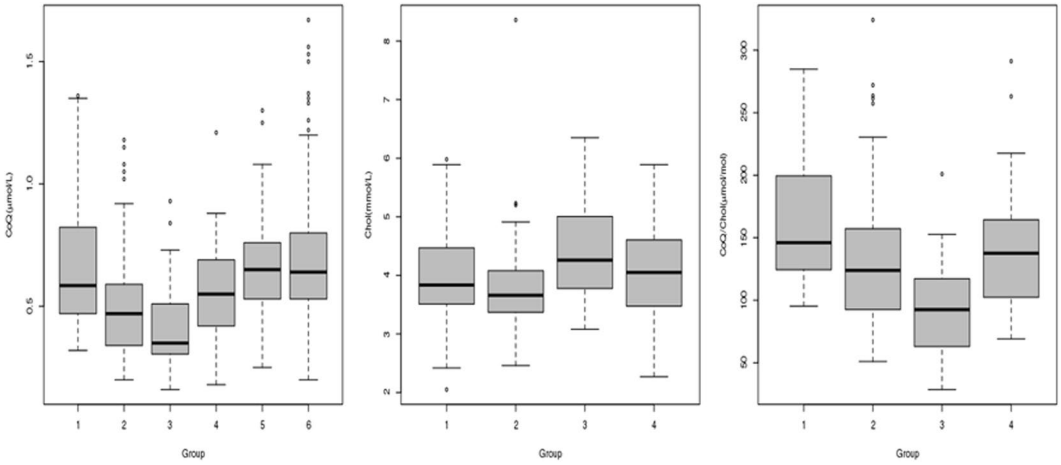

Figure 2. Box plot representation of: Left panel. Plasma CoQ values (µmol/L) in the 6 subject groups. *Middle panel*. Serum Chol (mmol/L) and Right panel CoQ/Chol values (µmol CoQ/mol Chol) from controls, PKU, MPS and IEM patients. Low plasma CoQ concentrations are frequently observed in both PKU and MPS patients, while the IEM group show intermediate values. The length of the boxes indicates the interquartile space (p25–p75); the horizontal line into the box represents the median (p50), and the circles indicate outlier values. *X-axis groups: Group 1: healthy participants. Group 2: phenylketonuric. Group 3: patients with mucopolysaccharidoses. Group 4: patients with other inborn errors of metabolism. Group 5: patients with neurogenetic diseases. Group 6: individuals with neurological diseases with no genetic diagnosis.

Group (I)	Groups (J)	Average difference (I-J)	95% confidence interval
CoQ			
Controls	PKU	0.164	0.067–0.261 ($p < 0.0001$)
	MPS	0.252	0.127–0.377 ($p < 0.0001$)
	IEM	0.100	−0.013–0.213 ($p = 0.139$)
CoQ/Chol			
Controls	PKU	31.6	11.4–51.8 ($p < 0.0001$)
	MPS	69.4	38.9–99.9 ($p < 0.0001$)
	IEM	24.1	0.4–47.8 ($p = 0.043$)
MPS	PKU	−37.8	−67.4 – −8.4 ($P = 0.003$)
	IEM	−45.3	−77.3–13.4 ($p = 0.001$)

Table 2. ANOVA with Bonferroni correction showed that plasma CoQ values were significantly lower in the PKU and MPS groups than in controls. IEM group showed intermediate values that were not significantly different from those of the controls. When we compared the CoQ/Chol ratios in these 3 groups, MPS values were significantly lower than controls, PKU, and IEM. In turn, IEM and PKU patients showed significantly lower values than controls.

Plasma CoQ, Chol, CoQ/Chol ratio values, and the number of cases with low plasma CoQ values in the different cohorts of patients are stated in Table 1 and Fig. 2. ANOVA with Bonferroni correction showed that plasma CoQ values were significantly lower in the PKU and MPS groups than in controls and neurological patients. IEM group showed intermediate values that were not significantly different from those of the controls (Fig. 2, Tables 1 and 2). When we compared the CoQ/Chol ratios in these 3 groups, MPS values were significantly lower than controls, PKU, and IEM (Tables 1 and 2). In turn, IEM and PKU patients showed significantly lower values than controls (Table 2). The highest percentage of patients with low-plasma CoQ and Q/Chol values belonged to the MPS group. In this group, low plasma CoQ value was a consistent feature in Sanfilippo patients, but it was also present in other MPS patients, except for Hurler-Scheie and Maroteaux-Lamy patients (data not shown). Even MPS patients with normal CoQ values displayed plasma CoQ concentrations close to the lowest limit of our reference interval.

In 65 PKU cases, we studied the correlation between CoQ concentration, the residual predicted PAH activity, and the Phe value at diagnosis (as a predictor of disease severity). As expected, residual PAH activity was found to be negatively correlated with the Phe level at diagnosis ($r = -0.448$; $p < 0.0001$) but positively correlated with the CoQ concentration ($r = 0.379$; $p = 0.003$). To further validate this observation, we used the assigned value study by classifying 89 PKU patients into 4 groups (2, 5, 8, and 9) according to the type of mutation (Supplementary Data Set). Chi-Square test showed a significant association between having low plasma CoQ values and belonging to group 2 (Chi square = 7.518; $p = 0.006$). Thus, from the 39 cases of group 2, 23 displayed low CoQ values, while

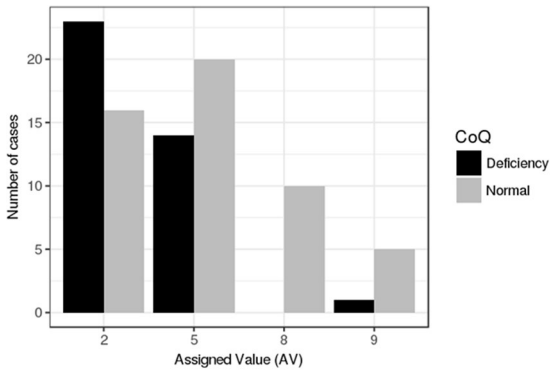

Figure 3. Graphic resprsentation of the assigned value (AV) study results in PKU patients. The black bars represent the number of PKU paints displaying low plasma CoQ concentrations in the different AV groups. The grey bars represent the number of PKU patients with normal plasma CoQ concentration. In AV groups 2 and 5 (classic PKU), the number of cases displaying low plasma CoQ values is higher than those belonging to AV groups 8 and 9 (milder PKU forms).

16 were normal. In the 34 patients classified as group 5, 14 showed low CoQ values and 20 were normal. From the 16 cases belonging to groups 8 and 9, only 1 displayed low CoQ concentrations. Results are represented in Fig. 3.

Regarding the patients with IEM, the percentage of cases with low CoQ values was lower than those of MPS and PKU patients (Table 1), and the frequency of low CoQ values was randomly distributed in homocystinurias, organic acidurias, and urea cycle defects. We compared (student t test) the plasma CoQ and CoQ/Chol values between patients with IEM and carbohydrate-restricted diet (galactosemias and fructosemias) with those taking protein-restricted diets (amino acid defects and organic acidemias), and no differences were observed. Only plasma CoQ levels tended to be lower ($p = 0.069$) in the protein-restricted group, but values normalized when they were related to Chol.

The 2 groups of neurological patients did not show any significant differences in plasma CoQ concentrations compared with the control group values, and the percentage of cases with low CoQ values was below 8% (Table 1, Fig. 2). NGS analysis in selected neurological patients with low plasma CoQ values failed to detect any pathogenic mutations in genes related to CoQ biosynthesis or associated with secondary CoQ deficiency. However, we found two unexpected findings: one patient with an epileptic encephalopathy diagnosed with a channelopathy (CACNA1A (NM_023035.2): p.Arg198Gln/c.593 G > A (heterozygous) and another case diagnosed with a Xq28 duplication syndrome (chrX:154125883–154562336 duplication [436Kb] [heterozygous]). The pathogenicity of both mutations was assessed following the recommendations of the American College of Medical Genetics[25].

Discussion

To our knowledge, this is the first work to analyze plasma CoQ and Chol status in a large cohort of pediatric patients. We first confirmed the previously published reference intervals for CoQ and CoQ/Chol values in a healthy population of 83 individuals. The concentration of plasma CoQ in healthy subjects has been reported by different authors in various populations, in general with a good agreement and such values close to those stated in the present study[26–29]. Since CoQ is known to bind to lipoproteins, the amount of CoQ in the plasma can also be related to the amount of Chol, and differences in total CoQ may be normalized to total Chol[29]. Another important observation was that neither plasma CoQ nor CoQ/Chol ratios correlated with the age in the control group (all were below 22 years), establishing only one reference interval. These results are in agreement with other studies[29] and indicate the stability of plasma CoQ status, at least in humans below 22 years.

Other remarkable observation of the present study is that low plasma CoQ values is a common biochemical feature in PKU and MPS patients and less frequent in the IEM group (in spite of having restricted diets that might lead to CoQ suboptimal status). In these groups, we also analyzed total Chol and calculated CoQ/Chol ratio to assess whether these values were concomitantly decreased. PKU is caused by a deficiency in the enzyme phenylalanine 4-hydroxylase (EC 1.14.16.1) due to mutations in the PAH gene. Plasma CoQ status is decreased in around one-third of the PKU patients compared to an age-matched reference population, as demonstrated previously[12], as well as in the present study. Several hypotheses may explain this deficiency, although none of them have yet been demonstrated in humans, and a combination of different factors is the most plausible explanation: Firstly, PKU patients avoid foods that are rich sources of CoQ. Secondly, the availability of tyrosine is essential for the synthesis of CoQ. Tyrosine may be low in PKU patients, but no association has been reported between the lowered plasma tyrosine concentration of patients and their serum CoQ level[30]. Thirdly, other factor associated with the decreased plasma CoQ level of PKU patients may be the elevated blood phenylalanine concentrations[30], since experimentally induced hyperphenylalaninemia in mice has been reported to inhibit the activities of the brain and liver enzymes, 3-hydroxy-3-methylglutaryl-CoA reductase, and mevalonate-5-pyrophosphate decarboxylase[31], which are both essential for CoQ and Chol biosynthesis. Interestingly, plasma CoQ and Chol values were decreased in PKU patients, indicating that inhibition of the mevalonate pathway may be the common cause of the deficit in CoQ and Chol status. In all likelihood, the factors responsible for the CoQ deficiency in PKU are multifactorial, but the demonstration of an association between the predicted PAH residual activity or the type of *PAH* mutations and the CoQ status of the patients supports the hypothesis that the more severe the degree of

hyperphenylalaninemia, the lower the level of plasma CoQ. Therefore, the inhibition of the mevalonate pathway by high Phe values[30,31] would be the most plausible explanation for decreased concentrations of both Chol and CoQ.

Regarding the MPS group, only 2 reports demonstrate that a noticeable percentage of patients with a genetic diagnosis of MPS present with plasma CoQ deficiency[15,32]. Those studies did not conduct etiologic investigations but raised several hypotheses regarding the presence of low plasdma CoQ values: (1) Impaired liver function in MPS, which can cause low plasma CoQ levels; (2) Nutritional problems, although this was unlikely since other lipophilic and hydrophilic vitamins were normal in these patients[15,32]; (3) the most plausible explanation for the deficit in plasma CoQ concentrations is related to vitamin B_6, which can show low blood levels in MPS patients[32]. The active form of vitamin B_6, pyridoxal 5-phosphate, is required for the transamination of tyrosine into 4-hydroxyphenylpyruvic acid for CoQ biosynthesis. In this context, a correlation between blood CoQ and vitamin B_6 status has been demonstrated[33]. It is also unknown whether CoQ deficiency is also present in tissues and how this may contribute to the pathophysiology of MPS. In the present study, this group displayed the highest frequency and most profound low plasma CoQ concentrations, while, in contrast to the PKU group, Chol values were not reduced in parallel. Furthermore, the low CoQ values was present in all types of MPS patients except for Hurler-Scheie and Maroteaux-Lamy patients. A hypothesis that may account for the CoQ subpotimal status in MPS is that heparan sulphate (and probably other mucopolysaccharides), may create adducts with pyridoxal 5-phosphate, leading to a loss of vitamin B_6 and consequently low CoQ concentrations[32]. However, this has yet to be confirmed or refuted and requires further investigation.

Few reports have stated the association between other IEM under restricted dietary treatments and CoQ status. CoQ deficiency has been reported to be associated with propionic and type II glutaric acidurias[14,34,35], mainly in muscle biopsy. We demonstrate here that none of the groups investigated showed a more consistent CoQ deficiency compared with the others. In fact, the IEM group displayed intermediate values between those for MPS, PKU, and the controls or the neurological patients. A limitation of the present study is that the IEM group is heterogeneous, and one hypothesis that can be drawn is that the contribution of the restricted diets to the plasma CoQ concentration would be expected to be limited (lack of differences between the CoQ values in carbohydrate-restricted diet group with that of the normal CoQ availability vs protein-restricted diet group that would be at risk of low CoQ intake). Thus, the contribution of dietary sources, which has been reported to be up to 25% of the total plasma CoQ values[3], in the absence of other etiologic mechanisms such as in PKU, would explain these intermediate values. Interestingly, the degree of low CoQ values in this group was higher than that in the neurological patients, advocating the value of plasma CoQ monitoring in IEM patients, considering that these patients will be chronically treated.

Surprisingly, neither the neurogenetic group (including, for example, mitochondrial disorders patients) nor the non-diagnosed neurological patients showed reduced plasma CoQ values. Furthermore, the average plasma CoQ values in these 2 groups of patients were almost identical to those of the control group. Moreover, the NGS assessment of those selected cases with a complex neurological phenotype and CoQ deficiency failed to detect pathogenic mutations in the candidate genes, confirming that plasma CoQ status is quite stable, probably tightly regulated, and may not be a good biomarker to reflect systemic (brain, muscle) CoQ status. Thus, plasma CoQ would not be a good biomarker to demonstrate genetic diseases associated with CoQ deficiency. In fact, a high percentage of mitochondrial disease patients and those having primary genetic disturbances in CoQ biosynthesis show a CoQ deficient status in tissues (fibroblast, muscle) but not in plasma[36]. However, it has been demonstrated after a genome-wide association study that serum CoQ levels identify susceptibility loci linked to neuronal disease (Alzheimer's disease, autism, and schizophrenia)[37]. No patients with these diseases were investigated in the present study, and this would explain these differences.

CoQ values may be influenced by several genetic and environmental conditions, including changes in dietary habits and cholesterol patterns. Since, therefore the variation amongst individuals may be great, single and isolated CoQ determinations in patients are probably no sufficient for detecting a real CoQ suboptimal status or for indicating CoQ supplementation.

In conclusion, low plasma CoQ values is neither a common finding in most of neuropediatric patients nor a good biomarker to predict genetic conditions leading to primary CoQ deficiency. On the contrary, plasma CoQ monitoring in selected groups of patients with different IEM (especially in PKU and MPS patients, but also in organic acidemias and aminoacidopathies under protein-restricted diets) seems advisable to prevent the possibility of a chronic low blood CoQ values in such groups of patients.

Data Availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Acknowledgements

This work was supported by grant from the Instituto de Salud Carlos III (ISCIII-FIS PI17/00109, PI17/00021, PI17/01286, PI15/00166 and PI15/01082), the FEDER Funding Program from the European Union, CIBERER-ISCIII and Departamento de Ciencia, Tecnología y Universidad del Gobierno de Aragón (Grupos de Referencia B33_17R). The Department of Genetic and Molecular Medicine is part of the CIBERER-ISCIII and 'Centre Daniel Bravo de Diagnòstic i Recerca en Malalties Minoritàries'.

Author Contributions

R. Montero, D. Yubero, M.C. Salgado, J. Maynou, G. Fernandez, J. Montoya, E. Ruiz-Pesini, V. Neergheen, P. Navas and I. Hargreaves were in charge of the biochemical, genetic and bioinfomatic analysis in the cohort of patients. J. González, J. Campistol, M. O'Callaghan, M. Pineda, V. Delgadillo, S. Meavilla and A. García-Cazorla were the pediatricians in charge of the patients. R. Artuch designed and supervised the work. Every one of the authors has participated sufficiently in the study, meeting the appropriate authorship criteria, and each has seen, reviewed and approved this version of the manuscript and takes full responsibility for it. We all agree to its submission for publication. Nobody who qualifies for authorship has been omitted from the list of authors. All the authors have complete access to the study data.

Additional Information

Supplementary information accompanies this paper at https://doi.org/10.1038/s41598-018-37542-2.

Competing Interests: The authors declare no competing interests.

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6.4. Síntesis de resultados 4

- En 65/113 casos de pacientes PKU, se estudió la correlación entre la concentración de CoQ10, la actividad residual de PAH y el valor de Phe en el momento del diagnóstico (como predictor de la severidad de la enfermedad). Se objetivó una correlación negativa entre la actividad residual de PAH y el nivel de Phe en el momento del diagnóstico ($p < 0.0001$), pero una correlación positiva con el concentración de CoQ10 ($p = 0.003$).
- Los niveles plasmáticos de CoQ10 fueron significativamente más bajos en los grupos PKU y MPS que en los controles y en los pacientes neurológicos (ANOVA con corrección de Bonferroni).
- Los valores del índice CoQ10/Col fueron significativamente más bajos en el grupo MPS que en el resto (PKU, EIM y GC)
- El mayor porcentaje de pacientes con valores bajos de CoQ10 y CoQ10/Col en plasma pertenecían a grupo MPS, sobretodo los niveles plasmáticos bajos CoQ10 en los pacientes con Sanfilippo, excepto en pacientes con Hurler-Scheie y Maroteaux-Lamy. Incluso los pacientes con MPS con valores normales de CoQ10 mostraron concentraciones plasmáticas de CoQ10 cercanas al límite más bajo de nuestro intervalo de referencia.
- Respecto a los pacientes con EIM, el porcentaje de casos con valores bajos de CoQ10 fue menor que los de MPS y pacientes PKU, y la frecuencia de valores bajos de CoQ10 se distribuyó aleatoriamente en homocistinurias, acidurias orgánicas y defectos del ciclo de la urea.
- Se comparó los valores de CoQ10 en plasma y CoQ10/Col entre pacientes con EIM y dieta restringida en carbohidratos (galactosemias y fructosemias) con aquellos que toman dietas restringidas en proteínas (defectos de aminoácidos y acidemias orgánicas) y no se observaron diferencias significativas.

DISCUSIÓN

7. Discusión por temas

7.1. Caracterización de los determinantes de las complicaciones neurológicas y del comportamiento en los pacientes PKU seguidos a largo plazo.

Existe un consenso general en la literatura sobre una ligera disminución de la capacidad intelectual de pacientes con PKUDP (Smith et al. 1990; Waisbren et al. 2007; Moyle et al. 2007; DeRoche et al. 2008). Además, muestran algunos déficits, especialmente en funciones ejecutivas (Gassió et al. 2005; Christ et al. 2010; Welsh et al. 1990; Diamond et al. 1997). Todos estos déficits están relacionados con el inicio del tratamiento, la calidad y la duración del control dietético (Smith et al. 1991; Burgard et al. 2000; Waisbren et al. 2000). En un estudio previo de Gassió et al. (2005), de 37 pacientes PKUDP (con 29 sujetos como grupo control), se observó un CI medio de 100, que es significativamente más bajo que el encontrado en el grupo de control (CI = 111), en nuestro estudio la media del CI fue similar (100) en este grupo de pacientes PKUDP (n=92). En este estudio, los pacientes PKUDT mostraron una media de CI de 62, de ellos el 46.3% fueron pacientes con discapacidad intelectual (DI) leve a profunda, 28.5% con CI límite y sólo 25% con CI dentro de la normalidad (Mallolas et al. 2000). Esta proporción de pacientes con DI en PKUDT es baja en comparación con otras como, 96-98% tienen un CI inferior a 50 según Tourian et al. (1982) y 84% según en Pitt et al. (1991). Una posible explicación de esto puede ser la mayor proporción de mutaciones leves en los países mediterráneos (Desviat et al. 1997; Mallolas et al. 1999) y la otra es que la mayoría de nuestros pacientes PKUDT (65%) que iniciaron la restricción de la dieta baja en Phe mantuvieron un buen control, y esto podría haber tenido un efecto beneficioso sobre su CI incluso en pacientes PKUDT (Koch et al. 1999).

El CI fue similar entre pacientes diagnosticados y tratados durante el primer mes y aquellos diagnosticados y tratados durante el segundo mes de vida. El CI descendió bruscamente después del segundo mes y siguió disminuyendo después del primer año de vida.

La calidad del ICD, especialmente durante los primeros 6 años de vida, además determinó el CD/CI de los pacientes de diagnóstico precoz, como se ha observado en estudios previos (Gassió et al. 2005; Vilaseca et al. 2010; Burgard et al. 2000).

En cuanto a las complicaciones neurológicas, estas fueron más frecuentes en los pacientes PKUDT, la presencia de epilepsia fue del 26%, similar a la reportada por otros autores (25%) (Tourian et al. 1982; Smith et al. 1996; Brenton et al. 2000), y este también fue el caso para el temblor y la torpeza (Brenton et al. 2000; Perez-Dueñas et al. 2006). Sin embargo, incluso algunos pacientes PKUDP y con buen control metabólico mostraron temblor y torpeza.

La prevalencia de alteraciones de la SB en la RMC es alta, especialmente en pacientes con pobre control metabólico (Anderson et al. 2010). Estas se han asociado con concentraciones de Phe altas en plasma y en el SNC valorado con la RMC con espectroscopía (Thompson et al. 1993), y parecen ser reversibles con optimización del control metabólico. Detectamos estos patrones anormales en un alto porcentaje de pacientes con diagnóstico temprano y tardío en la presente serie, aunque la mayoría de estos pacientes están asintomáticos.

Los problemas de comportamiento en los pacientes PKU, incluyen síntomas internalizantes como ansiedad, depresión y baja autoestima (Weglage et al. 1996; Pietz et al. 1998; Smith et al. 2000) y síntomas externalizantes como la hiperactividad e impulsividad (Stemerdink et al. 2000; Brumm et al. 2010; Antshel et al. 2010; Arnold et al. 2004). Los síntomas de externalización, son los más prevalentes en nuestra serie. No observamos una proporción diferente según sexo, en contraste a los resultados de otros autores (Stemerdink et al. 2000).

7.2 Alteraciones de la microestructura de la sustancia blanca

Las presencia de alteraciones de SB en la PKU son frecuentes, y se observan como hiperintensidades en la secuencia T2 de la RMC, sobretodo en áreas posteriores (Villasana et al. 1989; Thompson et al. 1990; 1993), en especial en regiones periventriculares parieto-oc-

cipitales (Bick et al. 1991; 1993; Cleary et al. 1994; Phillips et al. 2001), similar a lo encontrado en nuestro estudio, además sólo 3 pacientes (20%) mostraron una RMC en T2 normal.

Además en este trabajo se ha evaluado la integridad de la microestructura de la SB de los tractos cerebrales, a través de la RMC-TD. La cual a través de los paramétros AF, DM y DR permite cuantificar mejor las alteraciones de la SB en relación a un grupo control (GC). Se observó que todos los pacientes PKUDP mostraron una disminución de los valores de DM bilateral en relación al GC en diferentes estructuras de SB como cuerpo calloso, fascículo longitudinal superior, corona radiata y en la región posterior de la cápsula interna, como en estudios previos (Vermathen et al. 2007; White et al. 2010; 2013; Antenor-Dorsey et al. 2013; Peng et al. 2014; Wesonga et al. 2016; Hood et al. 2015; Ding et al. 2008; Kono et al. 2005). Los valores DR también siguieron un patrón similar y coincidiendo a lo reportado por otros autores (Peng et al. 2014; Hood et al. 2016).

Nuestro estudio estudió 3 paramétros (AF, DM, DR) de la RMC-TD, como en el estudio de Peng et al. (2014), donde se estudia una serie más pequeña de pacientes pediátricos y adultos, y sus resultados concuerdan con los nuestros. En otro estudio de Hood et al. (2016) estudiaron una serie de 10 pacientes pediátricos PKUDP y 12 controles, que también mostró una disminución en los valores de DM y DR, pero analizaron 2 regiones puntuales, el área parieto-occipital posterior (POP) y centro semioval (CSO) relacionadas con áreas de memoria de trabajo, y procesamiento estratégico verbal y no verbal; no observaron diferencias significativas en los valores de AF respecto al GC, como en nuestro estudio.

La asociación entre la disminución de los valores de DM y los niveles de Phe fue descrita por otros autores (Vermathen et al. 2007; Peng et al. 2014; Wesonga et al. 2016; Hood et al. 2015). Nuestro estudio demostró que la media de las medianas del último año y la Phe concurrente, se correlacionan significativamente con la disminución de ambos valores, DM y DR. La disminución de los valores de DM se ha correlacionado con el incremento de la edad en niños PKU (White et al. 2010; Peng et al.2014; Wesonga et al. 2016), lo cual refuerza los resultados de este estudio.

La diferencia se observa en investigaciones en poblaciones sanas que demuestran que los valores de DM disminuyen con la edad durante la niñez, (por el proceso de mie-

linización, la SB está más compacta), mientras todavía está madurando (Scantlebury et al. 2014). Mientras que, durante la edad adulta, los valores de DM tienden a aumentar por los procesos que afectan la integridad de la SB (Madden et al. 2009, Benitez et al. 2018). En cambio la AF disminuye a lo largo de la edad adulta. La disminución de valores de DM y DR se asocian a un mejor estado de la microestructura de la SB (Basser et al. 2014; 2015; Song et al. 2002; 2005). Sin embargo, en poblaciones PKU como las estudiadas en este trabajo, los valores reducidos de DM y DR parecen estar más relacionados con el aumento de los niveles de Phe (Peng et al. 2014; Hood et al. 2015). Es decir, que en los pacientes PKU la reducción de los valores de DM y DR, no refleja que la SB está más preservada, sino que, representa el daño de SB ya que los niveles elevados de Phe inhiben la biosíntesis y estabilidad de la mielina en los oligodendrocitos. Por lo tanto, se postula que en poblaciones sanas, la correlación negativa entre los valores de DM y DR con la edad refleja una mejor preservación del proceso de mielinización (Scantlebury et al. 2014), mientras que en la PKU, los resultados refuerzan la hipótesis que los niveles elevados de Phe producen un daño acumulativo en la microestructura de la SB y por eso disminuyen los valores de DM.

Sería interesante para estudios prospectivos tener en cuenta que para identificar las vías dañadas de la SB en los pacientes PKU, crear y usar plantillas de tensor de difusión para edad de GC. Si bien estas plantillas se pueden obtener de atlas existentes, sugerimos que, para evitar sesgos entre escáneres, sería útil tener una plantilla específica hecha para cada escáner y centro hospitalario. Además se podrían usar para comparar las lesiones microestructurales de la sustancia blanca de cada paciente de forma individual con el grupo de control del hospital (Crawford et al. 1998, 2012).

En relación a los metabolitos de neurotransmisión en orina (5-HIAA, HVA), no encontramos correlaciones significativas con los valores de difusividad. Aunque ambas vías (de la dopamina y serotonina) están afectas en la PKU, estos no resultaron ser buenos biomarcadores. Nuestra investigación apoya aún más que, el nivel plasmático de Phe sigue siendo el biomarcador más fiable en el seguimiento de estos pacientes.

Estudios previos sugieren que la velocidad de procesamiento (VP) lenta se relaciona con daño de la SB de los pacientes PKU, (Janos et al. 2012; Anderson et al. 2012). Sin embar-

go a pesar del daño microestructural, no se observa afectación de la VP en este grupo. Una explicación podría ser que la población reclutada para el estudio en general tuvo un buen ICD. Posiblemente sean necesarios niveles más altos de Phe para producir mayor daño de la SB y consecuentemente de la neurotransmisión, y esto a su vez se vería reflejado en un rendimiento más lento en la VP.

7.3. Trastornos de sueño en la PKU y su correlación con marcadores de neurotransmisión

La prevalencia de los trastornos del sueño notificados en diferentes países es de entre el 13% y el 27% en niños de 4 a 12 años (Guideline 2009). Nuestro estudio es el primero que analiza los trastornos del sueño en pacientes PKUDP pediátricos y adolescentes; el 12.5% tenía trastornos del sueño, ligeramente similar a la del grupo control (15.6%) y a la prevalencia reportada en niños sanos (Guideline 2009).

Los pacientes PKU, son en teoría una población de riesgo para presentar trastornos del sueño debido a las alteraciones en la síntesis de neurotransmisores y melatonina. Inicialmente los estudios publicados abordaron interrogantes relacionados con los patrones de sueño registrados en el electroencefalograma (EEG) en lugar de estudiar la calidad del sueño. Giorgis et al. (1996), encontraron un retraso en la maduración de los patrones de "trazado alterante" y husos de sueño en 16 lactantes PKUDP en los primeros meses de vida, durante la normalización de los niveles de Phe. En 1973, Schulte et al. hipotetizaron que los pacientes PKU pueden presentar alteraciones de sueño debido a alteraciones del metabolismo del Trp, estudiaron 22 pacientes PKU sin tratamiento (rango de edad 16 días de vida a 3.75 años de edad) y no encontraron diferencias en la distribución entre sueño REM, sueño no-REM e indiferenciado, en comparación con los GC (controles sanos y PKUDP). Concluyeron que en condiciones crónicas, las concentraciones reducidas de serotonina y sin disminuciones abruptas, podrían mantener una secuencia normal o casi normal del sueño REM y sueño no-REM a pesar de un descenso la serotonina en sangre y sistema nervioso como puede ocurrir en la PKU.

En los últimos años, el interés en evaluar el sueño en pacientes con PKU ha aumentado, y se centran en la población adulta. En un estudio previo de nuestro grupo (Gassió et al. 2013), de 25 pacientes PKUDP (18-31 años, 52% con buen ICD) fueron evaluados con la escala de calidad de sueño (Pittsburg), todos los pacientes mostraron subjetivamente buena calidad del sueño, 15 muy buena y 10 bastante buena. Por el contrario, Bruinnenberg et al. (2017) encontraron una mayor incidencia de trastornos de sueño (insomnio y alteraciones del ritmo circadiano del sueño) y menor calidad de sueño en 25 pacientes PKUDP adultos (edad media: 30±9 años) comparados con un GC, pero no evaluaron el ICD ni la edad de inicio del tratamiento. Bilder et al. (2017), informaron una prevalencia de trastornos de sueño del 14.4% en una serie de mayor de 3714 pacientes PKU adultos (rango de edad: 20 a > 80 años, edad media: 38.5 años) versus 6.9% en controles de población general, los mismos disminuyeron a 9% en el rango de edad de 20-39 años (n=2247).

En la PKU adulta, los trastornos del estado de ánimo son más comunes que en niños, y deben tenerse en cuenta, ya que podrían afectar al sueño independientemente del estado de la neurotransmisión (Brumm et al. 2010; Bilder et al. 2017). Las concentraciones de serotonina plaquetaria y melatonina urinaria son buenos indicadores a largo plazo de estas en plasma circulante (Ormazábal et al. 2005; Pääkkönen et al. 2006). En nuestra muestra, el 43.3% de pacientes con PKU tenía niveles bajos de melatonina y 43.8% niveles bajos de serotonina, pero estos valores no se correlacionaron con la presencia de trastornos del sueño. Estos pacientes podrían tener un déficit crónico de serotonina y de melatonina, y según la hipótesis de Schulte et al. (1973), explicaría porque no hay correlación con los trastornos del sueño, ya que quizá sea necesaria una mayor exposición a niveles elevados de Phe a largo plazo para producir un mayor déficit de la síntesis de neurotransmisores y consecuentemente para inducir trastornos de sueño. Otra posibilidad es que la melatonina urinaria sea una estimación indirecta de la excreción de melatonina pineal y las diferencias en el metabolismo hepático de la melatonina explicarían las diferencias interindividuales.

7.4. Coenzima Q10 en la PKU y otros EIM

Este es el primer trabajo para analizar el estado de CoQ_{10} y colesterol en plasma en una gran cohorte de pacientes pediátricos. Se conoce que la CoQ_{10} se une a las lipoproteínas, la cantidad de CoQ_{10} en el plasma también puede ser relacionado con la cantidad de Colesterol (Col), y las diferencias en CoQ_{10} total pueden normalizarse a Col total (Molyneux et al. 2005). Las proporciones de CoQ_{10} en plasma y el índice de CoQ_{10}/Col no se correlacionaron con la edad en el GC, estableciendo solo un intervalo de referencia. Estos resultados están de acuerdo con otros estudios (Molyneux et al. 2005) e indican la estabilidad del estado de CoQ_{10} en plasma.

Además se observó que los valores de CoQ_{10} en plasma están disminuídos de forma característica en pacientes PKU y con MPS, y con menos frecuencia en el grupo de EIM (a pesar de tener dietas restringidas). El estado de CoQ_{10} en plasma disminuye en alrededor de un tercio de los pacientes con PKU en comparación con una población de referencia de la misma edad, como se demostró anteriormente (Artuch et al. 1999), así como en el presente estudio. Varias hipótesis pueden explicar esta deficiencia, aunque ninguna de ellas aún se han demostrado en humanos, y una combinación de diferentes factores es la explicación más plausible. En primer lugar, los pacientes con PKU evitan los alimentos que son ricos en CoQ_{10}. En segundo lugar, la disponibilidad de tirosina es esencial para la síntesis de CoQ_{10} (la tirosina puede ser baja en la PKU), pero no se ha demostrado ninguna asociación entre la disminución de la concentración plasmática de tirosina de los pacientes y su nivel de CoQ_{10} sérico (Artuch et al. 2001). En tercer lugar, otro factor asociado con la disminución del nivel de CoQ_{10} en plasma de los pacientes con PKU pueden ser las concentraciones elevadas de Phe en sangre (Artuch et al. 2001), dado que se ha reportado que la hiperfenilalaninemia inducida experimentalmente en ratones inhibe las actividades de la enzimas cerebrales y hepáticas, 3-hidroxi-3-metilglutaril-CoA reductasa y mevalonato-5-pirofosfato descarboxilasa (Castillo et al. 1988), esenciales para la biosíntesis de CoQ_{10} y Col. Curiosamente, los valores de CoQ_{10} y Col en plasma disminuyeron en pacientes con PKU, lo que indica que la inhibición de la vía del mevalonato puede ser la causa común del déficit en el estado de

CoQ_{10} y Col. Con toda probabilidad, los factores responsables de la deficiencia de CoQ_{10} en pacientes PKU son multifactoriales, pero la demostración de una asociación entre la actividad residual de PAH o el tipo de las mutaciones de PAH y el estado de CoQ_{10} de los pacientes respalda la hipótesis de que cuanto más grave es el grado de hiperfenilalaninemia, menor es el nivel de CoQ_{10} en plasma. Por lo tanto, la inhibición de la vía del mevalonato por valores altos de Phe (Artuch et al. 2001; Castillo et al. 1988) sería la explicación más plausible para la disminución de las concentraciones de Col y CoQ_{10}.

Con respecto al grupo de MPS, solo 2 investigaciones previas demuestran que un porcentaje notable de pacientes con MPS que presenten una deficiencia de CoQ_{10} en plasma (Delgadillo et al. 2011; Yubero et al. 2016). En el presente estudio, este grupo mostró mayor frecuencia y concentraciones más bajas de CoQ_{10} en plasma, mientras que, en contraste con el grupo PKU, los valores de Col no se redujeron en paralelo.

Pocos estudios han mostrado la asociación entre otros EIM bajo tratamientos dietéticos restringidos en proteínas y la deficiencia de CoQ_{10} como lo descrito en acidurias glutárica tipo II y, propiónica (Neergheen et al. 2018; Gempel et al. 2007; Baruteaus et al. 2014). En este estudio ninguno de los grupos investigados mostró una deficiencia consistente de CoQ_{10} en comparación con GC. La contribución de las dietas, se ha informado que es de hasta el 25% de los valores totales de CoQ_{10} en plasma (Weber et al. 1997), en ausencia de otros mecanismos etiológicos como en la PKU, explicaría estos valores intermedios. El grado de valores bajos de CoQ_{10} en este grupo fue mayor que en los pacientes neurológicos, abogando por el valor de la monitorización de CoQ_{10} en plasma en pacientes con EIM, considerando que estos pacientes serán tratados crónicamente.

CONCLUSIONES

8. Conclusiones

1. Nuestro estudio confirma el gran impacto que tienen el diagnóstico precoz y el control adecuado del tratamiento sobre el coeficiente intelectual y la incidencia de complicaciones neurológicas y del comportamiento en los pacientes PKU.
2. Los pacientes PKUDP muestran valores significativamente disminuidos de DM y DR, comparados con los controles sanos. Este daño está asociado con valores elevados de Phe y con la edad de estos pacientes. Estos resultados confirman el compromiso de la SB, incluso en los pacientes PKUDP, con un buen control metabólico, por lo cual la utilización sistemática de esta técnica de neuroimagen debería estar indicada. A pesar del daño microestructural de la sustancia blanca, no se observa afectación de la velocidad de procesamiento en los pacientes con buen control metabólico.
3. La prevalencia de trastornos del sueño en pacientes pediátricos y adolescentes PKU son similares a las observadas en el grupo control, a pesar de tener un déficit en los valores de serotonina y melatonina. Sin embargo, no debemos descartar completamente la mayor incidencia de trastornos del sueño dado el pequeño tamaño de muestra que estudiamos y el hecho de que solo se utilizaron cuestionarios como herramienta para evaluar los trastornos del sueño. Creemos que es importante hacer un seguimiento de las características del sueño en pacientes con PKU, especialmente en aquellos que son mayores o tienen un peor control metabólico.
4. La monitorización del CoQ10 plasmático en grupos seleccionados de pacientes con diferentes EIM (especialmente en pacientes con PKU y MPS, pero también en acidemias orgánicas y aminoacidopatías con dietas restringidas en proteínas) parece aconsejable para prevenir la posibilidad de una deficiencia crónica de CoQ10 en sangre en este grupo de pacientes.

ESTUDIOS FUTUROS

9. Estudios futuros

- Estudios volumétricos de ganglios de la base (estructuras de sustancia gris) y correlación con las alteraciones de la motricidad fina.
- Estudios prospectivos de evaluación a largo plazo de la microestructura de la sustancia blanca con tensor de difusión en series más grandes de la unidad de seguimiento de la PKU. Comparándolos con una plantilla control de mayor número de pacientes control de nuestro centro.

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ANEXOS

11. Anexos:

11.1. Otras publicaciones relacionadas con el tema:

1. **González MJ**, Gassió R, Artuch R, Campistol J. Impaired Neurotransmission in Early- treated Phenylketonuria Patients. Semin Pediatr Neurol. 2016;23(4):332–340. doi:10.1016/j.spen.2016.11.007
2. Campistol J, **González MJ,** Gutiérrez AP, Vilaseca MA; Grupo Colaborativo de Unidades de Seguimiento Españolas. Tratamiento y control de los pacientes con fenilcetonuria: resultados del Grupo Colaborativo de Unidades de Seguimiento en España [Treatment and control of patients with phenylketonuria: results from the Collaborative Group of Spanish Follow-up Units]. *Med Clin (Barc)*. 2012;138(5):185–191. doi:10.1016/j.medcli.2011.03.037
3. Gassió R, **González MJ**, Colomé R, Sans O, Sierra C, Vilaseca MA, et al. Health-related quality of life, sleep and behavioural emotional functioning in early-treated phenylketonuric adult patients. J Inherit Metab Dis 2013;36(Suppl. 2):S127.

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Original

Tratamiento y control de los pacientes con fenilcetonuria: resultados del Grupo Colaborativo de Unidades de Seguimiento en España

Jaume Campistol [a,b,*], María Julieta González [a,b], Alfonso Pablo Gutiérrez [a,b] y María Antònia Vilaseca [b], Grupo Colaborativo de Unidades de Seguimiento Españolas [◇]

[a] *Servicio de Neurología, Hospital Sant Joan de Déu, Universitat de Barcelona, Esplugues, Barcelona, España*
[b] *Unidad Seguimiento de la PKU, Hospital Sant Joan de Déu, Universitat de Barcelona, Esplugues, Barcelona, España*

INFORMACIÓN DEL ARTÍCULO

Historia del artículo:
Recibido el 10 de enero de 2011
Aceptado el 3 de marzo de 2011
On-line el 26 de julio de 2011

Palabras clave:
Fenilcetonuria
Tratamiento
Seguimiento
Registro
Tetrahidrobiopterina
Control dietético

RESUMEN

Fundamento y objetivo: Conocer el control de la fenilcetonuria (PKU) en las unidades de seguimiento españolas y realizar un registro de pacientes.
Pacientes y método: Pacientes con PKU diagnosticados y/o seguidos en España, con fenilalanina previa al tratamiento > 360 μmol/L. Cuestionarios: datos anonimizados incluidos, aquellos aportados por las unidades durante el año 2010.
Resultados: Se han recogido datos de las 18 unidades de seguimiento españolas. El 83% muestran una composición multidisciplinaria y todas controlan pacientes de todas las edades, con criterios de tratamiento, en general, uniformes. Se han registrado datos de 688 pacientes con PKU, con una mediana de edad de 14 años (extremos 1 mes-53 años); un 41,5% eran mayores de 18 años. Un 71,8% se diagnosticaron precozmente. Un 15,8% tienen una PKU leve, el 26% una forma moderada y el 51,5% la forma clásica. Un 78,6% de los pacientes son tratados con dieta restringida en proteínas, el 9,3% con tetrahidrobiopterina (BH4) y dieta libre y un 7,8% con BH4 y dieta. El control dietético es bueno en el 58,6% de pacientes, regular en el 26% y malo en un 15,4%. La mediana del coeficiente intelectual del total de pacientes con PKU es de 97 (extremos 25-145). El porcentaje de pacientes de diagnóstico tardío con complicaciones neurológicas y conductuales es significativamente mayor al de los diagnosticados precozmente. El 13,3% de adultos han cursado estudios universitarios y el 37,5% tiene pareja estable.
Conclusiones: Este estudio permite evaluar el funcionamiento de las unidades de seguimiento de la PKU en España, así como registrar y analizar, por primera vez, los datos de los pacientes con PKU controlados en ellas. Se demuestra la necesidad de unidades de enfermedades metabólicas de adultos y el valor del diagnóstico precoz en el pronóstico de los pacientes con PKU.

Treatment and control of patients with phenylketonuria: results from the Collaborative Group of Spanish Follow-up Units

Keywords:
Phenylketonuria
Treatment
Management
RegistryTetrahydrobiopterin
Dietary control

ABSTRACT

Background and objective: To evaluate the management of phenylketonuria (PKU) in Spanish metabolic units and to develop a patients registry.
Patients and methods: PKU patients diagnosed and/or followed up in Spain, with phenylalanine values before treatment > 360 μmol/L. Registered anonimous data are those yielded by the units during 2010.
Results: Data from the 18 Spanish Follow-up Units were collected. Eighty-three per cent of Units are multidisciplinary, all of them corresponding to control patients of all ages, with uniform management criteria. Data of 688 PKU patients were registered (median: 14 years [1 month-53 years], 41.5% are presently > 18-year-old. 71.8% patients came from neonatal screening; 15.8% have mild-PKU, 26% moderate-PKU and 51.5% classic-PKU. 78.6% patients are treated with protein-restricted diet, 9.3% with

* Autor para correspondencia.
Correo electrónico: campistol@hsjdbcn.org (J. Campistol).
◇ Los componentes del grupo están relacionados en el anexo 1.

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doi:10.1016/j.medcli.2011.03.037

BH4 and free diet and 7.8% with BH4 and diet. Dietary control was good in 58.6% patients, intermediate in 26% and poor in 15.3%. Median (range) intellectual quotients was 97 (25-145). The number of neurological complications in late diagnosed patients was three-times higher than those of neonatal screening patients. 13.3% of adults had university studies and 37.5% had a stable couple.
Conclusions: This study allows for the first time the evaluation of the PKU management by Spanish PKU Follow-up Units, as well as the analysis and registry of controlled PKU patients. The study makes evident the need of adult Follow-up Units and the importance of neonatal screening for PKU patients prognosis.

Introducción

La fenilcetonuria (PKU) es un error congénito del metabolismo de la fenilalanina (Phe) causado por la deficiencia enzimática de fenilalanina hidroxilasa (PAH, EC 1.14.16.1), la enzima hepática que sintetiza tirosina a partir de Phe, siendo el cofactor de la reacción enzimática la tetrahidrobiopterina (BH4). La acumulación de Phe en plasma y tejidos y la disminución de la síntesis de tirosina parecen estar implicadas en la patogénesis de la enfermedad[1,2]. El tratamiento clásico de la PKU consiste en la restricción de Phe de la dieta, lo que implica una dieta con bajo contenido en proteínas naturales, suplementada con una fórmula especial exenta de Phe y enriquecida en tirosina, y que contenga los demás aminoácidos y micronutrientes (vitaminas, minerales, oligoelementos y ácidos grasos esenciales en algunas de ellas) necesarios para evitar deficiencias nutricionales[3,4]. El tratamiento alternativo con BH4 también logra disminuir las concentraciones de Phe en los pacientes que responden al mismo[5]. El tratamiento precoz de la PKU evita el daño neurológico grave[1]. Sin embargo, puede aparecer una ligera reducción del coeficiente intelectual respecto a la población general, así como déficits específicos de las funciones ejecutivas, especialmente si el control metabólico no es el adecuado[6,7]. La calidad del control dietético es determinante en el pronóstico de estos pacientes[7,8].

En 1963 Guthrie y Susi publicaron un método de cribado de la PKU mediante la determinación de la Phe en sangre que debía ser utilizado en toda la población en el período neonatal, lo que permitía el tratamiento precoz de la PKU y evitaba el retraso mental de los pacientes tratados precozmente[9]. En España se inició en Granada en 1968, en Barcelona en 1970 y en Madrid en 1973, siendo la cobertura actual superior al 99% de la población de recién nacidos[2]. El cribado neonatal de la PKU ha dado lugar al diagnóstico de un creciente número de pacientes con esta enfermedad que han requerido una confirmación diagnóstica y un complejo seguimiento clínico, bioquímico y nutricional de por vida[3,4]. Esto ha determinado la creación de unidades de seguimiento que han generado sus propios protocolos para el tratamiento y control de dichos pacientes[10–15]. No obstante, no se ha logrado aunar criterios para los requerimientos del buen funcionamiento de estas unidades[16,17], ni se han unificado los protocolos básicos para el seguimiento de estos pacientes, aun cuando a través de la Asociación Española para el Estudio de los Errores Congénitos del Metabolismo (AECOM) se consensuó una pauta de diagnóstico y seguimiento[18]. Por todo ello, resulta indispensable la recogida de datos sobre el funcionamiento actual de las unidades y la realización de un registro de pacientes con PKU que nos permita conocer el estado actual de los casos diagnosticados y/o tratados en el país a lo largo de estos años[19].

Los objetivos de este estudio han sido conocer el funcionamiento de las unidades de seguimiento de PKU en España, evaluar el seguimiento de la enfermedad en las mismas y realizar una base de datos con un registro de pacientes con PKU, en la que se recojan y analicen las características clínicas, bioquímicas y genéticas de los mismos.

Pacientes y método

Unidades de seguimiento

Se han incluido en el estudio las 18 unidades de seguimiento de PKU situadas en diferentes comunidades autónomas españolas.

Pacientes

Criterios de inclusión

a) Pacientes con PKU diagnosticados y/o seguidos en centros españoles, con deficiencia de PAH confirmada por diagnóstico diferencial y/o análisis mutacional del gen *PAH*; b) pacientes con PKU con concentraciones de Phe previas al tratamiento superiores a 360 μmol/L.

Criterios de exclusión

a) Pacientes con PKU diagnosticados tardíamente y que han rechazado el tratamiento; b) pacientes que han cambiado de residencia a otras zonas del país y se controlan actualmente en otras unidades de seguimiento (estos pacientes se incluyen en la unidad de seguimiento actual); c) pacientes extranjeros que fueron diagnosticados en España pero han retornado a su país de origen y no son controlados actualmente en las unidades de seguimiento españolas; y d) hiperfenilalaninemias leves.

Cuestionarios de manejo de fenilcetonuria

En febrero de 2009 se remitió a los centros españoles de seguimiento de PKU un cuestionario (base de datos Excel) compuesto de dos partes destinadas a: 1) conocer el funcionamiento de las unidades y el seguimiento global de los pacientes, y 2) registrar los pacientes diagnosticados y/o tratados en las unidades de seguimiento españolas. Los datos incluidos en el estudio son los aportados por las unidades hasta junio de 2010. El cuestionario sobre el funcionamiento de las unidades consta de un total de 14 preguntas cerradas, que incluyen datos codificados del centro y de los criterios de seguimiento de los pacientes (periodicidad de los controles, concentraciones máximas de Phe recomendadas e índice de control dietético utilizado en el seguimiento). El cuestionario sobre los pacientes consta de 41 preguntas cerradas, que incluyen datos personales codificados (5 preguntas), datos dietéticos (10 preguntas), evolución del desarrollo y complicaciones (19 preguntas) e inserción social (7 preguntas).

Entre las variables utilizadas en los datos dietéticos se deben definir:

- El test de sobrecarga de BH4 es la prueba que permite valorar la respuesta a este cofactor, es decir, el porcentaje de descenso de la concentración de Phe al suplementar al paciente con BH4[20].

- La tolerancia es la cantidad máxima de Phe que el paciente tolera para mantener la concentración plasmática dentro del intervalo recomendado[2].
- Índice de control de la dieta (ICD) es el cálculo (media, mediana o media de medianas) de las concentraciones de Phe durante un período de control del paciente[21] (el último año en este estudio).

Análisis estadístico

El análisis estadístico se ha realizado usando el programa SPSS (versión 17.0). Dada la naturaleza del estudio, se ha utilizado un análisis descriptivo para muchos de los datos, calculando únicamente el porcentaje de respuestas y la mediana (extremos) de las variables cuantitativas. La prueba de Chi al cuadrado se ha utilizado para comparar las variables cualitativas y el test ANOVA con la corrección de Bonferroni para comparar las variables cuantitativas (ICD, coeficiente intelectual o de desarrollo). El test de Pearson se ha usado para determinar la correlación entre variables cuantitativas (Phe al diagnóstico frente a respuesta a la sobrecarga de BH4 y edad frente a índice de control de la dieta). Se ha aceptado significación estadística para $p < 0,05$.

Protección de datos y confidencialidad

Los autores han seguido los protocolos establecidos por sus respectivos centros sanitarios para acceder a los datos de las historias clínicas y poder realizar esta publicación con la finalidad de investigación y divulgación científica. Los datos registrados tanto de centros como de pacientes han sido anonimizados mediante códigos de centros y de pacientes. La realización del registro ha sido aprobada por la Agencia Española de protección de datos y AECOM figura como responsable final del registro de pacientes.

El estudio ha sido aprobado por el Comité de Ética del Hospital Sant Joan de Déu de Barcelona y se ha realizado de acuerdo con las normas éticas de Helsinki de 1964, revisadas en Seúl en octubre de 2008.

Resultados

Unidades de seguimiento

Se han recogido los datos sobre la organización y manejo de pacientes de las 18 unidades de seguimiento españolas de PKU a las que se envió el cuestionario.

La creación de estas unidades se remonta a 1977. Entre 1977-1980 se crearon cuatro unidades, entre 1981-1990 empezaron a funcionar 6 unidades, de 1991-2000 tres unidades y entre 2001-2005 tres unidades (dos centros no han contestado a esta cuestión). Las unidades están formadas básicamente por pediatras (83%), nutricionistas (72%), dietistas (67%), bioquímicos (94%), neuropediatras (55%) y psicólogos (83%), aun cuando algunos especialistas pueden no formar parte de la unidad propiamente dicha, sino actuar como consultores externos. Todas las unidades controlan pacientes con PKU de todas las edades.

Confirmación del diagnóstico y control de pacientes

Diagnóstico diferencial

En 17/18 centros (94,4%) se realiza el diagnóstico diferencial de los pacientes con hiperfenilalaninemia mediante la determinación de pterinas, de la actividad dihidropteridina reductasa (DHPR) en sangre seca y el análisis de aminoácidos. Sólo 10/18 unidades (55,5%) realizan el test de sobrecarga de BH4 al diagnóstico, 7 unidades no lo realizan (38,9%) y un centro no contesta. Todas las unidades coinciden en tratar a los pacientes con PKU cuyas concentraciones de Phe son superiores a 360 μmol/L.

Seguimiento de pacientes

El seguimiento clínico se realiza según las diferentes especialidades integrantes de la unidad con una periodicidad mensual o trimestral (nutrición/dietética y pediatría), o anual (neurología, psicología).

El seguimiento bioquímico generalmente es mensual. El análisis de Phe se realiza en sangre seca recogida en el domicilio en 17/18 unidades (94,4%) y, además, por venopunción en 6 unidades (33,3%).

La evaluación del control de Phe se realiza calculando el índice de control de la dieta de los pacientes en base a la media (3 centros), la mediana (3 centros), la media de medianas de las concentraciones de Phe (3 centros), otras medidas (un centro) y no consta en 8 centros (ver datos dietéticos en material y método).

Los valores máximos de Phe que las unidades consideran como aceptables en el seguimiento de estos pacientes según los grupos de edad son inferiores a 600 μmol/L para la edad adulta (intervalo 480-900 μmol/L) y de 360 μmol/L en niños, lactantes (< 1 año) y embarazadas.

Pacientes

Sujetos de estudio

Se han analizado los datos de 703 pacientes con PKU. Se han excluido 15 pacientes de los que no se disponía de muchos de los datos solicitados en el cuestionario. Finalmente se han registrado los datos de 688 pacientes con PKU, 328 varones y 356 mujeres (en 4 se desconoce el sexo), con edades comprendidas entre 1 mes y 53 años (mediana 14 años). La distribución por edades se representa en la tabla 1.

Diagnóstico

De los 688 pacientes, 494 se diagnosticaron precozmente (< 2 meses) y actualmente tienen una mediana de edad de 10 años (extremos 0-39 años) y 171 se diagnosticaron tardíamente y actualmente tienen una mediana de edad de 30 años (extremos 1-53 años) (en 23 pacientes no consta la fecha de nacimiento o de diagnóstico). El 70,2% de los pacientes se diagnosticaron antes del mes de vida, el 6% entre 1-2 meses, el 3,6% entre 2 meses y un año, mientras que el 20,2% eran mayores de un año en el momento del diagnóstico.

La mediana de concentración de Phe plasmática en el momento del diagnóstico en el conjunto de pacientes era de 1.267 μmol/L (extremos 138-4.500 μmol/L). Algunos valores de Phe eran inferiores a 360 μmol/L cuando se diagnosticó al paciente, porque se trataba de valores muy iniciales en hermanos de pacientes diagnosticados previamente. Teniendo en cuenta los valores de Phe previos al tratamiento, se podía clasificar a 109 (15,8%) de ellos como una PKU leve, a 179 (26%) como una PKU forma moderada y a 354 (51,5%) como una forma severa o PKU clásica. En 46 pacientes no constan los valores previos al tratamiento.

En relación con el estudio genético, de los 688 pacientes dicho estudio se realizó en 508. En 496 se hallaron dos mutaciones y en 52 sólo una mutación. En la tabla 2 se describen las mutaciones de mayor frecuencia alélica.

Tabla 1
Edad actual de los pacientes con fenilcetonuria

Grupo de edad	< 6 años	6-11 años	12-18 años	> 18 años	No consta
Número de pacientes	165	136	98	286	3
Porcentaje	23,9%	19,7%	14,2%	41,4%	0,6%

Tabla 2
Espectro de mutaciones de mayor frecuencia alélica en pacientes con fenilcetonuria

Mutación en el gen *PAH*	Número de alelos detectados	Frecuencia alélica
IVS10-11G>A*	107	10,5%
I65T*	80	7,8%
V388M*	79	7,7%
R261Q*	72	7,0%
IVS4+5G>T*	48	4,7%
S349P*	38	3,7%
R408W*	29	2,8%
Y414C*	26	2,5%
R243X*	25	2,4%
R158Q*	23	2,2%
R243Q*	23	2,2%
E280K	20	1,9%
L48S	17	1,6%
IVS8 -7A>G	16	1,5%
IVS10 int546	15	1,4%
P281L	14	1,3%
R252W	13	1,2%
IVS12+1G>A	12	1,1%
R68S	11	1,0%
L348V	10	0,9%
R261X	10	0,9%
IVS1+5G>T	9	0,8%
G46S	7	0,6%
P122Q	6	0,5%
F55fs	6	0,5%
R111X	6	0,5%
Y198fs	6	0,5%

Con asterisco las de mayor frecuencia alélica.

Tratamiento

El test de sobrecarga de BH4 se realizó a 411 (59,7%) de los pacientes. En 89 de ellos (12,9%) la respuesta fue buena (disminución de la Phe basal > 50%), en 84 (12,2%) fue regular (30-50%) y en 238 (34,6%) fue mala (< 30%). Se observó una relación significativa negativa entre la concentración de Phe al diagnóstico y la respuesta a la sobrecarga de BH4 (r = -0,520; p < 0,0001). En la figura 1 se representan las concentraciones de Phe al diagnóstico en relación con la respuesta al test de sobrecarga de BH4.

De los 688 pacientes, 541 (78,6%) están tratados con dieta restringida en proteínas y fórmula, 64 (9,3%) con BH4 y dieta libre y 54 (7,8%) con BH4 y dieta. En 29 pacientes no consta el tratamiento. La mediana de la tolerancia a la Phe en los pacientes tratados con dieta y fórmula es de 390 mg/día (extremos 180-600 mg/día) y la mediana de los pacientes tratados con BH4 es de 1.000 mg/día (extremos 250-2.700 mg/día) (fig. 2).

El control dietético se consideró bueno (en relación a los valores recomendados para cada grupo de edad) en 403 pacientes (58,6%), regular en 158 (26%) y malo en 105 pacientes (15,3%) (no consta en 22 pacientes) (fig. 3). La mediana del índice de control dietético del último año es de 390 μmol/L (extremos 66-2.310 μmol/L). En la figura 4 se representan los índices de control de la dieta en los grupos etarios estudiados. Se observa una relación significativa entre la edad y el índice de control de la dieta (r = 0,475; p < 0,0001), con un peor cumplimiento a medida que ésta se incrementa. De los 688 pacientes, 586 mantienen el tratamiento (85%), mientras que 68 lo abandonan a una mediana de edad de 12,0 años (extremos 1-38 años).

Seguimiento neurológico

Los tests de inteligencia más frecuentemente realizados en los pacientes mayores de 3 años son WISC III-IV, K-BIT, K-ABC y RAVEN. El coeficiente intelectual (CI) o de desarrollo (CD) del total de los pacientes (mediana 97 [extremos 25-145]) se expresa en la tabla 3. El CI o CD presenta una mediana de 100 (extremos 100-143) en los

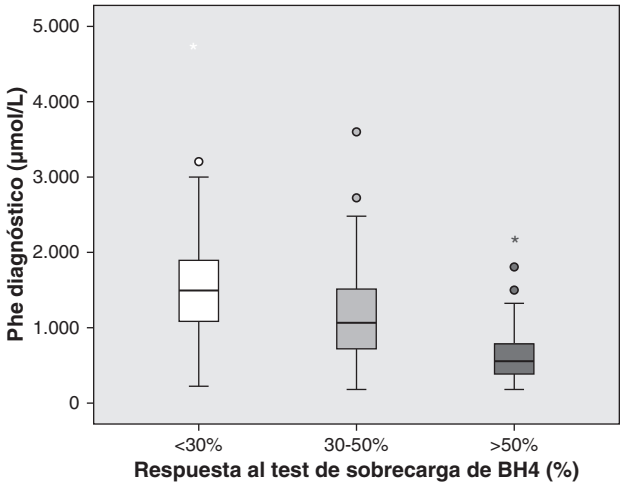

Figura 1. Valores de fenilalanina (Phe) al diagnóstico en relación con la respuesta al test de sobrecarga de tetrahidrobiopterina (BH4). La línea media de las cajas representa la mediana (P_{50}), la base inferior el percentil 25 (P_{25}) y la superior el 75 (P_{75}). Los trazos horizontales corresponden a valores adyacentes, máximo y mínimo; los puntos representan los valores exteriores y la estrella el valor alejado (ambos, valores anómalos).

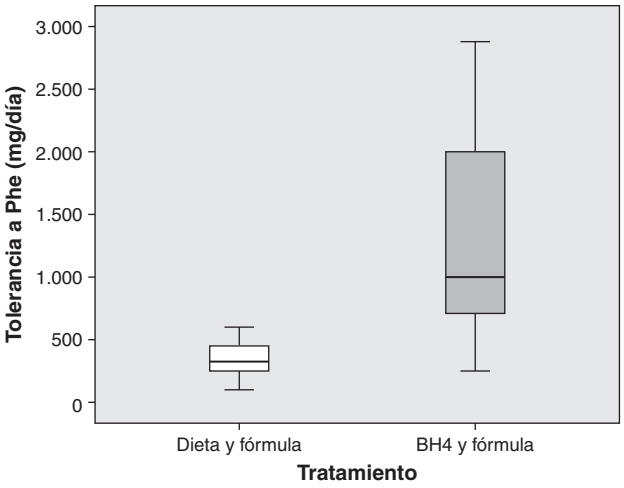

Figura 2. Tolerancia a fenilalanina (Phe) (mg/día) en pacientes con fenilcetonuria (PKU) agrupados según su tratamiento. La línea media de las cajas representa la mediana (P_{50}), la base inferior el percentil 25 (P_{25}) y la superior el 75 (P_{75}). Los trazos horizontales corresponden a valores adyacentes, máximo y mínimo. BH4: tetrahidrobiopterina.

pacientes con diagnóstico precoz (< 2 meses de edad) (n = 494) y una mediana de 75,5 (extremos 25-118) en los pacientes diagnosticados tardíamente (n = 171) (p < 0,0001). Se representan dichos coeficientes en relación con la edad actual de los pacientes en la figura 5.

El porcentaje de pacientes de diagnóstico tardío con complicaciones neurológicas y trastornos del comportamiento es significativamente mayor al de los diagnosticados precozmente (tablas 4 y 5).

Inserción social de los pacientes

Escolarización. Del conjunto de los pacientes, sólo consta que 594 han estado o están escolarizados. De ellos, 392 en una escuela ordinaria (66%), mientras que 56 lo están en escuela ordinaria con adaptación curricular (9,4%) y 63 en una escuela de educación especial (10,6%). Actualmente, de los 234 pacientes en edad escolar

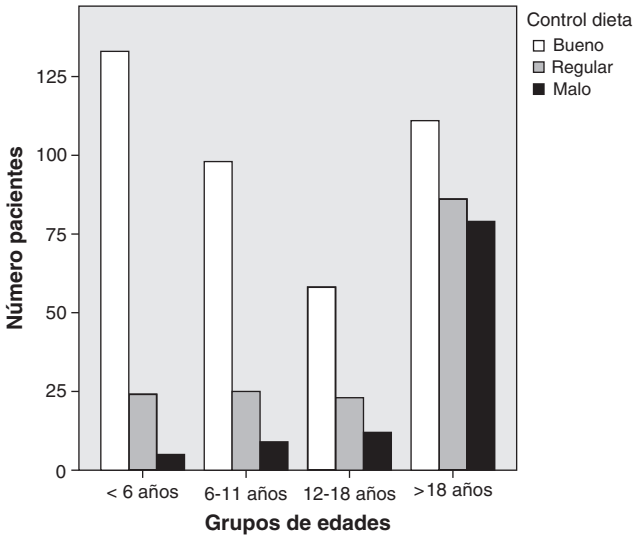

Figura 3. Control de la dieta según grupos de edades.

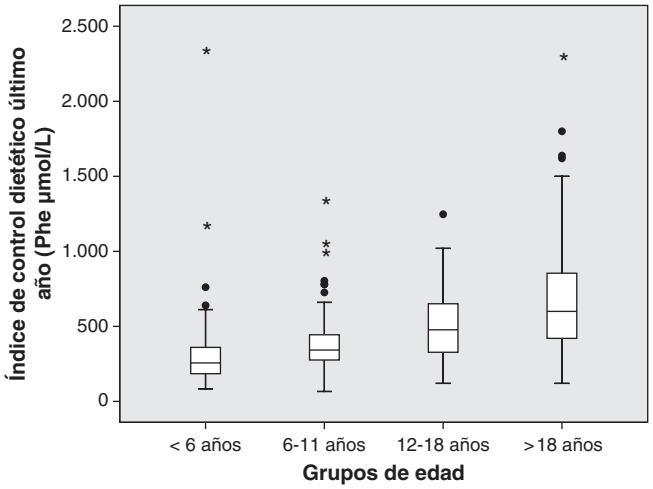

Figura 4. Índice de control de la dieta según los grupos de edad. La línea media de las cajas representa la mediana (P_{50}), la base inferior el percentil 25 (P_{25}) y la superior el 75 (P_{75}). Los trazos horizontales corresponden a valores adyacentes, máximo y mínimo; los puntos negros representan los valores exteriores y las estrellas los valores alejados (ambos, valores anómalos).

(6-17 años), estudian en una escuela ordinaria 186 (79%), con adaptación curricular 19 (8,1%) y educación especial 8 (3,4%) (en 21 pacientes no disponemos de datos). Considerando la edad al diagnóstico, se observan diferencias significativas entre el número de pacientes con PKU que están o han estado escolarizados en una escuela ordinaria o con soporte y los que han sido diagnosticados precoz o tardíamente ($p < 0{,}0001$) (fig. 6).

Estado actual de pacientes adultos. De los 688 pacientes registrados, 286 son mayores de 18 años (41,4%). De ellos, 38 cursan o han

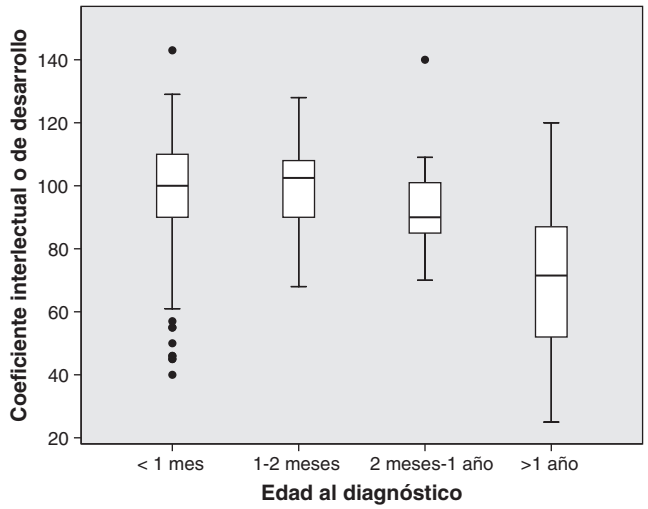

Figura 5. Coeficiente de desarrollo o intelectual en los diferentes grupos de edad al diagnóstico.

cursado estudios universitarios (13,3%). Los 12 (> 24 años) que han completado los estudios universitarios están insertados laboralmente.

Inserción laboral del conjunto de pacientes > 18 años. De 286 pacientes mayores de 18 años, constan los datos de 155 (54,2%). De éstos, 113 tienen trabajo estable, 17 con ayuda para inserción laboral, 5 trabajan en el hogar y 20 están actualmente en el paro.

Inserción social. Respondieron 205 pacientes mayores de 18 años, de los que 77 tienen pareja estable (37,5%) y 128 (62,5%) están solteros. Diez mujeres con PKU tienen un hijo sano y 4 mujeres tienen 2 o más hijos sanos, mientras que 13 madres con PKU tienen un hijo con embriopatía, 4 tienen dos hijos afectados y una madre tiene 4 hijos afectados.

Discusión

La necesidad del seguimiento de los pacientes con PKU y otras enfermedades metabólicas por parte de unidades multidisciplinarias especializadas en dichos trastornos se ha hecho evidente en los últimos años[16]. No obstante, son pocos los estudios dedicados a la evaluación del funcionamiento de estas unidades[16,17]. Por ello, es importante la realización de este estudio, que ha permitido conocer el funcionamiento de las unidades españolas, así como registrar y analizar los datos de los pacientes con PKU controlados por ellas.

Un 83% de nuestras unidades son multidisplinarias, algo que se considera imprescindible en este momento[16]. Estos datos contrastan con los recientemente publicados por van Spronsen y Burgard en un estudio de 165 unidades en 23 países europeos, donde únicamente un 12% de ellas disponen de un equipo completo[16]. La mayoría de las unidades españolas están lideradas por pediatras, situadas físicamente en departamentos de pediatría u hospitales pediátricos. Este problema se pone en evidencia en este estudio,

Tabla 3
Coeficiente de desarrollo o intelectual en los pacientes con fenilcetonuria

CI/CD	Retraso mental grave	Retraso mental moderado	Retraso mental leve	Límite	Normal	Normal-alto
Número de pacientes	11	15	34	65	292	51
Porcentaje (%)	2,4	3,2	7,3	13,9	62,4	10,9

Basado en la Scale of Mental Retardation of American Association 1992.
CI/CD: coeficiente intelectual o de desarrollo.

Tabla 4
Complicaciones neurológicas en pacientes españoles con fenilcetonuria

Complicaciones neurológicas	Diagnóstico precoz	Diagnóstico tardío	Significación estadística
Epilepsia	1,9	19,2	$p < 0,0001$
Temblor	4,7	39,7	$p < 0,0001$
Torpeza motriz	5,7	56,9	$p < 0,0001$
Retardo mental	3,8	66,2	$p < 0,0001$
Otras alteraciones neurológicas	7,1	17,3	$p < 0,015$

Datos expresados en % de pacientes.
Significación estadística: prueba de Chi al cuadrado.
Otras alteraciones neurológicas: alteraciones en la resonancia magnética craneal, alteraciones en el electroencefalograma y potenciales evocados visuales.

Tabla 5
Trastornos del comportamiento en pacientes españoles con fenilcetonuria

Trastornos del comportamiento	Diagnóstico precoz	Diagnóstico tardío	Significación estadística
Déficit de atención	19,1	59,2	$p < 0,0001$
Hiperactividad	13,6	28,2	$p < 0,001$
Impulsividad	13,0	27,6	$p < 0,001$
Depresión	3,6	25,6	$p < 0,0001$
Ansiedad	7,8	30,9	$p < 0,0001$
Fobias	1,9	15,7	$p < 0,0001$
Baja autoestima	7,2	25,0	$p < 0,0001$
Otros trastornos	4,1	13,8	$p < 0,002$

Datos expresados en % de pacientes.
Significación estadística: Prueba de Chi al cuadrado.
Otros trastornos: trastornos del espectro autista, alteraciones del lenguaje, agresividad y autoagresividad.

donde se observa que la mediana de edad actual de los pacientes con PKU españoles ya alcanza los 14 años, con un 41,4% de ellos mayores de 18 años, que continúan controlándose en unidades pediátricas. Esta situación es similar en Europa, donde sólo existen tres unidades de adultos[16], con un 1,9% de médicos dedicados al cuidado de pacientes metabólicos adultos en los 163 centros estudiados. Esto conduce a la primera conclusión importante, que es la necesidad de unidades de enfermedades metabólicas para adultos, estrechamente coordinadas con las unidades pediátricas actuales, que conozcan la problemática de estos pacientes y cubran las necesidades de los adultos de una forma adecuada.

Se observa un elevado cumplimiento de las unidades en cuanto a la realización del diagnóstico diferencial (94,4%), mientras que el test de sobrecarga de BH4 se realiza en una proporción menor (55,5%), similar a la del conjunto de centros europeos (53,5%)[17]. Destaca también la concordancia de los valores de Phe por encima de los cuales se inicia el tratamiento, así como la de los valores

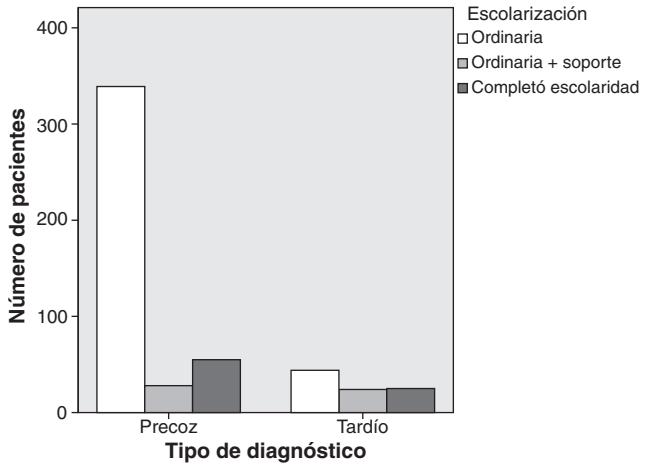

Figura 6. Pacientes escolarizados en escuela ordinaria, ordinaria con soporte y que completaron escolarización en relación con la edad al diagnóstico.

máximos recomendados de Phe en el control de los pacientes, sobre todo en la edad pediátrica y embarazo, a diferencia de la gran discrepancia observada en los estudios europeos[16,17].

En cuanto a los pacientes con PKU, más del 70% de ellos se han diagnosticado antes del mes de vida, lo que mejora claramente el pronóstico de la enfermedad mediante una introducción temprana de la dieta y un seguimiento y detección precoz de las complicaciones que pueden presentarse.

La elevada proporción de formas leves y moderadas (42%) representa una mayor facilidad para el buen control de estos pacientes, muchos de los cuales se benefician ya, o pueden hacerlo en un futuro próximo, del tratamiento con dihidrocloruro de sapropterina, un derivado sintético de la BH4 (KUVAN®)[22], así como de otras opciones terapéuticas basadas en la acción de chaperona de futuras terapias[23].

La mutación más prevalente es el cambio IVS10-11G>A, tal como se había ya descrito en estudios anteriores[24,25]. Destaca la gran heterogeneidad genética observada en pacientes españoles en relación con los de otras áreas geográficas.

El control dietético de los pacientes es bueno o regular en una gran proporción de ellos, siendo la edad un factor determinante en el buen control. La utilidad del cálculo de un índice de control de la dieta (ver material y métodos) se evidencia en los estudios de evaluación de los resultados del control a corto y largo plazo[21]. Aunque la adolescencia y edad adulta permitan una cierta relajación de la dieta (siempre y cuando no exista la posibilidad de embarazo en las mujeres), numerosos estudios muestran la necesidad de una restricción dietética de por vida, para evitar una posible regresión neurológica[8]. Esto hace necesarias las unidades para adultos que puedan atender a los pacientes una vez superada la edad pediátrica.

En cuanto a la situación actual de los pacientes con PKU en España, hay que resaltar el impacto del diagnóstico precoz sobre el coeficiente intelectual de los mismos, así como en la existencia de complicaciones neurológicas y conductuales. El diagnóstico precoz ha permitido que la escolarización de los pacientes sea prácticamente normal en la mayor parte de ellos, así como su inserción laboral y social.

En resumen, hay que destacar el esfuerzo realizado por las unidades de enfermedades metabólicas pediátricas para constituirse en grupos multicéntricos de seguimiento de los pacientes y la importancia de la futura creación de unidades de adultos. Hay que resaltar también la importancia del diagnóstico precoz de los pacientes para la buena evolución neuropsicológica de los mismos y en su inserción social. Queremos destacar la importancia de haber podido realizar este registro de pacientes españoles gracias a la colaboración de todos los centros de seguimiento y la necesidad de actualizarlo periódicamente, para reunir la información que nos permita evaluar el seguimiento y las necesidades de los pacientes con PKU españoles. Por último, mencionar la necesidad de reunir los registros de PKU europeos para generar protocolos internacionales basados en datos objetivos.

Conflicto de intereses

Este estudio ha sido posible gracias a las becas de AECOM y Nutricia.

Agradecimientos

A la gran colaboración mostrada por todas las unidades de seguimiento PKU de España.

Anexo 1.

Grupo Colaborativo de Unidades de Seguimiento Españolas: Alcalde C (Hospital Universitario de Valladolid), Aldámiz K (Hospital de Cruces, Bilbao), Baldellou A (Hospital Miguel Servet, Zaragoza), Balmaseda E (Hospital Universitario de Albacete), Bélanger-Quintana A (Hospital 12 de Octubre, Madrid), Blasco J (Hospital Carlos Haya, Málaga), Bousoño C (Hospital de Oviedo), Bueno M (Hospital Virgen del Rocío, Sevilla), Cáceres C (Hospital Infanta Cristina, Badajoz), Couce ML (Hospital Clínico Universitario de Santiago), Dalmau J (Hospital La Fe, Valencia), García I (Hospital Miguel Servet, Zaragoza), Gil D (Hospital Virgen de la Arrixaca, Murcia), González-Lamuño D (Hospital Nacional Marqués de Valdecilla, Santander), Lambruschini N (Hospital Sant Joan de Déu, Barcelona), Martínez-Pardo M (Hospital 12 de Octubre, Madrid), Muro JM (Hospital Universitario de Valladolid), Peña-Quintana L (Hospital Materno-Infantil de Las Palmas), Pérez M (Hospital Virgen del Rocío, Sevilla), Ruiz A (Hospital Son Dureta, Palma de Mallorca), Ruiz-Pons M (Hospital Virgen de la Candelaria, Tenerife), Sánchez-Valverde F (Hospital Virgen del Camino, Pamplona), Sanjurjo P (Hospital de Cruces, Bilbao), Sierra C (Hospital Carlos Haya, Málaga), Vitoria I (Hospital La Fe, Valencia).

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Impaired Neurotransmission in Early-treated Phenylketonuria Patients

María Julieta González, MD,[*] Rosa Gassió, MD, PhD,[*]
Rafael Artuch, MD, PhD,[†,‡] and Jaume Campistol, MD, PhD[*,‡]

Cerebral neurotransmitter (NT) deficiency has been suggested as a contributing factor in the pathophysiology of brain dysfunction in phenylketonuria (PKU), even in early-treated phenylketonuric patients. The study aimed to review dopamine and serotonin status in PKU, and the effect of the impaired neurotransmission. Several mechanisms are involved in the pathophysiology of PKU, primarily characterized by impaired dopamine and serotonin synthesis. These deficits are related to executive dysfunctions and social-emotional problems, respectively, in early treated patients. Blood phenylalanine is the main biomarker for treatment compliance follow-up, but further investigations and validation of peripheral biomarkers may be performed to monitor NT status. The development of new therapies is needed not only for decreasing blood and brain phenylalanine levels but also to improve NT syntheses.
Semin Pediatr Neurol 23:332-340

Introduction

Phenylketonuria (PKU; OMIM 261600) is an inborn error of metabolism of phenylalanine (Phe) caused by mutations in the gene coding for the enzyme phenylalanine hydroxylase (PAH) (EC 1.14.16.1). PAH locus is on chromosome 12q22-12q24.1. To date, more than 950 mutations have been described (http://www.biopku.org). This enzyme catalyses the hydroxylation of Phe to tyrosine, and uses tetrahydrobiopterin (BH4) as a cofactor. The mutations in the PAH gene lead to a partial or total enzyme activity lost, and produce a Phe accumulation and decreased tyrosine biosynthesis in the liver and consequently in other organs and biological fluids.[1] If PKU is untreated, it leads to intellectual disability, seizures, and microcephaly, among other severe neurologic disorders. The diagnosis of newborns in screening programs and early treatment allows virtually normal physical and intellectual development in patients with PKU.[1] The classic treatment for PKU is a restricted diet in Phe, which means low natural proteins, supplemented by special medical foods enriched in tyrosine and other amino acids and in nutrients (vitamins, minerals, and essential fatty acids) necessary to avoid nutritional deficits.[1] In the past decade, there has been a new treatment for some patients with PKU harboring mild-to-moderate mutations. This is sapropterin dihydrochloride, a synthetic form of BH4 that acts as a chemical chaperone improving PAH residual activity. This effect leads to an increase in Phe transformation to tyrosine, therefore, decreasing plasma Phe levels and, allowing full or partial withdrawal of the restrictive diet in these patients.[2-4] There is an extensive research conducted on new therapeutic options like enzyme and gene therapy, and a glycomacropeptide-based diet (a natural Phe-free protein). The main aim of PKU treatment, whether the patient is undergoing dietary treatment or BH4 therapy, consists of keeping blood Phe concentrations in a safe range for the central nervous system. There are several recommendations, but no consensus, about the limits of this range. A common practice is that blood Phe should be lower than 360 μmol/L until 12 years of age and should remain lower than 600 μmol/L in patients older than 12 years. These values are 3-5 times more elevated that normal

From the *Department of Neurology, Institut de Recerca Pediàtrica Hospital Sant Joan de Déu, Hospital Sant Joan de Déu, Barcelona, Spain.
†Department of Biochemistry, Institut de Recerca Pediàtrica Hospital Sant Joan de Déu, Hospital Sant Joan de Déu, Barcelona, Spain.
‡Centre for Biomedical Research on Rare Diseases (CIBERER), Institute of Health Carlos III, Madrid, Spain.
This work was supported by grants from the Spanish Ministerio de Economia y Competitividad FIS PI12/01469 and FEDER Funding Program from the European Union. The "Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER) is an initiative of the Instituto de Salud Carlos III.
Address reprint requests to María Julieta González, MD, Department of Neurology, Institut de Recerca Pediàtrica Hospital Sant Joan de Déu, Hospital Sant Joan de Déu, Santa Rosa 39-57, 08950 Esplugues de Llobregat, Barcelona, Spain. E-mail: mjgonzalez@hsjdbcn.org

 http://dx.doi.org/10.1016/j.spen.2016.11.007
1071-9091/16/

Table The hypotheses of different mechanisms underlying neurotoxicity in Phenylketonuria

Brain high levels of Phe concentrations
Neurotransmitter biosynthesis defect
Abnormal brain myelination
Abnormal synthesis of proteins in brain
Cerebral tyrosine and tryptophan deficiency
Neurotoxicity of Phe metabolites
Cholesterol biosynthesis defects
Increase oxidative stress
Altered DNA methylation
Bioenergetics deficit
LCPUFA deficiency

LCPUFA, long-chain polyunsaturated fatty acid.

Phe values (35-75 μmol/L) because the protein requirements of the organism makes the complete removal of Phe (an essential amino acid) from the diet impossible.[5,6]

These treatments prevent the severe neurologic complications of patients with PKU, when started early in newborns. However, it has been demonstrated that early treated patients with PKU may present some neurologic and neuropsychological problems. Intellectual quotient scores are normal, but usually lower than intellectual quotient scores of control groups. Patients display deficits in executive functions (attentional control, planning or goal setting and problem solving, cognitive flexibility of thought and action, concept formation or abstraction, information processing, and social cognition), and deficits in fine-motor skills.[5,7-10] These complications, despite being mild, can negatively affect school performance.[11] There is also a high incidence of anxiety, depression, and attention deficit hyperactive disorder (ADHD) in these patients.[12,13]

Another consequence of high levels of Phe in the brain is impaired white matter (WM) myelination. The prevalence of such WM disturbances is high in patients with high Phe concentrations, especially in older patients and in those with poor treatment compliance. These disturbances may be detected through brain magnetic resonance imaging studies, which show areas of increased signal in T2 sequences, predominantly in the parieto-occipital periventricular WM.[14] A new magnetic resonance imaging technique (diffusion tensor imaging) shows evidence of abnormalities in microstructural WM integrity in different brain regions of patients with early treated PKU (lower mean diffusion), even when WM hyperintensity is not present.[15]

In this article, we will review current knowledge about neurotransmitter (NT) dopamine and serotonin status in PKU, and the effect of impaired neurotransmission in cognitive, emotional, behavioral and social skills of patients with early treated PKU. New therapies will also be discussed.

Pathophysiology of Cerebral Dysfunction in PKU

It is now recognised that there is minimal, but demonstrable brain damage in patients with early treated PKU and the causal factors are not entirely understood. Although PAH is a liver enzyme, the target organ of hyperphenylalaninaemia is the brain. The hypotheses of different mechanisms underlying neurotoxicity in PKU are listed in the Table.[1,16-20]

It has been postulated that hyperphenylalaninaemia-related neurotoxicity could be explained in part by Phe sharing the same L-type amino acid carrier (LAT1, SLC7A5) with other large neutral amino acids (LNAA) (mainly tyrosine and tryptophan), and competes with these to cross the blood-brain barrier (BBB).[21] Tyrosine and tryptophan are precursors of NTs, and its relative brain deficit may contribute to a reduced synthesis of serotonin, dopamine, and noradrenaline. Moreover, the high Phe values in the brain may produce an inhibition of tyrosine and tryptophan hydroxylases activities, causing a further reduction of dopamine and serotonin biosynthesis[17,18] (Fig.).

These potential neurotransmission deficits are relevant, as the dopamine is essential for a proper functioning of the prefrontal cortex (PFC), which in turn controls the executive functions. Serotonin is involved both in the cognitive processes

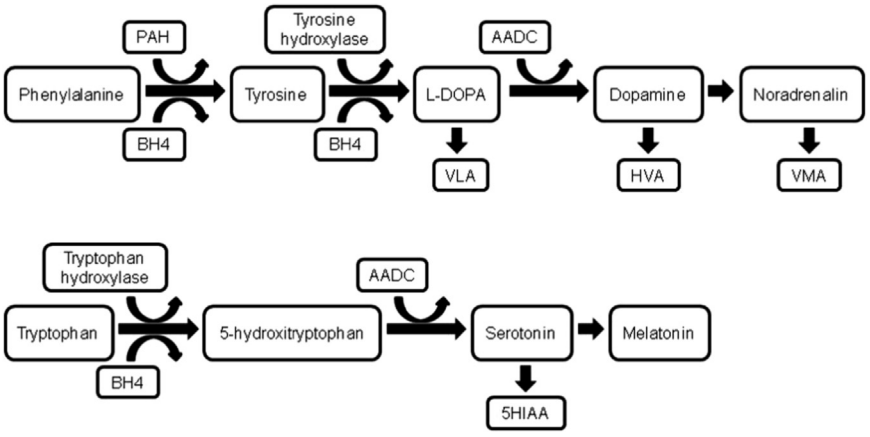

Figure Schematic representation of the synthesis of serotonin and dopamine and their metabolites (biogenic amines). AADC, aromatic L-amino acid decarboxylase; BH4, tetrahydrobiopterin; L-DOPA, L -3,4 dihydroxyphenylalanine; 5HIAA, hydroxyindoleacetic acid; HVA, homovanillic acid; PAH, phenylalanine hydroxylase; VLA, vanillactic acid; VMA, vanillylmandelic acid.

mediated by the orbitofrontal cortex and in the regulation of mood, emotions, and behavior.[22] It is important to consider the relative deficit of tyrosine in PKU, because this amino acid becomes an essential one as its biosynthesis is severely decreased. Once again, a high Phe value is the main pathophysiological factor, as Tyr supplementation in the presence of toxic Phe values does not prevent neurologic damage in PKU.[1] Although the special diets are supplemented with Tyr, it has been demonstrated that blood tyrosine concentrations fluctuate throughout the day, and may decrease after prolonged fasting.[23]

Another pathophysiological mechanism in PKU is the low bioavailability of LNAA that causes a decrease in brain protein synthesis, as it has been proved in the PAH^{enu2} mice model.[24] In studies performed in patients with PKU, it was demonstrated that cerebral protein synthesis, which is necessary for the development of cerebral functions, was inversely proportional to plasma Phe concentrations.[25] Myelin basic protein biosynthesis, which is essential for myelin formation, may be affected if there are low brain concentrations of some essential amino acids. This may also affect other brain proteins, such as tyrosine and tryptophan hydroxylase enzymes and some cerebral receptors such as the glutamate receptor. This reduced protein synthesis may result in decreased synaptic plasticity and axonal growth.[17] Recently, Horling et al[26] revealed disturbances in the presynaptic and postsynaptic protein expression in the hippocampal area of PAH^{enu2} mice. This finding was associated with a dysfunction in synaptogenesis and in synaptic transmission, and with reduced microglial activity, observed especially when the blood Phe levels were elevated.

Other pathophysiological factor is that the elevated concentrations of Phe may inhibit the cholesterol biosynthesis. In the PAH^{enu2} mice, there is consistent evidence that the alterations in cerebral WM are due to the inhibition of the rate-limiting enzyme of cholesterol biosynthesis, the 3-hydroxymethylglutaril-CoA reductase, that impairs the ability of oligodendrocytes to produce and maintain the myelin.[27] It was suggested that the absence of myelin in patients with PKU secondarily affects the synthesis of NTs,[28] and produce disruptions in the interconnectivity among different cerebral areas.[29] This inhibition of cholesterol biosynthesis would be related to other common observations in PKU, for example, a coenzyme Q10 deficiency,[30] as both molecules are synthesised through the mevalonate pathway.

Other factors implicated in the neuronal damage are antioxidant system abnormalities, which may adversely affect the maturation of the developing brain. These abnormalities in patients with PKU are secondary to nutritional deficits owing to the treatment with a diet restricted in animal protein. Furthermore, elevated concentrations of Phe can inhibit antioxidant enzyme activities, which occur with catalase and glutathione peroxidase activities, or may decrease coenzyme Q10 biosynthesis.[30]

Studies have claimed that long-chain polyunsaturated fatty acid deficiency due to low dietary intake may be an additional deleterious factor for the brain. Long-chain polyunsaturated fatty acids have a critical role as a structural component of the phospholipids in the plasma membranes. Docosahexaenoic acid is the main lipid inside the neuronal membranes and in retinal cells. Thus, it is essential for brain development in general and for retina and visual circuits in particular.[31]

It is known that toxic exposures can alter DNA methylation. It has been shown that high Phe levels in patients with PKU may lead to aberrant DNA methylation that secondarily produces gene dysregulation in the brain and leucocytes.[19]

Brain energy metabolism disturbances have also been described in both animal models and patients with PKU owing to decreased glucose oxidation and impaired activities of different enzymes, such as mitochondrial respiratory chain complexes, Krebs cycle enzymes, and creatine kinase.[20]

Therefore, from these studies, we can conclude that the neurologic damage in PKU may be due to multifactorial mechanisms, which might be involved to a greater or lesser degree in the pathophysiology of the disease.

Impaired Neurotransmission in PKU

The first descriptions of the alteration in NT synthesis in untreated patients with PKU date from the late 1950s and initially was involved only serotonin. Pare et al in 1957,[32] demonstrated lower concentrations of serotonin in the blood and 5-hydroxindolacetic acid (5HIAA) in the urine of these patients, and Yuwiler et al[33] demonstrated decreased brain serotonin in mice with high levels of Phe. McKean[34] reported reduced concentrations of serotonin, dopamine, noradrenaline, tyrosine, and tryptophan in the brains of untreated patients with PKU, with major reduction in the caudate nucleus, and increased 5HIAA and homovanillic acid (HVA) in cerebrospinal fluid (CSF) when the blood Phe values decreased. Butler et al,[35] also described an increase of 5HIAA and HVA in the CSF of patients with untreated PKU after beginning the dietary treatment. These findings led to the hypotheses that elevated Phe levels may inhibit tyrosine hydroxylase and tryptophan hydroxylase activities[36] together with a decrease in the neuronal uptake of tyrosine and tryptophan.[37] These hypotheses were later confirmed. Pascucci et al,[38] demonstrated in PAH^{enu2} mice a severe cerebral depletion of serotonin, with mild reduction in the brain level of tryptophan, and major deficits in the brain level of 5-hydroxytryptophan (5-HTP). These findings also suggested that an excess of brain Phe might interfere with tryptophan hydroxylase activity, thus reducing availability of 5-HTP.

It was also suggested that elevated blood Phe interferes with the transport of LNNA through the BBB.[39] The affinity of the LAT-1 transporter for Phe is high, therefore a subtle increase of Phe (within 200-500 μmol/L) may produce a substantial increment of the transport of this amino acid across the BBB and limit the passing of other LNNA such as tyrosine and tryptophan.[21] This effect occurs not only in the BBB but also in synaptosomal plasma membrane vesicles.[1]

After this initial observation, the studies were conducted in early treated patients. A disturbance in the NT synthesis and in LNNA transport through BBB was also observed. Giovaninni et al[40] detected in patients with PKU without treatment and in patients with good metabolic control decreases in norepinephrine and platelet serotonin values, and suggesting that even a slight increase in plasma Phe could inhibit peripheral synthesis of serotonin and noradrenaline. Paans et al[41] demonstrated with positron emission tomography (PET) scans low availability of brain tyrosine in adult patients with PKU with Phe values higher than 700 μmol/L. In patients with plasma Phe levels between 0.47 and 2.24 mmol/L, a correlation between the brain and plasma Phe concentrations was shown through magnetic resonance spectroscopy,[42] although these findings have not been confirmed by other studies in adult early treated patients.[43] Burlina et al,[44] described decreased NT metabolite concentrations in CSF (HVA and 5HIAA) in early treated adolescents and young adults with WM abnormalities (hyperintensity in T2-weighed images) in the presence of Phe levels higher than 700 μmol/L. Landvogt et al,[45] showed in a PET scan study a severely impaired influx and distribution of 6-[^{18}F] fluoro-L-DOPA (FDOPA) throughout the striatum (41% reduction in the rate of use of FDOPA) in early treated adult patients without strict dietary treatment and with mean serum Phe levels of 1260 μmol/L. The competitive inhibition of the LNAA transporter by high plasma Phe values explains this abnormality.

Groot et al[46] studied cerebral protein synthesis in patients with PKU using PET scan and magnetic resonance spectroscopy. They found that increased blood Phe concentrations were strongly associated with reduced L-[1-^{11}C]-tyrosine (^{11}C-Tyr) transport from blood to brain, which was associated in turn with reduced ^{11}CTyr cerebral protein incorporation, and inverse association between brain Phe concentrations and the rate of ^{11}C-Tyr incorporation into cerebral proteins.[47]

Animal models studies confirmed the NT alteration synthesis in different brain areas. Diamond et al,[48] in rat pups with mild plasma Phe elevations, found reductions in HVA values in 2 frontal cortical areas (the medial PFC and the anterior cingulate cortex). The 5-HIAA values were decreased in all brain regions examined, and there was no effect on norepinephrine. Puglisi-Allegra et al[49] demonstrated in the brains of PAH^{enu2} mice a marked decrease in serotonin, norepinephrine, dopamine, and their metabolite levels in different cerebral areas (PFC, cingulate cortex, nucleus accumbens, caudate-putamen, hippocampus, and amygdala), with the PFC and amygdala being the most affected anatomic structures. A marked decreased in the dopamine reuptake was also observed in the PFC, accumbens nucleus and cingulate cortex. Serotonin appeared to be more affected than dopamine, given that inhibition of dopamine synthesis needs a Phe concentration 3 times higher than those needed for inhibition of serotonin synthesis. Other studies in PAH^{enu2} mice[50] revealed decreased concentrations of striatal 5-HIAA; 3,4-dihydroxy-phenylacetic acid (a dopamine metabolite); and 5-HIAA/5-HT ratios.

Pascucci et al[51-54] studied in PAH^{enu2} mice several aspects of NT synthesis and its effect in cognitive and behavioral development. Biogenic amines (serotonin, dopamine, and noradrenaline) were implicated in the formation and maintenance of synapses during brain postnatal maturation. Their deficits during critical brain developmental phases can produce neurodevelopmental disturbances. They demonstrated deficits of the brain amines levels in pups with PKU between postnatal days 14 and 35. In another study, they evaluated amine release in adult PAH^{enu2} mice in response to a psychogenic stressor, and observed no dopaminergic and serotoninergic responses in prefrontal cortical terminals. Introduction of a Phe-free diet promoted recovery of the serotonin response only, and after administration of 5-hydroxytryptophan a frontal cortical serotonin response was detected. They concluded that high Phe levels interfere with the ability of the mature PFC to respond to psychological challenges. They also showed a deficient tyrosine hydroxylase expression and reduced cortical activity in the medial PFC. Interestingly, low L-DOPA doses restored dopaminergic transmission in the PFC. In a study in different forms of HPA (mild-HPA, mild-PKU, and classic-PKU), demonstrated increasing severity of executive disabilities and brain aminergic deficits in mild-to-severe HPA forms, with the PFC and amygdala as the most affected brain areas. These data confirmed the high susceptibility of brain serotonin metabolism to increased Phe levels.

In 1999, Dyer[28] hypothesized another cause of cognitive abnormalities in patients with PKU. As previously mentioned, in the PAH^{enu2} mouse, moderately elevated Phe concentrations inhibited 3-hydroxy-3-methylglutaryl-coenzyme A reductase, which is a key enzyme for cholesterol biosynthesis. This fact is related to the inability to produce or maintain myelin by the oligodendrocytes, leading to an immature state that secondarily decreased dopamine production in neurons. Joseph and Dyer[55] found in PAH^{enu2} mice a decreased level of dopamine in the PFC cortex and striatum. After the introduction of a low Phe diet, decreased levels of Phe and increased tyrosine values in the blood and brain (frontal cortex and striatum) were detected, leading to an increment in dopamine and basic myelin protein content in different cerebral areas. These findings supported the hypothesis of the role of myelination in the regulation of dopamine synthesis.

Besides alterations in aminergic transmission, alterations in glutamatergic synaptic transmission have also been reported, which could be involved in the cognitive deficits of patients with PKU. Glutamate is the main excitatory NT *par excellence* of the brain cortex and its specific receptors have an important role in the functional and morphologic plasticity of synapses. Martynyuk et al[56] reported that high Phe concentrations in PAH^{enu2} mice depressed glutamatergic synaptic transmission by a combination of presynaptuic and postsynaptic actions, and an attenuation of NT release. Exposure to prolonged periods of high Phe values also caused compensatory changes in glutamatergic synaptic transmission, such as an increase in the expression and density of glutamate receptors.

Neuropsychological Abnormalities Related to Neurotransmission in PKU

Executive functions are frequently affected in patients with early treated PKU. For an adequate acquisition of executive functions, a proper anatomical and functional development of the PFC and related brain areas (basal ganglia, anterior cingulate cortex, and posterior parietal cortex) is required. Therefore, executive functions would be impaired by the dopamine deficiency and by disruptions in the functional connectivity of the PFC.[29] Welsh et al[57] hypothesized that prefrontal dysfunction was a cause of executive deficits in early treated patients.

The relationship between decreased NT biosynthesis and neuropsychological alterations has been described in patients with early treated PKU. Krause et al[58] reported in children and young adults with PKU (6-24 years), most of them early treated, that with blood Phe > 1300 μmol/L, the patients showed prolonged performance times on neuropsychological tests of higher integrative function. They found an inverse correlation between plasma phenylalanine and urine HVA values. Lou and Lykkelund[59-61] confirmed these results when they studied adolescent and young adults, and suggested that bad dietary compliance would lead to decreased NT biosynthesis, thus interfering with cognitive functions. The reaction time variability increased in the vigilance test, when blood Phe levels were higher than 1200 μmol/L and CSF concentrations of HVA and 5-HIAA were low.

Diamond et al[48,62] investigated the mechanisms of cognitive impairment in rat pups. Mild plasma Phe elevations produced impaired on a task dependent on the frontal cortex (delayed alternation), and the reduced levels of HVA observed in the medial PFC were significantly correlated with poor performance in the delayed alternation task. These results showed that slight increase in plasma Phe levels could affect cognitive processes dependent on the PFC, and would also decrease HVA levels in the frontal cortex. Slightly decreased concentrations of cerebral tyrosine may have a significant effect on dopaminergic neurons from the PFC. These neurons are especially sensitive to the decrease of dopamine biosynthesis, probably owing to high dopamine turnover.[63]

A decreased dopamine biosynthesis in the striatum has been reported both in patients with PKU and animal models.[45,48,50,55] As one of the main brain dopaminergic tracts is the nigrostriatal pathway, which is involved in motor planning, it is not surprising that impaired coordination of fine-motor skills is observed in some patients with early treated PKU.[8,64-68]

Deficits of other cognitive functions have been reported, including visual-spatial and visual-constructive disabilities, and impaired sensitivity of visual contrast. The dopaminergic neurons in the retina are also very sensitive to decreased tyrosine availability and would explain in part some of the cognitive skills alteration.[69,70]

WM disturbances may also cause a noticeable effect in fine-motor and visual-spatial skills, and in the slowing processing speed of patients with PKU.[70]

In recent years, a higher prevalence of ADHD in patients with early treated PKU has been reported in the literature, with between 13% and 32% of PKU patients having ADHD (with a higher incidence of predominantly inattentive presentation) when compared with 5% and 7% of the general population.[13,71-75] ADHD is a neurobiological disorder caused by a dysfunction of the PFC and its connections with subcortical regions (basal ganglia and cerebellum) and posterior cortical areas, which also affect executive dysfunction. In this disorder, prevalent symptoms are inattention, hyperactivity, and impulsivity.[76] Children with ADHD and PKU share a common pathophysiological process, as PFC dysfunction secondary to a hypofunction of catecholaminergic circuits, in the PKU because of NT deficit, and in ADHD because of a dysregulation of NT systems.[77]

Executive dysfunction is one of the disorders most commonly reported in patients with early treated PKU; however, there are also other studies focusing on the increased incidence of socioemotional problems in these patients, produced in part by serotonin deficit without forgetting the role it can play the emotional stress associated with any chronic health disorder or secondary to executive dysfunction. Mood disorders (anxiety and depression) are seen more frequently in adolescents and adults, and these symptoms are related with concurrent and lifetime Phe levels, and behavioral disorders in patients who are off diet or with poor metabolic control of the disease.[12,78-85] These problems are beginning to be taken more into account, and in the last management guidelines, follow-up is recommended to improve the emotional state and quality of life, and the cognitive development of these patients.[80,86,87]

Between 1% and 2% of patients with PKU, without or with poor dietary treatment compliance, may have an atypical outcome. They do not display cognitive deficits or have only a very slight intellectual disability. In these cases, in spite of clearly high blood Phe values, brain Phe values remain in a safe range.[88] The most plausible explanation is the presence of allelic variants in the LAT-1 transporter that would impair the Phe transport through the BBB.[1]

Therapeutic Approaches to Improve NT Disturbances

LNAA (tyrosine and tryptophan, as well as other amino acids) was proposed as a complementary treatment to restricted diet, with the rationale of increased cerebral NT concentrations and brain essential amino acid availability.[89] Isolated tyrosine supplementation has been tested with controversial results. Although in some studies, an increment in dopamine biosynthesis and improvement in visual reaction time[60] has been documented, whereas in others no improvement in cognitive functions could be established.[90,91] When supplementation was based on LNAA, a decrease in brain Phe levels was observed,[92-94] together with an improvement in executive functions.[95] The present recommendations can be applied as a therapeutic approach only for adult patients with PKU with poor treatment compliance. In patients with good metabolic control, the benefits of LNNA treatment are probably

limited,[96] and it has not been tested in children younger than 11 years of age.

Pascucci et al,[52] based on their studies of catecholamine metabolism in PAH^{enu2} mice, proposed treatment with low L-DOPA doses together with 5-hydroxytryptophan,[53] with the rationale of increasing the cortical dopamine neurotransmission and improving the frontal cortical serotonin response even in the presence of high Phe levels in the blood and brain. Previously, Ullrich et al[97] did not find improvement in visual evoked potentials and in different neuropsychological tests in untreated adult patients with PKU under L-DOPA therapy.

In a recent study,[98] significantly lower concentrations of urine and blood melatonin (a serotonin metabolite), and urine dopamine, in adult patients with PKU were observed when compared with controls. After supplementation with LNAA, patients demonstrated significantly increased levels of these NTs in blood and urine compared with the placebo group. These results support the statement that blood and urine melatonin monitoring may serve as biomarkers reflecting brain serotonin synthesis in patients with PKU.

Novel therapies for patients with PKU beyond dietary treatment are being tested for decreasing Phe values by different mechanisms. In a recent review,[99-101] these alternative therapies were detailed and included treatment with the cofactor BH4 in adults and paediatric population groups as a chaperone, and the enzyme replacement therapy with phenylalanine ammonia lyase (PAL EC 4.3.1.5) as a potential enzyme substitution therapy for patients with PKU, among others. It has been consistently demonstrated in follow-up studies that BH4 resulted in reductions in the levels of blood phenylalanine, increased intake of dietary phenylalanine, and preserved neurocognitive performance.[4,102] PAL degrades Phe into transcinnamic acid and ammonia and reduces Phe levels, but more research is needed to demonstrate efficacy and safety for patients.

Ormazabal et al[103] found decreased platelet serotonin concentrations in patients with PKU and restored platelet serotonin values by BH4 supplementation. They postulated that this increase might be owing to a direct effect of BH4 on peripheral tryptophan hydroxylase. In 38 children and adults with PKU and ADHD, sapropterin therapy significantly improved inattention symptoms and executive functioning in the first 4 weeks of treatment, and improvements were maintained throughout 26 weeks of treatment.[71] Douglas et al[104] showed a significant increase in HVA after 1 month of sapropterin administration in patients with PKU. Van Vliet et al[105] compared blood prolactin values and blood phenylalanine concentrations among the BH4 responders of patients with PKU. Blood prolactin concentrations positively correlated with blood phenylalanine concentrations, suggesting reduced cerebral dopamine availability, and median blood prolactin concentrations were significantly lower while patients were undergoing therapy, especially at increasing BH4 dose. Denecke et al[106] reported controversial results regarding this issue. Pituitary prolactin secretion is inhibited by dopamine, and it is postulated as a parameter of brain dopamine availability.

In PAH^{enu} mice, the study of monoamine NT turnover has been evaluated after enteral BH4 administration.[107] Sapropterin treatment was associated with a statistically significant increase in brain total biopterin content, but the greatest and most consistent effect was seen only with high doses of sapropterin (100 mg/kg body weight). Saproterin therapy did not alter the dopamine and serotonin status in the brain, but it was associated with increased HVA and 5HIAA concentrations. These results suggest that BH4 therapy may stimulate synaptic NT release and subsequent monoamine metabolism, but the transport of BH4 through BBB is probably limited, and thus these potential effects of BH4 therapy on NT merit further investigation.

Novel pharmacotherapeutic approaches for PKU are being investigated in PAH^{enu2} mice. For example, selected non-physiological amino acids (2-aminoisobutyrate and N-methyl-2-aminoisobutyrate), acting as competitive inhibitors of brain amino acid transporters, may reduce Phe deposit in the brain.[108-110] In PKU^{enu2} mice, gene therapy restores blood Phe levels to a safe range, and locomotor activity, exploratory behavior, and neurogenic amines recovered after liver-targeted gene transfer with recombinant adeno-associated virus vectors. After 8 weeks of gene therapy, aminergic deficits were markedly ameliorated, and catecholamine synthesis was restored to approximately 80%-90% and serotonin synthesis to 60%-70%. A marked recovery was also observed in the brain weight.[111] Improved behavioral phenotype (hyperactivity and reduced digging behavior) and brain NT concentrations were demonstrated after the application of the glycomacropeptide diet.[112] Oral nitisinone therapy has been proposed as an adjunct treatment. Nitisinone is a reversible inhibitor of 4-hydroxyphenylpyruvate dioxygenase (HPD, EC 1.13.11.27), an enzyme in the tyrosine degradation pathway. After administration of nitisinone, pharmacologic inhibition of tyrosine degradation increased blood and brain tyrosine, decreased blood and brain Phe, and increased dopamine and its metabolites in the brain. Brain serotonin content was unchanged.[113]

Conclusions

Several mechanisms are involved in the neuropathophysiology of PKU, primarily characterized by impaired dopamine and serotonin status. These neurotransmission deficits are closely related with executive dysfunctions and social-emotional problems that may appear in patients with PKU, even in early treated cases with good metabolic control. At present, blood Phe values are the main biomarker for follow-up of treatment compliance, as most cases are directly related to brain Phe levels. A major limitation for the monitoring of NT status is the need for CSF to analyse the most informative biochemical parameters. Thus, further investigation and validation of peripheral biomarkers (such as prolactin and melatonin) may be promising to evaluate NT status in PKU. The development of new therapies is effective not only for decreasing blood and brain Phe levels but also to improve NT status.

Acknowledgements

This work was supported by grants from the Spanish Ministerio de Economia y Competitividad FIS PI12/01469 and FEDER Funding Program from the European Union. The Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER) is an initiative of the Instituto de Salud Carlos III.

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11.2. Otras publicaciones en las que ha colaborado la doctoranda:

1. Cassis L, Cortès-Saladelafont E, Molero-Luis M, et al. Review and evaluation of the methodological quality of the existing guidelines and recommendations for inherited neurometabolic disorders [published correction appears in Orphanet J Rare Dis. 2016 Nov 3;11(1):147]. *Orphanet J Rare Dis*. 2015;10:164. Published 2015 Dec 30. doi:10.1186/s13023-015-0376-9
2. Vidal S, Xiol C, Pascual-Alonso A, O'Callaghan M, Pineda M, Armstrong J. Genetic Landscape of Rett Syndrome Spectrum: Improvements and Challenges. *Int J Mol Sci*. 2019;20(16):3925. Published 2019 Aug 12. doi:10.3390/ijms20163925
3. Vidal S, Brandi N, Pacheco P, et al. The utility of Next Generation Sequencing for molecular diagnostics in Rett syndrome. *Sci Rep*. 2017;7(1):12288. Published 2017 Sep 25. doi:10.1038/s41598-017-11620-3
4. Kölker S, Valayannopoulos V, Burlina AB, et al. The phenotypic spectrum of organic acidurias and urea cycle disorders. Part 2: the evolving clinical phenotype [published correction appears in J Inherit Metab Dis. 2015 Nov;38(6):1157-8. Garcia Cazorla, Angeles [corrected to Garcia-Cazorla, Angeles]]. *J Inherit Metab Dis*. 2015;38(6):1059–1074. doi:10.1007/s10545-015-9840-x
5. Chakrapani A, Valayannopoulos V, Segarra NG, et al. Effect of carglumic acid with or without ammonia scavengers on hyperammonaemia in acute decompensation episodes of organic acidurias. *Orphanet J Rare Dis*. 2018;13(1):97. Published 2018 Jun 20. doi:10.1186/s13023-018-0840-4

11.3. Comunicaciones científicas a congresos:

1. Poster: **Comparación de funciones cognitivas entre pacientes PKU de diagnóstico precoz en tratamiento con BH4 Vs pacientes PKU tratados con dieta**. XI Congreso Nacional de Errores Congénitos del Metabolismo. Pamplona 14-16 octubre de

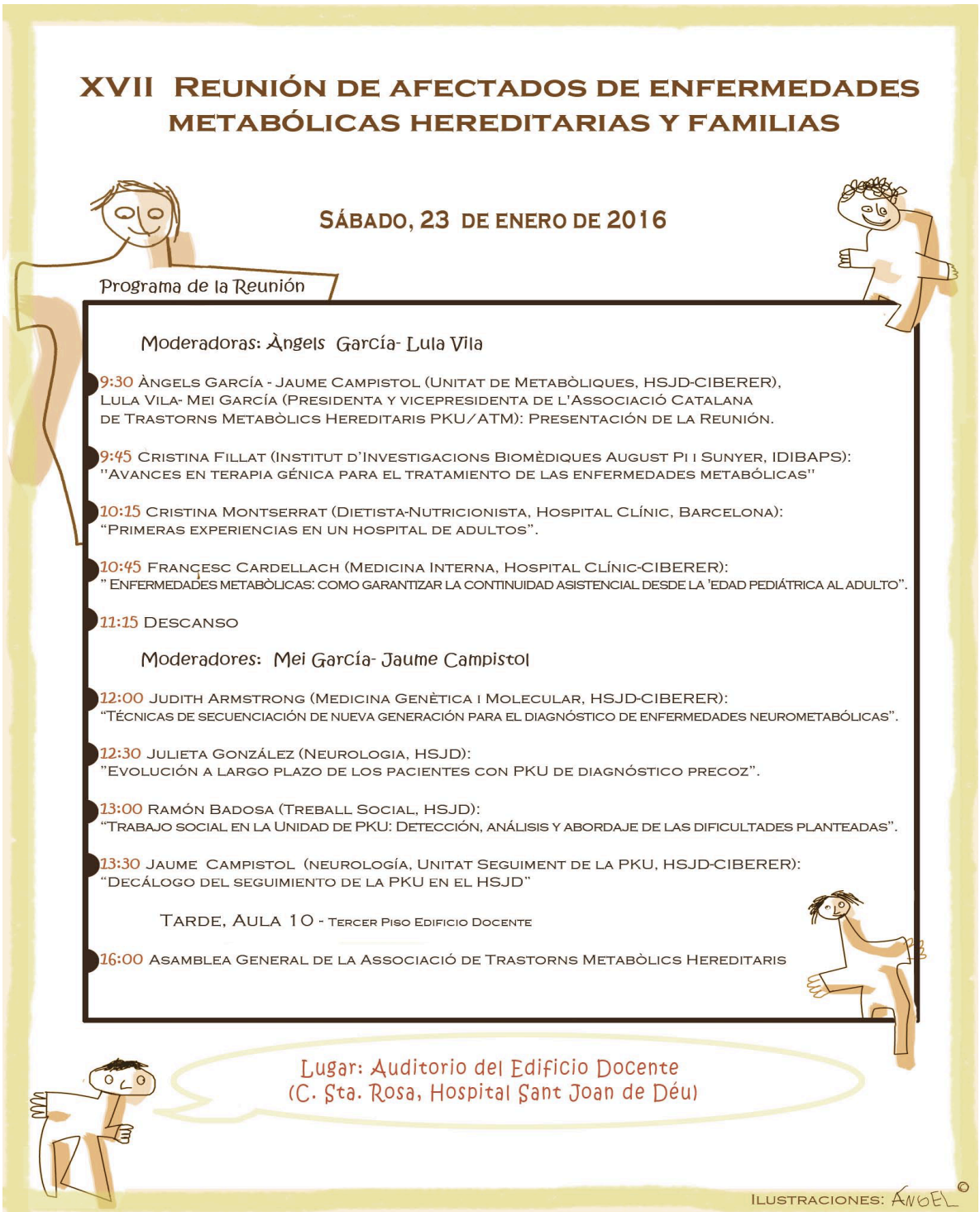
2015. Autores: **González MJ,** R Gassió, R Colomé, R Artuch E Castejón, S Meavilla, J Campistol.

2. Comunicación oral de **Evolución neurológica a largo plazo de población de pacientes fenilcetonúricos de diagnóstico precoz**, Autores: **MJ González**, RGassió, M Tondo, JMuchart, E Fuste, I Alonso Colmenero, N Lambruschini, N Egea, R Artuch, J Campistol. Reunión Científica conjunta de la AINP – SENEP, Valencia España, 10-14 de septiembre de 2013.
3. Poster presentation: **Neurological outcomes in early-treated phenylketonurics adults patients**, **MJ González**, R Gassió, M Tondo, J Muchart, E Fuste, I Alonso Colmenero, N Lambruschini, N Egea, R Artuch, J Campistol, 12th International Congress of Inborn Errors of Metabolism, 2013.
4. Poster presentation: **Health-related quality of life, sleep and behavioural and emotional functioning in early- treated phenylketonuric adults patients**, R Gassió, **González MJ**, R Colomé, O Sans, C Sierra, MA Vilaseca E Castejón, AP Gutierrez, R Artuch, J Campistol, 12th International Congress of Inborn Errors of Metabolism, 2013.
5. Poster presentation: **Epilepsy debut in late diagnosed PKU patients after introduction phenylalanine restricted diet**. **Gonzalez M J**, Itzep D, Lambruschini N, Artuch R, Campistol J. SSIEM 2015, Lyon.
6. Poster: **Comparación de funciones cognitivas entre pacientes PKU de diagnóstico precoz en tratamiento con BH4 Vs pacientes PKU tratados con dieta**. XI Congreso Nacional de Errores Congénitos del Metabolismo. Pamplona 14-16 octubre de 2015. Autores: **González MJ,** R Gassió, R Colomé, R Artuch E Castejón, S Meavilla, J Campistol..
7. Poster: **Estudio de Motricidad fina en pacientes PKU de diagnóstico precoz. XI Congreso Nacional de Errores Congénitos del Metabolismo.** Pamplona 14-16 octubre de 2015. Autores: **González MJ,** R Gassió, R Colomé, A Mesas, C Sierra, E Castejón, S Meavilla, J Campistol.

8. **Comunicación Oral: Evolución neurológica a largo plazo de pacientes Adultos con fenilcetonuria de diagnóstico precoz**. XVII reunión de pacientes afectos de enfermedades metabólicas hereditarias y familias. 23 de enero de 2016. HSJD.
9. Poster: **Sleep study in early treated phenylketonurics patients relationship with melatonin and serotonin status.** R Gassió , **MJ González**, O Sans, R Artuch, C Sierra, A Ormazabal, D Cuadras, J Campistol. EPNS 2017, Lyon, France.
10. Poster**: Advances in early treated Phenylketonuria: the first study with DTI in Spanish pediatric population. M.J. González** M. Rebollo P. Ripolles R.Gassió R. Artuch J. Campistol. EPNS 2017, Lyon, France

11.4. Reunión de pacientes y familiares con PKU y otros errors congénitos del metabolismo

11.4.1. La doctoranda participa como ponente de la charla:"Evolución a largo plazo de los pacientes con PKU de diagnóstico precoz"



XVII REUNIÓN DE AFECTADOS DE ENFERMEDADES METABÓLICAS HEREDITARIAS Y FAMILIAS

SÁBADO, 23 DE ENERO DE 2016

Programa de la Reunión

Moderadoras: Àngels García- Lula Vila

9:30 ÀNGELS GARCÍA - JAUME CAMPISTOL (UNITAT DE METABÒLIQUES, HSJD-CIBERER), LULA VILA- MEI GARCÍA (PRESIDENTA Y VICEPRESIDENTA DE L'ASSOCIACIÓ CATALANA DE TRASTORNS METABÒLICS HEREDITARIS PKU/ATM): PRESENTACIÓN DE LA REUNIÓN.

9:45 CRISTINA FILLAT (INSTITUT D'INVESTIGACIONS BIOMÈDIQUES AUGUST PI I SUNYER, IDIBAPS): "AVANCES EN TERAPIA GÉNICA PARA EL TRATAMIENTO DE LAS ENFERMEDADES METABÓLICAS"

10:15 CRISTINA MONTSERRAT (DIETISTA-NUTRICIONISTA, HOSPITAL CLÍNIC, BARCELONA): "PRIMERAS EXPERIENCIAS EN UN HOSPITAL DE ADULTOS".

10:45 FRANCESC CARDELLACH (MEDICINA INTERNA, HOSPITAL CLÍNIC-CIBERER): " ENFERMEDADES METABÒLICAS: COMO GARANTIZAR LA CONTINUIDAD ASISTENCIAL DESDE LA 'EDAD PEDIÁTRICA AL ADULTO".

11:15 DESCANSO

Moderadores: Mei García- Jaume Campistol

12:00 JUDITH ARMSTRONG (MEDICINA GENÈTICA I MOLECULAR, HSJD-CIBERER): "TÉCNICAS DE SECUENCIACIÓN DE NUEVA GENERACIÓN PARA EL DIAGNÓSTICO DE ENFERMEDADES NEUROMETABÓLICAS".

12:30 JULIETA GONZÁLEZ (NEUROLOGIA, HSJD): "EVOLUCIÓN A LARGO PLAZO DE LOS PACIENTES CON PKU DE DIAGNÓSTICO PRECOZ".

13:00 RAMÓN BADOSA (TREBALL SOCIAL, HSJD): "TRABAJO SOCIAL EN LA UNIDAD DE PKU: DETECCIÓN, ANÁLISIS Y ABORDAJE DE LAS DIFICULTADES PLANTEADAS".

13:30 JAUME CAMPISTOL (NEUROLOGÍA, UNITAT SEGUIMENT DE LA PKU, HSJD-CIBERER): "DECÁLOGO DEL SEGUIMIENTO DE LA PKU EN EL HSJD"

TARDE, AULA 10 - TERCER PISO EDIFICIO DOCENTE

16:00 ASAMBLEA GENERAL DE LA ASSOCIACIÓ DE TRASTORNS METABÒLICS HEREDITARIS

Lugar: Auditorio del Edificio Docente (C. Sta. Rosa, Hospital Sant Joan de Déu)

ILUSTRACIONES: ANGEL ©

11.4.2. La doctoranda participa con la ponencia: ”Resultados del estudio FIS en la PKU: sueño, neuroimagen y neurotransmisores

REUNIÓN PARA PACIENTES Y FAMILIARES
CON PKU Y OTROS ERRORES DEL METABOLISMO

QUERIDOS PACIENTES Y PADRES.
EL PRÓXIMO SÁBADO, 24 DE NOVIEMBRE 2018, HEMOS ORGANIZADO UNA JORNADA FORMATIVO-FESTIVA, DIRIGIDA A LOS PACIENTES Y A SUS PADRES, PARA PRESENTAR ALGUNAS NOVEDADES QUE AFECTAN A LOS TRASTORNOS DEL METABOLISMO. ESTE AÑO, EL PROGRAMA CONTIENE UNA SERIE DE CONFERENCIAS Y TALLERES DIVULGATIVOS SOBRE ESTE TIPO DE ENFERMEDADES EN GENERAL Y ALGUNAS ESPECÍFICAS, ASÍ COMO, DE SU MANEJO MULTIDISCIPLINAR. OS CONVOCAMOS Y PEDIMOS VUESTRA COLABORACIÓN Y PARTICIPACIÓN ACTIVA EN LA JORNADA, A VOSOTROS PADRES Y A VUESTROS HIJOS, EL PRÓXIMO SÁBADO 24 DE NOVIEMBRE 2018 EN EL EDIFICIO DOCENTE DEL HOSPITAL SANT JOAN DE DÉU D'ESPLUGUES DE LLOBREGAT. AGRADECE-MOS AL HOTEL DE ENTIDADES, Y, MUY ESPECIALMENTE, AL HOSPITAL SANT JOAN DE DÉU, POR FACILITAR LA CELEBRACIÓN DE LA JORNADA.

MUY CORDIALMENTE. UNIDAD DE SEGUIMIENTO DE LA PKU Y OTM

PROGRAMA DIRIGIDO A NIÑOS PEQUEÑOS PKU-ATM. *Aula 12*

9.30 -11 H. Cuentacuentos PKU-ATM (Els amics de la Claudia, Que te en Cesc? O La Muriel i el Corb mari)
A. Perayre - MA Vilaseca - HSJD
11-12H. Talleres con voluntarios HSJD. A. Perayre- HSJD
12 H. Talleres para niños con la participación de la familia Abate.
Las mil versiones del pan de Renato y Cristian. Coordina A. Gutiérrez- HSJD

PROGRAMA DIRIGIDO A PADRES, PACIENTES Y FAMILIARES. *Auditorio Edificio Docente*

9.30-11.30 H. Conferencias sobre temas interés general
- Actividad física y metabolopatías. Dra. A García Cazorla -HSJD
- Gestión de las emociones. Sra. Esther García Navarro – Universidad de Barcelona (UB)
- Dieta y gestación en EIM. D-N Cristina Montserrat, Dra. María Forga - HClínic
- Papel de Trabajo Social en una unidad EIM. Sra. Esther Lasheras - HSJD
- Resultados laboratorio en el portal del paciente, como acceder, ventajas, interpretación de resultados.
  Dres. Aida Ormazabal- Rafael Artuch- Cristina Sierra- HSJD
-¿Las ERN i las MetabXUEC que significan? .
  Dra. Angels García Cazorla- HSJD y Dra. Mireia del Toro- HVall d'Hebron

PROGRAMA DIRIGIDO A PADRES, PACIENTES Y FAMILIARES. *Sesiones Paralelas*

11.45-12.45 H. Conferencias específicas, diferentes metabolopatías

PKU *Auditorio edificio docente*

- Pegvaliasa (PEGPAL), una nueva opción de tratamiento de la PKU. Dra. María Forga- HClínic
- Resultados del estudio FIS PKU (JG, RG, JC) sueño, neuroimagen, neurotrasmisores .
  Dres. R.Artuch, MJ.Gonzalez, J. Campistol, R Gassio – HSJD
- Proyectos de futuro. Dr. J Campistol-HSJD

BETA OXIDACION *Aula 10*

- Actitud ante una descompensación .Dra. Mariela de los Santos, DN. Dolores García-Arenas-HSJD
- Afectación Neurológica . Dras Mar O'Callaghan, Antonia Ribes – HSJD, Dra. Mireia del Toro- HVall d'Hebron

CICLO UREA Y ACIDURIAS ORGANICAS *Aula 11*

- Afectación Neurológica. Dra. Angels GarcíaCazorla - HSJD
- Opciones terapéuticas en casos complicados. Dr.Guillem Pintos y Dr.Jesús Quintero – HVall d'Hebron

HIDRATOS DE CARBONO *Sala Nexus*

-Nutrición en los Errores Congénitos que afectan a los Hidratos de Carbono. Dra. Camila García Volpe , D-N Mireia Termes- HSJD

TALLERES COCINA Y ENTREGA DE PREMIOS (13-14H). *Espai La Cuineta, Aulas 11 y 12*

- Las recetas de Tere. Demostración de recetas, Tere Nonnato, chef de la cuineta HSJD. *Espai cuineta*
-Taller de cocina con Anna Grau, chef restaurante Kombu. *Aulas 11-12*
- Entrega de premios Nutricia 2018. *Aulas 11-12*

FIN DE LA JORNADA

14-15H Comida para padres (comedor Hospital , con ticket) y niños (previa inscripción a gastrodietetica@sjdhospitalbarcelona.org)
Coordinado por A.Gutiérrez y N.Egea.
16H. ASAMBLEA DE LA ASOCIACIÓN PKU-ATM *Auditori Edificio Docente*

11.5. Premio

Mejor poster del Congreso Europeo de Neuropediatría 2017 otorgado por la European Paediatric Neurology Society. Advances in early treated Phenylketonuria: the first study with DTI in Spanish pediatric population. M.J. González M. Rebollo P. Ripolles R.Gassió R. Artuch J. Campistol. EPNS 2017, Lyon, France.