UNIVERSITAT DE BARCELONA

Estudio de defectos en el transporte y el metabolismo de tiamina asociados a encefalopatías recurrentes en la infancia

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ESTUDIO DE DEFECTOS EN EL TRANSPORTE Y EL METABOLISMO DE TIAMINA ASOCIADOS A ENCEFALOPATÍAS RECURRENTES EN LA INFANCIA

TESIS DOCTORAL

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Barcelona, 2017

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Memoria presentada por el Licenciado en Medicina **Juan Darío Ortigoza Escobar** para optar por el grado de Doctor en Medicina bajo la dirección de

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Este trabajo ha sido realizado en el Hospital Sant Joan de Déu, Servicio de Neurología Pediátrica de la Universidad de Barcelona. Ha sido posible gracias a una ayuda de postgrado de la Agència de Gestió d'Ajuts Universitaris i de Recerca (2014FI_B 01225).

“La educación es el arma más poderosa que puedes usar para cambiar el mundo”.

Nelson Mandela

A mis padres, Juan y Ana, por dar siempre más de lo que tenían, por enseñarme el legado de la bondad para con otros, el auténtico significado de la felicidad y por preocuparse de que tuviese la mejor educación posible

A mi hermano Juan y mi hermana Laura, por haber sido mis mejores compañeros de infancia y por enseñarme las cosas que realmente importan en la vida

A David por ser un compañero inseparable y por abrirme las puertas de su familia como si fuese la mía

A Jessica, Martín y Fátima por la noble amistad que compartimos

A Belén, por su apoyo y su amistad y por la gran ayuda para llevar a buen puerto esta tesis

A los pacientes y sus familias, por instruirme en que las ganas de vivir y la alegría no deben perderse nunca, incluso en los momentos más devastadores de la vida

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Abreviaturas

BTRBGD	Enfermedad de ganglios basales que responde a tiamina y biotina
CRM	Cadena respiratoria mitocondrial
DNA	Ácido desoxirribonucleico
EII	Encefalopatía hipóxico-isquémica
FLAIR	Fluid attenuated inversion recovery
HPLC	Cromatografía de alta eficacia
LCR	Líquido cefalorraquídeo
MRS	Espectroscopia por resonancia magnética
OMIM	Online Mendelian Inheritance in Man
PCR	Polymerase chain reaction
PDH	Piruvato deshidrogenasa
PTT	Tiamina Trifosfato
RNA	Ácido ribonucleico
RM	Resonancia magnética
hTHTR1	Transportador de tiamina de tipo 1
hTHTR2	Transportador de tiamina de tipo 2
hTDPT	Transportador humano de TDP
hMTPPTR	Transportador mitocondrial de TDP
TDP	Tiamina Difosfato
TMP	Tiamina Monofosfato
TPK	Tiamina pirofosfoquinasa
TRMA	Anemia megaloblástica sensible a tiamina

Introducción

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En esta introducción abordaremos primero el metabolismo y transporte de la tiamina y la acción de sus derivados fosforilados, subrayando las características clínicas, bioquímicas y radiológicas de los defectos genéticos que interfieren en su transporte y metabolismo, con especial énfasis en la afectación del sistema nervioso central.

1. La tiamina o vitamina B1

a. Estructura química

La tiamina es una vitamina hidrosoluble del complejo B (vitamina B1) aislada por primera vez en 1926 [Duran et al., 1985] que está implicada en varios procesos del metabolismo energético cerebral. Es considerada un nutriente esencial dado que los humanos no podemos sintetizarla. Su estructura química consiste en un anillo tiazol y otro de aminopirimidina, unidos por un puente de metileno (C12H17N4OS) (**Figura1**).

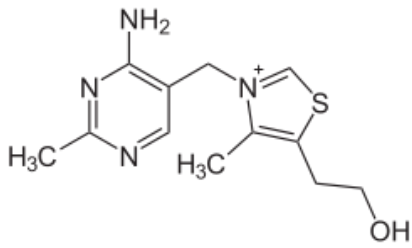

Figura 1. Estructura química de la tiamina

b. Derivados fosforilados

Los derivados de fosfato de tiamina: (Monofosfato de tiamina [TMP], difosfato de tiamina [TDP] - también conocido como un pirofosfato de tiamina [TPP] - y el trifosfato de tiamina [PTT]) están involucrados en múltiples reacciones celulares [Gangolf et al., 2010]. La esterificación con pirofosfato se realiza en la cadena lateral de alcohol del anillo tiazol [Brown, 2014].

2. Metabolismo y transporte de la tiamina

a. Recomendaciones dietéticas diarias

La tiamina se encuentra en una amplia variedad de alimentos a bajas concentraciones, siendo las fuentes dietéticas más importantes: los cereales enteros, la carne y el huevo. Las recomendaciones dietéticas diarias (RDA) de vitamina B1 según la Academia Nacional de Ciencias, varían de 0,2 mg en neonatos a 1-1,2 mg en adultos [Institute of Medicine, 1998].

b. Difusión pasiva y transporte activo

Las isoformas de tiamina o vitámeros, tiamina libre y TMP, se absorben en el intestino delgado por dos transportadores específicos: el transportador de tiamina de tipo 1 (hTHTR1, codificado por el gen *SLC19A2*) y el transportador de tiamina de tipo 2 (hTHTR2, codificado por el gen *SLC19A3*) [Brown, 2014]. En la barrera hematoencefálica y en los plexos coroideos, el hTHTR2 se expresa en los pericitos que rodean endotelial células, mientras que el hTHTR1 se localiza en el lado luminal [Kevelam et al., 2013]. Las fosfatasas intestinales convierten la tiamina de la dieta y sus derivados fosforilados en tiamina-libre. La mucosa del duodeno tiene la tasa más alta de absorción de tiamina [Hoyumpa et al., 1982]. En la absorción de tiamina también participan otros transportadores como: *SLC19A1*, *SLC35F3*, *SLC44A4* y *OCT1* [Zhao et al., 2002; Zhang et al, 2014; Nabokina et al., 2014; Chen et al., 2014], así por ejemplo, la TMP es absorbida principalmente por el transportador *SLC19A1* [Zhao et al., 2002].

La captación de tiamina es dependiente de energía y de temperatura, es sensible al pH (el transporte a través de *SCL19A3* aumenta con el pH con una actividad máxima a pH 7,5) [Rajgopal et al., 2001] y por último, es independiente del sodio. La tiamina se

transporta tanto en eritrocitos como en plasma [Institute of Medicine, 1998]. Además de la absorción por transportadores, hay evidencia de absorción por difusión pasiva [Brown, 2014].

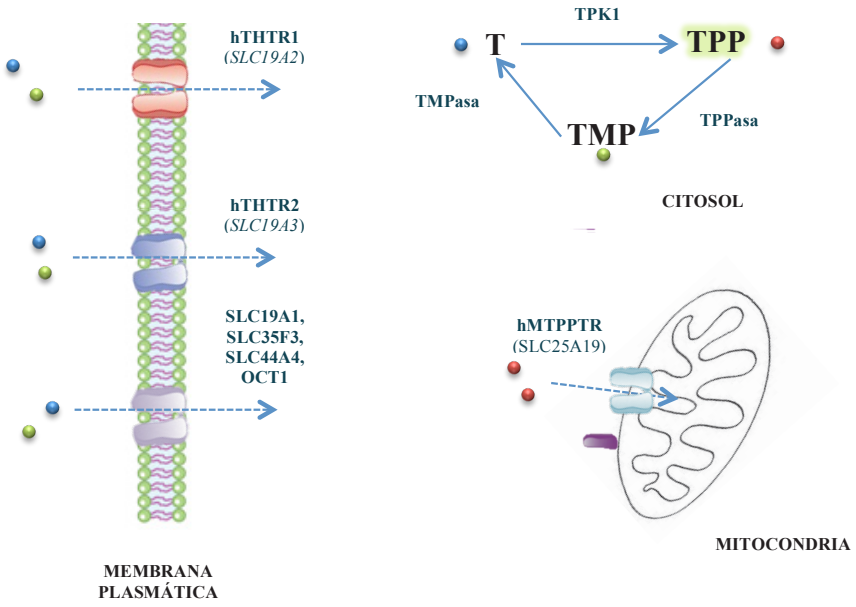

Figura 2. Metabolismo y transporte de la tiamina. A) Las isoformas de tiamina: TMP y tiamina-libre atraviesan la *membrana plasmática* a través de transportadores codificados por *SLC19A1*, *SLC19A2*, *SLC19A3*, *SLC35F3*, *SLC44A4* y *OCT1*. B) En el *citosol*, la tiamina-libre es fosforilada a TPP por la enzima *TPK1*. La TPP puede convertirse en TMP o tiamina libre por acción de la TPPasa y de la TMPasa. C) La TPP ingresa a la *matriz mitocondrial* utilizando el transportador *SLC25A19*.

Dentro de la célula, la tiamina-libre se convierte en TDP por acción de una quinasa citosólica específica (tiamina pirofosfoquinasa, TPK, EC 2.7.4.15, codificada por el gen *TPK1*). A partir de este momento, el transportador mitocondrial de TDP, codificado por el gen *SLC25A19*, permite su captación intramitocondrial. La TDP es un cofactor de varias enzimas: 1) en el citosol: de la transcetolasa (EC, 2.2.1.1), enzima que interviene en la conversión de glucosa a ribosa, la cual es esencial para la síntesis de los ácidos nucleicos, DNA y RNA 2) en los peroxisomas: de la 2-hidroxiacil-CoA liasa (EC, 4.1.2.n2), que interviene en la alfa-oxidación y por último, 3) en la mitocondria: de la

piruvato deshidrogenasa (PDH) (EC, 1.2.4.1); de la 2-oxoglutarato deshidrogenasa (EC, 1.2.4.2), y de la deshidrogenasa de alfa-cetoácidos de cadena ramificada (EC, 1.2.4.4); enzimas que intervienen en la producción de energía del conocido ciclo de Krebs y en el metabolismo de los aminoácidos de cadena ramificada.

3. Defectos genéticos en el transporte y metabolismo de la tiamina: Fenotipos clínicos y diagnóstico diferencial

Se conocen patologías producidas por los defectos genéticos de cuatro genes implicados en el metabolismo y transporte de la tiamina: *SLC19A2*, *SLC19A3*, *SLC25A19*, y *TPK1.,* En 1999, se identificó el primer defecto genético del transporte de tiamina: mutaciones del gen *SLC19A2* que codifica el hTHTR1 en pacientes con anemia megaloblástica sensible a tiamina [Labay et al., 1999; Fleming et al., 1999; Diaz et al., 1999]. Más adelante, Zeng et al., 2005 identificaron un defecto en el hTHTR2, codificado por el gen *SLC19A3* en pacientes con enfermedad de ganglios basales que responde a tiamina y biotina (BTRBGD), previamente descritos por Ozand et al., 1998. Un año más tarde, Lindhurst et al., 2006 describen la microcefalia de tipo Amish causada por mutaciones en el gen *SLC25A19*, que codifica al transportador mitocondrial de TDP. Por último, Mayr et al., 2011 identifican un defecto en tiamina pirofosfoquinasa (*TPK1*) en niños con ataxia, retraso psicomotor, distonía progresiva y acidosis láctica.

En resumen, los fenotipos descritos son los siguientes, con una respuesta variable a la suplementación con tiamina y biotina:

(i) *SLC19A2*, síndrome de anemia megaloblástica sensible a tiamina (TRMA);

(ii) *SLC19A3*, enfermedad de los ganglios basales que responde a tiamina y biotina, síndrome de Leigh, espasmos infantiles con acidosis láctica, y encefalopatía de Wernicke;

(iii) *TPK1*, síndrome de Leigh; y

(iv) *SLC25A19*, microcefalia de tipo Amish o necrosis estriatal bilateral con polineuropatía progresiva.

El síndrome de Leigh está producido por defectos genéticos nucleares y mitocondriales que codifican componentes del sistema de oxidación y fosforilación o del metabolismo del piruvato, causando enfermedades devastadoras con clínica y características radiológicas similares. Entre pacientes con síndrome de Leigh, es de vital importancia pensar siempre en los defectos del transporte y metabolismo de tiamina, puesto que la suplementación con altas dosis de vitaminas puede revertir el fenotipo y estabilizar el metabolismo del paciente previniendo la aparición de futuras recurrencias.

En este trabajo hacemos una revisión de los aspectos clínicos, bioquímicos y las características radiológicas de estos defectos. Realizamos, a continuación, una descripción de todos los casos reportados de cada defecto genético, incluyendo la edad de inicio de la enfermedad, los síntomas clínicos asociados, los datos bioquímicos relevantes y las dosis de suplementación de vitaminas.

3.1. *SLC19A2*

El gen *SLC19A2* se identificó en el año 1999 [Labay et al., 1999; Diaz et al., 1999; Fleming et al., 1999]. El gen está situado en el cromosoma 1q23.3 y contiene seis exones (22,5 kb) que codifica una proteína de 497 aminoácidos (55.400 Da) con 12 dominios transmembrana. El transportador de tiamina tipo 1 (*SLC19A2*) se expresa en una amplia gama de tejidos humanos, incluyendo la médula ósea, hígado, colon, intestino delgado, páncreas, cerebro, retina, corazón, músculo esquelético, riñón, pulmón, placenta, linfocitos y fibroblastos [Thameen et al., 2001; Setoodeh et al., 2013; Tahir et al., 2015]. El síndrome de anemia megaloblástica sensible a tiamina (TRMA)

(OMIM 249270), también conocido como Síndrome de Rogers [Porter et al., 1969] está causado por mutaciones del gen *SLC19A2*. Este síndrome se caracteriza por una tríada de (i) anemia megaloblástica con sideroblastos anillados, (ii) diabetes mellitus no autoinmune, y (iii) sordera neurosensorial de inicio temprano [Banka et al., 2014].

El *SLC19A2* es el único transportador de tiamina conocido en médula ósea, células beta pancreáticas y en el subgrupo de células cocleares ciliares internas; lo que justifica las manifestaciones clínicas de anemia, diabetes y sordera observadas en esta enfermedad [Bergmann et al., 2009]. Los hallazgos cardinales de la tríada pueden aparecer en cualquier momento entre la infancia y la adolescencia. Desde la primera descripción de la enfermedad por Rogers et al. [Porter et al., 1969], se han descrito aproximadamente 80 casos en la literatura. Los elementos de la tríada no se observan en todos los pacientes, así existen dos pacientes que no manifestaron diabetes mellitus [Onal et al., 2012; Lui et al., 2014], cuatro pacientes sin pérdida auditiva [Bergman et al., 2009; Onal et al., 2012; Agliadioglu et al., 2012; Mathews et al., 2009] y un paciente sin anemia.

La diabetes mellitus puede desarrollarse incluso desde el período neonatal [Shaw-Smith et al., 2012] y junto con la anemia, suelen ser las primeras manifestaciones de la enfermedad. Se trata de una diabetes mellitus con deficiencia en la secreción de insulina, por lo que los pacientes suelen requerir la administración de la misma [Onal et al., 2009; Aycan et al., 2011]. Algunos pacientes pueden mantener una concentración péptido-C detectable hasta 24 años después del diagnóstico [Lagarde et al., 2004]. La deficiencia de tiamina causa una reducción en la secreción de enzimas digestivas por parte de las células acinares e intolerancia a la glucosa a causa del deterioro de la síntesis y secreción de insulina por parte de las células beta [Srikrupa et al., 2014].

Se asocian con la enfermedad la anemia megaloblástica o sideroblástica y la anemia aplásica. Todos estos tipos de anemia son sensibles a la suplementación con tiamina. El tipo más común de anemia es la megaloblástica, que se presenta en la infancia y la adolescencia [Liu et al., 2014; Aycan et al., 2011]. La deficiencia intracelular de tiamina afecta al sistema eritropoyético mediante dos mecanismos: (i) la alteración de la síntesis de *novo* de ácidos nucleicos, que está catalizada por la enzima transcetolasa de la vía de la pentosa fosfato, que resulta en un defecto de la división celular y macrocitosis y (ii) la afectación de la alfa-cetoglutarato deshidrogenasa que suministra metabolitos al ciclo de Krebs, que produce la succinil-CoA, que es precursora del grupo hemo, causando una eritropoyesis ineficaz con sideroblastos. El examen de la médula ósea revela anemia megaloblástica con eritroblastos y mitocondrias llenas de hierro formando los llamados sideroblastos en anillo [Srikrupa et al., 2014; Pichler et al., 2012; Beshlawi et al., 2014; Gritli et al., 2001].

El hTHTR1 (*SLC19A2*) es esencial para la función y supervivencia de las células cocleares ciliares internas. Los ratones homocigotos *SLC19A2* -/-, muestran una pérdida completa de las células cocleares ciliares internas y una pérdida parcial de las células cocleares ciliares externas; mientras que los ratones heterocigotos +/- conservan las células cocleares ciliares internas y muestran una pérdida ocasional de células cocleares ciliares externas en la zona del ápex coclear [Srikrupa et al., 2014]. Esto podría explicar por qué los heterocigotos compuestos con mutaciones *missense* pueden presentar pérdida de la audición neurosensorial de inicio tardío o solo estar mínimamente afectados [Bergmann et al., 2009].

Otros síntomas asociados con el defecto del hTHTR1 son: convulsiones, ataxia, retraso del desarrollo psicomotor, ictus, síntomas oculares (retinopatía pigmentaria, anomalías del nervio óptico, distrofia de conos y bastones, y amaurosis congénita de Leber), talla

baja, malformaciones cardíacas congénitas y defectos de conducción cardíaca (fibrilación auricular, comunicación interauricular, anomalía de Ebstein, defecto del relieve endocárdico, arritmia auricular, taquicardia supraventricular), cardiomiopatía, *situs inversus*, criptorquidia, síndrome de ovario poliquístico, tiroiditis inmunitaria, hepatomegalia, reflujo gastroesofágico, nódulos de cuerdas vocales, trombocitopenia y neutropenia [Bergmann et al., 2009; Mikstiene et al., 2015; Akbari et al., 2014; Mozzillo et al., 2013].

En resumen, el diagnóstico de mutaciones en el gen *SLC19A2* debería ser considerado en pacientes con: (i) diabetes mellitus insulino-dependiente no tipo 1 con anticuerpos negativos contra la insulina, antiGAD65, antiIA2 o transglutaminasa y sordera, (ii) anemia megaloblástica refractaria al tratamiento con concentraciones séricas de folato y vitamina B12 normales, (iii) síndrome de Wolfram-*like* (diabetes mellitus, sordera, diabetes insípida, y atrofia óptica) sin confirmación genética (gen *WFS1, CISD2*), (iv) síndrome de Almström-*like* (pérdida progresiva de la visión y la audición, miocardiopatía dilatada, obesidad, diabetes mellitus tipo 2, y estatura baja) sin confirmación genética (gen *ALMS1*), y (v) trastornos mitocondriales, incluyendo los síndromes de Pearson y Kearns-Sayre [Lagarde et al., 2004; Mikstiene et al., 2015].

3.2. *SLC19A3*

El gen *SLC19A3* se encuentra en el cromosoma 2q36.3, contiene cinco exones (32,8 kb) que codifican una proteína de 496 aminoácidos (55665) que se expresa ampliamente en todo el cuerpo, pero más abundantemente en placenta, riñón e hígado. Esta proteína comparte homología de su secuencia aminoácidica con los transportadores *SLC19A1* y *SLC19A2* en un 39% y 48%, respectivamente [Rajgopal et al., 2001].

La deficiencia de hTHTR2 (OMIM 607483) es una enfermedad recesiva causada por mutaciones en el gen *SLC19A3*. Los primeros pacientes fueron descritos por Ozand et al., 1998 en Arabia Saudita. Los pacientes presentan un desarrollo psicomotor normal hasta que desarrollan episodios agudos y recurrentes de encefalopatía, a menudo desencadenados por fiebre, trauma o vacunación. Además de la encefalopatía, asocian distonía, disartria, oftalmoplejía externa, convulsiones y muchos otros síntomas, junto con lesiones simétricas del caudado, putamen, región dorso-medial talámica, diferentes áreas de la corteza cerebral, y, con menor frecuencia del tronco cerebral y del cerebelo [Ozand et al., 1998; Kevelam et al., 2013; Gerards et al., 2013]. Algunos pacientes muestran aumento de biomarcadores de disfunción mitocondrial, como lactato, 2-oxoglutarato o alfa-alanina en fluidos biológicos (sangre y LCR) y pico de lactato en la espectroscopia [Kevelam et al., 2013; Gerards et al., 2013; Serrano et al., 2012; Distelmaier et al., 2014; Haack et al., 2014].

3.3. *SLC25A19*

El gen *SLC25A19* se localiza en el cromosoma 17q25.1 [Rosenberg et al., 2002], contiene nueve exones (16,5 kb) que codifican una proteína de 320 aminoácidos (35.511 Da). Las concentraciones más altas de la proteína se detectan en colon, riñón, pulmón, testículo, bazo y cerebro. La deficiencia de este transportador mitocondrial está asociada a dos fenotipos diferentes: (i) la microcefalia de tipo Amish (OMIM 607196), caracterizada por microcefalia congénita letal que puede ser evidente a partir de las 21 semanas de gestación por ecografía, retraso psicomotor severo, malformaciones del sistema nervioso central (lisencefalia, agenesia parcial del cuerpo calloso, y disrafia de médula espinal), encefalopatía episódica con acidosis láctica y aciduria alfa-cetoglutárica [Kelley et al., 2002; Siu et al., 2010] y (ii) necrosis estriatal bilateral con polineuropatía progresiva (OMIM 613,710), caracterizada por episodios de

encefalopatía recurrente de inicio en la infancia y parálisis flácida, desencadenadas por enfermedades febriles y polineuropatía axonal crónica lentamente progresiva. El perímetro cefálico y el desarrollo psicomotor suelen ser normales en este último fenotipo, a diferencia de los pacientes con microcefalia de tipo Amish [Banka et al., 2014; Spiegel et al., 2009].

En la microcefalia de tipo Amish, se puede observar aciduria alfa-cetoglutárica aunque este marcador es poco sensible durante las crisis metabólicas y puede no estar presente al nacer [Siu et al., 2010]. La acidosis láctica aparece durante las descompensaciones en ambos fenotipos [Kelley et al., 2002; Siu et al., 2010; Spiegel et al., 2009]. El fenotipo bioquímico puede ser atribuible a la actividad disminuida de las tres enzimas mitocondriales que requieren TDP como cofactor: la piruvato deshidrogenasa, la 2-oxoglutarato deshidrogenasa y la deshidrogenasa de alfa-cetoácidos de cadena ramificada.

3.4. *TPK1*

La tiamina pirofosfoquinasa (TPK, EC 2.7.4.15) es una proteína de 243 aminoácidos (27.265 Da; NM_022445.3) codificada por el gen de la *TPK1*, que se localiza en el cromosoma 7q34-q35 y contiene nueve exones (420 kb) [Nosaka et al., 2001; Zhao et al., 2001]. La expresión génica es más elevada en tejidos implicados en la absorción (intestino delgado) y reabsorción (riñón) de tiamina, siendo más baja en los demás tejidos [Zhao et al., 2001].

La primera descripción de esta enfermedad fue realizada por Mayr et al., 2011 quien reportó cinco individuos afectados en tres familias diferentes. Los primeros análisis bioquímicos señalaron la existencia de un posible defecto en la vía de oxidación mitocondrial del piruvato. Sin embargo, el análisis de inmunoblot no mostró cambios en

el contenido de proteínas E1α, E1 β, E2, E3, que son subunidades de la PDH. Más adelante el análisis de mutaciones identificó cambios patológicos en el gen *TPK1*, lo que condujo al diagnóstico final de estos pacientes.

En 2014, se describieron dos hermanos chinos homocigotos para una nueva mutación *missense* (c.604T> G) en el gen *TPK1* por Fraser et al., 2014. Al mismo tiempo, Banka et al., 2014 describió a dos nuevos pacientes con dos nuevas mutaciones c.479C>T y c.664G>C con afectación de residuos altamente conservados del gen *TPK1*. Actualmente, se han descrito 10 mutaciones diferentes en este gen.

Clínicamente, la sintomatología inicial de estos pacientes se evidencia entre los 18 meses y 4 años de edad, por lo general después de un período de desarrollo psicomotor normal o sólo levemente alterado. Las características comunes de estos pacientes incluyen ataxia episódica, regresión psicomotriz, distonía y espasticidad. Durante las crisis metabólicas los estudios bioquímicos revelaron concentraciones elevadas de lactato en sangre en cinco de nueve pacientes y en LCR, en tres de nueve pacientes. También se observó aumento de la excreción de alfa-cetoglutarato en orina en ocho de nueve pacientes [Banka et al., 2014; Mayr et al., 2011; Fraser et al., 2014]. Se ha propuesto la detección de alfa-cetoglutarato en orina como un posible biomarcador para esta enfermedad.

3.5. *SLC35F3*

En 2014, un transportador previamente insospechado de tiamina (*SLC35F3*) fue descrito como un nuevo locus de susceptibilidad para la hipertensión arterial [Zhang et al., 2014]. El gen *SLC35F3* está localizado en el cromosoma 1q42.2 y contiene siete exones (419 kb) que codifican una proteína de 421 aminoácidos (46.817 Da). Esta proteína se

expresa ampliamente en el organismo. Hasta ahora, no se han reportado pacientes con mutaciones en este gen, por lo que el fenotipo clínico todavía es desconocido.

3.6. *SLC44A4*

El transportador humano de TDP (hTDPT; producto del gen *SLC44A4*) es responsable de la absorción de TDP generado en el intestino grueso por la microbiota. El gen *SLC44A4* se encuentra en el cromosoma 6p21.33 (15,8 kb), y codifica una proteína de 710 aminoácidos (79.254 Da). El hTDPT se expresa exclusivamente en el colon, y se localiza en el dominio de la membrana apical del epitelio colónico [Nabokina et al., 2016]. No se ha detectado expresión en SNC y, hasta la fecha, no se ha reportado ningún paciente con mutación de este transportador.

4. Suplementación con tiamina

4.a. Eficacia y seguridad

4.a.1. Pacientes con deficiencia de SLC19A2

Hay más de 46 mutaciones diferentes descritas en pacientes con TRMA [Manimaran et al., 2016]. La mayoría de las mutaciones son *nonsense* o *frameshit* y se encuentran en el exón 2, mientras que sólo unas pocas son de tipo *missense* [Mikstiene et al., 2015]. Estas mutaciones conducen a la ausencia de síntesis proteica y por lo tanto a la deficiencia completa del hTHTR1. Algunos autores han demostrado que la captación específica en las células mutantes hTHTR1 varía entre el 2% [Manimaran et al., 2016] y el 3% [Gritli et al., 2001]. A pesar de estas observaciones, la suplementación de tiamina en los pacientes con mutación del gen *SLC19A2* produce una mejoría exitosa de los síntomas, consiguiendo un control adecuado de la anemia y la glucemia y, de ese modo, prolonga significativamente la esperanza de vida de estos pacientes [Mikstiene et al.,

2015]. La suplementación con dosis altas de tiamina ralentiza la aparición de la diabetes [Aycan et al., 2011] y disminuye la necesidad de insulina [Tahir et al., 2015]. Sin embargo, y a pesar de esta suplementación se produce una progresión lenta de la insuficiencia de células beta pancreática, y los pacientes se vuelven dependientes de insulina a partir de la adolescencia [Valerio et al., 1998; Ricketts et al., 2006]. La retirada de la suplementación con tiamina induce a un aumento de las necesidades de insulina [Borgna-Pignatti et al., 1989] o cetoacidosis diabética [Kurtoglu et al., 2008]. También existe una respuesta hematopoyética casi inmediata tras la suplementación con tiamina. La respuesta completa de la anemia se observa 1 o 2 meses después del inicio de la suplementación con tiamina, mientras que la trombocitopenia y la neutropenia mejoran en las primeras semanas. Esta respuesta es sostenida hasta la adolescencia o la edad adulta, cuando los pacientes requieren nuevamente de transfusiones sanguíneas [Ricketts et al., 2006]. Los eritrocitos permanecen macrocíticos [Agladioglu et al., 2012] e incluso existen sideroblastos en anillos hasta 2 años después del inicio de la suplementación con tiamina [Gritli et al., 2001]. La anemia reaparece cuando se retira la suplementación con tiamina [Viana et al., 1978; Borgna-Pignatti et al., 1989].

La administración de suplementos de tiamina no impide la pérdida de la audición neurosensorial en niños [Akin et al., 2011]. En modelos experimentales de ratones SLC19A2 -/-, la suplementación con tiamina no restaura la función auditiva [Liberman et al., 2006]. Stagg et al., 1999 postula que una explicación de este hallazgo sea que los requerimientos de tiamina de las células cocleares sean más altos que la de los fibroblastos. Los pacientes se benefician con implantes cocleares, mejorando su percepción y el habla [Hagr et al., 2014; Mikstiene et al., 2015].

La administración de tiamina no mejora la talla baja [Setoodeh et al., 2013] o las manifestaciones neurológicas [Akbari et al., 2014]. En cuanto a las manifestaciones

psiquiátricas, la administración de tiamina mejora el trastorno de conducta explosiva y la agresividad, pero no los trastornos del estado de ánimo o las ideaciones paranoides [Wood et al., 2014].

4.a.2. Pacientes con deficiencia de SLC19A3

Se han descrito más de 22 mutaciones diferentes en pacientes con defectos del hTHTR2 (*SLC19A3*). En la primera descripción de la enfermedad, Ozand et al., 1998 reportó una buena respuesta terapéutica de los pacientes suplementados con biotina (5 mg/kg/día). Más tarde, cuando Zeng et al., 2005 descubrió que la proteína codificada por el gen *SLC19A3* es un transportador de tiamina, se indicó la suplementación con tiamina de estos pacientes. Hasta la fecha, hay pruebas abrumadoras de que la administración temprana de tiamina puede revertir las anomalías clínicas y radiológicas y así mejorar el pronóstico neurológico [Serrano et al., 2012; Distelmaier et al., 2014; Haack et al., 2004M; Kono et al., 2009; Debs et al., 2010; Alfadhel et al., 2013; Pérez-Dueñas et al., 2013; Tabarki et al., 2013; Brown et al., 2014]. Si estos pacientes no reciben tratamiento o si se inicia tardíamente, los pacientes pueden presentar discapacidad cognitiva severa o incluso morir [Gerards et al., 2013; Kohrogi et al., 2015]. Muchos autores describen una respuesta dramática a la administración de tiamina, con una mejoría completa dentro de pocas horas o días. La administración de suplementos de tiamina restaura los niveles de tiamina en LCR e intracelular, tal como se ha comprobado en esta tesis.

La mayoría de los autores prefieren la administración oral, pero en pacientes graves, con alteración del nivel de conciencia es más útil la administración intravenosa [Debs et al., 2010]. Las dosis de tiamina son muy variables pero, generalmente se encuentran dentro del rango de 10-40 mg/kg/día. La dosis máxima terapéutica reportada es de 1500mg/día. Se han descrito descompensaciones metabólicas tras 30 días de la retirada

de tiamina en pacientes con déficit de SLC19A3 [Kono et al., 2009]. La suplementación con tiamina evita en la mayoría de casos nuevas descompensaciones y la progresión de la enfermedad [Distelmaier et al., 2014].

Una vez más, el mecanismo biológico que explica la eficacia de la suplementación con tiamina en pacientes con defectos del gen *SLC19A3*, es incierto. Es posible que los pacientes con mutaciones *missense*, con transportadores con alguna función residual sean capaces de transportar tiamina en condiciones de aumento de las concentraciones plasmáticas. Por otra parte, en pacientes con mutaciones con pérdida de función del transportador, la absorción de tiamina probablemente se compensa por el aumento de los transportadores alternativos. Como he mencionado, existen otros transportadores de tiamina descritos como el transportador de folato reducido (RFC1, también conocido como *SLC19A1*), o el transportador de cationes orgánicos (OCT1), recientemente identificado. Estos transportadores podrían contribuir a la absorción de tiamina y, por lo tanto, compensar el transporte en pacientes con deficiencia de hTHTR2.

La administración de biotina en la deficiencia de *SLC19A3* es controversial. Unos pocos pacientes reportados recientemente tratados solo con biotina no mejoraron [Yamada et al., 2010; van der Knaap et al., 2014], a diferencia de los pacientes descritos inicialmente por Ozand et al., 1998 y Debs et al., 2010. Por otra parte, un tercio de los pacientes en el estudio de Alfadhel et al., 2013 demostraron una recurrencia de descompensaciones, cuando recibían suplementación solo con biotina.

Así, Tabarki et al., 2015 compararon la combinación de biotina y tiamina (Grupo 1) vs. la administración de solo tiamina (Grupo 2) en una serie de pacientes con la mutación c.1264A>G en homocigosis del gen *SLC19A3*. Las dosis media de biotina y tiamina en este estudio fueron 5 y 40 mg/kg/día, respectivamente. Se observó una recuperación

más rápida de las descompensaciones agudas en el Grupo 1 (2 días; 1,80±0,63) vs. el Grupo 2 (3 días; 2,90±0,87; p=0,005). Sin embargo, la combinación de biotina y tiamina no fue superior a solo tiamina en cuanto al número de recurrencias, las secuelas neurológicas, o los cambios en la RM en los siguientes 30 días de seguimiento. Dos limitaciones principales de este estudio fueron el seguimiento a corto plazo y el número reducido de pacientes incluidos. Estos autores recomiendan el uso de la combinación de biotina y tiamina en las descompensaciones agudas para facilitar una recuperación más rápida y tiamina sola en el tratamiento a largo plazo.

Las dosis de biotina reportadas varían de 1-5 mg/kg/día [Ozand et al., 1998] a 5-10 mg/día [Serrano et al., 2012; Pérez-Dueñas et al., 2013], siendo las dosis máximas reportadas de hasta 600 mg/día [Debs et al., 2010]. No se conoce con certeza el mecanismo por el que la biotina puede contribuir a la estabilidad metabólica de estos pacientes. En ese sentido se han planteado varias hipótesis: la biotina puede aumentar la expresión de los niveles de hTHTR2 que aún conserven alguna actividad residual [Haack et al., 2014]. Esto podría explicar las diferencias de respuesta a la biotina en estos pacientes, dependiendo del tipo de mutación, y porque no es eficaz en el caso de mutaciones sin sentido. La biotina es un cofactor esencial para un número de enzimas que participan en el metabolismo energético mitocondrial, incluyendo la varias carboxilasas: piruvato carboxilasa, propionil-CoA carboxilasa y 3-metilcrotonil-CoA carboxilasa [Brown et al., 2014]. Finalmente, las dosis altas de biotina permiten al piruvato pasar por alto el ciclo de Krebs mediante el uso de la piruvato carboxilasa, que produce oxaloacetato, un sustrato de este ciclo en lugar de acetil-CoA [Kohrogi et al., 2015]. Se ha descrito el uso de varios fármacos antiepilépticos para el tratamiento de las convulsiones de estos pacientes. También se ha descrito el uso de trihexifenidilo y diazepam intravenoso para el tratamiento del status distónico [Serrano et al., 2012].

4.a.3. Suplementación de tiamina en pacientes con deficiencia de *SLC25A19*

El fenotipo de microcefalia de tipo Amish está asociada a la mutación c.530G>C en homocigosis [Kelley et al., 2002; Siu et al., 2010] del gen SLC25A19, mientras que el fenotipo de necrosis estriatal bilateral con polineuropatía está producido por la mutación c.373G>A en homocigosis [Spiegel et al., 2009]. Los pacientes con el primer fenotipo muestran una mejoría de la hiperlactacidemia observada durante las crisis metabólica si se administra dieta cetogénica. El retraso del desarrollo psicomotor y la microcefalia no responden a la suplementación con tiamina [Siu et al., 2010]. Los pacientes con el fenotipo de necrosis estriatal bilateral con polineuropatía presentan disminución de la frecuencia de las crisis encefalopáticas tras la suplementación con tiamina, como se ha podido comprobar en los resultados de esta tesis.

4.a.4. Suplementación de tiamina en pacientes con deficiencia de TPK1

En el primer estudio que describe a pacientes con deficiencia de TPK, Mayr et al., 2011 suplementó con 100-200mg/día de tiamina a tres de sus cinco pacientes. Dos de ellos se estabilizaron e incluso mostraron una ligera mejoría clínica. Uno de estos pacientes presentó con el tiempo un desarrollo psicomotor normal. El tercer paciente tratado no presentó mejoría tras 2 años de suplementación. Con estos datos, Mayr et al., 2011 llegaron a la conclusión de que los suplementos de tiamina deben ser aumentados para ajustar la concentración favoreciendo la actividad residual TPK y evitando el agotamiento de esta vitamina. Por otra parte, en su trabajo han descrito que las concentraciones de TDP alcanzaron valores normales en fibroblastos de un paciente tras la suplementación del medio de crecimiento con 10,7 mmol/l de tiamina.

Fraser et al., 2014 describió a dos hermanos con deficiencia de TPK. El primer hijo de la familia murió a la edad de 29 meses. Su diagnóstico no se hizo hasta después de que

el hermano fuera diagnosticado a los 18 meses. Este paciente manifestó regresión neurológica. Tras el diagnóstico, se trató con dieta cetogénica, suplementos de tiamina (10 mg/kg/d, en 3 dosis), biotina (5 mg, 3 veces/día) y ácido α-lipoico (5 mg/kg/d, en 3 dosis). La dieta cetogénica (Ketocal 3:1) administrada a través de sonda nasogástrica se inició para reducir la demanda metabólica a través de la PDH. Este es el primer estudio que reportan los beneficios de la dieta cetogénica junto con suplementación de vitaminas en un paciente que presentación neurológica grave.

En paralelo, Banka et al., 2014 trató a sus pacientes con 500 mg de clorhidrato de tiamina. Sólo uno de ellos, de 8 años de edad, respondió al tratamiento. Este paciente no volvió a presentar episodios de encefalopatía, incluso durante episodios febriles, mostrando una lenta pero gradual mejoría del desarrollo psicomotor, la comprensión, la interacción social, las habilidades lingüísticas y las habilidades motoras. Asimismo, por mejoría de la deglución, pudo retirarse la sonda nasogástrica. Actualmente este paciente asiste a la escuela con refuerzo escolar. Su vocabulario es reducido y tiene espasticidad en las extremidades inferiores. El otro paciente de esta cohorte tratado presentaba una afectación severa al diagnóstico a 7 años de edad. El control cefálico era pobre y no presentaba sedestación. En su caso, la suplementación de tiamina fue ineficaz.

En conjunto, estos resultados muestran algunos pacientes con deficiencia de TPK responden a la suplementación con tiamina, lo que convierte a esta enfermedad en un error innato del metabolismo potencialmente tratable. Los pacientes con un diagnóstico tardío no responden a la suplementación de tiamina, haciendo hincapié en la importancia del diagnóstico y tratamiento precoz para obtener mejores resultados.

4.b. Efectos adversos de la suplementación con tiamina

Se ha informado de anafilaxia durante la administración intravenosa de tiamina, sin que los mecanismos de este efecto secundario sean conocidos [Morinville et al., 1998; Stephen et al., 1992; Brown et al., 2014].

Existe un reporte sobre la seguridad del clorhidrato de tiamina en administración IV (bolo 100 mg) en 989 pacientes consecutivos (1070 dosis) [Wrenn et al., 1989]. En este trabajo, se notificaron un total de 12 (1,1%) reacciones adversas, consistentes en 11 reacciones leves (irritación local transitoria) y un efecto adverso mayor que consistió en prurito generalizado. Los estudios farmacocinéticos de suplementación de tiamina muestran que aunque se desconoce las dosis óptimas para el tratamiento beneficioso con tiamina, altas dosis (de hasta 3000 mg) administradas por periodos extensos no tienen ningún efecto perjudicial. Por otra parte, se ha descrito que el mecanismo de absorción, regulado por una combinación de un transportador activo y pasivo, no es saturable hasta 1500 mg [Smithline et al., 2012].

No se ha estudiado la toxicidad de la biotina en humanos sanos [Sawamura et al., 2007]. En un estudio de 20 pacientes con esclerosis múltiple tratados con dosis altas de biotina (100 a 300 mg/día), solamente se observó diarrea transitoria como efecto secundario en dos pacientes [Sedel et al., 2015]. Sawamura et al., 2007 reportaron que la ingesta alimentaria de altas dosis de biotina inhibe la espermatogénesis en ratas jóvenes [Sawamura et al., 2015] y que no había efectos adversos en dosis equivalentes a 38,4 mg/kg/día en estas ratas [Sawamura et al., 2007].

4.c. Dosificación y duración de la suplementación con tiamina

El tratamiento de pacientes con defectos en el transporte y metabolismo de la tiamina se centra en la suplementación de por vida con tiamina y biotina. Las dosis de tiamina varían de acuerdo al defecto genético: 1) *SLC19A2*, la dosis habitual es de 25-200

mg/día (aproximadamente 1-4 mg/kg/día); 2) *SLC19A3*, 10-40 mg/kg/día, 3) *SLC25A19*, 400 mg/día y para 4) *TPK1*, 30 mg/kg/día. En el caso de *SLC19A2* y *SLC19A3*, la dosis que produce efectos beneficiosos no está definida. La distinta afinidad de los transportadores alternativos a la tiamina pueden explicar en parte estas diferencias. En los pacientes con un defecto en la fosforilación citoplasmática por la enzima (*TPK1*), se recomiendan dosis más altas de tiamina para forzar la actividad residual de la TPK.

Con respecto a la biotina, se recomienda el tratamiento en defectos genéticos de *SLC19A3* y *TPK1*. Las dosis varían de acuerdo con el momento de la enfermedad en el que se indican (episodio agudo o tratamiento crónico) y el tipo de mutación.

4.d. Monitorización durante la suplementación de tiamina

4.d.1. Monitorización durante la suplementación de tiamina en pacientes con deficiencia de *SLC19A2* y *SLC19A3*

Los pacientes con defectos en los transportadores hTHTR1 (*SLC19A2*) y hTHTR2 (*SLC19A3*) muestran niveles plasmáticos normales de tiamina, lo que sugiere que los transportadores alternativos compensan la absorción intestinal de la tiamina [Brown et al., 2014]. Por lo tanto, la cuantificación de tiamina plasmática no es un biomarcador útil para el diagnóstico de estos defectos.

El reto principal del tratamiento de los pacientes con defectos en el transportador *SLC19A3* y sintomatología neurológica es restaurar la concentración de tiamina en el SNC. En ese sentido, la cuantificación de las isoformas de tiamina en el LCR se correlaciona con la concentración de tiamina a nivel del cerebro. Uno de los objetivos de esta tesis es la comprobación de los cambios bioquímicos se producen tras la suplementación con tiamina.

4.d.2. Monitorización durante la suplementación de tiamina en pacientes con deficiencia de *TPK1*

No existe información sobre el seguimiento y monitorización de pacientes con defectos de TPK hasta la realización de esta tesis.

4.d.3. Monitorización durante la suplementación de tiamina en pacientes con deficiencia de *SLC25A19*

Desde un punto de vista bioquímico, durante el seguimiento de estos los pacientes se constata la normalización de la acidosis láctica y de los ácidos orgánicos en orina. Previamente, no existían estudios sobre el seguimiento clínico de estos pacientes hasta la realización de esta tesis.

5. La tiamina y sus isoformas como biomarcadores de estos defectos.

Mayr et al., 2011 estudió a cinco individuos de tres familias con ataxia, retraso psicomotor, distonía progresiva y acidosis láctica. La investigación del metabolismo energético mitocondrial mostró una reducción de la oxidación de piruvato, pero con actividad normal de PDH en presencia de exceso de TPP. Este autor describe una concentración reducida de TPP en el músculo y la sangre, y propone su utilización como biomarcador de la enfermedad. Más tarde, Banka et al., 2014 presenta dos pacientes con nuevas mutaciones en homocigosis del gen *TPK1* (paciente 1, p.Ser160Leu y paciente 2, p.Asp222His). El paciente 2 de este trabajo presentó un síndrome de Leigh junto con un retraso global del desarrollo. En este trabajo se realizaron ensayos para la medir de la actividad enzimática de la tiamina pirofosfoquinasa y se realizó la cuantificación de TPP en músculo y sangre congelados confirmando que TPP podría utilizarse como biomarcador de esta enfermedad.

En pacientes con deficiencia de *SLC19A2*, solo existen reportes aislados de medición de la concentración de tiamina y sus isoformas en sangre. Todas estas determinaciones se han realizado en pacientes turcos con fenotipo de anemia megaloblástica sensible a tiamina. Estos reportes son de Ozdemir et al., 2002 que describe concentraciones bajas de tiamina total y de TMP en 1 paciente con inicio de síntomas a los 5 años de edad; Yesilkaya et al., 2008 que reportan una leve disminución de la tiamina en sangre (22 ug/dl para valores de referencia: 25 – 75 ug/dl) en un paciente con inicio de síntomas a los 2 años de edad; Aycan et al., 2011 que reporta valores de tiamina total de 20 g/dL (valor de referencia 25-75 g/dl) en un niño con inicio de síntomas a los 4 meses de vida y finalmente Agladioglu et al., 2012 que informa de niveles levemente disminuidos de tiamina de 19.5 g/dl (valor de referencia 25-75 g/dl) en un paciente con inicio de síntomas a los 2 años de edad.

Justificación de la Unidad Temática

En el conjunto de trabajos que forman parte de esta tesis doctoral, hemos intentado caracterizar el espectro fenotípico y genotípico de los pacientes con deficiencia de los genes *SLC19A2*, *SLC19A3*, *TPK1* y *SLC25A19*, implicados en el transporte y metabolismo de tiamina.

Para ello, nos propusimos realizar una revisión bibliográfica extensa de todos los pacientes reportados hasta la fecha. Con ello, sentamos las bases para que se conociesen mejor las manifestaciones clínicas y radiológicas de estos pacientes, con especial interés en el defecto del gen *SLC19A3,* ya que es el defecto más frecuente y el que mejor responde a la suplementación con vitaminas. Precisamente, nos propusimos averiguar sobre la eficacia y la seguridad reportadas de la suplementación con biotina y tiamina, los efectos adversos de la suplementación, así como la dosificación y el seguimiento de estos pacientes durante el tratamiento.

En general, la literatura publicada previamente sugiere un claro beneficio de la suplementación temprana con tiamina y biotina, con resultados menos eficaces cuando estas se administran a pacientes con afectación severa o de forma tardía. Por ello, uno de nuestros objetivos ha sido el de desarrollar un biomarcador que nos permitiera el diagnóstico precoz de pacientes con deficiencia de hTHTR2 antes incluso de los estudios genéticos. De este modo, analizamos las isoformas de tiamina mediante cromatografía líquida de alto rendimiento en sangre total y LCR de controles pediátricos, pacientes con síndrome de Leigh, pacientes con otros trastornos neurológico y, finalmente, en pacientes con mutación del gen *SLC19A3*, en quienes también hemos realizado estudios en fibroblastos. Asimismo, la determinación de isoformas de tiamina en sangre total también puede ser útil para monitorizar el

tratamiento de estos pacientes, dado que no siempre puede asegurarse una adherencia correcta.

Nuestro siguiente objetivo fue el de describir la historia natural de los defectos genéticos del transporte y metabolismo de la tiamina en la mayor cohorte panétnica de pacientes recopilados hasta la fecha. Específicamente, nos propusimos demostrar como el tratamiento con vitaminas modifica la historia natural de estos defectos. Para ello realizamos un análisis sistemático de las características clínicas y radiológicas, y de las anormalidades bioquímicas en el debut de la enfermedad. También investigamos nuevos factores pronóstico de evolución a largo plazo. Por último, comparamos las curvas de supervivencia de pacientes no tratados y de pacientes tratados con vitaminas, y de pacientes tratados con otras causas de síndrome de Leigh.

Las causas genéticas del síndrome de Leigh son heterogéneas, con una pobre correlación genotipo-fenotipo. Por lo tanto, otro objetivos de esta tesis ha sido el de establecer el diagnóstico diferencial con otras causas de Síndrome de Leigh. De este modo, comparamos los hallazgos de pacientes con mutaciones en *SLC19A3* con pacientes reportados con defectos del complejo I mitocondrial, la causa más frecuente de síndrome de Leigh y en especial con el defecto *NDUFS4*.

En resumen, en esta tesis se ha realizado un estudio exhaustivo de los aspectos clínicos, bioquímicos, radiológicos y genéticos de pacientes con deficiencias de *SLC19A2*, *SLC19A3*, *TPK1* y *SLC25A19*, implicados en el transporte y metabolismo de tiamina, de la modificación de la historia natural de estos defectos tras la suplementación con vitaminas y, en consecuencia, del mejor pronóstico en comparación con otros defectos genéticos asociados con el síndrome de Leigh. También hemos establecido las bases para la monitorización terapéutica. Como consecuencia, los resultados y conclusiones

de esta tesis doctoral demuestran cómo la investigación coordinada clínica y básica puede trascender en la mejoría de la salud, y ser la base de la medicina translacional actual.

Hipótesis de trabajo

La hipótesis de esta tesis doctoral postula que los defectos del transporte y metabolismo de la tiamina corresponden a errores innatos del metabolismo que se benefician de la suplementación precoz con vitaminas. La determinación de isoformas de tiamina en sangre, LCR y fibroblastos demostrará que la deficiencia es mayor a nivel del propio sistema nervioso central, produciendo sintomatología predominantemente neurológica. La diferente concentración de isoformas en estos compartimentos permitirá el diagnóstico definido de los pacientes y ayudará a la monitorización durante el tratamiento. Hipotéticamente, la suplementación con vitaminas modifica la historia natural de estos pacientes, a pesar de que existen factores diferenciales genéticos (mutación) o radiológicos (mayor extensión de las lesiones cerebrales) que explican la respuesta diferente a la suplementación con vitaminas. El conocimiento de otras enfermedades que se manifiestan con el fenotipo de Síndrome de Leigh es fundamental para establecer el diagnóstico diferencial con la deficiencia de tiamina.

Objetivos

El objetivo principal de esta tesis ha sido el de profundizar en el conocimiento del metabolismo y transporte de la tiamina y en los estados patológicos ocasionados por las alteraciones genéticas que condicionan su deficiencia y, muy especialmente en los cuadros de encefalopatía producidos por mutaciones del gen *SLC19A3*.

Con esto, se marcaron los siguientes objetivos específicos:

1. Caracterizar el espectro fenotípico y genotípico de pacientes con deficiencia de los genes *SLC19A2*, *SLC19A3*, *TPK1* y *SLC25A19*.
2. Proporcionar predictores clínicos de supervivencia y describir la historia natural de la enfermedad así como estimar su modificación tras la suplementación con vitaminas.
3. Comparar estos hallazgos con el de pacientes reportados con defectos del complejo I mitocondrial, la causa más frecuente de síndrome de Leigh y en especial con el defecto *NDUFS4*. Además, discutir los posibles indicios clínicos y radiológicos para la distinción de pacientes con mutaciones en el gen *SLC19A3* de otras causas de síndrome de Leigh.
4. Establecer los valores de referencia para las isoformas de tiamina en sangre, LCR y fibroblastos, su utilidad como biomarcador para el diagnóstico precoz en los defectos genéticos del transporte y metabolismo de tiamina así como establecer causas de deficiencia secundaria de tiamina en LCR en pacientes con síndrome de Leigh y otras patologías neurológicas genéticas o adquiridas
5. Puntualizar la utilidad de la determinación de las concentraciones de tiamina en sangre total para la monitorización de estos pacientes con suplementación de tiamina.

Con el fin de facilitar la organización y la compresión esta tesis, los apartados de materiales y métodos, así como la presentación de los artículos y la discusión se detallaran siguiendo el orden de los objetivos específicos.

Materiales y métodos

1) Sujetos de Estudio
   a) Pacientes
   b) Controles

2) Aspectos éticos

3) Materiales y métodos
   (1) Revisión de la literatura
   (2) Estudios bioquímicos en sangre, LCR y fibroblastos
      (a) Fibroblastos y condiciones de cultivo
      (b) Análisis por HPLC de isoformas de tiamina
   (3) Estudios funcionales
   (4) Estudios genéticos
   (5) Estudios radiológicos
   (6) Análisis estadístico

Materiales y métodos

1) Sujetos de Estudio

a. Pacientes

En el conjunto de trabajos que se incluyen en esta tesis han participado un total de 79 pacientes con defectos del transporte y metabolismo de tiamina (incluidos 70 pacientes con mutaciones del gen *SLC19A3* - 3 de ellos en seguimiento periódico en consultas externas de neurología pediátrica del Hospital Sant Joan de Déu; 4 pacientes con mutaciones del gen *TPK1* y 5 pacientes con mutaciones del gen *SLC25A19*. En el marco de esta tesis, se han diagnosticado dos nuevos pacientes, un paciente con diagnóstico *postmorten* de mutación del gen *SLC19A3* y otra paciente con defecto del *SLC25A19*. Si bien se ha incluido a los pacientes con defectos del *SLC19A2* en la revisión de la literatura, no se ha incluido pacientes con este defecto en la tesis dado que casi no presentan manifestaciones neurológicas.

b. Controles

Las características de los pacientes que participaron en cada parte del estudio, así como los criterios de inclusión y exclusión, se describen con más detalle en cada uno de los trabajos publicados.

En el trabajo Nº6 “La tiamina libre es un biomarcador potencial de la deficiencia del transportador de tiamina de tipo 2: una causa tratable de Síndrome de Leigh” se analizaron 106 muestras de sangre total y 38 muestras de LCR de sujetos controles pediátricos. También se analizaron 10 muestras de LCR de pacientes con síndrome de Leigh y 49 pacientes con enfermedades neurológicas genéticas o adquiridas. Además se analizaron seis líneas celulares de fibroblastos humanos de pacientes con otros errores

innatos del metabolismo que se utilizaron como controles. Los valores de concentración de tiamina en 106 muestras de sangre total, también se utilizaron en el trabajo Nº2 "Deficiencia del transporte de tiamina de tipo 2: seguimiento y monitorización del tratamiento"

2) Aspectos éticos

Las muestras de sangre, LCR, biopsia muscular y fibroblastos de los pacientes incluidas en estudio se recogieron tras firma de consentimientos informados de los tutores legales de los pacientes. Toda la investigación llevada a cabo contó con la aprobación de la Junta de Ética Institucional del Hospital Sant Joan de Déu, respetando los principios fundamentales de declaración de Helsinki, del Convenio de Consejo de Europa relativo a los derechos humanos y la biomedicina y de la declaración universal de la UNESCO sobre el programa Genoma Humano y Derechos Humanos.

El trabajo Nº6 "La tiamina libre es un biomarcador potencial de la deficiencia del transportador de tiamina de tipo 2: una causa tratable de Síndrome de Leigh" se realizó siguiendo las directrices de STARD de precisión diagnóstica (primera versión oficial, enero de 2003) como se recomienda para los biomarcadores de fluidos en los trastornos neurológicos [Gnanapavan et al., 2014].

3) Materiales y métodos

3.1. Revisión de la literatura

Se ha realizado revisión de la literatura en el trabajo Nº1 "Tratamiento de los defectos genéticos del transporte y metabolismo de tiamina", Nº2 "Deficiencia del transporte de tiamina de tipo 2: seguimiento y monitorización del tratamiento" y Nº3 "Errores innatos del metabolismo tratables por defectos del transporte de vitaminas".

Para la revisión sistemática de la literatura de esta sección, se realizaron búsquedas en MEDLINE (a través de PubMed) usando la siguiente palabras claves: #1 *SLC19A2*, #2 *SLC19A3*, #3 *SLC25A19*, #4 *TPK1*, #5 Leigh encephalophaty, #6 thiamine transporter type-2, #7 thiamine transporter type-1, #8 hTHTR2, #9 hTHTR1, #10 Thiamine responsive megaloblastic anemia, #11 Amish microcephaly, #12 striatal necrosis y #13 Biotin responsive basal ganglia disease. El número de hallazgos a fecha 02 de enero de 2014 fue de #2: 50, #5: 44, #6: 190, #8: 5, y #13: 14, respectivamente. Un total de 15 estudios clínicos (4 informes de casos, 11 series cuantitativas) y 1 guía de práctica clínica fueron finalmente seleccionado. En una actualización de esta tesis, a fecha 10 de enero de 2017, incluyendo además la ampliación de palabras claves, el número de hallazgos fue de #1: 262, #2: 97, #3: 28, #4: 112, #5: 608, #6: 7, #7: 10, #8: 17, #9: 20, #10: 112, #11: 18, #12: 400 y #13: 23. Los resultados de la interpretación de la revisión bibliográfica de esta sección de han presentado en la sección de Introducción.

También se ha realizado revisión de la literatura en el trabajo Nº4 “Síndrome de Leigh por defecto de NDUFS4: reporte de un caso y revisión de la literatura”. Para la revisión de literatura de esta sección se identificaron 198 pacientes reportados con deficiencia del complejo I de la cadena respiratoria mitocondrial (CRM) debida a un defecto nuclear de alguna de sus subunidades.

3.2. Estudios bioquímicos en sangre, LCR y fibroblastos

Los aminoácidos y ácidos orgánicos se analizaron mediante cromatografía de intercambio iónico con detección de ninhidrina (Biochrom 30, Pharmacia Biotech, Biochrom, Cambridge, Science Park, Inglaterra) y cromatografía de gases/espectrometría de masas (Agilent Technologies Inc., Santa Clara, CA) siguiendo procedimientos previamente informados [Moyano et al., 1998, Blau et al., 2008]. Los

análisis de lactato de plasma y CSF se realizaron mediante procedimientos espectrométricos automatizados (Architect ci8200, Abbott).

Para el estudio del paciente el trabajo Nº4 "Síndrome de Leigh por defecto de NDUFS4: reporte de un caso y revisión de la literatura", se determinó la actividad PDH en cultivos de fibroblastos y en tejido muscular según el procedimiento descrito en [Guitart et al., 2009]. Las velocidades de oxidación del sustrato se analizaron en fibroblastos midiendo la producción de $^{14}CO2$ a partir de la oxidación de [1-^{14}C]-piruvato, [2-^{14}C]piruvato y [^{14}C]-glutamato [Willems et al., 1978]. La concentración total se determinó utilizando HPLC con detección electroquímica [Montero et al., 2005]. Se realizó BN-PAGE para aislar complejos de proteínas intactas de tejido muscular. El ensamblaje de los cinco complejos OXPHOS se examinó utilizando la técnica blue native/SDS-PAGE de dos dimensiones. Los geles se transfirieron y se incubaron con cinco anticuerpos específicos para cada complejo mitocondrial. La biopsia muscular *pre-morten* se tomó con técnica abierta y las muestras de músculo fueron teñidas utilizando procedimientos estándares.

3.2.a. Fibroblastos y condiciones de cultivo

Los fibroblastos de los pacientes y los controles se analizaron entre los pasajes 5 y 8. Estos fibroblastos se mantuvieron en una atmósfera humidificada de 5% de CO2 y 95% de aire. Las células se removieron enzimáticamente utilizando tripsina-EDTA al 0,25%, durante 10 min, a 37ºC. Se realizó la división 1:4, y posteriormente se centrifugó durante 10 min a 252xg y se subcultivó en matraces de cultivo celular (área de crecimiento de 9.6 cm^2, Nunclon delta, Nunc, Dinamarca). Los fibroblastos se cultivaron en dos medios diferentes: 1) DMEM o Medio de Eagle modificado por Dulbecco: este medio presenta bajas concentraciones de tiamina (2,8 nmol/l) y 2) MEM

o Medio esencial mínimo (Sigma, Saint Louis, MO, USA) con concentraciones normales de tiamina (304,3 nmol/l). Ambos medios se suplementaron con 10% de suero bovino fetal (que contenía 0,017 mg/l de tiamina), 100 unidades/mL de penicilina y 100 µg/mL de estreptomicina. El medio de cultivo se cambió cada 3 a 4 días. Después de 10 días de incubación, las células se tripsinizaron y se lavaron dos veces con solución salina. Las células sedimentadas se resuspendieron con 300 µl de solución salina tamponada con fosfato (PBS), pH 7,4 y se sonicaron una vez durante 5s. Los homogeneizados se utilizaron para determinar las concentraciones de tiamina y sus derivados por HPLC (Waters 2690, Milford, MA, EE.UU.) con detección flourimétrica (Kontron Instruments, Zurich, Suiza). Los homogenados también se usaron para medir la concentración de proteína total con un kit de ensayo de proteínas (Bio-Rad Laboratories, Hercules, CA, EE.UU.) basado en el método de Lowry. Los fibroblastos se analizaron por duplicado en tres experimentos independientes.

3.2.b Análisis por HPLC de derivados de tiamina en muestras de sangre, CSF y fibroblastos

Los derivados de tiamina se analizaron con ligeras modificaciones siguiendo un procedimiento previamente descrito [Mayr et al., 2011]. Se desproteinizó un total de 200 µl de sangre entera con 200 µl de ácido tricloroacético al 10%. Después de la incubación durante 15 min en hielo, las muestras se centrifugaron durante 10 min a 1500xg y 4ºC, y se recogieron 150 µl del sobrenadante. A continuación se añadió 1 ml de dietiléter (DEE) al sobrenadante para separar la fracción orgánica, del agua. Las muestras se centrifugaron durante 10 min (1500 x g a 4ºC), separándose la fracción orgánica. Posteriormente se añadió 1 ml de DEE y se mezcló la solución y se centrifugó nuevamente. Antes del análisis de HPLC, se derivatizaron 90 µl de soluciones que contenían tiamina mediante la adición de 10 µl de una solución recién preparada de

hexacianoferrato de potasio 10 mM (III) en NaOH al 15%, mezclándose esta solución inmediatamente. El procesamiento de 100 µl de cada muestra de LCR se realizó de la misma manera que para las muestras de sangre total. Se adquirieron los estándares para tiamina-libre, TMP y TDP de la casa comercial Sigma (referencias T4625, T8637 y C8754, respectivamente, Sigma Chemical Company, St Louis, EE.UU.) y se diluyeron en agua destilada hasta obtener soluciones de 50 nmol/L. Se desproteinizaron cien microlitros de homogeneizado de fibroblastos mediante la adición de 100 ml de ácido tricloroacético (TCA) al 10% como se ha descrito anteriormente. Las muestras se colocaron en un autoamplificador protegido de la luz y se inyectaron 20 µl en un sistema HPLC equipado con una columna analítica de fase inversa (Teknokroma, Barcelona, C18 250 mm x 4,6 mm, tamaño de partícula 5 µM) y una precolumna C18 (Teknokroma, Barcelona, 581372-U). Inicialmente, se normalizó el mismo método cromatográfico para el análisis de las isoformas de tiamina en las muestras de sangre y LCR. Las condiciones de elución de este método fueron las siguientes: 0 - 6 min, 80% A; 6 - 12 min, 20% A; y 12-20 minutos, 80% A. La fase móvil A consistía en 100% de fosfato de potasio 25 mmol/L (pH 7,0) y la fase móvil B consistía en 100% metanol de grado HPLC. Dado que la tiamina-libre se consideró la forma más sensible para la identificación de la deficiencia de hTHTR2 en el LCR, nuevamente se normalizó un procedimiento nuevo más rápido de HPLC para el análisis de tiamina-libre en LCR. En este nuevo método se utilizó 50% de metanol y 50% de fosfato de potasio 25 mmol/L (pH 7,0) como fase móvil. El caudal para ambos métodos fue de 1,0 ml/min. El detector de fluorescencia se ajustó con una longitud de onda de excitación de 375 nm y una longitud de onda de emisión de 435 nm. La TDP, TMP y la tiamina-libre en las muestras de sangre y LCR se eluyeron a 4,8, 6,8 min y 12,4 min, respectivamente, para el método original, y además la tiamina-libre en LCR se eluyó a 4,4 min para el método

rápido. Los datos cromatográficos se procesaron usando el software Breeze GP (Waters, MA, EE.UU.).

3.3.Estudios funcionales: análisis funcional de la mutación SLC19A3 c.980-14A> G

Para investigar la implicación de la mutación c.980-14A>G idenficada en dos pacientes con deficiencia de hTHTR2 en el procesamiento del mRNA, se contruyó un mini gen basado en el sistema *pSPL3 exon-trapping vector* (Exon Trapping System, Gibco, BRL,Carlsbad, CA, USA). Los fragmentos del exón 4 del gen *SLC19A3* fueron amplificados del paciente 2 y de fibroblastos controles y clonados en el vector pGEMT (Promega Corporation, Madison, WI, USA). Después de la escisión con EcoRI (Roche Diagnostic GmbH, Roche Applied Science, Nonnenwald, Penzberg) y la purificación con el kit de extracción QIAEX II, se insertaron en el vector *pSPL3*, utilizando el kit de ligamiento rápido (Roche Diagnostics GMBH, Roche Applied Science, Mannheim, Germany). Se realizó la transfección de los mini-genes conteniendo los insertos normales y mutados en células HEK293T. La extracción de ARN y el análisis por RTPCR se realizaron como se describió previamente en Fernández-Guerra, et al., 2010. Los transcritos derivados de mini-gen de empalme se amplificaron usando los cebadores específicos de pSPL3: SD6 y SA2 (Exon Trapping System). Se realizó la caracterización de las secuencias de las diferentes especies moleculares después de clonar los productos amplificados por PCR en el vector pGEMT.

3.4 Estudios genéticos

El análisis molecular del gen *SLC19A3* se realizó en las muestras de sangre de pacientes con síndrome de Leigh (RefSeq NM_025243.3_ [ARNm]). Las regiones exónicas codificantes y las regiones que flanquean la unión intrón-exón se amplificaron a través de PCR con cebadores basados en la entrada del Ensembl genome browser:

ENSG00000135917. Los amplicones se secuenciaron y se analizaron como se describe en [García-Cazorla et al., 2014]. Las nomenclaturas de las mutaciones se realizaron según http://www.hgvs.org./mutnomen/.

Para el estudio del paciente el trabajo Nº4 "Síndrome de Leigh por defecto de *NDUFS4*: reporte de un caso y revisión de la literatura", se extrajo el ADN total de muestras de sangre usando el sistema MagnaPure (Roche Applied Science, IN, EE.UU.). El análisis genético de genes nucleares implicados en enfermedades mitocondriales fue realizado a través de la secuenciación de exoma utilizando un panel dirigido TruSight (Illumina) con la técnica que se describe en [Vega et al., 2016A, 2016b].

Para el diagnóstico de la paciente con mutación *SLC25A19* del trabajo Nº4 "Supervivencia y predictores del tratamiento en pacientes con defectos de tiamina", en un estudio prospectivo a lo largo de un año, aplicamos un panel dirigido (NGS) de 78 genes vinculados a necrosis estriatal, utilizando HaloPlex de Agilent Genomics.

3.5.Estudios radiológicos

Se realizó un análisis de las imágenes de RM siguiendo un protocolo que recoge las siguientes variables de estudio: a) localización de las lesiones a nivel de las siguientes estructuras: sustancia blanca y córtex de cerebro, cerebelo, vermis cerebeloso, núcleo dentado, cuerpo calloso, tálamo, putamen, núcleo caudado, globo pálido, sustancia *nigra*, sustancia gris periacueductal, mesencéfalo, protuberancia, bulbo y médula cervical; b) simetría/asimetría de la alteración de señal; c) alteración del volumen (atrofia/edema); d) restricción del coeficiente de difusión de estas áreas. También se describió la impresión global de la neuroimagen (atrofia/normal/edema) y la presencia de pico de lactato en la espectroscopia. La RM cerebrales realizadas en nuestro centro utilizaron un equipo General Electric de 1.5T (Signa Excite HD, Milwaukee, WI, USA).

El protocolo estándar constaba de las siguientes secuencias: T1-sagital, SPGR 3D T1 axial, fast-spin echo with fluid-attenuated inversión recovery (FLAIR) coronal, DWI axial y FSE DP-T2-axial. Se procesó la adquisición de espectroscopia con el software LCModel v6.2 (Stephen Provencher©). También se han analizado un total de 9 RM cerebrales enviadas por colaboradores internacionales.

3.6 Análisis estadístico

El análisis estadístico se realizó utilizando el software IBM SPSS Statistics 23 (IBM Corp., Armonk, Nueva York, EE.UU). La prueba de Kolmogorov-Smirnow se utilizó para evaluar la distribución de los datos. Las variables cuantitativas se reportaron de acuerdo a su distribución normal con media, error estándar de la media (SE) o desviación estándar (SD) y rango; o con mediana y rango interquartil (IQR). Se aplicó la prueba de Mann-Whitney para la evaluación de las diferencias en las variables numéricas entre los grupos. La prueba de Chi-cuadrado y la prueba exacta de Fisher se utilizaron para probar la asociación entre variables categóricas.

En el trabajo Nº4 "Supervivencia y predictores del tratamiento en pacientes con defectos de tiamina", se realizó un análisis de regresión logística múltiple para investigar más a fondo la relación entre la variable de respuesta binaria y potenciales predictores de supervivencia. Se utilizó el análisis de supervivencia de Kaplan-Meier para comparar las tasas de supervivencia para los pacientes con deficiencia de *SLC19A3* y pacientes con deficiencia del complejo I. Las diferencias en la supervivencia entre los grupos se evaluó con la prueba de log-rank.

En el trabajo Nº5 "La tiamina libre es un biomarcador potencial de la deficiencia del transportador de tiamina de tipo 2: una causa tratable de Síndrome de Leigh", debido a que los datos no siguieron una distribución gaussiana, se aplicaron diferentes pruebas no

paramétricas. Se utilizó la prueba de correlación simple de Spearman para determinar las correlaciones entre isoformas de tiamina de sangre total o LCR y la edad de los pacientes. Se utilizaron las pruebas de U de Mann-Whitney y Kruskal-Wallis para probar las diferencias significativas en las concentraciones de las isoformas de tiamina en los diferentes grupos de edad.

Todas las pruebas estadísticas se llevaron a cabo con un nivel de significancia de $p.<0.05$.

Investigación y resultados

Los resultados del estudio que hacen referencia al objetivo (1) se presentan en los trabajos:

- Treatment of genetic defects of thiamine transport and metabolism. "Tratamiento de los defectos genéticos del transporte y metabolismo de tiamina". Expert Rev Neurother. 2016 Jul;16(7):755-63.
- Thiamine transporter-2 deficiency: outcome and treatment monitoring. "Deficiencia del transporte de tiamina de tipo 2: seguimiento y monitorización del tratamiento" Orphanet J Rare Dis. 2014 Jun 23;9:92.
- Treatable Inborn Errors of Metabolism Due to Membrane Vitamin Transporters Deficiency. "Errores innatos del metabolismo tratables por defectos del transporte de vitaminas". Seminars in Pediatric Neurology (in press)

Los resultados del estudio que hacen referencia al objetivo (2) se presentan en el trabajo:

- Survival and treatment predictor in thiamine defects. "Supervivencia y predictores del tratamiento en pacientes con defectos de tiamina". Annals of Neurology (submitted)

Los resultados del estudio que hacen referencia al objetivo (3) se presentan en el trabajo:

- *NDUFS4* related Leigh syndrome: A case report and review of the literature. "Síndrome de Leigh por defecto de *NDUFS4*: reporte de un caso y revisión de la literatura". Mitochondrion. 2016 May;28:73-8.

Los resultados del estudio que hacen referencia a los objetivos (4) se presentan en el trabajo:

- Free-thiamine is a potential biomarker of thiamine transporter-2 deficiency: a treatable cause of Leigh syndrome. “La tiamina libre es un biomarcador potencial de la deficiencia del transportador de tiamina de tipo 2: una causa tratable de Síndrome de Leigh”. Brain. 2016 Jan;139(Pt 1):31-8.

Los resultados del estudio que hacen referencia al objetivo (5) se presentan en el trabajo:

- Thiamine transporter-2 deficiency: outcome and treatment monitoring. “Deficiencia del transporte de tiamina de tipo 2: seguimiento y monitorización del tratamiento” Orphanet J Rare Dis. 2014 Jun 23;9:92.

Treatment of genetic defects of thiamine transport and metabolism.

"Tratamiento de los defectos genéticos del transporte y metabolismo de tiamina".

Expert Rev Neurother. 2016 Jul;16(7):755-63.

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En este trabajo realizamos una revisión bibliográfica de todos los casos reportados de pacientes con defectos genéticos del transporte y metabolismo de tiamina. Los trabajos publicados hasta el momento solo ofrecían observaciones parciales de los dos defectos genéticos más frecuentes, *SLC19A2* y *SLC19A3*. El objetivo de este trabajo ha sido el de caracterizar el espectro fenotípico y genotípico de pacientes con deficiencia de los genes *SLC19A2*, *SLC19A3*, *TPK1* y *SLC25A19*. Además, en este trabajo se comenta la eficacia y la seguridad del tratamiento con biotina y tiamina, los efectos adversos de la suplementación, así como la dosificación y el seguimiento de estos pacientes durante el tratamiento. En resumen, en este trabajo se han evaluado por primera vez en una sola revisión, todos los defectos conocidos del transporte y metabolismo de tiamina. Con todo ello, sentamos la bases para la descripción que se realiza más delante de la historia natural de algunos de estos defectos genéticos.

EXPERT REVIEW OF NEUROTHERAPEUTICS, 2016
VOL. 16, NO. 7, 755–763
http://dx.doi.org/10.1080/14737175.2016.1187562

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REVIEW

Treatment of genetic defects of thiamine transport and metabolism

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ABSTRACT

Introduction: Thiamine is a key cofactor for energy metabolism in brain tissue. There are four major genetic defects (*SLC19A2, SLC19A3, SLC25A19* and *TPK1*) involved in the metabolism and transport of thiamine through cellular and mitochondrial membranes. Neurological involvement predominates in three of them (*SLC19A3, SCL25A19* and *TPK1*), whereas patients with *SLC19A2* mutations mainly present extra-neurological features (e.g. diabetes mellitus, megaloblastic anaemia and sensori-neural hearing loss). These genetic defects may be amenable to therapeutic intervention with vitamins supplementation and hence, constitutes a main area of research.

Areas covered: We conducted a literature review of all reported cases with these genetic defects, and focused our paper on treatment efficacy and safety, adverse effects, dosing and treatment monitoring.

Expert commentary: Doses of thiamine vary according to the genetic defect: for *SLC19A2*, the usual dose is 25–200 mg/day (1–4 mg/kg per day), for *SLC19A3*, 10–40 mg/kg per day, and for *TPK1*, 30 mg/kg per day. Thiamine supplementation in *SLC19A3*-mutated patients restores CSF and intracellular thiamine levels, resulting in successful clinical benefits. In conclusion, evidence collected so far suggests that the administration of thiamine improves outcome in *SLC19A-2, SLC19A3*- and *TPK1*-mutated patients, so most efforts should be aimed at early diagnosis of these disorders.

ARTICLE HISTORY
Received 11 February 2016
Accepted 5 May 2016
Published online 23 May 2016

KEYWORDS
SLC19A3; SLC19A2; SCL25A19; TPK1; SLC35F3; SLC44A4; thiamine; biotin; Wernicke encephalopathy; Leigh syndrome

1. Introduction

Thiamine, a water-soluble vitamin of the B complex (vitamin B1), is a key cofactor involved in energy metabolism in brain tissue. Because humans cannot synthesize thiamine, it is an essential nutrient. Thiamine chemical structure consists of an aminopyrimidine and a thiazole ring linked by a methylene bridge ($C_{12}H_{17}N_4OS$). Thiamine phosphate derivatives (thiamine monophosphate [TMP], thiamine diphosphate [TDP] – also known as a thiamine pyrophosphate [TPP] – and thiamine triphosphate [TTP]) are involved in many cellular processes.

Thiamine isoforms, free-T and TMP, are absorbed in the small intestine by two specific transporters: human thiamine transporter-1 (hTHTR1, encoded by *SLC19A2*) and human thiamine transporter-2 (hTHTR2, encoded by *SLC19A3*). The mucosa of the duodenum has the highest rate of thiamine uptake [1]. After absorption, thiamine is converted into TDP by a specific cytosol kinase (thiamine phosphokinase, *TPK*, EC 2.7.4.15). Then, the mitochondrial TPP carrier encoded by *SLC25A19* mediates the uptake of TDP into the mitochondria [2]. TPP is a cofactor of various enzymes in the cytosol (transketolase, EC, 2.2.1.1), in peroxisomes (2-hydroxyacyl-CoA lyase, EC, 4.1.2.n2), and in mitochondria (pyruvate dehydrogenase [PDH], EC, 1.2.4.1; 2-oxoglutarate dehydrogenase, EC, 1.2.4.2, and branched-chain alpha-keto acid dehydrogenase, EC, 1.2.4.4).

There are four known genetic defects (*SLC19A2, SLC19A3, SLC25A19*, and *TPK1*) involved in the metabolism and transport of thiamine with a variable response to the administration of thiamine and biotin. These may present with the following phenotypes: (i) *SLC19A2*, thiamine-responsive megaloblastic anemia (TRMA) syndrome; (ii) *SLC19A3*, biotin–thiamine-responsive basal ganglia disease, Leigh syndrome, infantile spasms with lactic acidosis, or Wernicke encephalopathy-like syndrome; (iii) *TPK1*, Leigh syndrome; and (iv) *SLC25A19*, Amish microcephaly or bilateral striatal degeneration and progressive polyneuropathy.

In this paper, we review the clinical, biochemical, and radiological characteristics of these defects, and we discuss their biochemical diagnosis, treatment response, and treatment monitoring. A description of all reported cases of each genetic defect, including age at disease onset, associated clinical symptoms, biochemical data, mutation type, thiamine doses, and outcome is reported in Supplementary Table 1 (SLC19A2 defects), Table 2 (SLC19A3 defects), and Table 3 (TPK1 defects).

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Supplemental data for this article can be accessed here.

2. Genes involved in thiamine metabolism and transport

2.1. SLC19A2

SLC19A2 gene was identified in the year 1999 [3–5]. It is located on chromosome 1q23.3 and contains six exons (22.5 kb), encoding a protein of 497 amino acids (55,400 Da) and 12 transmembrane domains. SLC19A2 is expressed in a wide range of human tissues, including bone marrow, liver, colon, small intestine, pancreas, brain, retina, heart, skeletal muscle, kidney, lung, placenta, lymphocytes, and fibroblasts [6–8].

TRMA syndrome (OMIM 249270), also known as Rogers' syndrome [9], is caused by mutations in *SLC19A2*. TRMA is characterized by a triad of (i) megaloblastic anemia with ringed sideroblasts, (ii) nonautoimmune diabetes mellitus, and (iii) early-onset sensori-neural deafness [10]. SLC19A2 is the only known transporter in marrow, pancreatic beta cells, and a subgroup of cochlear cells; therefore, anemia, diabetes, and deafness are the consequences of SLC19A2 deficiency [11]. Cardinal findings manifest at any time between infancy and adolescence. Since the first description of the disease by Rogers et al. [9], approximately 80 new cases have been reported. Two cases without diabetes mellitus [12,13], four without hearing loss [11,12,14,15], and one without anemia have been described (see Supplementary Table 1).

Diabetes mellitus may develop as early as the neonatal period [16], and together with anemia, these are the first manifestations of the disease. It is a non-type 1 diabetes with insulin secretion deficiency, with patients requiring insulin at the end of puberty [12,17]. Some patients can maintain a measurable C-peptide level as long as 24 years after diagnosis [18]. Thiamine deficiency causes a reduction in the secretion of digestive enzymes by acinar cells and glucose intolerance because of impairment of insulin synthesis and secretion by beta cells [19].

Megaloblastic, sideroblastic, and aplastic anemia are associated with the disease; all of them respond to thiamine supplementation. The most common type of anemia is megaloblastic anemia, occurring between infancy and adolescence [13,17]. Intracellular thiamine deficiency affects the erythropoietic system by two mechanisms: (i) impairment of *de novo* synthesis of nucleic acids, which is catalyzed by the enzyme transketolase from the pentose phosphate pathway, resulting in a defect in cell division and macrocytosis and (ii) involvement of the alpha ketoglutarate that supplies metabolites to the Krebs cycle, which finally produces the heme precursor succinyl-CoA and causes ineffective erythropoiesis of the sideroblastic type. Examination of bone marrow reveals megaloblastic anemia with erythroblasts, often containing iron-filled mitochondria (ringed sideroblasts) [19–22].

SLC19A2 is essential for the function and survival of the cochlear inner hair cells (IHCs). Homozygous SLC19A2 −/− mice show complete loss of IHCs and partial loss of outer hair cells (OHCs), whereas heterozygous SLC19A2 +/− mice have preserved IHCs and occasional loss of OHCs at the cochlear apex [19]. This could explain why compound heterozygote missense mutations in humans may present with late-onset or a minimal sensori-neural hearing deficit [11].

Manimaran et al. [23] hypothesized that SLC19A2 may have additional signaling functions (G-protein-coupled receptors family 1 signature) in addition to thiamine transport, highlighting the disease pathology of retinitis pigmentosa in some TRMA patients.

Other symptoms associated with the disease are seizures, ataxia, developmental delay, stroke-like episodes, ocular symptoms (pigmentary retinopathy, abnormalities of the optic nerve, cone–rod dystrophy, and Leber's congenital amaurosis), short stature, congenital cardiac malformations with conduction defects (atrial fibrillation, secundum atrial septal defect, Ebstein anomaly, endocardial cushion defect, atrial dysrhythmia, and supraventricular tachycardia), cardiomyopathy, situs inversus, cryptorchidism, polycystic ovarian syndrome, immune thyroiditis, hepatomegaly, gastroesophageal reflux, vocal cord nodules, thrombocytopenia, and neutropenia [11,24–26].

In summary, the diagnosis of SLC19A2 defect should be considered in patients with: (i) non-type 1 insulin-dependent diabetes negative for antibodies (against insulin, antiGAD65, antiIA2, and transglutaminase) and deafness, (ii) refractory megaloblastic anemia despite normal serum folate and vitamin B12 concentrations, (iii) Wolfram-like syndrome (diabetes, deafness, diabetes insipidus, and optic atrophy) with no genetic confirmation (*WFS1* or *CISD2* gene), (iv) Almström-like syndrome (progressive loss of vision and hearing, dilated cardiomyopathy, obesity, type 2 diabetes mellitus, and short stature with no genetic confirmation (ALMS1 gene), and (v) some mitochondrial disorders, including Pearson and Kearns–Sayre syndrome [18,24].

2.2. SLC19A3

SLC19A3 gene is located on chromosome 2q36.3. It contains five exons (32.8 kb) encoding a protein of 496 amino acids (55665) that is widely expressed but most abundant in placenta, kidney, and liver. Human SLC19A3 shares 39% and 48% amino acid sequence identity with human SLC19A1 and SLC19A2, respectively [27].

hTHTR2 deficiency (OMIM 607483) is a recessive inherited disease caused by mutations in *SLC19A3*. Patients were first described by Ozand et al. [28] in Saudi Arabia. Normally developing children present with acute and recurrent episodes of encephalopathy (often triggered by febrile illness, trauma, and vaccines), dystonia, dysarthria, external ophthalmoplegia, and seizures, in association with symmetrically distributed brain lesions in caudate nuclei, putamen, medial thalami, and, less frequently, cerebral cortex, brainstem, and cerebellum [28–30]. The following clinical phenotypes have been described in SLC19A3 patients: (i) biotin–thiamine-responsive basal ganglia disease, (ii) Leigh and Leigh-like encephalopathy, (iii) Wernicke-like syndrome, and (iv) infantile spasms (see Supplementary Table 2). Some patients show nonspecific biomarkers of mitochondrial dysfunction (i.e. increases of 2-oxoglutarate, lactate, and alanine in

biological fluids and a lactate peak on spectroscopy) [29–33]. Recently, a remarkable free-T deficiency in cerebrospinal fluid (CSF) and fibroblasts of SLC19A3 patients was described [2].

In a recent review of 69 patients [34], symptoms appeared before the age of 12 years in 80% (age of onset: 3.5 ± 4.6 years [mean ± standard deviation], range: 1 month–20 years). Trigger events (fever, vaccination, trauma, etc.) were reported in 40 of 69 patients. Symptoms included encephalopathy and lethargy, seizure (myoclonic jerks, epileptic spasms, focal and generalized seizure, epilepsia partialis continua, and status epilepticus), generalized and focal dystonia, opisthotonus, rigid akinetic syndrome, tremor, chorea, jitteriness, dystonic status, ataxia, bulbar dysfunction (dysarthria, anarthria, and dysphagia), pyramidal signs, abnormal ocular movements (nystagmus, oculogyric crisis, oculomotor nerve palsy, ophthalmoplegia, and sunset phenomenon), developmental delay, dysautonomia, ptosis, rhabdomyolysis, and facial dyskinesia [34].

2.3. SLC25A19

SLC25A19 gene is located on chromosome 17q25.1 [35]. It contains nine exons (16.5 kb) encoding a protein of 320 amino acids (35,511 Da). The highest levels of the protein are detected in colon, kidney, lung, testis, spleen, and brain.

Mitochondrial TPP carrier deficiency is associated with two different phenotypes: (i) Amish microcephaly (OMIM 607196), characterized by severe infantile lethal congenital microcephaly that may be evident from 21 weeks' gestation on ultrasound, profound global developmental delay, CNS malformations (lissencephaly, partial agenesis of corpus callosum, and closed spinal dysraphic state), episodic encephalopathy associated with lactic acidosis and alpha-ketoglutaric acidurias [36,37] and (ii) bilateral striatal degeneration and progressive polyneuropathy (OMIM 613710), characterized by childhood-onset recurrent episodes of encephalopathy, flaccid paralysis, and febrile illnesses and slow chronically progressive axonal polyneuropathy. Normal head circumference and normal early neurodevelopment differentiate this phenotype from the Amish microcephaly [10,38].

In Amish microcephaly, alpha-ketoglutaric aciduria appears during a time of metabolic stability but can be normal at birth and during metabolic crises [37]. Lactic acidosis appears during illnesses in both phenotypes [36–38]. The biochemical phenotype may be attributable to decreased activity of the three mitochondrial enzymes that require TDP as a cofactor: PDH, 2-oxoglutarate dehydrogenase, and branched-chain alpha-keto acid dehydrogenase.

2.4. TPK1

Thiamine pyrophosphokinase (TPK, EC 2.7.4.15) protein consists of 243 amino acids (27,265 Da; NM_022445.3) encoded by the *TPK1* gene, which is located on chromosome 7q34-q35 and contains nine exons (420 kb) [39,40]. Gene expression levels are high in tissues involved in thiamine absorption (small intestine) and re-absorption (kidney) and very low in a variety of other tissues [40].

In 2011, Mayr et al. [41] reported five individuals (P1–P5) affected by a TPK deficiency from three different families. The first biochemical analysis pointed to the existence of a possible defect in the mitochondrial pyruvate oxidation pathway. However, immunoblot analysis showed no changes of the protein content of E1α, E1 β, E2, or E3 PDH subunits. Finally, mutation analysis revealed distinct mutations in the *TPK1* gene, leading to the final diagnosis of the affected patients. Patients P1 and P2 were compound heterozygous (c. [148A>C]+[501+4A>T] (p.[Asn50His]+[Val119_Pro167del]), P3 and P4 were homozygous for the c.119T>C (p.Leu40Pro) mutation, and P5 was a compound heterozygous for two other mutations (c.[179_182delGAGA]+[656A>G] (p.[Arg60LysfsX52]+[Asn219Ser]).

In 2014, two Chinese siblings homozygous for a new missense mutation (c.604T>G (p.Trp202Gly) in the *TPK1* gene were described [42]. At the same time, two new patients with two novel mutations c.479C>T (p.Ser160Leu) and c.664G>C (p.Asp222His) affecting highly conserved residues were reported (see P8 and P9 in Supplementary Table 3) [10]. Clinically, the onset of symptoms appeared between 18 months and 4 years, after a period of normal development. Common features were episodic ataxia, psychomotor arrest, dystonia, and spasticity. Some patients presented with developmental delay and hypotonia [10,41,42]. Biochemical studies revealed elevated concentrations of lactate in blood (five out of nine) and CSF (three out of nine) during metabolic crisis. Increased excretion of alpha-ketoglutarate in urine (eight out of nine) was also reported [10,41,42]. Detection of alpha-ketoglutarate in urine was proposed as a possible biomarker for this disease. Some patients had reduced TPP concentrations in blood and muscle.

2.5. SLC35F3

In 2014, a previously unsuspected thiamine transporter *(SLC35F3)* was described as a new hypertension susceptibility locus [43]. *SLC35F3* gene is located on chromosome 1q42.2. It contains seven exons (419 kb) encoding a protein of 421 amino acids (46,817 Da), and it is widely expressed. So far, no patients with mutation of this gene have been described, making the clinical phenotype as yet unknown.

2.6. SLC44A4

Human TPP transporter (hTPPT; product of the SLC44A4 gene) is responsible for absorption of the microbiota-generated TPP in the large intestine. SLC44A4 gene is located on chromosome 6p21.33 (15.8 kb), encoding a protein of 710 amino acids (79,254 Da). The highest levels of the protein have been found in colon, kidney, lung, testis, spleen, and brain. The hTPPT is highly expressed in the colon but not in other regions of the intestinal tract and is localized exclusively at the apical membrane domain of epithelia [44]. There had been no protein expression in the CNS and, to date, no patients have been reported with this mutation carrier.

3. Treatment efficacy and safety

3.1. SLC19A2 deficiency

There are more than 33 different mutations described in patients with TRMA [23]. Most mutations are nonsense or frame shift mutations at exon 2, whereas only a few of them are missense [24]. These mutations lead to the absence of protein synthesis and thus a complete impairment of cellular thiamine transport. Some authors have demonstrated that specific uptake in the mutants' SLC19A2 cells varies between 2% [23] and 3% of control values [22]. In spite of these observations, thiamine supplementation in SLC19A2 patients can successfully improve clinical symptoms, and proper control of anemia and blood sugar significantly prolongs the life expectancy of these patients [24].

High doses of thiamine supplementation slow down the onset of diabetes [17] and decrease the requirement for insulin [8]. However, there is a slow progression of pancreatic B cell insufficiency, and patients become insulin dependent during adolescence [45,46]. Withdrawal of thiamine treatment induces an increase in insulin requirements [47] or leads to diabetic ketoacidosis [48].

Similarly, there is an almost immediate hematopoietic response after a few days of thiamine supplementation. Complete response can be seen 1 or 2 months after thiamine treatment is initiated. Thrombocytopenia and neutropenia recover within the first weeks of treatment (see Supplementary Table 1). This response is sustained until adolescence or adulthood, when patients may require blood transfusions [45]. Erythrocytes remain macrocytics, [14] and ringed sideroblasts can still be present 2 years after thiamine therapy [22]. Anemia may recur when thiamine is withdrawn [47,49].

Thiamine supplementation does not prevent sensori-neural hearing loss in children [50]. Similarly, in experimental models (SLC19A2 –/– mice), thiamine fails to restore auditory function [51]. Stagg et al. [52] postulated that thiamine requirements of cochlear or acoustic nerve cells are substantially higher than those of fibroblasts. In fact, initial observations of children with SLC19A2 defects and normal hearing could correspond to incomplete phenotypes [12,47,50]. Patients usually benefit from cochlear implantation with positive hearing and speech perception effects [24,53].

Thiamine supplementation does not improve short stature [7] or neurological manifestations [25]. Regarding psychiatric manifestations, thiamine administration improves explosive and aggressive behaviors but not mood disorders or paranoid ideations [54].

The biological mechanism of thiamine treatment is not yet known. Especially in the case of nonsense or frameshift mutations, a residual functional SLC19A2 transporter is not likely an option for cellular thiamine uptake. Possibly, alternative transport pathways can be exploited by increasing plasma thiamine concentrations after oral thiamine supplementation.

3.2. SLC19A3 deficiency

More than 16 different mutations have been described in SLC19A3-mutated patients, some of them associated with specific phenotypes [2].

In the first description of the disease, Ozand et al. [28] reported good responses of SLC19A3-mutated patients to biotin supplementation (5 mg/kg/day). Later, Zeng et al. [55] discovered that SLC19A3 is a thiamine transporter, and thiamine has been used to treat patients since then. To date, there is overwhelming evidence that early administration of thiamine can potentially reverse clinical and radiological abnormalities and improve neurological outcome [10,31–33,56–61]. If left untreated or if treatment is initiated late, patients can experience severe intellectual disability or even death [30,62].

Some authors describe a dramatic response to the administration of thiamine, with complete improvement within a few hours or days of the administration. Thiamine supplementation in these children restores CSF and intracellular thiamine levels [2]. Most authors prefer oral doses, but intravenous thiamine is used in some cases with severe presentations [57]. Thiamine doses are very variable but generally within the range of 10–40 mg/kg/day. Maximum reported therapeutic doses of thiamine are 1500 mg/day (see Supplementary Table 2). Metabolic decompensation may recur within 30 days of thiamine withdrawn [56]. Despite thiamine supplementation not improving the sequelae of the disease when treatment is initiated late, it may prevent further disease progression [32].

Again, the biological mechanism for thiamine treatment in SLC19A3 defects is uncertain. It is possible that missense mutations leading to some residual transporter function may be able to take up thiamine with increasing plasmatic thiamine concentrations. Alternatively, in patients carrying loss-of-function mutations in both alleles, thiamine uptake is probably compensated by the upregulation of an alternative transport system. Other human thiamine transporters, such as the reduced folate carrier (RFC1, also known as SLC19A1), thiamine transporter SLC19A2, or organic cation transporter (OCT1), recently identified as an important contributor to the uptake of thiamine from blood to tissues, could compensate for the thiamine transport in SLC19A3-deficient patients.

The administration of biotin in SLC19A3 deficiency is controversial. A few reported patients did not improve with biotin [63–64], as opposed to the initial description by Ozand et al. (1998) [28] and Debs et al. (2010) [57]. Moreover, one-third of patients in the study by Afadhel et al. (2013) showed a recurrence of acute crisis while on biotin therapy alone. After the addition of thiamine, crisis did not recur in these cases. Tabarki et al. [65] compared the combination of biotin plus thiamine (group 1) to thiamine alone (group 2) in a series of patients homozygous for the mutation c.1264A>G in the SLC19A3 gene. Mean biotin and thiamine doses in this study were 5 and 40 mg/kg/day, respectively. They observed a faster recovery from the acute attack or crisis in the group receiving both thiamine and biotin (2 days; 1.80 ± 0.63) than the group receiving only thiamine (3 days; 2.90 ± 0.87; $p = 0.005$). However, the combination of biotin and thiamine was not superior to thiamine alone in the number of recurrences, neurological sequel, or brain MR changes for at least a 30-month period. Two major limitations of this study were the short-term follow-up and small number of patients included. The authors recommend using a combination of biotin and

thiamine in the acute crisis for faster recovery and thiamine alone for life-long-term treatment.

Doses of biotin vary from 5 mg/kg/day [28] to 5–10 mg/day [31,59]. Maximum reported therapeutic doses are 600 mg/day [57]. Authors hypothesize that biotin can increase the expression levels of hTHTR2 mutants with some residual activity [33]. This might explain the differences in response to biotin in SLC19A3 patients, depending on the nature of the mutations, as it might not be effective in case of nonsense mutations. Moreover, biotin is an essential cofactor for a number of enzymes involved in mitochondrial energy metabolism (including propionyl coenzyme A [CoA] and pyruvate carboxylases and 3-methylcrotonyl CoA carboxylase) [61]. Finally, high doses of biotin enable pyruvate to bypass the tricarboxylic acid cycle by using pyruvate carboxylase, which produces oxaloacetate, a substrate in the tricarboxylic acid cycle instead of acetyl CoA [62].

Several antiepileptic drugs have been described for treating seizures in SLC19A3 patients. Status dystonicus was treated with drugs used in severe dystonias (e.g. trihexyphenidyl, intravenous diazepam) [31].

3.3. SLC25A19 deficiency

Amish microcephaly phenotype is associated with the homozygous mutation c.530G>C [36,37], whereas Spiegel et al. [38] reported the bilateral striatal necrosis phenotype with the homozygous c.373G>A mutation. Severe lactic acidosis during metabolic crisis responds to treatment with a high-fat diet. Developmental delay and microcephaly do not respond to thiamine treatment [37].

3.4. TPK1 deficiency

In the first study describing TPK-deficient patients, Mayr et al. [41] treated three out of five patients (P3, P4, and P5; see Supplementary Table 3) with 100–200 mg of thiamine per day. Two of them (P4 and P5) stabilized and even slightly improved clinically after thiamine supplementation. One patient achieved normal development. The third treated individual (P3) did not present clear improvement after 2 years of treatment. They concluded that the dosage of thiamine should be increased to adjust the substrate concentration for the residual TPK and to prevent any depletion of this vitamin. Moreover, they found that TPP concentrations reached normal values in fibroblasts after supplementing the growth medium with 10.7 mmol/l of thiamine in P3.

Fraser et al. [42] described two siblings with TPK deficiency. The first child (P6) died at the age of 29 months. Her diagnosis was not made until her brother (P7) was genetically diagnosed at the age of 18 months. P7 manifested a neurological regression and was treated with an aggressive approach because of the severe clinical presentation of his sister. Treatment consisted of a ketogenic diet, thiamine supplementation (10 mg/kg, 3 times/day), biotin (5 mg, 3 times/day), and α-lipoic acid (5 mg/kg, 3 times/day). The ketogenic diet (Ketocal 3:1), through nasogastric tube feeding, was initiated to reduce metabolic demand through PDH. Although Banka et al. [10] reported negative effects of the implementation of a ketogenic diet [10], in this case and after almost 9 months of treatment, both the cofactor supplements and ketogenic diet continue to be well tolerated, and the family is extremely reticent to change any medical intervention. This is the first study to report the benefits of dietary management together with cofactor supplementation in a patient presenting severe TPK mutations.

In parallel, Banka et al. [10] treated their patients (P8 and P9 see Supplementary Table 3) with 500 mg of thiamine hydrochloride. Only one of them (P8) responded to treatment at the age of 8 years. Encephalopathic episodes stopped, even during infectious illnesses, and showed a slow but gradual developmental progression and improvement in understanding, social interaction, language skills, and motor abilities. Moreover, the patient's nasogastric tube could be removed. Currently, P8 is attending school with extra support; however, he has unclear speech and spasticity in the four limbs. P9 was already severely affected when she was diagnosed at the age of 7 years. She presented poor head control and poor ability to sit. In her case, thiamine supplementation was ineffective.

Altogether, these results showed that TPK deficiency can be added to the list of thiamine-responsive disorders and is a potentially treatable inherited metabolic disorder. Late-diagnosed patients showed no response to thiamine supplementation, emphasizing the importance of an early diagnosis and treatment to reach a better outcome. However, it is important to take into consideration that no results after long-term treatment are available, and we still do not know if thiamine treatment would stop the natural course of the disease. Thus, long-term studies are needed.

4. Adverse effects

There have been some reports of anaphylaxis during intravenous administration of thiamine since 1938, but the mechanisms for this side effect are uncertain [61,66,67]. Some authors have reported the safety of thiamine hydrochloride given at 100-mg IV bolus in 989 consecutive patients (1070 doses) [68]. A total of 12 (1.1%) adverse reactions were reported, 11 minor reactions consisting of transient local irritation and only one major reaction consisting of generalized pruritus. Pharmacokinetic studies in thiamine supplementation showed that, although the optimum dosing for the beneficial effects is still unknown, high doses (up to 3000 mg) for extended periods of time have no deleterious effect. Moreover, it has been described that the absorption mechanism, regulated by a combination of an active transporter and a passive process, is not saturable up to 1500 mg [69].

Biotin toxicity in healthy humans has not been studied [70]. In a study of 20 patients with multiple sclerosis treated with high doses of biotin (100–300 mg/day), only transient diarrhea was observed as a side effect in two patients [71]. Sawamura et al. reported that dietary intake of high-dose biotin inhibits spermatogenesis in young rats [72] and that there were no adverse effects at 38.4 mg/kg body weight per day in rats [70].

5. Dosing and duration of treatment

Treatment focuses on lifelong use of pharmacological doses of thiamine or biotin, when recommended in affected individuals. Doses of thiamine vary according to the genetic defect: for SLC19A2 defects, the usual dose is 25–200 mg/day (approximately 1–4 mg/kg/day), for SLC19A3, 10–40 mg/kg/day, and for TPK1, 30 mg/kg/day. In the case of SLC19A2 and SLC19A3 thiamine transporters, the variance in the doses of thiamine that are beneficial for patients is not clear. The affinity for thiamine of the alternative transporters may in part explain these differences. In patients with a defect in the cytoplasmic phosphorylation enzyme *(TPK1)*, higher doses of thiamine are recommended to force the residual TPK activity.

With regard to biotin, treatment is recommended in SLC19A3 and TPK1 genetic defects (see section of efficacy of thiamine and biotin treatment in these defects). The doses vary according to the time of illness (acute episode or chronic treatment) and mutation.

6. Thiamine monitoring during treatment

6.1. SLC19A2 and SLC19A3 genes

Patients with ThTR1 and THTR2 defects show normal blood thiamine levels, suggesting that alternative transporters compensate for the intestinal absorption of thiamine [61]. Hence, blood thiamine quantification is not a useful biomarker for the diagnosis of these defects.

In SLC19A3 patients with prominent neurological dysfunction, the restoration of thiamine levels in the CNS is a major challenge. Quantification of thiamine isoforms in CSF is directly representative of thiamine status in brain. A lumbar puncture was performed in one patient with ThTR2 deficiency who was receiving oral thiamine, and CSF analysis showed values for the thiamine derivatives considerably above the upper limit of reference range, whereas thiamine was severely decreased in patients before the initiation of treatment [2]. Five patients in this report were stable and had not experienced complications since the initiation of treatment, suggesting that thiamine restored thiamine availability in brain tissue.

Measurement of thiamine concentrations is required for treatment monitoring and for the analysis of the safe and effective doses. However, a lumbar puncture is not recommended in children who are compensated under thiamine supplementation, for ethical reasons. An alternative measurement for treatment monitoring is the analysis of TDP in whole blood, the most concentrated vitamer in this peripheral fluid [34]. Patients treated with 10–40 mg/kg/day of thiamine were clinically compensated, and whole blood TDP values were above the upper limit of the reference range. Conversely, one patient with poor treatment compliance had persistent acidosis and low whole blood TDP levels [34]. TDP values increased, and lactic acid normalized when treatment was strictly followed.

Mitochondrial biomarkers, such as plasma and CSF lactate and amino acids, and the excretion of organic acids in urine, are useful in Leigh syndrome patients, including those with mutations in *SLC19A3* [2]. However, the majority of patients with ThTR2 deficiency shows normal values for these mitochondrial biomarkers [28–30,32,55–57,60,63,73].

6.2. TPK1 gene

Few patients have been diagnosed with TPK deficiency [10,41]. Authors recommended administrating high doses of thiamine to force the residual TPK activity and increase TPP production for the different thiamine-dependent dehydrogenases. However, not all the patients had a positive response. So far, no studies on follow-up of TPK-deficient patients exist.

6.3. SLC25A19 gene

From a biochemical point of view, the monitoring of these patients consists of normalizing lactic acidemia and the excretion of organic acids in urine. No studies exist about the follow-up of patients with alterations in *SLC25A19* gene.

7. Expert commentary

Evidence collected so far suggests that the administration of thiamine improves outcome in *SLC19A-2, SLC19A-3,* and *TPK1*-mutated patients, so most efforts should be aimed at early diagnosis of these disorders. Patients presenting with Leigh syndrome should be promptly treated with a vitamin cocktail including thiamine and biotin, and a lumbar puncture should be performed before the empirical administration of vitamins because children with hTHTR2 deficiency have remarkable free-T deficiency in CSF. Empirical thiamine administration is also recommended in patients with a combination of at least two of the following: diabetes mellitus, megaloblastic anemia, and sensori-neural hearing loss.

8. Five-year view

The next 5 years may provide the initial events in finding definitive therapies for patients with genetic defects of thiamine metabolism and transport. In the near future, we expect that patients with thiamine defects will be promptly treated with vitamins to improve their short- and long-term outcomes. The time frame from disease onset to thiamine administration strongly influences the outcome. Hence, treatment protocols for children presenting with acute encephalopathy and Leigh-like phenotype should include early administration of thiamine and biotin as a major recommendation. Rapid genetic testing is essential to switch from empirical to gene-specific treatment. Thiamine supplementation is a treatment for life; therefore, efforts should be made to establish a safe dose of thiamine capable of allowing normal development and preventing further decompensations.

Key issues

- Genetic defects of metabolism and transport of thiamine may present as: 1) *SLC19A2*, thiamine-responsive megaloblastic anaemia syndrome, 2) *SLC19A3*, biotin–thiamine-responsive basal ganglia disease, Leigh syndrome or Wernicke encephalopathy, 3) *TPK1*, Leigh syndrome and 4)

SLC25A19, Amish microcephaly or bilateral striatal degeneration and progressive polyneuropathy.

- Considering the presenting phenotypes and accumulating evidences for treatment, biotin and thiamine are empirically recommended in all patients with Leigh syndrome. Thiamine administration is also advisable in patients with a combination of at least two of the following: diabetes mellitus, megaloblastic anaemia and sensori-neural hearing loss.
- Doses of thiamine vary according to the genetic defect; usual doses are *SLC19A2* 25–200 mg/day (1–4 mg/kg per day); *SLC19A3* 10–40 mg/kg per day and *TPK1* 30 mg/kg day. Rapid genetic testing is essential to switch from empirical to gene-specific treatment.
- Children with *SLC19A3* mutations have remarkable free-T deficiency in CSF and fibroblasts. Thiamine supplementation restores CSF and intracellular thiamine levels and has a dramatic and sustained clinical response in early treated patients.
- Most of the *SLC19A2*-mutated, treated patients show a significant improvement in haematopoiesis and glycaemia control. Hearing function may not be preserved by thiamine treatment.
- *TPK1*-mutated patients may benefit from treatment with thiamine, biotin, niacin, alpha-lipoid acid and ketogenic diet.
- *SLC25A19*-mutated patients usually do not respond to thiamine treatment, but a ketogenic diet may improve the acidosis.

Declaration of interests

This research was supported by the Instituto de Salud Carlos III-FEDER (FIS PI12/02010, FIS PI15/00287); Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), an initiative of the Instituto de Salud Carlos III (Ministerio de Ciencia e Innovación, Spain); and Agència de Gestió d'Ajuts Universitaris i de Recerca-Agaur, (2014FI_B 01225) (JD Ortigoza-Escobar). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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Supplementary Table 1. Literature review of SLC19A2 defects.

Reference	N patient /origin	Age at onset			Other clinical features associated	Hb (g/dl)	Hto (%)	Reticulocytes	Platelets	WBC/ neutrophils	SLC19A2 mutations (homozygosis)	Thiamine therapy / Age at onset of thiamine treatment	Outcome
		Diabetes mellitus	Megaloblastic anemia	Deafness									
Haworth, 1982[1]	1 patient Pakistan	22 mo	22 mo	8 mo	na	na	na	na	na	na	c.484C>T (hom)	25 mg/d	Post-thiamine marrow aspirate showed a considerable improvement in both the megaloblastic and sideroblastic changes and anemia resolves.
Borgna-Pignati, 1989[2]	2 patients Italy	2 – 3 y	4 – 9 y	3 – 4 y	Optic atrophy	na	na	na	na	na	c.515G>A (hom)	na	Hematologic findings had returned to normal and the insulin requirements had decreased. Withdrawal of thiamine repeatedly induced relapse of the anemia and an increase in insulin requirements.
Akinci, 1993[3]	1 patient Turkey	20 mo	4y	na	na	na	na	na	na	na	na	75 mg/d	Insulin requirement decreased and even ceased, and macrocytic anemia improved
Valerio, 1998[4] */ Poggi, 1984*[5]	2 patients Italy	P1: 2 y P2: 3 y	P1: 7.5 mo P2: 3 y	P1: 2.5 y P2: 1.5 y	No	na	na	na	na	na	na	25 – 100mg/d	Diabetes and anemia improved
Fleming, 1999[6]	2 families Alaska Turkish-Kurdish	na	na	na	na	na	na	na	na	na	c.885delT(hom) c.1147delGT(hom)	na	na
Labay, 1999[7]	6 families F1 Italy, F2,3 Israel F4 India F5 Pakistan F6 Japan	na	na	na	na	na	na	na	na	na	F1 c.515G>A F2, 3 c.724delC F4 c.750G>A F5, 6 c. 484C>T	na	na
Diaz, 1999[8]	5 patients Iran	na	na	na	na	na	na	na	na	na	F1 c.242insA (hom -2 patients) F2 c.429–430delTT (hom - 3 patients)	na	na

Raz, 2000[9]	Lebanon France Brazil Italy Pakistan Turkey India	na	na	na	na	na	na	na	na	na	c.1223+1G>A (hom) c.277G>C c.428C>T c.515G>A c.196G>T c. 484C>T c. 750G>A	na	na
Scharfe, 2000[10]	1 patient Turkey	3 y	5 mo	18 mo	Hepatomegaly, splenomegaly, short stature, retinal degeneration	8.9	na	2.4	na	na	c.1074G>A (hom)	200 mg/d	Lactate levels were increased in serum and CSF. TPP was reduced (20.1 ng/ml, N 30-90 ng/ml). Lactate levels dropped to normal values and insulin requirements reduced.
Villa, 2000[11]	1 patient Italy	2 y	7 mo	2y	Ischemic stroke	na	na	Na	na	na	na	50 mg/d	Insulin was discontinued because of frequent episodes of hypoglycemia until puberty. Anemia improved.
Meire, 2000[12]	1 patient Turkey	1 y	7 y	1y	Cerebral vascular occlusion, cone-rod dystrophy	4.3	22	na	156,000	4,700/na	na	100 mg/d	Diabetes did not improve. She was deaf and blind.
Gritli, 2001[13]	2 patients Tunisia	P1: 5 y	P1: 8 mo P2: 1mo	P1: 2y P2: 1mo	P1 and P2: secundum atrial septal defect, atrial dysrrithmia, P1: dilated cardiomyopathy	P1: 2 P2: na	P1:na P2:na	P1:na P2:na	P1:15,000 P2: na	P1: occasionally neutropenia P2: pancytopenia at onset	c.287del (hom)	P1: 25 mg/d P2: 25 mg/d	P1: treatment only few days before her death P2: pancytopenia and diabetic control improved at 1 wk., macrocytosis persist
Bappal, 2001[14]	2 patients India	P1: 18 mo P2: 5 mo	P1: 18 mo P2: 5 mo	P1: 18 mo P2: 5 mo	P2: atrial defect	P1: 2.7 P2: 6.9	P1: 15 P2: na	P1: 0.2 P2: 0.3	P1: 42,000 P2: 124,000	P1: 2,900/290 P2: 12,300 / 3,690	P1 and P2: na	P1: 100 – 200 mg/d P2: 200 mg	P1: There was a rapid fall in insulin requirement followed by cessation of insulin requirement within 3 weeks, after thiamine stepped to 200 mg/d P2: diabetes and anemia improved
Ozdemir, 2002[15]	1 patient Turkey	5y	5y	5y	Optic atrophy, retinitis pigmentosa, paroxysmal atrial tachycardia, hepatomegaly, splenomegaly, autoimmune thyroiditis	4,4	18	1	106,000	10,000/ 6,000	c.697C >T (hom)	100 mg/d	Serum thiamine and TMP levels were found to be low. Two weeks after institution of thiamine therapy, the hemoglobin concentration increased. The patient's blood sugar became normal with- out administration of insulin.

Kipioti, 2003 [16]	1 patient Indian	11 y	na	2 y	Cone-rod dystropohy	na	na	na	Na	na	na	na	na
Lagarde, 2004 [17]	1 patient USA, African-American	13 mo	12 mo	2.5 y	Hepatomegaly, paroxysmal atrial tachycardia, optic atrophy, retinitis pigmentosa, autoimmune thyroiditis, psychotic episode	9,5	30	na	19,600	na	c.152C>T (hom)	75 mg/d	With initiation of thiamine therapy, reticulocyte count increased
Ricketts, 2006 [18]	13 patients Pakistan	P1: 1.5 y P2: 12 y P3: 2 y P4: 0.5 y P5: 2 y P6: 10 y P7: 2 y P8: 8 mo P9: 1 y P10: 3 y P11: 2 y P12: 2 y P13: 2.5 y	P1: 1.5 y P2: 2 y P3: 2 y P4: 0.5 y P5: 2 y P6: 10 y P7: 2 y P8: na P9: 1 y P10: 3y P11: 2y P12: 2y P13: 2.5y	P1: 0.7y P2: 2 y P3: birth P4: 0.7y P5: 1 y P6: 1.5y P7: 2y P8: na P9: 0.5y P10: 3y P11: birth P12: 2y P13: 0.5y	P4, P5, P9 and P10: short stature P1 optic atrophy	na	na	na	Na	na	P1: c.750 G>A P3: c.484 C>T P4: c.196 G>T P8: c.196 G>T P11: c.239insA P12: c.196 G>T P13: c.473C>G	All patients received 25 mg/d; except P12 250 mg/d and P9 and P10 150 mg/d	The anemia and diabetes mellitus responded, but during puberty supplements became ineffective, and almost all patients require insulin therapy and regular blood transfusions in adulthood. There was no evidence that treatment above 25 mg/d offered any additional benefit.
Alzahrani, 2006 [19]	1 patient Arabia Saudi	9 mo	2 y	< 1 y	na	3	na	na	14,000	na	c.514G>C (hom)	Thiamine hydrochloride 100 mg iv BID	After 3 days, platelets and reticulocyte counts started to increase reaching their maximum responses. The response of hemoglobin was slower reaching a maximum level about 2 months of thiamine therapy. HbA1c level decreased from 12% before starting thiamine therapy to 8.6% 5 months later. No noticeable changes occurred in the deafness.
Olsen, 2007 [20]	3 patient Denmark	P1: 10 mo P2: 12 mo P3: 8 wk.	P1: 10 mo P2: 16 mo P3: no	P1: 2 y P2: 12mo P3: 4mo	na	na	na	na	Na	na	c.196G>T (hom)	All patients Thiamine 200 mg/d	Thiamine treatment normalized anemia in all patients, glucose control en 2/3 (1/3 stop insulin treatment); and had no effect on hearing loss

Yesilkaya, 2008[21]	1 patient Turkey	2y	2y	2y	No	7.6	22	0.5	Na	na	c.697C>T (hom)	100 mg/d	Serum thiamine was found to be slightly decreases (22 ug dl^{-1} N: 25 – 75). Diabetes and anemia improved
Tinsa, 2009[22]	1 patient Tunisia	4y	4y	4y	Absent P waves, mitral and tricuspid insufficiency, retinitis pigmentosa, nystagmus, developmental delay, ischemic lesion on brain MRI	6.9	na	0.5	169,000	2,700/426	na	250 mg/d	At the follow up, the thiamine therapy was interrupted and the diabetes reappeared.
Mathews, 2009[23]	1 patient India	10y	7y	No	Retinitis pigmentosa	8,7	na	na	na	Na	na	Thiamine 75 mg/d	Her insulin requirement has decreased and anemia has improved.
Onal, 2009[24]	1 patient Turkey	no	1 mo	no	no	6,2	19,7	1%	21,000	5,200/900	c. 242insA	100 mg intravenous on day 15th	After administration of thiamine, the levels of Hb and platelets began to elevate. Three weeks after thiamine therapy, anemia ameliorated completely
Bergmann, 2009[25]	9 patients P1:Korea P2:India P3:Leban P4: Honduras (2) P5: Italy P6: Caucasian P7: Portugal	P1: 3,5y P2: 10y P3: 6,5 y P4: 5 / 1,5y P5: 11y P6: 6y P7: 6y	P1: 11mo P2: 7y P3: 1y P4: 11 /4 y P5: 1y P6: 7y P7: 1y	P1: 3y P2: normal P3: 2y P4: 5 / 5y P5: 30 y P6: 8mo P7: 6y	na	P1: 9,6 P2: na P3: 5 P4: na P5: 10 P6: 11 P7: 5,4	na	na	na	na	P1: 121G>C (hom) P2: 413G>A /758delT P3: 54_458del GGCATinsTA P4: 515 G>A / 760insT P5: 515G>A / 1002G>A P6: 602C>T /688A>T P7: 1172insCAT	na	P1: off insulin after initiation of thiamine P2: Transfusion-dependent until thiamine and reduced insulin requirement after initiation of thiamine P3: na P4: Megaloblastosis on Thiamine, insulin discontinued after 1 year of thiamine **
Bay, 2010[26]	1 patient/ Turkey	7 mo	5 mo	8 mo	Hepatomegaly	5.8	na	na	164,000	5,200/na	c.242dup (hom)	Thiamine 100 mg/day	The amount of insulin needs was decreased dramatically and insulin was stopped at 15th day of admission. At the end of third week with thiamine treatment, anemia and thrombocytopenia improved completely.
Raju, 2011[27]	3 patients India	P1: 12 mo P2: 2 mo P3: 1 y	P1: 12 mo P2: 2 mo P3: 1 y	P1: 12 mo P2: 2 mo P3: 1y	P3: Secundum atrial septal defect	P1: 4.3 P2: 5 P3: 2.5	na	na	P1: 20,000 P3: 22,000	P1: normal	na	P1: 75 mg/d P2: 150 mg/d P3: 150 mg/d	P1: Diabetes, thrombocytopenia and anemia improved **

Akin, 2011[28]	1 patient Turkey	4 mo	4 mo	20 mo	No	6.6	20.6	1	50,000	7,000	c.566_567delGCinsTCT	100 mg/d	She became deaf at 20 months, even when treatment was initiated at 4 months of life.
Aycan, 2011[29]	1 patient/ Turkey	4mo	na	1y	Atrial standstill	9.3	26	na	329,000	7,000/na	c. 1147delG>T (hom)	Thiamine Hydrochloride (100mg orally daily).	Plasma thiamine concentration was decreased to 20 g/dL (normal range, 25 to 75 g/dL). On the third day of thiamine therapy, all cardiac findings improved. After 4 months his anemia improved. The child gained weight, his height improved and the requirement of insulin decreased.
Shaw-Smith, 2012[30]	5 patients P1: Sudan P2: Arabia Saudi P3: Irak/Kurd P4: UK P5: Kashmiri	P1: at birth P2: 6w P3: 26w P4: 7mo P5: 32 w	P1: 5mo P2: 4w P3: 6mo P4: 5mo P5: at birth	P1: 5 mo P2: 18 mo P3: 6 mo P4: 5y P5: no	P1: stroke at 2y, supraventricular tachycardia P2: short stature P3: severe developmental delay due to meningo-encephalitis at 6 mo. P4: maculopathy P5: spastic quadriplegia, cerebellar atrophy	na	na	na	na	na	P1: c.327_334del P2: c.428C>T P3: c.237C>A P4: c.1001G>A/c. 1148_1 149del P5: c.196G>T	na	na
Habeb, 2012[31]	1 patient/ Saudi Arabia	6y	Normoblastic anemia	Yes, na.	No cardiac defects	na	na	na	na	na	c.428C>T	na	She was started on thiamine at 14 months old which resolved the anaemia and reduced her insulin requirement.
Pichler, 2012[32]	1 patient Austria	10 mo	10 mo		Hepatomegaly	4.7		3		2,500/ 1,000	c.484 C>T / c.1001 G>A	100 mg/d	Within 2 weeks of therapy, reticulocytes rose to 30‰, hemoglobin to 104 g/L, and platelets to 412×109/L, respectively; and within 6 months, the blood counts and HbA1c levels had completely normalized.
Agladioglu, 2012[33]	1 patient Turkey	2y	2y	no	Atrial septal defect	9.2	29.2	na	178,000	na, referred as normal.	c.95T>A (hom)	Thiamine 75 mg/d	Decreased serum thiamine 19.5 g/dl (VR 25-75 g/dl). Normal glucose level 1 week after thiamine treatment was initiated , without insulin administration
Ganie, 2012[34]	1 patient India	23 mo	4y	4y	Patent ductus arteriosus, retinopathy	4.8	na	na	na	na	na	75 mg/d	Diabetes and anemia improved

Ghaemi, 2013[35]	3 patients Iran	P1: 16 mo P2: 3.9 y P3: 1y	P1: 16 mo P2: 3.9 y P3: 6 mo	P1: 6mo P2: 3.9 y P3: 1y	No	P1: 10.1 P2: 11.5 P3: 8.7	na	na	na	na	All patients: c.382G>A	At onset 200 mg/d, later 25 – 50 mg/d	P1 and P3: Anemia and diabetes improved P2: Anemia improved required insulin.
Mozillo, 2013[36]	2 patients Italy	20 - 27 mo	7 y	18 - 20 mo	P1: Retinitis pigmentosa, optic atrophy	7.5 / 11,5	na	na	na	na	c.242insA/ c.1370delT	P1: Thiamine 200 mg/day; P2: 100 mg/day	P1: 25years old: blind, deaf, still on insulin treatment, P2: Blood parameters and glucose control improved after 3 month
Dua, 2013[37]	1 patient India	3y	2y	3y	Patent ductus arteriosus	4	na	na	15,000	3,900/819	c.1002_1004delTGG (hom)	75 mg/d	Anemia and glucose control improved. Anemia recur when thiamine was withdrawn
Setoodeh, 2013[38]	4 patients Persia	P1: 8mo P2: 16 mo P3: 2y P4: 7y	P1: 8mo P2: 3mo P3: 1y P4: 5 y	P1: 10 mo P2: congenital P3: congenital P4: congenital	P1: P2:Seizures, stroke P3: P4:	P1:5.6 P2:8.1 P3:5 P4:7.5	na	P1:0.4 P2:0.6 P3:1.4 P4:1.2	P1: 30,000 P2: 214,000 P3: 320,000 P4: 284,000	P1:4,800 P2:6,400 P3:6,700 P4:8,600	c.697C>T(hom)	P1:100mg P2:300mg P3:300mg P4:100mg	Remarkably effectiveness in correction of anemia, and hemoglobin and hematocrit increased to normal levels within a few Weeks. The thrombocytopenia of P-1 was corrected. There were not improving in the hearing problem. Diabetes in P-1 improved after 2 months and therefore insulin was stopped.
Abdulsalam, 2014[39]	1 patient Iraq	27 mo	27 mo	27 mo	Cardiomyopathy	7.1	na	na	195,000	4,000/1.560	na	na	na
Akbari, 2014[40]	2patients Iran	4y	4y	4y	Ebstein anomaly	3,9	12	1	106,000	10,100/ 6.363	c.697C > T (hom)	P1: Thiamine, doses not reported.	P1: On the four week of thiamine therapy, anemia improved; blood sugar became normal without the administration of insulin. One year later, the hemoglobin concentration was 13 g/dl, hematocrit 40%, and platelets 292,000/mm3.
Wood, 2014[41]	2 patients USA	5 y	19 y	4 y	P1: Myopia, astigmatism, aggressive/assaulting behavior, paranoid ideations	na	na	na	na	na	c.63_71delACCGCTC (hom)	P1: Thiamine initially 100 mg, later decreased to 50 mg P2: na	P1: Thiamine decreased the frequency and severity of his explosive/aggressive episodes P2:Died at 18 y (heart problem)

Srikrupa, 2014[42]	1 patient India	3,5y	3.5y	1y	Leber's Congenital Amaurosis	na	na	na	na	Na	c.314G>A (hom)	25–75 mg/day	Anemia corrected after treatment
Liu, 2014 [43]	1 patient China	No	2 – 8 y	2 – 8 y	No	P1: 5.2 P2:6.4 P3:9.8 P4:7	na	P1: 1.6 P2:2.3 P3:1.8 P4:1.3	na	Na	P1: c.277G>C (hom) P2:c.508A>C (hom) P3:c.1148T>A (hom) P4:c.1322T>C(hom)	P4:na	P4:slightly improved of anemia
Beshlawi, 2014[44]	6 patients Egypt	P1: 23mo P2:20mo P3:9mo P4:no P5: 13mo P6: 18mo	P1: 17mo P2: 19 mo P3:6w P4:10 mo P5: 9mo P6:6 mo	At birth	P3: Uhl cardiac anomaly	P1: 3 P2: 4 P3: 3 P4: 5 P5: 6 P6: 4	na	na	na	na	Deletion of 5,224 bp consisting of 3.7-kb distal part of intron 3, exon 4, intron 4, exon 5, intron 5, and 1.5-kb proximal part of exon 6.	100 mg/day	The death of the two eldest females underscores the potentially lifesaving effect of the early diagnosis and treatment. All treated patients maintained a normal hemoglobin within 1 month of therapy while, their insulin requirements were reduced. Upon doubling the dose to 200mg/day, 2/9 patients were rendered insulin independent.
Tahir, 2015[45]	1 patient Portugal	10 mo	10 mo	13 mo	na	6.5		54	29,000	10,700 / 600	na	75 mg daily	Insulin requirements decreased
Manimaran, 2015 [46]	1 patient India	1.5 y	4y	7mo	Retinitis pigmentosa	na	na	na	Na	na	c.1232delT /ter422 (hom)	na	na
Mikstiene, 2015[47]	1 patient Lithuania	11 mo	2 y	7 mo	Bilateral maculopathy	9.8	na	na	245,000	na / 5,740	c.205G>T (hom)	100 mg/day	Patients showed borderline elevation of plasma branched amino acids Val, Leu, Ile. Marked improved of hematopoiesis, 4 days after treatment initiation with normal erythrocyte, after 1.5 months. The control of glycemia also improved.

na = not available, WBC = white blood cells; Uhl cardiac anomaly = (total absence of right ventricular myocardium with apposition of endocardium and pericardium. N = normal. We do not include patients in Viana et al,[48] 1978; Abboud et al, [49] 1985, Lo Curto et al, [50] 1989, Grill et al 1991, [51] Naeem et al, [52] 2008, Kurtoglu et al, [53] 2008, Bouyahia et al, [54] 2009, Dogan et al, [55] 2013 and Ach et al, [56] 2013. Partial data from Akinci et al, [3] 1993, and Kipioti et al, [16] 2003 were included.

Supplementary Table 2. Literature review of SLC19A3 defects.

Reference	N patient /origin /Age at onset	Phenotype	Clinical features	Biochemical data (blood, urine, CSF)/ PDH/RCC	MRI	SLC19A3 mutations (homozygosis)	Biotin or thiamine therapy (doses)/Timeframe between the onset of encephalopathic episode and thiamine supplementation	Outcome
Ozand, 1998[1] *and Zeng, 2010*[2]	19 Yemen and Saudi 3-14y	**BBGD**	Confusion, dystonia, quadriplegia, cranial nerve palsy, pyramidal signs, tonic clonic and clonic partial seizures, hypertension, chorea, truncal titubation, facial motor dyspraxia, akinetic mute state,	N/-/-	Central necrosis of the head of the caudate bilaterally and complete, or partial, involvement of the putamen	c.68G>T (hom) c.1264A>G (hom)	P1: Thiamine (no reported), L-dopa, carnitine, biotin (5mg/kg/day) – 4mo P2: biotin (5mg/kg/day) - days P3: biotin (5mg/kg/day) – 3 mo P5: biotin (5mg/kg/day) – 1 mo P6: biotin (5mg/kg/day) – 1y P7: biotin (not reported) - days P8: biotin (not reported) - days P9: biotin (5mg/kg/day) – 6mo P10: biotin (5mg/kg/day) - days	P1: improvement with biotin, walking independently 6 months later, and speech reappeared within 1 month, mild left hemiparesis remained, with no rigidity or dystonia, IQ 95. Discontinued biotin at 8y. Within 1 month, she developed grand mal seizures, rigidity and dystonia. IQ 75 P2: asymptomatic P3: improve dramatically within 24 h, walking talking and swallowing P5: Within 72 h, she started to walk and talk P6: for 7 years then, and has had no acute episodes P7: symptoms disappeared promptly within 3 days P8: responded partially P9: marked improvement within days, IQ 74 → IQ 85 P10: Within days, she started to behave normally, talk, walk and regain her memory.
Kono, 2009[3]	2 siblings Japan 2nd decade	**Wernicke's like encephalopathy**	Confusion, ataxia, ophtalmoplegia, partial *Status epilepticus*	N/-/-	High-intensity signals in the bilateral medial thalamus and periaqueductal region on fluid-attenuated inversion recovery images	c.130A>G/c.958G>C	Thiamine (600 → 100 mg/d) - days	High dose thiamine improved the seizures within 24 hours, although the ophthalmoplegia, nystagmus, and ataxia continued for several weeks. Subacute ophthalmoplegia with nystagmus and ataxia occurred repeatedly within several months after the discontinuation of 100 mg of thiamine per day.
Yamada, 2010[4]	4 cousins Japan 1-11mo	**Brain atrophy with basal ganglia and thalamic lesions**	Psychomotor retardation, pyramidal signs, atypical infantile spasms	N/-/-	Cerebral and cerebellar atrophy (4/4), brainstem atrophy (1/4), abnormal signal	c.958G>C (hom)	1 out of 4, biotin (5mg/kg/day) - 8 mo	Neurological symptoms and brain MRI findings did not improve

					in caudate, putamen and thalamus (4/4), none in cortical or subcortical and periaqueductal region			
Debs, 2010[5]	2 siblings Portugal 7-12 y	**BBGD**	Confusion, dystonia, cranial nerve palsy, partial/generalized seizures	N/-/ Normal RCC	Bilateral abnormalities of the putamen and caudate nuclei	c.74dupT/ c.980-14A>G	P1: Biotin 100 mg 3 times daily – 7 days → 600 mg/d – 10 day after admission P2: 300 mg/d for 3 days and then increased to 600 mg/d for 3 subsequent days, not improvement → Thiamine (500 mg iv)	P1: MRI and clinical improvement after biotin 600 mg/d P2: marked and rapid improvement within the following 24 hours of thiamine treatment
Serrano, 2012[6]	2 siblings Spain 4-8 y	**BBGD**	Confusion, dystonia, cranial nerve palsy, partial seizures	Lactate on MRS (1 p.)/ Normal PDH in fibroblasts/ Normal RCC	P1: symmetrical bilateral hyperintense lesions in the striatum and midline nuclei of the thalami and diffuse cortical involvement in a gyriform pattern P2: high T2 signal in the striatum and midline nuclei of the thalamus and a high lactate peak on spectroscopy	c.74dupT/ c.980-14A>G	P1: dystonic storm, requiring intensive general support and treatment with trihexyphenidyl (up to 10 mg 3 times per day iv) and diazepam (50 mg iv) for several days. Thiamine (300mg/d) - 9 y after first episode. P2: Thiamine (150 mg/8 h), 1 year after the first episode	P1: significant clinical improvement within the first days of therapy. A subsequent MRI, 1 month later, showed striatal atrophy and resolution of the widespread cortico-subcortical lesions P2: full cognitive and speech recovery and normal ambulation within 48 hs. Dystonia disappeared completely after 1 mo.
Pérez-Dueñas 2013[7]	1 patient Marocco 1month	**Infantile Leigh syndrome**	Neonatal hyper-lactacidemia, with irritability, opisthotonus, hipertonicity, no seizures	Lactic acidosis ↑ Leu, Ileu and Ala, ↑αKG urine/-/-	Bilateral and symmetric cortico-subcortical lesions involving the perirolandic area, bilateral putamina, and medial thalami	c.68G>T (hom)	Thiamine (100 mg/day), biotin (10 mg/day), and carnitine (300 mg/day) started at onset	Dramatic clinical and biochemical improvement in the hours after initiation of therapy: irritability and feeding difficulties ceased within 24 hours of treatment. Thiamine was maintained at 20 mg/kg/day
Tabarki 2013[8]	10 patients Saudi Arabia 3-12 y	**BBGD**	Subacute encephalopathy (N=9): ataxia, dysarthria, dystonia, spasticity, ophtalmoplegia, progressive dystonia (N=1), rhabdomyolysis and exitus (N=1), seizure (N=8)	Lactate on MRS (1 p.)N blood, urine and CSF/-/-	100% striatum; 70% medial dorsal thalamus; 50% brainstem; 80% cortex; 70% cerebellum; 10% periventricular region of III ventricle Vasogenic (no cytotoxic) edema	c.1264A>G (hom)	Thiamine (100-300mg) and biotin (2-3mg/kg/d)	7 early treated patients: minimal or no sequels (mild dystonia or dysarthria)
Tabarki 2013[9]	1 patient Saudi Arabia 10 y	**BBGD**	4-month history of abnormal gait and dysarthria, acute encephalopathy, dystonia and ophtalmoplegia. No seizures	N Thiamine levels normal /- /-	Cerebellum, BBGG, medial nucleus of the thalamus, cerebral cortex, vasogenic edema	c.1264A>G (hom)	Thiamine and biotin (doses not reported)	Marked improvement during following 4 days and residual gliosis in the basal ganglia
Gerards 2013[10]	3 families from Marroco	**Fatal infantile Leigh syndrome**	Jitteriness, opisthotonus, apnea, seizures, roving eye movements, sunset	Lactic acidosis, ↑ Leu, Ileu and Ala ↑αKG, 2OHGA, GA, succinate and 2-ketoadipic / Family C: ↓	**Leigh-like:** Basal ganglia, thalamus, brainstem, cerebellum	c.20C>A (hom)	Family A and B: no treatment Family C: thiamine	Family C: Thiamine response (1p) and clinical deterioration despite thiamine (1p) (retrospective

	3-5wks		phenomenon, neonatal seizures	oxigen consumption with pyruvate but N with succinate and ascorbate N/↑ PDH and αKGDH activity with TPP and ↓ PDH activity without TPP/ Family A: Slight ↓ C IV muscle (1 p.) Family C: COX-neg. fibers and normal RCC (1 p.)				diagnosis by exome sequencying)
Kevelam,[11] *2013*	7 patients Origin not reported 2-5 mo	**Fatal infantile Leigh syndrome**	Irritability, lethargy, arching posture, regression milestones Infantile spasms, myoclonic jerks, other seizures	↑lactate in blood (3.3-4.6) and CSF (2.3-2.6), ↑pyruvate (28-53) Normal organic acids/ Normal PDH activity and RCC in fibroblasts Normal lactate/pyruvate ratio/ Increased lipid and glycogen in muscle, Slightly low RCC mtDNA/nDNA ratio 61% depleted in muscle	**Leigh-like** Swollen brain progressing to cystic degeneration and atrophy:cerebellum, thalamus, striatum, dentate nuclei, brain stem, cerebral cortex..	c.68G>T/r.1173_1314del c.895_925del (hom) c.541T>C/c.1154T>G c.527C>A/c.507C>G c.1332C>G (hom)	Death (4-58 mo) without thiamine. 2/7 received biotin.	No improvement with biotin alone
Alfadhel,[12] *2013*	18 patients from 13 families Saudi Arabia 14 mo – 23 y	**BBGD**	Subacute encephalopathy, ataxia (n= 18), seizures (n= 13) dystonia (n=12) ,dysarthria (n= 9), quadriparesis and hyperreflexia (n=9)	Normal	Abnormal signal intensity with swelling in the basal ganglia during acute crises (n= 13/13) and atrophy of the basal ganglia and necrosis during follow up (n= 13/13)	c.1264 A>G (hom)	Variable doses: biotin 5 – 10 mg/kg/d, thiamine 200 - 300 mg/d. Most of them, treated at onset or delayed treated (9/18 patients, treated 1 to 8 y after onset). 2 patients died before treatment.	One-third of the present patients showed the recurrence of acute crises while on biotin therapy alone, but after the addition of thiamine, crises did not recur. On follow up, 4 patients died, 2 had spastic quadriplegia, 6 had normal outcome and the rest had speech and motor dysfunctions. P6 received biotin only with recurrence of crises, no thiamin was added until he die at 26 y.
Fassone,[13] *2013*	1 patient Indian 15y	**Leigh-like**	Rapid onset ptosis, ophthalmoplegia, subacute encephalopathy, difficulties in swallowing, episodic vomiting, a sensation of incomplete bladder emptying and constipation	Normal	Symmetrical basal ganglia and mid-brain lesions	c.517A>G (hom)	Initial treatment: thiamine 150 mg and biotin 10 mg → thiamine 100 mg twice a day and biotin 10 mg twice a day	Symptoms improved dramatically the next day. After 6 months of therapy she reported facial twitching and the biotin dose was increased to 100 mg twice a day (5 mg/kg/day).
Distelmaier,[14] *2014*	1 patient Moroccan 6y	**BBGD**	Somnolence, dystonia, seizure and dysarthria	Normal	Symmetric basal ganglia lesions, diffuse cortical and subcortical changes	c.1264G>A (hom)	Biotin (10 mg/kg/day), thiamine (100 mg/day)	Dramatically improvement of symptoms.
Schänzer,[15] *2014*	2 patients German	**BBGD**	P1: somnolence, generalized dystonia, hypoventilation, and epilepsia partialis continua	Organic acids in urine, amino acids and lactate in plasma N	P1: bilateral cystic swelling and increased signal intensities on T2-weighted images of the	c.280T>C (hom)	P1: Biotin (7 mg/kg per day) and thiamine (100 mg per day) P2: not treated	P1: At age of 9 years: Improved alertness and reduced seizure frequency. P2: initially, clinical symptoms

			P2: facial dyskinesia and dysarthria, generalized dystonia and rigidity with hypoventilation, dysphagia and epilepsia partialis continua		putamen and caudate nuclei P2: residual bilateral cystic lesions in the caudate head			resolved completely without specific treatment over 2 weeks. Boy deceased 4 weeks after onset of symptoms in the 2nd episode.
Haack, [16] *2014*	2 patients Turkish	**Leigh syndrome**	irritability, seizures and vomiting	↑lactate in blood Biochemical diagnostics in fresh muscle tissue revealed a globally reduced mitochondrial ATP production rate. However, there was no disturbance of a specific respiratory chain complex. Biochemical investigation of cultured fibroblasts was normal. Laboratory testing revealed increased blood lactate of maximal 7.0 mmol/l (normal range 51.6 mmol/l) and CSF lactate of 4.0 mmol/l (normal range 52.8 mmol/l).	symmetrical basal ganglia and brainstem lesions and lactate peak on magnetic resonance spectroscopy	c.982del (hom)	biotin (10 mg/kg/day) and additional thiamine (15 mg/kg/day)	Died at 2 mo old. This medication clearly improved his clinical status within _2 weeks. Blood lactate dropped to normal levels and seizures, as well as irritability, subsided completely. During medical follow-up, the boy showed adequate developmental progress. Brain MRI at the age of 4 months revealed a substantial regression of lesions in basal ganglia, brainstem, and subcortical regions
Ortigoza-Escobar, [17] *2014*	4 patients (3 previously reported in Pérez-Dueñas (2013) and Serrano (2012) Spanish 13 mo	**BBGD**	Irritability, continuous crying, hypotonia, status dystonicus, ophistotonus, tremor, dysphagia, nystagmus, strabismus, ataxia, weight loss, hepatomegaly, jaundice	Plasma lactate 2,3 mmol/L (normal range 0,7 – 2,2 mmol/L); High excretion of 2-hydroxy acids, isobutyric, 2-hydroxy-isovaleric acid, 2,4-dihydroxybutyric	Bilateral and symmetrical involvement of the putamina and medial thalamic nuclei	c.1079dupT/ c.98014A>G	Thiamine was empirically initiated 6 days before he died (initial doses of 150 mg daily, followed later by 1 g, twice a day).	Patient died.
Tabarki, [18] *2015*	20 patients Saudi Arabia 1 – 12 y	**BBGD**	Subacute encephalopathy, ataxia, seizures, dystonia, mutism, dysarthria,	n.a.	BG, thalami, Brain stem, cerebellar, cortical and subcortical changes	c.1264A>G (hom)	Patients are randomized for treatment either with a combination of biotin (5 mg/kg/day) plus thiamine (40 mg/kg/day), group 1: 10 patients, or thiamine (40 mg/kg/day) alone, group 2: 10 patients.	Comparison of pre- and postreatment BFMDS scores revealed improvement in both groups, however, there is no significant difference between group 1 and group 2 (mean improvement: 71.90% in group 1 and 69% in group 2; p = 0.84). The only difference was the duration of the acute crisis: group 1 had significantly faster recovery (2 days; 1.80 ± 0.63), versus 3 days (2.90 ± 0.87) in group 2; p = 0.005.

Kohrogi, [19] *2015*	1 patient Japan 6 mo	**BBGD**	Irritability, dystonia, respiratory failure	↑lactate in blood (27.6 mg/dl) ↑lactate in CSF (25.4 mg/dl) OA and AA normal	Bilateral and symmetric cortico-subcortical lesion, bilateral putamen and medial thalami, low apparent diffusion coefficient values	c.464C>T/c.1196A>T	Thiamine 100 mg/d Biotin 20 mg/d	When the patient was discharged from the hospital on day 24 after admission, he was able to smile and could recognize his parents. The boy is now 1 year and 1 month old, and is capable of rolling over and sitting down for a while. Although the boy still cannot speak, his development is catching up to other children his age.
Ortigoza-Escobar, [20] *2016*	6 patients (3 previously reported), Spain, Marocco, Sweden 1mo-15y	**BBGD**	n.a.	↑lactate in blood (2/6; 2.3-8.6) ↑lactate in CSF (1/6; 7.1) ↑αKG urine (1/6) ↑ Leu, Ileu and Ala (1/6) Low Free-T CSF (5/6)	n.a.	c.68G>T (hom) c.1264A>G(hom) c.126A>G(hom) c.1079dupT/980-14A>G c.153A>G/c.157A>G c.74dupT/c.980-14A>G	No doses available/3 days – 11 years	Death (1), mild motor delay (1), dystonia (3), normal neuro-development (2), dysarthria (1), spasticity(1), epilepsy (1)

Supplementary Table 3. Literature review of TPK1 defects.

Reference	N patient /origin	Age at onset	Other clinical features associated	Blood lactate RV 0.5-2.2 mmol/l	CSF lactate RV 1.1-2.4 mmol/l	Blood TPP RV 132-271 mmol/l	Organic acid/Other studies	TPK1 mutations	Type of mutation	Thiamine therapy
Mayr, 2011[1]	P1	>1y	Developmental delay, Lethargic, hypotonic and lost ability to walk. Encephalopathy	3.5	2.7	n.a	-lactic acidosis -Elevated 2-KGA	c.[148A>C]; [501+4A>T] p.[Asn50His]; [Val119_Pro167 del]	-The splice-site mutation decreases the splice efficiency of exon 7 -Missense mutation	-n.a -Died at 8 1/2y
Mayr, 2011[1]	P2	18mo	Truncal ataxia, unable to walk Mild dystonia of the upper limbs	1.3	2.4	n.a	-Elevated 2-KGA			-Thiamine 100–200 mg/day -Died at 3 1/2y
Mayr, 2011[1]	P3	>3y	Spasticity, progressive dystonic movement disorder. Lost the ability to speak and developed a symptomatic epilepsy	1.5 to 4	n.a	68	-Elevated 2-KGA and 3-hydroxyisovaleric acid	c.119T>C. (p.Leu40Pro)	-Missense mutation	-Thiamine 100 mg/day initially, then 200 mg/day -Fat-rich diet
Mayr, 2011[1]	P4	>3y	Progressive dystonia and had severe difficulties in walking	2.3 to 4.6	n.a	50.4	-Elevated 2-KGA			-Thiamine 100 mg/day initially, then 200 mg/day -Fat-rich diet
Mayr, 2011[1]	P5	2y	Dizziness and vertiginous, gait disturbance. Intermittent gait ataxia. Lost speech, a seizure with clonic jerks of his arms and gaze, dysarthria, intention tremor, confusion, episodic ataxia, ophthalmoplegia, nystagmus	4.4	3.3	96.9	n.a	c.[179_182delGAGA]; [656A>G] p.[Arg60LysfsX52]; [Asn219Ser]	-frameshift mutation -Missense mutation	
Fraser, 2014[2]	P6 Chinese descent	7mo	Developmental delay and hypotonia Intractable seizures with rapid progression to coma	n.a	n.a	n.a	-Elevated lactic acid, 2-KGA and fumaric acid	c.604T>G. (p.Trp202Gly)	Missense mutation In the thiamine binding domain	Died at 29mo
Fraser, 2014[2]	P7 Chinese descent	4mo	Hypotonia and a decreased level of alertness	n.a	n.a	n.a	-Elevated 2-KGA and glutaric acid. Mild elevation of lactic acid			-Thiamine 30mg/kg/day, biotin, niacin and alpha-lipoic acid. -Ketogenic diet
Banka, 2014[3]	P8 Indian descent	30mo	Lethargy, loss of ability to walk, brisk deep tendon reflexes and mild past pointing	normal	normal	60.9	-Elevated dicarboxylic acid and 2-KGA -Relatively lower activity of complex II	c.479C>T (p.Ser160Leu)	-Missense mutation -Prevents dimerization of the TPK enzyme	Thiamine 500mg /day
Banka, 2014[3]	P9 German descent	10mo	Muscle hypotonia Hypertonia of the extremities No speech development	3.9	n.a	85.4	-Elevated 2-KGA and 3, 4-dihydroybutryate -Decreased pyruvate oxidation in substrate oxidation analysis	c.664G>C p.Asp222His	-Missense mutation -Close to the binding site for thiamine	n.a

na = not available, 2-KGA – 2-ketoglutaric acid, CSF - cerebrospinal fluid, TPK – thiamine pyrophosphokinase; TPP – thiamine pyrophosphate

Síntesis de resultados

- En la revisión de literatura de todos los defectos genéticos del transporte y metabolismo de la tiamina se han incluido un total de 107 publicaciones.
- Los defectos genéticos del metabolismo y el transporte de tiamina pueden presentarse con los siguientes fenotipos: (i) *SLC19A2*, síndrome de anemia megaloblástica sensible a tiamina (TRMA); (ii) *SLC19A3*, enfermedad de los ganglios basales que responde a tiamina y biotina, síndrome de Leigh, espasmos infantiles con acidosis láctica y encefalopatía de Wernicke; (iii) *TPK1*, síndrome de Leigh; y (iv) *SLC25A19*, microcefalia de tipo Amish o necrosis estriatal bilateral con polineuropatía progresiva.
- Teniendo en cuenta estos fenotipos se recomienda la suplementación empírica con vitaminas (tiamina y biotina) en todos los pacientes con síndrome de Leigh. La administración de tiamina también es aconsejable en pacientes con una combinación de, al menos dos de los siguientes: diabetes mellitus, anemia megaloblástica y pérdida auditiva sensorio-neural.
- Las dosis de tiamina varían según el defecto genético: para los defectos de *SLC19A2*, la dosis habitual es de 25-200 mg/día (1-4 mg/kg/día), para *SLC19A3*, 10-40 mg/kg/día, *SLC25A19*, 400 mg/día y para *TPK1*, 30mg/kg/día.
- La evidencia recogida hasta ahora sugiere que la administración de tiamina mejora el resultado en los pacientes con *SLC19A2*, *SLC19A3*, *SLC25A19* con fenotipo de necrosis estriatal con neuropatía periférica y algunos pacientes con defectos de *TPK1*, por lo que la mayoría de los esfuerzos deben estar dirigidos a un diagnóstico precoz de estos trastornos.

- La mayoría de los paciente con mutación del gen *SLC19A2* tratados muestran una mejora significativa de la hematopoyesis y del control de la glucemia. Sin embargo, la suplementación con tiamina no previene la sordera neurosensorial.
- Los pacientes con mutación del gen *TPK1* pueden beneficiarse del tratamiento combinado de biotina, niacina, ácido alfa-lipoico y dieta cetogénica.
- Los pacientes con mutación del gen *SLC25A19* y fenotipo de microcefalia de tipo Amish no responden al tratamiento con tiamina, pero mejoran los episodios de acidosis metabólica con la administración de dieta cetogénica.
- La suplementación con tiamina y biotina es segura. Solo se han reportados casos aislados de anafilaxia leve tras la administración endovenosa.

Thiamine transporter-2 deficiency: outcome and treatment monitoring.

"Deficiencia del transporte de tiamina de tipo 2: seguimiento y monitorización del tratamiento"

Orphanet J Rare Dis. 2014 Jun 23;9:92.

Ortigoza-Escobar JD, Serrano M, Molero M, Oyarzabal A, Rebollo M, Muchart J, Artuch R, Rodríguez-Pombo P, Pérez-Dueñas B.

Son escasas las características clínicas que distinguen la deficiencia de hTHTR2 tratable de otras causas devastadoras de síndrome de Leigh. En este trabajo se ha realizado el seguimiento clínico, bioquímico y radiológicos de cuatro niños con deficiencia de hTHTR2 con fenotipo de síndrome de Leigh y de BTRBGD tras la suplementación con tiamina y biotina. Uno de nuestros pacientes presentó hiperlactacidemia persistente, por lo que se sospechó baja adherencia al tratamiento. Uno de los objetivos de este trabajo fue el desarrollar un biomarcador para monitorizar la adecuada suplementación con vitaminas. Para ello, se establecieron valores de referencia de tiamina en sangre total de pacientes controles y se compararon con los valores de pacientes con deficiencia de hTHTR2 suplementados con tiamina. Así mismo, en este trabajo se comparan los resultados clínicos y radiológicos de estos pacientes con los resultados de otros 69 pacientes con deficiencia de hTHTR2 reportados en la literatura. Con todo ello, se alcanzó una mejor definición de la enfermedad.

Ortigoza-Escobar et al. Orphanet Journal of Rare Diseases 2014, 9:92
http://www.ojrd.com/content/9/1/92

ORPHANET JOURNAL OF RARE DISEASES

RESEARCH Open Access

Thiamine transporter-2 deficiency: outcome and treatment monitoring

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Abstract

Background: The clinical characteristics distinguishing treatable thiamine transporter-2 deficiency (ThTR2) due to *SLC19A3* genetic defects from the other devastating causes of Leigh syndrome are sparse.

Methods: We report the clinical follow-up after thiamine and biotin supplementation in four children with ThTR2 deficiency presenting with Leigh and biotin-thiamine-responsive basal ganglia disease phenotypes. We established whole-blood thiamine reference values in 106 non-neurological affected children and monitored thiamine levels in *SLC19A3* patients after the initiation of treatment. We compared our results with those of 69 patients with ThTR2 deficiency after a review of the literature.

Results: At diagnosis, the patients were aged 1 month to 17 years, and all of them showed signs of acute encephalopathy, generalized dystonia, and brain lesions affecting the dorsal striatum and medial thalami. One patient died of septicemia, while the remaining patients evidenced clinical and radiological improvements shortly after the initiation of thiamine. Upon follow-up, the patients received a combination of thiamine (10–40 mg/kg/day) and biotin (1–2 mg/kg/day) and remained stable with residual dystonia and speech difficulties. After establishing reference values for the different age groups, whole-blood thiamine quantification was a useful method for treatment monitoring.

Conclusions: ThTR2 deficiency is a reversible cause of acute dystonia and Leigh encephalopathy in the pediatric years. Brain lesions affecting the dorsal striatum and medial thalami may be useful in the differential diagnosis of other causes of Leigh syndrome. Further studies are needed to validate the therapeutic doses of thiamine and how to monitor them in these patients.

Keywords: Thiamine transporter 2 deficiency, Biotin responsive basal ganglia disease, SLC19A3, Leigh syndrome, Lactic acidosis, Thiamine, Biotin, Striatal necrosis, Dystonia

Resumen

Antecedentes: Las características clínicas distintivas del déficit tratable del trasportador de tiamina tipo 2 (ThTR2) debido a defectos genéticos del *SLC19A3* de las otras causas devastadores del síndrome de Leigh son escasas.
(Continued on next page)

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Métodos: Presentamos el seguimiento clínico después de la administración de suplementos de tiamina y biotina a cuatro niños con deficiencia ThTR2 que presentaban fenotipos de *biotin-thiamine responsive basal ganglia disease* y síndrome de Leigh. Hemos establecido valores de referencia de tiamina en sangre total en 106 niños sin patología neurológica y monitorizamos los niveles de tiamina en pacientes con mutación del *SLC19A3* después del inicio del tratamiento. Hemos comparado nuestros resultados con los de 69 pacientes con deficiencia ThTR2 después de una revisión de la literatura.

Resultados: Al momento del diagnóstico , los pacientes tenían entre 1 mes a 17 años, y todos ellos mostraron signos de encefalopatía aguda, distonía generalizada, y lesiones cerebrales que afectan el cuerpo estriado dorsal y el tálamo medial. Un paciente murió de septicemia, mientras que el resto de pacientes evidenciaron mejoras clínicas y radiológicas poco después del inicio de la tiamina. Al seguimiento, los pacientes recibieron una combinación de tiamina (10–40 mg/kg/día) y biotina (1–2 mg/kg/día) y se mantuvieron estables, aunque con distonía y dificultades del habla residual. Después de establecer valores de referencia para los diferentes grupos de edad, la cuantificación de tiamina en sangre total demuestra ser un método útil para el seguimiento del tratamiento.

Conclusiones: La deficiencia ThTR2 es una causa reversible de la distonía aguda y síndrome de Leigh en la edad pediátrica. Las lesiones cerebrales que afectan el cuerpo estriado dorsal y tálamo medial pueden ser útiles en el diagnóstico diferencial de otras causas de síndrome de Leigh. Se necesitan más estudios para validar las dosis de tiamina y la monitorización terapéutica de estos pacientes.

Background

Acute encephalopathy with bilateral striatal necrosis in childhood includes several disorders of infectious, autoimmune, metabolic and genetic origin [1-5]. One of these diseases is thiamine transporter-2 deficiency (ThTR2, OMIM#607483), a recessive inherited defect due to mutations in the *SLC19A3* gene that cause acute and recurrent episodes of encephalopathy with dystonia, seizures and brain injury that respond extremely well to the early administration of thiamine and biotin [6-20]. However, biochemical or neuroimaging criteria for diagnosis are not available, and timely and effective treatment relies on a high index of clinical suspicion. Of particular interest is the distinction of ThTR2 from other untreatable causes of Leigh syndrome, such as defects in the nuclear and mitochondrial genes encoding components of the oxidative-phosphorylation system or the pyruvate metabolism, causing a devastating disorder with similar clinical and radiological features in the pediatric age.

We aim to describe the phenotypes of four children with mutations in the *SLC19A3* gene, comparing their clinical, biochemical, radiological and genetic data with all of the formerly reported patients and discussing the possible clinical and radiological clues for the distinction of ThTR2 from other causes of irreversible basal ganglia necrosis, especially Leigh syndrome. Moreover, we report the follow-up after thiamine and biotin supplementation and the utility of monitoring whole-blood thiamine concentrations.

Methods

Patients

Four children with *SLC19A3* gene mutations were diagnosed at a tertiary university children's hospital (Hospital Sant Joan de Déu, University of Barcelona) during the past 4 years.

Patients 1, 3 and 4 were diagnosed at the onset of acute encephalopathy and received early treatment with thiamine and biotin. A clinical description of these patients at diagnosis has been previously reported [11,19]. Patient 2 was identified by mutation screening for the *SLC19A3* gene in 11 children with Leigh syndrome who had normal respiratory chain enzyme analyses. Leigh patients were previously analyzed for mitochondrial DNA mutations and for candidate nuclear genes associated with Leigh syndrome, all with negative results. The patients were evaluated using a standardized protocol, including a complete physical and neurological examination and biochemical studies at diagnosis, at 4 weeks and every 6 months after the onset of encephalopathy. Brain MRIs were performed at diagnosis and at 6 to 12 months after the initiation of treatment. Samples were obtained in accordance with the Helsinki Declaration of 1964, as revised in October 2013 in Fortaleza, Brazil. Ethical permission for the studies was obtained from the Research & Ethics Committee of the Hospital Sant Joan de Déu.

Neuroimaging

MRI examinations were performed on a 1.5-T magnet system (Signa Excite HD, Milwaukee, WI, USA), obtaining a sagittal T1-weighted, axial fast-spin echo with fluid-attenuated inversion recovery (FLAIR) and T2-weighted imaging. Diffusion weighted imaging was performed in patient 1. Two pediatric neuro-radiologists reviewed the images.

Laboratory studies

Blood concentrations of lactate and pyruvate were measured by standard automated spectrometric procedures.

Plasma amino acids and urinary organic acids were analyzed following previously reported procedures [21,22].

The concentration of thiamine and its metabolites (thiamine monophosphate (TMP) and thiamine diphosphate (TDP)) were analyzed in whole-blood EDTA samples by high-performance liquid chromatography (HPLC) with fluorescence detection (Perkin Elmer, series 200, Norwalk, CT, USA) according to a modified reported procedure [23]. Whole-blood thiamine, TMP and TDP reference values were established in 106 children (59% males) referred to our hospital for minor surgical interventions. Exclusion criteria were the presence of chronic diseases, malnutrition and special diets. Whole-blood thiamine was quantified in patients 1, 2 and 3 at 6 months and 12 months after the onset of treatment.

Molecular analysis of the *SLC19A3* gene

Genomic DNA from the blood samples of 11 patients with Leigh syndrome were used for the mutation analysis of the *SLC19A3* gene (RefSeq accession number NM_025243.3_ [mRNA]). The coding region and the flanking intron-exon boundaries were PCR amplified with primers based on the Ensembl genome browser entry ENSG00000135917. The amplicons were sequenced and analyzed as previously described [24]. The mutation nomenclature used follows that described at http://www.hgvs.org./mutnomen/.

Systematic review of the literature

We searched MEDLINE (through PubMed) using the following keywords: #1 SLC19A3, #2 thiamine transporter-2, #3 Leigh encephalopathy, #4 ThTR2 and #5 biotin responsive basal ganglia disease. The number of hits at 02/01/2014 was 50, 190, 44, 5, and 14, respectively. A total of 15 clinical studies (4 case reports, 11 quantitative series) and 1 guideline/clinical practice proposal were finally selected [6-20].

Results

Patients

Table 1 summarizes the clinical, biochemical and genetic data of the four patients with *SLC19A3* defects.

Four patients suffering *SLC19A3* mutations had no relevant family history for neurological diseases and were normally developing children until the onset of symptoms (mean age 3 years, range 1 month - 8 years).

Table 1 The clinical, biochemical and genetic data of the four patients with thiamine transporter-2 deficiency

Patients	1	2	3	4
Origin	Morocco	Spain	Spain	Spain
Mutation *SLC19A3* gene	c.68G > T in homozygosis	c.1079dupT/ c.980-14A > G	c.74dupT/ c.980-14A > G	c.74dupT/ c.980-14A > G
Phenotype	Leigh syndrome	Leigh syndrome	BTBGD	BTBGD
Sex and Onset	Male, 1 month	Male, 13 months	Female, 4 years	Male, 15 years
Encephalopathy	Lethargy, vomiting	Irritability, continuous crying	Agitation, lethargy	Agitation, coma
Extrapiramidal features	Hypotonia, jitteriness, dystonia, opisthotonus, tremor	Hypotonia, status dystonicus, ophistotonus, tremor	Paroxysmal dystonia, generalized dystonia, tremor	Status dystonicus, akinetic-rigid syndrome, tremor
Cranial nerves	Dysphagia	Dysphagia, nystagmus, strabismus	Anarthria, dysphagia	Nystagmus, ptosis, diplopia, dysarthria, vertigo, facial dyskinesias/hyposthesias
Others	Pyramidal signs	Ataxia, weight loss, hepatomegaly, jaundice	None	Pyramidal signs, rabdomyolisis, dysautonomia, generalized seizures
Plasma Lactate (RR 0,7 – 2,4 mmol/L)	8.6	2.3	1.6	1.2
Plasma Pyruvate (RR 0,03-0,1 mmol/L)	0.14	0.1	0.21	0.13
Lactate/Pyruvate ratio (RR 11–30)	19.1	23.4	11.5	18.2
Alpha Alanine (RR 167 – 439 μmol/L)	637	355	300	370
CSF Lactate (RR 1,1 – 2,2 mmol/L)	7.1	1.7	Not performed	1.8
Organic acid analysis in urine	High excretion of alpha-ketoglutarate (11463 mmol/mol creatinine)	High excretion of 2-hydroxy acids, isobutyric, 2-hydroxy-isovaleric acid, 2,4-dihydroxybutyric	Normal	Normal

BTBGD: Biotin-thiamine responsive basal ganglia disease.

A trigger condition before the neurological episodes was identified in patients 2 and 4 (gastroenteritis, trauma, strong physical exercise and an upper respiratory tract infection).

All of the patients showed signs of encephalopathy and focal or generalized dystonia. In all of the cases, dystonia progressed to be generalized, and 2 patients had associated *opisthotonus*. Patients 2 and 4 suffered *status dystonicus* and were transferred to the intensive care unit for profound sedo-analgesia. Patient 2 received diazepam, levomepromazine and chlorpromazine, followed by midazolam from day 27 to day 34, when he died. Patient 4 received trihexyphenidyl and diazepam for several days until the spasm and posture were under control.

Other clinical features at onset of the disease are reported in Table 1.

Clinical improvement was evidenced shortly after the initiation of thiamine in patients 1, 3 and 4 (the daily doses varied from 15 to 30 mg/kg/day and were given orally in two or three divided doses), combined with biotin in patient 1 (10 mg/day). Patient 2 suffered septicemia caused by *Enterobacter cloacae* and hepatic and cardiac failure and died 34 days after admission. Thiamine was empirically initiated 6 days before he died (initial doses of 150 mg daily, followed later by 1 g, twice a day).

Patient follow-up

Currently, patients 1, 3 and 4 are 25 months, 8 years and 23 years old, respectively. The median follow-up of these patients is 57 months (range 22 – 99 months). As of the last visit, they are receiving a combination of thiamine (10 – 40 mg/kg/d) and biotin (1 – 2 mg/kg/d) (Table 1), and they remain stable under this treatment and have not suffered any new episodes of encephalopathy.

Patient 1 developed independent gait at 19 months, and on his last examination at the age of 25 months he was walking, with occasional falls due to gait-induced dystonia. He plays and eats independently, but upper limb dystonia and thumb adduction partially interfere with his fine motor skills. He has oro-mandibular dystonia and expressive language delay, but his language comprehension and cognitive skills are in the average range for his age. A physical examination also showed pyramidal signs in the lower limbs. He has begun an intensive physiotherapy program.

Patient 3 is asymptomatic at 8 years old, and her neurological examination is normal. She attends a normal school and achieves good academic performance. She developed a nephrotic syndrome at 6 years old that was responsive to oral corticosteroids.

Patient 4 is 23 years old and has mild dysarthria and dysphagia. He shows intermittent facial dyskinesia and eye-blinking, as well as dystonic posturing of his right

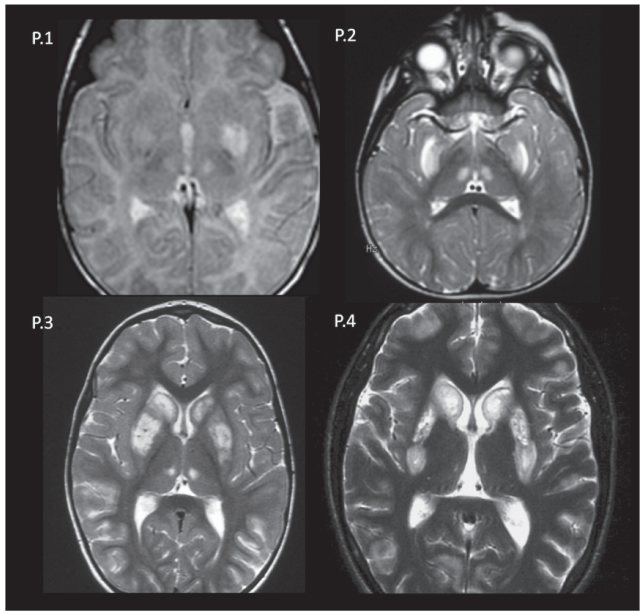

Figure 1 Axial T2 FSE demonstrated bilateral and symmetrical involvement of the putamina and medial thalamic nuclei in patients 1 (P.1), 2 (P.2), 3 (P.3) and 4 (P.4). In patients 2, 3 and 4, the head of the caudates were also affected.

upper limb and left foot. He has some difficulties with activities that require fine motor skills, such as buttoning, tying shoes or opening bottles. He is engaged in gainful employment and exercises regularly.

Neuroimaging

The brain MRIs of the four patients in the acute phase showed lesions in both the dorsal striatum and the medial thalamic nuclei (Figure 1). Putamen involvement was diffuse in patients 2, 3 and 4 and was limited to the posterior region in patient 1. A concentric lesion of the head of the caudate was observed in patients 2, 3 and 4. There was variable cortical and subcortical involvement of the hemispheres: in patient 1, lesions had a peri-rolandic distribution; in patients 2 and 4, they were patchily distributed across both cerebral hemispheres. Diffusion weighted imaging was performed in patient 1 showing low ADC values in the putamina and peri-rolandic cortex. High lactate peaks were detected in patients 2 and 4 on MR spectroscopy.

The follow-up MRIs performed at age 6 months (patient 1), 7 years (patient 3) and 20 years (patient 4) showed an improvement in the signal abnormalities in all of the patients (Figure 2). Residual abnormal signal intensity and volume loss were observed in the putamen (patients 1, 3 and 4) and head of the caudate (patients 3 and 4). The cortical and subcortical lesions disappeared in patient 3, but volume loss was observed at the peri-rolandic region in patient 1 (Figure 2).

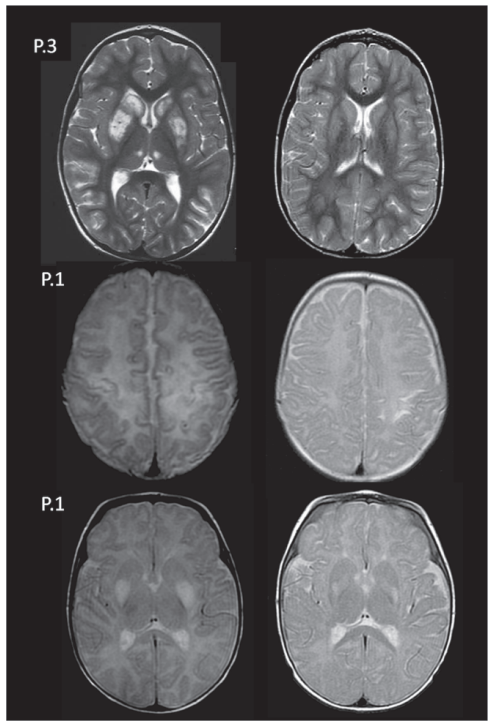

Figure 2 Axial T2 FSE of patient 3 (P.3) at the level of the basal ganglia and of patient 1 at the level of the peri-rolandic region (P.1) and the basal ganglia (P.1) before and after treatment. There is a dramatic improvement of the lesions after thiamine supplementation.

Laboratory studies

The biochemical analysis at diagnosis showed high lactate levels in patient 1 (Table 1). Patient 2 had normal lactate concentrations until he presented with septicemia, when lactic acid increased to 16 mmol/L. Alanine was increased only in patient 1. Organic acids showed high excretion of alpha-ketoglutarate in patient 1 and mild excretion of 2-hydroxy acids, isobutyric, 2-hydroxy-isovaleric acid and 2,4-dihydroxybutyric in patient 2.

The analysis of thiamine, TDP and TMP isoforms in whole-blood samples in the control patients showed that the TDP isoform represented 85% of the whole thiamine concentration. Therefore, TDP values were used for treatment monitoring. The reference whole-blood TDP values were stratified into two age groups, as a statistically significant negative correlation was observed between whole-blood TDP values and the age ($r = -0.290$; $p = 0.003$) (Figure 3).

On the last follow-up visit, Patient 3 was taking biotin (2 mg/kg/day) and thiamine (10 mg/kg/day) and patient 4 was taking biotin (2 mg/kg/day) and thiamine (15 mg/kg/day). Both patients had TDP values above the upper limit of our reference range (Figure 4). Patient 1 was receiving biotin (1.2 mg/kg/day) and thiamine (40 mg/kg/day), but his TDP concentrations did not reach the upper limit of the age reference range. Persistent lactic acidemia (mean 2.69 mmol/L, range 2.1–3.46) was detected in the follow-up of this patient.

Molecular studies

A Sanger sequencing of the *SLC19A3* gene in patients 1, 3 and 4 had identified missense, small duplication and splicing mutations, all of which were carried either in a homozygous or heterozygous fashion [11,19]. The mutation analysis in Patient 2 disclosed two different changes, both of which created premature stop codons in the ThTR2 protein sequence. One of the changes was the previously described c. 980-14A > G, and the other change was the novel duplication, c.1079dupT, with a predictable effect on the protein of p.Leu360Phefs*38. A schematic representation of the *SLC19A3* mutations present in our four patients and in all previously reported patients is shown in Figure 4.

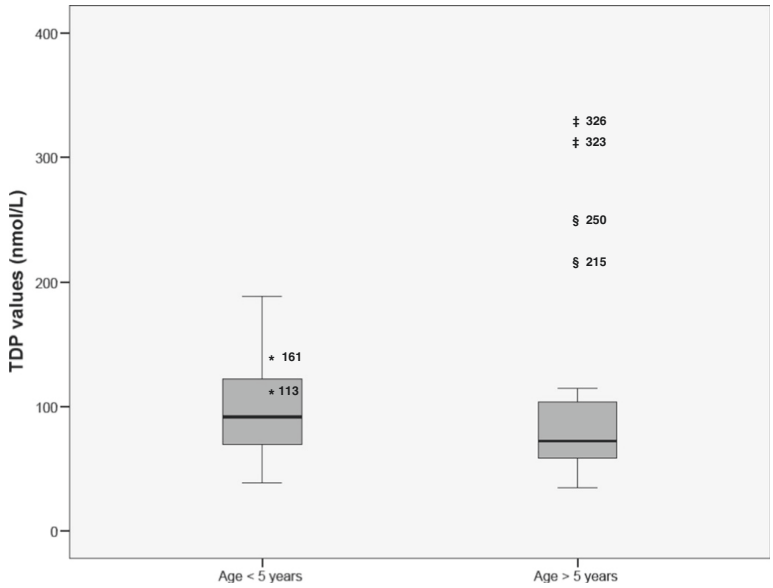

Figure 3 Box-plot representations of the whole-blood TDP concentrations divided into two intervals: < 5 years (n = 67): 90.3 nmol/L (38.8-188.4) (median, range); > 5 years (n = 39): 68.8 (34.2-114.8) (median, range). The Mann–Whitney U test showed significantly different values for TDP when comparing both groups (U = 731, p < 0.001). The TDP values of patients 1(*), 2 (‡) and 3 (§) under thiamine treatment are also represented in the figure. The length of the boxes indicates the interquartile space (P25-P75), the horizontal line represents the median (P50), and the bars indicate the range.

Review of the literature

A summary of the clinical data from the literature review in 69 patients is reported in Table 2. Most of the patients (80%) presented with symptoms before the age of 12 years (age onset 3.5 ± 4.6 years (mean ± SD), range 1 month – 20 years). The patients exhibited the following phenotypes: biotin-thiamine responsive basal ganglia disease (BTBGD) (N = 46), Leigh encephalopathy (N = 23) and Wernicke encephalopathy (N = 2). Figure 4 shows the age at onset of all of the formerly reported patients, as well as their clinical phenotypes and related genotypes.

In regard to treatment, there were some reports of using biotin alone (N = 2) [6,9,10], thiamine alone (N = 3) [8,13], and biotin combined with thiamine (N = 5) [10,11,14,16, 18,19]. In two studies on Leigh patients, the diagnosis was performed retrospectively, and treatment with vitamins was introduced late in the evolution of symptoms in a few patients, with very poor outcomes [12,13].

Discussion

We describe four patients with ThTR2 deficiency presenting with acute encephalopathic episodes and generalized dystonia between 1 month and 15 years of age. Their dystonia was improved when each of them were administered thiamine, with the exception of the patient treated late in the evolution of the disease. The data from the previously reported patients with *SLC19A3* mutations showed that either focal or generalized dystonia, in combination with decreased consciousness and seizures, were the most common clinical features at onset and were reported in more than fifty percent of the patients [6-20], reflecting that ThTR2 deficiency is an important cause of reversible dystonia in children. Hence, a trial with thiamine should be indicated in every case of acute dystonia. Patients also presented with other less common extrapyramidal and pyramidal features, cranial nerve palsy, dysautonomia, rhabdomyolisis, jaundice and other systemic symptoms.

The literature review showed that most patients with *SLC19A3* mutations experienced an onset of the disease between 1 month and 12 years of age. Two-thirds of the patients were classified as BTBGD and the remaining patients were classified as having Leigh and Wernicke encephalopathies. However, there is probably a clinical continuum among patients that, in view of the reported mutational spectrum, appears to be biologically more plausible. In fact, patient 2 with Leigh syndrome in our series carried the mutation c.980-14A > G, which has been previously described in children with a BTBGD phenotype [10,11], and patient 1 who also presented with infantile lactic acidosis and Leigh syndrome harbored the mutation c.68G > T, which has been previously

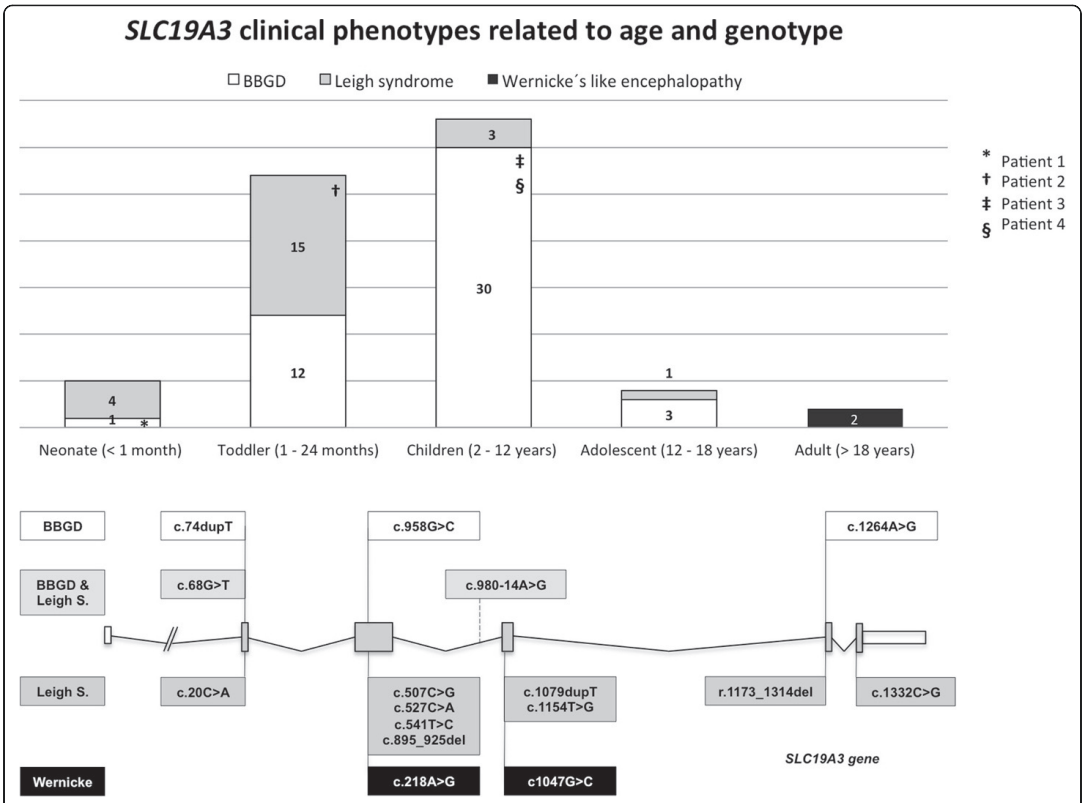

Figure 4 The upper figure shows a distribution of the age at onset in all reported patients with ThTR2 deficiency and the related clinical phenotypes. The lower figure shows a schematic representation of all reported *SLC19A3* mutations and the related clinical phenotypes.

associated with BTBGD [7]. We also observed that patients with the same mutations had different ages at onset (e.g., c.1264A > G [14,15] and c.20C > A [13]). Considering the dense genetic interaction network that sustains a disease phenotype, it is probable that a combination of yet unknown genetic and environmental factors may be responsible for the different age presentations and related phenotypes [25,26].

Despite the genetic heterogeneity of our patients and the wide age range of disease at onset, all of them presented symmetrical involvement of the dorsal striatum and medial thalamic nuclei. In the older patients, the head of the caudate was always affected, and the cortical and subcortical lesions showed a diffuse and patchy distribution in the cerebral hemispheres [14,15]. In the newborn, the caudate was not affected, and there was a selective involvement of the peri-rolandic area [19]. The differences in the distribution of the brain lesions observed in our patients probably depend on the regional variations in the energetic demands according to the different ages. Although this pattern of brain lesions may not be specific, it can be useful in suggesting the diagnosis of a *SLC19A3* defect. In line with our results, the literature review showed that the most frequent brain areas involved in ThTR2 deficient patients were, in order of frequency, the caudate, putamen and thalamus, followed by the cerebellum, brainstem and cerebral hemispheres [6-20].

Regarding the biochemical findings, lactic acidemia and high excretion of organic acids were detected during acute metabolic decompensations in two infants in our series. Thiamine is an essential cofactor of 3 mitochondrial enzymes: pyruvate dehydrogenase complex, alpha-ketoglutarate dehydrogenase, and branched-chain alpha-keto acid dehydrogenase. These enzymes are involved in the oxidative decarboxylation of pyruvate, alpha-ketoglutarate, and branched chain amino acids, respectively. The biochemical abnormalities detected in our patients could be due to the decreased activity of these thiamine-dependent mitochondrial enzymes [19]. The older children showed normal biochemical analyses for plasma, urine and CSF, and lactic acid accumulation was

Table 2 The clinical and radiological features of patients with thiamine transporter-2 deficiency reported in the literature

	Number of patients		Number of patients
Patients	69	Dead patients	23
Age (years ± SD)	3.5 ± 4.3	**Symptoms at follow up**	
Male/Female	36/33	Tetraparesia/Dystonia	32
Trigger events	40	Cognitive impairment	23
Symptoms at onset		Dysphagia	13
Encephalopathy/Lethargy	57	Epilepsy	11
Seizure	47	Dysarthria	10
Generalized and focal dystonia	38	Respiratory support	4
Dysarthria/Anarthria	28	Ataxia	3
Ataxia	25		
Dysphagia	21	**MRI**	
Pyramidal signs	19	Caudate	55
Abnormal ocular movement	17	Putamen	55
Developmental delay	12	Thalami	31
Opisthotonus	11	Cerebellum	22
Rigidity/Rigid akinetic syndrome	11	Brainstem	19
Tremor	4	Subcortical WM	16
Chorea	2	Cerebral cortex	13
Jitteriness	2	Globus pallidus	8
Dystonic status	2	Medulla	3
Dysautonomia	2	Lactate on spectroscopy	6
Ptosis	2		

The table shows a list of signs and symptoms at onset and at follow-up, as well as MRI abnormalities. Seizures include myoclonic jerks, epileptic spams, focal and generalized seizure, *epilesia partialis continua* and *status epilepticus*. Abnormal ocular movements include nystagmus, oculogyric crisis, oculomotor nerve palsy, ophtalmoplegia and sunset phenomenon. Symptoms reported only once: rhabdomyolisis, facial dyskinesia. SD: Standard Deviation.

detected only on MR spectroscopy. Similarly, other authors have described high amounts of lactic acid in the serum and high excretion of organic acids in the urine of patients with fatal infantile Leigh phenotypes (Table 1) [12,13] but normal biochemical profiles in children classified as having BTBGD phenotypes [8-10,14].

We observed a dramatic response to high doses of thiamine in the three patients who were treated during the first days of encephalopathy. Clinical follow-up showed a complete clinical and radiological recovery in one patient, but the other two patients showed residual dystonia, speech difficulties, and necrotic changes in the dorsal striatum and the frontal cortex.

Even though thiamine was initiated four weeks after the onset of symptoms, patient 2 died at 14 months of age. In other reported cases of fatal infantile Leigh and *SLC19A3* defects, the lesions progressed to cystic degeneration and severe atrophy, suggesting that the prognosis of these patients is poor and largely depends on the early administration of biotin and thiamine [8,10,15]. Although *SLC19A3* deficiency is considered to be a treatable entity, the literature review showed that sixty percent of previously reported patients with either BTBGD or a Leigh phenotype had poor outcomes, including early death, tetraparesis, dystonia or cognitive impairment. At the other end of the spectrum, patients with Wernicke encephalopathy showed lesions that selectively affected the periaqueductal grey matter, which disappeared when thiamine was initiated [8].

When establishing the whole-blood thiamine reference values, we found that TDP was the most concentrated thiamine isoform, similar to other studies [23,27]. For this reason, treatment monitoring relied on whole-blood TDP concentrations. Patients treated with 10 to 40 mg/kg/day of thiamine were clinically stable for a mean follow-up of 57 months. At these doses, TDP levels remained above the upper limit of the reference values in patients 3 and 4. Conversely, in patient 1, the TDP concentrations remained in the reference range, and he presented persistent acidosis. These data led to the suspicion of poor family adherence to the treatment, which was confirmed and corrected with the participation of a social worker in the follow-up program. This patient did not present any clinical relapse, even though lactic acid concentrations were persistently elevated, perhaps due to the absence of relevant trigger factors during follow-up.

Currently, there is no agreement in the long-term doses of vitamins that should be administered in *SLC19A3* deficient patients, and the documented doses of thiamine and biotin vary from 100 to 900 mg per day and from 2 to 12 mg/kg per day, respectively [6-20]. It is likely that higher doses are required when trigger factors, such as fever or trauma, are present [18]. Initial reports described a good response to biotin as a monotherapy [8]. However, a recent description by Tabarki et al. reported that a high proportion of patients treated with biotin only showed recurrences of encephalopathy compared with those who received biotin and thiamine simultaneously [14].

We detected pathogenic mutations in the *SLC19A3* gene in 1 of 11 patients with Leigh syndrome. Similarly, Gerards et al. reported that 2 of 17 Leigh patients were positive for *SLC19A3* mutations [13]. The MRI pattern of brain injury involving the dorsal striatum and medial thalamic nuclei in patients with SLC19A3 defect may be useful to distinguish this disorder from other causes of Leigh syndrome. Interestingly, some correlations have been described between MRI findings and specific genetic defects. In patients with *ATPase 6* mutations MRI typically shows necrosis in the putamina, demyelization in the corona radiata and cerebellar and brainstem atrophy in the final stages [28]. Patients with PDHc deficiency usually present with lesions in the basal ganglia, brainstem and dentate nuclei, being the globus pallidus frequently involved [29]. A common pattern of brain MRI in patients with Complex I deficiency consists of brainstem and striatal lesions (putamina more frequently than the caudate and pallidum) [30]. MRS may show lactate peaks during the acute phase in *SLC19A3* defects and in other causes of Leigh syndrome [11].

In view of the overlapping phenotypes that may exist between ThTR2 deficiency and mitochondrial disorders causing Leigh encephalopathy, it seems advisable to initiate empirically biotin and thiamin in every patient with Leigh syndrome. However, it is concerning that in a recent report on the practice patterns of mitochondrial disease physicians in North America, only 3 of 32 medical doctors administered thiamine and other B complex vitamins [31].

In conclusion, thiamine transporter-2 deficiency is an inherited recessive disease that affects the central nervous system during development and may present as Leigh syndrome in infants, mimicking untreatable mitochondrial disorders. A characteristic MRI pattern of caudate, putamen and medial thalamus involvement, in association with lactic acid accumulation and high excretion of organic acids in urine in infants, suggests the diagnosis. It is of utmost importance to start early treatment with thiamine and biotin because the process may be at least partially reversible. Currently, there is an urgent need for validated tools for early diagnosis and treatment monitoring. In our experience, thiamine quantification by the HPLC method in whole-blood samples appears to be a useful method for the evaluation of the adherence to treatment. Further studies are needed to validate the therapeutic doses of thiamine and how to monitor them in these patients.

Abbreviations

DNA: Deoxyribonucleic acid; HPLC: High-performance liquid chromatography; MRI: Magnetic resonance image; MRS: Magnetic resonance spectroscopy; BTBGD: Biotin-thiamine responsive basal ganglia disease; FLAIR: Fluid attenuated inversion recovery; TMP: Thiamine monophosphate; TDP: Thiamine diphosphate; PCR: Polymerase chain reaction; ThTR2: Thiamine transporter type 2; CSF: Cerebrospinal fluid; HIE: Hypoxic ischemic encephalopathy.

Competing interests

The authors declare that they have no competing interest.

Authors' contributions

JDOE conceptualized and designed the study, drafted the initial manuscript, and approved the final manuscript as submitted. MS contributed to the analysis and interpretation of the clinical data, critically reviewed the manuscript, and approved the final manuscript as submitted. MM contributed to the analysis and interpretation of the biochemical studies, critically reviewed the manuscript, and approved the final manuscript as submitted. AO contributed to the analysis and interpretation of the molecular studies, critically reviewed the manuscript, and approved the final manuscript as submitted. MR contributed to the analysis and interpretation of the neuroradiological studies, critically reviewed the manuscript, and approved the final manuscript as submitted. JM contributed to the analysis and interpretation of the neuroradiological studies, critically reviewed the manuscript, and approved the final manuscript as submitted. RA contributed to the analysis and interpretation of the biochemical studies, critically reviewed the manuscript, and approved the final manuscript as submitted. PRP contributed to the analysis and interpretation of the molecular studies, critically reviewed the manuscript, and approved the final manuscript as submitted. BPD conceptualized and designed the study, contributed to the analysis and interpretation of the results, critically reviewed the manuscript, and approved the final manuscript as submitted.

Funding source

Supported by Fondo de Investigación Sanitaria Grant PI12/02010 and PI12/02078; Centre for Biomedical Research on Rare Diseases, an initiative of the Instituto de Salud Carlos III, Barcelona, Spain; Agència de Gestio' d'Ajuts Universitaris i de Recerca-Agaur FI-DGR 2014 (JD Ortigoza-Escobar).

Financial disclosure

All of the authors report no financial relationships relevant to this article.

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Received: 8 April 2014 Accepted: 13 June 2014
Published: 23 June 2014

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doi:10.1186/1750-1172-9-92
Cite this article as: Ortigoza-Escobar *et al.*: Thiamine transporter-2 deficiency: outcome and treatment monitoring. *Orphanet Journal of Rare Diseases* 2014 **9**:92.

Síntesis de resultados

- En el momento del diagnóstico, los cuatro pacientes de este trabajo tenían entre 1 y 17 años de edad, y mostraban signos de encefalopatía aguda, distonía generalizada y lesiones cerebrales que afectaban el caudado, putamen y la región dorso-medial talámica.
- Tras el seguimiento, los pacientes recibieron una suplementación combinada de tiamina (10-40 mg/kg/día) y biotina (1-2 mg/kg/día) permaneciendo estables, aunque con distonía residual y disartria leve.
- Un paciente murió en el contexto de una sepsis de origen enteral. El diagnóstico genético de este paciente se realizó *post-mortem*. Los demás pacientes evidenciaron mejoría clínica y radiológica poco después del inicio de la suplementación con vitaminas.
- El alfa-cetoglutarato no es un biomarcador sensible de los defectos del metabolismo y transporte de tiamina.
- Se establecieron valores de referencia de tiamina para diferentes grupos de edad en 106 pacientes controles. En este trabajo, la TDP fue la isoforma más concentrada en sangre total, similar a lo reportado en otros estudios. La determinación de tiamina de sangre total es un método útil para la monitorización de la adherencia al tratamiento en pacientes con deficiencia de hTHTR2 suplementados con tiamina.
- Las áreas del SNC más frecuente involucradas en pacientes con deficiencia de hTHTR2 fueron, en orden de la frecuencia: el caudado, el putamen y el tálamo, seguido por el cerebelo, el tronco cerebral y los hemisferios cerebrales
- La deficiencia de hTHTR2 es una causa importante de la distonía tratable en los niños. Por lo tanto la suplementación, a modo de ensayo terapéutico con tiamina,

debe indicarse en casos de distonía aguda, sobre todo cuando exista además lesión de ganglios basales.

- El fenotipo clínico reportado en la literatura es un *continuum*, más que formas clínicas definidas. En este sentido, algunos fenotipos comparten las mismas mutaciones y pacientes con mutaciones similares presentan diversidad en la edad de inicio de sintomatología.

Treatable Inborn Errors of Metabolism Due to Membrane Vitamin Transporters Deficiency.

"Errores innatos del metabolismo tratables por defectos del transporte de vitaminas".

Seminars in Pediatric Neurology (in press)

Ortigoza-Escobar JD, Pérez-Dueñas B

En este trabajo se ha realizado un resumen de la biología de la tiamina y de su transporte a través de la membrana plasmática y de la membrana mitocondrial. Así mismo se comenta sobre las isoformas predominantes en cada compartimento celular: extracelular (sangre, LCR) e intracelular. Nuestro objetivo en este trabajo ha sido el de presentar de forma resumida la edad de presentación, las características clínicas, el perfil bioquímico y los hallazgos radiológicos en cada defecto genético (*SLC19A2*, *SLC19A3* y *SLC25A19*) y además comentar el tratamiento empleado en cada defecto. En este trabajo, se presenta por primera vez, un esquema radiológico que evidencia la afectación principal del caudado, el putamen y la región dorso-medial del tálamo en los pacientes con deficiencia de hTHTR2.

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Treatable Inborn Errors of Metabolism Due to Membrane Vitamin Transporters Deficiency

Juan Darío Ortigoza Escobar, MD,*,† and Belén Pérez Dueñas, MD, PhD*,†

B vitamins act as cofactors for strategic metabolic processes. The *SLC19* gene family of solute carriers has a significant structural similarity, transporting substrates with different structure and ionic charge. Three proteins of this family are expressed ubiquitously and mediate the transport of 2 important water-soluble vitamins, folate, and thiamine. *SLC19A1* transports folate and *SLC19A2* and *SLC19A3* transport thiamine. *PCFT* and *FOLR1* ensure intestinal absorption and transport of folate through the blood-brain barrier and *SLC19A25* transports thiamine into the mitochondria. Several damaging genetic defects in vitamin B transport and metabolism have been reported. The most relevant feature of thiamine and folate transport defects is that both of them are treatable disorders. In this article, we discuss the biology and transport of thiamine and folate, as well as the clinical phenotype of the genetic defects.
Semin Pediatr Neurol ▮:▮▮▮-▮▮▮ © 2016 Elsevier Inc. All rights reserved.

Introduction

B vitamins are a class of water-soluble vitamins that play important roles in cell metabolism. Each B vitamin is either a cofactor (generally a coenzyme) for key metabolic processes or is a precursor needed to create one. As a cofactor, B vitamins participate in the metabolism of carbohydrates, amino acids, and fatty acids and have a major role in energy production. They are also involved in myelination, DNA synthesis, and neurotransmission.

B vitamins are found in whole unprocessed foods. Processed carbohydrates such as sugar and white flour tend to have lower B vitamin than their unprocessed counterparts. For this reason, the B vitamins thiamine, riboflavin, niacin, and folic acid are added back to white flour after processing in many countries.

There are several known genetic defects in vitamin B transport and metabolism causing disease in humans. For folate, riboflavin, and thiamine, genetic transport defects have been described in children. In this article, we will focus on thiamine and folate transporter defects.

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The folates and thiamine are metabolized to active forms that accumulate in cells where they sustain key metabolic reactions. They are transported into cells by a specific member of the SLC19 family.[1] SLC19A1 transports folate, and SLC19A2 and SLC19A3 transport thiamine. The proton-coupled folate transporter (PCFT; MIM*611672) is responsible for the intestinal absorption and the transport across the blood:choroid plexus (CP):cerebrospinal fluid (CSF) barrier. The folate receptor alpha (FOLR1) also mediates active transport to the brain using an endocytosis process. The mitochondrial thiamine pyrophosphate carrier (SLC25A19) enters the active form of thiamine to the mitochondria.

Two inborn errors affecting folate transport have been well studied: hereditary folate malabsorption (MIM 229050) due to mutations in PCFT[2] and cerebral folate transport deficiency (MIM 613068) due to defects in FOLR1.[3] Additionally, the following inherited defects of thiamine transport have been described: SLC19A2: thiamine-responsive megaloblastic anemia (MIM 249270),[4] SLC19A3: thiamine transporter-2 deficiency (biotin- or thiamine-responsive encephalopathy type 2) (MIM 607483),[5] SLC25A19: microcephaly Amish type (MIM 607196),[6] and SLC25A19: thiamine metabolism dysfunction syndrome 4 (progressive polyneuropathy type) (MIM 613710).[7]

Thiamine and folate transport defects across cell membranes share a common feature that is relevant from a therapeutic perspective: they are treatable disorders. In both cases, oral or intravenous supplementation or both leads to a significant and

http://dx.doi.org/10.1016/j.spen.2016.11.008

sustained clinical response and restores CSF and cellular concentrations in affected patients. The biological mechanism for this clinical response is unknown, although a major hypothesis is that alternative low affinity or residual transport pathways into the brain can be exploited by increasing plasma concentrations.

Folate Biology

Folate is a water-soluble B vitamin comprising several vitamers, which are compounds that act as coenzymes for cellular one-carbon metabolism.[8] Folate is essential for the synthesis of thymidine, purines, myelin, and neurotransmitters, and for the metabolism of amino acids such as homocysteine, methionine, serine, and glycine. Homocysteine remethylation to methionine leads to more than 100 methylation reactions via s-adenosylmethione.[9]

Impaired myelination of the central nervous system (CNS) is a common abnormality in inborn errors of folate transport or metabolism or both, such as hereditary folate malabsorption, FOLR1 deficiency, and severe 5,10-methylentetrahydrofolate reductase (MTHFR) deficiency. In these disorders, a relationship has been suggested between S-adenosylmethionine (SAM) deficiency and impaired myelination, the proposed mechanisms being related to a reduced methylation of lipids and proteins required for the formation and maintenance of the myelin sheaths.[100] SAM is the methyl donor in the synthesis of the key cell membrane component phosphatidylcholine from phosphatidylethanolamine. In rats, diet-induced folate deficiency depletes brain membrane phosphatidylcholine, which may be prevented by supplementation with L-methionine.[10] A reduced choline peak on spectroscopy in FOLR1 defects may be an estimation of reduced SAM and, consequently, of a decreased methylation capacity in the brain in cerebral folate transport deficiency.[11]

Early diagnosis and treatment of folate metabolism and transport defects can restore CSF 5MTHF concentrations and the methionine and S-adenosylmethionine pool within the brain, leading to myelin formation and brain growth.[3,12,13]

Folate Transport Across Cell Membranes and the CP

Folate is mainly obtained from fruits and vegetables in the form of polyglutamates that have to be transformed into monoglutamates to be transported into cells.

Two systems are responsible for the intestinal absorption of folate: the reduced folate carrier (RFC, encoded by the *SLC19A1* gene)[8] and the proton-coupled folate transporter (PCFT, encoded by the *SLC46A1* gene).[1] Both PCFT and RFC are expressed at the apical membrane of the intestinal epithelia, and the contribution of each system to total folate absorption depends on their expression and on the intestinal pH. The PCFT system acts in the proximal half of the small intestine, whereas the RFC system operates in the distal small intestine and the colon.[9] The identification of the first patients with pathogenic mutations in *SLC46A1*, which affects PCFT function, supports its key role in folate transport.[1,13]

Folate is converted to 5-methyltetrahydrofolate (5MTHF) by several enzymatic reactions. 5MTHF is the major biologically active form that functions as a cofactor in many methylation reactions. Different folate carriers and receptors participate in the cellular uptake of 5MTHF from the circulation to organs and cells.

The reduced folate carrier (RFC; SLC19A1) is an organic anion antiporter that exchanges 5MTHF with other inorganic or organic anions. It is ubiquitously expressed, and has a low affinity for folate, especially for the active-reduced forms. To date, no disease-causing mutations have been identified in the *SLC19A1* gene in patients with cerebral folate deficiency (CFD) syndrome.

Two glycosylphosphatidylinositol-anchored receptors, folate receptor alpha (FRα) and beta (FRβ), mediate endocytosis of folates after binding them with high affinity at neutral pH. FRα encoded by *FOLR1* gene is expressed in the apical border membrane of proximal renal tubular cells, in the retinal pigment epithelium, and in the CP.[2] Within the CP, PCFT is co-expressed with FOLR1 at the endosomal membrane, and it is likely that PCFT is required for FOLR1-mediated endocytosis. The fact that both genetic defects produce a failure to transport folates across the CP suggests that these transporters act in series; disruption of either of them results in the same defect.

Zhao et al[14] suggested that the PCFT might work in tandem with FRα-mediated endocytosis by exporting folate from the endosomes into the cytoplasm. Folate binding to FRα is followed by the invagination of the cell membrane containing the folate-receptor complex, the formation of an endosome, and trafficking of the vesicle in the endosomal compartment where it acidifies releasing folate from the receptor.

Recently, Grapp et al[15] elucidated the mechanism of folate transport through the CP and to the brain. They identified a unidirectional basolateral to apical transport of FRα and release of FRα from the apical membrane to the CSF. Within the CSF, FRα was found at the surface of exosomes, and FRα-exosome levels positively correlated with 5MTHF concentrations. Furthermore, FRα could be detected in the CSF of controls but was absent from patients with FOLR1 and Kearns-Sayre syndrome who had 5MTHF concentrations less than 5 nM (normal range: 40-120 nM). These findings suggest a link between CSF 5MTHF and CSF FRα and indicate a crucial role of the CP in the export and maintenance of 5MTHF and FRα in the CSF. Furthermore, these authors demonstrated that FRα-positive exosomes penetrate into brain parenchyma where they are internalized by astrocytes and neurons. These studies reveal a novel function of exosome as transport medium for folate, and that FRα-positive exosomes represent a particular attractive shuttle system for a broad variety of biomolecules and organic or inorganic compounds.

Hereditary Folate Malabsorption

Hereditary folate malabsorption is due to mutations in *PCFT*.[2] Biochemically, the disorder is characterized by profound blood

and CSF folate deficiency (CSF values usually less than <10 nmol/L) and an abnormal CSF:serum folate ratio.[16] The reduced availability of all forms of folate within the cells results in disturbances in several folate-related pathways within the first year of life. Decreased synthesis of the nucleic acids affects tissues with rapid turnover, such as blood and epithelial cells, thus producing megaloblastic anemia, pancytopenia, hypogammaglobulinemia with recurrent severe infections, diarrhea, oral ulcers, failure to thrive, and weight loss. Within the CNS, demyelination and intracranial calcifications are frequent, together with developmental delay, intellectual disability, seizures, and motor disturbances. Daily parenteral folinic acid administration improves symptoms and guarantees normal development. Also, it restores the normal CSF:serum folate at 3:1 ratio, which is decreased in this disorder.

Cerebral Folate Transport Deficiency Syndrome

CFD syndrome is a heterogeneous neurometabolic condition characterized by low concentration of 5-methyltetrahydrofolate (5MTHF) in the CSF.[17] Several unrelated processes can lead to 5MTHF depletion in the CSF. These can be divided into the following 2 main CFD syndromes: (1) a more common, milder form of deficiency identified in a broad spectrum of neurologic diseases[18] and (2) a severe form restricted to children with genetic conditions leading to impaired folate transport or metabolism.

In the latter group, Steinfeld et al[3] described etiologic mutations in the candidate gene *FOLR1* (MIM*136430) encoding the folate receptor alpha (FRα) in 3 children with profound CSF 5MTHF deficiency. The functional loss of FRα was associated to very low 5MTHF concentration in the CSF but normal plasma concentration of 5MTHF. As FRα was abundantly expressed in the CP, authors hypothesized that FRα provides the major route for the blood-CSF transport of 5MTHF. Thus, they named this entity cerebral folate transport deficiency. To date, a total of 19 patients with FRα defects have been published in the literature (Table 1).[11,12,15,19–22] Symptoms started between the age of 6 months and 5 years, following a period of normal development. Seizures, tremor, ataxia, chorea, and hypotonia were frequently reported. Some patients were referred for study of developmental delay, poor brain growth, and acquired microcephaly. Seizures were present in 18 of 19 children and started at a mean age group of 3-7 years (range: 8 months-11 years). Seizures were frequently myoclonic and tonic, causing drop-attacks and head injuries. They were drug resistant and caused *status epilepticus* in 5 patients. The electroencephalography activity deteriorated with disease evolution, and high-voltage spike and sharp wave activity and a slow high-amplitude background activity, multifocal epileptiform activity, and hypsarrhythmia were recorded. The cranial magnetic resonance imaging (MRI) most frequently showed delayed myelination or hypomyelination of the cerebral white matter and a slight cerebral but more pronounced cerebellar atrophy.[11,20,23] In other cases, MRI depicted progressive demyelination in the frontal and parietal lobes, which also extended into the brain stem.[22] One patient had normal signal intensity but white matter loss and calcifications.[20] Two patients developed a severe polyneuropathy.[23]

Most patients received oral supplementation of folinic acid at doses of 1-6 mg/kg/d. In patients with incomplete response, intravenous administration of folinic acid (100 mg) every 1-2 weeks was added to oral doses.[12] Authors reported a significant response to folinic acid administration in all cases. Seizure control in 1 or 2 months, and global improvement in social, language, and motor development were reported in most cases. Additionally, brain growth, improvement in white matter myelination, and increase of the choline peak on MR spectroscopy were described. 5MTHF concentrations normalized on a second lumbar puncture in most patients.

Loss-of-function mutations in FOLR1 cause a loss of FR-specific folate binding to patients' fibroblasts. Grapp et al[23] also demonstrated a reduction in folic acid surface binding of the FOLR1 mutants and a mistarget of the mutant protein to intracellular compartment where it partially colocalized with the endoplasmic reticulum.[24]

Thiamine Biology

Thiamine is an essential water-soluble B vitamin that acts as a cofactor in many cellular processes of which the most important has to do with energy production. There are several phosphate derivatives of thiamine in humans: thiamine monophosphate (TMP), thiamine diphosphate (TDP), and thiamine triphosphate. TDP is the biologically active form that functions as a cofactor of many enzymes as follows: (1) transketolase connects the cytosolic pentose phosphate pathway to glycolysis, metabolizing the excess of sugar phosphates into the main carbohydrate metabolic pathways; (2) 2-hydroxyacyl-CoA lyase catabolize phytanoic acid by alpha-oxidation in peroxisomes; (3) pyruvate dehydrogenase is the first component enzyme of mitochondrial pyruvate dehydrogenase complex linking the glycolysis metabolic pathway to the citric acid cycle and releasing energy (4) 2-oxoglutarate dehydrogenase is part of the mitochondrial oxoglutarate dehydrogenase complex or alpha-ketoglutarate dehydrogenase complex involved in citric acid cycle, lysine degradation, and tryptophan metabolism; and (5) branched-chain alpha-keto acid dehydrogenase catalyze the mitochondrial oxidative decarboxylation of branched, short-chain alpha keto acids.[25]

Thiamine Transport Across Cell Membranes and the CP

Humans lack biochemical pathways for thiamine synthesis, so cellular requirements are met via specific carrier-mediated uptake pathways.[26] Thiamine is found in a wide variety of foods at low concentrations, with whole grains, meat, and eggs being the most important dietary sources. Daily recommendations for dietary vitamin B1, according to the National

Table 1 Patients Reported With FOLR1 Mutations

References	Grapp et al[15]	Grapp et al[15]	Grapp et al[15]	Grapp et al[15]	Grapp et al[15]	Grapp et al[16,16]	Grapp et al[15]	Grapp et al[15]	Grapp et al[15]	Grapp et al[15]	Perez-Dueñas et al[18]	AlBaradie et al[19]	Toelle et al[12]	Ohba et al[20]	Dyment et al[21]	Dill et al[22]
N patients	1	1	1	1	1	1	1	1	1	1	1	2 (Siblings)	1	2 (Siblings)	2 (Siblings)	1
5MTHF in CSF (nmol/L)	5	<5	3	5	<5	5	<5	<5	1.4	2	2	7, 11	0	0	<10	<3
Age onset /symptoms	1 y DD	2 y DD, seizures	3 mo Microcephaly	3 y Ataxia	2 y Ataxia	1.5 y Developmental delay	2 y Developmental delay	2 y Speech delay	2.5 y Ataxia	2.5 y Tremor ataxia	2 y Tremor ataxia	4 and 5 y DD, seizures	2 y Ataxia, DD	1 and 2 y Ataxia, DD	2 y seizures	6 mo DD, ataxia
Epilepsy																
Age onset	16 mo	22 mo	8 mo	11 mo	4 y	2.5 y	6 y	3.5 y	3.3 y	None	21 mo	4 y	4 y	Epilepsy	2 y	6 y
Frequency	Daily	Daily	Daily	+	Daily	Daily	+	Daily	Daily		Daily	Daily	Daily	NR	NR	Daily
Status epileptic	+	−	−		−	+	+	−	−		+	−	−			+
Myoclonic	+	+	+		+	+	+	+	+		+	+	+		GTC	+
Drop-attacks	+		+		+	+					+	+	+			+
Ataxia-tremor/coreo-athetosis	+/+	+/−	+/−	+/−	+/−	+/+	+/−	+/−	+/+	−	+/+	+/−	+/+	+/−	NR	+/−
Autistic features/ intellectual disability	−	+	+	−	+	−	+	+	+	−	+	+	+	+	+	+
Hypomyelination-demyelination	+	+	+	+	+	+	+	+	+	−	+	+	+ (BBGG calcium)	WM atrophy calcium	+	+
Atrophy brain/ cerebellum	+/+	−/+	+/+	+/+	−/+	−/+	+/+	−/+	+/+	−/+	−/+	−/+	+/+	−/+	NR	+/?
Decreased choline on MRS	+	−	−	−	+	−	−	−	+	−	+	NR	+	NR	NR	+
Mutations (homozygozity)	p.C169Y (Hom)	p.C65W (Hom)	p.C169Y (Hom)	p.C169Y (Hom)	p.C169Y p.N222S	p.C169Y (Hom)	g.3576T4G (Hom)	p.K44_P49dup (Hom)	p.Q118X p.C175X	p.Q118X p.C175X	p.Cys105Arg (Hom)	p.Pro133His (Hom)	p.Arg204 (Hom)	p.R125L p.W156G	p.His43Arg (Hom)	p.R204X (Hom)

BBGG, basal ganglia; GTC, generalized tonic-clonic seizures; WM, white matter.

Academy of Sciences, vary from 0.2 mg in neonates to 1-1.2 mg in adults.[27]

Intestinal phosphatases convert dietary phosphate thiamine derivatives into free-T. At that moment, 2 specific transporters —thiamine transporter-1 (hTHTR1, encoded by *SLC19A2*) and thiamine transporter-2 (hTHTR2, encoded by SLC19A3) —mediate thiamine absorption in the upper small intestine. Both transporters are co-expressed but are differentially targeted in polarized cells, establishing a vectorial transport system.[26] The polarization decreases functional redundancy between transporter isoforms and allows for independent regulation of thiamine import and export pathways in cells.[28] hTHTR1 is expressed mainly at the apical brush-border membrane, playing a significant role in carrier-mediated thiamin uptake in human intestine.[29] Thiamine uptake is energy- and temperature-dependent, pH-sensitive (transport through *SCL19A3* increases with pH with a peak activity at pH 7.5),[30] Na+ independent, and saturable at both the nanomolar (apparent km, 30 ± 5 nM) and the micromolar (apparent Km, 1.72 ± 0.3 μM) concentration ranges.[31] Thiamin is transported in blood both in erythrocytes and plasma.[27]

Two-thirds of thiamine in plasma and CSF is TMP.[32] RFC (*SLC19A1*) can contribute to the transport of TMP in CNS.[33] Thiamine enters the CNS via a facilitated diffusion system at the blood:CSF barrier, and an active transport system in the CP (SLC19A2 and SLC19A3), which releases both free-T and TMP into CSF. There is a rapid turnover of total thiamine in brain and CSF, defined as the amount of vitamin per unit time that enters brain or CSF from plasma at steady state divided by the total vitamin content in the brain or CSF.[34] A specific cytosol kinase (thiamine phosphokinase, encoded by *TPK1*) converts thiamine into TDP, which is transported into the mitochondria by another carrier *SLC25A19*.[25] The kidneys play a critical role in regulating body thiamin homeostasis by salvaging the vitamin via reabsorption from the glomerular filtrate.[35] Elevated serum values result in active urinary excretion of the vitamin. After an oral dose of thiamin, peak excretion occurs in approximately 2 hours, and excretion is nearly complete after 4 hours. Mean elimination half-life of thiamine has been estimated as 1.8 days, and the biological half-life of the vitamin is probably in the range of 9-18 days.[27]

SLC19A2 encodes a protein of 497 amino acids (55,400 Da) with 12 transmembrane domains expressed in a wide range of human tissues, including bone marrow, liver, colon, pancreas, brain, and retina.[36] The delivery of the protein encoded by SLC19A2 to the cell surface is critically dependent on the integrity of the transmembrane backbone of the polypeptide so that minimal truncations abrogate cell surface expression of SLC19A2.[26] *SLC19A3* encodes a widely expressed protein of 496 amino acids (55,665 Da) that shares amino acid sequence identity with human SLC19A1 and SLC19A2 in 39% and 48%, respectively.[30] *SLC25A19* encodes a protein of 320 amino acids (35,511 Da) expressed in colon, kidney, lung, testis, spleen, and brain.

A summary of the clinical, biochemical, and radiological features, as well as treatment in each of these defects, are detailed in Table 2.

SLC19A2 Deficiency

Thiamine-responsive megaloblastic anemia is due to mutations in *SLC19A2*. There have been approximately 88 reported cases in all ethnic groups. Clinically, the disease is characterized by a triad of megaloblastic anemia, nonautoimmune diabetes mellitus, and sensorineural deafness. All these manifestations are because of impairment in energy production and de novo synthesis of nucleic acids and heme precursors in acinar and beta pancreatic cells, hematopoietic precursors, and cochlear inner hair cells. These manifestations may come out simultaneously or gradually from the time of birth up to the age of 26 years. Anemia and diabetes mellitus appear earlier than deafness in most patients. The average age groups of presentation of the 3 main manifestation of this disease are as follows: diabetes mellitus (2.68 ± 2.77 years; range: birth-12 years), megaloblastic anemia (2.55 ± 3.08 years; range: birth-19 years), and sensorineural deafness (2 ± 3.61 years; range: birth-30 years). There are additional symptoms of the disease described in Table 2. Blood thiamine can be normal or be slightly reduced.[37–39] Diabetes mellitus, anemia, and some psychiatric manifestations have an excellent response to thiamine supplementation.[40] Thiamine dose varies from 25-300 mg/d. Initial insulin requirements are usually high, but decrease with the onset of treatment with thiamine. Many patients may even do not require insulin until the pubertal period. Similarly, transfusion needs are reduced with thiamine therapy. Unfortunately, there is no improvement or prevention of deafness, short stature, or neurologic manifestations.[40,41]

SLC19A3 Deficiency

Mutations in *SLC19A3* are associated with the following phenotypes: (1) biotin-responsive basal ganglia disease, (2) Leigh syndrome, (3) Wernicke encephalopathy, and (4) infantile spams. To date, more than 75 patients have been reported. A few reported cases start in the first month of life; however, the most common clinical setting is of an acute encephalopathy proceeding by a trigger (fever, vaccinations, trauma, etc) in a previously healthy child. This episode of encephalopathy occurs with dystonia, dysarthria, ophthalmoplegia, or seizures or all of these. Radiological MRI pattern of symmetrically distributed brain lesions in caudate nuclei, putamen, and medial thalami is very suggestive of the disease (Fig.).[87–89] Free-T deficiency in CSF and fibroblasts can be found[80] as well as other nonspecific biomarkers (ie, increases of 2-oxoglutarate, lactate, and alanine in biological fluids, and a lactate peak on spectroscopy).[88–92]

The first patients reported were treated with high doses of biotin (5 mg/kg/d) with good response.[89] Thiamine (10-40 mg/kg/d) was used since the discovery that *SLC19A3* is a thiamine transporter.[93] Early administration of biotin and thiamine can improve the clinical and radiological abnormalities leading to a better neurologic outcome [81,90–98] Untreated patients suffer recurrent episodes of encephalopathy until their death.[82,90] Patients treated with the combination of thiamine and biotin show a faster recovery from an encephalopatic

Table 2 Characteristics of Patients With *SLC19A2*, *SLC19A3*, and *SLC25A19* Mutations

	SLC19A2	*SLC19A3*	*SLC25A19*
Phenotypes	(1) Thiamine-responsive megaloblastic anemia	(1) Biotin-responsive basal ganglia disease, (2) Leigh syndrome, (3) Wernicke encephalopathy, and (4) Infantile spams	(1) Amish microcephaly and (2) bilateral striatal necrosis with progressive axonal polyneuropathy
Number of patients reported	88	75	10
Reference	4,36–79	5,80–85	6,7,86
Age at onset			
	<1 mo 18.1%	<1 mo 7.2%	<1 mo 60%
	1-24 mo 52.2%	1-24 mo 39.1%	2-12 y 40%
	2-12 y 23.8%	2-12 y 48%	
		12-18 y 5.7%	
		>18 y 2.8%	
Clinical characteristics	Diabetes mellitus 92%	Encephalopathy 82.6%	Phenotype (1) microcephaly and developmental delay 100%
	Megaloblastic anemia 94.3%	Seizure 79.6%	*Other clinical manifestations*: irritability and inconsolable crying, failure to thrive, hepatomegaly, spasticity and, tonic-clonic seizures
	Deafness 89.7%	Generalized or focal dystonia 55%	Phenotype (2) encephalopathy 100% (1-3 episodes per patients), distal weakness 100%, contractures, and atrophy 50%
	Other clinical manifestations: epilepsy, ataxia, cognitive impairment, stroke, ocular symptoms (pigmentary retinopathy, cone-rod dystrophy, and Leber's congenital amaurosis), short stature, congenital cardiac malformations with conduction defects, cardiomyopathy, situs inversus, cryptorchidism, polycystic ovarian syndrome, immune thyroiditis, hepatomegaly, gastroesophageal reflux, vocal cord nodules, thrombocytopenia, and neutropenia	*Other clinical manifestations*: dysarthria/anarthria, ataxia, dysphagia, pyramidal signs, abnormal ocular movement, developmental delay, opisthotonus, rigid akinetic syndrome, tremor, chorea, jitteriness, dystonic status, dysautonomia, ptosis, rhabdomyolisis, and facial dyskinesia	*Other clinical manifestations*: sensory loss, dysphagia,
Biochemical profile	No lactic acidosis	High plasma lactate, alpha-alanine, and CSF lactate	Phenotype (1) lactic acidosis (6.7-16.7 mmol/L), elevated ammonia, ALT, and AST. Increase in pyruvic acid, 2-hydroxyisovaleric acid, 2-hydroxiglutaric, 2-ketoadipic acids, and alpha-ketoglutarate (>3700 mg/g creatinine, reference < 200)
	Normal urinary organic acid profile	High excretion of alpha-ketoglutarate	
	Normal or slightly decreased blood thiamine	Low free-T in CSF and fibroblast	
			Phenotype (2) increase in CSF lactate (2.9-4.2 mmol/L). Normal alpha-ketoglutarate in urine.
MRI findings	Ischemic stroke 5.6%	T2-hyperintensity in caudate and putamen 79.7%, thalami 44.9%, cerebellum 31.8%, and brainstem 27.5%. Other lesions are located in	Phenotype (1) agenesis of corpus callosum, large cisterna magna, enlarge lateral ventricles, hypoplastic cerebellar vermis,
	Cerebellar atrophy 1.1%		

Table 2 (*continued*)

	SLC19A2	*SLC19A3*	*SLC25A19*
		subcortical white matter, cerebral cortex, globus pallidus and medulla. Lactate on spectroscopy in some patients	lyssencephaly, hypoplastic pons, closed spinal dysraphism Phenotype (2) T2-hyperintensity in caudate and putamen 100%, medial posterior thalami 25%
Treatment	Thiamine 25-300 mg/d (1-4 mg/kg/d)	Biotin: 5-10 mg/kg/d or 5-10 mg/d Thiamine: 10-40 mg/kg/d, maximum: 1500 mg/d	Ketogenic diet

episode; however, the number of recurrences, neurologic sequel, and brain MRI changes are similar to those children treated with thiamine alone.[99,100] Likewise, biotin deficiency reduces the expression of SLC19A3.[86]

SLC25A19

Mutation in the mitochondrial thiamine transporter is associated with 2 different phenotypes as follows: (1) severe congenital microcephaly, cognitive impairment, CNS malformations (lyssencephaly, partial agenesis of corpus callosum, and closed spinal dysraphic state), recurrent episodes of encephalopathy,[6,86] and a characteristic facial appearance named Amish microcephaly and (2) bilateral striatal necrosis with progressive axonal polyneuropathy, recurrent episodes of encephalopathy, and flaccid paralysis during febrile illnesses. There are some clinical and biochemical differences between these 2 phenotypes: patients suffering Amish microcephaly phenotype usually show an occipitofrontal circumference of 6-12 standard deviations below the mean[6] and lactic acidosis and alpha-ketoglutaric aciduria between the episode, whereas patients with the striatal necrosis phenotype do not show microcephaly, have a normal IQ before the onset of the encephalopatic episodes and have a normal urinary organic acid profile.[7] Patients can develop mild hepatomegaly, body temperature instability, and irritability. Unfortunately, both phenotypes do not respond to treatment with thiamine, but ketogenic diet may be effective in reducing the metabolic crisis.[86]

Conclusion

Membrane vitamin transporter deficiencies are clinically, biochemically, and genetically heterogeneous disorders, for which effective therapies are currently available. A trial of folinic acid supplementation should be considered in children with megaloblastic anemia, recurrent severe infections, diarrhea, and failure to thrive (*PCFT* mutations) and in children with progressive ataxia, drug-resistant myoclonic and tonic seizures, and drop-attacks (*FOLR1* mutations). Also, thiamine plus biotin should be administered in children with Leigh syndrome of unknown etiology (*SLC19A3* mutations), and thiamine should be prescribed in patients with the triad of diabetes mellitus, megaloblastic anemia, and deafness

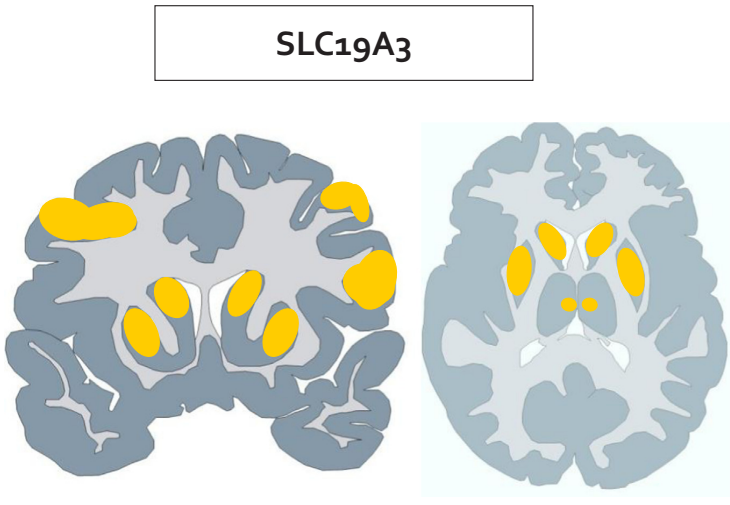

Figure Schematic representation of MRI changes in SLC19A3 patients. Lesions are distributed symmetrically affecting the cerebral cortex, striatum, and dorsomedial thalamic nuclei. (Color version of figure is available online.)

(*SLC19A2* mutations). Ketogenic diet should be initiated in patients suspected of *SCL25A19* mutations (severe microcephaly or striatal necrosis with polyneuropathy). Simultaneous analysis of blood and CSF concentrations of folate and thiamine before the empirical administration of these vitamins is highly important to rule out secondary dietary deficiencies and to identify specific cerebral transporter defects.

Acknowledgments

This work was supported by grants from the Spanish Ministerio de Economia y Competitividad (FIS PI12/02010, PI15/00287) and fondo Europeo de desarrollo regional (FEDER); Agència de Gestió d'Ajuts Universitaris i de Recerca-Agaur (2014FI_B 01225) (JD Ortigoza-Escobar); Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), an initiative of the Instituto de Salud Carlos III (Ministerio de Ciencia e Innovación, Spain).

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Survival and treatment predictor in thiamine defects.

"Supervivencia y predictores del tratamiento en pacientes con defectos de tiamina".

Annals of Neurology (submitted)

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En este trabajo se describe la historia natural de los defectos genéticos del transporte y metabolismo de la tiamina en la mayor cohorte panétnica de pacientes recopilados hasta la fecha y se comenta como el tratamiento con vitaminas modifica la historia natural de estos defectos. Se realiza un análisis sistemático de las características clínicas y radiológicas, y de las anormalidades bioquímicas en el debut de la enfermedad, confirmando que los defectos de tiamina se manifiestan más frecuentemente como lesión cerebral aguda en la primera década de la vida. También se presentan datos sobre la suplementación con tiamina, y se identifican nuevos factores pronóstico de la evolución de estos pacientes a largo plazo. Por último, se evalúa la discapacidad y se compara las curvas de supervivencia de estos pacientes con las curvas de supervivencia de pacientes con otras causas de síndrome de Leigh. Así, demostramos que los defectos de tiamina tienen una mejor tasa de supervivencia que otras encefalopatías mitocondriales. Los resultados que presentamos aquí serán de utilidad para optimizar las estrategias de tratamiento y ayudarán a medir el efecto de terapias futuras.

Thiamine Deficiency in Childhood with Attention to Genetic Causes: Survival and Outcome Predictors

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Primary and secondary conditions leading to thiamine deficiency have overlapping features in children, presenting with acute episodes of encephalopathy, bilateral symmetric brain lesions, and high excretion of organic acids that are specific of thiamine-dependent mitochondrial enzymes, mainly lactate, alpha-ketoglutarate, and branched chain keto-acids. Undiagnosed and untreated thiamine deficiencies are often fatal or lead to severe sequelae. Herein, we describe the clinical and genetic characterization of 79 patients with inherited thiamine defects causing encephalopathy in childhood, identifying outcome predictors in patients with pathogenic *SLC19A3* variants, the most common genetic etiology. We propose diagnostic criteria that will aid clinicians to establish a faster and accurate diagnosis so that early vitamin supplementation is considered.

ANN NEUROL 2017;82:317–330

Thiamine or vitamin B1 is a critical cofactor involved in energy metabolism and in the synthesis of nucleic acids, antioxidants, lipids, and neurotransmitters.[1,2] Thiamine is a water-soluble essential nutrient obtained from cereals, meat, eggs, legumes, and vegetables. In the absence of adequate thiamine intake, limited tissue storage may be depleted in 4 to 6 weeks.[3] Thiamine requires specific transporters for the absorption in the small intestine and for cellular and mitochondrial uptake (thiamine transporter-1, encoded by *SLC19A2*, thiamine transporter-2, encoded by *SLC19A3*, and mitochondrial thiamine diphosphate carrier, encoded by *SLC25A19*). Within the cellular compartment, thiamine is converted into thiamine diphosphate by thiamine phosphokinase

View this article online at wileyonlinelibrary.com. DOI: 10.1002/ana.24998

Received Nov 4, 2016, and in revised form Jul 9, 2017. Accepted for publication Jul 12, 2017.

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This article was published online on 30 August 2017. After online publication, updates were made to an author degree and author contributions. This notice is included in the online and print versions to indicate that both have been corrected on 10 September 2017.

Additional supporting information can be found in the online version of this article.

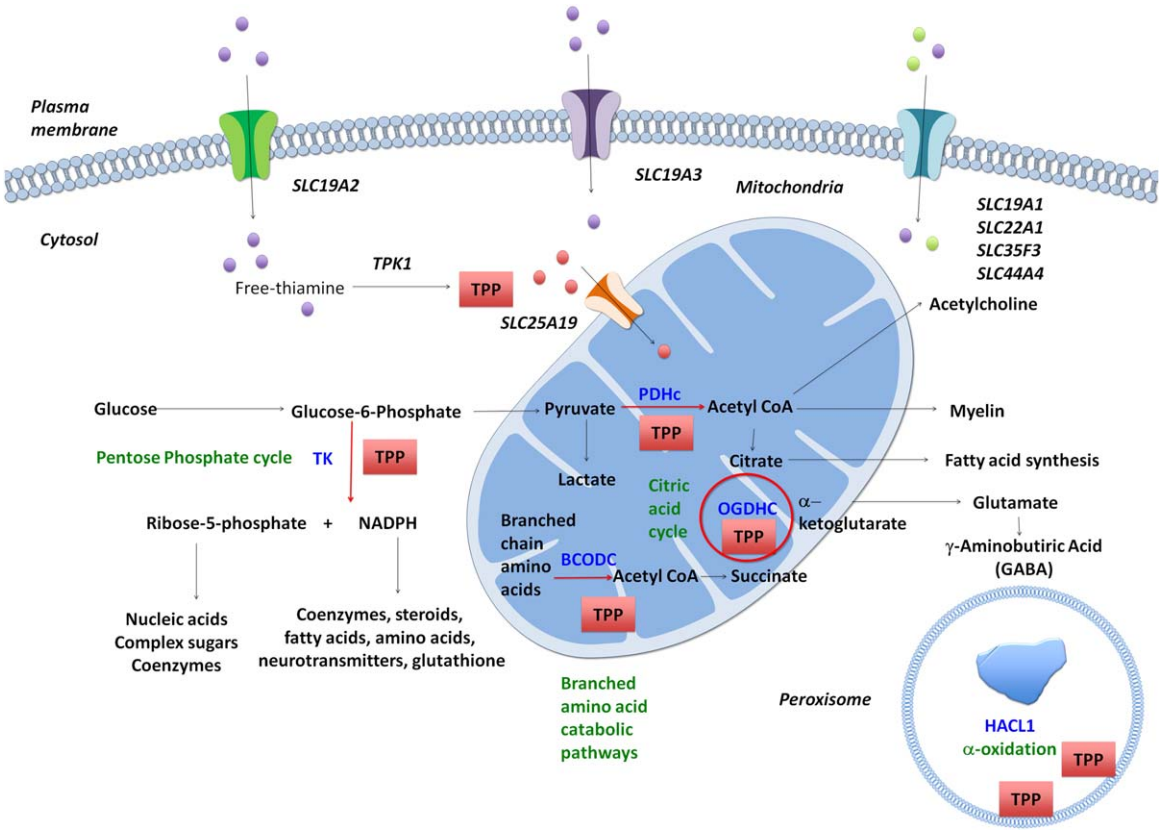

FIGURE 1: Schematic layout of the thiamine transport and metabolism. There are four known forms of thiamine in humans, free nonphosphorylated thiamine (free-T, purple balls) and its phosphate esters: thiamine monophosphate (TMP, green balls), thiamine diphosphate (TDP, red balls), and thiamine triphosphate (TTP, nonrepresented). Although, at high concentrations, thiamine absorption is by passive diffusion, thiamine is absorbed in the small and large intestine and transported across the blood–brain barrier using several well-known transporters: *SLC19A1* (folate transporter); *SLC19A2* (thiamine transporter-1); *SLC19A3* (thiamine transporter-2); *SLC44A4* (human TDP transporter); *SLC22A1* (OCT1, organic cation transporter 1); and *SLC35F3*. At that point, intracellular free-T is converted to TDP, which is the metabolically active form of the vitamin, by *TPK1* (thiamine pyrophosphokinase) and transported inside the mitochondria by the *SLC25A19* (mitochondrial TDP transporter). Human TDP-dependent enzymes comprise TK (transketolase), HACL1 (2-hydroxyacyl-CoA lyase 1), PDHc (pyruvate dehydrogenase complex), OGDHC (oxoglutarate dehydrogenase complex), and BCODC (branched chain 2-oxo acid dehydrogenase complex). NADPH = nicotinamide adenine dinucleotide phosphate. [Color figure can be viewed at www.annalsofneurology.org]

(*TPK1*), the metabolically active form of thiamine, which acts as a cofactor of several thiamine-dependent enzymes in the cytosol, peroxisomes, and mitochondria (Fig 1). Specifically, in the mitochondria, thiamine diphosphate (TDP) acts as a cofactor of the PDHc (pyruvate dehydrogenase complex), OGDHC (oxoglutarate dehydrogenase complex), and BCODC (branched chain 2-oxo acid dehydrogenase complex).

Infantile beriberi and Wernicke's encephalopathy are rare life-threating and reversible causes of secondary thiamine deficiency that still occur in vulnerable populations.[4] Infantile beriberi presents in infants breastfed by mothers with inadequate intake of thiamine[5] or receiving low thiamine-content formula,[6] whereas Wernicke's encephalopathy is described in sick children that undergo medical or surgical procedures such as gastrointestinal resections, parenteral nutrition, chemotherapy, etc.[7]

In recent years, genetic defects in thiamine transport and metabolism have been described in childhood, with overlapping clinical, biochemical, and radiological features to those observed in secondary forms, and a good response to vitamin supplementation. Well-defined clinical phenotypes have been recognized in the following defects:[8] (1) *SLC19A2* (thiamine transporter-1) causes Roger's syndrome or thiamine responsive megaloblastic anemia (OMIM 249270); *SLC19A3* (thiamine transporter-2; ThTR2) is responsible for biotin thiamine responsive basal ganglia disease (BTRBGD; OMIM 607483); Leigh's syndrome (LS); infantile spasms with lactic acidosis; and Wernicke-like encephalopathy; (3) *TPK1* causes LS (OMIM 614458); and, finally, (4) *SLC25A19* (mitochondrial thiamine pyrophosphate carrier) produces Amish microcephaly (OMIM 607196) and bilateral striatal degeneration and progressive polyneuropathy (OMIM 613710). Interestingly, three of

these genotypes (*SLC19A3*, *TPK1*, and *SLC25A19*) present with acute encephalopathy, basal ganglia lesions, and lactic acid accumulation attributed to brain energy failures.[9–12] These clinical and radiological manifestations are indistinguishable from LS, a severe neurological disorder of brain energy production, caused by more than 88 genetic variants.[13,14]

In this study, we provide a global overview of the numerous genetic and acquired etiologies of thiamine deficiency in childhood, with specific attention to inherited defects of thiamine transport and metabolism. We analyze the clinical features and long-term outcomes in a multiethnic cohort of 79 *SLC19A3*, *SLC25A19*, and *TPK1* patients, evaluate how thiamine/biotin treatment modifies the natural history of *SLC19A3* patients, and identify the clinical parameters that may help predict neurological outcome and guide further therapeutic interventions. We propose fundamental features to suspect inherited thiamine defects against external or secondary causes of thiamine deficiency, and suggest diagnostic criteria that will help clinicians to establish faster and accurate diagnosis so that early vitamin supplementation is considered.

Materials and Methods

Study Design

We conducted a multicenter cohort study by reviewing data from patients with inherited defects in thiamine transport and metabolism. We invited 44 investigators that had published patients with inherited thiamine defects and/or patients with LS and mitochondrial disorders in PubMed. In total, 21 investigators accepted to participate, 17 centers did not have patients to include, and, last, six colleagues did not answer or refused to participate in the study.

A systematic analysis in MEDLINE (through PubMed) was performed to search for secondary causes of thiamine deficiency. We included the following keywords: #1 beriberi, #2 Wernicke's encephalopathy, and #3 secondary thiamine deficiency, from January 2010 to February 2017. We analyzed predisposing factors, consanguinity, clinical, biochemical and radiological features, mortality, treatment, recovery, and neurological and radiological sequelae.

Study Population

We included patients with two pathogenic variants of the *SLC19A3*, *TPK1*, and *SLC25A19* genes. Patients with *SLC19A2* mutations were excluded from the study, because they did not have significant involvement of the central nervous system (CNS). The responsible clinician at each collaborating center collected data via a questionnaire that consisted of 131 items including: demographic data, family and perinatal history, genetic defects, gene-related phenotype, early developmental milestones, age of disease onset, triggering events, clinical neuroimaging and biochemical data at disease onset, thiamine and biotin supplementation and follow-up. In surviving patients presenting with dystonia, disability was evaluated using part b of the Burke–Fahn–Marsden scale (BFMDS). This questionnaire evaluates the dystonic patient's ability to perform everyday activities and has been used previously in SLC19A3 patients.[15] One hundred twenty-nine patients with nuclear encoded complex I deficiency and 324 patients with PDHc deficiency were collected through a review of the research literature in order to perform a comparative survival analysis. The time of death of nuclear encoded complex I patients was quantified according to methods described by Ortigoza-Escobar et al.[16] References of patients collected with PDHc deficiency included for comparative survival analysis appear in the Supplementary Material.

Standard Protocol Approvals, Registration, and Patient Consent

This study was approved by the Ethics Committee of the Hospital Sant Joan de Déu, Barcelona, Spain. Informed consent was obtained from all patients.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics 23 software (IBM Corp., Armonk, NY). The quantitative variables were reported either in terms of the normal distribution mean, standard error of the mean (SEM), and the range; or in terms of the median and interquartile range (IQR). The Mann–Whitney *U* test was applied to evaluate differences in numerical variables between groups. The chi-square test and Fisher's exact test were used to test the association between categorical variables. Multiple logistic regression analysis was performed to further investigate the relationship between the binary response variable and potential predictors of survival. The Kaplan–Meier survival analysis was used to compare the survival rates of the *SLC19A3*-deficient patients, patients with PDHc, and nuclear-encoded complex I-deficient LS. Differences in survival between the groups were evaluated using the log rank test. All statistical tests were two-sided and performed at a 0.05 significance level.

Results

Inherited Thiamine Defects

We identified 70 patients with *SLC19A3* disease, 4 patients with *TPK1* disease, and 5 patients with *SLC25A19* disease. The patients were diagnosed at 21 centers: UK (n = 4); United States, Germany, and Finland (n = 3); Saudi Arabia (n = 2); and The Netherlands, Spain, Israel, France, and Sweden (n = 1). Genotypes were established in all patients, including P76 who had a similar disease course to his sibling with *TPK1* deficiency and in whom the same mutations were confirmed using residual DNA. Complete clinical data sets were available in 65 of 70 patients with the *SLC19A3* mutation and in all patients with *SLC25A19* and *TPK1* mutations. Magnetic resonance imaging (MRI) data were available in all except 7 *SLC19A3* patients who were diagnosed postmortem (P39–P45). None of the patients were found to have additional clinical or biochemical abnormalities suggestive of other genetic diseases.

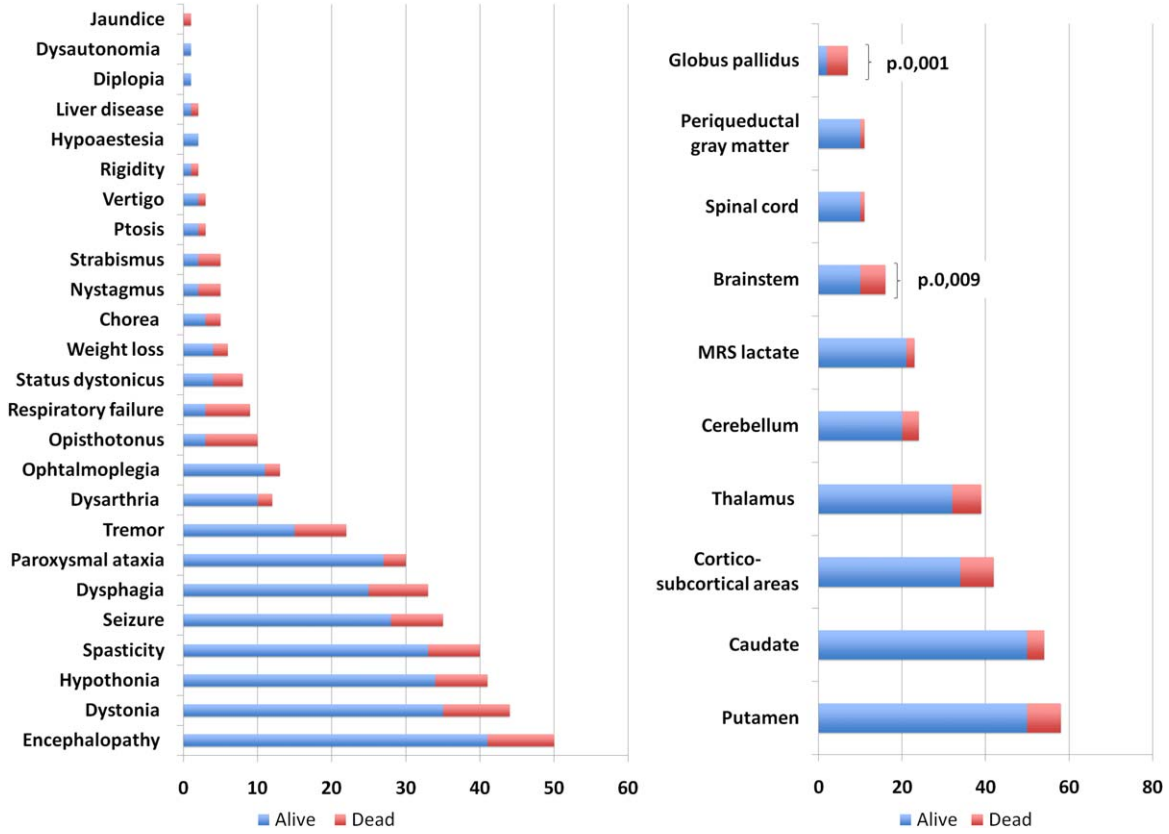

FIGURE 2: Major clinical features and neuroimaging results in 70 SLC19A3-deficient patients. (A) Encephalopathy defined as lethargy, irritability, agitation, vomiting, continuous crying, coma leading to ventilatory support, etc. Status dystonicus defined as the need of specific management, such as admission to pediatric intensive care unit, sedation and ventilatory support, benzodiazepines, baclofen, clonidine, anticholinergic, chloral hydrate, DBS, etc. Spasticity includes hyper-reflexia and signs of Babinski reflex. Liver disease defined as increased liver enzymes, liver failure, or hepatomegaly. The number of patients is plotted on the x-axis and the symptoms and signs are plotted on the y-axis. **(B)** Most patients presented a characteristic radiological pattern with hyperintensities in the caudate, putamen, ventromedial region of thalamus, and diffuse corticosubcortical areas. Statistical analysis indicated that deceased patients had more-frequent involvement of the globus pallidus (3 of 15 [20%] vs 3 of 55 [5%]; $p = 0.001$) and brainstem (4 of 15 [26%] vs 10 of 55 [18%]; $p = 0.009$) than surviving patients. [Color figure can be viewed at www.annalsofneurology.org]

SLC19A3. The 70 patients with *SLC19A3* deficiency (mean age at assessment ± SEM, 9.5 ± 0.9 years; range, 1 month–40 years old; 36 males; 51%) were born between 1975 and 2015. Of these, 63 (90%) had been previously reported. Consanguinity was reported in 51 (73%) patients and 44 (62%) had other affected family members. Arabs formed the largest ethnic group (58 of 70, 82%: Saudi Arabian n = 41, Moroccan n = 11, Iraqi n = 3, Kurdish n = 2, and Kuwaiti n = 1), followed by white European (10 of 70, 14%: Spanish n = 3, Portuguese n = 2, German n = 2, Finnish n = 2, and Hispanic n = 1), and African/Afro-Caribbean (2 of 70, 2.8%; Supplementary Table 1).

Clinical Phenotype

Supplementary Table 1 summarizes the demographic, genetic, clinical, and radiological features in the entire patient cohort. The frequency of the main clinical features appears in Figure 2. Fetal distress was noted in P2, P29, and P61 and acute presentation during the newborn period (around 4 weeks of age in all cases) was reported in 9 (12%) patients. In the vast majority, the developmental milestones were average, except in 7 cases (P28, P29, P53, P57, P60, P61, and P66)

The median age at disease onset was 3 years, the range was 1 month to 34 years, and the IQR was 1 to 2.8 years. The trigger events (39 of 70 patients; 55%) were viral (n = 30) or bacterial (n = 4) infection, trauma (n = 3), profuse exercise, and vaccination (n = 1, each). Fifteen (21%) patients were classified as LS and 53 (75%) as biotin thiamine responsive basal ganglia disease (BTRBGD) attributed to their positive responses to thiamine/biotin treatment. Twins (P35 and P36) with a positive family history (siblings of P34) were identified before the onset of symptoms.

Twenty-six patients experienced more than one encephalopathic episode before the initiation of vitamin supplementation (2 episodes n = 12, 3 episodes n = 7,

4 episodes n = 4, and 5 or more episodes n = 3 [P32, P33, and P49]). Most episodes of neurological deterioration were triggered by infection or stress. Intensive care was required in 5 of the patients (P1, P3, P48, P68, and P69).

A minority of patients (10 of 62; 16%) had an insidious onset of symptoms characterized by psychomotor regression, hyperactivity and attention deficit, unsteady gait, toe walking, or stiffness of the limbs.

Systemic features of mitochondrial disease (cardiomyopathy, cardiac conduction defects, renal tubulopathy, or facial dysmorphism) were absent in our series. P4 developed a steroid-sensitive nephrotic syndrome.

Seizures were classified according to the International League Against Epilepsy as follows: generalized seizures (n = 31); focal seizures (n = 6, including 2 patients [P30 and P31] with *epilepsia partialis continua*); epileptic spasms (P64, n = 1); and West syndrome (P48, n = 1). Thiamine and biotin treatment controlled seizures in patients effectively, except in P38, P53, and P59, who also received antiepileptic drugs, and P49, P61, and P64, who developed drug resistant epilepsy.

Ancillary Testing

Few patients showed increased cerebrospinal fluid (CSF) lactate (5 of 29 patients; mean ± SEM, 3.7 ± 1.0mmol/l; range, 2.1–7.1; normal voiding [NV] < 2), increased blood lactate (20 of 40; mean ± SEM, 4.1 ± 0.4mmol/l; range, 2.1–8.6; NV < 2), metabolic acidosis (6 of 36) and increased blood alanine (4 of 15; mean ± SEM, 666 ± 129μmol/l; range, 450–1,037; NV < 439). The lactate/pyruvate ratio was below the normal limit in 2 patients. Increased blood lactate levels were negatively correlated with the age of onset in the *SLC19A3* patients ($p < 0.001$).

Abnormal organic acid profiles were recorded in 8 of 50 patients, with high excretion of isobutyric, 2-OH-isovaleric and 2,4-di-OH-butyric (P1), alpha-ketoglutaric (P2), lactic (P34 and P52), 3-OH-butyric (P37), 2-OH-glutaric, glutaric, succinic, and 2 keta adipic acid (P41), and 4-OH-phenyllactic (P46).

MRI and magnetic resonance spectroscopy (MRS) were available in 61 of 70 (87%) and 43 of 70 (61%) patients, respectively. All symptomatic patients had lesions at onset, involving bilateral caudate (n = 55), putamen (n = 57), cortico/subcortical areas of the cerebral hemispheres (n = 40), ventromedial region of the thalamus (n = 38), cerebellum (n = 23), brainstem (n = 14), periaqueductal region (n = 12), spinal cord (n = 11), and the globus pallidus (n = 6; Figs 2 and 3). Acute MRIs indicated swelling and chronic MRIs indicated volume loss and necrotic changes. MRS detected a lactate peak in 55% (24 of 43) patients within the affected areas. Stroke-like lesions or mammillary body lesions were not identified.

Statistical analysis showed that deceased patients had more frequent involvement of the globus pallidus and brainstem than surviving patients (3 of 15 vs 3 of 55; Mann–Whitney U test, $p = 0.001$; and 4 of 15 vs 10 of 55; Mann–Whitney U test, $p = 0.009$, respectively).

Oxidative phosphorylation (OXPHOS) activity was normal in the muscle and skin biopsies of 6 patients, with the exception of P41 who showed 56% of complex IV activity in fibroblasts. None of the patients had ragged red fibers.

Treatment and Outcome

Fifty-one patients received vitamin supplementation during the acute encephalopathic episode. The time from disease onset to vitamin initiation was very broad (median, 14 days; IQR, 4–180). Forty-four patients had a significant clinical recovery within hours or days of vitamin initiation: They regained alertness, improved feeding, had a better control of seizures, and gradually recovered previously acquired milestones. Four more patients showed a mild improvement, and 3 patients did not improve at all.

Fifty-five patients were alive at the time of recruitment (mean follow-up, 5.2 ± 0.7 years; range, 2 weeks–22 years). All of them received thiamine (thiamine hydrochloride; mean dose, 20mg/kg/day; range, 5–55) and 47 received biotin (mean dose, 5mg/kg/day; range, 1–30). Both vitamins were administered orally in most patients, although some patients received intravenous supplementation in the acute episode. The neurological examination was normal in 26 patients at the time of assessment and they were symptom-free, whereas 27 had developed some neurological sequelae (Supplementary Table 1). No further decompensating episodes of encephalopathy, dystonia, or other neurological symptoms were recorded after vitamin supplementation in these patients, except for P61 who received inadequate vitamin doses. Additionally, blood alanine levels and the organic acid profiles in urine were normal in patients receiving vitamin supplementation. Only P52 had slightly elevated blood lactate (2.3mmol/l). The twins (P35 and P36) who were treated presymptomatically with thiamine alone were symptom free at the time of assessment (5 years).

Fifteen patients (21%) died, the majority of them from central respiratory failure (6.1 ± 1.9 years at death; range, 4 weeks–20 years). Deceased patients were younger at onset compared to surviving patients (mean age ±

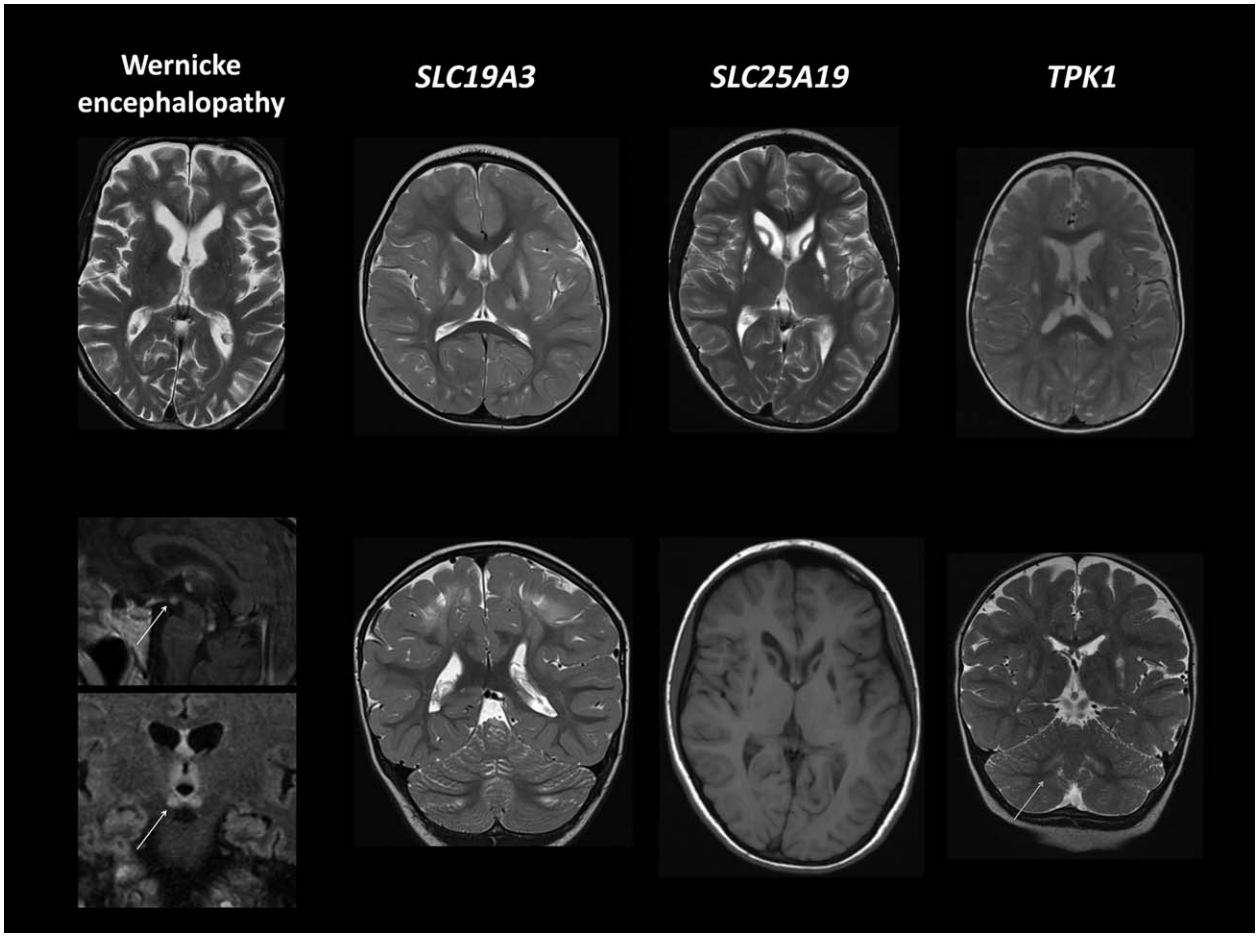

FIGURE 3: MRI patterns in patients with secondary and inherited thiamine defects. Wernicke encephalopathy. Axial T2W, sagittal and coronal FLAIR images show bilateral symmetric involvement of dorsal medial thalamus, periaqueductal gray matter, mammillary bodies (white arrow), and patchy cortical and subcortical hyperintensities. SLC19A3. Axial and coronal T2W images show bilateral symmetric involvement of the putamen and thalamus along with patchy cortical and subcortical hyperintensities. SLC25A19. Axial T2W and T1W images show cystic necrosis of the caudate and putamen. TPK1. Axial and coronal T2W SE images show involvement of the posterior putamen and dentate nuclei (gray arrow). FLAIR = fluid-attenuated inversion recovery; MRI = magnetic resonance imaging; SE = spin-echo; T1W/T2W = T1 and T2 weighted.

SEM, 2.4 ± 1.1 vs 5.4 ± 0.9 years; Mann–Whitney *U* test, $p = 0.005$). Among the 15 deceased patients, 4 had received vitamin supplementation (P1, P30, P41, and P49).

Disability Score

The BFMDS questionnaire was administered to 34 *SLC19A3* patients with dystonia (9.8 ± 1.8 points [mean ± SEM]; range, 0–30). Higher BFMDS scores were identified in patients who had a previous history of developmental delay (19.5 ± 4.1 vs 7.7 ± 1.7; Mann–Whitney *U* test, $p = 0.017$) and in patients with disease onset before 6 months of age (23.7 ± 2.8 vs 7.9 ± 1.7; Mann–Whitney *U* test, $p = 0.01$). A positive, and almost significant, correlation was observed between the BFMDS scores and the time from disease onset to thiamine initiation (Pearson correlation, $r = 0.340$; $p = 0.053$; Fig 4D).

Survival Analysis

In the Kaplan–Meier analysis (Fig 4), treated *SLC19A3* patients had a longer mean survival length than nontreated patients (A; 28.99 vs 17.23 years; log rank test, $p < 0.0001$). Additionally, mean survival length was longer in homozygous c.1264A>G *SLC19A3* patients than in patients with other mutations (B; 29.88 vs 15.52 years; log rank test, $p < 0.0001$). Homozygous c.1264A>G patients were comparable to patients with other mutations with respect to age at disease onset and age at treatment initiation. However, a significant difference was observed between both groups in the number of treated patients (39 of 44 [88%] treated c.1264A>G patients vs 13 of 22 [59%] treated patients with other mutations; $p = 0.006$). Mean survival length was longer in the 70 *SLC19A3* patients than in 129 patients with nuclear-encoded complex I deficiency (C; 28.0 vs 11.5 years; log rank test, $p < 0.001$). Similar results were obtained

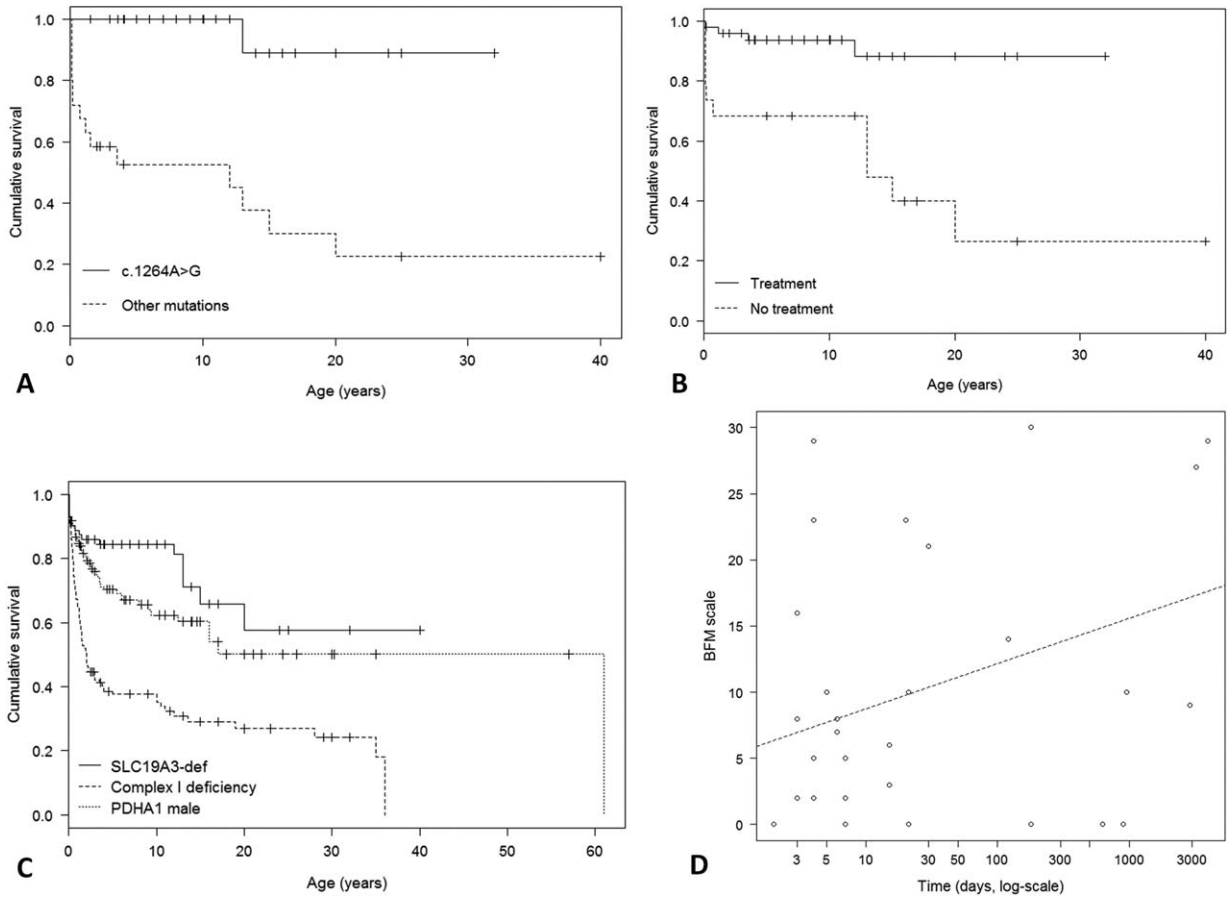

FIGURE 4: The figure shows Kaplan-Meier survival curves (A, B, C) and the correlation between the Burke-Fahn-Marsden Disability Scale and the time elapsed between disease onset and thiamine supplementation in *SLC19A3* patients (D). (A) Comparison between treated (n = 51) vs untreated (n = 19) SLC19A3-deficient patients (log rank test, p < 0.0001). (B) Comparison between c.1264A>G homozygous mutation (n = 44; 39 [88%] treated patients) vs other mutations (n = 22; 13 [59%] treated patients) in SLC19A3-deficient patients (log rank test, *p* < 0.0001). (C) Comparison between *SLC19A3* patients (n = 70), nuclear-encoded complex I deficient Leigh syndrome (n = 129), and male PDHc-deficient patients attributed to PDHA1 deficiency (n = 145). When comparing *SLC19A3* and nuclear-encoded complex I deficient Leigh syndrome, differences reach statistical significance (log rank test, *p* < 0.001). When comparing *SLC19A3* and male PDHc patients, differences did not reach statistically significance (log rank test, *p* = 0.06). (D) Correlation between the BFMDS (y-axis) and the time elapsed between disease onset and thiamine supplementation (x-axis, days, log-scale) in *SLC19A3* patients (r = 0.34; *p* = 0.053).

when the *SLC19A3* and Complex I patients were divided into two age groups: those with disease onset before 6 months (11.3 vs 2.9 years; log rank test, $p = 0.004$) and those with a later onset (33.3 vs 22.6 years; log rank test, $p = 0.003$). No significant differences were observed in the mean survival length between 70 *SLC19A3* and 324 PDHc patients in both age groups. However, an almost significant difference was observed when selecting male PDHA1 patients.

SLC19A3 *Gene Mutations*

We identified 13 *SLC19A3* pathogenic mutations (Supplementary Table 1; Fig 4) in the 70 patients. Five of these mutations were novel (c.91T>C, c.157A>G, c.503_505delCGT, c.516_delC, and c.833T>C). Fifty-nine patients were homozygous for the following missense mutations: c.1264A>G (n = 47); c.20C>A (n = 7); c.157A>G (n = 3); c.68G>T (n = 1); and c.541T>C (n = 1). Eleven patients were compound heterozygotes. The most frequently occurring mutation in our cohort, c.1264A>G, was present in patients with Arab ethnic backgrounds, including Saudi Arabian, Moroccan, Kurdish, and Kuwaiti patients. The next most common mutation, c.20C>A, occurred exclusively in subjects from the province of Al Hoceima in Northern Morocco (n = 7; 3 pedigrees).[9] he splice mutation, c.980-14A>G, was observed in 5 compound heterozygote individuals, all of them of white European origin.

SLC25A19. We recruited 4 consanguineous Arabic patients from Israel (homozygous for c.373G>A)[12] and a new white European German patient diagnosed by our group (P75). The phenotype of this girl, aged 21 years, was similar to previously reported cases (Supplementary Table 1). The symptoms

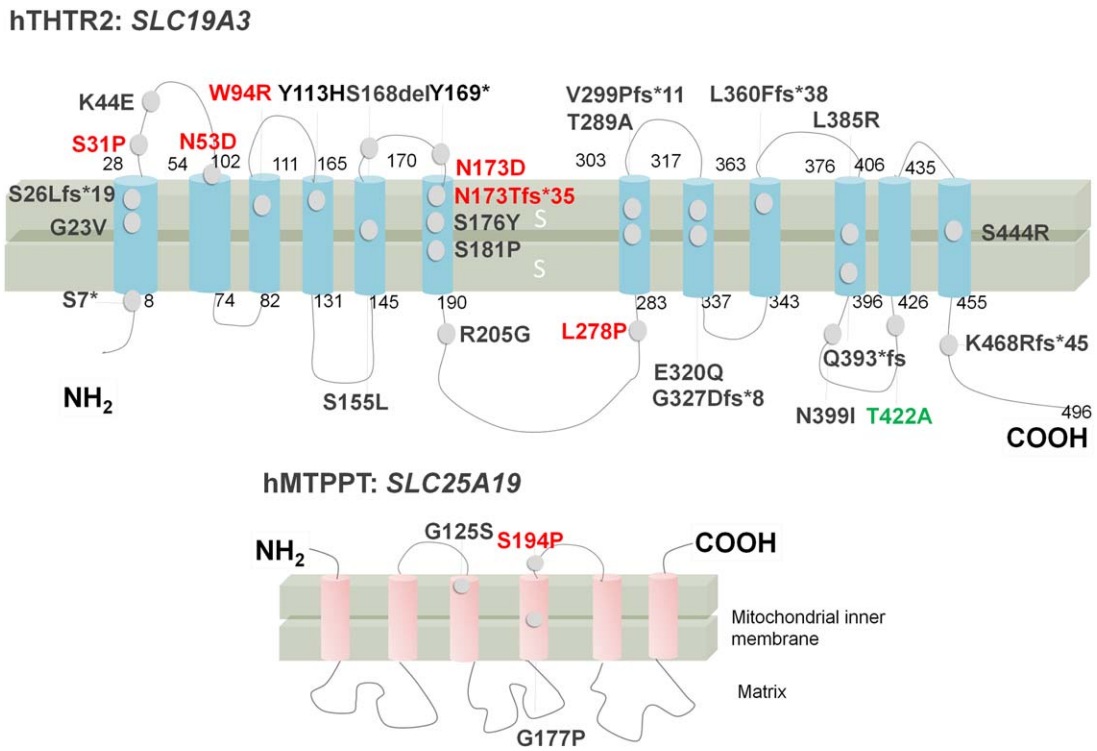

FIGURE 5: Pathogenic mutations in the human thiamine transporter type-2 (*SLC19A3*) and the human mitochondrial thiamine pyrophosphate transporter (*SLC25A19*). A schematic diagram of these proteins illustrating 25 and three mutations reported to date. †Novel unreported mutations identified in this study; ‡most common mutation. [Color figure can be viewed at www.annalsofneurology.org]

were triggered by febrile illness between 20 months and 6.5 years and consisted of acute encephalopathy, dysarthria, and episodic flaccid weakness. They had elevated levels of lactate in the CSF at onset (2.9–4.2mmol/l; NV < 2), but normal systemic mitochondrial biomarkers. MRI showed T2 hyperintensity and necrosis in the caudate and putamen in all patients. Additionally, P74 had T2 hyperintensity (cavitated lesion) in the medial thalamus.

Thiamine treatment (400–600mg/day) administered 3 years after onset led to substantial improvement in peripheral neuropathy and gait in P71 and P72, whereas P74 and P73, treated 9 and 12 years later, respectively, continued to have significant ambulatory impairment. All patients are currently alive. They have mild-to-severe bilateral pes equinus, axonal polyneuropathy, and dystonia. The disease severity was clearly milder in P71, P72, and P75, supporting the efficacy of thiamine treatment in preventing further disease progression.

Interestingly, P73 stopped thiamine treatment because of an apparent lack of benefit. Four years later, a severe episode of flaccid paralysis occurred with fever, and intravenous thiamine (1.500mg) led to clinical recovery within 24 hours.

TPK1. Four *TPK1* patients (P76, P77, and P79, previously reported)[17,18] suffered from LS triggered by febrile illness between 1 month and 2.5 years of age (Supplementary Table 1). Brain lesions developed in the cerebellum and dentate nuclei (n = 4), striatum (n = 3), thalamus, globus pallidus (n = 2), brainstem, and spinal cord (n = 1). P76 showed lactic acidosis (3.0mmol/L; NV < 1.77) whereas none had increased lactic acid in the CSF. Increased excretion of organic acids was recorded in 3 of 4 patients: lactic acid (P76), glutaric acid (P77), and mildly increased alpha-ketoglutaric acid and dicarboxylic acid (P79). P76 and P78, who did not receive thiamine supplementation, died at the age of 29 and 6 months, respectively. P77 and P79 are currently aged 4 and 7 years, respectively. P77 receives a combination of thiamine (15mg/kg/day), biotin (1mg/kg/day; P77), and ketogenic diet, and P79 receives thiamine (500mg/day) alone. Both patients show severe neurological sequelae, with spasticity, hypotonia, dystonia, developmental delay, and high scores on the BFMDS (27 and 14, respectively).

Secondary Thiamine Deficiency

A total of 153 patients (beriberi, N = 88; Wernicke's encephalopathy, N = 65) were collected, aged between 2 weeks and 17 years of life.[4,19–42] A summary of the main characteristic features collected in our database is provided in Figure 6. Predisposing factors were reported in

	A. Primary causes (*SLC19A3* N=70; *SLC25A19* N=5; *TPK1* N=4)	B. Secondary causes (beriberi N=88; Wernicke encephalopathy N=65)
Age at onset	***SLC19A3***: 1 month – 34 years, ***SLC25A19***: 20 months – 6.5 years and ***TPK1***: 1 month – 2.5 years	**Beriberi** (two weeks- 1 year), **Wernicke's encephalopathy** (19 months – 17 years)
Consanguinity	***SLC19A3*** and ***TPK1***: 73 -75%, ***SLC25A19***: 100%	Non-consanguinity reported
Triggers or predisposing factors	Trigger events in 55% of patients: viral or bacterial infections, trauma, profuse exercise and vaccination	Predisposing factor in 100% patients. **Beriberi**: breastfeeding by mothers with inadequate intake of thiamine or feeding with low thiamine-content formula. **Wernicke's encephalopathy** gastrointestinal surgical procedures, prolonged total parenteral nutrition without thiamine, malignancies, organ transplantation, pancreatitis, restricted diet (soy milk, autism spectrum disorder)
Clinical pictures	Recurrent encephalopathy (Leigh syndrome phenotype) is common in all three genetics defects. No cardiovascular or respiratory symptoms are observed. Other symptoms: **SLC19A3:**dystonia, seizure, spasticity, dysphagia, dysarthria, ophtalmoplegia, nystagmus, strabismus, akinetic-rigid syndrome, psychomotor regression and ataxia, ***SLC25A19***: flaccid tetraparesis and peripheral neuropathy and ***TPK1***: ataxia, dystonia and developmental delay	**Beriberi symptoms:** systemic (fever, vomiting, diarrhea and anorexia), neurologic (altered sensorium, persistent hoarse cry, ptosis, nystagmus, seizure, hypotonia, divergent squint, peripheral and central neuropathy), cardiovascular (shock, tachycardia and edema) and respiratory symptoms (acidotic breathing and respiratory distress syndrome) **Wernicke's encephalopathy triad:** acute or subacute ataxia, altered consciousness, and ophthalmoparesis
Biochemical abnormalities	***SLC19A3***: metabolic acidosis (16%), increased lactate in blood (50%) and CSF (17%) and normal thiamine blood levels, and increased CSF lactate ***SLC25A19*** 100% and ***TPK1*** 0%	84% (11/13) series report metabolic acidosis, increased lactate in blood, urine and/or CSF. 100% (16/16) detect low thiamine blood levels.
MRI abnormalities (in order of frequency)	***SLC19A3***: putamen, caudate, cerebral cortex, ventromedial thalamus, cerebellum, brainstem, periaqueductal region, spinal cord and globus pallidus, ***SLC25A19***: putamen and caudate and ***TPK1***: basal ganglia, cerebellum and dentate nuclei.	Common (>50% series): dorsalmedial thalamus, periaqueductal region, mammillary bodies, caudate, putamen, and tectal plate. Uncommon (<50%): cranial nerve nuclei, cerebral cortex, and hypothalamus and cerebellum.
Treatment and response to treatment	***SLC19A3*** (Td: 5-55 mg/kg/d): 86% presented clinical recovery in the following hours or days, 3 patients did not improve at all. ***SLC25A19*** (Td: 400 – 600 mg/day): avoid decompensation and ***TPK1*** (Td: 15 mg/kg/d): variable response.	Initially Td 50 - 1500 mg/day (oral or intra-venous), then oral treatment for 2-3 weeks. Complete biochemical and clinical improvement in a few hours.
Sequels	***SLC19A3***: 38% mild to moderate intellectual disability, ataxia, seizure and dystonia, ***SLC25A19***: polyneuropathy, and ***TPK1***: 100% severe sequels	<6% mild to severe intellectual and motor disability, ataxia, seizures. Radiological improvement in 2 - 3 months in the majority of cases.
Mortality	***SLC25A19*** - BSDPP phenotype: 0%, ***SLC19A3***: 21% and ***TPK1***: 50%	5- 20%

FIGURE 6: Clinical, biochemical, and radiological characteristics of patients with inherited thiamine defects (N = 79) compared to secondary thiamine deficiency (N = 153). CSF = cerebrospinal fluid. [Color figure can be viewed at www.annalsofneurology.org]

100% of cases. Systemic, cardiovascular, and respiratory symptoms were recorded in all patients with beriberi and a few cases with Wernicke encephalopathy. The majority of cases showed increased blood (53 of 55; 96%) and CSF lactate 12 of 13; 92%) and metabolic acidosis (17 of 34; 50%). Low blood thiamine levels were detected in 100% of cases that were analyzed. The thalamus, periaqueductal region, and mammillary bodies were more frequently involved, as opposite to patients with inherited thiamine defects, who showed more-frequent alterations in the caudate and putamen. Clinical improvement after thiamine supplementation was constant, and less than 20% showed mortality or neurological sequel.

Discussion

Thiamine diphosphate, the metabolically active form of thiamine, is essential for energy production in the CNS. In brain regions with high metabolic demands, thiamine deficiency attributed to exogenous (nutritional) or endogenous (genetic) defects can trigger a metabolic crisis, and Wernicke's encephalopathy is a model of cerebral thiamine deficiency.[43] Primary and secondary conditions leading to thiamine deficiency have overlapping features in children, both presenting with acute episodes of encephalopathy, bilateral symmetric brain lesions, and biomarkers of mitochondrial dysfunction, such as lactic acid accumulation in different tissues and high excretion of organic acids. In both scenarios, early thiamine supplementation may lead to clinical recovery within a few hours or days, and brain lesions may be reversible on MRI.

In our international study group of 79 patients with inherited thiamine defects, the vast majority of children were born to consanguineous patients; they presented between the age of 1 and 6 years in the context of a febrile illness, with acute/recurrent encephalopathy, basal ganglia lesions, dystonia, hypotonia, spasticity, ataxia, and seizures. Lactic acid accumulation in the CNS was identified by MRS in half of the *SLC19A3* patients, and in all *SLC25A19* cases by CSF analysis, suggesting brain energy failure, as in other cases of Leigh's encephalopathy.[13] Also, abnormal organic acids were identified in a few *SLC19A3* and *TPK1* cases. These included organic acids that were specific of thiamine-

dependent mitochondrial enzymes, such as lactic acid (PDHc), alpha-ketoglutarate (OGDHC), and branched chain keto-acids (BCODC), and other nonspecific organic acids caused by generalized mitochondrial dysfunction. These biomarkers did not predict outcome in our series.

Fundamental features to suspect primary thiamine defects against secondary etiologies were the frequency of consanguineous families, the absence of predisposing factors for beriberi and Wernicke's encephalopathy, the lack of cardiovascular or respiratory features, and the sparing of mammillary bodies on MRI (Fig 6).

A characteristic feature that distinguished patients with mutation in *SLC25A19* from the other genetic defects was the presence of peripheral neuropathy. This phenotype was initially described in Spiegel et al,[12] and, to our knowledge, no further patients have been reported on since then. We identified a novel missense variant in the *SLC25A19* gene in a 20-year-old woman with striatal necrosis and peripheral neuropathy, thus providing clinical evidence to the recognition of this phenotype. In contrast to *SLC19A3* and *SLC25A19*, patients with mutation in *TPK1* had an earlier onset of symptoms, they showed variable response to thiamine supplementation, and developed a more-severe phenotype with higher morbidity and mortality.

Based on the frequency of the main clinical and radiological features, and on specific biomarkers, we propose diagnostic criteria for the three known inherited thiamine defects with prominent neurological involvement (Table 1). Total thiamine levels in blood are reduced in patients with secondary deficiencies, but are normal in genetic conditions,[44–46] in which the quantification of thiamine isoforms, either in blood, CSF, fibroblasts, or muscle cells, is critical for the diagnosis.[13,47]

More than one third of *SLC19A3* patients had several recurrent encephalopathic episodes before vitamin supplementation. Most of them were born after the first description of the disease by Ozand et al in 1998,[48] suggesting that this is an underdiagnosed disorder. Thiamine and biotin supplementation led to prompt and significant clinical recovery in most *SLC19A3* patients. Survival analyses showed that the mean survival length was longer in patients who received thiamine and biotin compared to nontreated patients. No further episodes of encephalopathy, dystonia, or other neurological disturbances were noted after vitamin supplementation. Additionally, patients had effective control of seizures without the need of antiepileptic drugs and nonspecific biomarkers of mitochondrial dysfunction remained within normal limits. More important, half of treated patients had no disability at all, and neurological examination was normal at the time of assessment. A positive correlation was observed between the disability scores and the time spent between disease onset and thiamine initiation, meaning that the earlier the therapeutic intervention, the lower the sequel observed, although these data did not reach statistical significance. It was likely that the deceased patients with severe disabilities were missing relevant data, representing an important bias against a significant correlation in this analysis.

We identified clinical parameters that may help predict neurological outcomes before the initiation of vitamin supplementation. Patients with symptoms presenting within the first 6 months of life had a shorter survival curve and higher scores on the disability scale. Moreover, the distributions of brain lesions in the deceased *SLC19A3* patients were more diffuse, with a significantly higher involvement of the globus pallidus and brainstem. This is in accord with previous reports that indicate a subgroup of patients with thiamine transporter 2 deficiency presenting with fatal infantile LS, who died very early in the disease course, or were left with severe neurological sequel and extensive brain injury despite the initiation of vitamin treatment.[9,49] In our cohort, 4 children died despite vitamin supplementation. Pretreatment predictors of poor responses in these patients were extensive brain involvement (P49), early onset of the disease (P41), or the co-occurrence of septicemia in a child receiving intensive care (P1).

Based on these data, we believe that patients presenting with LS should be treated with a vitamin cocktail including thiamine and biotin, given that the phenotype of inherited thiamine defects may be clinically indistinguishable from other genetic disorders leading to Leigh's encephalopathy. However, therapeutic interventions should be individualized in those cases presenting clinical predictors of poor neurological recovery.

SLC19A3 patients showed longer survival length than patients with complex I deficiency, with more than 60% of the *SLC19A3* patients surviving at 20 years of age. Previous studies have demonstrated poor survival rates in LS patients.[50–55] Factors associated with poor prognosis in LS patients were the onset of symptoms within the first 6 months, the presence of cardiomyopathy,[56] and brainstem involvement.[50] As opposite to these features, *SLC19A3* patients in our series had a median age at disease onset of 3 years, they did not show cardiac involvement, and brainstem lesions were observed in a minority of cases. More important, thiamine supplementation prolonged survival in *SLC19A3* patients, whereas no effective treatment is available for complex I deficiency patients. We did no observe differences on survival between *SLC19A3* and PDHc patients; however, an almost significant difference

TABLE 1. Suggested Criteria for the Diagnosis of Inherited Thiamine Defects With Prominent Neurological Involvement

Required
1. ***Clinical criteria***
a. ***SLC19A3***: Acute or recurrent episodes of encephalopathy (decreased consciousness, irritability) with two or more of the following: (1) dystonia, (2) hypotonia, (3) bulbar dysfunction, (4) ataxia, and (5) seizures. Of note, 16% of patients may have an insidious onset of symptoms (psychomotor regression, clumsy or abnormal gait, and stiff limbs).
b. ***SLC25A19***: Acute or recurrent episodes of encephalopathy with: (1) progressive peripheral neuropathy or (2) severe congenital microcephaly with brain malformations.
c. ***TPK1***: Acute or recurrent episodes of encephalopathy, with two or more of the following: (1) dystonia, (2) hypotonia, (3) ataxia, (4) seizures, and (5) developmental delay. Of note, some patients may have a nonepisodic early-onset global developmental delay.
2. ***Biochemical criteria***
a. Normal total thiamine blood levels
b. Low free-thiamine in CSF and/or fibroblasts (*SLC19A3*)
c. Low TDP in blood, muscle, and/or fibroblasts (*TPK1*)
d. High excretion of alpha-ketoglutaric acid in urine (common in *TPK1* and *SLC25A19*, rare in *SLC19A3*).
3. ***Radiological criteria***
a. MRI pattern compatible with Leigh's syndrome (*SLC19A3*, *SLC25A19*, *TPK1*) or Wernicke's encephalopathy (*SLC19A3*)
i. ***SLC19A3***: Symmetrical T2W hyperintensity of caudate, putamen, cortico/subcortical areas, and/or ventromedial thalamus. No involvement of mammillary bodies.
ii. ***SLC25A19***: Symmetrical T2W hyperintensity in the caudate and putamen.
iii. ***TPK1***: Symmetrical T2W hyperintensity in basal ganglia and cerebellum (dentate nuclei).
4. ***Therapeutic criteria***
a. Clinical improvement after thiamine supplementation.
Supportive
1. Consanguinity
2. Trigger event (infection, vaccination, trauma, intense physical activity, etc.).
3. Absence of predisposing factors of beriberi or Wernicke's encephalopathy.
4. Absence of systemic features of mitochondrial disease (cardiomyopathy, arrhythmia/conduction defects, renal tubulopathy, or dysmorphic features).
5. Increased lactate in blood and/or CSF.
6. Normal OXPHOS and PDHc activity in muscle and fibroblast.
7. Increased lactate on MRS.

CSF = cerebrospinal fluid; TDP = thiamine diphosphate; MRI, magnetic resonance imaging; T2W = T2 weighted; OXPHOS = oxidative phosphorylation; PDHc = pyruvate dehydrogenase complex; MRS = magnetic resonance spectroscopy.

was observed when selecting male patients with PDHA1 deficiency, who are known to have a poorer survival than females. PDHc patients benefit from ketogenic diet[57] and thiamine supplementation,[58] which reduce neurological features and the frequency of hospitalizations.

A high proportion of *SLC19A3* patients in our cohort were homozygous for the missense variant, c.1264A>G, all of them belonging to the Arab ethnic group. These patients had a longer survival than patients with other mutations. The higher proportion of patients

receiving thiamine and biotin in the group of homozygous c.1264A>G patients compared to patients with other mutations may account for these differences. In line with this observation, transfection studies demonstrated that the c.1264A>G mutation led to a protein with null-transport activity,[59] which is difficult to correlate with a more-benign phenotype.

Among patients from European countries, we identified 4 compound heterozygous cases with c.74dupT/c.980-14A>G that had in common a late disease onset, 3 of them in adolescence and adulthood, with an excellent response to thiamine.[60,61] Likewise, a recently described patient with the same mutations presented an adult-onset subacute leukoencephalopathy with an effective response to thiamine.[62]

Our results confirm previous reports on a characteristic pattern of brain injury in inherited thiamine defects[9–12,17,19,45,60,61,63] that will help in the early recognition and differential diagnosis. The majority of *SLC19A3* patients showed striatal lesions in combination with other affected brain areas, including cortico/subcortical regions of the cerebral hemispheres, ventromedial thalamic nuclei, cerebellum, brainstem, periaqueductal region, spinal cord, and globus pallidus, in order of decreasing frequency. As previously mentioned, deceased patients had a greater involvement of the brainstem and globus pallidum. Lesions in the respiratory centers, located in the medulla oblongata and pons, may be responsible for acute respiratory failure, the most frequent cause of death in this cohort, as in other LS patients. Moreover, bilateral lesions in the globus pallidus were also related to severe dystonia and poor prognosis in patients with glutaric aciduria type I and in carbon monoxide intoxication.[64,65] Isolated striatal necrosis was a common feature of *SLC25A19* patients. In contrast, TPK1 patients showed basal ganglia lesions in combination with involvement of the cerebellum and dentate nuclei.

The small size of the sample was an important limitation for this study. Also, recruitment was restricted to published cases and individual centers, leading to a possible selection bias toward more-severe presentations. These are common limitations for research in rare diseases, where the limited number of patients hampers the recognition of the full spectrum of severities. Also, a more-accurate assessment on neurodevelopment should be warranted in future studies.

In summary, this international study describes the natural history of 79 patients with inherited defects in thiamine transport and metabolism. We confirm that thiamine defects manifest with acute brain injury in the first decade of life in the vast majority of patients. We demonstrate how vitamin supplementation modifies the survival curve of *SLC19A3* patients and identify statistically significant predictors of neurological outcome that may guide clinicians in further therapeutic interventions. Our study indicates a better prognosis than other causes of LS, encouraging clinicians to suspect the disease and to make an early diagnosis and accurately prescribe treatment. We also contribute with diagnostic criteria for inherited thiamine defects and help differentiate them from secondary causes of thiamine deficiency.

Acknowledgment

This work is funded by the "Plan Nacional de I+D+I and Instituto de Salud Carlos III–Subdirección General de Evaluación y Fomento de la Investigación Sanitaria", project PI12/02010, PI15/00287, J.D.O.E. (Rio Hortega—CM16/00084), and the European Social Fund (ESF). R.M. and R.W.T. are supported by the Wellcome Centre for Mitochondrial Research (203105/Z/16/Z), the Medical Research Council (MRC) Centre for Translational Research in Neuromuscular Disease, Mitochondrial Disease Patient Cohort (UK) (G0800674), the Lily Foundation, and the UK NHS Highly Specialized Service for Rare Mitochondrial Disorders of Adults and Children.

We acknowledge the contributions of the clinical scientists from the referring hospitals for their help with data processing and the statistical assistance of Daniel Cuadras Pallejà (Statistician, Foundation Sant Joan de Déu, Barcelona, Spain).

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The following authors contributed to the study concept and design (J.D.O.E., B.P.D.), analysis of data (J.D.O.E., M.A., M.M.L., N.D., R.S., I.F.deC., M.G., R.A., M.N., P.R.P., B.T., and B.P.D.), and drafting of the manuscript and figures (J.D.O.E., B.P.D., M.M.L., R.A., and P.R.P.). Authors who participated in data acquisition were included into the Thiamine Deficiency Study Group: Felix Distelmaier, Andreas Hahn, Eva Morava, Siddharth Banka, Rabab Debs, Jamie L. Fraser, Pirjo Isohanni, Tuire Lähdesmäki, John Livingston, Yann Nadjar, Elisabeth Schuler, Johanna Uusimaa, Adeline Vanderver, Jennifer R. Friedman, Michael R. Zimbric, Robert McFarland, Saikat Santra, Evangeline Wassmer, Laura Martí-Sanchez, and Alejandra Darling. All authors participated in editing and approving of the manuscript.

Potential Conflicts of Interest

A.V. reports nonfinancial support from Illumina, personal fees from Shire, nonfinancial support from Lilly, and nonfinancial support from Gilead, outside the submitted work. The authors have no other relevant affiliations or financial involvement with any organization or

entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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Supplementary material

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Supplementary TABLE 1. Demographic, Clinic, Genetic, and Radiological Features of 78 Patients With *SLC19A3*, *SLC25A19*, and *TPK1* deficiency

*Other sibling affected not included in this study; NA = not available, **SYMPTOMS AT ONSET/ OUTCOME:** Encephalopathy (E), Paroxysmal Ataxia (A), Hypotonia (H), Tremor (T), Dystonia (D), Status dystonicus (Sd), Chorea (Ch), Opistothonus (O), Rigidity (Akinetic-rigid syndrome) (Ak), Spasticity (S), Nystagmus (N), Strabismus (Sb), Ptosis (Pt), Ophtalmoplegia (Oph), Diplopia (D), Vertigo (V), Hypoaesthesia (Hy) Dysarthria (Dth), Dysphagia (Dph), Weight loss (W), Respiratory failure (Res), Liver disease (Hep), Rhabdomyolisis (Rab), Dysautonomia (Dys), Jaundice (J), Seizure—including focal, generalized, and infantile spasms (Sz), Peripheral neuropathy (Pn), Developmental arrest (Rg), Movement disorder (MD), Spasticity (S), Intellectual disability (ID), Scoliosis (Sch), microcephaly (m). **ABNORMAL REGIONS ON NEUROIMAGING:** Caudate (C), Putamen (P), Globus pallidus (Gp), Thalamus (T), Corticosubcortical (Cs), Cerebellum (Cb), Brainstem (B), Periqueductal (Pq), Spinal cord (SC), Lactate on MRS (Lactate), Cerebral atrophy (CA), Cerebellar atrophy (CbA). **PHENOTYPES** Leigh Syndrome (LS), Biotin responsive basal ganglia disease (BTRBGD), Asymptomatic.

CASE	REF	CONSANGUINITY-FAMILY HISTORY (Yes (Y), No (N))	ETHNICITY	ONSET/ CURRENT AGE (Years)	MUTATION ALLELE 1	MUTATION ALLELE 2	PHENOTYPES	SYMPTOMS at onset	TRIGGER EVENT at onset	ABNORMAL REGIONS ON NEUROIMAGING	OUTCOME	DISABILITY SCORE
P1	27	N	White European-Spanish	13mo/-	c.1079dupT/p.L360Ffs*11	c.980-14A>G/p.G327Dfs*8	LS	E, H, T, D, Ch, O, N, J, Hep, W, Res, Dph, A	Viral infection	C,P,T,Cs, Lactate	Deceased at 14 mo	—
P2	27	Y	Arab-Moroccan	1mo/3y	c.68 G>T/p.G23V	c.68 G>T/ p.G23V	LS	E, H, T, D, O, S, Dph	None	P, T, Cs, Lactate	Alive-MD, E, S, M	16
P3	27	N-sibP4	White European-Spanish	17y/25y	c.74dupT/p.S26Lfs*19	c.980-14 A>G/p.G327Dfs*8	BTRBGD	E, H, T, D, Sd, Ak, S, N, Rab, Dys, Dth, Hy, V, D, Pt	Profuse exercise	C,P,T,Cs	Alive-normal, D	5
P4	27	N-sibP3	White European-Spanish	4y/4y	c.74dupT/ p.S26Lfs*19	c.980-14 A>G/p.G327Dfs*8	BTRBGD	E, H, T, D, Dph, Dth	None	C,P,T, Lactate	Alive-normal	1
P5	7	Y-sibP6	Arab-Saudi Arabian	2y/7y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	E, A, H, T, D, S	Viral infection	C,P,T, Cs, Lactate	Alive-normal	0
P6	7	Y-sibP5	Arab-Saudi Arabian	8y/12y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	E, A, H, T, D, S, Oph, Dth, Dph, Sz	Viral infection	C,P,T, Cs, Cb, B, Pq, SC, Lactate	Alive-normal	3
P7	7	Y-sibP14	Arab-Saudi Arabian	13y/-	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	E, H, T, D, S, Oph, Dth, Dph, Res, Rab, Sz	Viral infection	C,P,T, Cs, Cb, B, Pq, SC, Lactate	Deceased at 13 y	—
P8	7	Y	Arab-Saudi Arabian	13y/16	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	E, H, T, D, S Oph, Dth, Dph, Res, Sz	Viral infection	C,P,T, Cs, Cb, B, Pq, Lactate	Alive-S, ID, D	29
P9	7	Y-sibP10	Arab-Saudi Arabian	6y/10y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	E, A, H, T, D, S, Dth, Dph	Viral infection	C,P, Lactate	Alive-ID, D	10
P10	7	Y-sibP9	Arab-Saudi Arabian	-/16y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	NA	NA	C,P,T, Cs, Cb, B, Pq, SC, Lactate	Alive-not known	—
P11	7	Y	Arab-Saudi Arabian	50mo/8y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	E, A, H, T, D, S, Oph, Dth, Dph, Sz	None	C,P,T, Cs, Cb, B, Pq, SC, Lactate	Alive-normal	0
P12	7	Y	Arab-Saudi Arabian	-/7y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	NA	NA	C, P, T, B, SC	Alive-normal	—
P13	7	Y	Arab-Saudi Arabian	8y/12y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	E, A, H, D, S, Oph, Dth, Dph, Sz	Viral infection	C,P,T, Cs, Cb, B, SC, Lactate	Alive-normal	0
P14	7	Y-sibP7	Arab-Saudi Arabian	11y/32y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	E, A, D, S, Dth	None	C, P	Alive-S, ID, D	10
P15	7	Y	Arab-Saudi Arabian	11y/13y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	E, A, H, D, S, Oph, Dth, Dph, Sz	Viral infection	C,P,T, Cs, Cb, SC	Alive-normal, D	6
P16	7	Y	Arab-Saudi Arabian	2y/5y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	E, A, H, D, S, Dth, Dph, Sz	Viral infection	C,P,T, Cs, Cb, B, SC, Lactate	Alive-S, ID, D	8
P17	7	Y	Arab-Saudi Arabian	3,5y/15y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	E, A, H, D, S, Dth, Dph, Sz	Viral infection	C, P	Alive-ID, D	8
P18	7	Y	Arab-Saudi Arabian	11y/13y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	E, A, H, T, D, S, Oph, Dth	Viral infection	C,P,T, Cb	Alive-normal	2
P19	7	Y-*	Arab-Saudi Arabian	3y/6y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	E, A, H, D, S, Dth, Dph, Sz	Trauma	C,P,T, Cs	Alive-normal	2
P20	7	Y-P21	Arab-Saudi Arabian	8y/11y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	E, A, H, D, S, Oph, Dth, Dph, Sz	Viral infection	C, P, T, Cs, Cb, Pq, Lactate	Alive-normal	2
P21	7	Y-P20	Arab-Saudi Arabian	9y/13y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	E, A, H, D, S, Dth, Sz	Trauma	C, P, T, Cs, Cb, Pq, Lactate	Alive-normal	2
P22	7	Y	Arab-Saudi Arabian	1y/4y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	E, A, H, D, S, Dth, Dph, Sz	Viral infection	C, P	Alive-H, ID, D	14
P23	7	Y-P24	Arab-Saudi Arabian	5,5y/6y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	E, A, H, T, D, S, Dth, Dph, Sz	Viral infection	C, P, T, Cs, Cb, Pq, SC	Alive-S, ID	21
P24	7	Y-P23	Arab-Saudi Arabian	47mo/4,08y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	E, A, H, D, S, Dth, Dph	Viral infection	C, P, T, Cs	Alive-normal	0
P25	7	Y	Arab-Saudi Arabian	5y/10,08y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	E, A, H, D, S, Dth, Dph, Sz	Viral infection	C, P, T, Cs, Cb, Lactate	Alive-A	2
P26	7	Y	Arab-Saudi Arabian	1y/5y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	E, A, H, D, S, Dth, Dph, Sz	Viral infection	C, P, T, Cs, Cb, B, Pq, SC, Lactate	Alive-m, H, ID, D	28
P27	7	Y	Arab-Saudi Arabian	15y/20y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	E, A, H, D, S, Oph, Dth	Trauma	C, P, T	Alive-normal, D	7
P28	22	N-P29	White European-Portuguese	29y/25y	c.74dupT/p.S26Lfs*19	c.980-14 A>G/p.G327Dfs*8	BTRBGD	E, D, N, Dth, Dph, Sz	Urinary tract infection	C, P, GP, Cs	Alive-D, S, Dth	5
P29	22	N-P28	White European-Portuguese	34y/40y	c.74dupT/p.S26Lfs*19	c.980-14 A>G/p.G327Dfs*8	BTRBGD	E, D, O, S, Oph, Dth, Dph, W	Viral infection	C, P, T, Cs, B	Alive-D, S, Dth, ID	23
P30	28	N-P31	White European-German	3y/-	c.280 T>C/p.W94R	c.1173-3992_1314+41del4175/ p.Q393*fs	BTRBGD	E, A, Sd, O, S, Dth, Dph, Hep, Sz, D	Viral infection	C, P, T	Deceased at 12y	—
P31	28	N-P30	White European-German	9y/-	c.280 T>C/p.W94R	c.1173-3992_1314+41del4175/ p.Q393*fs	BTRBGD	E, A, Sd, Ch, S, Dth, Dph, Res, Sz	Viral infection	C, P, T	Deceased at 13y	—
P32	NR	Y-P32	Arab-Moroccan	3.5y/9y	c.1264G>A/p.T422A	c.1264G>A/p.T422A	BTRBGD	E, H, T, Dth, Sz	None	C, P, T, Cs, Lactate	Alive-S, ID, D	10
P33	NR	Y-P33	Arab-Moroccan	1.5y/13y	c.1264G>A/p.T422A	c.1264G>A/p.T422A	BTRBGD	E, H, T, Sz	Gastroenteritis	C, P, Cs	Deceased at 13y	—
P34	29	N-P35, P36	Arab-Iraqi	19mo/4y	c.157A>G/p.N53D	c.157A>G/p.N53D	LS	E, A, H, D, Dth, Sb,	Pneumonia	C, P, T, Cs, Cb, Pq, Lactate	Alive-normal	0
P35	NR	N-P34, P36	Arab-Iraqi	Asymptomatic/ 0,16y	c.157A>G/p.N53D	c.157A>G/p.N53D	Asymptomatic	Asymptomatic	NA	NA	Alive-normal	—
P36	NR	N-P34, P35	Arab-Iraqi	Asymptomatic/ 0,16y	c.157A>G/p.N53D	c.157A>G/p.N53D	Asymptomatic	Asymptomatic	NA	NA	Alive-normal	—
P37	29	Y-P37	Arab-Kurdish	2y7mo/7y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	LS	E, H, T, S, Dph, Dth, A	Viral infection	C, P, T, Lactate	Alive-ID	—
P38	29	Y-P38	Arab-Kurdish	3mo/14y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	E, H, D, Dph, W,	None	C, T, Cs	Alive-ID, D	28
P39	2	Y-P40, P41	Arab-Moroccan	1mo/0,08y	c.20C>A/p.S7*	c.20C>A/p.S7*	LS	NA	NA	NA	Deceased at 1mo	—
P40	2	Y-P39, P41	Arab-Moroccan	1mo/0,08y	c.20C>A/p.S7*	c.20C>A/p.S7*	LS	NA	NA	NA	Deceased at 1mo	—
P41	2	Y-P39, P40	Arab-Moroccan	1mo/0,11y	c.20C>A/p.S7*	c.20C>A/p.S7*	LS	E, H, T, D, O, Ak, S, N	None	NA	Deceased at 6w	—

P42	2	Y-P43	Arab-Moroccan	0,08y	c.20C>A/p.S7*	c.20C>A/p.S7*	LS	NA	NA	NA	Deceased at 1mo	—
P43	2	Y-P42	Arab-Moroccan	0,08y	c.20C>A/p.S7*	c.20C>A/p.S7*	LS	NA	NA	NA	Deceased at 1mo	—
P44	2	N-*	Arab-Moroccan	1mo/20y	c.20C>A/p.S7*	c.20C>A/p.S7*	LS	NA	NA	NA	Deceased at 20y	—
P45	2	N-*	Arab-Moroccan	1mo/15y	c.20C>A/p.S7*	c.20C>A/p.S7*	LS	NA	NA	NA	Deceased at 15y	—
P46	NR	N-P47	African/Afro-Caribbean	1mo/2mo	c.91T>C/p.S31P	c.516Het_delC/p.D173Tfs*35	LS	E, H, D, O, S	None	P, GP, T, Cs, B	Deceased at 2mo	—
P47	NR	N-P46	African/Afro-Caribbean	1mo/0,75y	c.91T>C/p.S31P	c.516Het_delC/p.D173Tfs*35	LS	E, H, T, D, O, S	None	P, GP, T, Cs, Cb, B	Deceased at 9mo	—
P48	NR	N	White European-Finnish	1mo/2y	c.541T>C/p.S181P	c.541T>C/p.S181P	LS	E, H, T, D, Sd, Ch, O, S	Viral infection	P, T, Cs, Cs, Lactate	Alive-H, m, D, ID	23
P49	NR	N	White European-Finnish	3,5mo/3,5y	c.541T>C/p.S181P	c.833T>C/p.L278P	LS	E, H, T, D, Sd, O	None	C, P, GP, T, Cs, Cb, B	Deceased at 3.5y	—
P50	26	Y-*	Arab-Saudi Arabian	3y/7y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	E, A, Dth,	Viral infection	C, P, Cs	Alive-normal	—
P51	26	Y	Arab-Saudi Arabian	3.5y/5y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	A	Viral infection	C, P, Cs	Alive-normal	—
P52	26	Y	Arab-Saudi Arabian	3mo/3y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	E, H, Sz	None	C, P, T, Cs, Cb, B	Alive-H, ID, Sz	—
P53	26	Y-*	Arab-Saudi Arabian	14mo/17y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	D, S, Dth, Sz	None	C, P	Alive-m, S, Sz, ID, D	9
P54	26	Y-*	Arab-Saudi Arabian	5mo/14y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	E, D, S, Dth, Sz	None	C, P, Cs, Cb	Alive-m, S, Sz, ID, D	26
P55	26	N	Arab-Saudi Arabian	1y/7y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	E, D, S, Sb, Sz, Dth	None	C, P, T, Cs	Alive-status not known	—
P56	26	Y	Arab-Saudi Arabian	5mo/4y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	E, H, Ch	Vaccination	C, P, Cb	Alive-A, ID, H	—
P57	26	Y	Arab-Saudi Arabian	3y/13y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	E, H, A, Dth, Sz	None	C, P, Cs, Pq	Alive-S, Sch, D	—
P58	26	N-*	Arab-Saudi Arabian	2y/24y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	E, S	Viral infection	C, P	Alive-D	—
P59	26	Y-*	Arab-Saudi Arabian	4y/25y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	E, A, Dth, Sz	None	C, P	Alive-D, Sz	—
P60	26	Y-*	Arab-Saudi Arabian	6y/13y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	S, Sz	None	C, P, Lactate	Alive-normal	—
P61	26	Y-*	Arab-Saudi Arabian	21mo/12y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	D, S, Dth, W, Sz	None	C, P, GP, Lactate	Alive-S, Sz, H, ID, D	29
P62	26	Y-*	Arab-Saudi Arabian	3y/4y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	A	Viral infection	C, P	Alive-normal	—
P63	26	Y-*	Arab-Saudi Arabian	3.5y/8y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	A, Dth, MD	None	C, Cs	Alive-normal	—
P64	26	Y	Arab-Saudi Arabian	4mo/18mo	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	E, S, Dph, Hep, Sz	Meningitis	C, P, Cs, Cb	Alive-Sz, ID, D	—
P65	26	Y	Arab-Saudi Arabian	23mo/4y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	Rg, S, Dth, Dph	Viral infection	C, P, CA, CbA	Alive-A, S, ID	—
P66	26	Y-*	Arab-Saudi Arabian	3y/5y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	Dth	None	NA	Alive-normal	—
P67	26	Y	Arab-Saudi Arabian	3y/3y7mo	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	Rg	None	NA	Alive-normal	—
P68	30	Y-*	Arab-Moroccan	3.5y/10y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	E, H, D, Sd, Ch, Sz, Dth	Viral infection	C, P, Cs, Lactate	Alive-ID	0
P69	30	Y	Arab-Kuwaiti	9.5y/14y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	E, Oph, D, Sd, Dth	Viral infection	C, P, T, Cs, Cb, SC	Alive-normal	0
P70	NR	N	White-Hispanic	23mo/27mo	c.74dupT/p.S26Lfs*19	c.503_505delCGT/p.Y169*	BTRBGD	E, T, Pt, V, Dph, Dth, Dys, S	Viral infection	C, P, Gp, T, Pq, Lactate	Alive-normal	—
P71	5	Y-P72, P73, P74	Arab-Israeli	6y/14y	c.373G>A/p.G125S	c.373G>A/p.G125S	Bilateral striatal necrosis and neuropathy	E, H, Dph, Dth	Febrile illness	C, P,	Alive-Pn, ADHD,	1
P72	5	Y-P71, P73, P74	Arab-Israeli	6.5y/14y	c.373G>A/p.G125S	c.373G>A/p.G125S	Bilateral striatal necrosis and neuropathy	E, H,	Febrile illness	C, P,	Alive-Pn, ADHD,	1
P73	5	Y-P71, P72, P74	Arab-Israeli	3.5y/26y	c.373G>A/p.G125S	c.373G>A/p.G125S	Bilateral striatal necrosis and neuropathy	E, H, Sd, Dph, Dth	Febrile illness	C, P,	Alive-Pn, MD	6
P74	5	Y-P71, P72, P73	Arab-Israeli	4y/24y	c.373G>A/p.G125S	c.373G>A/p.G125S	Bilateral striatal necrosis and neuropathy	E, H, Sd, Dph, Dth	Febrile illness	C, P, T	Alive-Pn, MD	3
P75	NR	Y	White European-German	20mo/21y	c.580T>C/p.S194P	c.580T>C/p.S194P	Bilateral striatal necrosis	E, T, D, O, V, A, Dph	Otitis media	C, P, Gp, Cs	Alive -normal	0
P76	9	Y-P77	Asian-Chinese	6mo/-	c604T>G/p.W202G	c604T>G/p.W202G	LS	E,H,S, Hy, Dph, Res, Sz	Viral infection	C, P, GP, T, B, Pq, SC, Cb-dentate	Deceased at 29 mo	—
P77	9	Y-P76	Asian-Chinese	4mo/4y5mo	c604T>G/p.W202G	c604T>G/p.W202G	LS	E, H, D, S, Sb, Hy, Dph	Febrile illness	P, T, Cb-dentate	Alive – S, H, ID	23
P78	NR	N-*	White European-Finnish	1mo/-	c.365T>C/p.I122T	c.365T>C/p.I122T	LS	H, Hep, Sz	N	P, Gp, Cb-dentate	Deceased at 6 mo	—
P79	10	Y	Indian	2,5y/7y	c.479C>T/p.S160L	c.479C>T/p.S160L	LS	S	Viral infection	Cb-dentate	Alive – S, MD, M, ID	14

Reference of patients with PDHc deficiency

To identify previously published PDHc deficiency cases, a systematic literature search was conducted in PubMed in January 2017 using the search terms "Pyruvate dehydrogenase complex deficiency", "pyruvate dehydrogenase complex", "PDHA1", "PDHB", and "PDHX". The search was limited to studies in humans published after January 1, 1990 (considering availability in our electronic library). We restricted the search to articles in English. Seventy-five full-text records were retrieved, describing 324 cases with PDHc deficiency. The cases were used to ascertain genotypes, sex, and for Kaplan– Meier survival analysis.

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Sintesis de resultados

- La gran mayoría de los niños con mutación del gen *SLC19A3* presenta su debut entre los 1 y 6 años de edad en contexto de una enfermedad febril, con signos de encefalopatía (disminución de la conciencia, hipotonía global), distonía, disartria, disfagia y convulsiones.
- En los pacientes con mutación del gen *SLC19A3,* las manifestaciones más frecuentes fueron: encefalopatía aguda (78%), distonía (74%) y lesiones simétricas del caudado y/o putamen (93%).
- Algunos pacientes (15%) con mutación en el gen *SLC19A3* presentaron un inicio insidioso de síntomas, con regresión psicomotora, marcha torpe u anormal, ataxia y espasticidad, por lo que se debería investigar esta patología en niños con estas características y lesiones de los ganglios basales o del tálamo.
- El aumento de lactato en sangre o LCR no es útil para predecir la supervivencia o severidad de estos pacientes. Así mismo, el alfa-cetoglutarato no es biomarcador sensible en estos pacientes.
- La suplementación con tiamina y biotina conduce a una pronta recuperación en los pacientes con mutaciones del gen *SLC19A3*, en quienes no se registran nuevos episodios encefalopáticos.
- La duración media de supervivencia es más larga en los pacientes con mutaciones en el gen *SLC19A3* tratados que en los pacientes no tratados (28,9 vs. 17,2 años, p <0,0001) y en los pacientes la mutación c.1264A>G en homocigosis vs. pacientes otras mutaciones (29,88 vs. 15,52 años, p <0,0001). Por lo tanto, la suplementación con vitamina mejora la supervivencia y modifica el curso natural de los pacientes con mutación del gen *SLC19A3.*

- Otros predictores de mal resultado son la historia de retraso psicomotor previa al inicio de síntomas, el inicio de síntomas en los primeros 6 meses de vida y la afectación radiológica del globo pálido y del tronco encefálico.
- Se identificaron seis nuevas mutaciones, incluyendo un paciente con necrosis estriatal y fenotipo de neuropatía periférica debido a mutación del gen *SLC25A19*.

NDUFS4 related Leigh syndrome: A case report and review of the literature.

"Síndrome de Leigh por defecto de NDUFS4: reporte de un caso y revisión de la literatura".

Mitochondrion. 2016 May;28:73-8.

Ortigoza-Escobar JD, Oyarzabal A, Montero R, Artuch R, Jou C, Jiménez C, Gort L, Briones P, Muchart J, López-Gallardo E, Emperador S, Pesini ER, Montoya J, Pérez B, Rodríguez-Pombo P, Pérez-Dueñas B.

Las causas genéticas del síndrome de Leigh son heterogéneas, con una pobre relación genotipo-fenotipo. En este trabajo se ha realizado el diagnóstico genético y la descripción clínico-radiológica de una paciente con mutación en el gen *NDUFS4,* ampliando de esta forma el espectro clínico y bioquímico de la enfermedad. Los trabajos publicados hasta el momento no habían reportado el defecto combinado de las actividades de los complejos I y III, de la CoQ y de la PDH hallados en esta paciente. Como explicación de estos hallazgos se hipotetizaba el montaje inadecuado del complejo I, para lo cual se realizó el análisis de este complejo mediante electroforesis en gel de poliacrilamida azul (BN-PAGE). También se realiza la descripción de los hallazgos de la biopsia muscular. Así mismo, en este trabajo se ha realizado por primera vez una revisión extensa de pacientes reportados (198 pacientes con 24 defectos genéticos diferentes) con deficiencia del complejo I.

Mitochondrion 28 (2016) 73–78

Contents lists available at ScienceDirect

Mitochondrion

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

Ndufs4 related Leigh syndrome: A case report and review of the literature

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ARTICLE INFO

Article history:
Received 4 January 2016
Received in revised form 31 March 2016
Accepted 1 April 2016
Available online 11 April 2016

Keywords:
Leigh syndrome
Respiratory chain complex I
NDUFS4
BN-PAGE
Next generation sequencing

ABSTRACT

The genetic causes of Leigh syndrome are heterogeneous, with a poor correlation between the phenotype and genotype. Here, we present a patient with an *NDUFS4* mutation to expand the clinical and biochemical spectrum of the disease. A combined defect in the CoQ, PDH and RCC activities in our patient was due to an inappropriate assembly of the RCC complex I (CI), which was confirmed using Blue-Native polyacrylamide gel electrophoresis (BN-PAGE) analysis. Targeted exome sequencing analysis allowed for the genetic diagnosis of this patient. We reviewed 198 patients with 24 different genetic defects causing RCC I deficiency and compared them to 22 NDUFS4 patients. We concluded that NDUFS4-related Leigh syndrome is invariably linked to an early onset severe phenotype that results in early death. Some data, including the clinical phenotype, neuroimaging and biochemical findings, can guide the genetic study in patients with RCC I deficiency.

1. Introduction

Leigh syndrome is a progressive neurodegenerative condition of childhood characterized by lesions of the basal ganglia, thalamus, brainstem, and less frequently, the cerebellum. The symptoms of Leigh syndrome are highly variable, but usually include psychomotor arrest or regression, hypotonia, dystonia, seizures, ocular movements, respiratory failure and vomiting. Biochemically, elevated lactate levels in the blood and cerebral spinal fluid are frequently encountered (Anderson et al., 2008). Recently, reversible causes of Leigh syndrome were described to involve a thiamine transporter type 2 deficiency (Ortigoza-Escobar et al., 2014; Lake et al., 2015). Therefore, an analysis of thiamine derivatives in the CSF and early treatment with thiamine and biotin are recommended (Ortigoza-Escobar et al., 2016).

RCC I (CI- NADH-ubiquinone reductase) deficiency is the most frequently observed abnormality and accounts for ~30% of the cases of Leigh syndrome (Fassone and Rahman, 2012; Hoefs et al., 2012; Pagniez-Mammeri et al, 2012; Pagniez-Mammeri et al., 2009). CI is the largest multimeric enzyme of the mitochondrial RCC and has been shown to oxidize NADH, transfer electrons to CoQ and pump protons across the mitochondrial membrane (Scheffler, 2015; Mimaki et al., 2012; Antonicka et al., 2003).

The aim of this report was to describe a Moroccan infant with fatal early Leigh syndrome and a combination of PDH, RCC and CoQ deficiencies in muscle tissue, who was identified to have a mutation in the *NDUFS4* gene using massive parallel sequencing (MPS). Blue-Native polyacrylamide gel electrophoresis (BN-PAGE) revealed a complete absence of the fully assembled RCC I in the muscle tissue, thereby confirming the crucial role of *NDUFS4* in the assembly of functional RCC I (Anderson et al., 2008; Assereto et al., 2014; Budde et al., 2003; Hinttala et al., 2005, Leshinsky-Silver et al., 2009, Lombardo et al., 2014; Papa et al., 2001, Petruzzella et al., 2005). To better characterize the phenotype of patients with NDUFS4-related Leigh syndrome, we compared

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http://dx.doi.org/10.1016/j.mito.2016.04.001
1567-7249/

their phenotype to the phenotype of 198 patients with RCC 1 deficiency. Our results confirm that children with *NDUFS4* mutations show a homogeneous early onset and severe, lethal course of the disease in contrast to the broad clinical spectrum described in RCC 1 defects.

2. Methods

Blood samples, a muscle biopsy and fibroblasts from the patient were collected with the approval of the Institutional Review Board at Hospital Sant Joan de Déu.

The PDHc activity was determined in cultured fibroblasts and muscle tissue as previously reported (Guitart et al., 2009). The substrate oxidation rates were analysed in fibroblasts by measuring $^{14}CO_2$ production from [^{14}C]-pyruvate and [^{14}C]-glutamate (Willems et al., 1978). The total CoQ concentration was determined using HPLC with electrochemical detection (Montero et al., 2005).

We performed BN-PAGE to isolate intact protein complexes from the skeletal muscle. The assembly of the five oxidative phosphorylation complexes was examined using two-dimension blue native/SDS-PAGE. The gels were blotted and incubated with five antibodies specific to each mitochondrial complex.

Pre-mortem open biopsies were taken and muscle specimens were stained using standard procedures.

Total DNA was extracted from blood samples using the MagnaPure system (Roche Applied Science, IN, USA). Genetic analysis of nuclear DNA-encoded genes involved in mitochondrial disorders was achieved through targeted exome sequencing using the TruSight One Sequencing Panel (Illumina) as previously described (Vega et al., 2016a, 2016b).

3. Results

3.1. Case report

The patient was a female born after spontaneous vaginal delivery at 40-weeks of gestation with a birth weight of 2860 g and head circumference of 34 cm. Her Apgar scores were 9 and 10 at 1 and 5 min, respectively. Her prenatal history was unremarkable, except for pyelectasis, which resolved spontaneously. Her Moroccan parents were consanguineous (first cousins).

She presented at 37 days of age with paroxysmal abnormal ocular movements consisting of conjugate down-gaze deviation, convergent strabismus and horizontal nystagmus. Her neurological examination was otherwise normal, and a cranial ultrasound disclosed no abnormalities. At 2 months of age, she was admitted to the hospital with vomiting, lethargy alternating with irritability and severe axial hypotonia with increased muscle tone in the four limbs. Cranial tomography showed bilateral and symmetric basal ganglia hypointensity. Brain MRI demonstrated bilateral and symmetric T2 signal hyperintensity in the globus pallidus, putamen, cerebral peduncles, medulla oblongata and cervical spinal cord. Swelling and restricted diffusion of the affected basal ganglia, together with a prominent lactate peak in the magnetic resonance spectroscopy of the left basal ganglia, suggested acute damage caused by Leigh syndrome (Fig. S1). The patient became less responsive and more hypotonic, despite treatment with biotin and thiamine, and developed an abnormal respiratory pattern leading to progressive respiratory failure requiring ventilator support. She presented with brief generalized seizures that responded well to diazepam, but the progression of symptoms led to her death 5 days after admission.

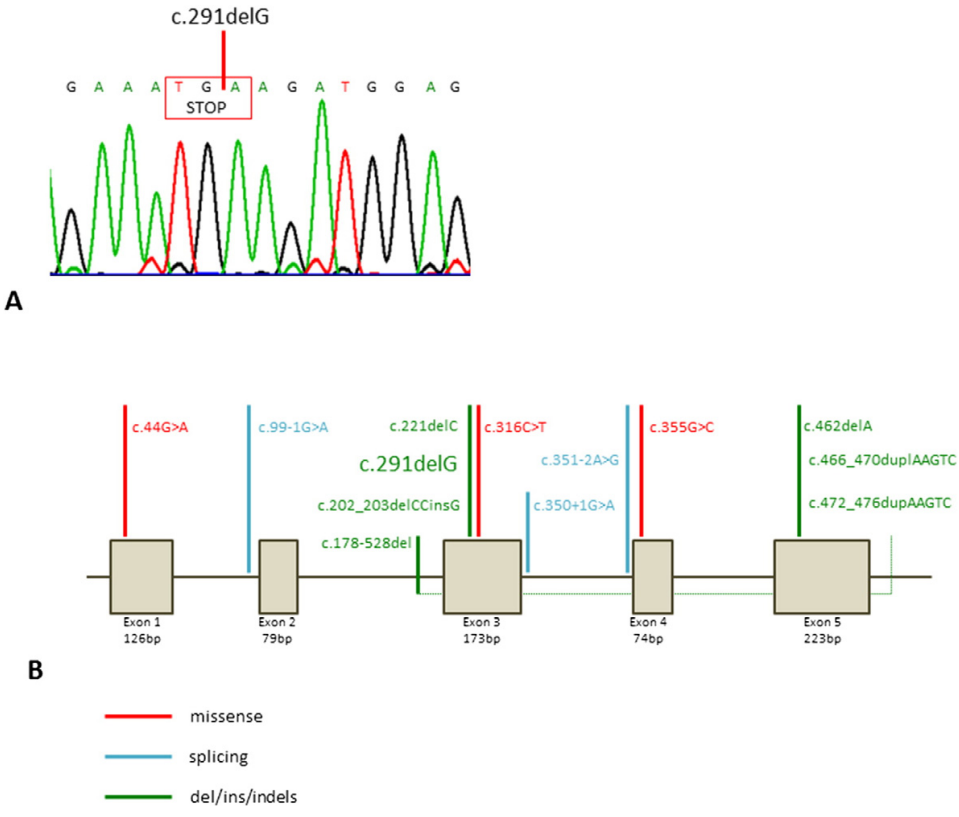

Fig. 1. (A) Sanger confirmation of the homozygous mutation, c.291delG (p.Trp97Ter), in the *NDUFS4* gene and (B) all human *NDUFS4* (NM_002495.2) described mutations.

The plasma lactate levels ranged from 3.9 to 5.8 mmol/L (normal < 2.2) over the 5-day period, and the ratio of plasma lactate to pyruvate was 17 (normal < 25). The urinary organic acids and plasma amino acids were normal. The patient showed a significant reduction in PDHc activity in fibroblasts (0.22 nmol/min/mg protein, control values 0.34–2.6) and muscle tissue (0.5 nmol/min/mg protein, control values 0.8–3.4) and a reduction in oxidation of pyruvate in fibroblasts (Table 2).

RCC analysis in muscle tissue showed a 50% reduction in the residual activity of RCC I and a 22% reduction in RCC III activity compared to the control values. The muscle CoQ activity was low relative to citrate synthase (CS) (2.2 nmol/U CS, reference values 2.7–8.4) (Table 2).

BN-PAGE analysis showed a complete absence of the fully assembled RCC I (~1 MDa) (Fig. S2A) in contrast to control cells. Two-dimension blue native/SDS-PAGE showed a complete absence of assembled RCC I in the patient's muscle tissue compared to control samples (Fig. S2B), whereas other oxidative phosphorylation complexes remained normal (Fig. S2C).

Histopathological investigation showed mild variability in the muscular fibre size. The oxidative stains (SDH and COX) showed immature patterning with isolated COX-negative fibres. The fibres showed an increase in the number and size of lipid droplets.

MPS revealed a previously described homozygous mutation c.291delG (p.Trp97Ter) in the *NDUFS4* gene (NM_002495.2) (Budde et al., 2000 and Scacco et al., 2003), which was further confirmed using Sanger sequencing (Fig. 1). All human *NDUFS4* described mutations are also shown in Fig. 1. No other biallelic disease-causing mutations were detected in the nuclear DNA-encoded genes targeted using a virtual panel based on the human MitoCarta (https://broadinstitute.org/pubs/MitoCarta). Unfortunately, a familial segregation analysis of the mutation could not be performed, as both parents were unavailable for DNA analysis.

3.2. Literature review

A total of 198 patients with RCC I deficiency due to nuclear DNA-encoded subunits have been reported: 50% supernumerary subunits, 18% assembly factors (*NDUFAF1–6*), 16% N module subunits and 16% Q module subunits. In addition, 61% of RCC I patients harboured mutations in one of the following genes: *NDUFS1*, *NDUFS4*, *NDUFS8*, *NDUFV1*, *NDUFV2*, *NDUFAF2* and *NDUFAF4*.

Clinical presentation included Leigh syndrome or Leigh-*like* syndrome, leukoencephalopathy, fatal infant lactic acidosis (FILA), progressive external ophthalmoplegia, histiocytoid cardiomyopathy and encephalomyopathy. The age at onset of symptoms was in the first decade of life (median ± SD, range) (2.9 ± 5.0 months, 24 h – 9 years of age), and the age of death occurred during childhood (6.2 ± 8.18 months, 24 h – 36 years of age). Biochemically, elevation of lactate in plasma (3.5 ± 5.3 mmol/L; 1 to 23.5 times the upper reference value) and CSF (3.2 ± 2.12 mmol/L, 1.3 to 9.2 times the upper reference value) was highly variable. Similarly, RCC I deficiency in muscle (13 ± 8%; 1–50%) and fibroblasts (33 ± 19%; 2–60%) showed significant fluctuation (Table 1).

The findings from 22 patients with NDUFS4 defects have been published, including the present case. All reported patients presented with Leigh syndrome in the first few months of life (4.5 ± 4.44 months, 4 days to 22 months), and the age of death was within the first 3 years of life (10 ± 7.66 months, 3 to 27 months). Hypotonia, developmental arrest or regression; ocular abnormalities; and apnoeic episodes were reported in approximately half of the patients. These features, together with lesions affecting the brain stem and basal ganglia, may help to guide the genetic diagnosis of NDUFS4 patients (Tables 1 and 2).

Biochemically, the patients consistently showed lactic acid accumulation in plasma (4.1 ± 2.75 mmol/L) and CSF (4.7 ± 1.98 mmol/L) and variable defects of RCC I in muscle (30 ± 21%; 3–74%) (Table 1).

4. Discussion

Mutation in the *NDUFS4* gene is a relevant genetic cause of RCC I deficiency and has been previously reported to account for 11% (22/198) of all RCC I deficient patients. *NDUFS4* is a nuclear DNA-encoded CI 'supernumerary' subunit located in a strategic region of the complex. It is involved in several processes, including RCC I assembly, mitochondrial import and activation of the complex (Assereto et al., 2014). The role of *NDUFS4* in the RCC I function may explain the severe phenotype described in this defect compared to other causes of RCC I deficiency (Table 1).

To date, *NDUFS4* mutations have been described in 22 patients from 18 families with symptom onset between 5 days and 4 months of life (Haack et al., 2012; Van den Heuvel et al., 1998; Budde et al., 2000, 2003; Lebre et al., 2011; Petruzzella et al., 2001; Scacco et al., 2003; Bénit et al., 2003; Assouline et al., 2012; Rötig et al., 2004; Anderson et al., 2008; Leshinsky-Silver et al., 2009; Calvo et al., 2010). The most frequent symptoms were hypotonia, abnormal ocular movements and visual impairment (nystagmus, strabismus, ophthalmoplegia, ptosis, absence of visual fixating), psychomotor arrest or regression and episodes of respiratory failure. MRI was abnormal in all *NDUFS4* mutated patients, and there was involvement of the brainstem, basal ganglia, and less frequently, the cerebral cortex, which is in line with other RCC I defects (Assouline et al., 2012). The prognosis for individuals with *NDUFS4* mutations is poor and patients typically die before 3 years of age (Lombardo et al., 2014) (Table 1).

As in previous reports concerning *NDUFS4* mutations, our patient suffered a rapid neurological deterioration starting in the second month of life, with signs of basal ganglia and brain stem involvement, such as global hypotonia, rigidity, abnormal ocular movements and respiratory failure. The patient presented with high lactate levels in plasma and lactic acid accumulation in the magnetic resonance spectroscopy of the brain. Moreover, the residual activity of RCC I and RCC III was reduced to 50% and 78%, respectively, of the minimal control values.

Previously, variable reductions in the enzymatic activity of RCC I (1–74% in the muscle tissue of 18 patients reported and 16–82% in the fibroblasts of 12 patients) have been previously reported for patients with *NDUFS4* mutations (Haack et al., 2012; Van den Heuvel et al., 1998; Budde et al., 2000; 2003; Lebre et al., 2011; Petruzzella et al., 2001; Scacco et al., 2003; Bénit et al., 2003; Assouline et al., 2012; Rötig et al., 2004; Anderson et al., 2008; Leshinsky-Silver et al., 2009; Calvo et al., 2010) (Table 2). RCC III deficiency has also been reported in some NDUFS4 patients. Interestingly, our patient associated a secondary deficiency of CoQ and PDH activity that has not previously been reported in patients with *NDUFS4* mutations. In addition, secondary CoQ deficiency has been reported in mitochondrial (MELAS, Kearns-Sayre syndrome) and other neurological diseases (glutaric aciduria type IIC, ataxia-oculomotor apraxia syndrome-1) (Yubero et al., 2015). RCC I is closely related to CoQ in the mitochondria and the loss of RCC may lead to a secondary CoQ deficiency, as is reported in other mitochondrial defects. The combination of PDH and RCC deficiency is characteristic of patients with mitochondrial iron-sulphur (Fe-S) cluster biosynthesis defects *(NFU1, BOLA3)* (Navarro-Sastre et al., 2011; Cameron et al., 2011; Ahting et al., 2015) and has been observed in some cases of RCC I deficiency due to *NDUFS2* mutations (Tuppen et al., 2010) and in inherited defects of valine metabolism due to *HIBCH* mutations (Ferdinandusse et al., 2013).

Our patient showed an inappropriate assembly of RCC 1 with BN-PAGE analysis. Previous studies have described a total absence of fully assembled RCC I with the accumulation of the 830-KDa subcomplex in *NDUFS4* mutant fibroblasts (Ugalde et al., 2004; Assouline et al., 2012; Iuso et al., 2006; Leshinsky-Silver et al., 2009; Leong et al., 2012; Breuer et al., 2013), although this was not the case in our patient. This difference may be due to the different tissue assessed in this study.

Histopathological studies of the muscle tissue revealed an increased size of lipid droplets in our patient. Nonspecific alterations in muscle

Table 1
Clinical, biochemical and radiological features of reported RCC I deficiency patients.

	NDUFA1	NDUFA2	NDUFA4	NDUFA9	NDUFA10	NDUFA11	NDUFA12	NDUFA13	NDUFAF1	NDUFAF2	NDUFAF3	NDUFAF4
References	1,2,3,4	5	6	7	4, 8	9	10	11	12,13,14,15	4, 16, 17, 18, 18, 20, 21	22	4, 22
Number of patients	8	1	4	1	2	6	1	2	6	12	5	10
Age at onset (range in months)	4–48	0.2	0.03	0.01	2	0.03	20	8–13	0.03–11	0.01–20	0.03–3	0.03
Age at death (range in months)	14–19	11	8–26	1	23	0.19–6	na	na	0.23–12	12–156	3–6	0.06–18
Current age of living patients (years)	35	No	34	No	No	0.5	10	No	20	No	No	7
Clinical phenotype	L	L	FILA/L	FILA/L	L	FILA/L	L	L	FILA/L	FILA/L	FILA/L	FILA/L/Leu
Symptoms and sign (%)												
Hypotonia – Encephalopathy	75	100	na	na	100	na	100	100	<25	<50	<25	<25
Intra-uterine growth retardation	na	na	na	na	na	50	na	na	<25	<25	na	na
Failure to thrive	na	na	50	na	na	na	100	100	<25	<25	na	<25
Psychomotor delay	>50	na	100	na	na	50	100	na	<25	<25	na	<25
Cognitive regression	<25	na	na	na	50	na	na	na	na	<25	na	na
Movement disorder (dystonia, etc.)	<25	<75	na	100	na	50	100	50	<25	na	na	<25
Pyramidal signs	na	na	50	100	na	na	na	100	<50	<25	na	<25
Cerebellar ataxia	<25	na	50	na	na	na	na	na	na	<25	na	na
Hypertrophic cardiomyopathy	na	100	na	na	50	>50	na	na	<25	<25	na	<25
Seizure/Epilepsy	<50	100	25	na	50	<25	na	50	na	<25	<50	<25
Other symptoms and signs	na	na	e	na	na	i	na	k	e, f, g, l	h, i	j	na
Neuroimaging (CT-scan /MRI)(%)												
Brainstem/Basal ganglia	<50/25	na	50/25	100/100	50/100	na/na	na/100	na/na	na/na	>25/na	na	<25/<25
Cortical atrophy/Subcortical white matter	na/25	100	0/100	100/na	na/na	na/na	na/na	100/na	na/>75	na/<25	na	<25/<25
Cerebellum/Spinal cord	>25/<25	na	25/na	na/na	na/na	na/na	na/100	100/na	100/na	na/<25	na	<25/<25
MR spectroscopy – lactate peak	<25	na	25	na	na	na	na	na	na	Na	<25	<25
Others	na	a,b,c	na	na	d	c	na	na	b	b	b, d	a
*Biochemical investigation**												
Lactate in plasma (range)	1.9	na	1.2–4.2	1.2	3.9	1.4–6.8	2.3	1.6–2.2	2.1–7.5	1.2–4.5	12.2	17.2
Lactate in CSF	na	na	3.2	na	2.7	na	1.3	na	na	6	na	na
RCC (% of residual activity)												
CI deficiency in muscle (range)	4–30	20	na	11–29	7	4–39	11	7–14	10–25	12–36	26–40	5.5–17
CI deficiency in fibroblast (range)	17–70	36	na	na	28	45	60	na	2–57	<20–60	18–39	32–67

	NDUFB3	MDUFB9	NDUFB11	NDUFS1	NDUFS2	NDUFS3	NDUFS4	NDUFS6	NDUFS7	NDUFS8	NDUFV1	NDUFV2
References	23, 24, 25	4	26,27	4, 28, 29, 30, 31, 32, 33, 34, 35, 36	4, 29, 33, 37, 38, 39	23, 40	4, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, current case.	4, 53, 54	29, 55,56,57	23, 39, 58, 59, 60	20, 28, 29, 37, 61, 62, 63, 64, 65, 66, 67, 68	39, 69, 70
Number of patients	2	1	5	28	17	2	22	8	6	11	28	10
Age at onset (range in months)	3y	<6	0.01–6	1–12	0.01–12	9y	0.17–22	0.06–6	4–15	0.01 m – 7y	0.03–4	0.03–10
Age at death (range in months)	36y	na	na	5y–10.5y	4d–3.5y	10.5y	3–28	0.2–0.3	5 m – 5y	2–14	0.1	0.3–3
Current age of living patients (years)	29	No	na	No	9	No	No	No	No	13	15	32
Clinical phenotype	FILA/L	E	HC	L/Leu	FILA/L	L	L	FILA/L	L	PEO/L/FILA/Leu	FILA/L/Leu	L
Symptoms and sign (%)												
Hypotonia – Encephalopathy	50	100	<50	<50	<50	50	100	<75	50	<75	<75	<25
Intra-uterine growth retardation	50	na	<25	<25	<25	na	<50	na	na	na	na	<25
Failure to thrive	na	na	<25	<25	<25	na	na	na	na	<25	<25	na
Psychomotor delay	50	na	<25	25	<25	50	50	na	<25	na	na	na
Cognitive regression	na	na	na	>25	<25	na	na	na	<25	na	na	na
Movement disorder (dystonia, etc.)	na	na	na	<25	<25	50	<25	na	<25	<50	>50	na
Pyramidal signs	na	na	na	>25	<25	50	<50	na	50	na	na	25
Cerebellar ataxia	na	na	na	na	<25	<25	na	na	na	na	na	na
Hypertrophic cardiomyopathy	na	na	100	na	<25	na	<25	na	na	<25	na	<50
Seizure/Epilepsy	na	na	<25	<25	<25	na	<50	na	<25	<25	na	<50
Other symptoms and signs	na	na	j	h, i, j	h	na	h, i	f	h	na	i, j	i
Neuroimaging (CT-scan /MRI)(%)												
Brainstem/Basal ganglia	na/na	na/na	na/na	<25/<25	<25/<25	50/50	<75/<50	na/na	50/50	na/<25	25/25	<25/na
Cortical atrophy/Subcortical white matter	na/na	na/na	na/na	na/<25	na/<25	na/50	<25/na	na/na	<25/<25	na/<25	<25/<50	<25/na
Cerebellum/Spinal cord	na/na	na/na	na/na	na/na	<25/<25	na/na	na/na	na/na	<25/na	na/na	na/na	na/na

Table 1 (*continued*)

	NDUFB3	MDUFB9	NDUFB11	NDUFS1	NDUFS2	NDUFS3	NDUFS4	NDUFS6	NDUFS7	NDUFS8	NDUFV1	NDUFV2
MR spectroscopy – lactate peak	na	na	na	<25	<25	na	na	<25	na	na	25	na
Others	na	na	b	B, quist	na	na	na	na	na	na	na	na
Biochemical investigation												
Lactate in plasma (range)	na	na	na	1.2–2.4	1.6–11.4	2–2.7	1–9.5	3–12	2.8–6.2	1.5–23.5	na	na
Lactate in CSF	na	na	na	2.13	5.5	na	na	9.2	1.5	2.8	na	na
RCC (% of residual activity)												
CI deficiency in muscle (range)	6	50	na	10–98	13–27	20–76	1–74	7–56	14–77	8–39	na	50
CI deficiency in fibroblast (range)	12–21	na	na	23–39	60	36	16–82	na	34–71	52–69	na	15

a — demyelination of corticospinal tracts; b — agenesis/hypoplasia of corpus callosum; c — optic nerve atrophy; d — periaqueductal substantia nigra; na — not available; e — alteration in EMG/NCV; f — elevated creatine kinase; g — dysmorphic features; h — hepatomegaly; i — microcephaly; j — macrocephaly; k — auditory neuropathy; l — peripheral neuropathy; FILA — Fatal infantile lactic acidosis; L — Leigh or Leigh-like syndrome; Leu — leucodystrophy; PEO — Progressive external ophthalmoplegia; HC — histiocytoid cardiomyopathy; E — encephalomyopathy; * blood and CSF lactate values are expressed as the patient's value divided by the higher value of the normal range for each laboratory.

Table 2
The clinical, biochemical and radiological features of patients with NDUFS4 mutations reported in the literature.

Patients (families)	22 (18)	Signs and symptoms during evolution	Number of patients
Age at onset - months, media ± SD (range)	4.5 ± 4.44 (0.16–22)	Hypotonia	22
Age at death - months, media ± SD (range)	10 ± 7.66 (3–27.5)	Developmental arrest-regression	11
Male/female	11/8	Ocular abnormalities*	11
Biochemical abnormalities	(media ± SD, range)	Absence of eye contact	10
Lactate elevation in plasma (mmol/L) (N = 16)	(4.1 ± 2.75, 1–9.5)	Apneic episodes	10
Lactate elevation in CSF (mmol/L) (N = 11)	(4.7 ± 1.98, 3–7.6)	Feeding problems/failure to thrive	8
Lactate: Pyruvate ratio (N = 8)	(38 ± 17.76, 20–59)	Pyramidal signs	6
CI deficiency muscle (media ± SD, range %) (n = 18)	(30.6 ± 21.4, 3–74)	Hypertrophic cardiomyopathy	5
CI deficiency fibroblasts (media ± SD, range %) (N = 12)	(50.2 ± 22.91, 16–82%)	Epilepsy/seizures	4
MRI		Movement disorder	2
Brainstem lesions	14	Microcephaly	1
Basal ganglia lesions	9	Ragged red fibres and lipid accumulation on muscle biopsy	4
Cortical atrophy	3		

* Ocular abnormalities include nystagmus, strabismus, ptosis and ophthalmoplegia. Symptoms reported only once: microcephaly. SD: Standard deviation.

biopsies, such as lipid accumulation and ragged red fibres, have been reported in a few NDUFS4 patients (Assouline et al., 2012).

The combined biochemical defects observed in the muscle biopsy of our patient, including the partial deficiencies in RCC I, RCC III, PDH and CoQ, were misleading, and conventional sequence analysis of the large number of candidate nuclear DNA-encoded genes was not possible. In this context, MPS resulted in the discovery that this patient had a homozygous mutation, c.291delG (p.Trp97Ter), in the *NDUFS4* gene. This mutation has previously been described as a founder mutation in North African populations (Algeria, Morocco) and has been demonstrated to produce a truncated protein with a loss of the cAMP-dependent protein kinase A phosphorylation consensus site (RVSTK, AA 171-175) (Assouline et al., 2012). To date, 13 genetic alterations to the DNA sequence have been reported in the *NDUFS4* gene, including missense mutations (Leshinsky-Silver et al., 2009), nonsense mutations (Petruzzella et al., 2001), splicing mutations (Bénit et al., 2003), microdeletions (Calvo et al., 2012) and large deletions (Assouline et al., 2012), all of which were associated with Leigh syndrome (Fig. 1).

In conclusion, the present case and the literature review demonstrates that NDUFS4 patients have a homogeneous phenotype, characterized by early onset of the disease and a clinical presentation of hypotonia, psychomotor regression, abnormal ocular movements and respiratory failure, in combination with brainstem and basal ganglia lesions leading to fatality. Biochemically, a combined defect in PDH and RCC activity was observed in our patient, together with a mild CoQ deficiency. These deficiencies were most likely due to an inappropriate assembly of RCC 1, which was confirmed using BN-PAGE analysis.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.mito.2016.04.001.

Acknowledgements

This work was supported by grants from the Spanish Ministerio de Economia y Competitividad (FIS PI12/02010, PI15/00287, PI12/02078, PI14/00005, PI14/00028 and PI14/00070); Agència de Gestió d'Ajuts Universitaris i de Recerca-Agaur (2014FI_B 01225) (JD Ortigoza-Escobar); FEDER Funding Program from the European Union and Fundación Ramón Areces (CIVP16A1853) (Pilar Rodríguez-Pombo); Departamento de Ciencia, Tecnología, Instituto de Investigación Sanitaria de Aragon (Grupos Consolidados B33) (Julio Montoya); Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), an initiative of the Instituto de Salud Carlos III (Ministerio de Ciencia e Innovación, Spain). We are in debt to the Biobanc de l'Hospital Infantil Sant Joan de Déu per a la Investigació, part of the Spanish Biobank Network of ISCIII for sample procurement.

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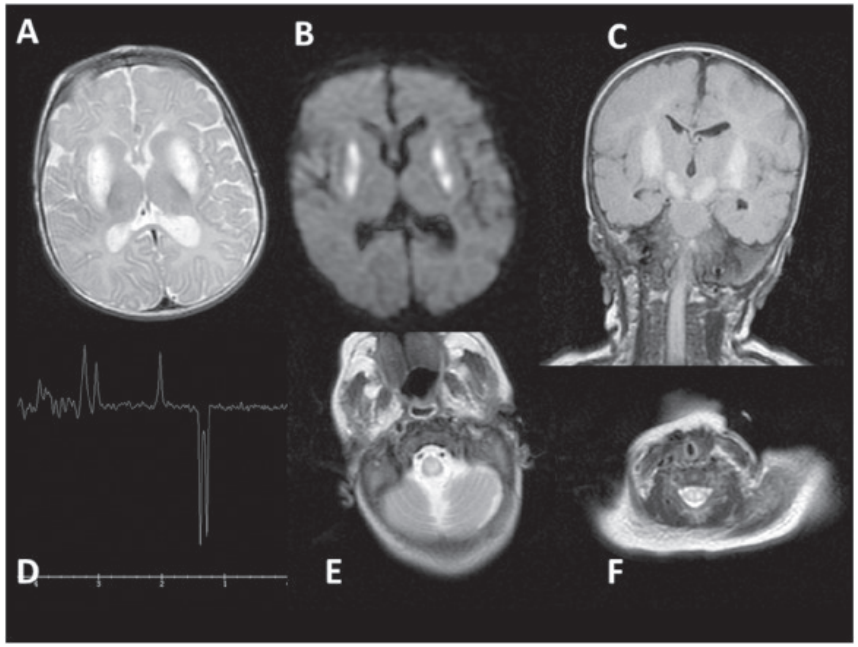

Fig. S1, supplementary material.
Brain magnetic resonance imaging (MRI) of the NDUFS4 patient. (A) The axial FSE T2-weighted image and (B) DWI shows swelling, hyperintensity and restricted diffusion symmetrically involving the putamen nuclei. (C) The coronal FLAIR T2-weighted image shows hyperintensity of the putamen, globus pallidus and cerebral peduncles. (D) MR spectroscopy (TE = 135 m) of the left basal ganglia shows a prominent lactate peak. (E, F) The axial FSE T2 –weighted images show brain stem (medulla oblongata) and spinal cord involvement, both with restricted diffusion (not shown).

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Ndufs4 related Leigh syndrome: A case report and review of the literature

Mitochondrion, Volume 28, 2016, 73–78

http://dx.doi.org/10.1016/j.mito.2016.04.001

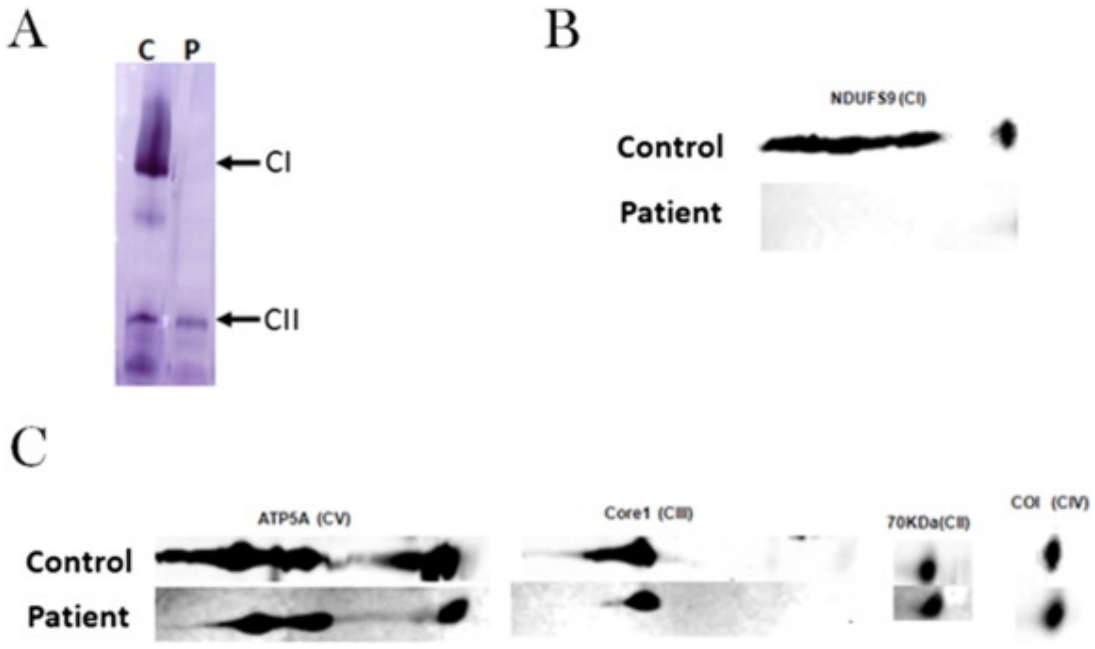

Fig. S2, supplementary material.
A. The BN-PAGE profile in the muscle biopsy shows a complete absence of the fully assembled RCC I (~ 1 MDa) in the patient (P) compared to the control (C) cells. B. A complete absence of the assembled RCC I in the patient's muscle compared to the control sample. C. Other oxidative phosphorylation complexes remained normal.

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Sintesis de resultados

- El déficit de complejo I es la causa más frecuente de Síndrome de Leigh. A su vez, las mutaciones en el gen *NDUFS4* son una causa genética frecuente de deficiencia del complejo I y corresponden al 11% (22/198) de los pacientes revisados en este trabajo. Con todo ello, estos defectos genéticos deberían incluirse en el diagnóstico diferencial de los defectos del transporte y metabolismo de la tiamina.
- Los síntomas de los pacientes con mutaciones en el gen *NDUFS4* se inician entre los 5 días y los 4 meses de vida. La RM cerebral es anormal en todos los pacientes, con mayor afectación del tronco cerebral y los ganglios basales y, en menor frecuencia, de la corteza cerebral.
- El pronóstico de los pacientes mutaciones del gen *NDUFS4* es malo, dado que generalmente mueren antes de los 3 años de edad.
- Se observó en esta paciente una actividad residual de los complejos I y III del 50% y del 78%, respectivamente, en comparación con los controles normales. Esta paciente asociaba, además, una deficiencia secundaria de PDH y de CoQ, que no habían sido previamente reportadas.
- El análisis BN-PAGE en tejido muscular mostró una alteración del ensamblaje del complejo I. Los estudios histopatológicos del tejido muscular revelaron un incremento del tamaño de gotas de lípidos.

Free-thiamine is a potential biomarker of thiamine transporter-2 deficiency: a treatable cause of Leigh syndrome.

"La tiamina libre es un biomarcador potencial de la deficiencia del transportador de tiamina de tipo 2: una causa tratable de Síndrome de Leigh"

Brain. 2016 Jan;139(Pt 1):31-8.

Ortigoza-Escobar JD, Molero-Luis M, Arias A, Oyarzabal A, Darín N, Serrano M, García-Cazorla A, Tondo M, Hernández M, Garcia-Villoria J, Casado M, Gort L, Mayr JA, Rodríguez-Pombo P, Ribes A, Artuch R, Pérez-Dueñas B.

La deficiencia de hTHTR2 es causada por mutaciones en el gen *SLC19A3*. Recientemente, varios estudios se han centrado en el creciente espectro fenotípico de la deficiencia de hTHTR2 y en las intervenciones terapéuticas. En general, la literatura publicada previamente sugiere un claro beneficio de la suplementación temprana con tiamina y biotina, con resultados menos eficaces cuando el tratamiento se administra a pacientes con afectación severa o de forma tardía. Por lo tanto son necesarios nuevos biomarcadores para ayudar al diagnóstico y a la intervención terapéutica precoz. En este trabajo se han analizado las isoformas de tiamina mediante cromatografía líquida de alto rendimiento (HPLC) en sangre total y en LCR de controles pediátricos, de pacientes con Síndrome de Leigh, de pacientes con otros trastornos neurológicos y, finalmente, de pacientes con mutación del gen *SLC19A3*. Ningún trabajo publicado hasta el momento había descrito un biomarcador para esta patología, por tanto, con la descripción de un biomarcador nos aseguramos de que pueda realizarse un diagnóstico precoz, con la consiguiente disminución de la morbilidad de los pacientes. Así, en este trabajo se evalúa por primera vez la utilidad de la tiamina-libre como biomarcador de la deficiencia de hTHTR2. Además se comprueba que tras la suplementación de tiamina se restauran los valores de tiamina en fibroblastos (intracelular) y LCR (SNC).

doi:10.1093/brain/awv342 BRAIN 2016: 139; 31–38 | 31

BRAIN
A JOURNAL OF NEUROLOGY

REPORT

Free-thiamine is a potential biomarker of thiamine transporter-2 deficiency: a treatable cause of Leigh syndrome

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Thiamine transporter-2 deficiency is caused by mutations in the *SLC19A3* gene. As opposed to other causes of Leigh syndrome, early administration of thiamine and biotin has a dramatic and immediate clinical effect. New biochemical markers are needed to aid in early diagnosis and timely therapeutic intervention. Thiamine derivatives were analysed by high performance liquid chromatography in 106 whole blood and 38 cerebrospinal fluid samples from paediatric controls, 16 cerebrospinal fluid samples from patients with Leigh syndrome, six of whom harboured mutations in the *SLC19A3* gene, and 49 patients with other neurological disorders. Free-thiamine was remarkably reduced in the cerebrospinal fluid of five *SLC19A3* patients before treatment. In contrast, free-thiamine was slightly decreased in 15.2% of patients with other neurological conditions, and above the reference range in one *SLC19A3* patient on thiamine supplementation. We also observed a severe deficiency of free-thiamine and low levels of thiamine diphosphate in fibroblasts from *SLC19A3* patients. Surprisingly, pyruvate dehydrogenase activity and mitochondrial substrate oxidation rates were within the control range. Thiamine derivatives normalized after the addition of thiamine to the culture medium. In conclusion, we found a profound deficiency of free-thiamine in the CSF and fibroblasts of patients with thiamine transporter-2 deficiency. Thiamine supplementation led to clinical improvement in patients early treated and restored thiamine values in fibroblasts and cerebrospinal fluid.

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Received March 1, 2015. Revised September 11, 2015. Accepted October 2, 2015. Advance Access publication December 11, 2015

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Keywords: thiamine transporter-2 deficiency; biotin thiamine responsive basal ganglia disease; *SLC19A3* gene; Leigh syndrome; striatal necrosis; mitochondrial disorders

Abbreviations: free-T = free-thiamine; TMP = thiamine monophosphate; TDP = thiamine diphosphate; TPK = thiamine pyrophosphokinase; hTHTR2 = thiamine transporter-2

Introduction

Thiamine is a major cofactor involved in energy metabolism in brain tissue. In humans, at least four forms of thiamine are known, namely free-thiamine (free-T), thiamine monophosphate (TMP), thiamine diphosphate (TDP), and thiamine triphosphate (TTP) (Gangolf *et al.*, 2010). Both free-T and TMP forms are absorbed in the small intestine by two specific transporters: thiamine transporter-1 (hTHTR1, encoded by *SLC19A2*) and thiamine transporter-2 (hTHTR2, encoded by *SLC19A3*) (Rajgopal *et al.*, 2001; Subramanian *et al.*, 2006; Mayr *et al.*, 2011). In the blood–brain barrier and the choroid plexus, hTHTR2 is expressed in the pericytes surrounding endothelial cells, while hTHTR1 is localized to the luminal side; this supports their role in the transport of thiamine into the CNS (Kevelam *et al.*, 2013).

Thiamine is converted into TDP, the metabolically active form of thiamine, by a specific kinase (thiamine phosphokinase, TPK, EC 2.7.4.15) (Tallaksen *et al.*, 1991; Zhao *et al.*, 2002; Banka *et al.*, 2014). TDP may act as a cofactor in the cytosol (transketolase, EC, 2.2.1.1), in peroxisomes (2-hydroxyacyl-CoA lyase, EC, 4.1.2.n2), or it can enter the mitochondria through another transporter encoded by the *SLC25A19* and be a cofactor for pyruvate dehydrogenase (EC, 1.2.4.1), 2-oxoglutarate dehydrogenase (EC, 1.2.4.2), and branched-chain alpha-keto acid dehydrogenase (EC, 1.2.4.4) (Mayr *et al.*, 2011).

Thiamine transporter-2 deficiency (hTHTR2 deficiency) (OMIM#607483) is a recessive inherited disease caused by mutations in *SLC19A3*. It presents in normally developing children as episodes of acute and recurrent encephalopathy, dystonia, seizures and brain lesions in the cerebral cortex, basal ganglia, thalami, brainstem and cerebellum (Ozand *et al.*, 1998; Gerards *et al.*, 2013; Kevelam *et al.*, 2013). Early administration of biotin and thiamine in patients with hTHTR2 deficiency can potentially reverse the clinical and radiological abnormalities and improve neurological outcome (Kono *et al.*, 2009; Debs *et al.*, 2010; Serrano *et al.*, 2012; Alfadhel *et al.*, 2013; Pérez-Dueñas *et al.*, 2013; Tabarki *et al.*, 2013; Distelmaier *et al.* 2014; Haack *et al.*, 2014).

To date, non-specific biochemical abnormalities (i.e. increases of 2-oxoglutarate, lactate, and alanine in biological fluids, and a lactate peak on spectroscopy) have been reported in hTHTR2 patients (Serrano *et al.*, 2012; Kevelam *et al.*, 2013; Gerards *et al.*, 2013; Distelmaier *et al.*, 2014; Haack *et al.*, 2014). Because hTHTR2 deficiency is a potentially treatable disorder, there is an urgent need for a robust biomarker to allow prompt diagnosis and treatment monitoring of this disease.

The aims of this study were: (i) to establish reference values for thiamine derivatives in blood and CSF; (ii) compare these results with a cohort of children with Leigh syndrome, six of them harbouring mutations in the *SLC19A3* gene, and children with other acquired and genetic neurological conditions; and (iii) establish assays for thiamine derivatives in fibroblast cultures to confirm the diagnosis of patients with mutations in *SLC19A3*.

This study was conducted with the approval of the institutional review boards of the Hospital Sant Joan de Déu, Hospital Clinic and the Autonomous University of Madrid. Written informed consent was obtained from the parents or guardians of all enrolled patients and participants. This study was conducted following the STARD guidelines of diagnostic accuracy (first official version, January 2003) as recommended for fluid biomarkers in neurological disorders (Gnanapavan *et al.*, 2014).

Materials and methods

Study population

Blood and CSF samples from control subjects

Reference values for free-T, TMP and TDP were established in 106 whole blood samples and 38 CSF samples from paediatric control subjects (Supplementary material). CSF/whole blood ratios for free-T, TMP and TDP were determined in nine subjects for whom lumbar puncture and venous puncture were performed on the same day. The CSF samples were frozen at −80°C and protected from light until analysis.

Patients with mutations in *SLC19A3*

We studied CSF thiamine derivatives in six patients with Leigh encephalopathy and *SLC19A3* mutations (Table 1). Lumbar puncture was performed before thiamine treatment in all patients except in Patient 3, who was receiving 24 mg/kg/day of thiamine. A blood sample before thiamine treatment was only available for Patient 6.

Five patients (Patients 1, 2, 3, 4 and 6) were treated with thiamine after 3 to 28 days following the onset of acute encephalopathy (mean age 8 years, range 1 month to 15 years). Patients 1, 3, 4 and 6 showed a dramatic improvement of symptoms in the short term, whereas Patient 2 evolved into

Table 1 Laboratory findings in the six *SCL19A3* mutated patients and reference values

Patients (Sex, age at lumbar puncture) *SLC19A3* mutations	CSF TDP (nmol/l)	CSF TMP (nmol/l)	CSF free-T (nmol/l)	Lactate (mmol/l)		α-ketoglutaric acid (mmol/mol creatine)		Amino acids		Skeletal muscle RCC activities	Time frame between the onset of encephalopathic episode and thiamine supplementation / current age and outcome
				Plasma (0.77–2.44)	CSF (1.11–2.22)	Urine	CSF	Plasma	CSF		
Reference values	**< 1 year: 8 (2–22) ⩾ 1 year: 8 (5–10)**	**< 1 year: 44 (20–77) ⩾ 1 year: 30 (17–40)**	**< 1 year: 74 (28–106) 1–6 years: 47 (22–98) 7–15 years: 26 (11–47)**								
Patient 1: Male, 1 month c.[68G > T];[68G > T] p.[Gly23Val]; [Gly23Val]	13.8	**10.3**	**2.8**	8.6	7.1	High excretion	n.a.	Ala, Val, Ile ↑	N	n.a.	3 days / 3 years: mild motor delay and dystonia
Patient 2: Male, 13 months c.[1079dupT]; [980-14A > G] p.[Leu360Phefs*38]; [Gly327Aspfs*8]	14.2	28.9	**0.6**	2.3	1.7	N	N	N	N	N	28 days / death
Patient 3[a]: Male, 19 months c.[153 A > G]; [157 A > G] p.[Ile51Met]; [Asn53Asp]	**13**	42	**179**	0.8	1.3	N	n.a.	N	n.a.	N	21 days / 2.5 years: normal neuro-development
Patient 4: Male, 4 years c.[1264A > G]; [1264A > G] p.[T422A]; [T422A]	9.0	19.9	**3.9**	0.8	1.2	N	n.a.	N	N	N	9 days / 7.5 years: normal neuro-development
Patient 5: Male, 6 years c.[1264A > G]; [1264A > G] p.[T422A]; [T422A]	6.3	21.2	**9.3**	1.4	1.7	N	n.a.	N	n.a.	N	11 years / 13 years: Improved alertness, severe dystonia, spasticity and epilepsy
Patient 6: Male, 15 years c.[74dupT]; [980-14A > G] p.[Ser26Leufs*19]; [Gly327Aspfs*8]	4.70	**14.8**	**4.8**	1.2	1.8	N	N	N	N	N	4 days / 24 years: mild dysarthria and dystonia

[a]Patient 3 was on thiamine treatment when the lumbar puncture was performed for CSF analysis.
RCC = respiratory chain complex. N = normal; n.a. = not analysed.

a drug-resistant *status dystonicus* and died from septicaemia. Patient 5, the older brother of Patient 4, who had a disease onset at 3 months of life, was treated at age 11 with no significant changes on his motor disability but with improved alertness as perceived by his parents. His follow-up MRI 13 months after thiamine supplementation showed regression of the cortical-subcortical changes in cerebral hemispheres and cerebellum. Clinical description of Patients 1, 2 and 3 has been previously published in Ortigoza-Escobar *et al.* (2014).

Biomarkers of mitochondrial dysfunction were analysed in blood, urine and CSF samples, when available, and compared with reference values that were previously established in our laboratory (Artuch *et al.*, 1995).

Patients with Leigh syndrome and other disorders of the CNS

To test the specificity of CSF thiamine as a biomarker for *SLC19A3* defects, we analysed CSF samples obtained from 10 patients with Leigh syndrome who were not on thiamine treatment (Table 2). We also included 49 patients with several acquired or genetic neurologic diseases (Supplementary material).

Fibroblast samples

We analysed nine human fibroblast cell lines from three patients with *SLC19A3* mutations (Patients 1, 2 and 6) and six patients with other inborn errors of metabolism that were used as controls.

Compliance with ethical guidelines

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Declaration of Helsinki of 1975, as revised in 2000. Informed consent for participation in the study was obtained from all patients.

Procedures

Procedures are detailed in the Supplementary material and Supplementary Fig. 1.

Results

Reference values for thiamine derivatives in whole blood and CSF samples

Within-run and between-run imprecision data, quantification limits, detection limits and analytical intervals for free-T, TMP and TDP are reported in Supplementary Table 1.

Reference values for free-T, TMP and TDP in blood were established. A negative correlation was observed between TDP concentration and age ($r = -0.290$, $P = 0.003$). Consequently, blood values were stratified into two different age groups (Fig. 1A).

A negative correlation between free-T and age was observed in the CSF ($r = -0.64$, $P < 0.01$); thus, three reference intervals were established (Table 1 and Fig. 1B).

Table 2 Laboratory findings in 10 patients with Leigh syndrome

Patients (Sex, current age) Reference values	Mitochondrial disease criteria Morava *et al.*, 2006	CSF Free-Thiamine (nmol/l) <1 year: 74 (28–106) 1–6 years: 47 (22–98) 7–15 years: 26 (11–47)	Lactate (mmol/l)		α-Ketoglutaric acid (mmol/mol creatine) Urine <1 year: 18–147	Amino acids		Skeletal muscle mitochondrial assays / mutations
			Plasma (0.7–2.4)	CSF (1.1–2.2)		Plasma	CSF	
Patient 1 (F, 1 month)	Probable	37.4	5.2	1.1	2432	↑ Ala, Gly, Tyr, Glu	↑ Orn, Tyr	N
Patient 2 (F, 5 months)	Definite	65.1	5.14	5.4	N	↑ Glu, Leu	N	↓ C II + III
Patient 3 (M, 8 months)	Definite	23.6	5	4.9	N	↑ Ser, Gly, Ala	N	N
Patient 4 (M, 8 months)	Definite	50.3	1.3	1.2	N	N	N	↓ C II + III + ↓CII + ↓Q10
Patient 5 (F, 9 months)	Definite	41.2	3	5.9	N	N	N	↓ substrate oxidation rates. ↓ C II + III, ↓ C II ↓ C I + III, ↓ C IV
Patient 6 (F, 1 year)	Definite	15.3	4.3	3.9	N	N	N	↓C I + III, ↓C II + III
Patient 7 (F, 1 year)	Definite	21.8	1.72	2.4	N	↑ Ala, Lys	N	↓C IV
Patient 8 (F, 3 years)	Confirmed	56	1.78	2.9	N	N	↑ Val	mtDNA (T8993G)
Patient 9 (M, 5 years)	Probable	11.8	2.19	0.9	N	↑ Val, Ile, Leu	n.a.	N
Patient 10 (F, 8 years)	Definite	25.2	2.4	1.6	High excretion	N	N	N

M = male; F = female; N = normal; C I-IV = mitochondrial respiratory chain complex I-IV; Q10 = coenzyme Q10.

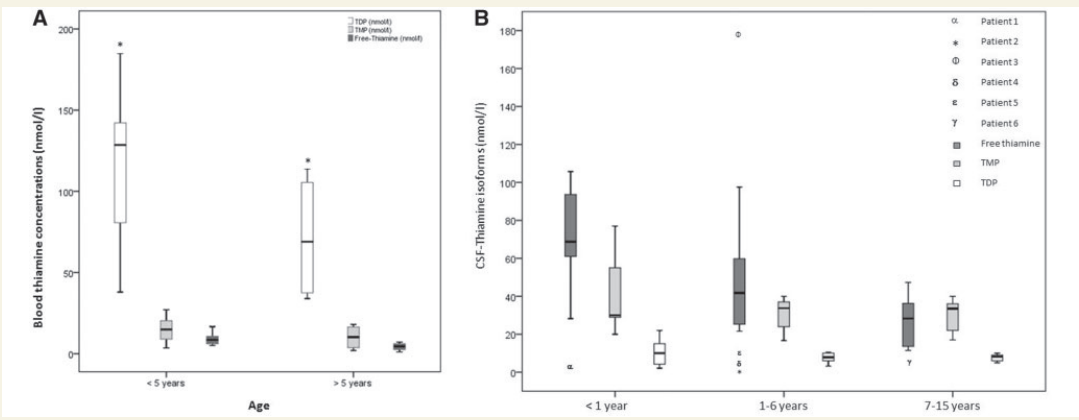

Figure 1 Thiamine concentrations in blood and the CSF. (**A**) Box-plot representation of whole-blood TDP, TMP and free-T concentrations in control subjects separated into two age groups. *$P < 0.001$ for TDP values between age groups (Mann-Whitney U-test). (**B**) Box-plot representation of CSF TDP, TMP and free-T concentrations in control subjects separated into three age groups. *$P < 0.01$ for free-T values between the three age groups ($X^2 = 18.89$, Kruskal-Wallis test). TDP and TMP are defined in three age groups in the present figure although two reference ranges were established for these derivatives (Table 1). The symbols represent the CSF free-thiamine of Patients 1–6 who all have *SLC19A3* mutations. Box length indicates the interquartile space (P25–P75), the horizontal line represents the median (P50), and the bars indicate the range. Patient 3 was on thiamine supplementation.

Significant correlations were found between the CSF and whole blood concentrations of TDP (r = 0.746, $P = 0.001$) and TMP (r = 0.601, $P = 0.039$). The median CSF/whole-blood ratios for TDP, TMP and free-T in the control samples were 0.04 (range: 0.01–0.09), 3.2 (range: 1.3–7.0) and 1.8 (range: 0.9–3.6), respectively, indicating the most prevalent thiamine form in each fluid.

Thiamine forms in whole blood and CSF from patients with mutations in *SLC19A3*

Before thiamine treatment, very low concentrations of free-T were found in the CSF samples of five patients (Table 1 and Fig. 1B). The values of the different thiamine forms in the CSF of Patient 3, who was on thiamine supplementation, were above the reference range.

The concentrations of TDP (105 nmol/l), TMP (8.4 nmol/l), and free-T (21.5 nmol/l) in the whole blood of Patient 6 before thiamine supplementation were within the control range, but the CSF/blood free-T ratio was low (0.22 versus 0.9–3.6 for control population).

Thiamine forms in CSF from patients with Leigh syndrome and other disorders of the CNS

Molecular studies ruled out the presence of mutations in *SLC19A3* in the 10 patients with Leigh syndrome. Three of them had free-T values below the reference range, but deficiencies were milder than those of patients with *SLC19A3* mutations (Table 2). Similarly, 6 of 49 patients with other neurological conditions showed CSF free-T levels that were slightly below the lower range of reference (Supplementary Fig. 2): perinatal asphyxia (2 years, 20.9 nmol/l and 13 years, 12.3 nmol/l), genetic epileptic encephalopathy (2 years, 15.0 nmol/l and 1 year, 19.0 nmol/l), encephalitis (1 year, 12.0 nmol/l) and spastic paraparesia (1 year, 13.9 nmol/l).

Thiamine forms, pyruvate dehydrogenase complex activity and mitochondrial substrate oxidation rates in fibroblasts

When the fibroblasts of Patients 1, 2 and 6 were cultured for 10 days in a medium with a low concentration of thiamine (2.8 nmol/l), significant reduced intracellular concentrations of all thiamine derivatives were observed as compared to the control group (Fig. 2A). TDP was the main intracellular form, both in patients and controls, though differences among these groups were relevant ($P = 0.02$) (Fig. 2A). When fibroblasts were cultured in a medium containing thiamine (304.3 nmol/l), all the thiamine forms normalized (Fig. 2B). Surprisingly, substrate oxidation rates and pyruvate dehydrogenase complex activity were similar in fibroblasts from hTHTR2 patients and controls when analysed in low thiamine medium (Supplementary Table 2).

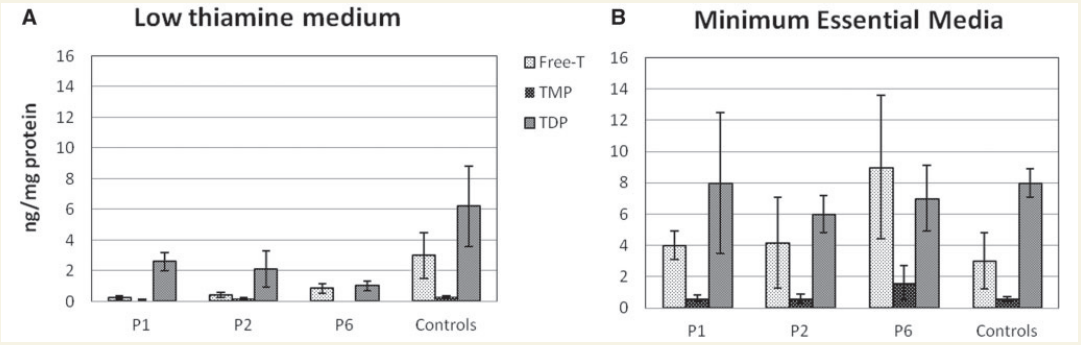

Figure 2 Thiamine concentration in fibroblasts. The figure shows the mean ± SD concentration of free-T, TMP and TDP in hTHTR2-deficient fibroblasts from Patients 1, 2 and 6, and in control fibroblasts, after repeating the experiments in three different cell lines for each sample. (**A**) Fibroblasts grown in a low thiamine medium (2.8 nmol/l). The significance for each patient and each form of thiamine as compared to controls was: Patient 1 (P1): free-T ($P = 0.02$), TMP ($P = 0,03$), TDP ($P = 0.02$); Patient 2 (P2): free-T ($P = 0.02$), TMP ($P = 0,1$), TDP ($P = 0.02$); Patient 6 (P6): free-T ($P = 0.3$), TMP ($P = 0.02$), TDP ($P = 0.02$). (**B**) Fibroblasts grown in minimum essential medium, which contains normal amounts of thiamine (304.3 nmol/l).

Discussion

hTHTR2 deficiency is a genetic disorder that leads to acute encephalopathy and brain damage in childhood, mimicking intractable causes of Leigh syndrome caused by mitochondrial respiratory chain defects (Gerards *et al.*, 2013; Kevelam *et al.*, 2013; Distelmaier *et al.*, 2014; Haack *et al.*, 2014).

Recently, several studies have focused on the increasing phenotypic spectrum of hTHTR2 deficiency and on therapeutic interventions. Overall, previously published literature suggests a clear benefit of early thiamine and biotin administration in the short-term, and less effective results of late treatment initiation when the patient is already severely affected (Gerards *et al.*, 2013).

In this setting, we underline the necessity of developing biochemical biomarkers for hTHTR2 defects that would allow early diagnosis and timely therapeutic intervention. For this purpose, we first established control values for thiamine derivatives in blood and CSF in a paediatric population. Subsequently, we determined blood and CSF thiamine concentrations in a cohort of patients with *SLC19A3* mutations and compared them with patients with Leigh syndrome who were negative for *SLC19A3* mutations and other neurological conditions.

Currently, several HPLC procedures have been published to measure the concentrations of thiamine and its esters (Tallaksen *et al.*, 1991; Körner *et al.*, 2009; Mayr *et al.*, 2011). Here, we present a modified HPLC procedure for free-T, TMP and TDP analyses.

We observed a strong correlation between CSF and whole blood concentrations of the different thiamine forms. Moreover, free-T and TMP were more concentrated in CSF than in whole blood. These forms may serve as a thiamine reservoir for the brain, and consequently, their measurement might be more sensitive for the identification of hTHTR2-deficient patients. In line with this hypothesis, free-T was severely reduced in the CSF of hTHTR2-deficient patients before the introduction of thiamine supplementation (Fig. 1B). In contrast, free-T concentrations were slightly reduced in 9 of 59 (15.2%) patients with acquired or inherited disorders of the CNS, three of them being Leigh syndrome patients. Secondary thiamine deficiency in these children may be due to a combination of several mechanisms, including increased oxidative stress and thiamine turnover, inflammatory cell activation and drug interactions. Similarly, CSF folate deficiency has been observed in children with mitochondrial disorders and other neurological conditions bearing no primary relation to folate transport or metabolism (Pérez-Dueñas *et al.*, 2011).

We then analysed the intracellular concentrations of thiamine in hTHTR2-deficient fibroblasts cultured in a low thiamine medium. Markedly reduced concentrations of all thiamine forms were observed when compared to controls (Fig. 2A). However, TDP concentrations were relatively preserved as compared to free-T and TMP, suggesting that the small amounts of thiamine that entered the cell were almost completely converted to TDP, probably due to the high-binding affinity of the TPK enzyme for its substrate, thiamine (Onozuka *et al.*, 2003). Remarkably, when fibroblasts were cultured in a medium containing normal amounts of thiamine all the thiamine forms normalized (Fig. 2B), but TMP remained lower than the other forms, reflecting its quick conversion TDP.

Surprisingly, pyruvate dehydrogenase complex (PDHc) activities and mitochondrial substrate oxidation rates in fibroblasts cultured in a low thiamine medium were similar

to those in controls (Supplementary Table 2). We speculate that residual TDP concentrations were sufficient to normalize the thiamine-dependent enzymatic activities in standard conditions, but would not be able to do so under stress situations, as those that trigger acute decompensations in hTHTR2 patients. In line with this hypothesis, fibroblasts from hTHTR2-deficient patients showed a substantially reduced capacity to increase *SLC19A3* expression in situations of hypoxia or acidosis (Schänzer *et al.*, 2014).

Mayr *et al.* (2011) analysed the thiamine forms in muscle, blood and fibroblasts of seven patients with TPK deficiency. They found a reduction of TDP in all tissue and blood samples from patients but normal levels of free-T and TMP. Recently, Banka *et al.* (2014) corroborated these results in frozen muscle biopsy samples from another TPK-deficient patient.

Taken together, these findings suggest that intracellular quantification of thiamine forms may be useful for distinguishing different genetic defects in thiamine transport and metabolism (Mayr *et al.*, 2011).

The *in vitro* analysis of aberrant pre-messenger RNA splicing due to the c.980-14A>G allele showed the total exclusion of exon 4 and a predicted severe impairment of hTHTR2 function (Supplementary material). Hence, in our two hTHTR2 patients carrying loss-of-function mutations in both alleles, thiamine uptake from fibroblasts is probably compensated by the upregulation of an alternative transport system. Other human thiamine transporters, such as the reduced folate carrier (RFC1) and the hTHTR1 (Zhao *et al.*, 2002), or the organic cation transporter (OCT1), recently identified as an important contributor to the uptake of thiamine from blood to tissues (Kato *et al.*, 2015), could compensate for the thiamine transport in hTHTR2-deficient patients.

Four patients with *SLC19A3* mutations in our series responded extremely well to thiamine overload during the acute encephalopathic episode and symptoms improved within hours or days (Table 1). All four patients are stable and have not experienced neurological recurrences since the initiation of treatment. Three of these patients (Patients 1, 3 and 4) harboured missense mutations in *SLC19A3*, whereas Patient 6 was heterozygous for two null mutations p.Ser26Leufs*19 and p.Gly327Aspfs*8. Previously, thiamine responsiveness was also reported in a Leigh-like encephalopathic infant that was homozygous for the p.Ala328Leufs*10 frameshift mutation in *SLC19A3* (Haack *et al.*, 2014).

A lumbar puncture was performed on Patient 3 who was receiving 24 mg/kg/day of thiamine, and CSF analysis showed free-T values high above the upper limit of reference range (Fig. 1B). Again, our findings suggest that thiamine supplementation can compensate the hTHTR2 defect and restore thiamine values in the CSF. Theoretically, CSF free-T could be used to monitor treatment and optimize thiamine dose in hTHTR2 patients showing poor clinical response. In contrast to this, whole-blood thiamine is useful to monitor adherence to therapy in patients who are metabolically compensated, in whom repeated lumbar punctures are not appropriate because of ethical reasons (Ortigoza-Escobar *et al.*, 2014).

Mitochondrial biomarkers were normal in all but one hTHTR2 patient who showed increased plasma and CSF levels of lactate, high plasma levels of alanine, leucine and isoleucine, and a high excretion level of alpha-ketoglutarate in urine (Table 1). Interestingly, that patient experienced the earliest onset of encephalopathy and showed a severe reduction of CSF thiamine levels. Other authors reported high lactate values and high organic acid excretion levels in infants with Leigh-like phenotypes (Gerards *et al.*, 2013; Kevelam *et al.*, 2013; Schänzer *et al.*, 2014) but normal values in older patients with the biotin-thiamine-responsive basal ganglia phenotype (Ozand *et al.*, 1998; Zeng *et al.*, 2005; Kono *et al.*, 2009; Debs *et al.*, 2010; Tabarki *et al.*, 2013; Distelmaier *et al.*, 2014). Therefore, the currently available mitochondrial biomarkers are not sensitive for all hTHTR2-related phenotypes.

In conclusion, children with hTHTR2 deficiency have remarkable free-T deficiency in CSF and fibroblasts. Thiamine supplementation in these children restores CSF and intra-cellular thiamine levels, probably through an alternative transport system. We recommend that patients presenting with Leigh syndrome be promptly treated with a vitamin cocktail including thiamine and biotin and that a lumbar puncture be performed before the empirical administration of vitamins. Very low values of free-T in the CSF and/or a good therapeutic response to thiamine supplementation should lead clinicians to consider a genetic analysis of the *SLC19A3* gene.

Funding

This research was supported by the Instituto de Salud Carlos III-FEDER (FIS PI12/02010, FIS PI12/01138 FIS PI12/02078 and PI14/00028); Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), an initiative of the Instituto de Salud Carlos III (Ministerio de Ciencia e Innovación, Spain); Agència de Gestio' d'Ajuts Universitaris i de Recerca-Agau, (2014FI_B 01225) (JD Ortigoza-Escobar); and Fundació Sant Joan de Déu per a la Recerca (*PFNR0042*) (M Molero-Luis).

All authors declare that they have no conflicts of interest to declare.

Supplementary material

Supplementary material is available at *Brain* online.

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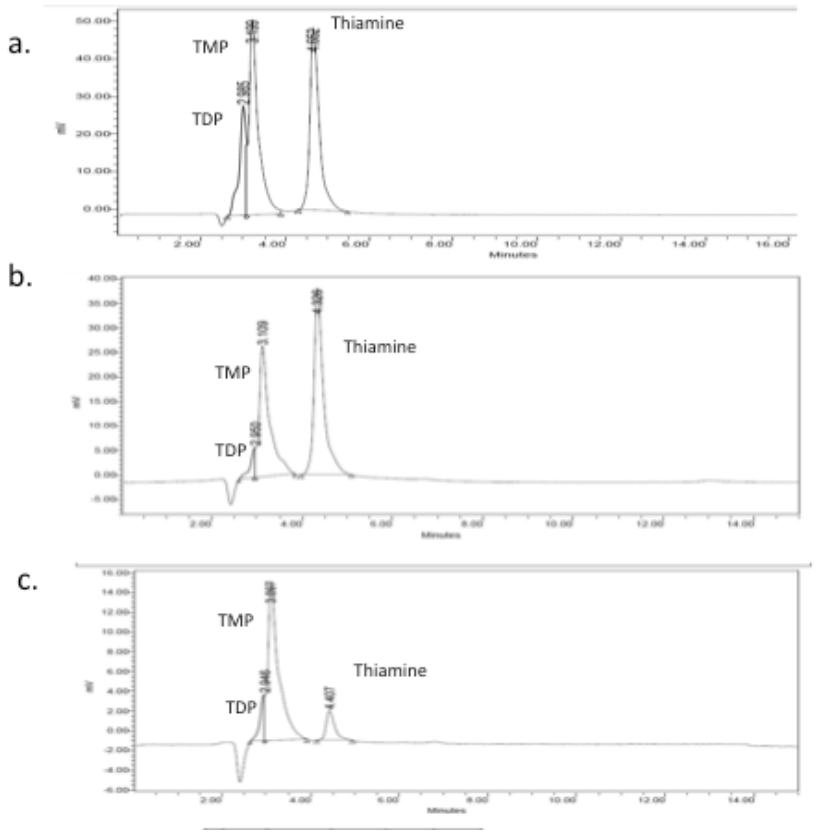

Figure 1.

CSF thiamine chromatograms.

CSF thiamine chromatograms for standard (a), control patient (b) and SCL19A3 deficient patient (c) by the fast method.

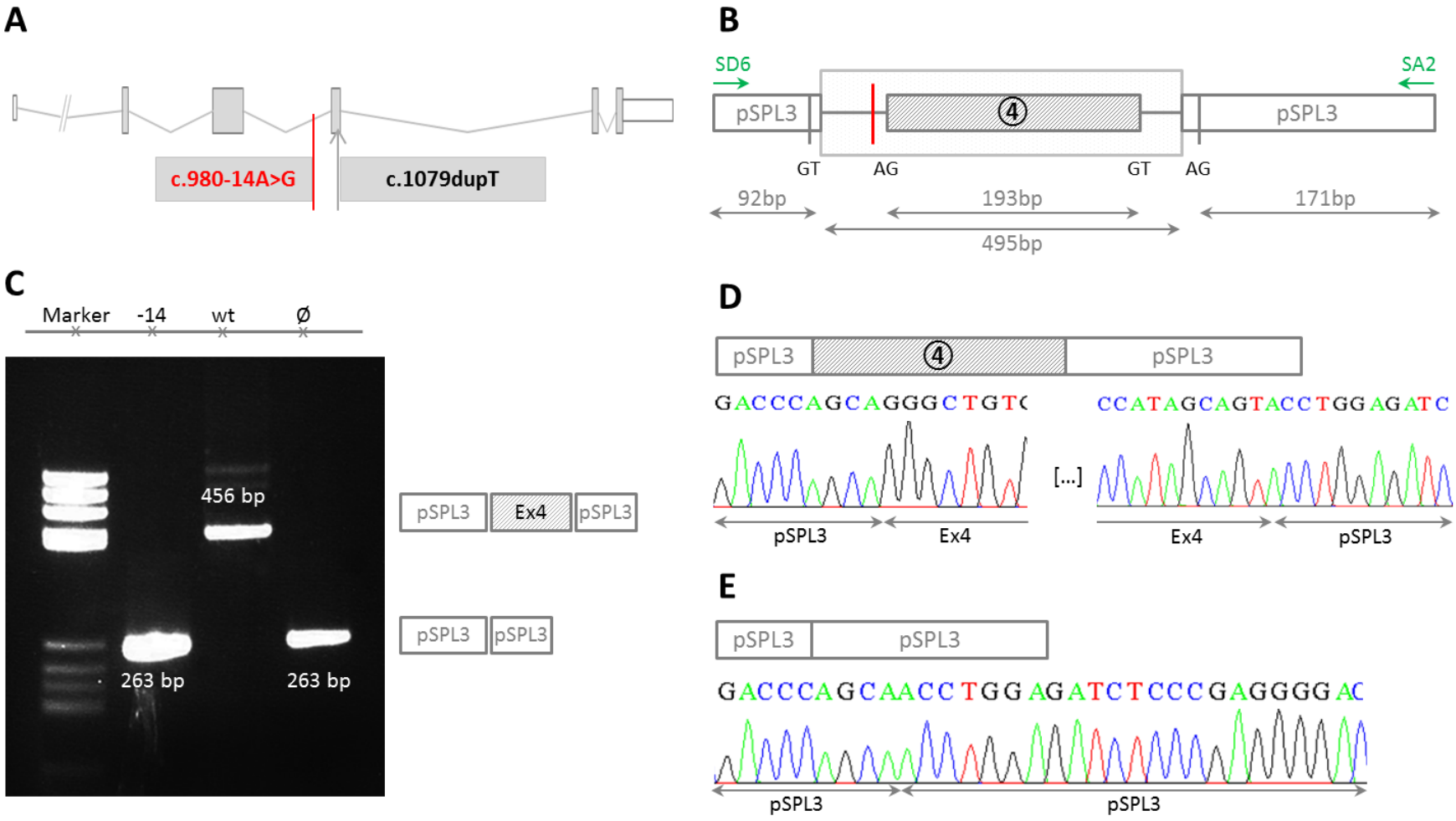

Figure 4

Splicing minigene reporter assay of c.980-14A>G allele.

A. Distribution of the mutations found on Patient 2 on *SLC19A3* gene. In red the c.980-14 A>G change. **B**. pSPL3 reporter minigene construct used in this functional assay and subcloning of the genomic SLC19A3 fragment from wild-type and mutant alleles. Green arrows show the primers used in reverse-transcriptase polymerase chain reaction (RT-PCR) experiments (SD6 and SA2). **C.** RT-PCR analysis of transcripts derived from the indicated reporter assay in HEK293T cells. Lanes from left to right: molecular weight marker, pSPL3/ SLC19A3 c.980-14 A>G mutant (-14), pSPL3/ SLC19A3 wild-type (wt) and pSPL3 mock vector (Ø). **D** and **E.** Sequence analysis of the molecular species contained in the RT-PCR from pSPL3/ SLC19A3 wt **(D)** or RT-PCR from pSPL3/ SLC19A3 mutant and mock vector **(E).** In both cases RT-PCR samples were first cloned in pGEMT easy vector.

Supplementary material 1.

Blood and CSF samples from control patients. Exclusion criteria

Reference values for whole blood free-T, TMP and TDP were established in 106 control subjects (mean age 5.7 years; range 1 day to 18 years; 52 males; 54 females) admitted to our Hospital from January to December 2013 for minor surgical interventions.

For CSF reference values, we analyzed CSF samples from 38 subjects (mean age: 3.8 years; range 1 day to 15 years; sex: 23 males; 15 females) whom viral or bacterial meningitis, encephalitis and other neurological conditions of non-metabolic origin were ruled out. White blood cells, glucose, proteins and neopterin levels, and viral and microbiological cultures were analyzed for each sample and all of them showed negative or normal results. These patients were admitted during the same period with a clinical suspicion of viral or bacterial central nervous system infection.

CSF/whole blood ratios for free-T, TMP and TDP were determined in 9 subjects for whom lumbar puncture and venous puncture were performed on the same day.

The exclusion criteria for the control subjects were as follows: presence of chronic diseases, inborn errors of metabolism, special diets, and malnutrition or pharmacological treatments, including multivitamins and thiamine supplementation, at the time of the analysis. Patients with traumatic spinal punctures, CSF samples with inflammatory parameters, or positive bacterial or viral tests were excluded from the study.

The CSF samples were frozen at -80ºC and protected from light exposure until performing the analysis.

Supplementary material 2.

Molecular analysis of the *SLC19A3* gene

Genomic DNA isolated from blood samples of patients with Leigh syndrome was used for mutation analysis of *SLC19A3* (RefSeq accession number NM_025243.3_ [mRNA]). The coding region and flanking intron-exon boundaries were PCR-amplified with primers designed based on the Ensembl genome browser entry ENSG00000135917. All PCR products were sequenced using BigDye Terminator v.3.1 Mix (Applied Biosystem Foster City, CA, USA) and were analyzed by capillary electrophoresis using an ABI Prism 3700 Genetic Analyzer (Applied Biosystems). DNA mutation numbering was based on the cDNA reference sequence, with nucleotide +1 representing the A of the ATG translation initiation codon. The nomenclature used follows the recommendation of the Human Genome Variation Society (http://www.hgvs.org./mutnomen/).

Functional analysis of the SLC19A3 c.980-14A>G mutation.

Minigene construction based on the pSPL3 exon-trapping vector was used to investigate the implication of c.980-14A>G identified in two of the *SLC19A3* mutated patients on the mRNA processing. Gene fragments corresponding to exon 4 of the *SLC19A3* gene were amplified from the DNA of patient 2 and control fibroblasts and cloned into the pGEMT easy vector (Promega Corporation, Madison, WI, USA). After excision with EcoRI, (Roche Diagnostic GmbH, Roche Applied Science, Nonnenwald, Penzberg) and purification with the QIAEX II Gel Extraction kit, inserts where subsequently cloned into pSPL3 vector (Exon Trapping System, Gibco, BRL,Carlsbad, CA, USA) using the Rapid Ligation kit (Roche Diagnostics GMBH, Roche Applied Science, Mannheim, Germany). Minigenes containing the mutant and normal inserts were transfected into HEK293T cells. RNA extraction and RT-PCR analysis were performed as previously described in (Fernandez-Guerra, *et al*. 2010). Splicing minigene-derived transcripts were amplified using the pSPL3-specific primers SD6 and SA2 (Exon Trapping System). Sequence characterizations of the different molecular species were accomplished after cloning the amplified PCR products into pGEMT vector.

Supplementary material 3.

Fibroblasts and culture conditions

Fibroblasts of controls and patients were analyzed at passage numbers 5 – 8 and were maintained in a humidified atmosphere of 5% CO2 and 95% air. Cells were removed enzymatically (0.25% trypsin-EDTA, 10 min, 37°C), split 1:4, centrifuged for 10 min at 252×g and sub-cultured in plastic culture dishes (6-well plates, 9.6 cm^2 growth area, Nunclon delta, Nunc, Denmark). Fibroblasts were grown in two different media: 1) Dulbecco's modified Eagle's medium (DMEM) without thiamine and 2) minimum essential medium (MEM) (Sigma, Saint Louis, MO, USA – thiamine concentration 1 mg/L). Both media were supplemented with 10% fetal bovine serum (containing 0.017 mg/L thiamine), 100 units ml-1 penicillin and 100 µg ml-1 streptomycin. The culture medium was changed every 3 to 4 days. After 10 days of incubation, cells were trypsin zed and washed twice with saline. Pelleted-cells were re-suspended with 300 µL of phosphate buffered saline (PBS) solution, pH 7.4, and sonicated once for 5 s. The homogenates were used to determine the concentrations of thiamine and its derivatives by HPLC (Waters 2690, Milford, MA, USA) with flourimetric detection (Kontron Instruments, Zurich, Switzerland). The homogenates were also used to measure total protein by a Protein Assay kit (Bio-Rad Laboratories, Hercules, CA, USA) based on the Lowry method. Fibroblasts were analyzed in duplicate in two independent experiments.

HPLC analysis of thiamine derivatives in blood, CSF and fibroblast samples

Thiamine derivatives were analyzed following a previously reported procedure with slight modifications (Mayr 2011). A total of 200 µL of whole-blood was deproteinized with 200 µL 10% trichloroacetic acid. After incubation for 15 min on ice, the samples were centrifuged for 10 min at 1500 x g and 4ºC, and 150 µL of supernatant was collected. Then, 1 ml of diethylether (DEE) was added to the supernatant to separate the organic and water fractions. Once the samples were vortexed and centrifuged for 10 min (1500 x g at 4°C), the organic fraction was removed, 1 mL of DEE was added, and the solution was mixed and centrifuged again. Before HPLC analyses, 90 µL of the thiamine-containing solutions were derivatized by the addition of 10 µL of a freshly prepared solution of 10 mM potassium hexacyanoferrat (III) in 15% NaOH, and this solution was immediately mixed. The processing of 100 µL of CSF samples was performed in the same manner as for the whole-blood samples. Free-T, TMP and TDP standards were purchased from Sigma (references T4625, T8637 and C8754, respectively; Sigma Chemical Company, St Louis, USA) and were diluted in distilled water to attain working solutions of 50 nmol/L. One hundred microliters of fibroblast homogenate was de-proteinized by the addition of 100 ml of 10% trichloroacetic acid (TCA) as described above.

The samples were placed in an autosampler protected from light, and 20 µL were injected into an HPLC system equipped with a reversed phase analytical column (Teknokroma, Barcelona, C18 250 mm x 4.6 mm, 5 µM particle size) and a C18 precolumn (Teknokroma, Barcelona, 581372-U). Initially, we standardized a common chromatographic method for the analysis of the thiamine derivatives in the blood and CSF samples. The elution conditions of this method were as follows: 0–6 min, 80% A; 6-12 min, 20% A; and 12-20 min, 80% A. Mobile phase A was 100% 25 mmol/l potassium phosphate (pH 7.0) and mobile phase B was 100% methanol HPLC grade. As free-T was considered the most sensitive form for the identification of hTHTR2 deficiency in the CSF, we standardized a novel and fast HPLC procedure for the analysis of free-T in the CSF. This new method used 50% methanol and 50% 25 mmol/l potassium phosphate (pH 7.0) as the mobile phase in an isocratic manner.

The flow rate for both methods was 1.0 mL/min. The fluorescence detector was set to an excitation wavelength of 375 nm and an emission wavelength of 435 nm. TDP, TMP and free-T in the blood and CSF samples were eluted at 4.8, 6.8 min and 12.4 min, respectively, by the original method, and free-T in the CSF samples was eluted at 4.4 min by the fast method (see elution times in **Fig. 1**). The chromatographic data were processed using the Breeze GP software (Waters, MA, USA).

Mitochondrial biomarkers

Amino acids and organic acids were analyzed by ion exchange chromatography with nynhydrin detection (Biochrom 30, Pharmacia Biotech, Biochrom, Cambridge, Science Park, England) and gas chromatography/mass spectrometry (Agilent Technologies Inc., Santa Clara, CA, USA), respectively, following previously reported procedures (Moyano *et al*., 1998, Blau *et al*., 2008). Plasma and CSF lactate analyses were performed by automated spectrometric procedures (Architect ci8200, Abbott). Substrate oxidation rates were analyzed in fibroblasts by measuring 14CO2 production from the oxidation of [1-14C]-pyruvate, [2-14C]-pyruvate and [14C]-glutamate (Willems *et al*., 1978)

Supplemental material 4. Statistical analysis

The Kolmogorov-Smirnow test was used to assess the distribution of the data. Because the data did not follow a Gaussian distribution, different non-parametric tests were applied. The Spearman simple correlation test was used to determine the correlations between whole blood and CSF thiamine forms and patient age. The Mann-Whitney U and Kruskal-Wallis tests were used to test the significant differences in the concentrations of thiamine derivatives in the different age groups. Differences with p values <0.05 were considered statistically significant. Statistical calculations were performed using SPSS 20.0 software.

Table 3. Within-run and between-run imprecision data and analytical range for thiamine, TDP and TMP measured by HPLC with fluorescence detection.

Thiamine vitamer	Within-run CV	Between-run CV	Limit of detection
Thiamine	4.55 % (38 nmol/L)	9.99 % (102 nmol/L)	0.1 nmol/L
TDP	6.56 % (59 nmol/L)	8.97 % (93 nmol/L)	1.2 nmol/L
TMP	9.99 % (4.2 noml/L)	12.72 % (12.8 nmol/L)	1.1 nmol/L

Data representing coefficients of variation are expressed as percentages (average concentration in nmol/L). Limit of detection is the lowest analyte concentration likely to be reliably distinguished from the blank response (signal to noise ratio >3). The units are represented in nmol/L (in brackets).

Table 4. Concentrations of free-T, TMP and TDP and substrate oxidation rates in fibroblasts of hTHTR2 deficient patients and controls.

Thiamine isoforms in fibroblasts

nmol/ g protein	TDP	TMP	Free-T	TDP	TMP	Free-T
	Media without thiamine			Media with thiamine (MEM)		
Patient 1 (n=2)	53	4	3.3	71	3.8	15
Patient 2 (n=2)	23	0.1	0.1	48	0.1	32
Patient 6 (n=2)	51	0.1	0.1	55	0.1	8
Control mean ± SD	49.6 ± 6	7.7 ± 4.6	8.4 ± 6.2	55.1 ± 16.5	6.2 ± 3.6	18.5 ± 6.7
Control range	44 – 58	4 – 16	3 - 20	37– 81	3 – 13	9- 30

Substrate oxidation rates in fibroblasts

nmol/h/mg protein	**1-^{14}C-pyruvate**	**2-^{14}C-pyruvate**	**Glutamate**	**1-^{14}C- pyruvate**	**2-^{14}C-pyruvate**	**Glutamate**
	Media without thiamine			Media with thiamine (MEM)		
Patient 1	18.5	4.7	10.9	19	4.5	10.4
Parallel Control	11.6	2	7.2	15.9	4.2	7
Control range	8 - 36	1.9 - 12	4.5 - 25			

Síntesis de resultados

- La tiamina-libre y TMP se concentran más en el LCR que en la sangre total. Estas isoformas pueden servir como depósito de tiamina para el cerebro y, en consecuencia, su medición podría ser más sensible para la identificación de los pacientes con deficiencia de hTHTR2.
- Se observó una reducción severa de tiamina-libre en LCR en cinco pacientes con mutación del gen *SLC19A3* antes de la suplementación con vitaminas.
- Las concentraciones de tiamina-libre se redujeron solo ligeramente en un 15,2% (9/59) de los pacientes con trastornos neurológicos congénitos o adquiridos. Tres de estos pacientes presentaban fenotipo de síndrome de Leigh. La deficiencia secundaria de tiamina en estos niños puede ser debida a una combinación de varios mecanismos, incluyendo el aumento del estrés oxidativo, la activación de células inflamatorias y las interacciones medicamentosas.
- Se verificó la restauración de los valores de tiamina en LCR en un paciente con deficiencia de hTHTR2 tras la suplementación con tiamina.
- Se comprobó una deficiencia severa de tiamina-libre y niveles bajos de TDP en fibroblastos de pacientes con deficiencia de hTHTR2. Sin embargo, las concentraciones de TDP estaban relativamente conservadas en comparación con la tiamina-libre y TMP, lo que sugiere que las pequeñas cantidades de tiamina que entran en la célula son casi completamente convertidas a TDP, probablemente debido a la alta afinidad de unión de la enzima por su sustrato a TPK
- La actividad de la PDH y las tasas de OXPHOS en fibroblastos de pacientes con deficiencia de hTHTR2 se encuentran dentro de rangos normales. La

concentración de las isoformas de tiamina se normalizan tras la adición de tiamina al medio de cultivo.

- El análisis del mini gen c.980-14A>G predice una proteína truncada de menor tamaño.

Discusión conjunta

El síndrome de Leigh es una enfermedad de inicio habitualmente precoz y de curso progresivamente fatal del que se conocen actualmente más de 75 genes causantes, lo que implica una heterogeneidad clínica importante [Lake et al., 2016]. Varios defectos genéticos del transporte y metabolismo de la tiamina (*SLC19A3*, *SLC25A19* y *TPK1*) se manifiestan como síndrome de Leigh. Estos defectos genéticos presentan, en la mayoría de sus fenotipos, una respuesta favorable a la suplementación con vitaminas. Es por ello, que se hace indispensable distinguir a estos pacientes de los pacientes con Síndrome de Leigh de otras causas y por este motivo, se hace también obligatorio suplementar con vitaminas a todos los pacientes con Síndrome de Leigh. Sin embargo, esta es una tarea difícil teniendo en cuenta que ambos comparten características clínicas y bioquímicas muy similares.

Historia natural de pacientes con defectos en los genes *SLC19A3*, *SLC25A19* y *TPK1* a través de un estudio multicéntrico colaborativo.

Los pacientes con defectos de tiamina (*SLC19A3*, *TPK1* y SLC25*A*19) de esta cohorte presentaron episodios de encefalopatía aguda y recurrente, con lesión en ganglios basales, aumento de lactato en sangre y LCR, cumpliendo criterios diagnósticos de Síndrome de Leigh. La gran mayoría de niños presentan el debut de la enfermedad, signos de encefalopatía (disminución de la conciencia, hipotonía global), distonía, disartria, disfagia y convulsiones. Estas características son superponibles a otras causas de necrosis estriatal, que incluyen trastornos infecciosos y post-infecciosos [Ashtekar et al., 2003, Karagülle Kendi et al., 2008, Voudris et al., 2002]. Por lo tanto, para un diagnóstico diferencial rápido y preciso es necesario un alto índice de sospecha.

Los pacientes con defectos genéticos del metabolismo y transporte de la tiamina de esta cohorte mostraron anomalías bioquímicas del metabolismo energético mitocondrial. Una proporción significativa de los niños tenía acumulación de lactato en la espectroscopia y en el LCR, mientras que solo 20 pacientes, todos ellos menores de 5 años, presentaron acidosis láctica. Estos biomarcadores no fueron útiles para predecir la supervivencia o severidad de estos pacientes. Se observó una correlación negativa entre la acidosis láctica y la edad de debut de los pacientes. Ningún paciente de más de 5 años de edad presentó hiperlactacidemia en el debut. Solo ocho pacientes con mutación del gen *SLC19A3* y un paciente con mutación del gen *TPK1* tuvieron un perfil alterado de ácidos orgánicos en la orina. El aumento de alfa-cetoglutarato se identificó solo en dos pacientes de nuestra cohorte. Por lo tanto, a diferencia de proposiciones realizadas en estudios previos, no podemos considerar al alfa-cetoglutarato como un biomarcador sensible de los defectos del metabolismo y transporte de tiamina [Pérez-Dueñas et al., 2013].

El ácido láctico y los ácidos orgánicos urinarios se normalizaron después de la administración de tiamina, excepto en un paciente. Por lo tanto estos parámetros junto con la cuantificación tiamina de sangre total se podrían utilizar para la monitorización del tratamiento, tal como se explica en el apartado "**Utilidad de la determinación de las concentraciones de tiamina en sangre total para la monitorización de estos pacientes con suplementación de tiamina"**

Los resultados expuestos en esta tesis confirman reportes previos de un patrón radiológico característico de lesión cerebral en estos pacientes [Tabarki et al., 2013, van der Knaap et al., 2014], confirmando la afectación frecuente del caudado y putamen observado en el 93% de los mismos. Sólo una minoría (siete casos) mostró necrosis

aislada del estriado, mientras que la mayoría de los pacientes asoció además lesiones del córtex cerebral, el tálamo, el cerebelo, el tronco cerebral o la médula espinal.

Hay que destacar que los pacientes que fueron éxitus mostraron una distribución más difusa de lesiones cerebrales, con participación significativamente mayor del globo pálido y el tronco cerebral que los pacientes vivos. Hay ciertas razones para esperar un peor pronóstico en pacientes con lesiones que afectan estas estructuras. En primer lugar, los centros respiratorios están situados en el bulbo raquídeo y la protuberancia y la mayoría de los pacientes con síndrome de Leigh con compromiso del tronco encefálico fallecen de insuficiencia respiratoria central. Por otra parte, las lesiones bilateral del globo pálido observadas en hipoxia severa, intoxicación por monóxido de carbono o cianuro, y en algunas enfermedades metabólicas como la aciduria glutárica tipo 1 [Bekiesinska-Figatowska et al., 2013, Alquist et al., 2012] que asocian distonía severa, también presentan una alta morbilidad en los supervivientes.

Se ha comprobado diferencias con respecto a la neuroimagen de pacientes con mutación de los genes *SLC25A19* y *TPK1*, comparados con pacientes con mutación del gen *SLC19A3*. El cuerpo estriado (caudado y putamen) está afectado en la mayoría de los pacientes con los tres defectos genéticos previamente mencionados. Sin embargo, la mayoría de los pacientes con mutación del gen *SLC19A3* asocian lesiones cortico-subcorticales, una característica que no se encuentra en los demás defectos. En contraste, los cuatro pacientes con mutación del gen *TPK1* presentan lesiones de los núcleos dentados del cerebelo y, dos de ellos, también presentaban lesiones en el globo pálido, estructuras que estaban poco afectadas en pacientes con mutación del gen *SLC19A3*. Los pacientes con mutación del gen *SLC25A19* presentaron necrosis con cavitación de los núcleos caudados. Sin embargo, se necesitan más casos para verificar

la legitimidad de estas diferencias, pero son tal vez hallazgos radiológicos que pueden predecir el genotipo de estos pacientes.

Se suplementó con tiamina a la mayoría de los pacientes de esta cohorte. El retardo del inicio de la suplementación desde el inicio de la enfermedad fue muy variable: un 50% recibió el tratamiento en las primeras dos semanas, y el 75% en los primeros 6 meses desde el inicio de los síntomas. Las dosis de tiamina fueron variables, de 50-1000 mg/día. Estos suplementos tuvieron un fuerte impacto en los resultados, con una mejor curva de supervivencia en el grupo tratado vs. el grupo no tratado. Por otra parte, en el seguimiento de estos pacientes, no se observaron nuevos episodios de encefalopatía, se observó un mejor control de la epilepsia y se normalizaron los parámetros bioquímicos (acidosis metabólica, ácido láctico y alfa-alanina plasmática). Más importante aún, la mitad de estos pacientes no mostró ninguna discapacidad y presentaron un examen neurológico normal. Por desgracia, cuatro niños murieron a pesar de la administración de tiamina. La respuesta pobre o ineficaz del tratamiento en estos pacientes se puede explicar por el inicio temprano de la enfermedad (P41), la afectación cerebral extensa (P49) o el inicio del tratamiento en un contexto metabólico/infeccioso inoportuno, como la sepsis (P1).

La composición del "*cóctel mitocondrial*" que se administra a pacientes con sospecha de enfermedad mitocondrial no es uniforme y varía enormemente entre los diferentes centros. Por desgracia, la tiamina todavía no se incluye de forma regular en el "*cóctel mitocondrial*". Parikh et al., 2013 demostraron que sólo 14/32 (44%) de los médicos indican vitaminas en el "*cóctel mitocondrial*" inicial, de los cuales sólo 3/32 (9%) son vitaminas del complejo B. Teniendo en cuenta la presentación clínica de los defectos genéticos del transporte y metabolismo de la tiamina descritas en esta tesis, cumple con los criterios diagnósticos de síndrome de Leigh, y que son clínicamente indistinguible

de otros defectos de OXPHOS, es aconsejable indicar suplementos de tiamina en todos los pacientes con síndrome de Leigh. No obstante, los pacientes con mutación en *SLC19A3* con debut en los primeros 6 meses de vida tienen una peor curva de supervivencia y mayor discapacidad que los pacientes con debut a mayor edad. Así, la administración de tiamina en pacientes con sintomatología severa y daño cerebral difuso debe ser individualizada, ya que no se ha demostrado que puede evitar la muerte o la discapacidad grave.

La historia natural de pacientes con síndrome de Leigh, en una gran cohorte multicéntrica ha sido descrita previamente por Sofou et al, 2014. También se ha realizado el análisis de subgrupos específicos: *TMEM70* [Magner et al., 2015], *SURF1* [Wedatilake et al., 2013], *LRPPRC* [Debray et al., 2011], deficiencia del complejo I [Koene et al, 2012] y deficiencia de PDH [Patel et al., 2012]. En general, estos estudios demuestran una tasa de supervivencia baja. Debray et al., 2011 describió una tasa de mortalidad global del 82% en paciente con mutación del gen *LRPPRC*, con una edad promedio de muerte a los 1,6 años y Sofou et al., 2014 informó que el 39% de los pacientes con síndrome de Leigh de su cohorte habían muerto a la edad de 21 años, con una edad media de muerte de 2,4 años. En el apartado "**Pacientes reportados en la literatura con defectos nucleares del complejo I mitocondrial**" hemos presentado datos de cómo sólo 4 de los 23 defectos del complejos I codificadas por ADN nuclear sobreviven hasta llegar a la edad adulta.

Este este trabajo se demuestra que los pacientes con mutación en el gen *SLC19A3* tienen una mejor tasa de supervivencia que otros pacientes con otras causas genéticas de síndrome de Leigh, dado que más del 60% continúan vivos a los 20 años de edad. La principal razón para esta diferencia es que, en contraste con la mayoría de defectos genéticos causantes del síndrome de Leigh que no tienen ningún tratamiento específico,

los pacientes con mutación en el gen *SLC19A3* muestran una mejoría clínica significativa tras la suplementación con tiamina y biotina. Esto produce un impacto positivo que podemos comprobar con la normalización de los biomarcadores, buen control de las convulsiones sin nuevos episodios de encefalopatía e, incluso, el hecho de que un alto porcentaje de pacientes está libre por completo de síntomas.

Además del tratamiento existen otras razones que explican el mejor pronóstico observado en estos pacientes: la edad media de aparición de la enfermedad en nuestra cohorte fue de 3 años, y el 80% de los pacientes *SLC19A3* desarrolló síntomas después de la edad de 6 meses. En pacientes con síndrome de Leigh de otras causas, la aparición de síntomas antes de los 6 meses es un mal indicador de supervivencia [Sofou et al., 2014]. La miocardiopatía, ausente en pacientes con mutación del gen *SLC19A3* también se asocia a una mayor mortalidad en pacientes con Síndrome de Leigh [Holmgren et al., 2003]. Por último, las lesiones del tronco cerebral que aparecieron en una minoría de pacientes con mutación en el gen *SLC19A3* se han vinculado a mala supervivencia en pacientes con síndrome de Leigh [Sofou et al., 2014].

Hasta ahora, pocos estudios han cuantificado la discapacidad de los pacientes con síndrome de Leigh. Con estos resultados se demuestra que las secuelas en los pacientes con mutación del *SLC19A3* son menos severas que la de los pacientes con síndrome de Leigh de otras causas. En este estudio, la mayoría de los pacientes tenían una puntuación de 10 o menos en la parte b de la BFMDS. Por otra parte, la mitad de los pacientes mostró un examen neurológico completamente normal con ausencia de síntomas de enfermedad neurológica. Esto contrasta con las graves secuelas neurológicas descrita en los pacientes con mutación en *SURF1*, presentes en el 60% de los casos de los sobrevivientes de más de 10 años de edad [Wedatilake et al., 2013]. En cuanto a los pacientes con mutación *SLC25A19*, todos permanecen vivos con

discapacidad muy leve, mientras que los pacientes con mutación en *TPK1* sufren una enfermedad grave que conduce a la muerte prematura y a la discapacidad severa en los supervivientes.

Existe una alta proporción de pacientes homocigotos con mutaciones *missense* pertenecientes a la etnia árabe. La variante más común (c.1264A>G) en pacientes con mutación *SLC19A3* se asoció con un fenotipo más leve con una mayor tasa de supervivencia. Sorprendentemente, esta mutación está asociada a un actividad nula del transportador [Yamada et al., 2010], además de las mutaciones *missense* c.20C>A y c.68G>T [Gerards et al., 2013, Subramanian et al., 2006] que también se observó en esta cohorte. Por el contrario, una minoría de pacientes Blancos Europeos fueron heterocigotos combinados de mutaciones que conducen a proteínas truncadas. Se identificaron cuatro pacientes con genotipo c.74dupT/c.980-14A>G, los cuales presentaron un fenotipo BTRBGD de inicio tardío, tres de ellos en la adolescencia y la edad adulta, con una excelente respuesta a la tiamina [Serrano et al., 2012, Debs et al., 2010]. Del mismo modo, un paciente compuesto heterocigoto para las mismas mutaciones presentó una fenotipo de leucoencefalopatía subaguda en la edad adulta, también con una buena respuesta a la tiamina [Sgobbi de Souza et al., 2016]. En el apartado de **"Nuevo biomarcador en líquido cefalorraquídeo y fibroblastos para el diagnóstico de pacientes con mutaciones en el gen *SLC19A3"*** se describe el análisis-*in vitro* de la expresión del ARN mensajero de la mutación c.980-14A>G que resulta en la afectación severa de la función del hTHTR2. Es necesario el análisis de un mayor número de pacientes para comprender mejor la correlación genotipo-fenotipo.

Nuevo biomarcador en líquido cefalorraquídeo y fibroblastos para el diagnóstico de pacientes con mutaciones en el gen *SLC19A3*.

Actualmente, hay varios procedimientos de HPLC publicados para medir las concentraciones de tiamina y sus isoformas [Tallaksen et al., 1991, Mayr et al., 2011]. En esta tesis, presentamos un procedimiento modificado de HPLC para analizar las concentraciones de tiamina-libre, TMP y TDP.

Este trabajo muestra una fuerte correlación entre las concentraciones de tiamina y sus isoformas en LCR y sangre total. La tiamina-libre y TMP se concentran más en el LCR que en la sangre total. Estas isoformas pueden servir como depósito de tiamina para el cerebro y, en consecuencia, su medición podría ser más sensible para la identificación de los pacientes con deficiencia de hTHTR2. En línea con esta hipótesis la tiamina-libre estaba severamente reducida en el LCR de pacientes con deficiencia en hTHTR2 antes de la introducción de la suplementación de tiamina. En contraste, las concentraciones de tiamina libre se redujeron solo ligeramente en 9 de 59 (15,2%) pacientes con trastornos adquiridos o congénitos del SNC, tres de ellos con síndrome de Leigh. La deficiencia secundaria de tiamina en estos niños puede ser debida a una combinación de varios mecanismos, incluyendo el aumento estrés oxidativo, la activación de células inflamatorias y las interacciones medicamentosas. Del mismo modo, la deficiencia de folato en LCR se ha observado en niños con enfermedades mitocondriales y otras condiciones neurológicas sin relación primaria con el transporte o metabolismo del folato [Pérez-Dueñas et al., 2011].

A continuación se analizaron las concentraciones intracelulares de tiamina en fibroblastos de pacientes con deficiencia de hTHTR2 cultivados en un medio con baja concentración de tiamina. Se observaron concentraciones marcadamente reducidas de todas las isoformas de tiamina en comparación con los controles. Sin embargo, las concentraciones de TDP estaban relativamente conservadas en comparación con la tiamina-libre y TMP, lo que sugiere que las pequeñas cantidades de tiamina que entran

en la célula son casi completamente convertidas a TDP, probablemente debido a la alta afinidad de unión de la enzima por su sustrato a TPK [Onozuka *et* al., 2003]. Cuando los fibroblastos se cultivan en un medio que contiene concentraciones normales de tiamina, todas las concentraciones de las isoformas de tiamina se normalizan, pero TMP sigue siendo más baja que las otras isoformas, lo que refleja su conversión rápida a TDP.

Las actividades del complejo piruvato deshidrogenasa (PDH) y las tasas de oxidación de sustrato mitocondrial en fibroblastos cultivados en medio con baja concentración de tiamina fueron similares a los de los controles. Especulamos que las concentraciones residuales de TDP fueron suficientes para normalizar las actividades de las enzimas dependientes de la tiamina, aunque pensamos que no serían capaces sin embargo, de hacerlo bajo situación de estrés, como las que provocan descompensaciones agudas en pacientes con deficiencia de hTHTR2. De acuerdo con esta hipótesis, los fibroblastos de estos pacientes muestran una disminución sustancial de la capacidad para aumentar la expresión de *SLC19A3* en situaciones de hipoxia o acidosis [Schänzer et al., 2014). Mayr et al., 2011 analizó la concentración de los derivados de tiamina en músculo, sangre y fibroblastos de siete pacientes con deficiencia de *TPK1*, observando una reducción de TDP en todos los tejidos y muestras de sangre de estos pacientes, pero concentraciones normales de tiamina-libre y TMP. Recientemente, Banka et al., 2014 corroboró estos resultados en muestras de biopsia muscular de otro paciente con deficiencia de *TPK1*. En conjunto, estos hallazgos sugieren que la cuantificación intracelular de los derivados de tiamina puede ser útil para distinguir los diferentes defectos genéticos del transporte y metabolismo de la tiamina [Mayr et al., 2011].

El análisis in vitro de ARN pre-mensajero aberrante producto del alelo c.980-14A>G reveló la exclusión total del exón 4, lo que conlleva a una perdida severa de la función

de hTHTR2 ya predicha. La absorción de tiamina por los fibroblastos de nuestro dos pacientes con deficiencia de hTHTR2, portadores de esta mutación, es probablemente compensada por la regulación al alza de un sistema de transporte alternativo. En este transporte alternativo podrían participar otros transportadores comentados en la sección de introducción, tales como el portador de folato reducido (RFC1), el hTHTR1 [Zhao et al., 2002], o el transportador de cationes orgánicos (OCT1), recientemente identificado [Kato et al., 2015], compensando así la deficiencia de hTHTR2.

La punción lumbar realizada al paciente 3, en tratamiento con 24 mg/kg/día de tiamina, mostró concentraciones de tiamina libre por encima del límite superior del rango de referencia. Una vez más, nuestros hallazgos sugieren que la suplementación con tiamina puede compensar la deficiencia de hTHTR2 y restaurar los valores de tiamina en el LCR. Siguiendo esta hipótesis, la medición de tiamina – libre en LCR se podría utilizar para monitorizar el tratamiento y optimizar las dosis de tiamina en pacientes con deficiencia de hTHTR2 con mala respuesta clínica. Así mismo, y como se comentará más adelante en esta tesis, la concentración de tiamina en sangre total es útil para monitorizar la adherencia al tratamiento en pacientes metabólicamente compensado, en los que se repetir la punción lumbar podría no estar indicado por razones éticas.

Los biomarcadores mitocondriales fueron normales en todos, excepto un paciente de esta cohorte, quien mostró aumento de lactato en plasma y LCR, alanina, leucina e isoleucina en sangre, y una excreción aumentada de alfa-cetoglutarato en la orina. Otros autores reportaron elevación de lactato y aumento de la excreción de alfa-cetoglutarato en lactantes con fenotipo similar [Gerards et al, 2013.; Kevelam et al, 2013.; Schänzer et al., 2014], pero normalidad de estas determinaciones en pacientes mayores [Ozand et al, 1998;.. Zeng et al, 2005; Kono et al., 2009; Debs et al., 2010; Tabarki et al., 2013;

Distelmaier et al., 2014]. Por lo tanto, los biomarcadores mitocondriales disponibles no son sensibles en pacientes con deficiencia de hTHTR2.

Utilidad de la determinación de las concentraciones de tiamina en sangre total para la monitorización de pacientes con suplementación de tiamina.

Los datos de los pacientes previamente reportados con mutaciones en el gen *SLC19A3* demostraron que la distonía focal o generalizada, en combinación con la encefalopatía y las convulsiones, son las manifestaciones clínicas más frecuentes al debut. Todos estos síntomas se observaron en más del cincuenta por ciento de los pacientes [Ozand et al., 1998, Zeng et al., 2005, Kono et al., 2009, Yamada et al., 2010, Debs et al., 2010, Serrano et al., 2012, Kevelam et al., 2013, Gerards et al., 2013, Tabarki et al., 2013, Alfadhel et al., 2013, Fassone et al., 2013, Distelmaier et al., 2013, Tabarki et al., 2013. Pérez-Dueñas et al., 2013, Schänzer et al., 2014], lo que refleja que esta enfermedad es una causa importante de la distonía tratable en los niños. Por lo tanto la suplementación a modo de ensayo terapéutico con tiamina debe indicarse en casos de distonía aguda, sobre todo cuando exista además lesión de ganglios basales.

Los pacientes reportados en la literatura también presentaron con menor frecuencia otros síntomas, entre ellos: síntomas extrapiramidales, parálisis de pares craneales, disautonomía, ictericia, rabdomiólisis y síntomas sistémicos (pérdida de peso, hepatopatía, etc.). La revisión de la literatura de esta sección mostró que la mayoría de los pacientes con mutaciones *SLC19A3* inician síntomas entre el mes de vida y los 12 años de edad. Dos tercios de los pacientes fueron clasificados como BTRBGD; los demás pacientes presentaron fenotipo de Síndrome de Leigh y encefalopatía de Wernicke, siendo probable es que exista un *continuum* clínico entre los pacientes. Así, el paciente 2 con síndrome de Leigh, en nuestra serie, es portador de la mutación c.980-

14A>G, que se había descrito previamente en algunos pacientes con fenotipo BTRBGD [Debs et al., 2010, Serrano et al., 2012], y el paciente 1, que debutó con acidosis láctica infantil y síndrome de Leigh es portador de la mutación c.68G>T, también previamente descrita en el fenotipo BTRBGD [Zeng et al., 2005]. En este trabajo también comprobamos que pacientes con las mismas mutaciones presentan edades de inicio diferentes (Por ejemplo, c.1264A>G [Tabarki et al., 2013, Alfadhel et al., 2013] y c.20C>A [Gerards et al., 2013]). Es probable que una combinación de factores genéticos y ambientales todavía desconocidos pueden ser responsables de las desiguales edades de presentación, así como de los fenotipos diferentes [Chan et al., 2012, Lehner et al., 2007].

A pesar de la heterogeneidad genética de estos pacientes y el amplio rango de edad de inicio de la enfermedad, todos ellos presentan afectación simétrica de caudado, putamen y región dorso-medial del tálamo. En los pacientes de mayor edad, la cabeza del caudado siempre está afectada, y se observan lesiones corticales y subcorticales distribuidas de forma asimétrica en los hemisferios cerebrales [Tabarki et al., 2013, Alfadhel et al., 2013] a diferencia de los pacientes en periodo neonatal, donde puede no observarse afectación del caudado y la afectación cortical-subcortical es más selectiva de las áreas perirolándicas [Pérez-Dueñas et al., 2013]. Estas diferencias en la distribución de las lesiones cerebrales observadas en nuestros pacientes probablemente dependa de las variaciones de la demandas energéticas regionales, según las diferentes edades. Aunque este patrón de lesiones cerebrales podría no ser específico, puede ser útil como indicador en el diagnóstico de la deficiencia de hTHTR2.

En este trabajo se observó una respuesta dramática a altas dosis de tiamina en los tres pacientes que fueron tratados en los primeros días del episodio encefalopático. El seguimiento clínico mostró una recuperación clínica y radiológica completa en un

paciente, mientras que los otros dos pacientes presentaron distonía residual, disartria y lesiones necróticas en el estriado y la corteza frontal. Sin embargo, el paciente 2 murió a los 14 meses, cuatro semanas después del inicio de síntomas, a pesar de recibir suplementación con tiamina.

Al establecer los valores de referencia de tiamina encontramos que TDP es la isoforma más concentrada en sangre total, similar a la reportado en otros estudios [Mayr et al., 2011, Körner et al., 2009]. Por esta razón, la monitorización del tratamiento se basó en la medición de la TDP en sangre total. Los pacientes tratados con 10-40 mg/kg/día de tiamina se mantuvieron clínicamente estables durante una media seguimiento de 57 meses. Con estas dosis de suplementación, los niveles de TDP se mantuvieron por encima del límite superior de los valores de referencia en los pacientes 3 y 4. En cambio, en el paciente 1, las concentraciones de TDP se mantuvieron en el rango de referencia. Este paciente además presentaba acidosis persistente. Estos datos llevaron a sospechar la mala adherencia familiar al tratamiento, hecho que fue confirmado y corregido después con la inclusión de la figura del trabajador social en el seguimiento de este paciente. Este paciente no presentó recaída clínica, a pesar de que las concentraciones de ácido láctico estuvieron persistentemente elevadas, quizás debido a la ausencia de factores desencadenantes durante el seguimiento.

Es probable que se requieran dosis más altas en la fase inicial del tratamiento cuando factores desencadenantes, tales como fiebre o trauma, aún están presentes y el gasto metabólico en consecuencia es mayor [Tabarki et al., 2013]. Los reportes iniciales de pacientes con deficiencia de hTHTR2 describen una buena respuesta a la biotina en monoterapia [Kono et al., 2009]. Sin embargo, una descripción realizada por Tabarki et al., 2013 informa que una alta proporción de pacientes tratados solo con biotina

muestran más episodios de recurrencias de la encefalopatía en comparación con aquellos que recibían biotina y tiamina al mismo tiempo.

En este trabajo, hemos detectado mutaciones patógenas en el gen *SLC19A3* en 1/11 pacientes con síndrome de Leigh. De forma similar, Gerards et al., 2013 informaron que 2/17 pacientes con síndrome de Leigh de su cohorte eran portadores de mutaciones patógenas del gen *SLC19A3*.

Pacientes reportados en la literatura con defectos nucleares del complejo I mitocondrial.

Las mutaciones en el gen *NDUFS4* son una causa genética frecuente de deficiencia del complejo I, dado que se han identificado en el 11% (22/198) de los pacientes de esta serie. El NDUFS4 es una subunidad "supernumeraria" codificada por ADN nuclear que está situada en una región estratégica del complejo I. Está involucrado en varios procesos, incluyendo el ensamblaje del complejo I, su transporte intramitocondrial y su posterior activación [Assereto et al., 2014].

Al igual que en otros reportes de pacientes con mutaciones de *NDUFS4*, nuestra paciente sufrió un deterioro neurológico rápido a partir del segundo mes de vida, con afectación radiológica de los ganglios basales y del tronco cerebral, y sintomatología consistente en hipotonía, rigidez, movimientos oculares anormales e insuficiencia respiratoria. También presentó hiperlactacidemia y acumulación de lactato en ganglios basales, detectado por espectrometría. Además, la actividad residual de los complejos I y III estaba disminuida a 50% y 78%, respectivamente, en comparación con los controles normales.

Previamente, se había reportado una reducción variable de la actividad enzimática del complejo I (1-74% en tejido muscular de 18 pacientes y 16-82% en fibroblastos de 12

pacientes) en pacientes con mutaciones de *NDUFS4* [Haack et al, 2012; Van den Heuvel et al., 1998; Budde et al., 2000; 2003; Lebre et al., 2011; Petruzzella et al., 2001; Scacco et al., 2003; Benit et al., 2003; Assouline et al, 2012.; Rötig et al., 2004; Anderson et al., 2008; Leshinsky-Silver et al., 2009; Calvo et al., 2010]. La deficiencia del complejo III también se había reportado en algunos pacientes con mutaciones de *NDUFS4*. Curiosamente, nuestro paciente asociaba una deficiencia secundaria de la PDH y de la CoQ, que no habían sido previamente reportadas en pacientes con mutaciones *NDUFS4*. La deficiencia secundaria de CoQ sí se había informado en otras enfermedades mitocondriales (MELAS, Kearns-Sayre) y en otras enfermedades neurológicas (aciduria glutárica tipo I, síndrome de apraxia ataxia-oculomotora-1) [Yubero et al., 2015]. El complejo I está estrechamente relacionado con la CoQ en la mitocondria, por lo tanto, el defecto del complejo I puede conducir a una deficiencia secundaria de CoQ, tal y como se ha visto en otros defectos mitocondriales. Por otro lado, la combinación de la deficiencia de PDH y de uno o varios complejos mitocondriales se ha descrito en pacientes con defectos de la biosíntesis mitocondrial de clúster de hierro y azufre (Fe-S) (*NFU1*, *BOLA3*) [Navarro-Sastre et al., 2011; Cameron et al., 2011; Ahting et al., 2015], en defectos del complejo I debido a mutación de *NDUFS2* [Tuppen et al., 2010] y en los defectos del metabolismo de la valina debido a mutaciones de *HIBCH* [Ferdinandusse et al., 2013].

El análisis BN-PAGE de nuestra paciente mostró una alteración del ensamblaje del complejo I. Estudios previos en otros pacientes con mutaciones de *NDUFS4* han encontrado una ausencia total del complejo I ensamblado, con acumulación de sub-complejos de 830-kDa en fibroblastos de pacientes con mutación de *NDUFS4* [Ugalde et al, 2004; Assouline et al., 2012.; Iuso et al., 2006; Leshinsky-Silver et al., 2009; Leong et al, 2012.; Breuer et al., 2013].

Los hallazgos bioquímicos iniciales en tejido muscular de deficiencias parciales combinadas de los complejos I, III, PDH y CoQ hacían afanoso el análisis completo del gran número de genes probablemente causante de todas estas alteraciones. En este contexto, el análisis genético por NGS ha dado como resultado la mutación c.291delG (p.Trp97Ter) en homocigosis del gen *NDUFS4*. Esta mutación previamente descrita con efecto fundador en poblaciones del norte de África (Argelia, Marruecos) ha demostrado producir una proteína truncada con pérdida del sitio de fosforilación dependiente de cAMP [Assouline et al., 2012].

Bioquímicamente, en nuestro paciente se observó un defecto combinado en la actividad de la PDH, de la CoQ y de los complejos I y III. Estas deficiencias combinadas están dadas muy probablemente a un ensamblaje inadecuado del complejo I, tal cual se confirmó con el análisis de BN-PAGE.

Conclusiones

Los pacientes con defectos del transporte y metabolismo de tiamina se presentan más frecuentemente en la primera década de la vida, con lesiones agudas en ganglios basales, córtex cerebral, tronco y cerebelo, junto con episodios de encefalopatía recurrente que pueden estar desencadenados por procesos intercurrentes, cumpliendo los criterios diagnósticos de Síndrome de Leigh.

La suplementación con tiamina tiene un fuerte impacto en el pronóstico de los pacientes con mutaciones del gen *SLC19A3*, conduciendo a una estabilidad metabólica, a la reducción de la discapacidad y mejoría de la curva de supervivencias comparados con pacientes no tratados. Entre los factores pronósticos hemos identificado un subgrupo de pacientes *SLC19A3* homocigotos c.1264A>G con mejor pronóstico. Opuestamente, aquellos con lesiones cerebrales difusas que se extienden a tronco cerebral y núcleo pálido tienen peor pronóstico y supervivencia. Globalmente, los pacientes con defectos en SLC19A3 tienen un mejor pronóstico que otras causas de síndrome de Leigh, alentando a los médicos a sospechar la enfermedad con el fin de realizar un diagnóstico temprano y un tratamiento oportuno.

Los pacientes con variantes en el gen *SLC19A3*, que son el principal defecto genético tratado en esta tesis, presentan un déficit severo de tiamina-libre en LCR y fibroblastos. La suplementación con tiamina restaura los niveles intracelulares y en LCR, probablemente a través del transporte alternativo de la vitamina. Esto conlleva a una respuesta clínica dramática que además es sostenida, siendo el beneficio mayor cuando la suplementación se ha iniciado de forma temprana. Este trabajo también demuestra que la cuantificación de tiamina por el método de HPLC en muestras de sangre entera es un método útil para la evaluación de la adherencia al tratamiento de estos pacientes.

Estudios futuros

- Desarrollar un registro internacional de pacientes con defectos del transporte y metabolismo de tiamina. El objetivo de este registro será el de conocer el estado preciso de la patología y comprobar que los hallazgos de la historia natural sean similares a los hallazgos de esta tesis.

- La administración de suplementos de tiamina es un tratamiento de por vida. Por lo tanto, es necesario establecer la dosis segura de tiamina capaz de permitir el desarrollo normal del paciente y la prevención de más descompensaciones.

- Promoción de la inclusión de tiamina y biotina en los protocolos de tratamiento para los niños que presentan encefalopatía aguda y síndrome de Leigh.

- Análisis de isoformas de tiamina en un mayor número de muestras de LCR para estudiar otras causas de deficiencias secundarias y establecer con más precisión la especificidad y sensibilidad de este biomarcador en pacientes con deficiencia de hTHTR2.

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