# Marcadores genéticos y de neuroimagen en el Trastorno obsesivo-compulsivo de inicio en la infancia y la adolescencia

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# Marcadores genéticos y de neuroimagen en el Trastorno obsesivo-compulsivo de inicio en la infancia y la adolescencia

Tesis presentada por

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# PRÓLOGO

El presente trabajo de tesis doctoral contiene las siguientes publicaciones científicas:

**Estudio 1**: **Clinical significance of psychiatric comorbidity in child and adolescents with obsessive-compulsive disorder: Subtyping a complex disorder**

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**Estudio 4**: **Association between Genetic Variants of Serotonergic and Glutamatergic Pathways and the Concentration of Neurometabolites of the Anterior Cingulate Cortex in Pediatric Patients with Obsessive-Compulsive Disorder**

Autores: **Ortiz AE**, Gassó P, Mas S, Falcon C, Bargalló N, Lafuente A, Lázaro L.

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1. Social anxiety and early life events in university students (2012). Binelli C, **Ortiz A**, Muñiz A, Gelabert E, Ferranz L, Filho AS, Crippa JA, Nardi AE, Subirà S, Martín- Santos R. Rev Bras Psiquiatr. 34 Supl 1: S 69-71.

2. Effectiveness of long-acting injectable risperidone versus oral antipsychotics in the treatment of recent-onset schizophrenia: a case-control study (2013). Barrio P, Batalla A, Castellví P, Hidalgo D, Garcia M, **Ortiz A**, Grande I, Pons A, Parellada E. Int Clin Psychopharmacol. 28 (4): 164-170.

3. New evidence of heterogeneity in social anxiety disorder: defining two distinct personality profiles taking into account clinical, environmental and genetic riskfactors (2014). Binelli C, Muñiz A, Sanches S, **Ortiz A**, Udina M, Batalla A, López- Solà C, Crippa JA, Martín-Santos R. European Psychiatry 30 (1) 60-65.

4. White matter structural alterations in pediatric obsessive–compulsive disorder: Relation to symptom dimensions (2014). Lázaro L, Ortiz AG, Calvo A, **Ortiz AE**, Moreno E, Morer A, Calvo R, Bargalló. Progress in Neuro-Psychopharmacology & Biological Psychiatry, 54: 249-258.

5. Microstructural Brain Abnormalities and symptom dimensions in child and adolescent patients with obsessive-compulsive disorder: A diffusion tensor imaging study (2014). Lázaro L, Ortiz AG, Calvo A, **Ortiz AE**, Moreno E, Morer A, Calvo R, Bargalló N. Depression and Anxiety, 31 (12): 1007-1017.

6. Association between genetic variants related to glutamatergic, dopaminergic and neurodevelopment pathways and white matter microstructure in pediatric patients with obsessive-compulsive disorder. Gassó P, **Ortiz AE**, Mas S, Morer A, Calvo A, Bargalló N, Lafuente A, Lázaro L, under review.

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## LISTADO DE ACRÓNIMOS

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|---|---|
| **ADN** | Ácido desoxiribonucleico |
| **AMPA-R** | Receptor del glutamato ionotrópico ácido alfa amino-3-hidroxi-5-metil-4-isoxazolepropionico |
| **BDNF** | Factor neurotrófico derivado del cerebro |
| **CCA** | Córtex cingulado anterior |
| **CDI** | Inventario de depresión infantil |
| **Cho** | Componentes de la colina |
| **CETC** | Circuito córtico-estriado-tálamo-cortical |
| **COF** | Córtex órbito-frontal |
| ***COMT*** | Gen de la catecol-O-metiltransferasa |
| **Cr** | Creatina |
| **CY-BOCS** | Escala de Síntomas Obsesivo-Compulsivos Yale-Brown para niños |
| **DE** | Desviación estandard |
| ***DRD2*** | Gen del receptor de la dopamina D2 |
| ***DRD3*** | Gen del receptor de la dopamina D3 |
| ***DRD4*** | Gen del receptor de la dopamina D4 |
| **DSM-IV** | Diagnostic and Statistical Manual of Mental Disorders, 4th Edition |
| **DSM-5** | Diagnostic and Statistical Manual of Mental Disorders, 5th Edition |
| **DTI** | Imágenes tensor de difusión |
| **FA** | Fracción de anisotropía |
| **GABA** | Ácido gamma-aminobutírico |
| ***GAD2*** | Gen del glutamato descarboxilasa 2 |
| **Glx** | Glutamato+Glutamina |
| ***GRIA1*** | Gen del receptor ionotrópico de glutamato AMPA1 |
| ***GRIA3*** | Gen del receptor ionotrópico de glutamato AMPA3 |
| ***GRIK2*** | Gen del receptor del glutamato ionotrópico de tipo kainato |
| ***GRIN2B*** | Gen de la subunidad 2B del receptor del glutamato |

| | |
|---|---|
| ***HTR1B*** | Gen del receptor de la serotonina 1B |
| ***HTR2A*** | Gen del receptor de la serotonina 2A |
| **Ins** | Inositol |
| **ISRS** | Inhibidores Selectivos de la Recaptación de Serotonina |
| **IRS** | Inhibidor de la Recaptación de Serotonina |
| **K-SADS-PL** | The Schedule for Affective Disorders and Schizophrenia for School-age Children-Present and Life Time Version |
| **LCR** | Liquido cefalorraquídeo |
| **M** | Media artimética |
| ***MAOA*** | Gen de la mono-amino oxidasa A |
| **N** | Número de pacientes |
| **NAA** | N-acetil-aspartato |
| **OCI-V** | Inventario de Obsesiones y Compulsiones Versión niños |
| **RMN** | Resonancia magnética cerebral |
| **RMS** | Resonancia magnética espectroscópica |
| **SB** | Sustancia blanca |
| **SCARED** | Screen for Child Anxiety Related Emotional Disorders |
| ***SCL1A1*** | Gen del transportador del glutamato (de *solute carrier family 1*) |
| ***SCL18A1*** | Gen del transportador vesicular de monoaminas |
| ***SCL6A3*** | Gen del transportador de la dopamina (de *solute carrier family 6, miembro 3*) |
| ***SCL6A4*** | Gen del transportador de la serotonina (de *solute carrier family 6, miembro 4*) |
| **SNPs** | Polimorfismo de nucleótido único |
| **SG** | Sustancia gris |
| **TAG** | Trastorno de Ansiedad Generalizada |
| **TCC** | Terapia Cognitivo-Conductual |
| **TDAH** | Trastorno por Déficit de Atención e Hiperactividad |
| **TEA** | Trastorno Espectro Autista |

| | |
|---|---|
| **TOC** | Trastorno Obsesivo-Compulsivo |
| ***TPH2*** | Gen del triptófano hidroxilasa 2 |
| **TT** | Trastorno de Tourette |
| **VBM** | Voxel based morphometry |
| **VOI** | Volumen de interés |
| **Y-BOCS** | Escala de Síntomas Obsesivo-Compulsivos Yale-Brown |
| **$^{1}$H-RMS** | Resonancia magnética espectroscópica protón de hidrógeno |

# INTRODUCCIÓN

# 1. INTRODUCCIÓN

## 1.1. Epidemiología y características clínicas del TOC de inicio en la infancia y la adolescencia

El trastorno obsesivo-compulsivo (TOC) es un trastorno psiquiátrico caracterizado por obsesiones, definidas como pensamientos intrusivos, repetitivos, angustiantes, a menudo incontrolables, y compulsiones, definidas como la necesidad de involucrarse en conductas repetitivas que requieren mucho tiempo, estando vigentes para reducir, neutralizar o prevenir la angustia, experiencias o eventos temidos (APA, 2013).

Recientemente, los criterios diagnósticos han sido actualizados en el Manual diagnóstico del DSM-5 (APA, 2013) (pueden ser consultados en la tabla 1). Se ha propuesto un grupo denominado Trastorno obsesivo-compulsivo y otros trastornos afines que incluye además del TOC, el trastorno dismórfico corporal, el trastorno por acumulación, la tricotilomanía, el trastorno por escoriación, el TOC y otros trastornos relacionados inducidos por sustancias, el TOC y otros trastornos relacionados atribuibles a una enfermedad médica, y el TOC y otros trastornos relacionados no especificados. Así, en esta nueva edición, el TOC no ha sido incluido dentro de los Trastornos de ansiedad. Como aspectos favorables a la inclusión del TOC en el grupo de trastornos de ansiedad se apuntaban: la frecuente comorbilidad con otros trastornos de ansiedad, la frecuente asociación a síntomas ansiosos sin conformar un trastorno, y la similar respuesta del TOC y los trastornos de ansiedad a la terapia cognitivo conductual (TCC) y a los inhibidores selectivos de la recaptación de la serotonina (ISRS). Como aspectos en contra mencionar que el TOC y los trastornos relacionados no siempre se acompañan de síntomas ansiosos, que los hallazgos neurobiológicos indican la implicación de otras vías cerebrales además de las causantes de la ansiedad, y la existencia de subpoblaciones que tienen alta morbilidad con otros trastornos del neurodesarrollo como el trastorno por déficit de atención con hiperactividad (TDAH), el trastorno de Tourette (TT) y otros trastornos por tics, y los trastornos del espectro autista (TEA).

Tabla 1. Criterios para el diagnóstico del TOC según la versión actual del DSM-5

**TRASTORNO OBSESIVO-COMPULSIVO**

A. Presencia de obsesiones, compulsiones o ambas

Las obsesiones se definen como (1) y (2)

1. Pensamientos, impulsos o imágenes recurrentes y persistentes que se experimentan en algún momento del trastorno como intrusivos, inapropiados y que causan en la mayoría de individuos ansiedad o malestar.

2. La persona intenta ignorar o suprimir estos pensamientos, impulsos o imágenes, o bien intenta neutralizarlos mediante otros pensamientos o actos.

Las compulsiones se definen como (1) y (2):

1. Comportamientos repetitivos (p.e., lavado de manos, puesta en orden de objetos, comprobaciones) o actos mentales (p.e., rezar, contar o repetir palabras en silencio) que el individuo se ve obligado a realizar en respuesta a una obsesión o con arreglo a ciertas reglas que debe seguir estrictamente.

2. Los comportamientos o actos mentales tienen como objetivo la prevención o reducción de la ansiedad o el malestar, o la prevención de algún acontecimiento o situación temida; sin embargo, estos comportamientos o actos mentales o bien no están conectados de forma realista con aquello que pretenden neutralizar o prevenir, o bien resultan claramente excesivos.

Nota: Los niños pequeños pueden no ser capaces de articular el objetivo de estos comportamientos o actos mentales

B. Las obsesiones o compulsiones representan una pérdida de tiempo (p.e., suponen más de 1 hora por día) o causan malestar clínico significativo, o interfieren en el funcionamiento social o laboral, u otra área importante de funcionamiento

C. Los síntomas obsesivo-compulsivos no son atribuibles a los efectos fisiológicos de una sustancia (p.e., drogas o fármacos) u otra enfermedad médica

D. El trastorno no se explica mejor como síntomas de otro trastorno mental (p.e., preocupaciones excesivas en un trastorno de ansiedad generalizada; preocupación por la apariencia en un trastorno dismórfico corporal; dificultad para deshacerse o separarse de posesiones, como en un trastorno de acumulación; tirar del pelo como en la tricotilomanía; esterotipias, como en un trastorno por movimientos estereotipados; comportamiento alimentario ritualizado, como en un trastorno de la conducta alimentaria; preocupación por sustancias o juego como en un trastorno adictivo o un trastorno relacionado con substancias; preocupación por tener una enfermedad como en la hipocondría; fantasías o urgencias sexuales, como en los trastornos parafílicos; impulsos como en un trastorno perturbador del control de impulsos o un trastorno de conducta; rumiaciones de culpa como en un trastorno depresivo mayor; inserción de pensamientos o preocupaciones delirantes como en el espectro de la esquizofrenia u otros trastornos psicóticos; o patrones repetitivos del comportamiento como en un trastorno del espectro autista)

Especificar si:

Con buena conciencia de enfermedad: La persona reconoce que las creencias del trastorno obsesivo-compulsivo son definitivamente o probablemente no ciertas o que pueden o no pueden ser ciertas.

Con escasa conciencia de enfermedad: La persona piensa que las creencias del trastorno obsesivo-compulsivo son probablemente ciertas.

Sin conciencia de enfermedad/creencias delirantes: La persona está completamente convencida de que las creencias del trastorno obsesivo-compulsivo son ciertas.

Especificar si:

Está relacionado con tics: La persona tiene historia pasada o actual de trastorno por tics.

Fuente: American Psychiatric Association (2013).

Durante mucho tiempo, se creía que el trastorno era poco frecuente en niños y adolescentes, sin embargo su prevalencia oscila entre el 1% y el 3% (Fontenelle et al., 2006), similar a la descrita en estudios realizados en edad adulta. En un estudio epidemiológico en nuestro país con una muestra comunitaria de 1.514 niños, la prevalencia estimada fue del 1.8% para el TOC, el 5.5% para el TOC subclínico y el 4.7% para la sintomatología obsesiva-compulsiva (Canals et al., 2012). Este trastorno a menudo se inicia en la infancia y en la adolescencia y puede convertirse en crónico debido a las altas tasas de persistencia (Micali et al., 2010). Aproximadamente, el 40% de los casos que comienzan en la niñez continúan en la edad adulta, y el número aumenta al 60% cuando se consideran presentaciones subclínicas (Stewart et al., 2004).

La edad media de inicio del TOC en los adultos se establece alrededor de los 19,5 años (Ruscio et al., 2010), mientras que la edad media de inicio en personas jóvenes se establece sobre los 11,5 años (Bryńska and Wolańczyk, 2005). Diferentes autores proponen una distribución bimodal según la edad de inicio del TOC (Delorme et al., 2005; Geller et al., 1998; Swedo et al., 1989a), sugiriendo la existencia de diferencias en la distribución por sexo, la agregabilidad familiar o el patrón de comorbilidad entre el TOC de inicio temprano y el de inicio tardío.

A pesar de que el TOC constituye una entidad nosológica unitaria, no hay duda de que es un trastorno muy heterogéneo desde el punto de vista fenomenológico, presentando una elevada variabilidad en la expresión clínica inter e intra-paciente. Así, dos pacientes con el mismo diagnóstico pueden exhibir patrones sintomáticos completamente diferentes y, aunque está descrita cierta estabilidad sintomática en el mismo paciente, los síntomas pueden variar claramente a lo largo de su vida (Leckman et al., 1997; Mataix-Cols et al., 2005, 2002; Rettew et al., 1992). Leckman y colaboradores llevaron a cabo un análisis factorial de las obsesiones y compulsiones presentadas en un grupo numeroso de pacientes con TOC de distintas edades,

concluyendo en la existencia de 4 factores (Leckman et al., 1997) (tabla 2). Con posterioridad se han realizado otros estudios factoriales, habiendo sido todos agrupados en un meta-análisis en el que se incluyeron más de 5.000 pacientes: niños, adolescentes y adultos, donde se demuestra la robustez de la estructura factorial de los síntomas obsesivo-compulsivos a lo largo de la vida (Bloch et al., 2008). Las únicas diferencias entre las estructuras factoriales que afectan a adultos y niños son que las compulsiones de comprobación se agrupan fundamentalmente en el factor de pensamientos prohibidos en el adulto, y en el factor de simetría en el niño, mientras que las obsesiones somáticas se agrupan fundamentalmente en el factor de pensamientos prohibidos en el adulto y en el factor de limpieza en niños, apuntándose que podría ser debido a la ambigüedad de la categoría de comprobación de la Escala de Síntomas Obsesivo-Compulsivos Yale-Brown (*Y-BOCS Yale-Brown Obsessive-Compulsive Scale*). Algunos de estos estudios han demostrado que estas dimensiones sintomáticas se mantienen estables a lo largo del tiempo tanto en adultos como en niños y adolescentes.

Tabla 2. Dimensiones o factores del TOC

| |
|---|
| **Factor I**: obsesiones agresivas, sexuales y religiosas y compulsiones de comprobación; |
| **Factor II**: obsesiones de simetría y compulsiones de repetición, contaje y orden; |
| **Factor III**: obsesiones de contaminación y compulsiones de limpieza; |
| **Factor IV**: obsesiones y compulsiones de acumulación. |

Finalmente, conviene remarcar que estudios recientes con técnicas de neuroimagen y técnicas de análisis genético han proporcionado pruebas de la validez biológica de estas dimensiones, sugiriendo que estas dimensiones son útiles en el esfuerzo para entender la historia natural de la enfermedad, la genética, la neurobiología, la respuesta al tratamiento y la evolución del TOC.

Como se ha apuntado previamente el TOC es un trastorno heterogéneo con posibles diferencias fenotípicas, por este motivo, es de especial interés identificar subtipos más homogéneos. Dos de los subtipos potenciales de este trastorno propuestos para su inclusión en el DSM-5, han sido el TOC de inicio precoz y el TOC relacionado con tics (Leckman et al., 2009). Finalmente, el único subtipo reconocido en el texto es la existencia o no de historia actual o pasada de tics (Leckman et al., 2010). Además, el grupo de trabajo del DSM-5 concluyó que los subtipos de acuerdo a las dimensiones sintomáticas obsesivo-compulsivas no eran necesarias para diagnosticar el trastorno (Thomsen, 2013), pero este enfoque de rasgos fenotípicos tiene la potencialidad de avanzar en nuestra comprensión en el trastorno pudiendo ayudar en la identificación de endofenotipos más robustos (Leckman et al., 2009). Por otra parte, la investigación ha destacado la importancia de identificar subtipos de TOC homogéneos basados en características clínicas como edad de inicio, sexo y comorbilidad (Mathis et al., 2011; Miguel et al., 2005; Taylor, 2011).

El TOC de inicio temprano es un fenotipo diferenciado que podría constituir un subtipo de trastorno del neurodesarrollo (Geller et al., 2001; Rosenberg and Keshavan, 1998) además de representar un subtipo más grave de la enfermedad (Rosario-Campos et al., 2001). Pero no existe un claro acuerdo entre los investigadores del TOC infantil acerca de la edad que lo delimitaría. Se han realizado estudios en los que se utiliza para su definición el inicio del trastorno antes de los 15 años, antes de los 17 y antes de los 18 años. Incluso se ha establecido el punto de corte de 10 años para hablar de un inicio muy temprano (*very early onset*) de la

enfermedad cuando ocurre antes de esta edad. El TOC de inicio infantil difiere en distintos aspectos del de inicio en la edad adulta, destacando que el primero es más prevalente en el sexo masculino, tiene una elevada agregabilidad familiar y está asociado con una mayor prevalencia de trastornos por tics, tanto en los propios pacientes con TOC, como en sus familiares de primer grado. Los niños y adolescentes tienen algunas características clínicas específicas de su edad. Así, les puede ser difícil definir el objetivo de los comportamientos o actos mentales, siendo más probable que tengan más compulsiones que obsesiones, ocurriendo este hecho tanto más frecuentemente cuanto más pequeños son los niños. También es menos probable que reconozcan sus síntomas como egodistónicos, resistiéndose menos a la urgencia en la ejecución de la conducta compulsiva.

Aunque la agregación familiar es mayor en los pacientes que han comenzado el TOC en la primera década de la vida, los estudios familiares nos demuestran que el TOC de inicio infantil es etiológicamente heterogéneo pudiendo aparecer un subtipo “relacionado con tics”, otro familiar “no relacionado con tics”, así como casos esporádicos sin historia familiar evidente (Leckman et al., 2009). El tener un familiar de primer grado afecto de TOC es un factor de riesgo para padecer un TOC o un TOC subclínico. Muchos de estos niños padecen pensamientos obsesivos centrados en la seguridad de los miembros cercanos de la familia así como compulsiones de limpieza y contaminación. A diferencia del TOC de inicio en la infancia relacionado con tics, en este grupo se observa comorbilidad con trastornos afectivos y de ansiedad tanto en el paciente como en familiares de primer grado. Trastornos como el trastorno de ansiedad generalizada (TAG), el trastorno de pánico, la agorafobia, el trastorno por ansiedad de separación y el trastorno depresivo mayor son frecuentemente comórbidos si hay un familiar de primer grado diagnosticado de TOC. También parece ser que una porción de estos casos remiten antes de la edad adulta.

El TOC relacionado con tics se diagnostica en individuos con historia a lo largo de la vida de trastorno de tics crónicos. Este subtipo puede aparecer en un 10 a un 40% de los casos de inicio infantil, siendo altamente familiar, y se observan los tics tanto en el menor afectado como en uno o más de los familiares de primer grado (Grados et al., 2001). Estos pacientes, predominantemente varones, tienen síntomas obsesivo-compulsivos fundamentalmente de simetría, pensamientos prohibidos y almacenamiento, y por el contrario escasos síntomas de la dimensión de limpieza. Además, es mucho más probable que refieran la existencia de fenómenos sensoriales. Los fenómenos sensoriales incluyen las sensaciones, percepciones, sentimientos o urgencias inconfortables o inquietantes que pueden preceder o acompañar a conductas repetitivas como compulsiones o tics. De esta forma, los pacientes con TOC pueden repetir las compulsiones hasta que experimenten una sensación de alivio para estas sensaciones desagradables o aversivas, y puedan sentir que "ya está bien" (*just right*). Ejemplos de estos fenómenos son sensaciones cutáneas o músculo-esqueléticas, percepciones asociadas con estímulos visuales, táctiles o auditivos y sentimientos de "incompletud". Los fenómenos sensoriales pueden causar más malestar que las propias compulsiones. La evolución de este subtipo puede ser diferente al resto, caracterizándose por un pico inicial de gravedad sobre los 12 años de edad seguido de un incremento en la probabilidad de remisión.

La comorbilidad del TOC y los trastornos relacionados con tics, básicamente con el TT, ha sido ampliamente reconocido en la literatura clínica y parece ser bidireccional, afectando a un 20-30% de los individuos con cada trastorno (Franklin et al., 2012). Estas presentaciones clínicas comparten características que incluyen el inicio temprano, ocurrencia familiar y un curso crónico fluctuante (Coffey et al., 1998), comportamientos repetitivos, pensamientos intrusivos y déficit en la inhibición conductual (Lewin et al., 2010).

Además de la comorbilidad de los trastornos relacionados con tics, el TOC también presenta comorbilidad con otros trastornos del neurodesarrollo como el TDAH. Alrededor del

25-30% de los niños y adolescentes con TOC, en particular los niños con un inicio temprano, también cumple criterios diagnósticos para el TDAH (Geller et al., 1996; Masi et al., 2010), mientras que la tasa de TOC en los niños con TDAH se estima en alrededor del 12% (Sheppard et al., 2010). Con frecuencia algunos pacientes con TOC pueden presentar ambas comorbilidades, TT u otros trastornos por tics y TDAH (Cavanna et al., 2009; Lebowitz et al., 2012; Wanderer et al., 2012).

Otro grupo de trastornos con una alta comorbilidad con TOC son los trastornos de ansiedad y los trastornos depresivos. Entre un tercio y la mitad de los niños con TOC sufren o han sufrido un trastorno de ansiedad durante su evolución (Ivarsson et al., 2008; Langley et al., 2010). Los estudios sugieren que algunos trastornos de ansiedad, especialmente el TAG, son relativamente comunes en personas con TOC y sus familiares de primer grado (Bienvenu et al., 2012; Nestadt et al., 2001). Por lo que se refiere a los trastornos depresivos, las tasas descritas de niños y adolescentes con TOC que presentan depresión comórbida son muy variables, desde un 16% de los niños y adolescentes (Storch et al., 2012), hasta una prevalencia incluso del 73% (Ivarsson et al., 2008). Sin embargo, los estudios de seguimiento indican que este trastorno se presenta con mayor frecuencia en el curso del trastorno, y es uno de los diagnósticos comórbidos más frecuentes al llegar a la edad adulta (Denys et al., 2004b).

### 1.2. Correlatos neurobiológicos del TOC. Hallazgos en neuroimagen

Los modelos neurobiológicos del TOC intentan explicar la etiopatogenia del trastorno desde diferentes vertientes. Las primeras hipótesis neuroquímicas del trastorno defendían la existencia de una disfunción serotoninérgica primaria basándose en la eficacia antiobsesiva que mostraban los fármacos antidepresivos con potente acción serotoninérgica (Greist et al., 1995; Insel et al., 1982), así como el agravamiento sintomático que podía ser observado cuando eran administrados antagonistas serotoninérgicos a pacientes que permanecían

previamente en remisión clínica (Benkelfat et al., 1989). Pero a pesar de que los agentes serotoninérgicos son clara y diferencialmente eficaces en el tratamiento del TOC, no existen datos concluyentes que apoyen una disfunción serotoninérgica de base, dado que estos fármacos podrían estar compensando alteraciones primarias en otros sistemas de neurotransmisión.

Desde el punto de vista neuroanatómico, estudios de meta-análisis realizados con resonancia magnética estructural cerebral global utilizando técnicas *voxel-based morphometry* (VBM) han puesto de manifiesto que en comparación con sujetos control sanos, los pacientes con TOC presentan: una disminución regional bilateral de volumen de sustancia gris (SG) en la corteza órbitofrontal (COF), la corteza prefrontal dorsolateral y del córtex cingulado anterior (CCA); un aumento regional bilateral del volumen de SG en el núcleo lenticular que se extiende hacia el núcleo caudado, así y como en el córtex prefrontal anterior (Radua and Mataix-Cols, 2009; Rotge et al., 2010); y un aumento del volumen de la cápsula interna y reducción de la sustancia blanca (SB) a nivel frontal y parietal (Piras et al., 2013b). Pero los estudios de resonancia magnética estructural que comparan niños y adolescentes con TOC versus sujetos control sanos muestran anomalías de forma inconsistente. En cuanto a la SG, se ha encontrado un aumento del volumen en el COF, CCA, putamen (Szeszko et al., 2008), y tálamo (Gilbert et al., 2000), mientras que se ha observado una disminución del volumen en la circunvolución frontal superior y medial (Gilbert et al., 2008), el globo pálido (Szeszko et al., 2004), y la corteza parietal (Carmona et al., 2007; Lázaro et al., 2009). En cuanto a la SB, se han descrito aumentos de volumen en zonas órbitofrontales (Macmaster et al., 2010) y un menor volumen en lóbulo frontal (Carmona et al., 2007) y lóbulos parietales (Lázaro et al., 2009). Un estudio longitudinal de niños y adolescentes con TOC en comparación con sujetos control sanos, halló una reducción del volumen de SG y SB en regiones parietales en el caso de los pacientes, normalizándose este volumen después de seis meses de tratamiento farmacológico y mejoría clínica (Lázaro et al., 2009). Otro estudio más reciente realizado en 29 pacientes pediátricos

con TOC que no realizaban tratamiento farmacológico en el momento de la evaluación y 29 controles sanos encontró que la SG del COF y la SB de la cápsula externa aumentaron después de 16 semanas de TCC (Huyser et al., 2013). Estos cambios observados persistieron en el seguimiento a dos años (Huyser et al., 2014).

Otra técnica de neuroimagen estructural utilizada para examinar la microestructura de la SB en pacientes con TOC mediante la cuantificación de la direccionalidad y la coherencia de la auto-difusión del agua es la técnica de Imagen de Tensor Difusión (DTI). Una revisión sistemática de estudios con esta técnica, que implica sobre todo pacientes adultos con TOC (Piras et al., 2013a), concluyó que, además del circuito fronto-estriatal, también están involucradas en la fisiopatología del TOC otras regiones de SB. En particular, se han descrito alteraciones de la microestructura de la SB en el cíngulo y en la parte anterior y posterior de la extremidad de la cápsula interna (Fontenelle et al., 2011; Li et al., 2011), además de en el cuerpo calloso; regiones prefrontales; y áreas parietales, temporales y occipitales (Piras et al., 2013a). En pacientes pediátricos con TOC se han realizado cinco estudios con DTI que informan de alteración en las medidas de difusión en varios en varios tractos de SB, en los fascículos longitudinales inferior y superior, los tractos corticoespinales, el córtex cingulado izquierdo, el fascículo uncinado derecho, el cuerpo calloso y la SB de cerebelo y del tronco cerebral (Zarei et al., 2011). Otros autores han referido hallazgos similares, con mayor fracción de anisotropía (FA) en el córtex cingulado izquierdo, el cuerpo calloso, el tracto corticoespinal derecho, y el fascículo longitudinal inferior izquierdo (Gruner et al., 2012; Jayarajan et al., 2012; Lázaro et al., 2014a; Silk et al., 2013; Zarei et al., 2011).

En referencia a estudios funcionales cerebrales, inicialmente fueron realizados numerosos estudios utilizando la Tomografía por Emisión de Positrones (PET) o por emisión de fotones (SPECT), describiéndose de forma consistente un aumento en el metabolismo de la glucosa y del flujo sanguíneo cerebral, basal pre-tratamiento y post-tratamiento, durante la

realización de tareas cognitivas o durante la provocación de síntomas, en una o más de las siguientes regiones: COF, CCA, caudado y el tálamo (Baxter et al., 1987; Benkelfat et al., 1990; Saxena et al., 1999; Swedo et al., 1989b).

Así, varios estudios de investigación con neuroimagen funcional han hallado resultados que son en gran medida coincidentes con resultados de resonancia magnética nuclear (RMN) estructural, como es la alteración de la activación regional, durante la realización de tareas cognitivas y después de la provocación de síntomas, de las estructuras cerebrales mencionadas previamente (Brem et al., 2012; Del Casale et al., 2011; Rotge et al., 2008). En 2008, se realizó uno de los primeros estudios en pacientes con TOC de edad infanto-juvenil, observándose que en comparación con los controles, los pacientes con TOC presentaban, durante la realización de una actividad motora, una activación cerebral significativamente más alta de forma bilateral en la circunvolución frontal media y que después de 6 meses de tratamiento farmacológico y una clara mejoría clínica, la activación en la ínsula izquierda y el putamen izquierdo disminuían significativamente (Lázaro et al., 2008). Un estudio que realizaba una provocación experimental de síntomas encontró que los niños y adolescentes con TOC presentaban una actividad reducida en regiones neurales subyacentes al procesamiento emocional y cognitivo y del rendimiento motor, comparado con sujetos controles sanos (Gilbert et al., 2009). Además, se encontró una menor activación en amígdala/hipocampo en respuesta a un paradigma de reconocimiento facial en pacientes de edad infantil y adolescente con TOC comparado con controles (Britton et al., 2010).

Todos estos resultados han contribuido a que sea ampliamente aceptado el modelo neuroanatómico del TOC que postula la existencia de alteraciones del circuito córtico-estriado-tálamo-cortical.

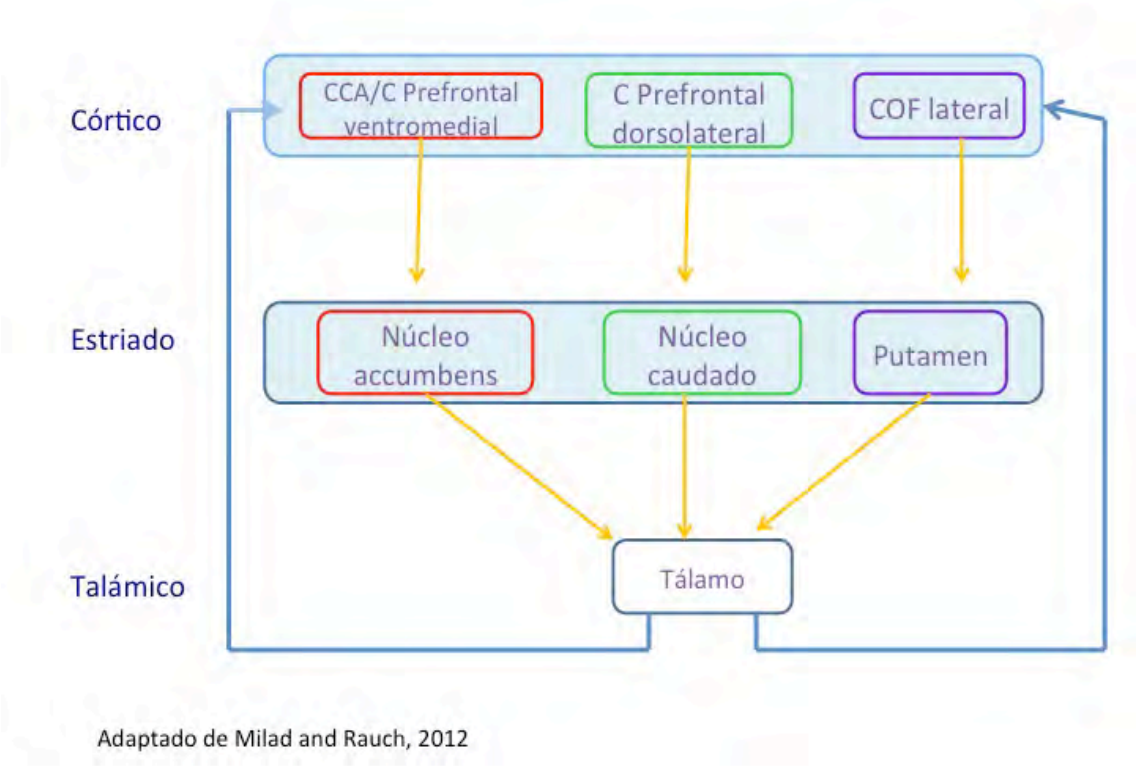
### 1.2.1 El circuito córtico-estriado-tálamo-cortical

El modelo neurobiológico del TOC radica en la sospecha de que la sintomatología se origina y se mantiene en varias áreas del cerebro que están interconectadas entre sí formando el circuito córtico-estriado-talámico-cortical (CETC) (Saxena and Rauch, 2000). La COF está implicada en funciones afectivas y motivacionales, en el control de los impulsos y en las repuestas de inhibición y regulación de la conducta. El estriado, además de regular las sensaciones y modular las funciones motoras, está implicado en las funciones afectivas y cognitivas. El caudado funciona como un filtro que únicamente deja pasar los estímulos relevantes a un nivel más consciente para que sean ejecutados. De este modo, el estriado regula el contenido de la información y facilita la calidad del procesamiento de forma inconsciente, reduciendo la carga de los sistemas de procesamiento conscientes. El tálamo recibe los impulsos que éste ha filtrado, los envía a la corteza frontal y envía el mensaje de que algún estímulo precisa una respuesta. Desde estas áreas cerebrales se organizan varios circuitos córtico-estriatales de diferente complejidad. Éstos son los que median en el aprendizaje implícito o inconsciente de procedimientos u actividades estereotipadas seriadas y estarían implicados en la patología obsesivo-compulsiva. La hipótesis planteada postularía que los circuitos de retroalimentación positiva entre la corteza y el tálamo podrían mediar los pensamientos circulares y repetitivos, mientras que el estriado mediaría los patrones de actividad fija en forma de conductas repetitivas o compulsiones.

La hiperactividad encontrada en estas áreas cerebrales puede ser explicada tanto por el modelo basado en una hiperactividad del caudado insuficiente para inhibir el tálamo como en que la hiperactividad del caudado actúe directamente excitando al tálamo. Es decir, por diferentes causas, la función del caudado estaría alterada, lo que permitiría que muchas sensaciones y pensamientos se hicieran conscientes, cuando normalmente estas ideas se suprimirían sin ningún esfuerzo consciente. Como estos pensamientos no son los que

habitualmente se tienen, se perciben como inapropiados y molestos y es el esfuerzo inútil por controlarlos lo que generaría el estado de ansiedad característico de la mayoría de los pacientes con TOC.

Figura 1. Circuito córtico-estriado-talámico-cortical




Adaptado de Milad and Rauch, 2012

Así, según este modelo existiría una modulación aberrante de varios de los subcircuitos que conectan las regiones corticales y subcorticales, cómo el córtex prefrontal, el córtex cingulado anterior, el cuerpo estriado, el globo pálido y el tálamo (Abramowitz et al., 2009; Baxter, 1992; Brennan et al., 2013; Milad and Rauch, 2012), existiendo una hiperactivación de este circuito que se atenúa con un tratamiento eficaz (Baxter, 1992).

### 1.2.2 La técnica de la resonancia magnética espectroscópica

La resonancia magnética espectroscópica de protón hidrógeno ($^{1}$H-RMS) permite la cuantificación in vivo de sustancias neuroquímicas específicas en varias regiones del cerebro. La $^{1}$H-RMS registra una serie de picos o resonancias, cada uno representando la concentración local de un neurometabolito diferente o una pequeña familia de neurometabolitos químicamente relacionados. Los compuestos que se pueden medir son el myo-inositol (Ins), los compuestos glutamatérgicos: glutamato + glutamina (Glx), colina (Cho), N-acetil-aspartato (NAA), y creatina (Cr). El Ins es considerado un marcador de células gliales que está íntimamente conectado a la osmorregulación de los astrocitos, por lo que su aumento probablemente refleja la activación glial; además, el Ins se considera un producto de degradación de la mielina (Starck et al., 2008). El Glx es una medida útil de la neurotransmisión glutamatérgica excitatoria (Starck et al., 2008), mientras que la concentración de Cho refleja los compuestos tales como la fosfocolina y la acetilcolina. La disminución de las concentraciones de Cho se han observado en las regiones de desmielinización aguda en pacientes con esclerosis múltiple y se cree que reflejan anormalidades de mielinización (Smith et al., 2003). El NAA es un marcador de la viabilidad neuronal y la disminución en el tejido neural antes de la pérdida neuronal se detecta mediante resonancia magnética estructural. Por lo tanto, las concentraciones disminuidas de NAA están asociadas con una función neuronal alterada (Birken and Oldendorf, 1989). Finalmente, concentraciones de creatina-fosfocreatina cerebral alteradas también podrían reflejar los cambios en el uso de energía del cerebro (Mirza et al., 2006).

Aunque la mayoría de estudios de RMS en población con TOC están realizados con muestras heterogéneas, con insuficiente poder estadístico y con baja sensibilidad para detectar diferencias debido a las diferentes tecnologías (1.5 T versus 3 T), algunos hallazgos se repiten como concentraciones reducidas de NAA en el CCA y el núcleo caudado, concentraciones reducidas de Glx en el CCA, concentraciones incrementadas de Glx en el

caudado y concentraciones incrementadas de Cho en el tálamo, SB parietal y el hipocampo (Brennan et al., 2013). Respecto a las concentraciones reducidas de NAA en el CCA, este hallazgo parece consistente con la disminución de volumen de SG en esta región (Radua and Mataix-Cols, 2009; Radua et al., 2010; Rotge et al., 2009). Estas concentraciones reducidas de NAA en el TOC pueden no estar reflejando una pérdida neuronal permanente, sinó anormalidades potencialmente reversibles tal y como sugieren dos estudios que describen la normalización de las concentraciones de NAA tras tratamiento con citalopram (Jang et al., 2006) o tras TCC (Whiteside et al., 2012). Respecto a las concentraciones incrementadas de Cho, diferentes estudios refieren aumento en pacientes con TOC en comparación con sujetos control sanos en el tálamo (Mohamed et al., 2007; Rosenberg et al., 2001; Smith et al., 2003), en SB parietal (Kitamura et al., 2006) y en el hipocampo (Atmaca et al., 2009)). Por el contrario, un estudio describió concentraciones disminuidas en el núcleo estriado izquierdo en pacientes con TOC pediátrico (Lázaro et al., 2012). Las alteraciones en la Cho y sus componentes, se han relacionado con un papel en la desmielinización y secundariamente con alteraciones descritas en la SB. Finalmente, los resultados respecto a las concentraciones de Glx en el TOC serán comentados específicamente en el siguiente apartado.

El hallazgo de resultados heterogéneos es frecuente en los estudios de neuroimagen psiquiátricos, principalmente debido a las diferentes técnicas de adquisición y análisis de las imágenes, el tamaño modesto de las muestras que podrían causar resultados incoherentes y mixtos y la heterogeneidad de las características clínicas de la muestra como lo son edad, duración de la enfermedad, gravedad, la existencia o no de comorbilidad y medicaciones concomitantes.

### 1.3. La hipótesis glutamatérgica en el TOC de inicio en la infancia y la adolescencia

El glutamato es el neurotransmisor excitador principal del sistema nervioso central y media la mayor parte de la transmisión excitadora rápida. Se estima que el 80% de las neuronas en el neocórtex son excitadoras, lo cual pone de manifiesto el papel clave que tiene este neurotransmisor sobre el funcionamiento cerebral (Orrego and Villanueva, 1993; Shepherd and Huganir, 2007). El glutamato regula la neurogénesis, el sobrecrecimiento de las neuritas, la sinaptogénesis y la apoptosis (Mattson, 2008) e interacciona con los factores neurotróficos en procesos de plasticidad neuronal (Lessmann, 1998).

En 1998, Rosenberg y Keshavan, fueron los primeros en proponer un papel para el glutamato en el TOC de inicio en la infancia. La existencia cada vez mayor de evidencia sobre modelos animales, genéticos, de neuroimagen y ensayos clínicos han apoyado esta hipótesis de disregulación de la neurotransmisión de glutamato como contribución a la fisiopatología del TOC (Ting and Feng, 2008). El glutamato es el neurotransmisor primario dentro del circuito córtico-estriado-talámico-cortical (Bronstein y Cummings, 2001), y la mayoría de los terminales de los axones en el cuerpo estriado son glutamatérgicos (Parent and Hazrati, 1995). Este modelo se basa en el equilibrio entre las vías directas e indirectas dentro del circuito CSTC (Saxena and Rauch, 2000). Según esta teoría, la interacción recíproca entre la vía directa (en última instancia conduce a la estimulación por parte del tálamo a la corteza) e indirecta (en última instancia conduce a la inhibición por parte del tálamo a la corteza) normalmente da como resultado un equilibrio dinámico sin predominio de una de las vías. Se piensa que la hiperactividad de la vía directa, o la hipoactividad de la vía indirecta pueda conducir a la desinhibición del circuito CSTC y la consiguiente liberación de conductas repetitivas (compulsiones) y cogniciones (obsesiones) que normalmente están bajo control. Tanto Rosenberg et al (2000) como otros investigadores al mismo tiempo (Carlsson, 2001) han

descrito que este exceso de actividad de la vía directa está relacionada con la hiperactividad asociada del glutamato, ya que es el principal neurotransmisor excitador en esta vía.

Por otra parte, los estudios en animales han demostrado que los receptores serotoninérgicos ejercen un control modulador muy complejo sobre la transmisión mediada por glutamato, que implica muchos subtipos de éstos receptores (Ciranna, 2006). Siguiendo esta línea de investigación, algunos autores han sugerido que la alteración del sistema glutamatérgico podría ser la de mayor contribución a la fisiopatología del TOC, y que las alteraciones serotoninérgicas y dopaminérgicas en el TOC podrían ser secundarias a alteraciones glutamatérgicas (Carlsson, 2000). En conjunto, estos resultados sugieren una posible disregulación tónico-fásica de compuestos glutaminérgicos dentro de los circuitos córtico-estriatal en el TOC, con compuestos glutaminérgicos reducidos en el CCA combinado con una hiperactividad en el cuerpo estriado y la corteza órbitofrontal (Rosenberg et al., 2004).

En concreto, se han llevado a cabo tres estudios de RMS centrados en el metabolismo del cuerpo estriado en adolescentes con TOC antes y después de someterse a tratamiento, así como un estudio transversal comparando tres grupos diagnósticos: TOC pediátrico, trastorno del estado de ánimo y un grupo control sano. En el primer estudio, se observó un aumento de la concentración Glx en el núcleo caudado previa al tratamiento farmacológico en 11 pacientes en edad pediátrica con TOC con una duración media de la enfermedad de 14,4 meses (DE = 15,4) en comparación con 11 sujetos control sanos; las concentraciones de Glx se normalizaron después de 12 semanas de tratamiento con el fármaco ISRS paroxetina 20-60 mg / día. Esta reducción en la concentración de Glx correlacionaba con la respuesta al tratamiento de acuerdo con puntuaciones en la Escala de Obsesiones y Compulsiones Yale-Brown para niños (CY-BOCS) (Rosenberg et al., 2000). En otro estudio, no se encontraron cambios en las concentraciones de Glx en el núcleo caudado izquierdo después de TCC en 21 pacientes con TOC en edad pediátrica aunque sin ser comparado a un grupo control. Estos pacientes

presentaban una duración media de la enfermedad de 38,88 meses (DE = 38,4) (Benazon et al., 2003). En 2004, un estudio de Rosenberg y colaboradores, en una muestra de 14 niños con TOC reveló concentraciones de Glx inferiores en el CCA sin importar si realizaban tratamiento farmacológico o no. La duración media de la enfermedad en este caso era de 46,44 meses (DE = 41,52 meses). Por último, un estudio transversal y longitudinal (antes y después de seis meses de tratamiento) no encontró diferencias significativas en la concentración del Glx en la corteza frontal medial, en el CCA o en los núcleos estriado izquierdo o derecho en 11 pacientes con TOC sin tratamiento farmacológico en comparación con 12 controles sanos, con una duración media de la enfermedad de 24 meses (Lázaro et al., 2012). Por otra parte, con respecto a los efectos de la farmacoterapia, estudios previos en pacientes adultos han sugerido que el hecho de recibir tratamiento psicofarmacológico no afecta a las variables medidas por la RMS (Yücel et al., 2008). En niños, las concentraciones de Glx en el CCA, después de realizar tratamiento eficaz con paroxetina (ISRS), disminuían en los pacientes TOC *naïve* de tratamiento en comparación con los controles (Rosenberg et al., 2000).

### 1.4. Papel de la genética en el TOC de inicio en la infancia y la adolescencia

Los estudios llevados a cabo en familias y con gemelos, así como los estudios genéticos de asociación apoyan la hipótesis de que el TOC tiene un importante componente familiar y que los factores genéticos juegan un papel clave en la manifestación de este trastorno. Se ha descrito que la prevalencia del TOC es mayor en familiares de primer grado de pacientes obsesivos que en familiares de individuos sanos (Nestadt et al., 2002). Además el riesgo de padecer el trastorno aumenta proporcionalmente con el grado de parentesco (Mataix-Cols et al., 2013). Cabe destacar que la influencia del componente genético en los casos de TOC de inicio temprano es mayor que en los de inicio de edad adulta (Van Grootheest et al., 2007). Partiendo de la base de que se trata de un trastorno poligénico, se ha sugerido que el TOC se

debe probablemente a una disregulación de varios genes que actúan interaccionando a nivel cerebral más que de genes individuales que simplemente van acumulando riesgo (Haber and Heilbronner, 2013).

Se han publicado más de 100 estudios de genes candidatos. La mayoría de éstos analizan polimorfismos de genes implicados en las vías dopaminérgica, serotoninérgica y glutamatérgica, y se han diseñado en base al conocimiento existente sobre los circuitos neuronales y los neurotransmisores involucrados en el TOC (Wu et al., 2012). Son muchos los ejemplos de asociaciones encontradas entre polimorfismos de genes implicados en éstos y otros sistemas y algún fenotipo relacionado con este trastorno. En cuanto al sistema dopaminérgico, variantes genéticas de los receptores de dopamina, incluyendo el polimorfismo Taq1A del gen del receptor de la dopamina D2 (*DRD2*) (Denys et al., 2006) o el 48-bp VNTR del gen del receptor de la dopamina (*DRD4*) se han asociado al riesgo de sufrir la enfermedad (Camarena et al., 2007). Cabe destacar también la asociación descrita en diferentes estudios entre una variante que provoca una disminución en la actividad enzimática de la catecol-o-metiltransferasa (*COMT*) y el riesgo incrementado que los hombres tienen de desarrollar TOC (Denys et al., 2006; Pooley et al., 2007).

Pero, probablemente, el sistema más estudiado sea el serotoninérgico debido a su clara implicación en el trastorno avalada por la reducción de la sintomatología que presentan los pacientes cuando son tratados con ISRS o un inhibidor de la recaptación de serotonina (IRS). Un polimorfismo en el gen del receptor de serotonina 1B (*HTR1B*) se ha asociado con la transmisión de la enfermedad en familias (Mundo et al., 2000); describiéndose también su implicación en la severidad de las obsesiones (Camarena et al., 2004). En un estudio llevado a cabo por nuestro grupo investigador en una población infanto-juvenil con anorexia y TOC se identificaron asociaciones con otros genes relacionados con este sistema, concretamente con el gen de triptófano hidroxilasa 2 (*TPH2*) y el gen del transportador vesicular de monoaminas (*SLC18A1*) (Mas et al., 2013). En ese mismo estudio se asoció el gen del receptor ionotrópico

de glutamato AMPA3 (*GRIA3*) que codifica para un tipo de receptor de glutamato. Pero éste no es el único receptor glutamatérgico que se ha relacionado con el TOC. Es importante destacar las asociaciones descritas entre la transmisión de la enfermedad y los genes del receptor del glutamato ionotrópico de tipo kainato (*GRIK2*) y de la subunidad 2B del receptor del glutamato (*GRIN2B*) (Arnold et al., 2004; Delorme et al., 2004; Sampaio et al., 2011). Éste último también se ha asociado con los valores de Glx en el CCA (Arnold et al., 2009) en población pediátrica y con determinados subfenotipos del TOC, concretamente con la presencia de obsesiones de contaminación y compulsiones de limpieza en población adulta (Alonso et al., 2012). No obstante, el transportador de glutamato SLC1A1 parece ser el componente de la vía glutamatérgica que más se ha asociado con la enfermedad. En diferentes estudios, varios polimorfismos de nucléotido único (SNPs) en la región 3' del gen se han asociado con el riesgo de TOC y también con la aparición de trastornos obsesivos en pacientes esquizofrénicos tratados con antipsicóticos (Kwon et al., 2009; Wu et al., 2013). Así mismo, se ha asociado un alelo de riesgo de un SNP del gen del transportador del glutamatato *SLC1A1* a un incremento en la probabilidad de resistencia al tratamiento (Real et al., 2013).

Éstos son sólo algunos de los genes candidatos relacionados con las vías citadas. Sin embargo, existen múltiples evidencias sobre otros genes clave en estas mismas vías y también sobre otros muchos genes relacionados con otras vías relacionadas con el GABA, las neurotrofinas y las neuroregulinas, entre otras. Es importante recalcar que existe cierta discrepancia en los resultados obtenidos en los diferentes estudios que podría ser atribuida a diversos factores. Uno de los principales problemas es la falta de poder estadístico para detectar efectos genéticos pequeños debido a los tamaños muestrales reducidos. Así, los meta-análisis pueden resolver en parte este problema. Un estudio reciente muestra los resultados de dos meta-análisis que incluyen 113 estudios con información suficiente para ser incluidos en este tipo de análisis, y en el cual se analizan un total de 220 polimorfismos (Taylor, 2013). Los resultados del primer análisis sugirieron que el TOC se asocia con polimorfismos en

dos genes relacionados con el sistema serotoninérgico, el gen del transportador de la serotonina de *solute carrier family 6, miembro 4* (*SLC6A4*) y el gen del receptor de la serotonina 2A (*HTR2A*), que codifican para el transportador y uno de los receptores de la serotonina, respectivamente. También se observaron asociaciones con las variantes de los genes de la *COMT* y monoaminooxidasa A (*MAOA*), las cuales parecen ser sexo-específicas ya que se asociaron únicamente en el sexo masculino con TOC. Además, se han observado tendencias aunque no significativas para las asociaciones con polimorfismos en dos genes relacionados con el sistema dopaminérgico, el gen del transportador de dopamina de *solute carrier family 6, miembro 3* (*SLC6A3*) y el gen del receptor de dopamina D3 (*DRD3*) y uno en un gen relacionado con el sistema glutamatérgico, el anteriormente mencionado transportador de glutamato *SLC1A1* (Taylor, 2013). En el segundo análisis, se observaron asociaciones significativas para polimorfismos relacionados con factores tróficos, ácido gamma-aminobutírico (GABA), glutamato, serotonina, bradicinina, acetilcolina, glicina, ubiquitina, factores inmunológicos y mielinización. De todas formas, estos resultados deben ser interpretados con cautela, ya que varios de ellos se basan en un único estudio.

A partir de los hallazgos previamente descritos, se puede concluir que el trastorno obsesivo-compulsivo, es un trastorno complejo, del que se precisan nuevas aproximaciones que permitan conocer mejor la etiopatogenia del trastorno, con el fin de poder definir nuevas dianas terapéuticas para una mejor evolución del mismo.

# HIPÓTESIS Y OBJETIVOS

## 2. HIPÓTESIS Y OBJETIVOS

***Hipótesis principales:***

1. Los pacientes con TOC de inicio temprano con diferente tipo de comorbilidad presentarán diferentes características clínicas, pudiendo la comorbilidad subtipificar el trastorno.

2. En comparación con sujetos control sanos, los pacientes con TOC de inicio temprano presentarán alteraciones en las concentraciones cerebrales de neurometabolitos, principalmente el glutamato, en la región del córtex cingulado anterior.

3. Los pacientes con TOC de inicio temprano presentarán diferencias en las frecuencias alélicas de polimorfismos de genes implicados en el trastorno.

4. La concentración de los neurometabolitos en estos pacientes estará asociada a las variantes genéticas estudiadas.

***Objetivos principales:***

1. Caracterizar sociodemográfica y clínicamente una muestra de niños y adolescentes con trastorno obsesivo-compulsivo.

2. Analizar las diferencias en las concentraciones de neurometabolitos, principalmente del glutamato, en la región del córtex cingulado anterior mediante técnicas de resonancia magnética espectroscópica entre dichos pacientes y una muestra de sujetos control sanos.

3. Identificar asociaciones entre variantes genéticas de los sistemas dopaminérgico, serotoninérgico y glutamatérgico, entre otros, y el riesgo de TOC de inicio temprano a través de un estudio de desequilibrio de transmisión realizado en familias.

4. Estudiar la relación existente entre las concentraciones de dichos neurometabolitos con las variantes genéticas analizadas.

***Objetivos secundarios:***

1.1. Valorar la influencia del sexo y la comorbilidad en una muestra de niños y adolescentes con TOC.

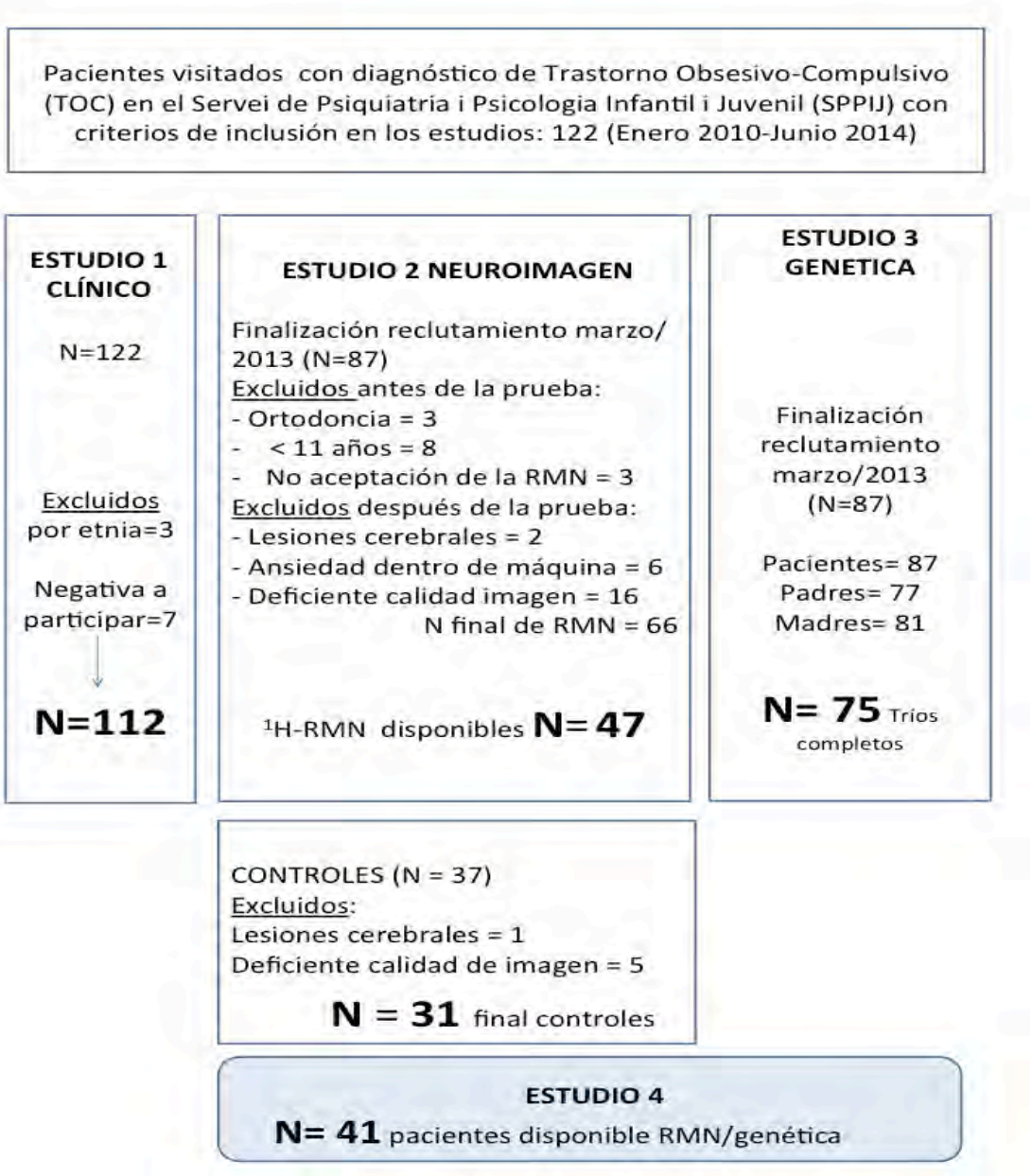
2.2. Conocer la influencia del tiempo de evolución de la enfermedad y el tratamiento farmacológico en las concentraciones de neurometabolitos medidos mediante resonancia magnética espectroscópica en los pacientes pediátricos con TOC.

# MATERIAL Y MÉTODOS

## 3. MATERIAL Y MÉTODOS

Las particularidades del material y métodos de cada estudio se describen de manera detallada en los estudios que componen el trabajo de tesis. A continuación se resumen los principales aspectos referentes a los participantes de cada estudio y a la metodología clínica y experimental utilizada.

### 3.1. Selección de sujetos y controles según el estudio



Los procedimientos fueron aprobados por el Comité de Ética del Hospital Clínic de Barcelona. Se informó verbalmente y se obtuvo el consentimiento informado firmado de todos los sujetos y familias. Todos los investigadores colaboradores estaban de acuerdo en preservar en todo momento el anonimato de los pacientes y los sujetos control sanos, utilizando la información recopilada exclusivamente para los fines indicados.

### 3.2. Evaluación clínica (Estudio 1)

Los pacientes presentaban TOC como diagnóstico principal según los criterios DSM-IV-TR (American Psychiatric Association, 2000). El reclutamiento fue de manera naturalística durante los últimos cuatro años, siendo incluidos los pacientes que ya estaban en tratamiento y los nuevos pacientes que se han ido incorporado en los últimos años a la unidad. La evaluación en los pacientes que se iban incorporando a la unidad, se realizó durante los cuatro meses siguientes a la primera visita del paciente. No se estableció una regla definida para establecer el momento de la inclusión en el estudio, sólo la aceptación de los niños y padres en un estudio más amplio que incluyó exploración genética y de neuroimagen. Se utilizó la entrevista K-SADS-PL (The Schedule for Affective Disorders and Schizophrenia for School-age Children-Present and Lifetime Version) (Kaufman et al., 1997) validada en español (Ulloa et al., 2006) para evaluar el diagnóstico actual predominante y los adicionales comórbidos. La entrevista se administró a ambos padres y al niño/adolescente. Todos los pacientes recibieron terapia cognitivo-conductual y el paciente que lo precisó, recibió tratamiento farmacológico naturalístico siguiendo la guía clínica de nuestra unidad basada en las guías clínicas internacionales (UK NICE 2008; AACAP 2012).

La evaluación diagnóstica se llevó a cabo por tres psiquiatras de la unidad de TOC con experiencia en el diagnóstico de TOC y en evaluaciones clínicas. Posteriormente, dos personas del equipo de investigación (una de ellas la doctoranda) con experiencia en el uso de

instrumentos de diagnóstico semi-estructurados administraron la K-SADS-PL para la evaluación de la comorbilidad actual y pasada. La información socio-demográfica y clínica se obtuvo de los pacientes y sus padres en la entrevista diagnóstica y en la evaluación clínica. El nivel socioeconómico de toda la muestra se estimó con la Escala Hollingshead Redlich (Hollingshead and Redlich, 1958). La edad de inicio se definió como la edad en que los pacientes mostraron malestar o deterioro significativo asociado a los síntomas obsesivo-compulsivos.

Para evaluar la gravedad de los síntomas del TOC, se utilizó la CY-BOCS (Scahill et al., 1997). Las respuestas de los pacientes con TOC en el CY-BOCS se clasificaron de forma dicotómica (presente / ausente). Además, se registró la obsesión y la compulsión más prominente. De acuerdo con el análisis factorial de Leckman (1997) de la CY-BOCS se han clasificado los pacientes según las cuatro dimensiones sintomáticas de síntomas obsesivo-compulsivos (obsesiones religiosas, sexuales, agresivas y compulsiones de comprobación; obsesiones de simetría, orden, el conteo, y compulsiones de organización; obsesiones de contaminación y compulsiones de limpieza; y acumulación). Además, se puntuó la severidad entre 0 y 40. Por otra parte, se administró el Inventario Obsesivo-Compulsivo Versión de niños (OCI-CV), cuestionario de 21 ítems basado en el Inventario de Obsesiones y Compulsiones revisado (OCI-R), que evalúa la presencia de síntomas obsesivo-compulsivo en niños y adolescentes durante el mes anterior (Foa et al., 2010).

La sintomatología depresiva se evaluó mediante un instrumento validado, el Inventario de Depresión Infantil (CDI) (Kovacs, 1985). Se trata de un autoinforme de 21 ítems para evaluar la presencia y severidad de la sintomatología depresiva con un rango de puntuación de 0 a 63 puntos.

El SCARED es un instrumento autoinformado para niños y padres utilizado para la detección de los trastornos de ansiedad, incluyendo el TAG, trastorno de ansiedad por separación, trastorno de pánico y el trastorno de ansiedad social. Además, evalúa los síntomas

relacionados con las fobias escolares. El SCARED consta de 41 ítems y 5 factores que son paralelos a la clasificación DSM-IV de los trastornos de ansiedad (Birmaher et al., 1997).

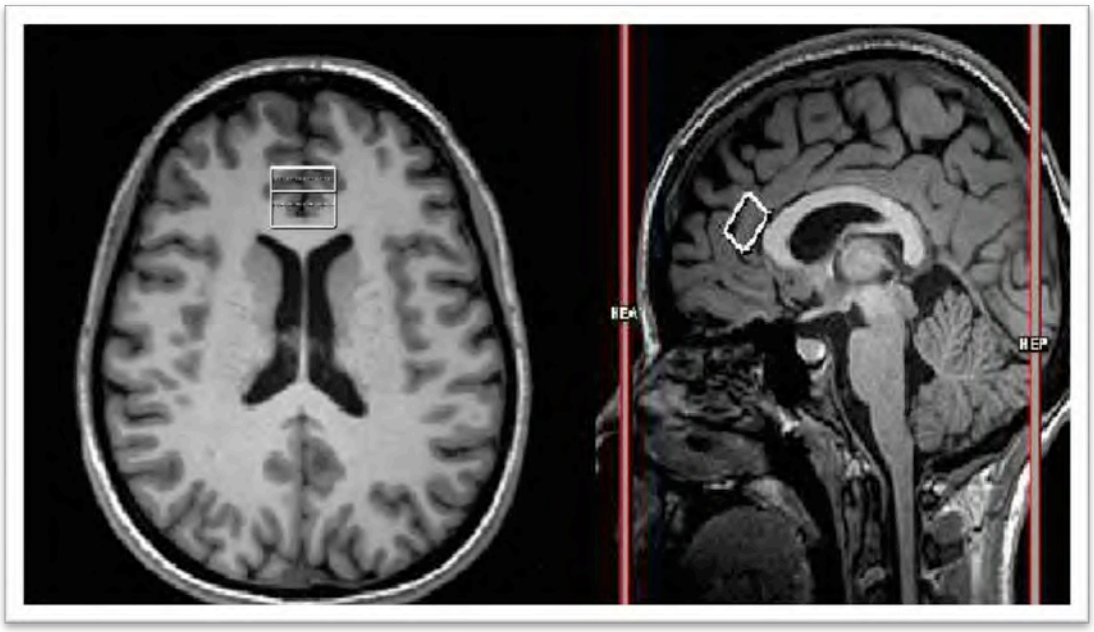
Se diseñaron tres grupos atendiendo al segundo diagnóstico además del TOC. Un primer grupo se definió como los pacientes con TOC sin comorbilidad, un segundo grupo se definió como aquellos con comorbilidad con un trastorno del neurodesarrollo: TT y otros trastornos de tics, y/o TDAH, y un tercer grupo de pacientes con comorbilidad internalizante: trastorno de ansiedad o trastorno de estado de ánimo. Se decidió incluir los pacientes con un trastorno alimentario (N = 6) en este grupo (Mitchell et al., 2014).

La entrevista Internacional neuropsiquiátrica MINI (Versión española 5.0.-DSM-IV) (Sheehan et al., 1998) se utilizó para evaluar los principales diagnósticos actuales del Eje I en los padres. Además, se realizó una entrevista especial para detectar trastornos psiquiátricos en parientes de segundo grado como parte de la entrevista clínica con los padres de los pacientes.

### 3.3. Medición de neurometabolitos mediante resonancia magnética cerebral (Estudios 2 y 4)

Las imágenes de RMS fueron adquiridas en un escáner 3T TIM TRIO (Siemens, Erlangen, Alemania), utilizando una bobina de canal de 32 cabezas. Se determinó un volumen de interés (VOI) de 3 $cm^3$ (15 x 20 x 10 $mm^3$) en el CCA. El vóxel fue colocado utilizando dos planos de la imagen ponderada obtenida previamente en T1 de alta resolución. En primer lugar, el vóxel se colocó en el plano sagital, por encima de la rodilla del cuerpo calloso y centrado en el CCA. A continuación se realizó la confirmación de que el vóxel estaba en el plano axial en el CCA. Este procedimiento se aplicó de la misma manera en todos los pacientes teniendo cuidado para asegurar la colocación estándar. La Figura 2 muestra la representación de la colocación del vóxel.

Figura 2. Colocación del VOI en el CCA en un paciente del estudio.



Los espectros se adquirieron con el uso de una técnica de espectroscopía de resolución de puntos (PRESS) con un TR = 200 ms y un TE = 35 ms. Las concentraciones de metabolitos fueron cuantificados por medio del programa de ajuste de dominio de frecuencia independiente (LCModel) (Provencher, 2001) versión 6.1-4A, aplicando la corrección de la corriente de Foucault y el uso del agua como señal de referencia interna para calcular las concentraciones de metabolitos absolutos; como se muestra el ejemplo de espectro medido por el software LCModel en la Figura 2 del estudio 2 (ver página 91). Algunos metabolitos son difíciles de cuantificar de forma independiente de los demás, por lo que se utilizó la suma de concentraciones. Los metabolitos base fijados para LCModel fueron inositol, glicerofosfocolina + fosfocolina, NAA + N-acetil aspartato glutamato (NAAG), Cr + Fosfo-Creatina y el glutamato + glutamina. Sólo fueron considerados los valores absolutos de metabolitos con un límite inferior de Cramer-Rao por debajo del 20%, lo que indicaba que estos metabolitos podían ser estimados con fiabilidad (Provencher, 2001), y una relación señal-ruido superior a 10. Se excluyeron 16 casos y cinco controles sanos por estas razones. En la misma sesión de exploración se registraba una imagen estructural adicional (3d secuencia MPRAGE en T1 con

vóxel isométrica de 1 x 1 x 1 $mm^3$). La imagen estructural fue segmentada utilizando mapas de probabilidad de tejido personalizados (MPTP) y siguiendo el modelo de la nueva segmentación proporcionada en SPM8 (Ashburner, 2009). Para generar los MPTP, las imágenes de SG y SB obtenidas de la segmentación estándar se normalizaron a MNI usando Dartel (Ashburner, 2007) con una resolución de 2 x 2 x 2 mm3, y promediados y suavizados con un kernel gaussiano de 8 mm. Las concentraciones de metabolitos fueron corregidas por las diferencias en el contenido del líquido cefalorraquídeo (LCR) en el VOI, utilizando un software creado por los investigadores. El porcentaje residual de SG en el VOI, tras ser eliminado el componente de LCR, fue utilizado como una variable de confusión en los análisis estadísticos (Guerrini et al., 2009).

Los parámetros de $^1$H-RMS utilizados para el estudio proporcionaron señales robustas, tanto para el grupo de sujetos control sanos, como para el grupo de pacientes con TOC. Específicamente, los sujetos sanos tenían una relación de señal-ruido de 14,45 (DE = 2,790) y un FWHM (anchura a media altura) de 0.041 ppm (DE = 0,010). Los pacientes con TOC tenían una relación de señal-ruido de 14,4 (DE = 2,390) y una FMHW de 0,042 ppm (DE = 0,009). Ninguna de estas medidas presentó diferencias entre los dos grupos (p = 0,936 y 0,505, respectivamente), lo que sugería que la calidad de los datos era comparable.

Un neuroradiólogo confirmó que todos los escáneres de RMN estuvieran libres de anomalías estructurales.

### 3.4. Selección de genes y SNPs (Estudios 3 y 4)

Se llevó a cabo una selección de 35 genes candidatos claves en los sistemas implicados en el TOC y otros trastornos psiquiátricos que incluyen el dopaminérgico, serotoninérgico, glutamatérgico, gabaérgico, de neurotrofinas y neuroregulinas, así como otros genes para los cuales se ha descrito alguna evidencia científica de interés en la patología (para más información de los genes seleccionados ver la tabla 1 del estudio 3, página 100).

En cuanto a los polimorfismos, se seleccionaron un total de 304 SNPs distribuidos entre los 35 genes candidatos siguiendo tres estrategias diferentes. Por un lado, se incluyeron polimorfismos para los que existe evidencia científica descrita en la literatura sobre su asociación con la susceptibilidad a padecer TOC u otras patologías psiquiátricas. Por otro lado, se escogieron SNPs con una frecuencia alélica mínima validada superior al 10% para la población caucásica, en base a su posible funcionalidad según su localización en la secuencia génica, y de acuerdo con la información publicada en la base de datos Ensembl (http://www.ensembl.org). Además, para los genes que fue posible, se siguió una tercera estrategia basada en la inclusión de SNPs marcadores (tagSNPs) informativos de la variabilidad existente a lo largo de toda la región genómica. La selección de tagSNPs se hizo a partir de la información del International Haplotype Mapping Project (http://www.hapmap.org) utilizando la población caucásica CEU como referencia (residentes en Utah de la muestra CEPH con antepasados del norte y oeste de Europa). Las regiones genómicas correspondientes a los genes de estudio se ampliaron 10kb por los extremos 5' y 3' para tener en cuenta la posible existencia de regiones reguladoras. La selección de los SNPs marcadores en las regiones seleccionadas se analizaron mediante el software Haploview 4.2 y se llevó a cabo teniendo en cuenta que los SNPs estuvieran en equilibrio de Hardy-Weinberg, tuvieran una frecuencia alélica mínima del 10%, no hubieran SNPs vecinos a menos de 20 pares de bases, y que el 90% de los individuos estuvieran correctamente genotipados. Los marcadores que cumplían los requisitos anteriores se sometieron a una estrategia de tagging agresivo utilizando haplotipos de dos y tres marcadores cubriendo, como mínimo, el 85% de la variabilidad genética. Siempre que fue posible, se forzó la inclusión de los SNPs seleccionados mediante las otras estrategias en el análisis de tagging. Cuando la estrategia de tagging proporcionó más de 20 tagSNPs, los SNPs para el gen candidato se seleccionaron únicamente en base a la evidencia científica existente y su funcionalidad predicha.

### 3.5. Preparación de la muestra y genotipado (Estudios 3 y 4)

Las muestras de sangre de los participantes se recogieron en tubos con EDTA (tubos K2 EDTA BD Vacutainer con EDTA; Becton Dickinson, Franklin Lakes, Nueva Jersey). El aislamiento del ADN se llevó a cabo a partir de 500 µl de sangre mediante el sistema automático MagnaPureLC2.0 (Roche Diagnostics GmbH, Mannheim, Alemania). La concentración y la calidad del ácido desoxiribonucleico (ADN) se determinaron por absorbancia mediante un NanoDrop™2000 (Thermo Fisher Scientific, Surrey, Reino Unido). El ADN aislado se conservó a -80º C hasta el momento de su utilización. Para el posterior genotipado, las muestras de ADN se diluyeron con agua miliQ (SigmaAldrich® ChemieGmbH, Steinheim, Alemania) hasta una concentración de 20 ng/µl.

Del total de SNPs seleccionados, 7 se rechazaron por incompatibilidades con la metodología del ensayo. Los 297 SNPs restantes se genotiparon mediante la tecnología MassArray de Sequenom en el Centro Nacional de Genotipado Español de Santiago (CeGen). Este proceso de genotipado se basa en una extensión de una única base (Single Base Extension, SBE) que genera una diferencia de masa en el producto de la reacción en función de la base añadida, por lo que puede utilizarse la espectrometría de masas MALDI-TOF para la discriminación alélica. Como control de calidad, se genotiparon 13 muestras por duplicado para todos los SNPs analizados, con una tasa de concordancia del 100%. Posteriormente, al genotipado se excluyeron varios SNPs porque los genotipos estaban presentes en menos del 90% de las muestras, mostraron errores mendelianos, o porque eran monomórficos. Finalmente, se utilizaron para los análisis de asociación genética hasta 265 SNPs validados (Tabla S1 del estudio 3, página 108).

# RESULTADOS

# 4. RESULTADOS

Los resultados se describen de manera detallada en los estudios que componen el trabajo de tesis. A continuación se resumen los principales hallazgos derivados de cada uno de los estudios.

## 4.1. Características clínicas de una muestra de pacientes con TOC de inicio en la infancia y la adolescencia. Valor clínico de la comorbilidad psiquiátrica (Objetivos 1 y 1.1)

La muestra compuesta por 112 pacientes con una edad media de 14,7 años (DE = 2,5), siendo la edad media de inicio del trastorno de 12,1 años (DE = 2,7). Había 57 pacientes de sexo masculino (50,9%) y 55 pacientes de sexo femenino (49,1%). La duración media de la enfermedad cuando se llevó a cabo la evaluación fue de 23,9 meses (DE = 24,2) con un intervalo de 6 a 137 meses. La media de la escala de severidad de los síntomas en el momento de la evaluación medida con el CY-BOCS fue 17,5 $\pm$ 7,7, mientras que la puntuación en el momento de mayor severidad del trastorno había sido de 26,2 $\pm$ 6,5. Con respecto a la terapia farmacológica, el 22,3% (n = 25) de los pacientes con TOC no estaban recibiendo ningún psicofármaco, mientras que el 77,2% (n = 87) estaban tratados con ISRS [34,8%] o IRS [clomipramina, 12,5% (n = 14)] y 3 pacientes estaban recibiendo una combinación de fluoxetina + clomipramina.

Respecto a la comorbilidad psiquiátrica, se encontró otro diagnóstico en el Eje I en el 67% de los pacientes, siendo dichos diagnósticos los siguientes: Trastorno de Tourette (8%, n = 9), trastorno por tics crónicos (1,8%, n = 2), trastorno de tics transitorios (1,8, n = 2), TDAH (8,9%, n = 10), TAG (26,8%, n = 30), trastorno de ansiedad social (4,5%, n = 5), trastorno de pánico (1,8%, n = 2), trastorno depresivo mayor (3,6%, n = 4), trastorno depresivo no especificado (1,8%, n = 2), hipomanía (1,8%, n = 2), trastorno bipolar no especificado (0,9%, n = 1), anorexia nerviosa (4,5%, n = 5) y bulimia nerviosa (0,9%, n = 1). Veintiséis pacientes (23,2%)

presentaron un tercer diagnóstico. El segundo y el tercer diagnóstico no estaban en fase aguda.

Respecto a los tres grupos de pacientes según su diagnóstico comórbido, encontramos diferencias significativas respecto al sexo ($p = 0.031$), edad ($p<0.000$) y edad de inicio ($p<0.000$). La historia familiar de TOC fue significativamente mayor en los pacientes con trastornos del neurodesarrollo comórbido respecto a los otros grupos ($p = 0.049$). Encontramos diferencias significativas respecto a las dimensiones entre los tres grupos ($p = 0.022$): la dimensión simetría y orden ($p=0.021$) fue más frecuente en el grupo de trastornos del neurodesarrollo comórbido y la dimensión evitación del daño y comprobación ($p = 0.016$) fue más frecuente en el grupo de trastorno comórbido internalizante. La tasa de hospitalización ($p=0.011$) era mayor en ambos grupos de comorbilidad respecto al grupo sin comorbilidad. En la tabla 1 del estudio se pueden consultar las diferencias entre estas y otras variables testadas (página 85).

A pesar de que no había una relación significativa entre la edad de inicio y el sexo (sexo masculino: $11.9 \pm 2.5$; sexo femenino: $12.4 \pm 3.0$; $t = -0.902$; $p = 0.369$), podemos ver en la figura del estudio (página 84) la diferente distribución de la densidad de los casos según la edad de inicio, separado por sexo, con un pico de presentación de casos en el sexo masculino en la infancia, en contraste con una presentación continua en el sexo femenino en la infancia y la adolescencia. Se encontró una tendencia a presentar una menor edad de los primeros síntomas (sexo masculino: $9.5 \pm 3.7$, sexo femenino: $10.8 \pm 3.3$, $t = -1.878$; $p = 0.063$) y una mayor duración del trastorno en el sexo masculino (sexo masculino: $28.1 \pm 24.7$, sexo femenino: $19.7 \pm 23,1$, $t = 1.858$, $p = 0.066$).

**Estudio 1:** Clinical significance of psychiatric comorbidity in children and adolescents with obsessive-compulsive disorder: Subtyping a complex disorder. European Archives of Psychiatry and Clinical Neuroscience (Under review)

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## Clinical significance of psychiatric comorbidity in children and adolescents with obsessive-compulsive disorder: Subtyping a complex disorder

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| Abstract: | A promising approach in relation to reducing phenotypic heterogeneity involves the identification of homogeneous subtypes of OCD based on age of onset, gender, clinical course and comorbidity. This study aims to assess the sociodemographic characteristics and clinical features of OCD patients in relation to gender and the presence or absence of another comorbid disorder. The sample comprised 112 children and adolescents of both sexes and aged 8-18 years, all of whom had a diagnosis of OCD. Overall, 67% of OCD patients had one comorbid diagnosis, 23.2% had two such diagnoses and 4.5% had three comorbid diagnoses. The group of OCD patients with a comorbid neurodevelopmental disorder had significantly more family history of OCD in parents (p=.032), as compared with the no comorbidity group and the group with a comorbid internalizing disorder, and they also showed a greater predominance of males (p=.013) than did the group with a comorbid internalizing disorder. The group of OCD patients with internalizing comorbidity had a later age of onset of OCD (p=.001) compared with both the other groups. Although the initial severity was similar in all three groups, the need for pharmacological treatment and for hospitalization due to OCD symptomatology was greater in the groups with a comorbid neurodevelopmental disorder (p=.038 and p=.009, respectively) and a comorbid internalizing disorder (p=.008 and p=.004 respectively) than in the group without comorbidity. Our findings suggest that two subtypes of OCD can be defined on the basis of the comorbid pathology presented. The identification of different subtypes according to comorbidity is potentially useful in terms of understanding clinical variations, as well as in relation to treatment management and the use of therapeutic resources. |

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**Clinical significance of psychiatric comorbidity in children and adolescents with obsessive-compulsive disorder: Subtyping a complex disorder**

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**Introduction**

Obsessive-compulsive disorder (OCD) is a neuropsychiatric disorder characterized by recurrent and persistent thoughts, urges or images that are experienced as intrusive and unwanted, and behaviours or mental acts that an individual feels driven to perform in response to an obsession or according to rules that must be applied rigidly [1]. For many years the disorder was thought to be rare in children and adolescents, but in fact its prevalence is in the range of 1-3% [2], similar to that reported for adults. OCD often starts in childhood and adolescence and can develop into a chronic disorder with high rates of persistence [3]. Approximately 40% of cases starting in childhood continue into adulthood, and the figure increases to 60% when subclinical presentations are considered [4].

OCD is a heterogeneous disorder with possible phenotypical differences. Brain-imaging and genetic studies [5,6] have provided evidence for the biological validity of OCD symptom dimensions [7], and this line of research may aid in the identification of more robust endophenotypes [8]. Another promising approach in relation to reducing phenotypic heterogeneity involves the identification of homogeneous subtypes of OCD based on sociodemographic or clinical characteristics such as age of onset, gender, clinical course and comorbidity [9-11].

The mean age at onset of OCD in adults is around 19.5 years [12], while the corresponding figure for young people is about 11.5 years [13]. Several authors have proposed a bimodal distribution for age at OCD onset [14-16], suggesting differences between early-onset and late-onset groups in terms of gender distribution, familial loading or the pattern of comorbidity. Early onset of OCD is more common in males, more familial, and is associated with a high prevalence of tic disorders in both OCD patients and in their first-degree relatives [15,21,22]. These findings have led some authors to suggest that childhood-onset OCD may be a neurodevelopmental subtype of the disorder [18,19], or a different phenotype of OCD [23,24].

The comorbidity of OCD and tic disorders, principally Tourette syndrome, has long been recognized in the clinical literature and appears to be bidirectional, affecting 20-30% of individuals with each disorder [25]. Clinical correlates shared by OCD and tic disorders include early onset, familial occurrence and a chronic fluctuating course [26], as well as repetitive behaviours, intrusive thoughts and sensations, and deficits in behavioural inhibition [27].

Recently, the presence or otherwise of a current or past history of tics had been recognized in DSM-5 as a subgroup of OCD [1]. However, OCD also presents comorbidity with other neurodevelopmental disorders such as attention deficit hyperactivity disorder (ADHD). Around 25-30% of children and adolescents with OCD, particularly boys with an early onset, also satisfy diagnostic criteria for ADHD [28,29], while the rate of OCD among children with ADHD is estimated to be around 12% [30]. In addition, patients with OCD frequently present both comorbidities, that is, Tourette syndrome or other tic disorders and ADHD [31-33].

Anxiety disorders and depressive disorders also show high comorbidity with OCD. Between a third and a half of children with OCD suffer from an anxiety disorder during the course of their OCD [34,35]. Studies suggest that some anxiety disorders, especially generalized anxiety disorder (GAD), are relatively common in people with OCD and their first-degree relatives [36-37]. As regards comorbidity with depression, the reported rates of this disorder among children and adolescents with OCD vary widely, from 16% [38] to as high as 73% [35]. Whatever the figure, follow-up studies indicate that a depressive disorder frequently accompanies the course of OCD, and it is one of the most prevalent comorbid diagnoses upon reaching adulthood [39].

Current research suggests that OCD and two of the most frequent neurodevelopmental disorders, ADHD and tic disorders, could have both unique and shared genotypic and phenotypic characteristics [28,40,41]. In this context, it is also worth noting one of the most influential contemporary models of psychiatric comorbidity, the internalizing/externalizing model, which organizes diagnoses on the basis of their associations with underlying liabilities. One of the higher-order liability factors, termed 'internalizing', represents mood and anxiety psychopathology [42], two of the disorders most frequently comorbid with OCD. In addition, studies that have examined gender in relation to OCD indicate that boys predominate among those with early onset, and also that whereas neurodevelopmental disorders are more common in males, internalizing disorders are more frequent in females. In light of these findings, the aim of this study was to examine the sociodemographic characteristics and clinical features of OCD patients in relation to the presence or absence of another comorbid disorder. To this end we assessed clinical variables such as age at onset, duration of illness, family history of OCD or other psychiatric disorders, severity of symptoms, symptom dimensions, functional impairment,

and pharmacological treatment and need for hospitalization. Our a priori hypotheses were that OCD patients with a comorbid neurodevelopmental disorder would have an earlier age at onset, more family history of OCD, present the symmetry and ordering dimension, show poorer functional impairment, and require more pharmacological treatment than would OCD patients without comorbidity. In addition, patients with comorbid internalizing disorders would have an older age at onset, present the harm avoidance and checking dimension, show poorer functional impairment, and require more pharmacological treatment and hospitalization than would patients without comorbidity. Finally, boys would have an earlier age of onset than girls and would more frequently present a comorbid neurodevelopmental disorder.

**Methods**

**Participants**

The sample comprised 112 children and adolescents of both sexes and ranging in age from 8 to 18 years. They had all been diagnosed with OCD according to DSM-IV criteria [43] following referral to a specialist OCD unit at the Hospital Clinic of Barcelona. OCD was the main diagnosis in all cases. The sample was recruited naturalistically over the last four years, and included patients already under treatment and new patients who entered treatment during this period. New patients were recruited within four months of their first being seen at the OCD unit, although no fixed criterion was established for the time of inclusion; this was because all the children and parents in the present sample had already been recruited for a larger study that included genetic and neuroimaging scan, and thus it was merely necessary to obtain their consent for inclusion in the study reported here.

The Schedule for Affective Disorders and Schizophrenia for School-age Children-Present and Lifetime Version (K-SADS-PL) interview [44] validated in Spanish [45] was used to confirm the main and any additional diagnoses. This interview was administered with both parent(s) and the child as informants. All patients received cognitive-behavioural therapy (CBT) and/or naturalistic pharmacological treatment following our own clinical guidelines, which are based on internationally recognized clinical guidelines [46,47].

The procedures were approved by the hospital's Ethics Committee. Written informed consent was obtained from all subjects and families once the procedures involved had been

explained to them. All researchers who collaborated in the study were obliged to preserve at all times the anonymity of patients and controls, and to use the information collected solely for the purposes indicated.

**Clinical assessment**

Diagnostic assessment was first conducted by three experienced psychiatrists. Then, two researchers with experience in the use of semi-structured diagnostic instruments administered the K-SADS to assess past and current comorbidity. Detailed sociodemographic and clinical information was gathered from patients and their parents during the diagnostic and assessment interview. Socioeconomic status of the whole sample was estimated with the Hollingshead Redlich Scale [48]. Age at onset was defined as the age at which patients first displayed significant distress or impairment associated with obsessive-compulsive symptoms.

The severity of OCD symptoms was assessed by means of a semi-structured interview, the *Children's Yale-Brown Obsessive-Compulsive Scale (CY-BOCS)* [49]. The responses of OCD patients on the CY-BOCS were categorized dichotomously (present/absent). The principal or most prominent obsession and compulsion were also recorded. In accordance with the factor analysis carried out by Leckman et al. [50] we computed CY-BOCS data for four separate obsessive-compulsive symptom dimensions (aggressive, sexual, and religious obsessions and checking compulsions; symmetry, ordering, counting, and arranging obsessions and compulsions; contamination obsessions and cleaning compulsions; and hoarding) for each subject. A total severity score, ranging from 0 to 40, was also obtained, with a higher score indicating greater severity. As a further measure of OCD we administered the *Obsessive-Compulsive Inventory Child Version (OCI-CV),* a 21-item questionnaire which assesses the presence of obsessive-compulsive symptoms in children and adolescents over the past month [51].

Depressive symptomatology was assessed by a validated instrument with proven reliability, the *Children's Depression Inventory (CDI)* [52]. This 21-item self-report measure assesses the severity of depressive symptoms in children and adolescents, yielding a score between 0 and 63.

The *Screen for Childhood Anxiety Related Emotional Disorders (SCARED)* is a child and parent self-report instrument used to screen for childhood anxiety disorders including general anxiety disorder, separation anxiety disorder, panic disorder and social anxiety disorder. It also assesses symptoms related to school phobias. The *SCARED* consists of 41 items and 5 factors that parallel the *DSM-IV* classification of anxiety disorders [53].

Three groups were established according to the primary comorbid diagnosis accompanying that of OCD. The first group was defined as OCD alone, in other words, patients without comorbidity. In the second group, the comorbid diagnosis concerned a neurodevelopmental disorder: Tourette syndrome and other tic disorders and/or ADHD. Finally, the third group of patients presented a comorbid internalizing disorder: anxiety disorder or mood disorder. Patients with a comorbid eating disorder (N=6) were included in this third group, based on the hypothesis that eating disorders would load onto the internalizing latent factor [54].

The *MINI International Neuropsychiatric Interview* (Spanish Version 5.0 - DSM-IV) [55] was used to assess current major Axis I diagnoses in parents. In addition, the parents of patients were asked during the clinical interview about psychiatric disorders in second-degree relatives.

**Statistical Analyses**

A database was created in SPSS 18.00 (SPSS Inc., Chicago ILL, USA) in order to collect all the sociodemographic and clinical data of patients. Results are presented as means and standard deviations (±SD). Chi-squared tests were used to compare categorical variables between groups, while independent sample t-tests were employed for the analysis of continuous variables. An ANOVA was used to explore the relationship between the clinical continuous variables and the three different groups of comorbidity. Post-hoc comparisons were adjusted using the Bonferroni correction. The relationship between the clinical categorical variables and the three different groups of comorbidity was explored by means of a non-parametric test (chi-squared). A density plot was used to examine the frequency of patients by gender and age at onset. A *P*-value < .05 was considered statistically significant for all comparisons.

**Results**

**Clinical characteristics of OCD patients**

The mean age of the sample was 14.7 years (SD = 2.5) and the mean age at onset of OCD was 12.1 years (SD = 2.7). There were 57 boys (50.9%) and 55 girls (49.1%). The mean duration of the disorder prior to assessment was 23.9 months (SD = 24.2), with a range from 6 months to 137 months. The mean symptom severity score at the time of assessment with the CY-BOCS was 17.5 ± 7.9, as compared with 26.2 ± 6.5 in the initial visit to the OCD unit. Regarding OCD dimensions, in 67 cases (59.8%) the principal dimension was harm avoidance and checking, in 26 cases (23.2%) it was cleanliness and washing, and in 19 cases (17%) it was symmetry and ordering. In terms of family history, 55.4% of patients had no family history of OCD (first or second degree), 42.9% had a first-degree relative with a history of OCD, and 18.8% had a second-degree relative with a diagnosis of OCD. As for family psychiatric history aside from OCD, 53.6% of patients had a first-degree relative with a history of mental illness, 49.1% had a second-degree relative with such a history and 25.9% had no family history of psychiatric disorders.

Another Axis I diagnosis was observed in 67% of patients: Tourette syndrome (8%, n=9), chronic tic disorder (1.8%, n=2), transient tic disorder (1.8%, n=2), attention deficit hyperactivity disorder (8.9%, n=10), generalized anxiety disorder (26.8%, n=30), social anxiety disorder (4.5%, n=5), panic disorder (1.8%, n=2), major depressive disorder (3.6%, n=4), depressive disorder not otherwise specified (1.8%, n=2), hypomania (1.8%, n=2), bipolar disorder not otherwise specified (0.9%, n=1), anorexia nervosa (4.5%, n=5), and eating disorder not otherwise specified (0.9%, n=1). Twenty-six patients (23.2%) had a second comorbid diagnosis: 1 (0.9%) ADHD, 11 (9.9%) anxiety disorder (3 GAD, 1 separation anxiety disorder, 1 panic disorder, 2 simple phobia, 1 agoraphobia, 2 social phobia and 1 post-traumatic stress disorder), 2 (1.8%) major depressive disorder, 6 (5.4%) anorexia nervosa, 5 (4.5%) oppositional defiant disorder and 1 (0.9%) enuresis. Five patients (4.5%) had a third diagnosis (2 major depressive disorder, 1 panic disorder, 1 simple phobia and 1 oppositional defiant disorder). The second and third diagnoses were not in an acute phase of the disorder. Patients with an eating disorder were not underweight. The six patients had marked anxious and depressive symptomatology, as evidenced by their scores on the psychopathological scales administered

(CDI: mean = 16.5, SD = 9.13; SCARED: mean = 37.75, SD = 16.82), which were in line with those obtained in the group of internalizing comorbidity as a whole. The patients with a mood disorder were stable in terms of psychopathology. Patients whose second comorbid diagnosis was oppositional defiant disorder were assigned according to their first comorbid diagnosis. The Venn diagram in Figure 1 shows the comorbidities in the sample.

With regard to pharmacological therapy, 22.3% (n=25) of patients were not receiving any medication, while 77.2% (n=87) were receiving SSRIs [34.8% (n=39) fluoxetine, 17% (n=19), sertraline, 9.8% (n=11) fluvoxamine and 0.9% (n=1) escitalopram] or serotonin reuptake inhibitors (SRIs) [clomipramine, 12.5% (n=14)]; three patients were receiving a combination of fluoxetine + clomipramine. Forty-four patients (39.3%) were receiving adjunctive psychotropic medication in addition to SSRIs or SRIs: 25% (n=28) were receiving antipsychotics, 9.8% (n=11) benzodiazepine, 0.9% (n=1) lithium carbonate and 3.6% (n=4) methylphenidate.

**Comparison of OCD patients without comorbidity, with externalizing comorbidity and with internalizing comorbidity**

Demographic and clinical characteristics of the three groups are shown in Table 1. There were significant differences between the groups with regard to gender (p=.031), age (p<.000) and age at onset (p<.001). A family history of OCD was significantly more common in patients with comorbid neurodevelopmental disorders than in the other groups (p=.049). There were also significant differences between the three comorbidity groups in terms of OCD dimensions (p=.022). Psychopharmacological treatment was prescribed significantly more often in patients with comorbidity than in those without comorbidity (p=.012). The need for hospitalization due to the interference and limitations produced by OCD symptoms was significantly greater in the two groups with comorbidity than in the group without it (p=.011). Post-hoc comparisons adjusted by Bonferroni correction and between-group comparisons of categorical variables indicated the direction of these differences (Table 1).

**Relationship between gender and other clinical variables**

There was no significant relationship between age at onset and sex (boys: 11.9 ± 2.5, girls: 12.4 ± 3.0, t = -.902; p = .369). Figure 2 show a density plot with the distribution of the

patients according to gender and age at onset. On the other hand, a trend among males for lower age at onset of first symptoms (boys: 9.5 ± 3.7, girls: 10.8 ± 3.3, t=-1.878; p=.063) and a longer duration of the disorder (boys: 28.1 ± 24.7, girls: 19.7±23.1, t=1.858, p=.066) were found. No significant differences by gender were observed for the other clinical variables: severity of symptoms, family history of OCD or other psychiatric disorders, symptom dimensions or treatment needed.

**Discussion**

Our findings highlight the high rates of comorbidity in a paediatric sample of OCD patients diagnosed by means of a semi-structured clinical interview. Overall, 67% of these young patients presented comorbidity; 23.2% of them had two comorbid diagnoses and 4.5% three such diagnoses besides OCD. Regarding the kind of comorbidity, 20.5% of patients presented a comorbid neurodevelopmental disorder: Tourette syndrome or other tic disorders and/or ADHD. This group had a higher proportion of males, a significant family history of OCD in parents, and symmetry and ordering as the most common symptom dimension. A second group, comprising 46.4% of patients, presented internalizing comorbidity, most frequently anxiety disorders, and especially GAD. In this group, the age of onset of OCD was later, and harm avoidance and checking was the most common symptom dimension. Although the initial severity was similar in all the groups, the need for pharmacological treatment and hospitalization due to OCD symptomatology was greater in groups with some form of comorbidity than in the group without it.

Numerous studies have reported higher levels of comorbidity in younger age-at-onset OCD patients [19,28,31,32,34,35,56,57]. However, the present study indicates not only a higher prevalence among these patients but also more comorbid diagnoses in a substantial number of them, highlighting the complexity of this disorder. It is clearly important to identify possible explanations for the high rates of comorbidity with OCD. One possibility is that OCD shares a common aetiology with these disorders, or that they are all epiphenomena of the same core condition that is expressed in a variety of ways. Alternatively, OCD may increase vulnerability for the development of additional disorders [58,59].

One fifth of the sample presented a neurodevelopmental disorder. The proportion of boys and the presence of a family history of OCD were both greater in this subgroup of comorbidity, as has been previously reported in the literature [18-20]. Brain imaging studies of tic disorder and childhood-onset OCD consistently point to involvement of cortico-striato-thalamocortical (CST) circuits in both disorders [60-62]. Moreover, family genetic studies suggest there are likely shared genetic vulnerabilities between OCD and Tourette syndrome [63,64], and the fact that the two disorders have similar psychopathological and neuropsychological aspects supports the idea that are closely affiliated [65,66]. Attention deficit hyperactivity disorder is also commonly observed in subjects with early-onset OCD. Geller et al. [67] wondered whether inattention in children with OCD might be an artefact of intrusive obsessional thoughts or anxiety rather than true ADHD. However, they and other authors concluded that ADHD symptoms were a true comorbid state independent of the OCD diagnosis, not least as they usually precedes the onset of OCD [67,68]. It has been hypothesized that OCD-ADHD may represent one of the most significant differential features in early-onset OCD [29], and comorbidity of ADHD in early-onset OCD seems to predict increased severity of OCD and greater persistence of obsessive-compulsive symptoms over a prospective follow-up period [68], suggesting that this comorbid status is clinically meaningful. As in the case of tic disorders, structural and functional imaging studies have revealed converging abnormalities in ADHD and OCD, with a failure of CST circuit function responsible for processes of cognitive control and performance monitoring [69]. Thus, OCD, tic disorders and ADHD are highly comorbid because they at least partially share a common aetiology. Hence, it has been suggested that OCD, ADHD and Tourette syndrome constitute a group of developmental basal ganglia disorders [70].

Our results also show that nearly a half of the sample presented anxiety or mood disorders, with prevalence being higher for the former; moreover, the presence of more than one comorbid anxiety disorder was relatively common. Although patients did fulfil the diagnostic criteria for several diagnoses, the assignment of two or three diagnoses at such an early age is far from satisfactory. Indeed, it is possible that at these ages, the co-existence of OCD and anxiety disorders, especially GAD, represents symptom overlap or proximity of the two diagnoses rather than true comorbidity, and the finding could be partially due to a failure in our artificial symptom breakdown and classification of disorders. Although GAD is characterized by

uncontrollable worries and OCD by obsession and compulsion, they also have common features. In both disorders, thoughts are exaggerated, persistent, unwanted and unbidden, and patients must make an effort to resist them. Young people with either disorder may also be more likely to exhibit hypervigilance with respect to their thoughts and to interpret their thoughts in a superstitious manner. Disorders that include hyperfocus on thoughts or the avoidance of cognitive stimuli are often difficult to separate. It should also be noted that GAD occurs more frequently among relatives of OCD patients, regardless of whether OCD is also present in these relatives [37]. This finding suggests that there is a common familial aetiology for OCD and GAD and that both may be part of the same phenotypic spectrum [38]. Patients with OCD may also have increased liability for depression due to the demoralizing effects of the disorder, and depression may therefore emerge secondary to OCD for some individuals. However, some comorbid depressive or bipolar disorders along the spectrum may also be conditions with a shared genetic susceptibility.

Our patients with internalizing comorbidity were, on average, older than those in the other groups, a finding that could be due to the fact that rates of comorbid anxiety disorders increase steadily over the course of adolescence and adulthood [71]; this is similar to the case of comorbid depression, which as follow-up studies [39] show, also occurs more often in the course of OCD. There is a temporal order of comorbid conditions in OCD across the lifespan, and the periods in which these conditions started may have an impact on the clinical profile and future development of each disorder. Tic disorders, ADHD and separation anxiety tend to precede the onset of OCD, whereas anxiety disorders, mood disorders and eating disorders, among others, tend to exacerbate later on during the OCD course [72].

Our findings indicate that comorbidity in OCD is associated with higher rates of pharmacological treatment. In addition, the need for admission due to the severity of OCD and the interference caused by it was greater in patients with comorbid disorders, regardless of the type of comorbidity. Given that a substantial proportion of comorbid disorders are observed among children and adolescents with OCD, our findings could have important clinical and therapeutic implications and could inform the clinician's decision-making process. Although the presence of anxiety or depressive disorders does not have a dramatic impact on the choice of pharmacological treatment, the presence of other disorders such as ADHD, tic disorders or

bipolar disorder can substantially influence the decision. Moreover, most children will likely need combined pharmacotherapy as a complement to CBT. In this context, it is important that the comorbid disorder is first stabilized before implementing CBT for the OCD, as failure to do so could undermine the benefits of psychotherapy. Regarding OCD patients with a disruptive behaviour disorder, the fact that this is frequently related with a greater accommodation of rituals represents a handicap for standard treatment [73]. Furthermore, the presence of depressive disorders may be associated with reduced anxiety habituation during exposure, as well as decreased hope that treatment might work or diminished motivation to engage in exposure [74]. From a clinical point of view, more systematic research on the role of comorbidity in clinical resistance profiles would be useful and could help to modify therapeutic protocols so as to include individualized options for different comorbidities. Likewise, the accurate identification of each syndrome could lead to a more successful treatment approach for children and adolescents with comorbid disorders.

Finally, regarding gender, although we observed a peak of boys in the pre-pubertal period, no significant differences by sex were found in the age of onset; there was merely a trend towards younger age at onset of first symptoms in boys compared with girls. It should be noted, however, that the recorded age at onset of first symptoms may not be accurate in many patients, since it does not necessarily coincide with the date of formal diagnosis. Moreover, the group of patients with comorbid neurodevelopmental disorders was mostly comprised of males, whereas females made up a larger proportion of the group with internalizing disorders. Nestadt et al. [37] found that males are over-represented in the OCD comorbid tic-related class, while females are more likely to present OCD with comorbid affective-related conditions, it being plausible that there are aetiologically distinct forms of male-related and female-related OCD.

**Limitations and Strengths**

This study has a number of limitations that should be taken into account before attempting to extrapolate the results. First, the children and adolescents in this sample had been referred to a specialist OCD clinic and this may have produced an ascertainment bias, such that the results might not be generalizable to community samples. The second potential limitation is that the data analysed were collected as part of a wider genetic and neuroimaging study and this meant

that subjects were included at different points in the course of their disorder. Third, some relatively frequent types of comorbidity, such as autism spectrum disorder, have not been reported in this study. In these cases it was considered that the main diagnosis was always this disorder and not the OCD. In addition, comorbidity with other neurodevelopmental disorders such as learning disorders was not studied because these were not included in the diagnostic interview used. The main strengths of this study are the use of a semi-structured clinical interview to support all diagnoses. Furthermore, the interdisciplinary team were experienced in the use of the structured interview for adults, which in this study was conducted with parents.

**Conclusions**

The results of this study involving young patients with early-onset OCD suggest that two subtypes can be defined on the basis of comorbid pathology. One OCD subtype is characterized by childhood onset, a predominance of males, high familial aggregation and comorbid neurodevelopmental disorders. The second subtype is phenotypically more related to anxiety/depressive disorders, is more common in females and has a later onset during adolescence. Unifying comorbidity criteria across OCD studies would help to clarify specific subgroups. Longitudinal studies across the life-span are required to identify other adult-related comorbidities and to correlate them with those presented at earlier stages. The identification of different subtypes according to comorbidity is potentially useful not only for understanding clinical variations but also with respect to tailoring treatment and the use of therapeutic resources. Future research trials of new treatments should aim to include real OCD populations, without excluding comorbidities, in order to obtain more useful clinical conclusions and propose definitions of resistance to treatment based on comorbidities.

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**Ethical standards**

All human studies have been approved by the ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

**Conflicts of interest**

No authors have any conflict of interest related to this study.

Figure 1. Venn diagram of comorbidities in our pediatric OCD sample

OCD, obsessive-compulsive disorder; TD, Tourette's disorder, chronic tic disorder and transient tic disorder; ADHD, attention-deficit/hyperactivity disorder ; AxD, anxiety disorders; AfD affective disorders; En, enuresis; AN, anorexia nervosa; BN, bulimia nervosa; ODD, oppositional defiant disorder.

Figure 2. Distribution of age at onset in our sample divided by gender

X-axis: age at onset (years); Y-axis: density.

Figure 1
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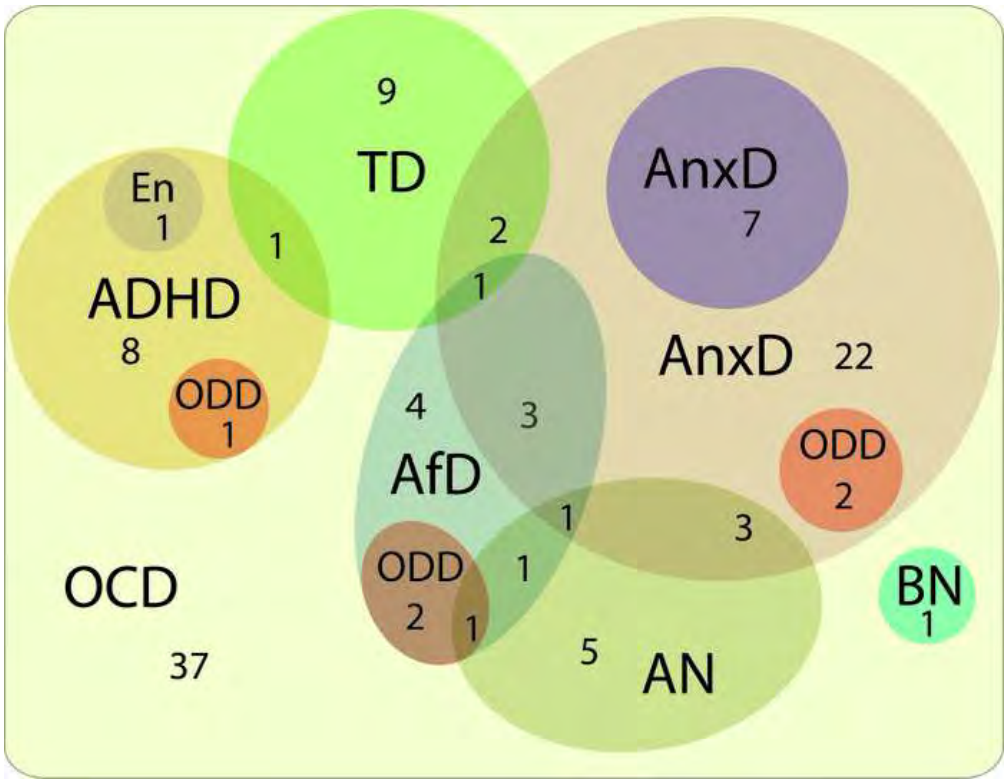


Figure 2
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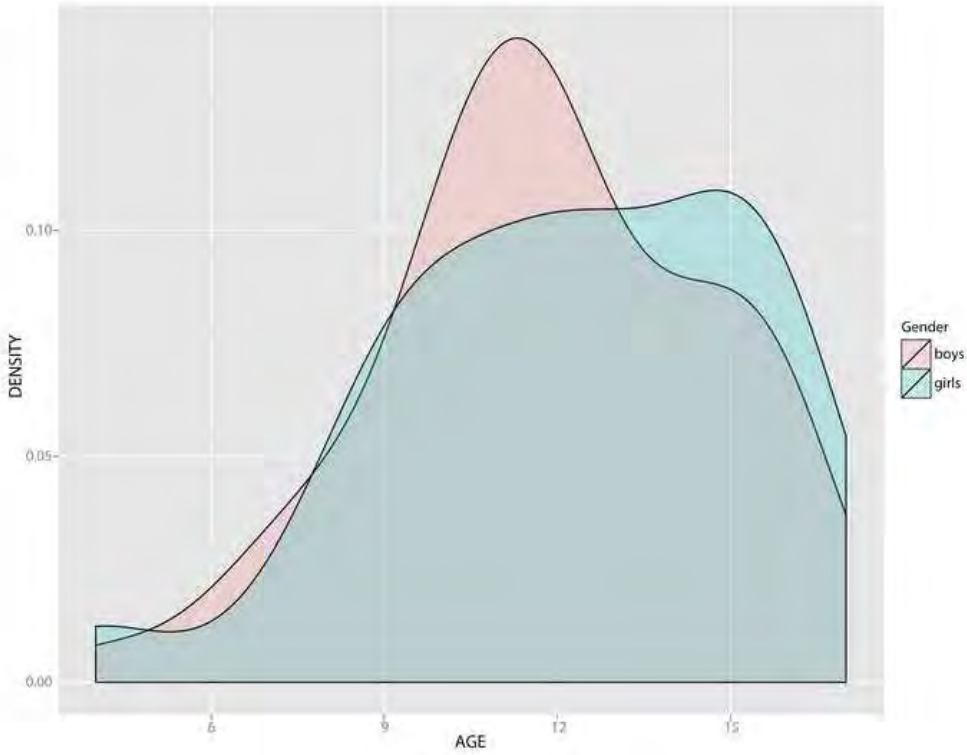

Table 1

Table 1. Comparison of demographic and clinical characteristics of patients with OCD without comorbidity, neurodevelopmental disorder comorbidity and internalizing disorders comorbidity.

| | Without comorbidity (n=37) Group A | | Neurodevelopment disorders comorbidity (n=23) Group B | | Internalizing comorbidity (n=52) Group C | | ANOVA / $X^{2*}$ *P* | | Post-hoc |
|---|---|---|---|---|---|---|---|---|---|
| | Mean (SD) | N (%) | Mean (SD) | N (%) | Mean (SD) | N (%) | | | |
| Gender (boys) | | 21 (56.7) | | 16 (69.5) | | 20 (38.4) | 6.933* | .031 | B>C .013 |
| Age | 13.7 (2.4) | | 13.9 (2.3) | | 15.8 (2.3) | | 10.350 | .000 | A<C .006; B<C .000 |
| Socioeconomic status (SES) | 48.5 (16.2) | | 50.1 (14.5) | | 46.4 (15.8) | | .450 | .639 | |
| Age of first OCD symptoms | 9.6 (3.5) | | 9.3 (3.1) | | 10.9 (3.6) | | 2.423 | .093 | |
| Age at onset | 11.2 (2.6) | | 11.4 (2.4) | | 13.2 (2.6) | | 7.656 | .001 | A<C .002; B<C .019 |
| Duration of disease | 22.1 (26.0) | | 23.1 (20.7) | | 25.7 (24.7) | | .254 | .776 | |
| Severity | | | | | | | | | |
| - CY-BOCS | | | | | | | | | |
| * Current | 17.5 (7.8) | | 17.3 (8.4) | | 17.6 (8.0) | | .015 | .985 | |
| * Major severity | 27.1 (7.1) | | 24.4 (5.1) | | 26.3 (6.6) | | 1.321 | .271 | |
| - OCI-CV | 14.0 (8.1) | | 12.6 (7.7) | | 15.3 (8.1) | | .766 | .468 | |
| - CDI | 12.1 (8.5) | | 12.2 (10.2) | | 16.2 (10.6) | | 2.170 | .120 | |
| - SCARED | 25.7 (12.6) | | 19.6 (12.8) | | 32.2 (13.9) | | 6.662 | .002 | B<C .002 |
| Family history | | | | | | | | | |
| - OCD of 1st degree | | 6 (16.2) | | 9 (39.1) | | 9 (17.3) | 6.038* | .049 | B>A .035; B>C .031 |
| - OCD of1st and 2nd degree | | 17 (45.9) | | 13 (56.5) | | 24 (46.1) | 1.198* | .549 | |
| - Other psychiatric disease (1st degree) | | 19 (51.3) | | 10 (43.4) | | 31 (59.6) | 1.686* | .430 | |
| - Other psychiatric disease (1st and 2nd degree) | | 26 (70.2) | | 18 (78.2) | | 38 (73.0) | .985* | .611 | |
| Main dimension | | | | | | | | | |
| - Washing and contamination | | 12 (32.4) | | 6 (26.0) | | 8 (15.3) | 2.718* | .257 | |
| - Harm avoidance and checking | | 20 (54.0) | | 9 (39.1) | | 38 (73.0) | 7.099* | .029 | C>B .005 |
| - Symmetry and ordering | | 5 (13.5) | | 8 (34.7) | | 6 (11.5) | 7.591* | .022 | B>C .017 |
| Treatment | | | | | | | | | |
| - Pharmacological treatment | | 23 (62.1) | | 20 (86.9) | | 45 (86.5) | 8.838* | .012 | B>A .038; C>A .008 |
| - Hospitalization | | 3 (8.1) | | 8 (34.7) | | 18 (34.6) | 9.108* | .011 | B>A .009; C>A .004 |
| - Day-patients | | 7 (18.9) | | 7 (30.4) | | 16 (30.7) | 1.745* | .418 | |

OCD: Obsessive-compulsive disorder; CY-BOCS: Children's Yale-Brown Obsessive-Compulsive Scale; OCI-CV: Obsessive-Compulsive Inventory-Child Version; CDI: Children's Depression Inventory; SCARED: Screen for Childhood Anxiety Related Emotional Disorders

### 4.2. Diferencias entre las concentraciones de neurometabolitos entre los pacientes TOC y los controles sanos. Diferencias en las concentraciones de glutamato según el tiempo de evolución de la enfermedad (Objetivos 2 y 2.1)

Se evaluaron las diferencias entre las concentraciones de neurometabolitos entre 47 pacientes TOC y 31 sujetos control sanos. Las variables demográficas y clínicas de los pacientes con TOC y los sujetos control sanos de la misma edad, sexo y CI estimado se muestran en la tabla 1 del estudio 2 (ver página 93). Como era esperable, los pacientes con TOC puntuaron más alto en las escalas de evaluación de la depresión y ansiedad que los sujetos control sanos.

Respecto a los casos, la media de la severidad de los síntomas evaluados con el CY-BOCS fue de 17,33 $\pm$ 9,4. Presentaron otro diagnóstico del Eje I en el 57,4% de los pacientes. Siete pacientes presentaban un segundo diagnóstico comórbido. Con respecto a la terapia farmacológica, el 21,3% (n =10) de los pacientes con TOC no estaban recibiendo ningún psicofármaco en el momento de la evaluación, mientras que el 78,7% (n = 37) eran tratados con ISRS [38,8% (n = 18) fluoxetina, 21,3% (n = 10), sertralina y 8,5% (n = 4) fluvoxamina] o IRS [clomipramina, 10,6% (n = 5)]. Diecisiete pacientes (37,7%) estaban recibiendo otro psicofármaco adyuvante además de ISRS o IRS: 13,3% (n = 6) risperidona, 8,9% (n = 4) quetiapina, 4,4% (n = 2) aripiprazol, 8,9% (n = 4) benzodiacepina y 2,2 % (n = 1), metilfenidato de liberación modificada.

La Tabla 2 del estudio (ver página 93) muestra los porcentajes de SG, SB y el LCR y las concentraciones de metabolitos cerebrales en el CCA de la muestra de pacientes TOC respecto a los controles. Las concentraciones de Ins ajustados por LCR (t= -2,31, DE = 76, p = 0,023) fueron menores en los pacientes con TOC que en los sujetos control sanos. Estas diferencias siguieron siendo significativas después de un análisis de covarianza utilizando como covariables la SG y la edad ($F_{1,74}$ = 5,686; p = 0,02; parcial eta cuadrado = 0,071). No se

encontraron diferencias significativas de los otros neurometabolitos ajustados por LCR en el área estudiada.

Cuando estudiamos las concentraciones de Glx ajustados por LCR en pacientes con o sin tratamiento farmacológico utilizando como covariables la SG y la edad, no se encontraron diferencias significativas ($F_{1,41}$ = 2,527, p = 0,120; parcial eta cuadrado = 0,058). Tampoco obtuvimos diferencias significativas cuando se separaron en grupos según el tipo de tratamiento farmacológico que recibía el paciente: ningún tratamiento, tratamiento antidepresivo o tratamiento antidepresivo y un fármaco antipsicótico como coadyuvante ($F_{2,40}$ = 2,733; p = 0,077; parcial eta cuadrado = 0,120).

Posteriormente realizamos un estudio específico según los meses de evolución de la enfermedad utilizando el punto de corte de 24 meses. Pese a que no hubo diferencias significativas en las concentraciones de Glx ajustados por LCR entre ambos grupos (< 24 meses; > 24 meses y el grupo control sano) utilizando las covariables SG y edad ($F_{2,73}$ = 3,949; p = 0,024; parcial eta cuadrado = 0,058); se realizó una comparación post-hoc aplicando corrección de Bonferroni, resultando que la concentración media de Glx en el CCA en el grupo de larga evolución (M = 10,27, DE = 1,52) fue significativamente menor que en el grupo de corta evolución (M = 11,91, DE = 2,32) [p = 0,019]. Los datos sociodemográficos y neuroquímicos de los grupos según los meses de evolución pueden ser consultados en la tabla 3 del estudio 2 (ver página 94).

**Estudio 2:** 1H-MRS of the anterior cingulate cortex in childhood and adolescent obsessive-compulsive disorder: A case-control study. European Neuropsychopharmacology (2015).

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# $^{1}$H-MRS of the anterior cingulate cortex in childhood and adolescent obsessive–compulsive disorder: A case-control study

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**Abstract**
Abnormal glutamate concentrations in the anterior cingulate cortex (ACC) have been identified in children and adults with obsessive–compulsive disorder (OCD). The purpose of the present study was to measure in vivo $^{1}$H-MRS neurometabolite concentrations in the ACC of children and adolescents with OCD, in order to identify metabolite abnormalities compared to healthy controls and to assess their relationship with clinical variables. 3 T proton-magnetic resonance spectroscopy was used to probe ACC biochemistry in 47 paediatric and adolescent OCD patients (11–18 years old) compared to 31 healthy subjects of similar age, sex and estimated intellectual quotient. There were no significant differences in the concentration of glutamate plus glutamine (Glx) adjusted for CSF between OCD patients and healthy controls [$F_{1,74}$=0.00; $P$=0.943], but there were significant differences in the concentration of Glx adjusted for CSF in paediatric and adolescent OCD patients according to duration of illness (less than or more than

*Abbreviations:* ACC, anterior cingulate cortex; ANOVA, analysis of variance; ANCOVA, analysis of covariance; CDI, child depression inventory; Cho, glycerophosphocholine plus phosphocholine; Cr, creatine plus phosphocreatine; CSF, cerebrospinal fluid; CY-BOCS, children's yale-brown obsessive-compulsive scale; DSM-IV, diagnostic and statistical manual of mental disorders, fourth edition; FWHM, full width at half maximum; Glx, glutamate plus glutamine; GM, grey matter; $^{1}$H-MRS, proton magnetic resonance spectroscopy; Ins, inositol; IQ, intelligence quotient; NAA, N-acetyl-aspartate plus N-acetyl aspartyl glutamate; SCARED, screen for childhood anxiety related emotional disorders; WM, white matter

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24 months) [$F_{2,73}$=3.95; $P$=0.024]. In addition, we found significantly lower levels of myo-inositol adjusted for CSF in the ACC [$F_{1,74}$=5.686; $P$=0.02] in patients compared with controls. The present findings do not confirm the hypothesis of differences in Glx concentrations in the ACC between children and adolescents with OCD and healthy controls; however, the observation of differences in the Glx concentration in children and adolescent OCD patients depending on the duration of illness is of interest.


## 1. Introduction

Obsessive–compulsive disorder (OCD) is a debilitating neuropsychiatric condition characterised by persistent intrusive thoughts (obsessions) and the expression of ritualistic repetitive behaviours (compulsions) which are generally enacted in an effort to alleviate the intense anxiety caused by specific obsessions. OCD is estimated to affect 1–3% of the population (Weissman et al., 1994).

Proton magnetic resonance spectroscopy ($^{1}$H-MRS) enables direct and non-invasive measurements of brain chemistry in vivo in vivo. MRS records a series of peaks or resonances, each representing the local concentration of a different neurometabolite or small family of chemically related neurometabolites. Glx is the abbreviation for glutamate (Glu) plus glutamine (Gln). Glutamine is a precursor to a metabolite of glutamate; glutamate is primarily found in neurons, and glutamine in glial cells. Glx is not only a measure of glutamatergic excitatory neurotransmission (Starck et al., 2008) but also a reflection of the protective function of astrocytes against brain injury.

An emerging body of evidence from animal models, genetics, neuroimaging and clinical trials supports the hypothesis that dysregulation of glutamate neurotransmission may contribute to the pathophysiology of OCD (Ting and Feng, 2008). Glutamate is the primary neurotransmitter within cortico-striatal-thalamic circuits (Bronstein and Cummings, 2001), and the majority of axon terminals in the striatum are glutamatergic (Parent and Hazrati, 1995). Animal studies have shown that 5-HT receptors exert a very complex modulatory control over glutamate-mediated transmission, involving many subtypes of 5-HT receptors (Ciranna, 2006). Following this line of research, some authors have suggested that the alteration of the glutamatergic system may make the greatest contribution to OCD pathophysiology, and that serotonergic and dopaminergic alterations in OCD may be secondary to glutamatergic alterations (Carlsson, 2000).

Rosenberg and Keshavan (1998) were the first to propose role for glutamate in OCD of childhood onset. Three $^{1}$H MRS studies focusing on striatal metabolism were conducted in OCD adolescents before and after undergoing treatment, as well as a cross-sectional study comparing paediatric OCD, mood depressive disorder and a healthy control group. In the first study, increased Glx concentration in the caudate nucleus was observed before treatment in 11 paediatric OCD patients with a mean duration of illness of 14.4 months (SD=15.4) compared to 11 healthy controls; Glx levels normalised after 12 weeks of paroxetine medication 20–60 mg/day. This reduction in Glx concentration correlated with treatment response according to CY-BOCS scores (Rosenberg et al., 2000). In another non-controlled study of 21 paediatric OCD patients, with a mean duration of illness of 38.88 months (SD=38.4), no changes were found after CBT treatment in any of the neurochemical compounds in the left caudate nucleus (Benazon et al., 2003). In 2004, Rosenberg et al.'s study of a sample of 14 children with OCD revealed lower Glx levels in the ACC in patients regardless of medication status. The mean duration of illness was 46.44 months (SD=41.52 months). A cross-sectional and longitudinal study by our group did not find significant differences in Glx compound concentration in the anterior cingulate–medial frontal cortex or in the right and left striata in 11 drug-naïve OCD patients with a mean duration of illness of 24 months either before or after six months of treatment in comparison with 12 healthy controls (Lázaro et al., 2012).

The anterior cingulate cortex (ACC) has been suggested to be involved in OCD, due to its role in error detection and monitoring and in the processing of conflicting information (Gehring et al., 2000; Van Veen and Carter, 2002). Glutamate has an important role in the ACC, where concentrations of Glu receptors are high compared with other neurotransmitter binding sites (Bozkurt et al., 2005). Taken together, these findings suggest a possible tonic-phasic dysregulation of glutamergic compounds within the cortico-striatal circuitry in OCD, with reduced tonic glutaminergic compounds in the ACC combined with phasic overactivity in the striatum and orbitofrontal cortex (Rosenberg et al., 2004). Moreover, with regard to the effect of pharmacotherapy, previous studies in adults have suggested that medication status does not affect MRS variables (Yücel et al., 2008). In children, Glx levels decreased in the ACC of psychotropic-naïve patients with OCD in comparison to controls after effective treatment with the selective serotonin reuptake inhibitor (SSRI) paroxetine (Rosenberg et al., 2000).

The purpose of the present study was to measure in vivo $^{1}$H-MRS 3-Tesla neurometabolite concentrations in the ACC of children and adolescents with OCD, in order to identify metabolite abnormalities in a region of fronto-subcortical circuits in comparison to children and adolescent healthy controls. We hypothesised that Glx levels in the ACC would be lower in OCD patients than in healthy controls. We also examined the relationship between concentrations of neurometabolites and duration of illness. We hypothesised that there would be no differences in the neurometabolite concentrations between child and adolescent OCD patients who received pharmacological treatment and those who did not.

## 2. Experimental procedures

### 2.1. Participants

We explored a total of 66 OCD patients and 37 controls. In three cases, the examination was terminated due to claustrophobia before the $^{1}$H MRS measurement was performed. One healthy control was excluded due to a low-grade glioma. We excluded 16 cases and 5 controls who presented low quality images. The final sample comprised 47 children and adolescents and 31 healthy controls of both sexes, ranging in age from 11 to 18 years. All patients had a current diagnosis of OCD according to DSM-IV criteria (American Psychiatric Association, (2000)). The Schedule for Affective disorders and Schizophrenia for School-age Children—present and lifetime version (K-SADS-PL) interview (Kaufman et al., 1996), validated in Spanish (Ulloa et al., 2006) and adapted to Spain by Cesar Soutullo (University of Navarre), was administered with both parent(s) and the child as informants. Exclusion criteria were psychiatric comorbidity with psychotic disorder, Tourette's syndrome, autism spectrum disorder, somatic or neurological illness, and intelligence quotient (IQ) $<$ 70. The standard score for vocabulary on the Wechsler Scale was used to estimate IQ. All patients received naturalistic pharmacological treatment with antidepressants and/or cognitive-behavioural therapy. None of the patients had participated in the previous proton magnetic resonance spectroscopy study conducted by our research group.

The healthy control group was recruited from several schools in the same geographical region. The standard score on the vocabulary subscale of the Wechsler Scale was used to estimate IQ. OCD cases and healthy controls were matched by age, sex, handedness and IQ. Subjects with personal history of neurological and psychiatric disorders were excluded. The neuroimaging protocol was the same as in OCD patients.

Patients with contraindications for magnetic resonance imaging (e.g., those with orthodontic braces) were also excluded.

The procedures were approved by the hospital's Ethics Committee. Written informed consent was obtained from all parents, and verbal informed consent was given by all subjects following explanation of the procedures involved. The researchers undertook to preserve the anonymity of patients and controls at all times and to use the information collected solely for the purposes indicated. All participants received reimbursement as compensation for their time.

### 2.2. Clinical assessment

Diagnostic assessment was conducted by three psychiatrists with experience of the OCD diagnosis and clinical evaluations, especially with regard to the use of semi-structured diagnostic instruments. The semi-structured interview cited above (the K-SADS) was administered to assess past and current comorbidity.

To assess OCD symptom severity, the Children's Yale-Brown Obsessive-Compulsive Scale (CY-BOCS) (Scahill et al., 1997), a semistructured interview, was used. The maximum score is 40 points: scores between 16 and 20 indicate mild impairment, 21 and 30 moderate, and 30 and 40 severe.

Depressive symptomatology was assessed with the Children's Depression Inventory (CDI) (Kovacs, 1985) a validated instrument with proven reliability. The CDI is 21-item self-report used to assess the presence and severity of depressive symptomatology with a score range from 0 to 63 points.

The Screen for Childhood Anxiety Related Emotional Disorders (SCARED) is a child and parent self-report instrument used to screen for childhood anxiety disorders including general anxiety disorder, separation anxiety disorder, panic disorder, and social anxiety disorder. It also assesses symptoms related to school phobias. The SCARED consists of 41 items and 5 factors that parallel the DSM-IV classification of anxiety disorders (Birmaher et al., 1997).

Psychiatric diagnosis was ruled out in control subjects by means of the same semi-structured interviews.

### 2.3. $^{1}$H-MRS study

$^{1}$H-MRS was acquired in a 3 T TIM TRIO scanner (Siemens, Erlangen, Germany) using a 32 head channel coil. A volume of interest (VOI) of 3 cm$^{3}$ ($15 \times 20 \times 10$ mm$^{3}$) was determined in the ACC. The voxel was placed using two planes from the high-resolution T1-weighted image obtained previously. First, the voxel was placed in the sagittal plane, above the genu of the corpus callosum and centred on the ACC. Confirmation that the ACC was in the voxel was then made in the axial plane. This procedure was applied in the same manner in all subjects and care was taken to ensure standard placement. Figure 1 shows the representative voxel placement. Spectra were acquired with the use of a double-spin echo point-resolved spectroscopy sequence (PRESS) with $TR$=200 ms and $TE$=35 ms.

Metabolite concentrations were quantified by means of the user-independent frequency domain-fitting programme (LCModel) (Provencher, 2001) version 6.1-4A, applying the eddy current correction and using internal water signal reference to calculate absolute metabolite concentrations; a typical LCModel spectrum and fitting are shown in Figure 2. Some metabolites are difficult to quantify independently from others, so the sum of concentrations was used. The metabolites of the basis set for LCModel were inositol (Ins), glycerophosphocholine (GPCh)+phosphocholine (PCh), NAA +N-acetyl aspartate glutamate (NAAG), Cr+PCR (Cr), glutamate

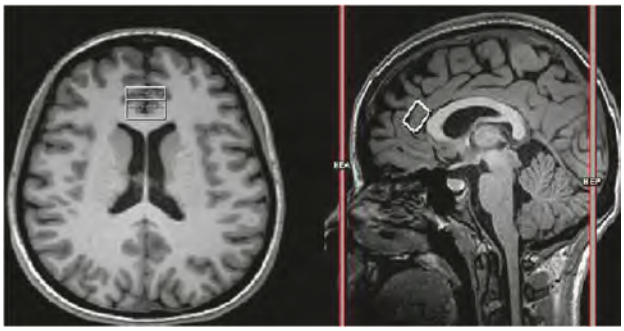

**Figure 1** A structural image (3d T1-weigthed MPRAGE sequence) indicating the representative voxel placement.

(Glu)+glutamine (Gln). We only considered the absolute metabolite values with a Cramer–Rao lower bound below 20%, indicating that these metabolites could be reliably estimated (Provencher, 2001), and a signal-to-noise ratio greater than 10. We excluded 16 cases and five healthy controls for these reasons. An additional structural image (3d T1-weighted MPRAGE sequence with isometric voxel of $1 \times 1 \times 1$ mm$^3$) was recorded in the same scanning session. The structural image was segmented using customised tissue probability maps (TPMs) and following the New Segmentation model provided in SPM8 (Ashburner et al., 2009). To generate customised TPMs, WM and GM images obtained from standard segmentation were normalised to MNI using DARTEL (Ashburner, 2007) at a resolution of $2 \times 2 \times 2$ mm$^3$, and averaged and smoothed with a Gaussian kernel of 8 mm. The metabolite concentrations were corrected for differences in CSF content of the VOI using home-made software. The residual percentage of GM in the VOI, after removing the CSF component, was used as a confounding variable in the statistical analyses (Guerrini et al., 2009).

The $^{1}$H MRS parameters used for the study provided robust signals for both the healthy control and OCD groups. Specifically, healthy controls had an ACC signal-to-noise ratio of 14.45 (SD=2.790) and a FWHM (full width at half maximum) of 0.041 ppm (SD=0.010). OCD patients had an ACC signal-to-noise ratio of 14.4 (SD=2.39) and a FMHW of 0.042 ppm (SD=0.009). None of these measures presented differences between the two groups ($p$=0.936 and 0.505 respectively), suggesting that the quality of the data was comparable.

A neuroradiologist confirmed that all MRI scans were free of gross structural abnormalities.

### 2.4. Statistical analysis

A database was designed using SPSS 18.00 (statistical analysis software, SPSS Inc., Chicago Il, USA) which included all the socio-demographic and clinical data of the patients. The results are presented as means and standard deviations ($\pm$SD). The student-$t$ test was used to compare continuous clinical and neurochemical variables between healthy controls and OCD patients. The effect on the metabolite measurements of drug use, sex, age, duration of illness (understood as time in months since the age at disease onset) and level of severity as measured by the CY-BOCS was studied using standard multiple regression. Sex, drug use, level of severity and months of duration of the disease had no effect on metabolite measurements. In contrast, age did have an effect on total Cho ($\beta$=0.564; $t$=3.7; $p$=0.001) and total Cr ($\beta$=0.384; $t$=2.4; $p$=0.022). In addition, many previous reports have demonstrated the age-dependent development of grey matter (e.g. Franke et al., 2012). For this reason, we used age as well as the percentage of GM in the ACC as covariate in the analysis. A $P$-value of 0.05 was considered statistically significant for all comparisons.

An ANCOVA was used to explore the relationship between the neurometabolite concentrations in OCD patients who received pharmacological treatment and those who did not. Finally, also using an ANCOVA, we further examined the differences in the neurometabolite concentrations between OCD patients with different duration of illness. Before studying the variable "months of duration of illness" as a continuous variable, we assessed its normality with the Kolmogorov-Smirnov statistic (Statistic=0.161; $p$=0.004). As it

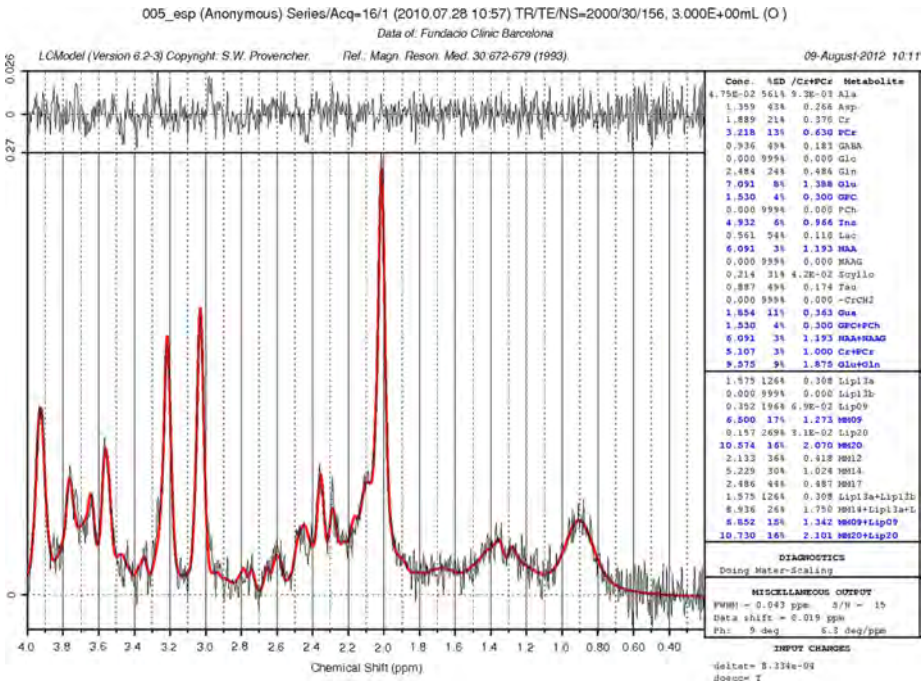


**Figure 2** Typical spectrum as analysed by LCModel. Note: This spectrum is from a voxel located in the ACC of subject number 6. The concentrations of the metabolites and their associated Cramer–Rao bounds are listed in the box on the right.

was not normal, we decided to explore the frequency using a histogram, which suggested the presence of two similar groups. We dichotomised the variable using a cut-off of two years of course of illness. The two subgroups did not differ significantly in terms of number (23 versus 24), age, sex and level of severity assessed by the CY-BOCS or IQ. Post-hoc comparisons were applied by Bonferroni's correction.

## 3. Results

### 3.1. Clinical characteristics of obsessive-compulsive patients

The mean age of the OCD group was 15.46 years (SD=2.23; range: 11.3–18.9) and mean age of onset was 11.93 years (SD=3.29; range: 4–16). The mean duration of disease until evaluation was 27.51 months (SD=23.6; range: 3–112). There were 24 boys (51.1%) and 23 girls (48.9%) in the case group. Regarding OCD categories, the principal dimensions were symmetry and ordering in seven cases (14.9%), harm avoidance and checking in 30(63.8%), cleanliness and washing in ten (21.3%). The mean of the symptoms severity assessed with the CY-BOCS was $17.33 \pm 9.4$.

Another Axis I diagnosis was found in 57.4% of the patients: generalised anxiety disorder (21.3%, $n=10$), attention-deficit/hyperactivity disorder (10.6%, $n=5$), anorexia nervosa (8.5%, $n=4$), hypomania (4.3%, $n=2$), panic disorder (2.1%, $n=1$), phobia simple (2.1%, $n=1$), enuresis (2.1%, $n=1$), bulimia nervosa (2.1%, $n=1$), major depressive disorder (2.1%, $n=1$) and oppositional defiant disorder (2.1%, $n=1$). Seven patients had a second co-morbid diagnosis: one had generalised anxiety disorder, one enuresis, two eating disorder not otherwise specified, one social phobia and one oppositional defiant disorder. The second and third co-morbid diagnoses were not in an acute phase of disease. Patients with an eating disorder were not underweight, and patients with a mood disorder were psychopathologically stable.

With regard to pharmacological therapy, 21.3% ($n=10$) of the OCD patients were not receiving any medication at the time of the evaluation, while 78.7% ($n=37$) were receiving SSRIs [38.8% ($n=18$) fluoxetine, 21.3% ($n=10$), sertraline and 8.5% ($n=4$) fluvoxamine] or serotonine reuptake inhibitors (SRI) [clomipramine, 10.6% ($n=5$)]. Seventeen patients (37.7%) were receiving another adjunctive psychopharmacological medication in addition to SSRI or SRI: 13.3% ($n=6$) were receiving risperidone, 8.9% ($n=4$) quetiapine, 4.4% ($n=2$) aripiprazole, 8.9% ($n=4$) benzodiazepine and 2.2% ($n=1$) methylphenidate.

### 3.2. Demographic and psychoclinical differences between obsessive-compulsive patients and healthy controls

The demographic and clinical variables of OCD patients and healthy controls are shown in Table 1. There were no differences in age or sex. As expected, OCD patients scored higher on the scales assessing depression and anxiety than healthy controls.

### 3.3. Differences in neurometabolite concentrations in the ACC between obsessive-compulsive patients and healthy controls

Table 2 shows the percentages of GM, WM and CFS in the VOI and MRS metabolite concentrations in the ACC. Concentrations of myo-inositol adjusted for CSF ($t=-2.31$, df=76; $P=0.023$) were all lower in OCD patients than in control subjects. These differences remained significant after a covariate analysis using GM and age ($F_{1,74}=5.686$; $P=0.02$; partial eta squared=0.071). No other significant differences in any other neurometabolites adjusted for CSF were found in the area studied.

### 3.4. The relationship between pharmacological therapy and clinical and neurochemical variables

There was a significant difference in age between OCD patients who received pharmacological treatment and those who did not ($t=-4.295$, $p<0.001$), but not in the other sociodemographic variables. When we studied concentrations of Glx adjusted for CSF in patients with or without pharmacological treatment controlling for the covariates GM and age, we found no significant differences ($F_{1,41}=2.527$; $P=0.120$; partial eta squared=0.058); nor were there significant differences when the type of the treatment was separated into no treatment, antidepressant treatment or antidepressant plus antipsychotic treatment ($F_{2,40}=2.733$; $P=0.077$; partial eta squared=0.120). No significant differences in the other neurometabolites adjusted for CSF were found in relation to psychopharmacological treatment.

### 3.5. Differences in neurometabolites between patients with a short or long duration of illness and the control group

The sociodemographic and neurochemical data of the groups are shown in Table 3. There were significant differences in the concentrations of Glx adjusted for CSF between the groups (<24 months; >24 months and a healthy control group) controlling for covariates GM and age ($F_{2,73}=3.949$; $P=0.024$; partial eta squared=0.058). Post-hoc comparisons using Bonferroni's correction indicated that the mean score of Glx in the ACC in the long term group ($M=10.27$, SD=1.52) was significantly lower than in the short term group ($M=11.91$, SD=2.32) [$P=0.019$]. The healthy control group ($M=11.08$, SD=1.86) did not differ significantly from either OCD group. No significant differences in the other neurometabolites adjusted for CSF were found in relation to the time of duration of illness.

## 4. Discussion

The main finding of the present study, which used improved $^{1}$H-MRS techniques in a large sample of childhood and adolescent OCD, disapproves our initial hypothesis: we found no differences in the concentration of Glx in the ACC between OCD children and adolescents and healthy controls. This lack of any difference remained when we controlled for the percentage of GM and age. The most important result of our study was that

**Table 1** Demographic and clinical characteristics in OCD patients and healthy control subjects.

| *Characteristics* | *OCD group (N=47) Mean (S.D.)* | *Healthy control group (N=31) Mean (S.D.)* | *Student-t (t)* | *P* |
|---|---|---|---|---|
| Sex-Males/female | 24/23 | 13/18 | ($\chi^2$=0.624) | 0.429 |
| Age (years) | 15.46 (2.23) | 15.92 (1.84) | −0.958 | 0.341 |
| CDI | 14.34 (9.99) | 3.59 (3.20) | 5.586 | **<0.001** |
| SCARED | 30.72 (14.96) | 10.04 (6.65) | 7.743 | **<0.001** |

OCD: Obsessive–Compulsive Disorder; S.D.:Standard deviation; CDI: Child Depression Inventory; SCARED: Screen for Childhood Anxiety Related Emotional Disorders. Significant results ($P<0.05$) are in bold type.

**Table 2** Per cent of grey matter (GM), white matter (WM) and cerebral spinal fluid (CSF) and metabolite concentrations adjusted for the amount of CSF metabolite concentrations in anterior cingulate cortex of OCD patients and healthy control subjects.

| | *OCD group (N=47) Mean (S.D.)* | *Healthy control group (N=31) Mean (S.D.)* | *Student-t* (df=76) | *P* |
|---|---|---|---|---|
| GM | 78.89(5.33) | 76.81(6.03) | 1.60 | 0.113 |
| VM | 4.47(2.98) | 5.55(5.85) | −0.95 | 0.347 |
| CSF | 16.64(5.24) | 17.64(5.58) | −0.80 | 0.424 |
| Ins[a] | **5.80(0.79)** | **6.30(1.12)** | **$F_{1,74}$=5.69** | **0.020** |
| Total Cho[a] | 1.55(0.25) | 1.55(0.21) | $F_{1,74}$=0.30 | 0.587 |
| Total NAA[a] | 7.38(0.66) | 7.47(0.65) | $F_{1,74}$=0.26 | 0.610 |
| Cr+PCr[a] | 6.15(0.67) | 6.40(0.56) | $F_{1,74}$=2.78 | 0.100 |
| Glx[a] | 11.11(2.12) | 11.08(1.86) | $F_{1,74}$=0.00 | 0.943 |

Ins: inositol; Total Cho: glycerophosphocoline+phosphocholine; Total NAA: N-acetyl-aspartate+N-acetyl aspartylglutamate; Cr+PCr: creatine+phosphocreatine; Glx: glutamate+glutamine.
Significant results ($P<0.05$) are in bold type.
[a]Neurometabolites adjusted for CSF (cerebrospinal fluid) and covaring by age and GM.

concentrations of Glx adjusted for CSF were significantly lower in OCD patients with a long duration of the condition (more than 24 months) than in patients with a shorter duration. Regarding pharmacological treatment, our data showed no differences in neurometabolites between OCD patients who received pharmacological treatment and those who did not; nor did the type of psychopharmacological treatment have an effect.

Several reports in the literature suggest an association between glutamatergic hyperactivity and OCD, possibly as a result of functional alterations of the different glutamate transporters (Carlsson, 2000; Rosenberg et al., 2004). It is possible that Glx may only be reduced in relation to certain clinical variables, such as duration of disorder. Although we did not find any differences in Glx levels between OCD patients and controls, we reported a presence of a lower Glx concentration in the ACC specifically in our subgroup with longer duration (45.17 months in the longer duration subgroup versus 10.83 months in the short duration subgroup), similar to the mean duration of illness [46.44 months] recorded in the paediatric sample conducted by Rosenberg et al., 2004. This finding may indicate that Glx concentration in the ACC tends to fall in paediatric patients with a longer course of disease. With regard to pharmacological treatment, our results suggest that treatment does not affect MRS variables in children and adolescents with OCD, corroborating previous studies in adults (Yücel et al., 2008). In relation to other psychiatric disorders such as schizophrenia, the majority of studies suggest that glutamatergic levels in medicated patients are similar to those in healthy control subjects (Poels et al., 2014). In addition, one recent study found significantly decreased medial prefrontal NAA and Glx levels specifically in subjects at the chronic stage of schizophrenia (Natsubori et al., 2013). Regarding depression, lower levels of Glx in ACC were found in the acute state of major depression disorder, suggesting the presence Glu-Glx cycle abnormalities during depressive episodes (Hasler et al., 2007), which seem to return to normal levels with full clinical recovery (Hasler et al., 2005). Thus, it appears that changes in glutamatergic neurotransmission in ACC are mood-dependent (Luykx et al., 2012).

Another possible explanation is that patients with mutations in genes that affect glutamatergic neurotransmission may present altered concentrations in these brain regions. Arnold et al. (2009) reported a correlation between a polymorphism in the "glutamate receptor, ionotropic, N-methyl-d-aspartate 2B gene" and decreased ACC glutamatergic concentrations with brain 1.5 T MRS in 16 psychotropic-naïve paediatric OCD patients. To our knowledge, this is the most recent report of the use of spectroscopic procedures to study the glutamatergic genes related to neurometabolites. More studies with this approach (genetics/$^{1}$H MRS) could also assess the impact of specific genes on the brain biochemistry of paediatric OCD, since this subtype has a high genetic aggregation and its study may well shed light on the pathophysiology of the condition.

On the other hand, we did not expect to find significantly decreased levels of myo-inositol (absolute and adjusted for

**Table 3** Differences between sociodemographic and neurochemical variables between the OCD patients with <24 months and <24 months of duration of illness and healthy control group.

| | *OCD <24 months of duration of illness (N=24) Mean (S.D.)* | *OCD>24 months of duration of illness (N=23) Mean (S.D.)* | *Healthy control group (N=31) Mean (S.D.)* | *Chi square/ t-test*[a] | *P* |
|---|---|---|---|---|---|
| Age | 15.10 (2.24) | 15.82 (2.21) | 15.92 (1.84) | −1.12 | 0.271 |
| Sex (Male/ Female) | 10/14 | 14/9 | 13/18 | 1.730 | 0.188 |
| CDI | 14.18 (9.38) | 14.53 (10.92) | 3.59 (3.20) | −0.11 | 0.914 |
| SCARED | 13.71 (2.92) | 16.69 (3.93) | 10.04 (6.65) | 0.49 | 0.647 |
| CY-BOCS | 19.22 (8.35) | 15.15 (10.23) | - | 1.44 | 0.159 |
| Pharmacotherapy (No/Yes) | 4/19 | 4/18 | - | 0.005 | 0.943 |
| Ins* | 5.68 (0.77) | 5.92 (0.80) | 6.30 (1.12) | $F_{2,73}=3.09$[b] | 0.051 |
| Total Cho* | 1.55 (0.26) | 1.55 (0.24) | 1.55 (0.21) | $F_{2,73}=0.51$[b] | 0.604 |
| Total NAA* | 7.40 (0.75) | 7.35 (0.56) | 7.47 (0.65) | $F_{2,73}=0.29$[b] | 0.744 |
| Cr+PCr* | 6.16 (0.68) | 6.13 (0.67) | 6.40 (0.56) | $F_{2,73}=1.59$[b] | 0.209 |
| Glx* | **11.90 (2.32)** | **10.27 (1.52)** | 11.08 (1.86) | $\boldsymbol{F_{2,73}=3.95}$[b] | **0.024** |

Ins: inositol; Total Cho: glycerophosphocoline+phosphocholine; Total NAA: N-acetyl-aspartate+N-acetyl aspartylglutamate; Cr+PCr: creatine+phosphocreatine; Glx: glutamate+glutamine.. Significant results ($P<0.05$) are in bold type.

*Neurometabolites adjusted for CSF.

[a] *t*-test/Chi square between OCD<24 months of duration of illness-OCD>24 months of duration of illness.

[b] ANCOVA between <24 months of duration of illness, >24 months of duration of illness and healthy control subjects, covaring by age and percentage of GM. Bonferroni's correction applicated.

CSF) in the ACC. This neurometabolite has mainly been used as a glial marker and has been closely connected with the osmoregulation of astrocytes (Govindaraju et al., 2000), but its exact significance remains unclear. Myo-inositol disturbances have been implicated in other mental illnesses including bipolar disorder (Davanzo et al., 2001) and attention deficit/hyperactivity disorder (Courvoisie et al., 2004). As such, this neurochemical abnormality is likely to reflect a general pathophysiology related to altered cellular homoeostasis, rather than one that is specific to OCD. To our knowledge, 11 studies in OCD patients have assessed ml versus healthy controls; one of them (Yücel et al., 2008) found significantly increased levels in right rostral and dorsal ACC in adult OCD, whereas one (Whiteside et al., 2006) found significantly decreased levels of Ins/Cr in the caudate. We stress that these two studies were conducted in adult populations on a single clinical 1.5 T MRI scanner, and the small sample size (15/20 subjects per group) may have restricted the power to detect differences.

Comparing these studies with ours is difficult due to these differences in design. For these reasons, we think that our serendipitous finding requires replication in an independent sample. However, glutamine and myo-inositol are both glial markers and the difference in these metabolites in controls and paediatric OCD patients may reflect not only a neurotransmission dysfunction in these patients but glial abnormalities as well. About two decades ago, several studies reported the role of myo-inositol as a synergic compound in the treatment of depressive disorder and OCD. Fux et al. (1996) reported that inositol alone is efficacious in the treatment of OCD patients. Nevertheless, in 1999, the same group reported that inositol did not reveal added benefit (Fux et al., 1999). Further studies in this area are needed to clarify these findings.

The present study has several limitations. First, $^{1}$H-MRS data were only acquired from the ACC, not from other brain regions which are also important in the neurobiology of OCD (Wu et al., 2012). The anatomical specificity of the present $^{1}$H-MRS findings is uncertain. MRS studies with other voxel locations are needed to examine additional brain circuits in the same subjects to identify the affected areas. Further $^{1}$H-MRS studies using special sequences that allow the separation of glutamate from glutamine signals, or an aggregate index, the Gln/Glu ratio, may gauge glutamatergic neurotransmission better because they reflect Glu release and the reciprocity of Glu and Gln (Brennan et al., 2013). Second, the voxel selection with PRESS is not ideal. Especially for the ACC location; here the CSF mostly arises from the centre of the voxel surrounded by GM and some WM, and the imperfect pulse profiles for voxel selection lead to the inclusion of more WM. Thus the CSF content might be overestimated, resulting in a biased correction; however, we did not correct this bias by means of the intercept of the regression line metabolite concentration versus CSF content, because the CSF content was quite similar in all subjects (mean=4.96; SD=0.75) and so the estimation of the intercept was not sufficiently reliable. Third, we included only OCD patients and controls and not considered other patient groups; therefore, our findings may not be specific.

Among the strengths of the study are its case-control design and the sample size (to our knowledge, this is the study with the largest number of patients and healthy controls in a childhood and adolescent population in 3 T MRS) and the adjustment of the concentration for GM and WM volume in order to avoid CSF contamination. In addition, the $^{1}$H MRS parameters used providest signals for both the healthy control and OCD groups. In the review by

Brennan et al. (2013), the median patient sample size in the studies was 13 – which, assuming equal numbers of healthy control subjects, yields 90% power for detecting only a very large effect size of approximately 1.3.

To our knowledge, this study is the first to specifically examine the relationship between the differences in neurometabolites and illness duration in paediatric OCD. There is a clear need for further research in this region with the use of more comprehensive and innovative approaches such as candidate genes or genome-wide association studies to confirm these preliminary findings.

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## Contributors

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## Conflict of interest

No authors have any conflict of interest related to this study.

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### 4.3. Genes de riesgo en el TOC de inicio en la infancia y la adolescencia (Objetivo 3)

En el estudio 3, se incluyeron un total de 75 tríos completos para llevar a cabo un test de desequilibrio de transmisión (se pueden observar los genes y las vías seleccionadas para este estudio en la figura 3). De los 266 SNPs analizados, 20 se transmitieron de forma superior de los padres a los individuos con TOC ($p < 0,05$). Estos 20 SNPs se localizan en diez genes diferentes, entre los que destacan los genes *HTR1B*, S*LC18A1*, *GAD1* y *GAD2* (gen del glutamato descarboxilasa 2), que mostraron un incremento en la frecuencia de transmisión de más de un SNP (se pueden consultar los resultados en la tabla 3 del estudio 3, página 102). Del total de polimorfismos estudiados, tres (rs2000292 (*HTR1B*), rs992990 y rs8190748 (*GAD2*)) se asociaron significativamente al riesgo de TOC después de aplicar las correcciones de Bonferroni ($p < 0,0002$).

Figura 3. Genes y vías seleccionadas para este estudio.

| Vías | Genes |
|---|---|
| **Dopamina** | *DRD2, DRD3, DRD4, SLC6A3, COMT, MAOA* |
| **Serotonina** | *NTR1B, HTR2A, HTR2C, SLC6A4, SLC18A1, TPH2* |
| **Glutamato** | *GRIK2, GRIN2B, GRIA1, GRIA3, SLC1A1, DLGAP3* |
| **GABA** | *GAD1, GAD2* |
| **BDNF** | *NTRK2, NTRK3, BDNF, AKT1, GSK3B* |
| **Neuroregulina** | *NGFR, ERBB4, NRG1, OLIG1, OLIG2* |
| **Otras** | *LMX1A, BDKRB2, CDH9, KCNN3, EFNA5* |

Al estratificar la muestra en función del sexo, ningún SNP superó dichas correcciones. Sin embargo, se observaron diferentes tendencias entre los dos sexos. En el sexo masculino, el rs2000292 (*HTR1B*) mostró un valor de p muy reducido ($p = 0,0006$) en comparación del encontrado en el sexo femenino ($p>0,05$), mientras que en éste fueron los SNPs en *GAD2*,

rs8190748 y rs992990, los que mostraron una p más reducida (p = 0,0006) que en el sexo masculino (p = 0,01).

Además del análisis de los SNPs individuales, se analizaron los bloques haplotípicos en los diferentes genes del estudio. Sólo se observó una cierta asociación para los genes identificados anteriormente en el análisis de los SNPs individuales. La estructura de los bloques haplotípicos para los genes *GAD2* y *HTR1B* se muestran en la figura 2 del estudio 3 (ver página 103). De los tres bloques haplotípicos construidos en *GAD2*, se asoció (p = 0.0002) un haplotipo que contenía tres de los cinco SNPs con valor de p < 0.05 identificados en el análisis individual de los SNPs, así como los dos SNPs de este gen que superaron las correcciones de Bonferroni. El haplotipo significativo es el único que contiene el alelo A del rs8190748, que corresponde al alelo que más se transmitió y que mostró el valor de p más bajo (para más información consultar la tabla 4 del estudio 3, página 104). Además tanto el haplotipo como el SNP individual muestran exactamente los mismos valores estadísticos. Para el gen *HTR1B*, se identificaron dos bloques haplotípicos. Uno de ellos contenía tres polimorfismos que mostraron un valor de p < 0.05 en el análisis individual de los SNPs, incluyendo el rs2000292 que se mantuvo significativo después de aplicar la corrección de Bonferroni. El haplotipo que mostró el menor valor de p (p = 0.0027) contenía el alelo A del rs2000292, que corresponde al alelo que más se transmitió de los padres a los hijos con TOC (ver tabla 4 del estudio 3, página 104).

**Estudio 3:** Role of *GAD2* and *HTR1B* genes in early-onset obsessive-compulsive disorder: results from transmission disequilibrium study. Genes, Brain and Behaviour (2014).

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# Role of *GAD2* and *HTR1B* genes in early-onset obsessive-compulsive disorder: results from transmission disequilibrium study

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**One of the leading biological models of obsessive-compulsive disorder (OCD) is the frontal-striatal-thalamic model. This study undertakes an extensive exploration of the variability in genes related to the regulation of the frontal-striatal-thalamic system in a sample of early-onset OCD trios. To this end, we genotyped 266 single nucleotide polymorphisms (SNPs) in 35 genes in 84 OCD probands and their parents. Finally, 75 complete trios were included in the analysis. Twenty SNPs were overtransmitted from parents to early-onset OCD probands and presented nominal pointwise $P < 0.05$ values. Three of these polymorphisms achieved $P < 2 \times 10^{-4}$, the significant *P*-value after Bonferroni corrections: rs8190748 and rs992990 localized in *GAD2* and rs2000292 in *HTR1B*. When we stratified our sample according to gender, different trends were observed between males and females. In males, SNP rs2000292 (*HTR1B*) showed the lowest *P*-value ($P = 0.0006$), whereas the SNPs in *GAD2* were only marginally significant ($P = 0.01$). In contrast, in females *HTR1B* polymorphisms were not significant, whereas rs8190748 (*GAD2*) showed the lowest *P*-value ($P = 0.0006$). These results are in agreement with several lines of evidence that indicate a role for the serotonin and γ-Aminobutyric acid (GABA) pathways in the risk of early-onset OCD and with the gender differences in OCD pathophysiology reported elsewhere. However, our results need to be replicated in studies with larger cohorts in order to confirm these associations.**

Keywords: Frontal-striatal-thalamic system, *GAD2*, *HTR1B*, obsessive-compulsive disorder, single nucleotide polymorphisms, transmission disequilibrium test

Obsessive-compulsive disorder (OCD) is a neuropsychiatric disease characterized by obsessions and/or compulsions that are distressing, time-consuming or significantly impairing. It is the fourth most common psychiatric illness, with a lifetime prevalence of 1–3% (Ruscio *et al.* 2010). The exact etiology of OCD is unknown, although there is evidence to suggest that the disorder arises from a complex combination of genetic and environmental factors (Taylor *et al.* 2010).

One of the leading biological models of OCD is the frontal-striatal-thalamic model, which emphasizes the role of the aberrant modulation of brain circuits in the prefrontal cortex, regions of the basal ganglia and the thalamus (Abramowitz *et al.* 2009). This aberrant modulation could be due to structural abnormalities and/or dysregulation of the neurotransmitters involved in these circuits [dopamine, serotonin, glutamate and γ-Aminobutyric acid (GABA)].

More than 80 genetic association studies of OCD covering 24 candidate genes have been published over the last decade but the findings are relatively inconsistent (Pauls 2010). This may be due to differences in statistical power across studies, but it may also be an indication of the etiologic heterogeneity of the condition; there is evidence of sex differences and also of distinct subtypes of the disorder. Patients with OCD have been subtyped according to age of onset (Taylor 2011): the condition has a bimodal distribution of age at onset with a peak of incidence in childhood (early-onset OCD) and another in mid-adulthood (late-onset OCD). The definition of age at onset varies, with some studies reporting the age at which the patient and/or family members first noted the presence of obsessive-compulsive symptoms (de Mathis *et al.* 2008), and others reporting the age at which the patient first met strict diagnostic criteria for OCD, when the symptoms displayed significant distress or impairment (Nakatani *et al.* 2011; Sobin *et al.* 2000). A recent meta-analysis indicated that compared with late onset, early onset is more likely to occur in males, is associated with greater OCD global severity and a higher prevalence of most types of OC symptoms, is more likely to be comorbid with tics and possibly with other putative obsessive-compulsive spectrum disorders and is associated with a greater prevalence of OCD in first-degree relatives (Taylor 2011).

This study undertakes an extensive exploration of the variability in genes related to the regulation of the

 

**Table 1:** Genes and pathways selected for this study

| Pathways | Genes |
|---|---|
| Dopamine | *DRD2, DRD3, DRD4, SLC6A3, COMT, MAOA* |
| Serotonin | *NTR1B, HTR2A, HTR2C, SLC6A4, SLC18A1, TPH2* |
| Glutamate | *GRIK2, GRIN2B, GRIA1, GRIA3, SLC1A1, DLGAP3* |
| GABA | *GAD1, GAD2* |
| BDNF | *NTRK2, NTRK3, BDNF, AKT1, GSK3B* |
| Neuroregulin | *NGFR, ERBB4, NRG1, OLIG1, OLIG2* |
| Others | *LMX1A, BDKRB2, CDH9, KCNN3, EFNA5* |

frontal-striatal-thalamic system in a sample of early-onset OCD trios. To this end, we genotyped 266 single nucleotide polymorphisms (SNPs) in 35 genes (Table 1) in 84 OCD probands and their parents.

## Materials and methods

### *Subjects*

Eighty-seven probands meeting DSM-IV (American Psychiatric Association 1994) diagnostic criteria for OCD and their parents ($N = 158$) were recruited from the Department of Child and Adolescent Psychiatry and Psychology at the *Hospital Clínic* in Barcelona. The age of onset was defined as the age at which patients first displayed significant distress or impairment associated with obsessive-compulsive symptoms. Non-Caucasian patients were excluded ($N = 3$). Ethnicity was determined by self-reported ancestries; cases and controls reported for the ethnicity of each grandparent. We also excluded subjects who mentioned non-European grandparents. Finally, 75 complete trios were included in the analysis.

Patients and their parents were interviewed with the Spanish version (Ulloa *et al.* 2006) of the semistructured diagnostic interview K-SADS-PL (Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime Version) (Kaufman *et al.* 1997) in order to assess current and past psychopathology. Sociodemographic and clinical characteristics are described in Table 1. Severity of OCD was measured with CY-BOCS (Children's Yale-Brown Obsessive Compulsive Scale) (Scahill *et al.* 1997). On the basis of previous factor analysis (Leckman *et al.* 1997), we computed scores for four obsessive-compulsive symptom dimensions for each subject (aggressive, sexual and religious obsessions and checking compulsions; symmetry, ordering, counting and arranging obsessions and compulsions; contamination obsessions and cleaning compulsions; and hoarding). For this, an algorithm derived from the notes of the CY-BOCS symptom checklist was used. Factor-analytic studies of OCD symptoms in children, adolescents and adults have also revealed highly consistent symptom structures, suggesting that the fundamental architecture of OCD symptoms remains consistent throughout the life span (Bloch *et al.* 2008; Mataix-Cols *et al.* 2008; Stewart *et al.* 2008).

All procedures were approved by the Hospital's Ethics Committee. Written informed consent was obtained from all parents and verbal informed consent was given by all subjects following an explanation of the procedures involved.

#### *Sample preparation*

Blood samples were collected from the individuals in ethylenediaminetetraacetic acid (EDTA) tubes (K2EDTA BD Vacutainer EDTA Tubes; Becton Dickinson, Franklin Lakes, NJ, USA) and genomic DNA was extracted using the MagNA Pure LC DNA Isolation Kit III and an LC MagNA Pure system (Roche Diagnostics GmbH, Mannheim, Germany). The DNA concentration was determined by absorbance (ND1000; NanoDrop, Wilmington, DE, USA).

#### *SNP selection, genotyping and quality control*

A total of 304 SNPs were selected in 35 candidate gene regions (covering target loci and upstream and downstream regions) following one of three strategies: (1) tagging analysis (as implemented in Haploview 4.2) at an $r^2$ threshold of 0.8 to capture 98% of the most common HapMap phase II variants based on the CEU panel (minor allele frequency > 0.1) (range 91–100% for individual genes); (2) suspected SNP functionality according to data published in Ensembl (http://www.ensembl.org), dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP/) and PupaSuite 3 (http://pupasuite.bioinfo.cipf.es/) databases, with a validated minor allele frequency > 0.1 in the Caucasian population; and (3) previous association reported in the literature either with OCD or with other psychiatric disease when the statistical power was sufficient to achieve the reported odds ratio (OR) or relative risk (RR). When tagging strategies using Hapmap provided more than 15 tagged SNPs, SNPs were selected according to strategies (2) and (3). We rejected seven SNPs prior to genotyping. The remaining 297 SNPs were genotyped by the MassArray assay with the Sequenom Genotyping System (Sequenom, San Diego, CA, USA) at the Santiago de Compostela Node of the Spanish National Genotyping Centre (CeGen). We excluded 25 SNPs whose genotypes were present in fewer than 90% of samples or showed inconsistent clustering, 5 SNPs showing Mendelian errors and 1 monomorphic SNP. The 266 validated SNPs (Table S1, Supporting Information) were used for the genetic association analysis. For quality control, 13 samples were genotyped in duplicate for all the SNPs analyzed, with a 100% concordance rate.

#### *Statistics*

Sample size and statistical power were calculated using Quanto1.2 software (http://hydra.usc.edu/gxe). Given the sample size, and assuming a 5% level of significance, we were able to detect OR values of >2.5 with >80% statistical power when polymorphisms with allele frequencies of >0.1 were analyzed. Analyses were completed with the transmission disequilibrium test (TDT) as implemented in Haploview 4.2, using the parentTDT option in order to include parents' diagnoses in the analysis. The TDT was used to conduct single marker and haplotype analysis by calculating *P*-values for individual SNPs and their associated haplotypes. The TDT assesses the distortion in the transmission of alleles from a heterozygous parent to an affected offspring. Under no association with the disease, both alleles of a given SNP have an equal chance of being transmitted from a heterozygous parent. If, however, one allele increases the risk of disease, this allele will be preferentially transmitted to the affected offspring. The *T*-test statistic considers all heterozygous parents, and compares the number of transmissions of each allele, in a McNemar's test. The *T* test has a $\chi^2$ distribution with one degree of freedom (Lewis 2002). Haploview was also used to define linkage disequilibrium (LD) and to identify haplotype blocks. To minimize the odds of false-positive associations, we applied Bonferroni correction for multiple testing (significant $P\text{-value} < 2 \times 10^{-4}$ after Bonferroni correction).

## Results

Table 2 shows the demographic and clinical characteristics of the early-onset OCD probands recruited for this study. Significant differences between males and females in comorbid diseases were observed ($P < 0.05$), with males having higher incidence of tics (8.5% males vs. 2.7% females) and females higher incidence of eating disorders (16.2% females vs. 0% males).

**Table 2:** Clinical data of the 84 early-onset OCD patients recruited for this study

| | Male | Female | Statistic$_{df}$, *P*-value | All |
|---|---|---|---|---|
| *N* | 47 | 37 | | 84 |
| Age (mean ± SD) | 15.13 ± 2.40 | 14.72 ± 2.79 | $T_{df=82}$ = 0.712, 0.478 | 14.95 ± 2.57 |
| Age at onset (mean ± SD) | 11.67 ± 2.96 | 12.03 ± 3.2 | $T_{df=82}$ = 0.515, 0.608 | 11.83 ± 3.09 |
| Current CY-BOCS (mean ± SD) | 16.98 ± 9.39 | 16.46 ± 8.01 | $T_{df=82}$ = 0.259, 0.796 | 16.74 ± 8.74 |
| Maxim CY-BOCS (mean ± SD) | 26.98 ± 7.83 | 25.71 ± 6.77 | $T_{df=82}$ = 0.752, 0.455 | 26.41 ± 7.35 |
| Dimensions, *N* (%) | | | $X^2_{df=3}$ = 2.30, 0.512 | |
| Washing/cleaning | 12 (25.5) | 7 (18.9) | | 19 (22.6) |
| Harm/checking | 26 (55.3) | 25 (67.5) | | 51 (60.7) |
| Symmetry/ordering | 9 (19.1) | 5 (13.5) | | 14 (16.7) |
| Comorbidities, *N* (%) | | | $X^2_{df=5}$ = 10.84, 0.048 | |
| ADHD | 7 (14.9) | 4 (10.8) | | 11 (13.1) |
| Anxiety disorder | 12 (25.5) | 10 (27.0) | | 22 (26.2) |
| Tics | 4 (8.5) | 1 (2.7) | | 5 (5.9) |
| Eating disorder | 0 | 6 (16.2) | | 6 (7.1) |
| ODD | 0 | 1 (2.7) | | 1 (0.1) |
| Family history of OCD in first- and second-degree relatives, *N* (%) | | | $X^2_{df=3}$ = 1.07, 0.783 | |
| First grade | 22 (46.8) | 17 (45.9) | | 39 (46.4) |
| Second grade | 5 (10.6) | 3 (8.1) | | 8 (9.5) |
| Complete trio, *N* (%) | 41 (87.2) | 34 (91.8) | | 75 (89.3) |

ADHD, attention-deficit hyperactivity disorder; ODD, oppositional defiant disorder

Of the 266 SNPs analyzed, 20 were overtransmitted from parents to OCD probands and presented nominal pointwise $P < 0.05$ values (Fig. 1). These SNPs mapped in 10 different genes. Among the genes with more than one significant SNP, *GAD2* and *HTR1B* each had five, and *SLC18A1* and *GAD1* showed two significant SNPs in each gene (Table 3). Three of these polymorphisms achieved $P < 2 \times 10^{-4}$, the significant *P*-value after Bonferroni corrections: rs8190748 and rs992990 localized in *GAD2* and rs2000292 in *HTR1B*.

When we stratified our sample according to gender, no SNP achieved a significant *P*-value after Bonferroni correction. However, different trends were observed between males and females. In males, SNP rs2000292 (*HTR1B*) showed the lowest *P*-value ($P = 0.0006$), whereas the SNPs in *GAD2*, such as rs8190748 and rs992990, were only marginally significant ($P = 0.01$). In contrast, in females *HTR1B* polymorphisms were not significant, whereas rs8190748 (*GAD2*) showed the lowest *P*-value ($P = 0.0006$).

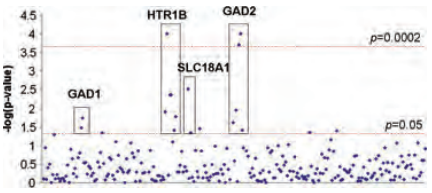


**Figure 1: Association results of 266 validated SNPs using parentTDT analysis in 75 early-onset OCD trios**. The *X*-axis indicates various SNPs ordered by gene and chromosome position. The horizontal lines at −log(*P*) 1.3 and 3.6 correspond to nominal $P = 0.05$ and $P = 0.0002$, respectively.

Figure 2 shows the haplotype block distribution of *GAD2* and *HTR1B*. In *GAD2*, three haplotype blocks were constructed (Fig. 2a), and a significant haplotype was identified in block 3 (Table 4). This haplotype contained three of the five SNPs with *P*-value < 0.05 and the two SNPs of this gene survived Bonferroni corrections. The significant haplotype carried the A allele of rs8190748, the overtransmitted allele of the SNP with the lowest *P*-value in the gene. This haplotype also showed the same ratio of transmitted and untransmitted alleles as rs8190748, and similar statistics. For *HTR1B*, two haplotype blocks were identified (Fig. 2b). Block 1 contained three polymorphisms with *P*-value < 0.05, including rs2000292, the SNP with a significant *P*-value after Bonferroni correction on this gene. The haplotype that contained the rs2000292 A allele, the overtransmitted allele of this SNP, was mainly transmitted from parents to OCD probands (Table 4). However, the *P*-value did not reach significance after correction for multiple testing.

## Discussion

The data presented here showed significant associations between SNPs in *GAD2* and *HTR1B* and early-onset OCD in a family-based study. The preferential transmission of these SNPs to OCD probands may be related to gender, because a non-significant trend was observed toward the overtransmission of *HTR1B* SNPs to male probands and the overtransmission of *GAD2* SNPs to female probands.

In agreement with several clinical studies that suggested that gender plays a relevant role in OCD phenotypic expression (Mathis *et al.* 2011), the associations reported here seem to be gender-specific. This finding is not new in the genetics of OCD, as several genes associated with the disease, such as *COMT*, *MAOA*, *DRD2*, *SLC1A1*, *5HT1D*, *5HT1A*,

**Table 3:** Significant associations with single marker SNPs using parentTDT analysis in 75 early-onset OCD trios

| | | | | From heterozygous parent | | | |
|---|---|---|---|---|---|---|---|
| Gene | SNP | Alleles* | Frequency | Transmitted | Untransmitted | Statistic | *P*-value† |
| *GRIN2B* | rs7301328 | **G**/C | 0.621 | 40 | 27 | 4.06 | 0.0440 |
| *SLC18A1* | rs6586896 | T/**C** | 0.106 | 20 | 5 | 8.76 | 0.0031 |
| *SLC18A1* | rs7013199 | C/**T** | 0.224 | 31 | 16 | 4.00 | 0.0455 |
| *GAD2* | rs2236418 | **A**/G | 0.842 | 29 | 10 | 5.12 | 0.0237 |
| *GAD2* | rs7908975 | **A**/C | 0.885 | 19 | 8 | 6.42 | 0.0113 |
| *GAD2* | rs992990 | **C**/A | 0.739 | 40 | 15 | 11.52 | **0.0002** |
| *GAD2* | rs8190748 | **A**/G | 0.727 | 43 | 15 | 13.47 | **0.0001** |
| *GAD2* | rs3781108 | **A**/G | 0.832 | 30 | 11 | 4.25 | 0.0394 |
| *NRG1* | rs7014762 | T/**A** | 0.248 | 29 | 18 | 4.41 | 0.0356 |
| *DLGAP3* | rs4652867 | **G**/T | 0.792 | 24 | 15 | 3.81 | 0.0508 |
| *5-HTR2A* | rs2296972 | G/**T** | 0.252 | 25 | 19 | 4.19 | 0.0407 |
| *HTR1B* | rs9359271 | A/**C** | 0.258 | 35 | 17 | 6.25 | 0.0124 |
| *HTR1B* | rs2000292 | G/**A** | 0.224 | 31 | 10 | 11.88 | **0.0001** |
| *HTR1B* | rs6296 | G/**C** | 0.202 | 28 | 11 | 8.07 | 0.0045 |
| *HTR1B* | rs6298 | C/**T** | 0.202 | 28 | 11 | 8.07 | 0.0045 |
| *HTR1B* | rs4140535 | C/**T** | 0.295 | 30 | 18 | 4.26 | 0.0389 |
| *GRIK2* | rs9390754 | A/**G** | 0.115 | 15 | 10 | 5.77 | 0.0164 |
| *DRD3* | rs2134655 | G/**A** | 0.211 | 27 | 14 | 4.00 | 0.0455 |
| *GAD1* | rs1420385 | C/**T** | 0.463 | 40 | 28 | 4.55 | 0.0330 |
| *GAD1* | rs3791860 | C/**G** | 0.422 | 43 | 31 | 5.54 | 0.0186 |

*In bold, analyzed allele.
† In bold, significant *P*-values after Bonferroni correction ($P < 2 \times 10^{-4}$).

*NTRK2* and *BDNF*, show differential effects in males and females (Mathis *et al.* 2011). Segregation analysis, used to assess the mode of inheritance of OCD, suggested that the most parsimonious model explaining the inheritance of OCD is a single gene (autosomal dominant) model with familial residual effects. Nonetheless, Mendelian factors only partially explain the familial aggregation of the phenotype and Nestadt *et al.* (2000) suggested that the genetic transmission of OCD differs in males and females. Similarly, on the basis of their segregation analysis, Cavallini *et al.* (1999) suggested a dominant model of transmission with different penetrance in males and females. Several hypotheses have been proposed to explain sex differences in behavior and in neuropsychiatric diseases. Recent studies have demonstrated that under stress, sex-related factors, such as the sex chromosome complement and X-chromosome genetic polymorphisms, influence the differential expression of genes linked to mood regulation between genders, including genes related to serotonin, GABA (including *GAD2*) and dopamine neurotransmission (Seney *et al.* 2013a,2013b). In addition, sex differences in serotonin neurotransmission have been associated with the differences in the hypothalamic-pituitary axis and response to stress (Goel & Bale 2010). Several authors have suggested a possible relationship between these sex differences and hormonal factors (Donner & Lowry 2013).

The 5-HT1D$\beta$ receptor (gene product of *HTR1B*) appears to be involved in the pathophysiology of OCD; it is a terminal autoreceptor involved in the regulation of 5-HT synthesis and release, and is expressed mostly in the limbic region and in the striatum. A recent study indicated that these receptors are necessary and sufficient to induce OCD-like behavior in mice, and that their desensitization by serotonin reuptake inhibitors is the key step to reduce OCD-like behavior (Shanahan *et al.* 2011). The acute administration of non-selective (i.e. mCPP) or selective (i.e. sumatriptan) ligands of the 5-HT1D$\beta$ receptor induces a transient worsening of OCD symptoms (Gross-Isseroff *et al.* 2004). Moreover, chronic administration of sumatriptan was found to improve symptoms in some OCD patients resistant to conventional pharmacotherapy (Stern *et al.* 1998). For these reasons, the 5-HT1D$\beta$ receptor gene has been considered as a candidate gene for OCD.

Our study replicates previously reported associations between *HTR1B* SNPs and OCD (Kim *et al.* 2009; Lochner *et al.* 2004; Mundo *et al.* 2002). Moreover, our male-specific association corroborates the results of Kim *et al.* (2009) in a sample of male adult OCD patients and Lochner *et al.* (2004) in an African subsample of male OCD patients. Also in agreement with our results, a recent meta-analysis by Taylor (2013) identified a significant trend for the involvement of *HTR1B* in OCD in interaction with age of onset. Other studies have found associations between genetic polymorphisms in *HTR1B* and OCD endophenotypes, such as orbitofrontal cortex volume measured by magnetic resonance image (Atmaca *et al.* 2010) and severity of obsessions measured by YBOCS score (Camarena *et al.* 2004). However, several studies reported non-significant associations between *HTR1B* and OCD (Di Bella *et al.* 2002; Dickel *et al.* 2007; Gratacòs *et al.* 2009; Hemmings *et al.* 2003; Liu *et al.* 2011; Walitza *et al.* 2004). This heterogeneity could be the result of differences in statistical power; however, these studies used similar sample sizes ranging from 44 to 187 OCD

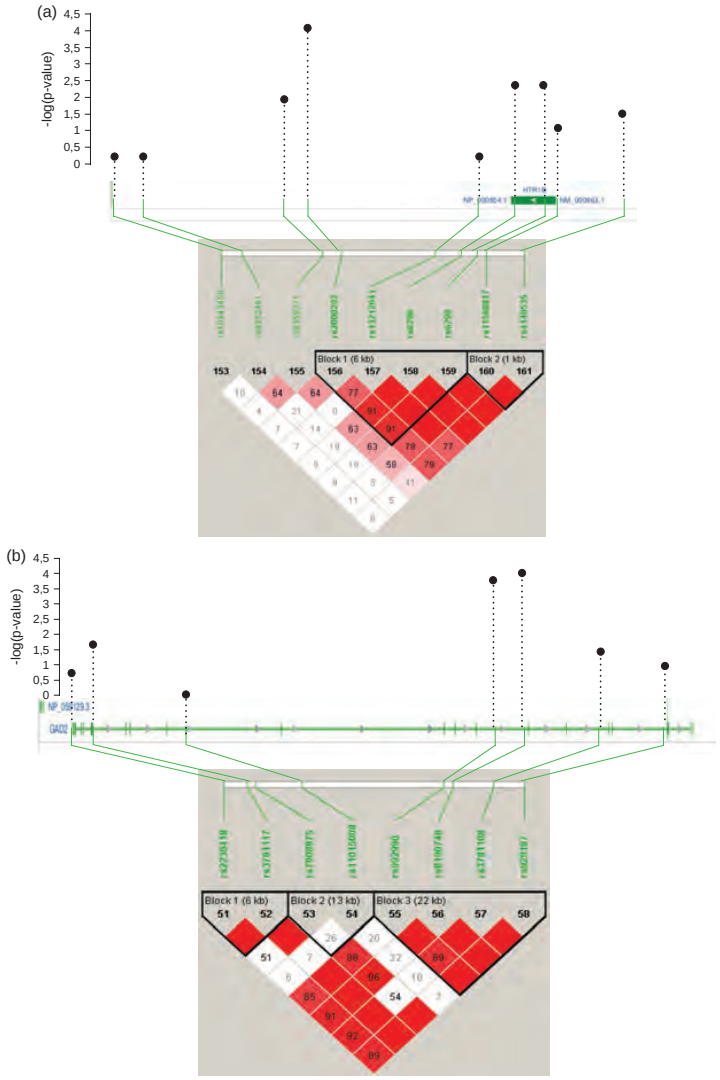


**Figure 2: Linkage disequilibrium pattern.** (a) *HTR1B* and (b) *GAD2*. The standard color scheme from Haploview is used to display logarithm of odds (LOD) and $D'$. Estimated statistics of $D'$ are shown in each box (they are not labeled if $D' = 1$). The gene structure is also illustrated. At the top, the –log(*P*-value) of each SNP obtained with single marker SNPs using parentTDT analysis in 75 early-onset OCD trios.

**Table 4:** Haplotype analysis of *GAD2* and *HTR1B* using parentTDT analysis in 75 early-onset OCD trios

| Gene | Haplotype block | SNPs | Frequency | From heterozygous parent | | Statistic | *P*-value |
|---|---|---|---|---|---|---|---|
| | | | | Transmitted | Untransmitted | | |
| *GAD2* | Block 1 | rs2236418-rs3781117 | | | | | |
| | Haplotype 1 | A-T | 0.71 | 38 | 23 | 1.11 | 0.2922 |
| | Haplotype 2 | G-T | 0.16 | 10 | 29 | 5.12 | 0.0237 |
| | Haplotype 3 | A-C | 0.13 | 16 | 12 | 1.26 | 0.2623 |
| | Block 2 | rs7908975-rs11015008 | | | | | |
| | Haplotype 1 | A-G | 0.49 | 36 | 31 | 0.14 | 0.7106 |
| | Haplotype 2 | A-A | 0.39 | 35 | 29 | 1.71 | 0.191 |
| | Haplotype 3 | C-A | 0.08 | 4 | 12 | 5.17 | 0.023 |
| | Haplotype 4 | C-G | 0.05 | 4 | 7 | 1.16 | 0.2808 |
| | Block 3 | rs992990-rs8190748-rs3781108-rs928197 | | | | | |
| | Haplotype 1 | C-A-A-T | 0.71 | 43 | 15 | 13.47 | **0.0002** |
| | Haplotype 2 | A-G-G-A | 0.16 | 11 | 26 | 2.47 | 0.1161 |
| | Haplotype 3 | A-G-A-T | 0.12 | 7 | 16 | 6.72 | 0.0095 |
| | Haplotype 4 | C-G-G-A | 0.01 | 0 | 3 | 3.00 | 0.0833 |
| *HTR1B* | Block 1 | rs2000292-rs13212041-rs6296-rs6298 | | | | | |
| | Haplotype 1 | G-T-G-C | 0.56 | 27 | 42 | 3.51 | 0.0612 |
| | Haplotype 2 | G-C-G-C | 0.22 | 20 | 26 | 0.94 | 0.3322 |
| | Haplotype 3 | A-T-C-T | 0.18 | 27 | 10 | 8.97 | **0.0027** |
| | Haplotype 4 | A-T-G-C | 0.02 | 3 | 2 | 0.14 | 0.7056 |
| | Haplotype 5 | G-T-C-T | 0.01 | 2 | 2 | 0.20 | 0.6539 |
| | Haplotype 6 | A-C-G-C | 0.01 | 3 | 0 | 3.99 | 0.0458 |
| | Block 2 | rs11568817-rs4140535 | | | | | |
| | Haplotype 1 | G-T | 0.50 | 27 | 38 | 3.04 | 0.0811 |
| | Haplotype 2 | T-T | 0.29 | 30 | 18 | 4.26 | 0.0389 |
| | Haplotype 3 | T-C | 0.21 | 21 | 22 | 0.02 | 0.9013 |

significant *P*-values after Bonferroni correction are provided in bold.

patients. Other causes are the heterogeneity of the disease, especially bearing in mind that we are using a specific sample of early-onset OCD with a high frequency of OCD antecedents and that the observed association seems to be male-specific. Among these 11 studies there are several differences in the percentage of males, ethnicity, age of onset and disease severity. Regarding age of onset, only one study was performed with early-onset OCD patients (Walitza *et al.* 2004), three studies used mixed populations of early- and late-onset OCD (Di Bella *et al.* 2002; Dickel *et al.* 2007; Hemmings *et al.* 2003) and the vast majority were conducted with adult OCD patients (Gratacòs *et al.* 2009; Kim *et al.* 2009; Liu *et al.* 2011; Lochner *et al.* 2004; Mundo *et al.* 2002). In addition, these previous studies genotyped only a synonymous polymorphism named G861C or rs6296. In our study, this SNP achieved a *P*-value < 0.05, but was not significant after Bonferroni correction. It was in high LD with rs2000292, and both SNPs were in the same haplotype block. However, the single marker analysis of rs2000292 provided better statistics than the haplotype analysis. These results may indicate that rs2000292 will be in higher LD with a causal variant than rs6296. Taking into account the localization of these SNPs in *HTR1B*, this causal variant is probably localized in the 5′-end of the gene affecting transcriptional regulatory elements. Differences in LD pattern between rs2000292 and rs6296 in different populations, and the lack of a more exhaustive analysis of the 5′-end of the gene, could help to explain these controversial results.

Several lines of evidence indicate that alterations in GABA neurotransmission could have a role in OCD (Rosenberg *et al.* 2000). On one hand, abnormalities in cortical inhibitory processes have been reported in OCD patients (Richter *et al.* 2012). On the other hand, GABA modulates cortical glutamatergic neurons (Gonzalez-Burgos & Lewis 2008) and could be related to abnormalities in cortical excitatory processes found in OCD (Maia *et al.* 2008). Direct alterations of GABA neurotransmission have recently been reported: using proton magnetic resonance spectroscopy, Simpson *et al.* (2012) found lower levels of medium prefrontal cortex GABA in OCD patients than in healthy controls, and Richter *et al.* (2012), using transcranial magnetic stimulation, found evidence of cortical inhibitory dysfunctions in OCD patients. However, little attention has been paid to this neurotransmitter in genetics studies of OCD, which have only included a few GABA receptors (Gratacòs *et al.* 2009; Richter *et al.* 2009; Zai *et al.* 2005). To our knowledge, our study is the first to explore the genetic variability of *GAD1* and *GAD2* in OCD. These genes encoded, respectively, two isoforms of the rate-limiting enzyme glutamate decarboxylase (GAD), GAD67 and GAD65. Both GAD isoforms are significantly expressed only in central GABA-containing cells, often inhibitory interneurons, making them a widely used cellular marker for GABAergic neurons. Although GAD67 and GAD65 are expressed widely across many cell types and both are

usually co-expressed in the same neuron, there is increasing evidence that they have differential expression distributions in subcellular compartments. GAD67 is mainly present in the cytoplasm and synthesizes GABA for cell metabolism and tonic, non-vesicle GABA release, whereas GAD65 is preferentially localized in axon terminals associated with membrane and synaptic vesicles, and is responsible for GABA synthesis in activity-dependent, vesicle GABA release during intense synaptic activities (Betley *et al.* 2009). The significant association between *GAD2* and OCD identified in our study is in agreement with a dynamic function of this GAD isoform in adaptive responses to cell activity and environmental stimulation. This association seems to be female-specific. This is in accordance with differences in GABA neurotransmission between males and females (Simpson *et al.* 2012). The dynamic expression of *GAD2* required a complex regulation of its transcription (Pan 2012), which seems to be under control of estrogen receptors (Hudgens *et al.* 2009) and could be modulated by sex steroids (Noriega *et al.* 2010).

Our study identified that two SNPs in *GAD2*, rs8190748 and rs992990, significantly overtransmitted to OCD probands. The haplotype block that includes the risk alleles of these SNPs was also transmitted preferentially from parents to OCD patients. These SNPs are intronics and do not seem to affect any regulatory elements, but the haplotype block defined covered a region of *GAD2* with several exons. Further studies are needed to explore this region and identify functional variants that might explain the associations observed.

Our study has a number of shortcomings. The main one is the sample size used, which limits the statistical power of the study and makes it difficult to detect small or modest effects of common variants. However, we achieved sufficient statistical power to identify a number of significant associations. Given that the study was hypothesis-driven, and owing to the small sample size, our results should be seen as preliminary and should be considered as exploratory findings in need of further confirmation. Moreover, the sample size was not large enough for the stratification into subgroups according to clinical variables such as comorbidities. However, it should be noted that our sample comprised early-onset OCD patients, and so the sample represented a homogeneous clinical population. Our study uses a candidate gene strategy. A disadvantage of this approach is that the objects of study are limited by our current understanding of the molecular mechanisms involved in the pathology of OCD; therefore, this method cannot identify hitherto unsuspected predictor genes. Moreover, in the selected genes, our study is limited to our current knowledge of their genetic variability and to the information regarding this variability that is available in public databases. Furthermore, given our incomplete knowledge of the pathophysiology of OCD, other candidate genes could be considered in the pathways selected in this study and elsewhere. Finally, the statistical power of our study is insufficient for us to rule out a contribution to OCD of the genes that did not yield significant results, as the discrete OR for association of their SNPs may not have been identified within our small sample.

In conclusion, bearing in mind the main limitations of this study, these results indicate a role for the serotonin and GABA pathways in the risk of early-onset OCD. However, our results need to be replicated in studies with larger cohorts in order to confirm these associations.

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## Supporting Information

Additional supporting information may be found in the online version of this article at the publisher's web-site:

**Table S1.** ParentTDT analysis in 75 early-onset OCD trios of 265 validated SNPs.

**Table S1.** Parent TDT analysis in 75 early onset OCD trios of 265 validated SNPs.

| SNPs | Allele | T:U, PA:PU | Chi square | P-value | Chr Position | Chr | Gene |
|---|---|---|---|---|---|---|---|
| rs4653108 | C | 19:17,10,0:6,0 | 0,074 | 0,7855 | 35327932 | 1 | DLGAP3 |
| rs4653110 | A | 18:8,6,0:6,0 | 2,5 | 0,1138 | 35328175 | 1 | DLGAP3 |
| rs11583978 | A | 24:22,12,0:8,0 | 0,056 | 0,8137 | 35330422 | 1 | DLGAP3 |
| rs7541937 | A | 27:26,17,0:8,0 | 0,727 | 0,3938 | 35341922 | 1 | DLGAP3 |
| rs7525948 | C | 7:5,1,0:3,0 | 1 | 0,3173 | 35353804 | 1 | DLGAP3 |
| rs6662980 | A | 28:20,11,0:3,0 | 0 | 1 | 35360018 | 1 | DLGAP3 |
| rs4259608 | - | 23:23,9,0:5,0 | 0,25 | 0,6171 | 35364357 | 1 | DLGAP3 |
| rs4652867 | A | 24:15,4,0:10,0 | 3,814 | 0,0508 | 35367190 | 1 | DLGAP3 |
| rs11264172 | C | 23:21,8,0:4,0 | 0,065 | 0,7995 | 35368331 | 1 | DLGAP3 |
| rs3587 | C | 37:32,11,0:11,0 | 0,248 | 0,6188 | 35373274 | 1 | DLGAP3 |
| rs7555884 | A | 36:32,12,0:11,0 | 0,091 | 0,763 | 35373778 | 1 | DLGAP3 |
| rs6694435 | A | 26:25,10,0:8,0 | 0,014 | 0,9055 | 35379059 | 1 | DLGAP3 |
| rs7532286 | C | 34:31,12,0:12,0 | 0,095 | 0,7582 | 154750716 | 1 | KCNN3 |
| rs6426929 | A | 34:31,12,0:12,0 | 0,095 | 0,7582 | 154750889 | 1 | KCNN3 |
| rs13376333 | A | 38:26,17,0:4,0 | 0,011 | 0,9174 | 154814253 | 1 | KCNN3 |
| rs4845396 | C | 35:33,7,0:9,0 | 0,186 | 0,6662 | 154828309 | 1 | KCNN3 |
| rs4387163 | C | 30:28,2,0:8,0 | 0,914 | 0,339 | 164995613 | 1 | LMX1A |
| rs4657411 | A | 33:32,10,0:9,0 | 0 | 1 | 165173449 | 1 | LMX1A |
| rs4657412 | C | 22:19,4,0:13,0 | 2,323 | 0,1275 | 165176933 | 1 | LMX1A |
| rs6668493 | A | 15:12,5,0:10,0 | 1,455 | 0,2278 | 165183867 | 1 | LMX1A |
| rs1978340 | C | 32:31,6,0:10,0 | 0,316 | 0,5737 | 171670021 | 2 | GAD1 |
| rs3791878 | C | 30:27,6,0:10,0 | 0,671 | 0,4126 | 171672091 | 2 | GAD1 |
| rs3749034 | C | 23:19,7,0:6,0 | 0,153 | 0,6961 | 171673375 | 2 | GAD1 |
| rs2241165 | C | 29:25,6,0:13,0 | 1,532 | 0,2159 | 171678279 | 2 | GAD1 |
| rs11542313 | A | 36:31,7,0:16,0 | 2 | 0,1573 | 171678525 | 2 | GAD1 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| rs1420385 | C | 40:28,6,0:15,0 | 4,546 | 0,033 | 171681883 | 2 | GAD1 |
| rs3791860 | C | 43:31,5,0:17,0 | 5,538 | 0,0186 | 171689321 | 2 | GAD1 |
| rs7561581 | C | 25:18,6,0:7,0 | 1,103 | 0,2935 | 171696372 | 2 | GAD1 |
| rs701492 | C | 24:17,6,0:7,0 | 1,143 | 0,285 | 171702380 | 2 | GAD1 |
| rs769395 | C | 22:18,7,0:7,0 | 0,286 | 0,593 | 171716703 | 2 | GAD1 |
| rs4297845 | C | 36:34,6,0:13,0 | 0,835 | 0,3608 | 171719439 | 2 | GAD1 |
| rs4439928 | A | 9:8,2,0:5,0 | 0,667 | 0,4142 | 171719465 | 2 | GAD1 |
| rs6435639 | C | 27:26,12,0:7,0 | 0,205 | 0,6506 | 212403223 | 2 | ERBB4 |
| rs4673628 | A | 31:29,12,0:16,0 | 0,353 | 0,5525 | 212543824 | 2 | ERBB4 |
| rs7598440 | A | 30:24,13,0:8,0 | 0,012 | 0,9126 | 212793098 | 2 | ERBB4 |
| rs839523 | A | 28:27,8,0:9,0 | 0,053 | 0,8185 | 212815989 | 2 | ERBB4 |
| rs707284 | A | 32:27,8,0:13,0 | 1,19 | 0,2752 | 212838946 | 2 | ERBB4 |
| rs7564590 | C | 29:26,8,0:13,0 | 0,762 | 0,3827 | 213387800 | 2 | ERBB4 |
| rs2087017 | A | 36:24,11,0:13,0 | 2,042 | 0,153 | 113841913 | 3 | DRD3 |
| rs2134655 | C | 27:14,7,0:10,0 | 4 | 0,0455 | 113858101 | 3 | DRD3 |
| rs3773679 | C | 35:29,9,0:13,0 | 1,064 | 0,3023 | 113869235 | 3 | DRD3 |
| rs3773678 | A | 13:11,5,0:2,0 | 0,03 | 0,8618 | 113869978 | 3 | DRD3 |
| rs167771 | A | 20:19,8,0:4,0 | 0,164 | 0,6858 | 113876175 | 3 | DRD3 |
| rs167770 | A | 33:26,11,0:9,0 | 0,287 | 0,5919 | 113879462 | 3 | DRD3 |
| rs9825563 | A | 26:17,8,0:9,0 | 1,562 | 0,2113 | 113900120 | 3 | DRD3 |
| rs4261913 | A | 35:28,7,0:11,0 | 1,391 | 0,2383 | 119546097 | 3 | GSK3B |
| rs7617372 | C | 25:24,10,0:5,0 | 0,242 | 0,6225 | 119619230 | 3 | GSK3B |
| rs9856817 | A | 31:26,10,0:7,0 | 0,05 | 0,8231 | 119625319 | 3 | GSK3B |
| rs6774210 | C | 27:12,9,0:8,0 | 3,062 | 0,0801 | 119680642 | 3 | GSK3B |
| rs4688054 | A | 23:16,7,0:5,0 | 0,455 | 0,5002 | 119733610 | 3 | GSK3B |
| rs11923854 | A | 17:13,4,0:9,0 | 1,8 | 0,1797 | 119746469 | 3 | GSK3B |
| rs17811061 | A | 24:18,4,0:4,0 | 0,72 | 0,3961 | 119821142 | 3 | GSK3B |
| rs27072 | C | 16:14,5,0:8,0 | 0,581 | 0,4458 | 1394422 | 5 | SLC6A3 |

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|---|---|---|---|---|---|---|---|
| rs27048 | C | 38:33,7,0:8,0 | 0,409 | 0,5224 | 1412545 | 5 | SLC6A3 |
| rs2042449 | C | 25:21,9,0:5,0 | 0 | 1 | 1416546 | 5 | SLC6A3 |
| rs464049 | C | 35:25,8,0:9,0 | 1,458 | 0,2273 | 1423805 | 5 | SLC6A3 |
| rs4975646 | A | 36:25,15,0:10,0 | 0,375 | 0,5403 | 1433301 | 5 | SLC6A3 |
| rs1048953 | A | 37:27,13,0:10,0 | 0,516 | 0,4726 | 1438074 | 5 | SLC6A3 |
| rs2455391 | C | 33:23,3,0:7,0 | 2,97 | 0,0848 | 1443398 | 5 | SLC6A3 |
| rs12652860 | A | 29:21,7,0:11,0 | 2 | 0,1573 | 1453672 | 5 | SLC6A3 |
| rs12654851 | C | 19:11,5,0:5,0 | 1,6 | 0,2059 | 1453904 | 5 | SLC6A3 |
| rs12658938 | A | 35:32,14,0:10,0 | 0,01 | 0,9215 | 26888360 | 5 | CDH9 |
| rs6870789 | C | 32:26,10,0:10,0 | 0,429 | 0,5127 | 26891908 | 5 | CDH9 |
| rs6895743 | A | 29:16,10,0:10,0 | 2,195 | 0,1385 | 26893349 | 5 | CDH9 |
| rs6885387 | A | 33:32,8,0:8,0 | 0,011 | 0,9146 | 26939078 | 5 | CDH9 |
| rs2054486 | A | 18:16,6,0:12,0 | 1,103 | 0,2935 | 26949541 | 5 | CDH9 |
| rs1366462 | - | 38:38,12,0:6,0 | 0,36 | 0,5485 | 26977680 | 5 | CDH9 |
| rs3792735 | A | 37:32,11,0:12,0 | 0,367 | 0,5445 | 27029789 | 5 | CDH9 |
| rs548294 | A | 33:27,10,0:10,0 | 0,419 | 0,5176 | 152868337 | 5 | GRIA1 |
| rs2195450 | C | 31:20,6,0:7,0 | 2,25 | 0,1336 | 152870909 | 5 | GRIA1 |
| rs1428920 | C | 33:32,9,0:10,0 | 0,043 | 0,8348 | 152876908 | 5 | GRIA1 |
| rs1864205 | C | 30:21,12,0:11,0 | 0,78 | 0,377 | 152884730 | 5 | GRIA1 |
| rs1994862 | A | 24:23,7,0:7,0 | 0,015 | 0,9028 | 152988810 | 5 | GRIA1 |
| rs10515697 | A | 24:23,7,0:7,0 | 0,015 | 0,9028 | 153010545 | 5 | GRIA1 |
| rs707176 | A | 35:27,10,0:6,0 | 0,19 | 0,6625 | 153029860 | 5 | GRIA1 |
| rs2963944 | A | 35:27,10,0:6,0 | 0,19 | 0,6625 | 153035425 | 5 | GRIA1 |
| rs2926835 | A | 18:14,7,0:4,0 | 0,021 | 0,884 | 153188830 | 5 | GRIA1 |
| rs10943458 | C | 27:21,8,0:5,0 | 0,143 | 0,7055 | 78161488 | 6 | HTR1B |
| rs9352481 | C | 34:27,15,0:6,0 | 0,044 | 0,833 | 78162436 | 6 | HTR1B |
| rs9359271 | C | 35:17,5,0:7,0 | 6,25 | 0,0124 | 78166020 | 6 | HTR1B |
| rs2000292 | C | 31:10,6,0:13,0 | 11,879 | 0,0001 | 78166845 | 6 | HTR1B |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| rs13212041 | A | 24:21,9,0:10,0 | 0,229 | 0,6326 | 78171024 | 6 | HTR1B |
| rs6296 | C | 28:11,6,0:11,0 | 8,067 | 0,0045 | 78172160 | 6 | HTR1B |
| rs6298 | C | 28:11,6,0:11,0 | 8,067 | 0,0045 | 78172892 | 6 | HTR1B |
| rs11568817 | A | 38:27,7,0:13,0 | 3,042 | 0,0811 | 78173282 | 6 | HTR1B |
| rs4140535 | C | 30:18,7,0:13,0 | 4,263 | 0,0389 | 78174952 | 6 | HTR1B |
| rs9390754 | C | 15:10,0,0:9,0 | 5,765 | 0,0164 | 101964814 | 6 | GRIK2 |
| rs2247215 | C | 29:27,15,0:7,0 | 0,429 | 0,5127 | 101966354 | 6 | GRIK2 |
| rs1556995 | A | 23:21,5,0:8,0 | 0,424 | 0,5151 | 102317245 | 6 | GRIK2 |
| rs1417182 | C | 35:29,8,0:11,0 | 0,91 | 0,3401 | 102333241 | 6 | GRIK2 |
| rs4921691 | C | 30:21,9,0:13,0 | 2,139 | 0,1436 | 19995617 | 8 | SLC18A1 |
| rs10099144 | A | 36:29,10,0:9,0 | 0,419 | 0,5176 | 19996760 | 8 | SLC18A1 |
| rs10088489 | A | 21:14,6,0:5,0 | 0,783 | 0,3763 | 19999129 | 8 | SLC18A1 |
| rs11204097 | A | 9:6,2,0:2,0 | 0,474 | 0,4913 | 20001394 | 8 | SLC18A1 |
| rs6586896 | C | 20:5,3,0:5,0 | 8,758 | 0,0031 | 20004581 | 8 | SLC18A1 |
| rs4922132 | A | 16:14,8,0:4,0 | 0,095 | 0,7576 | 20010058 | 8 | SLC18A1 |
| rs7013199 | C | 31:16,6,0:7,0 | 4 | 0,0455 | 20021557 | 8 | SLC18A1 |
| rs2132699 | - | 24:24,9,0:6,0 | 0,138 | 0,7098 | 20029164 | 8 | SLC18A1 |
| rs2279709 | A | 38:36,12,0:9,0 | 0,01 | 0,9223 | 20036133 | 8 | SLC18A1 |
| rs11783752 | C | 31:24,9,0:16,0 | 2,227 | 0,1356 | 20049424 | 8 | SLC18A1 |
| rs35753505 | C | 31:30,11,0:4,0 | 0,45 | 0,5023 | 31474041 | 8 | NRG1 |
| rs4623364 | C | 30:25,12,0:4,0 | 0,123 | 0,7255 | 31475173 | 8 | NRG1 |
| rs7014762 | C | 29:18,3,0:8,0 | 4,414 | 0,0356 | 31495568 | 8 | NRG1 |
| rs2439305 | A | 43:28,11,0:11,0 | 2,184 | 0,1394 | 32429364 | 8 | NRG1 |
| rs3924999 | C | 37:32,10,0:8,0 | 0,097 | 0,7557 | 32453258 | 8 | NRG1 |
| rs2439272 | C | 35:27,10,0:11,0 | 0,89 | 0,3454 | 32492992 | 8 | NRG1 |
| rs6988339 | C | 33:31,9,0:13,0 | 0,383 | 0,536 | 32545816 | 8 | NRG1 |
| rs3757930 | A | 35:17,18,0:3,0 | 0,103 | 0,7477 | 32589018 | 8 | NRG1 |
| rs10503929 | C | 20:15,8,0:2,0 | 0,021 | 0,884 | 32613883 | 8 | NRG1 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| rs3933331 | A | 19:18,11,0:7,0 | 0,153 | 0,6961 | 4389841 | 9 | SLC1A1 |
| rs7858819 | A | 26:17,10,0:9,0 | 0,941 | 0,332 | 4559792 | 9 | SLC1A1 |
| rs3780412 | C | 36:32,14,0:5,0 | 0,269 | 0,6041 | 4572380 | 9 | SLC1A1 |
| rs12682807 | C | 10:6,2,0:6,0 | 2,667 | 0,1025 | 4573922 | 9 | SLC1A1 |
| rs301430 | A | 30:25,10,0:10,0 | 0,309 | 0,5785 | 4576580 | 9 | SLC1A1 |
| rs301979 | - | 27:27,6,0:11,0 | 0,316 | 0,5737 | 4576751 | 9 | SLC1A1 |
| rs3087879 | - | 33:33,7,0:6,0 | 0,012 | 0,9115 | 4586708 | 9 | SLC1A1 |
| rs301443 | C | 30:22,13,0:12,0 | 0,576 | 0,4477 | 4594819 | 9 | SLC1A1 |
| rs1659400 | C | 34:32,8,0:11,0 | 0,275 | 0,6002 | 87325894 | 9 | NTRK2 |
| rs1187272 | C | 35:30,7,0:11,0 | 0,953 | 0,329 | 87403986 | 9 | NTRK2 |
| rs1822420 | C | 15:13,7,0:5,0 | 0 | 1 | 87467176 | 9 | NTRK2 |
| rs10868235 | A | 33:21,10,0:12,0 | 2,45 | 0,1175 | 87493655 | 9 | NTRK2 |
| rs2289658 | A | 6:4,2,0:3,0 | 0,6 | 0,4386 | 87563270 | 9 | NTRK2 |
| rs2378672 | C | 5:4,5,0:3,0 | 0,048 | 0,8273 | 87630934 | 9 | NTRK2 |
| rs2236418 | A | 29:10,7,0:4,0 | 5,12 | 0,0237 | 26505396 | 10 | GAD2 |
| rs3781117 | C | 16:12,3,0:6,0 | 1,256 | 0,2623 | 26511944 | 10 | GAD2 |
| rs7908975 | A | 19:8,5,0:11,0 | 6,422 | 0,0113 | 26514112 | 10 | GAD2 |
| rs11015008 | A | 35:33,15,0:12,0 | 0,01 | 0,9223 | 26527490 | 10 | GAD2 |
| rs992990 | A | 40:15,5,0:9,0 | 1,1521 | 0,0002 | 26567081 | 10 | GAD2 |
| rs8190748 | A | 43:15,5,0:9,0 | 1,3474 | 0,0001 | 26569655 | 10 | GAD2 |
| rs3781108 | A | 30:11,8,0:4,0 | 4,245 | 0,0394 | 26580993 | 10 | GAD2 |
| rs928197 | A | 26:11,8,0:4,0 | 2,469 | 0,1161 | 26589525 | 10 | GAD2 |
| rs3758653 | A | 24:17,4,0:8,0 | 2,2 | 0,138 | 636299 | 11 | DRD4 |
| rs11246234 | C | 35:26,4,0:6,0 | 1,704 | 0,1917 | 645236 | 11 | DRD4 |
| rs4074376 | C | 18:14,6,0:6,0 | 0,348 | 0,5553 | 648615 | 11 | DRD4 |
| rs925946 | C | 24:20,11,0:10,0 | 0,123 | 0,7255 | 27667102 | 11 | BDNF |
| rs10501087 | A | 28:22,8,0:9,0 | 0,671 | 0,4126 | 27670008 | 11 | BDNF |
| rs7124442 | C | 25:21,11,0:11,0 | 0,211 | 0,6464 | 27676941 | 11 | BDNF |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| rs6265 | A | 27:19,7,0:9,0 | 1,515 | 0,2184 | 27679830 | 11 | BDNF |
| rs11030101 | C | 21:19,8,0:5,0 | 0,019 | 0,8907 | 27680644 | 11 | BDNF |
| rs10835211 | A | 30:29,9,0:9,0 | 0,012 | 0,9115 | 27701305 | 11 | BDNF |
| rs16917237 | A | 26:22,8,0:9,0 | 0,352 | 0,5529 | 27702283 | 11 | BDNF |
| rs2049046 | A | 35:33,15,0:12,0 | 0,01 | 0,9223 | 27723675 | 11 | BDNF |
| rs962369 | C | 24:18,10,0:10,0 | 0,514 | 0,4733 | 27734320 | 11 | BDNF |
| rs908867 | - | 8:8,3,0:4,0 | 0,04 | 0,8415 | 27745664 | 11 | BDNF |
| rs1491850 | C | 37:33,14,0:10,0 | 0 | 1 | 27749625 | 11 | BDNF |
| rs2049048 | C | 14:11,3,0:10,0 | 2,5 | 0,1138 | 27750486 | 11 | BDNF |
| rs1491851 | C | 36:33,6,0:9,0 | 0,409 | 0,5224 | 27752663 | 11 | BDNF |
| rs1800497 | A | 30:17,7,0:5,0 | 2,051 | 0,1521 | 113270747 | 11 | DRD2 |
| rs2734841 | C | 28:18,6,0:8,0 | 2,182 | 0,1396 | 113281676 | 11 | DRD2 |
| rs1124491 | A | 40:33,9,0:5,0 | 0,101 | 0,7505 | 113281990 | 11 | DRD2 |
| rs6277 | A | 37:30,7,0:3,0 | 0,111 | 0,7389 | 113283359 | 11 | DRD2 |
| rs6275 | C | 26:20,6,0:8,0 | 0,97 | 0,3248 | 113283377 | 11 | DRD2 |
| rs1076560 | A | 24:11,6,0:4,0 | 2,689 | 0,1011 | 113283588 | 11 | DRD2 |
| rs2440390 | C | 19:11,8,0:5,0 | 0,581 | 0,4458 | 113286778 | 11 | DRD2 |
| rs1076563 | C | 22:16,8,0:7,0 | 0,439 | 0,5078 | 113295809 | 11 | DRD2 |
| rs17115583 | A | 12:8,3,0:6,0 | 1,69 | 0,1936 | 113308802 | 11 | DRD2 |
| rs4245147 | A | 37:29,8,0:9,0 | 0,91 | 0,3401 | 113317907 | 11 | DRD2 |
| rs7131056 | A | 35:22,12,0:9,0 | 1,163 | 0,2809 | 113329674 | 11 | DRD2 |
| rs4648317 | C | 16:14,7,0:8,0 | 0,191 | 0,6617 | 113331432 | 11 | DRD2 |
| rs11214613 | A | 23:10,6,0:4,0 | 2,814 | 0,0934 | 113335159 | 11 | DRD2 |
| rs1799732 | A | 9:6,2,0:2,0 | 0,474 | 0,4913 | 113346202 | 11 | DRD2 |
| rs12364283 | C | 10:7,6,0:4,0 | 0,037 | 0,8474 | 113346895 | 11 | DRD2 |
| rs10891556 | - | 12:12,7,0:3,0 | 0,444 | 0,505 | 113352661 | 11 | DRD2 |
| rs6589377 | A | 40:29,7,0:8,0 | 1,636 | 0,2008 | 113355636 | 11 | DRD2 |
| rs4522263 | - | 9:9,5,0:3,0 | 0,154 | 0,6949 | 13712375 | 12 | GRIN2B |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| rs3026174 | A | 16:13,10,0:3,0 | 0,348 | 0,5553 | 13713684 | 12 | GRIN2B |
| rs1805502 | A | 17:14,7,0:6,0 | 0,083 | 0,7728 | 13714081 | 12 | GRIN2B |
| rs890 | A | 42:33,9,0:8,0 | 0,667 | 0,4142 | 13715208 | 12 | GRIN2B |
| rs1806191 | A | 34:24,13,0:7,0 | 0,182 | 0,6698 | 13716538 | 12 | GRIN2B |
| rs1806201 | A | 27:25,6,0:6,0 | 0,062 | 0,8026 | 13717408 | 12 | GRIN2B |
| rs1806194 | C | 39:29,14,0:8,0 | 0,157 | 0,6921 | 13723067 | 12 | GRIN2B |
| rs10845840 | C | 30:27,8,0:7,0 | 0,051 | 0,8208 | 13930757 | 12 | GRIN2B |
| rs220599 | - | 31:31,8,0:4,0 | 0,211 | 0,6464 | 13975198 | 12 | GRIN2B |
| rs7301328 | A | 40:27,6,0:12,0 | 4,056 | 0,044 | 14018677 | 12 | GRIN2B |
| rs1019385 | A | 40:28,12,0:9,0 | 0,835 | 0,3608 | 14134743 | 12 | GRIN2B |
| rs3935748 | C | 14:11,7,0:3,0 | 0,027 | 0,8694 | 72325637 | 12 | TPH2 |
| rs4570625 | C | 19:14,8,0:3,0 | 0 | 1 | 72331823 | 12 | TPH2 |
| rs10748185 | C | 31:30,11,0:5,0 | 0,325 | 0,5688 | 72335755 | 12 | TPH2 |
| rs1386494 | A | 16:13,4,0:6,0 | 0,641 | 0,4233 | 72352443 | 12 | TPH2 |
| rs6582072 | C | 21:17,9,0:6,0 | 0,019 | 0,8907 | 72354377 | 12 | TPH2 |
| rs7305115 | A | 26:25,8,0:12,0 | 0,342 | 0,5584 | 72372762 | 12 | TPH2 |
| rs11179027 | A | 27:21,6,0:11,0 | 1,862 | 0,1724 | 72377212 | 12 | TPH2 |
| rs10879352 | C | 18:15,6,0:7,0 | 0,333 | 0,5637 | 72406858 | 12 | TPH2 |
| rs1487275 | A | 24:15,9,0:10,0 | 1,562 | 0,2113 | 72410192 | 12 | TPH2 |
| rs11179052 | C | 24:23,10,0:3,0 | 0,6 | 0,4386 | 72412601 | 12 | TPH2 |
| rs4290270 | A | 27:22,6,0:11,0 | 1,515 | 0,2184 | 72416135 | 12 | TPH2 |
| rs7333412 | C | 38:25,10,0:11,0 | 2,279 | 0,1311 | 47403260 | 13 | 5-HTR2A |
| rs3125 | C | 23:10,6,0:5,0 | 3,273 | 0,0704 | 47408751 | 13 | 5-HTR2A |
| rs6314 | A | 15:14,7,0:5,0 | 0,023 | 0,8788 | 47408934 | 13 | 5-HTR2A |
| rs9567736 | A | 14:7,3,0:4,0 | 2,133 | 0,1441 | 47420883 | 13 | 5-HTR2A |
| rs1923886 | - | 32:32,6,0:9,0 | 0,111 | 0,7389 | 47423191 | 13 | 5-HTR2A |
| rs2296972 | C | 25:19,3,0:14,0 | 4,188 | 0,0407 | 47428371 | 13 | 5-HTR2A |
| rs2770298 | C | 31:26,10,0:9,0 | 0,205 | 0,6506 | 47446747 | 13 | 5-HTR2A |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| rs731779 | A | 26:20,7,0:8,0 | 0,778 | 0,3778 | 47451938 | 13 | 5-HTR2A |
| rs1002513 | A | 15:14,6,0:6,0 | 0,021 | 0,884 | 47454340 | 13 | 5-HTR2A |
| rs927544 | - | 29:29,8,0:10,0 | 0,049 | 0,8252 | 47455951 | 13 | 5-HTR2A |
| rs9567746 | A | 25:20,7,0:8,0 | 0,581 | 0,4461 | 47456448 | 13 | 5-HTR2A |
| rs4941573 | A | 36:31,10,0:12,0 | 0,505 | 0,4772 | 47464757 | 13 | 5-HTR2A |
| rs6313 | A | 36:31,10,0:11,0 | 0,383 | 0,536 | 47469840 | 13 | 5-HTR2A |
| rs6311 | A | 36:31,10,0:11,0 | 0,383 | 0,536 | 47471378 | 13 | 5-HTR2A |
| rs17289394 | A | 30:24,11,0:12,0 | 0,563 | 0,453 | 47473120 | 13 | 5-HTR2A |
| rs945032 | A | 20:19,5,0:7,0 | 0,17 | 0,6803 | 96670561 | 14 | BDKRB2 |
| rs1799722 | A | 34:28,9,0:12,0 | 0,91 | 0,3401 | 96671039 | 14 | BDKRB2 |
| rs8016905 | C | 25:16,8,0:9,0 | 1,667 | 0,1967 | 96675833 | 14 | BDKRB2 |
| rs945039 | C | 27:14,10,0:7,0 | 1,724 | 0,1892 | 96684098 | 14 | BDKRB2 |
| rs2498804 | - | 29:29,8,0:7,0 | 0,013 | 0,9093 | 105232995 | 14 | AKT1 |
| rs2494731 | A | 32:30,7,0:8,0 | 0,114 | 0,7357 | 105237580 | 14 | AKT1 |
| rs1130233 | - | 23:23,6,0:5,0 | 0,017 | 0,8964 | 105239794 | 14 | AKT1 |
| rs2498794 | A | 35:32,10,0:10,0 | 0,097 | 0,7557 | 105245151 | 14 | AKT1 |
| rs2494743 | A | 15:8,6,0:4,0 | 0,758 | 0,3841 | 105251620 | 14 | AKT1 |
| rs10136000 | C | 23:21,8,0:4,0 | 0,065 | 0,7995 | 105253481 | 14 | AKT1 |
| rs1130214 | A | 26:25,9,0:6,0 | 0,057 | 0,8111 | 105259634 | 14 | AKT1 |
| rs3803300 | A | 10:9,7,0:3,0 | 0,31 | 0,5775 | 105269679 | 14 | AKT1 |
| rs1017412 | A | 34:28,5,0:9,0 | 1,316 | 0,2513 | 88412043 | 15 | NTRK3 |
| rs7176429 | A | 29:26,13,0:7,0 | 0,111 | 0,7389 | 88419324 | 15 | NTRK3 |
| rs999905 | A | 32:27,9,0:8,0 | 0,2 | 0,6547 | 88563771 | 15 | NTRK3 |
| rs4887348 | A | 32:24,8,0:5,0 | 0,362 | 0,5472 | 88571434 | 15 | NTRK3 |
| rs7180942 | C | 33:26,8,0:12,0 | 1,458 | 0,2273 | 88674476 | 15 | NTRK3 |
| rs1042173 | A | 33:28,10,0:7,0 | 0,048 | 0,8273 | 28524911 | 17 | SLC6A4 |
| rs2020936 | A | 23:15,7,0:9,0 | 1,724 | 0,1892 | 28550714 | 17 | SLC6A4 |
| rs2066713 | C | 31:28,6,0:10,0 | 0,636 | 0,425 | 28551565 | 17 | SLC6A4 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| rs7214991 | C | 25:22,6,0:7,0 | 0,25 | 0,6171 | 28572260 | 17 | SLC6A4 |
| rs2671689 | A | 11:5,4,0:3,0 | 1,087 | 0,2971 | 47563061 | 17 | NGFR |
| rs584589 | C | 16:13,1,0:8,0 | 2,5 | 0,1138 | 47563493 | 17 | NGFR |
| rs1035050 | C | 30:29,17,0:9,0 | 0,516 | 0,4726 | 47563912 | 17 | NGFR |
| rs2671687 | A | 33:24,12,0:13,0 | 1,136 | 0,2864 | 47564738 | 17 | NGFR |
| rs603769 | C | 31:28,9,0:12,0 | 0,4 | 0,5271 | 47569985 | 17 | NGFR |
| rs600120 | C | 32:26,17,0:9,0 | 0,043 | 0,8366 | 47577384 | 17 | NGFR |
| rs3785931 | A | 32:25,8,0:10,0 | 1,08 | 0,2987 | 47578018 | 17 | NGFR |
| rs2072445 | C | 10:9,5,0:3,0 | 0,037 | 0,8474 | 47587612 | 17 | NGFR |
| rs2072446 | A | 9:8,5,0:2,0 | 0,167 | 0,6831 | 47587719 | 17 | NGFR |
| rs1804011 | C | 15:8,5,0:9,0 | 2,951 | 0,0858 | 47591170 | 17 | NGFR |
| rs734194 | C | 14:12,6,0:10,0 | 0,783 | 0,3763 | 47591549 | 17 | NGFR |
| rs11701698 | C | 24:21,7,0:7,0 | 0,143 | 0,7055 | 34393845 | 21 | OLIG2 |
| rs1005573 | A | 34:21,8,0:6,0 | 1,704 | 0,1917 | 34398616 | 21 | OLIG2 |
| rs1059004 | A | 35:27,8,0:10,0 | 1,163 | 0,2809 | 34400363 | 21 | OLIG2 |
| rs13046814 | C | 37:33,8,0:6,0 | 0,047 | 0,8292 | 34401277 | 21 | OLIG2 |
| rs9653711 | A | 35:28,9,0:10,0 | 0,727 | 0,3938 | 34401849 | 21 | OLIG2 |
| rs762237 | C | 32:25,7,0:8,0 | 0,865 | 0,3524 | 34408077 | 21 | OLIG2 |
| rs2834072 | C | 38:25,8,0:7,0 | 1,8 | 0,1797 | 34410364 | 21 | OLIG2 |
| rs928736 | C | 27:18,9,0:8,0 | 0,941 | 0,332 | 34439789 | 21 | OLIG1 |
| rs7278735 | C | 19:12,5,0:3,0 | 0,641 | 0,4233 | 34444982 | 21 | OLIG1 |
| rs9606186 | C | 28:27,9,0:11,0 | 0,108 | 0,7419 | 19920259 | 22 | COMT |
| rs1800706 | C | 26:20,5,0:10,0 | 1,806 | 0,179 | 19927922 | 22 | COMT |
| rs4646310 | C | 19:18,7,0:4,0 | 0,071 | 0,7893 | 19928746 | 22 | COMT |
| rs737865 | C | 33:26,5,0:14,0 | 2,977 | 0,0845 | 19930021 | 22 | COMT |
| rs1544325 | C | 31:28,8,0:9,0 | 0,195 | 0,6587 | 19931568 | 22 | COMT |
| rs5993882 | C | 23:22,10,0:4,0 | 0,373 | 0,5413 | 19937433 | 22 | COMT |
| rs4646312 | C | 39:36,4,0:12,0 | 1,33 | 0,2489 | 19948237 | 22 | COMT |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| rs4818 | C | 39:36,4,0:12,0 | 1,33 | 0,2489 | 19951107 | 22 | COMT |
| rs165815 | A | 17:8,5,0:6,0 | 2,381 | 0,1228 | 19959373 | 22 | ARVCF |
| rs909525 | T | 13:12 | 0,04 | 0,8415 | 43553102 | X | MAOA |
| rs6323 | A | 19:13 | 1 | 0,2888 | 43590936 | X | MAOA |
| rs2064070 | A | 16:14 | 0,133 | 0,715 | 43608582 | X | MAOA |
| rs6609257 | A | 12:11 | 0,043 | 0,8348 | 43612608 | X | MAOA |
| rs3813928 | G | 9:08 | 0,059 | 0,8084 | 113818182 | X | HTR2C |
| rs3813929 | C | 9:07 | 0,25 | 0,6171 | 113818420 | X | HTR2C |
| rs518147 | A | 16:07 | 4 | 0,0606 | 113818482 | X | HTR2C |
| rs6318 | G | 16:13 | 0,31 | 0,5775 | 113965612 | X | HTR2C |
| rs3761555 | G | 17:15 | 0,125 | 0,7237 | 122316337 | X | GRIA3 |
| rs2269551 | C | 11:07 | 0,889 | 0,3458 | 122319817 | X | GRIA3 |
| rs2285127 | T | 14:13 | 0,037 | 0,8474 | 122336348 | X | GRIA3 |
| rs4825476 | G | 17:14 | 0,29 | 0,59 | 122441379 | X | GRIA3 |
| rs550640 | G | 8:03 | 2 | 0,1317 | 122528703 | X | GRIA3 |
| rs687577 | A | 15:14 | 0,034 | 0,8527 | 122579004 | X | GRIA3 |

### 4.4. Asociación entre genes relacionados con la serotonina y el glutamato con neurometabolitos medidos mediante resonancia magnética espectroscópica en pacientes con TOC de inicio en la infancia y la adolescencia (Objetivo 4)

Se incluyeron un total de 41 pacientes de los que se disponía de datos genéticos y espectroscópicos. El análisis genético de los 262 polimorfismos mostró varias asociaciones significativas, después de aplicar la corrección de Bonferroni, ($p \leq 0.00019$), entre la concentración cerebral de diferentes metabolitos y determinados SNPs. Estos resultados se pueden observar en la figura 1 del estudio, página 154.

La concentración de Ins se asoció con dos polimorfismos (rs6296 y rs6298) ubicados en el gen *HTR1B*. En concreto, la concentración de Ins fue menor en los pacientes portadores del alelo minoritario de los dos polimorfismos del gen *HTR1B* ($p = 0.000013$; se puede consultar en la tabla 1 del estudio 4, página 155).

Por otro lado, la concentración de Glx se asoció a un polimorfismo (rs6586896) del gen *SLC18A1*. En este caso, la concentración de Glx fue menor en los pacientes homocigotos para el alelo mayoritario del SNP ($p = 0.00011$; se puede consultar en la tabla 1 del estudio 4, página 155).

Finalmente, la concentración de Cho se asoció con dos polimorfismos (rs707176 y rs2963944) del gen del receptor ionotrópico de glutamato AMPA1 (*GRIA1*). En concreto, la concentración de Cho fue menor en los pacientes homocigotos para el alelo mayoritario de ambos polimorfismos ($p = 0.000053$; se puede observar en la tabla 1 del estudio 4, página 155).

Para el resto de concentraciones cerebrales de metabolitos, NAA o Cr, no se encontraron asociaciones genéticas significativas después de aplicar la corrección de Bonferroni.

**Estudio 4:** Association between genetic variants of serotoninergic and glutamatergic pathways and the concentration of neurometabolites of the anterior cingulate cortex in pediatric patients with obsessive-compulsive disorder. The World Journal of Biological Psychiatry (Under review).

**The World Journal of Biological Psychiatry**




# Association between Genetic Variants of Serotonergic and Glutamatergic Pathways and the Concentration of Neurometabolites of the Anterior Cingulate Cortex in Pediatric Patients with Obsessive-Compulsive Disorder

| | |
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| | |

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# Association between Genetic Variants of Serotonergic and Glutamatergic Pathways and the Concentration of Neurometabolites of the Anterior Cingulate Cortex in Pediatric Patients with Obsessive-Compulsive Disorder

**Linking genetics and neuroimage**

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**Abstract**

*Objectives:* The present study therefore aimed to assess the relationship between variability in genes related to the pathophysiology of OCD and the concentration of different neurometabolites in the anterior cingulate cortex (ACC). *Methods:* we concomitantly assessed neurometabolite concentrations by 3-T $^{1}$H-MRS and 262 single nucleotide polymorphism (SNPs) in 35 genes among 41 pediatric OCD patients. *Results:* There were significant associations, after Bonferroni's correction, between the concentration of inositol, glutamate and glutamine, and total choline and five polymorphisms located in genes related to serotonin and glutamate (i.e., the vesicular monoamine transporter 1 gene, *SLC18A1* [rs6586896]; the serotonin receptor 1B gene, *HTR1B* [rs6296 and rs6298]; and the glutamate receptor, ionotropic, AMPA1 gene, *GRIA1* [rs707176 and rs2963944]). *Conclusions:* The association observed between these polymorphisms and the neurometabolite concentrations could indicate the presence of a biological interaction between the serotonin and the glutamate pathways that could be involved in the pathophysiology of OCD. More studies with this methodology could increase our understanding of the etiology and pathophysiology of OCD in children.

***Keywords:*** Anterior cingulate cortex, Children and adolescents, Magnetic resonance spectroscopy, Obsessive-compulsive disorder, Single nucleotide polymorphism

**Abbreviations**

ACC, anterior cingulate cortex; ANOVA, analysis of variance; Cho, glycerophosphocholine plus phosphocholine; Cr, creatine plus phosphocreatine; CSF, cerebrospinal fluid; CSTC, cortico-striatal-thalamo-cortical; CY-BOCS, Children's Yale-Brown Obsessive-Compulsive Scale; DSM-IV, Diagnostic and Statistical Manual of Mental Disorders, fourth edition; EDTA, ethylenediamine tetraacetic acid; FWHM, full width at half maximum; Glx, glutamate plus glutamine, GM, gray matter; *GRIA1*, glutamate receptor, ionotropic, AMPA1; $^{1}$H-MRS, proton magnetic resonance spectroscopy; *HTR1B*, serotonin receptor 1B gene; Ins, inositol; IQ, intelligence quotient; MRI, magnetic resonance imaging; NAA: N-acetyl-aspartate plus N-acetyl aspartyl glutamate; OCD, obsessive-compulsive disorder; *SLC18A1,* vesicular monoamine transporter 1 gene,; SNP, single nucleotide polymorphism; VOI, volume of interest; WM, white matter.

## Introduction

Obsessive-compulsive disorder (OCD) is a psychiatric disorder characterized by distressing, intrusive, repetitive, and often incontrollable thoughts (obsessions) and the urge to engage in repetitive time-consuming behaviors (compulsions) that are enacted to reduce, neutralize, or prevent distress, dreaded experiences, or events (American Psychiatric Association, 2013). OCD is estimated to affect 1%–3% of the population (Heyman et al., 2001), with 30%–50% of patients developing it in childhood (Stewart et al., 2004), and it has been proposed that childhood onset of OCD might represent a more severe developmental subtype of the disorder (Rosario-Campos et al., 2001). Thus, it is essential that we clarify the neurobiology of OCD, characterize its subtypes, and improve the available treatment strategies in pediatric populations.

Although the exact etiology of OCD is unknown, there is evidence to suggest that the disorder arises from a complex combination of genetic and environmental factors (Taylor et al., 2010). More than 80 genetic association studies of candidate OCD genes have been published over the last decade, each of which could be relevant to the pathophysiology and pharmacology of OCD. However, despite this strong genetic background and high familiarity (Pauls, 2008; van Grootheest et al., 2005; Walitza et al., 2010), the identification of a single causal gene variant has remained elusive (Grünblatt et al., 2014).

The use of a variety of imaging techniques have led researchers to suggest that cortico-striatal-thalamo-cortical (CSTC) circuit dysfunction is a core pathophysiological feature of OCD (Saxena and Rauch, 2000). In OCD patients, the key components of this circuit include the orbitofrontal cortex (OFC), anterior cingulate cortex (ACC), and striatum (Brennan et al., 2013). The ACC has been suggested to be involved in OCD

because of its role in error detection and monitoring and role in the processing of conflicting information (Gehring et al., 2000; van Veen and Carter, 2002). Several studies have reported abnormal changes in the concentrations of neural metabolites in the ACC of patients with OCD (Ebert et al., 1997; Rosenberg et al., 2004, 2000; Tükel et al., 2014; Yücel et al., 2008).

Complementary approaches examining regional neurochemistry now hold the promise of additional insights into the neurobiology of OCD. In particular, proton magnetic resonance spectroscopy ($^{1}$H-MRS) permits in vivo quantification of specific neurochemicals in various brain regions. The technique records a series of peaks or resonances, each representing the local concentration of a different neurometabolite or small family of chemically related neurometabolites. Compounds that can be measured at present, include myo-inositol (Ins), glutamatergic compounds (Glx), Total choline (Cho), N-acetyl-aspartate (NAA) and creatine (Cr). Ins can be regarded as a glial cell marker that is intimately connected to the osmoregulation of astrocytes, so its increase probably reflects glial activation; in addition, Ins is considered a degradation product of myelin (Starck et al., 2008). Glx is a useful measure of glutamatergic excitatory neurotransmission (Starck et al., 2008), while Cho levels reflect choline-containing compounds such as phosphocholine and acetylcholine. Decreased Cho concentrations have been observed in regions of acute demyelinization in patients with multiple sclerosis and are believed to reflect abnormalities of myelination (Smith et al., 2003). NAA is a marker for neuronal viability and declines in neural tissue before neuronal loss is detected by structural magnetic resonance imaging (MRI). Decreased NAA concentrations are therefore associated with impaired neuronal function (Birken and Oldendorf, 1989). Altered brain creatine-

phosphocreatine concentrations might also reflect changes in brain energy use (Mirza et al., 2006). Despite the large amount of literature describing alterations in these neurometabolites in different areas of the brain OCD, most does not benefit from the addition of $^{1}$H-MRS. This is likely due to the reliance on small and heterogeneous study samples (age, illness duration, illness severity, comorbidity, and concomitant medications), together with the widely varying imaging methodologies used (Brennan et al., 2013).

Another way to clarify the genetic basis of OCD would be to identify the endophenotypes associated with OCD, including the identification of neuroimaging endophenotypes. This approach could delineate pathways linking risk genes to disorders (Meyer-Lindenberg and Weinberger, 2006), thereby increasing our understanding of the mechanisms of psychiatric diseases and identifying potential therapeutic targets. To our knowledge, only one previous genetic study of pediatric OCD used neuroimaging phenotypes (Arnold et al., 2009). That study reported an association between a polymorphism of the glutamate receptor and glutamate concentrations in the ACC. No other studies have been published in recent years that evaluate genetic and neurochemical variables concurrently.

This preliminary study aimed to assess the relationship between genetic variability associated with the pathophysiology of OCD and the concentration of different neurometabolites in the ACC. Therefore, we assessed neurometabolite concentrations by 3-T $^{1}$H-MRS against 262 single nucleotide polymorphism (SNPs) in 35 genes in 41 pediatric OCD patients. We hypothesized that altered neurometabolite concentrations in the ACC may be at least partly genetically determined.

## Materials and Methods

### *Subjects*

We recruited 87 patients meeting the DSM-IV diagnostic criteria for OCD from the Department of Child and Adolescent Psychiatry and Psychology at the Hospital Clinic in Barcelona (American Psychiatric Association, 2000). Non-Caucasian patients were excluded. Ethnicity was determined by self-reported ancestries, and we excluded participants with non-European grandparents. Genetic and $^{1}$H-MRS data were obtained from 75 and 47 patients, respectively. Finally, 41 patients, from which both genetic and $^{1}$H-MRS data were available, were included in the analysis. OCD severity was measured with CY-BOCS scale (Children's Yale-Brown Obsessive-Compulsive Scale) (Scahill et al., 1997).

The procedures were approved by our hospital's Ethics Committee. Written informed consent was obtained from all parents, and verbal informed consent was given by all participants following explanation of the procedures involved. The researchers undertook to preserve the anonymity of patients at all times and to use the information collected solely for the purposes indicated. All participants received reimbursement as compensation for their time.

### *$^{1}$H-MRS study*

The methods used for the $^{1}$H-MRS study have been previously reported (Ortiz et al., 2015). Briefly, $^{1}$H-MRS was acquired in a 3T TIM TRIO scanner (Siemens, Erlangen, Germany) using a 32-head channel coil. A volume of interest (VOI) of 3 cm$^{3}$ (15 × 20 ×10 mm$^{3}$) was determined in the ACC and the voxel was placed using two planes from the high-resolution T1-weighted image obtained previously. First, the voxel was placed in the sagittal plane, above the genu of the corpus callosum and centered on the ACC.

Confirmation that the ACC was in the voxel was then made in the axial plane. This procedure was applied in the same manner in all participants, and care was taken to ensure standard placement. Spectra were acquired with the use of a double-spin echo point-resolved spectroscopy sequence (PRESS) with a repetition time (TR) of 200 ms and an echo time (TE) of 35 ms.

Metabolite concentrations were quantified by a user-independent frequency domain-fitting program (LCModel, version 6.1-4A) (Provencher, 2001), applying the eddy current correction and using an internal water signal reference to calculate absolute metabolite concentrations. We only considered the absolute metabolite values with a Cramer-Rao lower bound below 20% and a signal-to-noise ratio greater than 10, so that these metabolites could be reliably estimated (Provencher, 2001). We excluded 16 participants for this reason. An additional structural image (3d T1-weighted MPRAGE sequence with isometric voxel of $1 \times 1 \times 1$ $mm^3$) was recorded in the same scanning session. The structural image was segmented using customized tissue probability maps (TPMs) and following the new segmentation model provided in SPM8 (Ashburner, 2009). To generate customized TPMs, white matter (WM) and gray matter (GM) images obtained from standard segmentation were normalized to MNI using DARTEL (Ashburner, 2007) at a resolution of $2 \times 2 \times 2$ $mm^3$, and averaged and smoothed with a Gaussian kernel of 8 mm. The metabolite concentrations were corrected for differences in the cerebrospinal fluid (CSF) content of the VOI using homemade software. The residual percentage of GM in the VOI, after removing the CSF component, was used as a confounding variable in the statistical analyses (Guerrini et al., 2009). A neuroradiologist confirmed that all MRI scans were free of gross structural abnormalities.

*Sample preparation*

Blood samples were collected from the individuals in ethylenediamine tetraacetic acid (EDTA) (K2EDTA BD Vacutainer EDTA tubes; Becton Dickinson, Franklin Lakes, New Jersey) and genomic DNA was extracted with the MagNA Pure LC DNA isolation Kit III and the LC MagNA Pure system (Roche Diagnostics GmbH, Mannheim, Germany). The DNA concentration was determined by absorbance (ND1000, NanoDrop, Wilmington, Delaware).

*SNP selection, genotyping, and quality control*

The method of SNP selection has been previously reported (Mas 2014). Briefly, SNPs were selected following one of three strategies: (1) tagging analysis (as implemented in Haploview 4.2) at an $r^2$ threshold of 0.8 to capture 98% of the most common HapMap phase II variants based on the CEU panel (minor allele frequency > 0.1) (range 91-100% for individual genes); (2) suspected SNP functionality according to data published in Ensembl (http://www.ensembl.org), dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP/), and the PupaSuite 3 (http://pupasuite.bioinfo.cipf.es/) databases, with a validated minor allele frequency > 0.1 in the Caucasian population; and (3) previous associations reported in the literature, either with OCD or other psychiatric diseases. SNPs were genotyped by the MassArray assay with the Sequenom genotyping system (San Diego, USA) at the Santiago de Compostela Node of the Spanish National Genotyping Centre (CeGen). For quality control, 13 samples were genotyped in duplicate for all the SNPs analyzed. There was a 100% concordance rate.

We excluded some SNPs from the analysis because they were incompatible with the genotyping design or monomorphics, because they showed Mendelian errors, inconsistent clustering, or low genotyping rates (fewer than 90% of samples), or because they were out of Hardy–Weinberg equilibrium. Finally, 262 validated SNPs in 35 candidate gene regions (covering target loci and upstream and downstream regions) were used for the genetic association analysis (Mas et al., 2014).

*Statistics*

Data were analyzed using IBM SPSS statistics 20 (IBM Corp., Chicago, Ill, USA). Means and standard deviations were computed for continuous variables. To identify variables associated with metabolite concentrations, the Spearman correlation, Student *t*-test, ANOVA, Mann–Whitney *U* test, or Kruskal–Wallis test were used according to the distribution and scales of the variables. Hardy–Weinberg equilibrium for each SNP was analyzed. To estimate the independent contribution of each SNP to determine metabolite concentration measurements, multivariate methods based on logistic regression analysis were performed under codominant, dominant, overdominant, recessive, and additive models. The analysis was adjusted for the variables identified in the univariate analysis. The best model was selected using Akaike information criteria (AIC). For those purposes, we used the SNPassoc R package (González et al., 2007). To avoid false positive results we applied Bonferroni correction for multiple testing (significant p-value < $1.9 \times 10^{-4}$ after Bonferroni correction).

**Results**

The mean age of the OCD group was 15.5 years (SD = 2.9) and the mean age at onset of OCD was 12.1 years (SD = 3.3). There were 21 boys (51.2%) and 20 girls (48.8%). The mean disease duration before evaluation was 25.2 months (SD = 20.9), with a range of 3–137 months. Most of the neurometabolite values obtained by $^{1}$H-MRS correlated with age. No other clinical variables were associated with the neurometabolites values. Therefore, the genetic analysis of neurometabolites was adjusted by age and the percentage of GM in the ACC (for conservativeness) as covariates.

The genetic analysis of the 262 validated polymorphisms showed that the concentration of Ins adjusted for CSF was associated with two polymorphisms (rs6296 and rs6298) located in the serotonin receptor 1B (*HTR1B*) gene. These SNPs showed significant p-values after Bonferroni correction (Figure 1). Particularly, the concentration of Ins adjusted for CSF was lower in patients that carried the minor allele frequency of the two polymorphisms of *HTR1B* (Table 1).

The concentration of Glx adjusted for CSF measured in our pediatric OCD population was associated with just one polymorphism in the vesicular monoamine transporter 1 (*SLC18A1*) gene (rs6586896). This showed significant p-values after Bonferroni correction (Figure 1). The concentration of Glx adjusted for CSF was lower in the major homozygous allele of *SLC18A1* rs6586896 ($p = 0.0001167$) (Table 1).

The concentration of Cho adjusted for CSF was associated with two polymorphisms in the glutamate receptor, ionotropic, AMPA1 (*GRIA1*) gene (rs707176 and rs2963944)). These also showed significant p-values after Bonferroni correction (Figure 1). The concentration of Cho adjusted for CSF was lower in the major homozygous allele of both *GRIA1* polymorphisms (Table 1).

No other significant genetic associations were found after Bonferroni correction, specifically for the concentrations of NAA and Cr neurometabolites.

**Discussion**

We conducted a preliminary analysis of the association between putative genetic polymorphisms related to OCD and the concentration of different neurometabolites in the ACC by $^{1}$H-MRS in children with OCD. Our results revealed significant associations, after Bonferroni correction, between the concentrations of Ins, Glx, and Cho and five polymorphisms in *SLC18A1* (rs6586896), *HTR1B* (rs6296 and rs6298), and *GRIA1* (rs707176 and rs2963944).

Differences in the concentration of Ins between controls and pediatric OCD patients may not only reflect dysfunctional neurotransmission in these patients but also glial abnormalities. In fact, this neurometabolite has been closely connected with the osmoregulation of astrocytes (Govindaraju et al., 2000), although its exact significance remains unclear. About two decades ago, several studies reported inconsistent findings in the role of Ins as a synergistic compound in the treatment of OCD (Fux et al., 1999, 1996). Several studies have assessed the concentrations of Ins in OCD patients versus healthy controls and have shown differences, including significantly increased concentrations in the right rostral and dorsal ACC (Yücel et al., 2008) and significantly decreased concentrations in the caudate (Whiteside et al., 2006). Interestingly, decreased concentrations of Ins in the ACC were previously found (Ortiz et al., 2015) in the same pediatric patients included in the present study when compared with healthy controls of the same age and sex. Now, we have associated the concentrations of this neurometabolite with two polymorphisms in the *HTR1B* gene.

Although they give rise to a synonymous change in the protein, these SNPs do seem to have some functional effect, being associated with altered response to antidepressant treatment (Villafuerte et al., 2009; Xu et al., 2012). Moreover, studies have reported associations between other *HTR1B* SNPs and OCD (Kim et al., 2009; Mundo et al., 2002) or other endophenotypes, such as the OFC volume (Atmaca et al. 2010) and severity of obsessions measured by the Y-BOCS score (Camarena et al., 2004). In fact, in a recent meta-analysis Taylor et al. (2013) identified a trend for the involvement of *HTR1B* in OCD. It is also noteworthy that this same gene, *HTR1B,* was also identified by our group in a transmission disequilibrium study of early-onset OCD, in which we included many of the same patients as in the present study (Mas et al., 2014).

The serotonin pathway became a leading target for investigation of the neurobiology of OCD largely because of the remarkable therapeutic effects of serotonin re-uptake inhibitors on obsessions and compulsions (Greist et al., 1995). This study identified that a polymorphism in another serotonin-related gene. Particularly, a polymorphism located in the vesicular monoamine transporter 1 gene, the *SLC18A1* (rs6586896), was associated with Glx concentrations in the ACC of pediatric OCD patients. The gene encoding *SLC18A1* is located on chromosome 8p21, a region implicated in linkage studies of schizophrenia, bipolar disorder, and anxiety-related phenotypes (Lohoff, 2010). In fact, several genetic case–control studies have documented an association between common missense variations in the *SLC18A1* gene and susceptibility to bipolar disorder and schizophrenia (Bly, 2005; Chen et al., 2007; Lohoff et al., 2006). Interestingly, this gene was also associated with anorexia nervosa and OCD in pediatric patients, some of whom were included in the present study (Mas et al., 2013).

There is growing evidence that disrupted glutamate neurotransmission within CSTC circuits is important in the pathogenesis OCD (Pittenger et al., 2006; Wu et al., 2012). Previous results by our group have also shown significant differences in Glx concentrations in the ACC in the same pediatric OCD patients (Ortiz et al., 2015). Although the meaning of Glx concentrations in $^{1}$H-MRS is a topic of debate, abnormal glutamate concentrations in the ACC have been identified in children and adults with OCD (Rosenberg et al., 2004, 2000; Yücel et al., 2007). Only one other study has assessed the associations between Glx concentrations in the ACC and genetic variants in pediatric patients with OCD, and this showed a significant association for a polymorphism of the glutamate receptor *GRIN2B* gene. Interestingly, two polymorphisms (rs707176 and rs2963944) of *GRIA1*, a gene encoding another glutamate receptor, were associated with the concentrations of Cho. Although they are synonymous and intronic SNPs, respectively, they could have some functional effect given their previous association with schizophrenia (Magri et al., 2006). It has to be noted that a third gene encoding yet another glutamate receptor, *GRIA3,* was also associated with anorexia nervosa and OCD in children (Mas et al., 2013).

Choline-containing compounds are components of cell membranes and increased Cho concentrations have been identified in several neurodegenerative disorders (Jenkins et al., 1993; Meyerhoff et al., 1994), perhaps reflecting membrane breakdown associated with neuronal loss. Thus, the occasional findings of increased Cho in OCD (Atmaca et al., 2009; Kitamura et al., 2006; Mohamed et al., 2007) might indicate myelin breakdown. This interpretation is strengthened by findings of WM abnormalities in OCD patients (Szeszko et al. 2005; Stewart et al. 2007; Lázaro et al. 2014a; Lázaro et al. 2014b) and the potential association between OCD and the genes

involved in myelination (Stewart et al., 2007). Conversely, Cho concentrations seem normal in other CSTC circuits in OCD (Bartha et al., 1998; Ebert et al., 1997; Rosenberg et al., 2000), which weighs against the demyelization hypothesis (Brennan et al., 2013). The concentrations of Cho in OCD have been well studied, as reported Brennan in 2013: some studies have found increased Cho in OCD versus healthy individuals in the thalamus (Mohamed et al., 2007; Rosenberg et al., 2001; Smith et al., 2003), parietal WM (Kitamura et al., 2006), and hippocampus (Atmaca et al., 2009), although one found decreased Cho in the left striatal area (Lázaro et al., 2012).

The association observed between the polymorphisms in *HTR1B*, *SCL18A1,* and *GRIA1* and the neurometabolite concentrations in the ACC indicate a role for the biological interaction between the serotonin and glutamate pathways in the pathophysiology of OCD. Several direct and indirect relationships have been described between these systems, especially in the fronto-striatal circuits (Drago et al., 2011; Marsh et al., 2009). In addition, molecular interactions suggest that there is a subcellular cross-talk between the two systems (Ciranna, 2006; López-Gil et al., 2010). Considerable attention has been paid to the symptomatic heterogeneity of OCD in recent years while attempting to find biological markers, genetic transmission mechanisms, or ways of predicting treatment response (Mataix-Cols et al., 2005). Due its heterogeneity and possible phenotypic differences, there has been increased interest in identifying subtypes that are more homogeneous, with greater focus on neuroimaging and genetic endophenotypes.

The present preliminary study has several limitations. First, $^{1}$H-MRS data were only acquired from the ACC, not from other brain regions that are similarly important in the neurobiology of OCD. Second, the sample size was modest, which limits the

statistical power of the study and makes it difficult to detect small or modest effects associated with common variants. Nevertheless, this is the largest study to be based on $^{1}$H-MRS data in patients with OCD.

To our knowledge, this study is the first to examine the relationship between differences in the concentrations of several neurometabolites and known genetic variants in children with OCD. Only one previous genetic study of pediatric OCD specifically examined glutamatergic concentrations in the brain (Arnold et al., 2009). More studies with this methodology could hold the promise of increasing our knowledge of the pathophysiology of OCD. Such investigations can offer insights into brain alterations that may clarify how genetic changes affect brain structure, chemistry, and function. Consequently, there is a clear need for further research with these innovative approaches to confirm our preliminary findings.

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**Statement of interest:**

None to declare.

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Figure 1. Association results for single nucleotide polymorphisms in candidate genes for the different models of inheritance. Genetic association with concentration of myo-inositol (Ins) (A), glutamergic compounds (Glx) (B) and total choline (Cho) (C). The Y-axis indicates –log of the likelihood ratio tests computed for 262 validated SNPs adjusting the analysis for age of onset or gender as appropriated. The X-axis shows SNPs ordered by gene. The horizontal lines at –log(p) 1.3 and 3.7 correspond to nominal p-values of 0.05 and 0.00019, respectively.

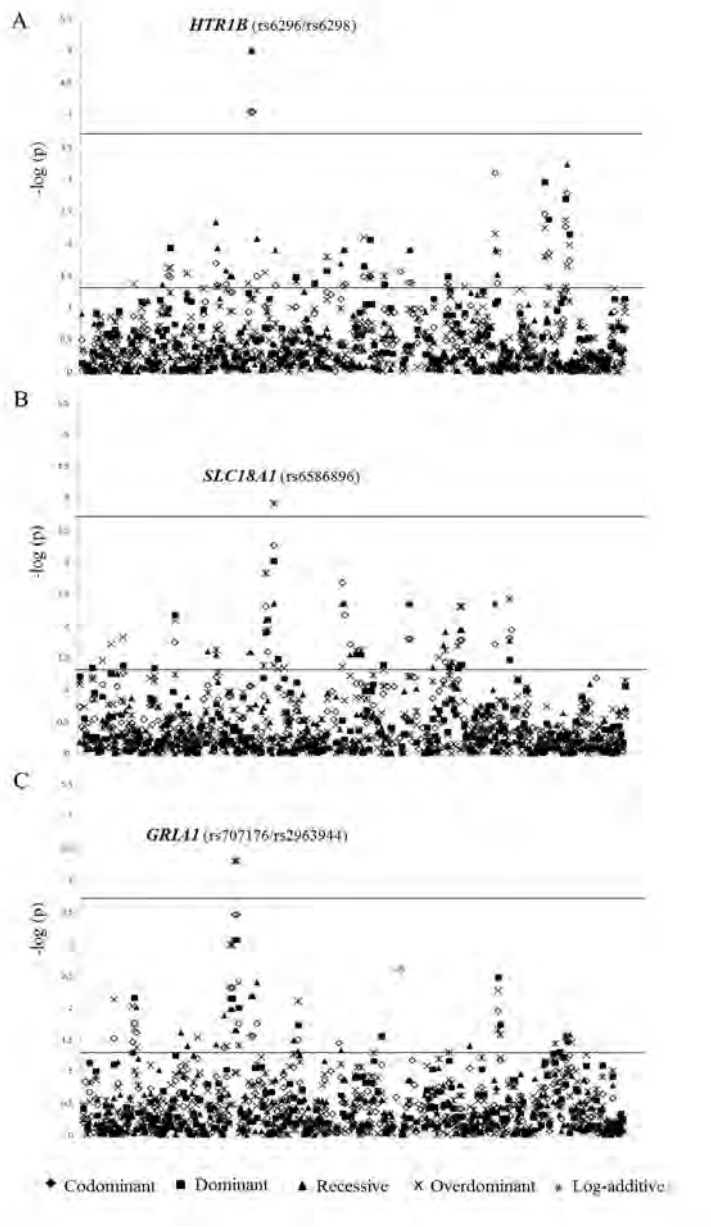

**Table 1.** Genetic Associations Obtained Between Polymorphisms and Neurometabolite Concentrations in the Anterior Cingulate Cortex

**Ins concentration [1] and genotype**

| Gene | Polymorphism | Genotype | N | Mean (SD) | *P*-value |
|---|---|---|---|---|---|
| ***HTR1B*** | rs6296/rs6298 | A/A-A/C | 39 | 5.90 (0.10) | 0.00001383 |
| | | C/C | 2 | 3.69 (0.21) | |

**Glx concentration [1] and genotype**

| Gene | Polymorphism | Genotype | N | Mean (SD) | *P*-value |
|---|---|---|---|---|---|
| ***SLC18A1*** | rs6586896 | A/A | 35 | 10.79 (0.32) | 0.0001167 |
| | | A/C | 5 | 13.03 (0.54) | |
| | | C/C | 1 | 16.79 (0) | |

**Cho concentration [1] and genotype**

| Gene | Polymorphism | Genotype | N | Mean (SD) | *P*-value |
|---|---|---|---|---|---|
| ***GRIA1*** | rs707176/rs2963944 | A/A | 16 | 1.53 (0.06) | 0.0000532 |
| | | A/C | 22 | 1.56 (0.06) | |
| | | C/C | 3 | 1.66 (0.12) | |

Ins: inositol; Glx: glutamate + glutamine; Cho: glycerophosphocholine + phosphocholine; [1] *neurometabolite concentrations adjusted for cerebrospinal fluid (CSF) with age and gray matter (GM) as covariates.* Mean concentrations obtained in each genotype group are expressed as means ± SD. Bonferroni corrected p-value < 0.00019.

# DISCUSIÓN

# 5. DISCUSIÓN

## 5.1. Valor clínico de la comorbilidad psiquiátrica. Subtipificando un trastorno complejo

Ha sido ampliamente descrita la existencia de un mayor nivel de comorbilidad en los pacientes con una edad de inicio más temprana de TOC (Coskun et al., 2012; Ivarsson et al., 2008; Langley et al., 2010; Lebowitz et al., 2012; Leonard et al., 1992; Masi et al., 2010; Wanderer et al., 2012); pero el presente estudio no indica sólo la alta prevalencia en estos pacientes, sino también el alto número de diagnósticos detectados en un grupo sustancial de pacientes con TOC, lo que indica la complejidad de este trastorno. Es importante buscar razones que expliquen la elevada comorbilidad. De entre ellas, destacan que el TOC puede tener una etiología común con varios trastornos psiquiátricos infantiles, que estos trastornos comórbidos pueden ser epifenómenos con una misma etiología pero con una expresión sintomática diversa; y que su existencia podría aumentar la vulnerabilidad al desarrollo de trastornos coexistentes (Hettema, 2008;. Nestadt et al, 2008).

Una quinta parte de la muestra presentó un trastorno del neurodesarrollo. Este grupo presentaba una tasa mayor de sexo masculino y una presencia mayor de historia familiar de TOC, lo que ya había sido descrito anteriormente en la literatura (Grados et al., 2001; Leonard et al., 1992; Riddle et al., 1990). Los estudios de neuroimagen del TT y del TOC de inicio infantil apuntan a la implicación del circuito CETC en ambos trastornos (Grados, 2010; Marsh et al., 2009; Rauch et al., 2001). Por otra parte, los estudios genéticos llevados a cabo en familias sugieren que probablemente existe una vulnerabilidad genética compartida entre el TOC y el TT (Davis et al., 2013; Grados, 2010), mientras que diferentes aspectos psicopatológicos y neuropsicológicos de ambos trastornos están estrechamente vinculados entre sí (Hanna et al., 2002; Müller et al., 2003). Además de los trastornos de tics, el TDAH es prevalente en los sujetos con una aparición temprana de TOC. Geller y colaboradores (2002) sugirieron la posibilidad de que la falta de atención en niños con TOC podría ser un artefacto originado por

los pensamientos obsesivos intrusivos o por la ansiedad existente y no ser un TDAH primario. No obstante, se considera que los síntomas del TDAH son un verdadero trastorno comórbido independiente del diagnóstico de TOC, que además suele precederle en su aparición (Geller et al., 2002; Walitza et al., 2008). Por una parte, se ha planteado que el TOC-TDAH puede representar una de las características diferenciales más significativas en el TOC de inicio temprano (Masi et al., 2010), y por otra parte, que la comorbilidad del TDAH en el TOC de inicio temprano parece predecir una mayor severidad del TOC y un mayor grado de persistencia del TOC a lo largo del seguimiento (Langley et al., 2010). Al igual que con los trastornos de tics, los hallazgos de imagen estructurales y funcionales han demostrado anomalías convergentes con un fallo de funcionamiento del circuito córtico-tálamo-estriatal responsable de los procesos de control y supervisión del rendimiento cognitivo, tanto en el TDAH como en el TOC (Brem et al., 2014). De esta forma el TOC, los trastornos relacionados con tics y el TDAH son altamente comórbidos por compartir, al menos parcialmente, una etiología común. Incluso se ha sugerido que el TOC, el TDAH y el TT presentan un solapamiento etiológico constituyendo un grupo de trastornos con afectación de los ganglios basales (Palumbo et al., 1997).

Por otro lado, casi la mitad de los pacientes con TOC presenta trastornos de ansiedad o trastornos del estado de ánimo con una mayor prevalencia de los primeros. A menudo, estos pacientes presentan más de un trastorno de ansiedad. Aunque los pacientes cumplían los criterios diagnósticos para varios diagnósticos, realizar dos o tres diagnósticos a una edad tan temprana no es lo ideal. Es posible que, alrededor de la adolescencia, la coexistencia de TOC y trastornos de ansiedad, especialmente el TAG, más que un verdadera comorbilidad sea una superposición de síntomas. Aunque el TAG se caracteriza por preocupaciones incontrolables y el TOC por obsesiones y compulsiones, ambos tienen otras características comunes. Los pensamientos en ambos trastornos son exagerados, persistentes, no deseados, aparecen de forma espontánea y, en ambos, los pacientes han de realizar un esfuerzo para resistirse contra

ellos. Los jóvenes con ambos trastornos también pueden ser más propensos a presentar hipervigilancia con respecto a sus pensamientos e interpretar sus pensamientos de una manera supersticiosa. Por otra parte, el TAG se presenta con mayor frecuencia entre los familiares de pacientes con TOC, independientemente de la presencia de TOC en estos parientes (Bienvenu et al., 2012). Este hallazgo sugiere la posible existencia de una etiología familiar común para el TOC y el TAG, pudiendo ser parte del mismo espectro fenotípico (Storch et al., 2012). Pero además, los pacientes con TOC pueden tener un aumento de las tasas de trastorno depresivo debido a los efectos desmoralizantes de la enfermedad que pueden surgir secundariamente al trastorno en algunos individuos.

Los pacientes con comorbilidad del subgrupo internalizante tienen una mayor edad que los otros subgrupos, lo que se puede explicar por el hecho de que las tasas de trastornos de ansiedad comórbidos van en constante aumento a lo largo de la adolescencia y la edad adulta (Piacentini et al., 2003), similar a las de depresión concomitante, la cual se produce más a menudo en el curso de TOC que durante su inicio, como indican los estudios de seguimiento (Denys et al., 2004a). Los trastornos comórbidos al TOC se presentan a lo largo de la vida temporalmente por orden y los períodos en los que estos trastornos se inician pueden tener un impacto en el perfil clínico y en el futuro desarrollo de cada trastorno. Los trastornos relacionados con tics, el TDAH y el trastorno por ansiedad de separación tienden a preceder a la aparición del TOC, mientras que el resto de trastornos de ansiedad, los trastornos del estado de ánimo y los trastornos de la alimentación, entre otros, tienden a aparecer posteriormente al TOC y exacerbar la clínica del TOC (de Mathis et al., 2013).

Los resultados presentados indican que la comorbilidad en el TOC se relaciona con mayores tasas de tratamiento farmacológico. Además, la necesidad de ingreso hospitalario debido a la gravedad del TOC y la interferencia causada por él, fue más frecuente en los pacientes con trastornos comórbidos, independientemente del tipo de comorbilidad. Así, los trastornos comórbidos, en una proporción sustancial de los niños y adolescentes con TOC,

tienen importantes implicaciones clínicas y terapéuticas y podrían guiar el proceso de toma de decisiones del terapeuta. A pesar de que la presencia de trastornos de ansiedad o trastornos depresivos no varía la elección farmacológica, la presencia de otros trastornos como TDAH, trastornos relacionados con tics o trastorno bipolar, pueden influir sustancialmente en la elección del tratamiento farmacológico. Por otra parte, la mayoría de los niños probablemente necesitará tratamiento farmacológico combinado, y sobre todo TCC. Es importante considerar en primer lugar, estabilizar el trastorno comórbido y, en segundo, realizar TCC en relación con el TOC. No estabilizar las condiciones comórbidas podrían limitar los beneficios de la psicoterapia. En cuanto a los pacientes con TOC con trastorno negativista-desafiante comórbido, el acomodamiento a los rituales representa una desventaja en el tratamiento estándar (Storch et al., 2008). Por otra parte, la presencia de trastornos depresivos y su desesperanza como síntoma primario del trastorno, puede asociarse con una menor habituación a la ansiedad durante las exposiciones, presencia de dudas acerca del funcionamiento del tratamiento y una menor motivación para participar en las exposiciones terapéuticas (Abramowitz, 2004). Desde el punto de vista clínico, la investigación sistemática del papel de la comorbilidad en la resistencia terapéutica puede ser útil y ayudar a la modificación de los protocolos terapéuticos, con el fin de incluir opciones individualizadas para las diferentes comorbilidades que presenta el paciente con TOC.

Por último, en relación con el sexo, aunque si se observa un pico de inicio en los niños en el período prepuberal, no se encontraron diferencias en la edad de inicio entre ambos sexos, sólo una tendencia a una edad más baja de los primeros síntomas en el sexo masculino en comparación con el sexo femenino. No obstante, la edad de inicio de los síntomas puede ser un dato no del todo preciso en el momento que hacemos la evaluación, ya que en muchos pacientes ha pasado tiempo, pudiendo aparecer un sesgo de memoria. Por otra parte, el grupo de pacientes con trastornos del neurodesarrollo de forma comórbido al TOC está en su mayoría compuesto por pacientes con sexo masculino frente a la predominancia de pacientes

de sexo femenino en el grupo con trastornos internalizantes. Nestadt y colaboradores (2008) encontraron que el sexo masculino está representado excesivamente en grupos con TOC relacionado con tics, mientras que el sexo femenino está representado en el TOC comórbido a trastornos afectivos, siendo plausible que haya formas etiológicamente distintas de TOC-masculino y de TOC-femenino.

### 5.2. Diferencias entre las concentraciones de inositol entre los pacientes con TOC y los controles sanos. Disminución de las concentraciones de glutamato según el tiempo de evolución de la enfermedad

Uno de los hallazgos del segundo estudio, utilizando técnicas de resonancia magnética espectroscópica en una amplia muestra de TOC de inicio en la infancia y la adolescencia, es que las concentraciones de Ins (absolutas y ajustadas por LCR) en el CCA estaban significativamente disminuidas respecto a los sujetos control sanos. Se ha descrito que este neurometabolito es principalmente un marcador de la glía y que están estrechamente relacionado con la osmorregulación de los astrocitos (Govindaraju et al., 2000), aunque su significado exacto aún no está claro, como en el trastorno bipolar (Davanzo et al., 2001) o el TDAH (Courvoisie et al., 2004). Esta anormalidad neuroquímica probablemente refleje una alteración de la fisiopatología general relacionada con la homeostasis celular, en lugar de una alteración específica del TOC. Hasta la fecha, 11 estudios en pacientes con TOC han evaluado el Ins frente a controles sanos; uno de ellos (Yücel et al., 2008) encontró un aumento significativo de las concentraciones en el CCA rostral y dorsal derecho en el TOC de adultos, mientras que otro (Whiteside et al., 2006) encontró una disminución significativa de la ratio Ins/Cr en el núcleo caudado. Es importante apuntar que estos dos estudios se llevaron a cabo en poblaciones de adultos utilizando una RMS de 1.5 Tesla y un tamaño muestral pequeño (15/20 sujetos por grupo), pudiendo este hecho limitar el poder estadístico para detectar diferencias. Estas diferencias metodológicas impiden realizar un análisis comparativo con el presente

estudio. Por otra parte, es importante destacar que el Ins, además de la glutamina, son marcadores gliales y la diferencia de estos metabolitos entre controles y pacientes pediátricos con TOC puede reflejar no sólo una disfunción de la neurotransmisión en estos pacientes, si no también anomalías gliales.

Respecto a su posible función terapéutica, hace aproximadamente dos décadas, varios estudios informaron del papel del Ins como un compuesto sinérgico en el tratamiento del trastorno depresivo y del TOC. En 1996, Fux y colaboradores informaron de que el Ins solo era eficaz en el tratamiento de pacientes con TOC (Fux et al., 1996), si bien, en 1999, el mismo grupo informó que el Ins no ofreció ningún beneficio añadido al tratamiento con ISRS (Fux et al., 1999). Dados los hallazgos presentados consideramos que se precisan más estudios en esta área para aclarar estos resultados.

Varios estudios sugieren una asociación entre la hiperactividad glutamatérgica y el TOC, posiblemente como resultado de alteraciones funcionales de los diferentes transportadores de glutamato (Carlsson, 2000; Rosenberg et al., 2004). Este hecho no ha sido corroborado en el presente trabajo. No obstante, uno de los resultados más importantes de nuestro estudio fue que las concentraciones de Glx ajustado por LCR fueron significativamente inferiores en los pacientes con TOC con una duración prolongada de la enfermedad (más de 24 meses) que en los pacientes con una duración menor. Es posible que el Glx sólo pueda estar reducido en relación con determinadas variables clínicas, como la duración del trastorno. Así, se ha observado la presencia de una menor concentración de Glx en el CCA específicamente en el subgrupo con mayor duración de la enfermedad (45,17 meses en el subgrupo de enfermedad con mayor duración frente a 10,83 meses en el subgrupo de enfermedad de corta duración), similar a la duración media de la enfermedad [46,44 meses] registrado en la muestra pediátrica descrita por Rosenberg y colaboradores en el 2004. Este hallazgo podría estar indicado que la concentración de Glx en el CCA tiende a disminuir en población de niños y adolescentes con TOC con un curso más largo de la enfermedad.

En cuanto al tratamiento farmacológico, nuestros resultados sugieren que el tratamiento no afecta a las concentraciones de neurometabolitos cerebrales, en niños y adolescentes con TOC, al igual que había sucedido en estudios previos en adultos (Yücel et al., 2008). En relación con otros trastornos psiquiátricos como la esquizofrenia, la mayoría de los estudios sugieren que las concentraciones de glutamato en pacientes que reciben tratamiento psicofarmacológico son similares a las concentraciones en sujetos control sanos (Poels et al., 2014). Además, un estudio reciente encontró disminuciones significativas de las concentraciones de NAA y Glx en el córtex prefrontal medial, específicamente en sujetos en fase crónica de la enfermedad. Al contrario, en los trastornos depresivos, se encontraron concentraciones más bajas de Glx en CCA en la fase aguda del trastorno depresivo, lo que sugiere la presencia de anomalías en el ciclo Glu-Glx (Hasler et al., 2007), volviendo a concentraciones normales una vez existe una remisión clínica completa (Hasler et al., 2005). Estos datos parecen indicar que los cambios en la neurotransmisión glutamatérgica depende de la fase evolutiva de la enfermedad (Luykx et al., 2012).

### 5.3. Variantes genéticas asociadas al TOC de inicio temprano

Los resultados del test de desequilibrio de transmisión llevado a cabo en familias para el estudio genético del riesgo de TOC de inicio temprano, mostraron asociaciones significativas con polimorfismos en los genes *GAD2* y *HTR1B*. La transmisión preferencial de estos SNPs a los pacientes con TOC podría estar relacionada con el sexo ya que, a pesar de que las diferencias no fueron significativas, se observaron tendencias diferentes al considerar los dos sexos por separado. Mientras que los polimorfismos de *GAD2* parecían ser los que se transmitían con mayor frecuencia en el sexo femenino, el SNP del *HTR1B* lo hacía en mayor medida en el sexo masculino. Estos resultados están en la misma línea que varios estudios clínicos que sugieren que el sexo juega un papel relevante en la expresión fenotípica del TOC (Mathis et al., 2011). Este hallazgo no es nuevo en la genética del trastorno, ya que varios genes asociados con la

enfermedad, como los genes *COMT*, *MAOA*, *DRD2*, *SLC1A1*, *HTR1D*, *HTR1A*, *NTRK2* y *BDNF*, muestran efectos diferenciales entre sexos (Mathis et al., 2011). Los estudios de ligamiento sugieren que el TOC sigue un modelo de herencia autosómico dominante con un importante componente familiar. Diferentes autores defienden que el modelo de transmisión genética del TOC puede ser diferente entre sexos (Nestadt et al., 2000) o alternativamente que el modelo de herencia dominante presenta diferente penetrancia entre hombres y mujeres (Cavallini et al., 1999). Estudios recientes han demostrado que bajo estrés, factores relacionados con el complemento cromosómico sexual pueden influir en la expresión de determinados genes relacionados con la serotonina, el GABA o la dopamina, de manera diferencial entre ambos sexos (Seney et al., 2013a, 2013b). Por otro lado, las diferencias entre sexos en cuanto a la neurotransmisión de la serotonina también se han asociado con las diferencias en el eje hipotálamo-hipofisario y la respuesta al estrés (Goel and Bale, 2010). Además, varios autores han sugerido una posible relación entre estas diferencias de sexo y los factores hormonales (Donner and Lowry, 2013).

El receptor 1B de la serotonina (producto del gen *HTR1B*) parece estar implicado en la fisiopatología del TOC. Este autorreceptor participa en la regulación de la síntesis y liberación de serotonina. Se expresa principalmente en la región límbica y en el cuerpo estriado. Un estudio reciente indica que estos receptores son necesarios y suficientes para inducir un comportamiento equivalente al TOC en ratones, y que su desensibilización por el tratamiento con ISRS es clave para reducir dicho comportamiento (Shanahan et al., 2011). La administración aguda de los ligandos no selectivos (es decir, meta-Clorfenilpiperazina) o selectivos (es decir, sumatriptán) del receptor de 1B de la serotonina induce un empeoramiento transitorio de los síntomas del TOC (Gross-Isseroff et al., 2004). Por otra parte, se encontró que la administración crónica de sumatriptán mejoraba los síntomas en algunos pacientes con TOC resistentes a la farmacoterapia convencional (Stern et al., 1998). Por estas razones, el gen *HTR1B* ha sido considerado como un gen candidato para el TOC.

El presente estudio replica asociaciones con polimorfismos del gen *HTR1B* descritas previamente (Kim et al., 2009; Lochner et al., 2004; Mundo et al., 2002). Además, la asociación observada en el sexo masculino está de acuerdo con los resultados de otros estudios realizados en pacientes varones adultos con TOC (Kim et al., 2009; Lochner et al., 2004). Además, un meta-análisis reciente, identificó una tendencia significativa en cuanto a la participación del gen *HTR1B* en el TOC en interacción con la edad de inicio (Taylor, 2013). Otros estudios han encontrado asociaciones entre los polimorfismos genéticos en *HTR1B* y endofenotipos del TOC, tales como el volumen de la COF analizada por RMN (Atmaca et al., 2010) o la gravedad de las obsesiones evaluada mediante la puntuación en la escala Y-BOCS (Camarena et al., 2004). De todas formas, hay que señalar que, otros estudios no han encontrado asociaciones significativas entre el gen *HTR1B* y el TOC (Di Bella et al., 2002; Dickel et al., 2007; Gratacòs et al., 2009; Liu et al., 2011; Walitza et al., 2004). Esta heterogeneidad podría ser el resultado de diferencias en el poder estadístico, ya que utilizan tamaños de muestra similares al nuestro, que van desde 44 hasta 187 pacientes. Otra posible causa podría ser la heterogeneidad de la enfermedad. En este estudio hemos analizado una muestra homogénea de TOC de inicio temprano con una alta frecuencia de antecedentes de TOC.

Entre los 11 estudios publicados existen varias diferencias respecto al porcentaje de varones, la etnia, la edad de inicio y gravedad de la enfermedad. En cuanto a la edad de inicio, sólo se ha realizado un estudio con pacientes con TOC de inicio temprano (Walitza et al., 2004), tres estudios utilizaron poblaciones mixtas de TOC de inicio temprano y tardío (DiBella et al, 2002; Dickel et al, 2007; Hemmings y otros, 2006), y la gran mayoría se llevaron a cabo con pacientes adultos con TOC (Gratacós et al, 2009; Kim et al, 2009; Liu et al, 2011; Lochner et al, 2004; Mundo et al, 2002). Además, en estos estudios sólo se genotipó un polimorfismo, el G861C o rs6296, que da lugar a un cambio sinónimo. En el presente estudio este SNP alcanzó un valor de $p < 0.05$ que no fue significativo después aplicar la corrección de Bonferroni. Este SNP se encuentra en gran desequilibrio de ligamiento con el SNP que hemos asociado, el

rs2000292, encontrándose ambos polimorfismos en el mismo bloque haplotípico. Sin embargo, el análisis individual del SNP rs2000292 proporcionó mejores resultados estadísticos que el análisis haplotípico. Estos resultados podrían indicar que el rs2000292 se encuentra en mayor desequilibrio de ligamiento con la variante causal que el rs6296. Teniendo en cuenta la localización de estos SNPs en el gen *HTR1B*, la variante causal probablemente se localiza en el extremo 5' del gen que afecta a elementos reguladores transcripcionales. Las diferencias en el patrón de desequilibrio de ligamiento entre rs2000292 y rs6296 en las diferentes poblaciones, y la falta de un análisis más exhaustivo del extremo 5' del gen, podrían ayudar a explicar la controversia en los resultados publicados.

Existen múltiples evidencias que indican que las alteraciones en la neurotransmisión de GABA podrían tener un papel clave en el TOC (Rosenberg et al., 2001). Por una parte, se han descrito ciertas anomalías en los procesos inhibitorios corticales en pacientes con esta patología (Richter et al., 2012). El GABA modula neuronas glutamatérgicas corticales (Gonzalez-Burgos and Lewis, 2008) y podría estar relacionado con anormalidades en los procesos de excitación cortical que se darían en el TOC (Maia et al., 2008). Mediante el uso de RMS, se han encontrado niveles más bajos del GABA en la corteza prefrontal de pacientes con TOC que en controles sanos (Simpson et al., 2012); y mediante estimulación magnética transcraneal, se han hallado evidencias de disfunciones inhibitorias corticales en pacientes con este trastorno (Richter et al., 2012). Sin embargo, en los estudios genéticos de TOC se ha prestado poca atención a este neurotransmisor incluyéndose únicamente algunos receptores gabaérgicos (Gratacòs et al., 2009; Richter et al., 2012; Zai et al., 2005). Por lo que sabemos, nuestro estudio es el primero en explorar la variabilidad genética de *GAD1* y *GAD2* en este trastorno. Estos genes codifican para GAD67 y GAD65, respectivamente, dos isoformas del enzima glutamato decarboxilasa. Ambas isoformas se expresan en las células que contienen GABA, a menudo interneuronas inhibitorias, por lo que representan un marcador celular ampliamente utilizado para las neuronas gabaérgicas. Aunque GAD67 y GAD65 se expresan

ampliamente en muchos tipos de células y generalmente se expresan conjuntamente en la misma neurona, cada vez existen más pruebas de que tienen distribuciones de expresión diferencial a nivel de los compartimentos subcelulares. GAD67 está presente principalmente en el citoplasma y se encarga de la síntesis y liberación de GABA no vesicular. GAD65 se localiza preferentemente en los axones y es responsable de la síntesis y de liberación de GABA dependiente de vesículas sinápticas (Betley et al., 2009). La asociación significativa entre GAD2 y TOC identificada en el estudio 3 está de acuerdo con una función dinámica de esta isoforma en respuestas de adaptación celular frente a la estimulación ambiental. Esta asociación parece ser específica del sexo femenino, lo cual estaría de acuerdo con las diferencias descritas en la neurotransmisión de GABA entre sexos (Simpson et al., 2012). La expresión dinámica de *GAD2* requiere una compleja regulación de su transcripción (Pan, 2012), lo que parece estar bajo control de los receptores de estrógeno (Hudgens et al., 2009) y podría estar modulada por los esteroides sexuales (Noriega et al., 2010).

En este estudio hemos identificado dos SNPs en *GAD2*, rs8190748 y rs992990, que se transmitieron de forma incrementada en los pacientes con TOC. El bloque haplotípico que incluye los alelos de riesgo de estos SNPs también se transmitió preferentemente de los padres a los pacientes con TOC. Estos SNPs son intrónicos y no parecen afectar a los elementos de regulación. No obstante, cabe destacar que el bloque haplotípico en el que se encuentran cubre una región de *GAD2* que incluye varios exones. Se necesitan más estudios para explorar esta región e identificar las variantes funcionales que podrían explicar las asociaciones observadas.

### 5.4. Asociación entre variantes genéticas de las vías serotoninérgicas y glutamatérgicas y las concentraciones de neurometabolitos en nuestra población con TOC

Los resultados del estudio de asociación entre los polimorfismos genéticos y la concentración de diferentes neurometabolitos en el CCA mediante RMS revelaron asociaciones significativas, después de aplicar corrección de Bonferroni, entre las concentraciones de Ins, Glx, y Cho y cinco polimorfismos localizados en tres genes: *SLC18A1* (rs6586896), *HTR1B* (rs6296 y rs6298), y *GRIA1* (rs707176 y rs2963944).

Se han descrito diferencias en la concentración de Ins entre sujetos control sanos y pacientes pediátricos con TOC que reflejan una disfuncionalidad en la neurotransmisión así como posibles alteraciones a nivel de las células gliales. De hecho, aunque la función exacta de este neurometabolito se desconoce, se relaciona estrechamente con la osmorregulación de los astrocitos (Govindaraju et al., 2000). Diversos estudios han evaluado las concentraciones de Ins en pacientes con TOC frente a sujetos control sanos y han mostrado diferencias, incluyendo un aumento significativo de las concentraciones en el CCA rostral y dorsal derecho (Yücel et al., 2008) y una disminución en el caudado (Whiteside et al., 2006). Es interesante el hecho de que en el estudio de espectroscopía comentado previamente (estudio 2), encontramos que las concentraciones de Ins estaban disminuidas en el CCA en los mismos pacientes incluidos en este análisis, en comparación con los controles sanos de la misma edad y sexo.

En el estudio de asociación, hemos relacionado las concentraciones de este neurometabolito con dos polimorfismos en el gen *HTR1B*. A pesar de que dan lugar a un cambio sinónimo en la secuencia aminoacídica de la proteína, estos SNP parecen tener algún efecto funcional, ya que previamente se han asociado con la alteración de la respuesta al tratamiento con antidepresivos (Villafuerte et al., 2009; Xu et al., 2012). Además, otros estudios también han descrito asociaciones entre otros SNPs del mismo gen *HTR1B* y el riesgo de TOC (Kim et al., 2009; Mundo et al., 2002) y la presencia de determinados endofenotipos,

tales como la reducción del volumen del córtex órbito-frontal (Atmaca et al 2010) y la gravedad de las obsesiones medida mediante la escala Y- BOCS (Camarena et al., 2004). De hecho, en un meta-análisis reciente, Taylor y colaboradores (2013) identificaron una tendencia en cuanto a la participación del gen *HTR1B* en el TOC. Es importante destacar que este mismo gen también fue identificado en el estudio de desequilibrio de transmisión presentado en esta tesis (estudio 3) y en el que se incluyeron la mayoría de los pacientes analizados en este estudio.

La vía de la serotonina se ha convertido en objeto principal en la investigación de la neurobiología del TOC debido en gran parte a los notables efectos terapéuticos de los ISRS en el tratamiento de las obsesiones y las compulsiones (Greist et al., 1995). En este estudio también se ha identificado un polimorfismo en otro gen relacionado con la serotonina. En concreto, se ha asociado un polimorfismo (rs6586896) localizado en el gen del transportador vesicular de monoaminas, el *SLC18A1*, con la concentración de Glx en el CCA de los pacientes. El gen *SLC18A1* se encuentra en el cromosoma 8p21, una región implicada en los estudios de ligamiento de la esquizofrenia, el trastorno bipolar y fenotipos relacionados con la ansiedad (Lohoff 2010). De hecho, varios estudios genéticos de casos y controles han documentado asociaciones entre variantes comunes del gen *SLC18A1* que provocan cambios no sinónimos en la proteína y la susceptibilidad a la esquizofrenia y el trastorno bipolar (Bly, 2005; Chen et al., 2007; Lohoff et al., 2006). Curiosamente, en un estudio previo llevado a cabo por nuestro grupo también se identificó este gen asociado con el riesgo de TOC y anorexia nerviosa en pacientes pediátricos, algunos de los cuales también han sido incluidos en este estudio (Mas et al., 2013). Existe una creciente evidencia de que las alteraciones de la neurotransmisión de glutamato en los circuitos CETC juegan un papel clave en la patogénesis del TOC (Pittenger et al., 2006; Wu et al., 2012). Aunque el significado de los cambios en la concentración cerebral de Glx es un tema de debate, se han identificado concentraciones anormales de glutamato en el CCA tanto en niños como en adultos con TOC (Rosenberg et al., 2000; Yücel et al., 2007).

Existe únicamente otro estudio que ha evaluado la asociación entre la concentración de Glx en el CCA y variantes genéticas en pacientes pediátricos con TOC. Los resultados de dicho estudio mostraron una asociación significativa de un polimorfismo del gen del receptor de glutamato *GRIN2B* (Arnold et al., 2009). Curiosamente, en nuestro estudio dos polimorfismos (rs707176 y rs2963944) de *GRIA1*, gen que codifica para otro receptor de glutamato, se han asociado con las concentraciones cerebrales de Cho. Aunque se tratan de un SNP sinónimo y otro intrónico, respectivamente, podrían tener algún efecto funcional dada su asociación descrita previamente con el riesgo de esquizofrenia (Magri et al., 2006). Cabe destacar que un tercer gen, el *GRIA3*, que codifica para otro receptor de glutamato, también se asoció con la anorexia nerviosa y el TOC infantil en el estudio de nuestro grupo comentado anteriormente (Mas et al., 2013).

Los compuestos que contienen Cho son componentes de las membranas celulares y se ha identificado un aumento de las concentraciones de este neurometabolito en varios trastornos neurodegenerativos (Jenkins et al., 1993; Meyerhoff et al., 1994), reflejando tal vez la presencia de ruptura de membranas celulares, lo cual estaría asociado con la pérdida neuronal. Por lo tanto, los hallazgos que mostraron un aumento de Cho en el TOC (Atmaca et al., 2009; Kitamura et al., 2006; Mohamed et al., 2007) podrían indicar una ruptura de la mielina. Esta interpretación se ve reforzada por los hallazgos que describen anomalías de la SB en los pacientes con TOC (Szeszko et al. 2005; Stewart et al. 2007; Lázaro et al. 2014a; Lázaro et al. 2014b) y la posible asociación entre la enfermedad y los genes implicados en la mielinización (Stewart et al., 2007). Por el contrario, las concentraciones de Cho parecen normales en otras partes del circuito CETC (Bartha et al., 1998; Ebert et al., 1997; Rosenberg et al., 2000), lo que va en contra de la hipótesis de desmielinización (Brennan et al., 2013). Las concentraciones de Cho en el TOC han sido bien estudiadas (Brennan et al., 2013). Algunos resultados muestran que los pacientes con TOC presentan mayores niveles de Cho en el tálamo (Milad and Rauch, 2012; Rosenberg et al., 2001; Smith et al., 2003), la SB parietal

(Kitamura et al., 2006), y el hipocampo (Atmaca et al., 2009) en comparación con individuos sanos. No obstante, resultados de nuestro grupo mostraron una disminución de Cho en el área del estriado izquierdo en población con TOC de inicio temprano (Lázaro et al., 2012).

La asociación observada entre los polimorfismos en *HTR1B*, *SCL18A1* y *GRIA1* y las concentraciones de los neurometabolitos en el CCA indican un papel clave de la interacción biológica que existe entre las vías serotoninérgica y glutamatérgica en la fisiopatología del TOC. Se han descrito varias relaciones directas e indirectas entre estos sistemas, especialmente en los circuitos fronto-estriatales (Drago et al., 2011; Marsh et al., 2009). Además, estas interacciones moleculares sugieren que existe una comunicación a nivel sub-celular entre los dos sistemas (Ciranna, 2006; López-Gil et al., 2010).

En los últimos años, se ha prestado una considerable atención a la heterogeneidad en la sintomatología del TOC, en un intento de encontrar marcadores biológicos de la enfermedad, factores de transmisión genética u otras formas de predecir la respuesta al tratamiento (Mataix-Cols et al., 2005). Es por dicha heterogeneidad y por las posibles diferencias fenotípicas que se ha incrementado el interés por identificar subtipos de la enfermedad que sean más homogéneos, especialmente endofenotipos genéticos y de neuroimagen.

La existencia de diferentes subtipos de TOC podría estar condicionada a una heterogeneidad en la etiología del trastorno determinada por la existencia de diferentes genes de riesgo dependiendo de las características específicas de la población de estudio. Por ello, puede resultar más fácil detectar asociaciones genéticas significativas si se estudian poblaciones más homogéneas como la incluida en nuestros estudios que incluye únicamente pacientes con TOC de inicio temprano, el cual además parece tener un mayor componente genético. Además, otra manera de esclarecer la base genética del TOC sería identificar los endofenotipos asociados a esta enfermedad, como endofenotipos de neuroimagen. Este

enfoque podría delinear las vías que unen genes de riesgo a trastornos (Meyer-Lindenberg and Weinberger, 2006), aumentando así nuestra comprensión sobre los mecanismos de las enfermedades psiquiátricas y la identificación de potenciales dianas terapéuticas. Hasta donde sabemos, existe únicamente un estudio previo al presentado en esta tesis doctoral en el que se analizó conjuntamente variantes genéticas y fenotipos de neuroimagen en pacientes con TOC en edad pediátrica (Arnold et al., 2009). En ese estudio se encontró una asociación entre un polimorfismo del receptor de glutamato y las concentraciones de glutamato en el CCA. Ningún otro estudio que evaluara variables genéticas y neuroquímicas simultáneamente en este trastorno y población pediátrica ha sido publicado en los últimos años.

Si observamos datos sobre la eficacia de los tratamientos utilizados actualmente, veremos que aún queda un largo camino hasta llegar a tener tratamientos ideales para todas las enfermedades. Según datos proporcionados por la OMS, sólo 1/4 de las patologías se tratan con una terapia adecuada y además 1/3 de las terapias adecuadas dan lugar a efectos adversos que pueden llevar al fracaso terapéutico. En el caso del trastorno que ocupa la presente tesis doctoral, los ISRS son los únicos fármacos aprobados por la *Food and Drug Administration* (FDA). Dichos fármacos son sólo efectivos en el 40-60% de los pacientes. En realidad, la respuesta al tratamiento es definida como reducción del 20-40% de los síntomas, es decir, muchos "respondedores" siguen presentando síntomas. Dada la persistencia de los síntomas y los limitados niveles de respuesta al tratamiento, parece claro que el paradigma serotoninérgico no explica por completo la neurobiología del trastorno.

Por eso consideramos que los estudios de las bases genéticas y moleculares para la identificación de nuevas dianas terapéuticas resultan imprescindibles. Los estudios de farmacogenómica se centran en el descubrimiento de estas dianas pero también buscan marcadores periféricos que ayudan al diagnóstico y pronóstico de las enfermedades. El estudio de riesgo de TOC que se presenta en esta tesis doctoral ha sido capaz de detectar tres polimorfismos que influyen en el riesgo de la enfermedad. Además en estos estudios

apuntamos a la participación de otras vías en la patofisiología del TOC como lo es la glutamatérgica. De hecho en la actualidad, se están ensayando diferentes fármacos con ácción glutamatérgica como agentes antiglutamatérgicos (Riluzole), antagonistas del receptor del glutamato (Memantina), aminoácidos que atenúan la neurotransmisión glutamatérgica (N-acetilcisteina) u otros (D-Cicloserina) en este y otros trastornos psiquiátricos.

Consideramos que serán necesarios más estudios, como los presentados en esta tesis doctoral, en los que se evalúen las características genéticas y de neuroimagen de los pacientes con TOC con el fin de ampliar el conocimiento sobre las bases biológicas de este complejo trastorno.

# LIMITACIONES Y PUNTOS FUERTES

## 6. LIMITACIONES Y PUNTOS FUERTES

Existen tres principales limitaciones en los estudios que componen la presente tesis doctoral.

En el primer estudio de caracterización clínica y comorbilidad en pacientes con TOC de inicio en la infancia y adolescencia, una limitación es la inclusión de los pacientes en diferentes momentos de la enfermedad. No obstante, todos habían completado un tiempo mínimo de tratamiento en la unidad de 4 meses.

En el segundo estudio, sólo fueron evaluados los neurometabolitos en un área cerebral, el CCA, y no en otras regiones del cerebro que también son importantes en la neurobiología del TOC. Se necesitarán otros estudios espectroscópicos que valoren la concentración de neurometabolitos en otras regiones cerebrales afectadas en este trastorno. Así mismo, serán necesarios otros estudios que utilicen secuencias especiales que permitan la separación de glutamato de las señales de glutamina, o un índice agregado, como la relación Gln / Glu, que puedan medir la neurotransmisión glutamatérgica al reflejar la liberación de Glu y la reciprocidad de Glu y Gln (Brennan et al., 2013). Además, la selección vóxel con PRESS no es ideal, especialmente para la ubicación del CCA. En esta zona el LCR surge principalmente del centro del vóxel rodeado de SG y SB, y los perfiles de pulso imperfectos de la selección del vóxel podría provocar la inclusión de más SB, por lo que el contenido de LCR podría ser sobreestimado, originando como resultado una corrección parcial. No obstante, nosotros no corregimos este sesgo (como realizan en otros estudios) por medio de la intersección en la línea de regresión de la concentración de metabolitos frente al contenido de LCR, debido a que el contenido de LCR fue bastante similar en todos los sujetos (M = 4,96; DE = 0,75), y por consiguiente, la estimación de la intercepción no era suficientemente fiable.

En el tercer estudio, el tamaño muestral representa una limitación, ya que ésta es una de las más frecuentes limitaciones de los estudios de asociación. El tamaño de la muestra

utilizada en el tercer estudio, puede limitar su poder estadístico dificultando la detección de efectos pequeños o moderados de las variantes estudiadas. Es por este motivo que tenemos que considerar los hallazgos de este estudio genético como exploratorios, los resultados del cual deberán ser confirmados en un futuro en una población más amplia.

En la literatura científica que analiza datos genéticos y de neuroimagen en trastornos psiquiátricos, frecuentemente se observa discrepancia en los resultados. En el trastorno objeto de la presente tesis doctoral, la inconsistencia de algunos resultados puede ser debida, al menos en parte, a la alta heterogeneidad del mismo y a algunas variables clínicas como el tiempo de evolución del trastorno. Por ello conviene destacar como punto fuerte, la homogeneidad clínica de la muestra estudiada, al tratarse específicamente de pacientes con TOC de inicio en la infancia y la adolescencia.

Entre los puntos fuertes del estudio espectroscópico es su diseño de caso-control y el ajuste de la concentración para el volumen de SG y SB con el fin de evitar la contaminación por LCR. Además, los parámetros espectroscópicos analizados presentaban señales robustas en ambos grupos. Así mismo, consideramos oportuno destacar que contamos con la muestra en población infanto-juvenil con TOC más amplia de los estudios de espectroscopía publicados hasta la actualidad.

A nuestro entender, el último estudio presentado en esta tesis doctoral es el primero en examinar la relación entre las diferencias en las concentraciones de varios neurometabolitos y variantes genéticas comunes en población infanto-juvenil con TOC. Sólo existe un estudio genético previo en TOC pediátrico que examinó, mediante técnicas espectroscópicas y de análisis genético, el papel del glutamato en la etiopatogenia del trastorno (Arnold et al., 2009). Y aunque dispongamos de un tamaño muestral escaso para un estudio de asociación, es importante señalar que hemos logrado suficiente poder estadístico para identificar una serie de asociaciones genéticas significativas.

Debido a la relevancia del trastorno y la población que lo sufre, serán necesarios más estudios que ayuden a aumentar el conocimiento actual sobre la fisiopatología del TOC, aportando más información sobre cómo variaciones genéticas pueden afectar a la estructura, la química y la función cerebral dando lugar al posterior en el desarrollo de trastornos psiquiátricos como el TOC.

# CONCLUSIONES

## 7. CONCLUSIONES

***Conclusión 1:*** Los pacientes con TOC en edad infantil y adolescente, presentan unas altas tasas de comobilidad. Alrededor del 67% de los pacientes de nuestra muestra presenta un trastorno comórbido al TOC y el 28.6% de ellos, dos o más diagnósticos.

***Conclusión 2:*** Existen dos subtipos de TOC de inicio en la infancia y la adolescencia, considerando la patología comórbida. Un subtipo de inicio más temprano, con predominio de sexo masculino, con alta agregación familiar y con comorbilidad de trastornos del neurodesarrollo; y otro subtipo fenotípicamente relacionado con trastornos de ansiedad y trastornos depresivos, más común en el sexo femenino y con un inicio más tardío, a lo largo de la adolescencia; además de un subgrupo de TOC "más puro".

***Conclusión 3:*** Encontramos niveles significativamente más bajos de inositol en el córtex cingulado anterior en pacientes con TOC de inicio temprano en comparación con sujetos sanos.

***Conclusión 4:*** Existen diferencias significativas en la concentración de glutamato en el córtex cingulado anterior de pacientes con TOC de inicio temprano en relación a la duración de la enfermedad (menor concentración a mayor duración de la enfermedad), es decir, más de 24 meses. Confirmamos que el tratamiento farmacológico no afecta a las concentraciones de neurometabolitos cerebrales en población con TOC de inicio en la infancia y adolescencia.

***Conclusión 5:*** Variantes genéticas en *HTR1B* (rs2000292) y *GAD2* (rs992990 y rs8190748) están asociadas al riesgo de TOC de inicio temprano según el estudio de desequilibrio de transmisión. Así mismo, parece existir un dimorfismo sexual en dichas asociaciones las cuales serían específicas del sexo masculino para el gen *HTR1B* y del sexo femenino para el *GAD2*.

***Conclusión 6:*** Polimorfismos en los genes *HTR1B* (rs6296 y rs6298), *SLC18A1* (rs6586896) y *GRIA1* (rs707176 y rs2963944) pueden determinar, en parte, las concentraciones de los neurometabolitos Ins, Glx y Cho, respectivamente, en el CCA de pacientes con TOC de inicio temprano.

***Conclusión 7:*** Alteraciones de las vías serotoninérgicas, glutamatérgicas y gabaérgicas pueden conferir un mayor riesgo de desarrollar TOC de inicio temprano.

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