Journal of Attention Disorders
1–10

DOI: 10.1177/1087054716647483
jad.sagepub.com
SAGE

Cingulate Cortical Thickness and Dopamine Transporter (*DAT1*) Genotype in Children and Adolescents With ADHD

Alberto Fernández-Jaén[1,2], Jacobo Albert[3], Daniel Martín Fernández-Mayoralas[1,2], Sara López-Martín[4,5], Ana Laura Fernández-Perrone[1], Mar Jimenez de la Peña[1], Beatriz Calleja-Pérez[6], Manuel Recio Rodríguez[1], and Sonia López Arribas[7]

Abstract

Objective: This study aimed to examine the influence of dopamine transporter gene (*DAT1*) *3′UTR* genotype on cingulate cortical thickness in a large sample of children and adolescents with ADHD. **Method:** Brain MRIs were acquired from 46 ADHD patients with homozygosity for the 10-repeat allele and 52 ADHD patients with a single copy or no copy of the allele. The cingulate cortex of each MRI scan was automatically parceled into sulci and gyri as well as into Brodmann areas (BA). **Results:** There were no group differences in age, gender, full-scale intelligence quotient, symptom severity, treatment status, comorbidity, or mean overall cortical thickness. Sulcus/gyrus- and BA-based analyses revealed that patients homozygous for the 10-repeat allele showed significantly greater thickness in right cingulate gyrus and right BA 24 compared with 9-repeat carriers. **Conclusion:** These findings suggest that thickness of cingulate cortex is influenced by the presence of the 10-repeat allele in ADHD. *(J. of Att. Dis. XXXX; XX(X) XX-XX)*

Keywords

ADHD, cortical thickness, cingulate cortex, *DAT1*

Introduction

ADHD is defined by developmentally inappropriate and impairing levels of hyperactivity, impulsiveness, and inattentiveness. It is estimated to affect 5% to 7% of school-age children when diagnosis was made according to *Diagnostic and Statistical Manual of Mental Disorders* (4th ed., text rev.; *DSM-IV-TR*; American Psychiatric Association, 2000) criteria (Willcutt, 2012). Convergent evidence from neuropsychological, structural, and functional imaging studies suggests that ADHD is associated with abnormalities in a number of distinct brain regions (Castellanos & Proal, 2012; Cortese et al., 2012). Among them, the cingulate cortex (CC; primarily anterior but also posterior) appears to be particularly important in the pathophysiology of the disorder (Bush, 2011; Makris, Seidman, & Valera, 2010; Nakao, Radua, Rubia, & Mataix-Cols, 2011). CC dysfunctions are thought to underlie a wide range of cognitive and affective deficits in ADHD, including those related to response inhibition, error processing, attention allocation, emotion regulation, and motivation (Bush, 2011).

Twin, adoption, and family studies have demonstrated that ADHD is a highly heritable condition with a mean heritability estimates between 60% and 90% (Faraone & Mick, 2010). Molecular genetic data suggest that ADHD is a complex genetic disorder in which multiple genes of small individual effects are involved (Gizer, Ficks, & Waldman, 2009). Genes that have been shown to be potentially associated with ADHD include, among others, those that code for dopamine (DA) receptors and transporters, catecholaminergic receptors, and catecholamine-metabolizing enzymes. Among them, the DA transporter (*SLC6A3/DAT1*) is one of the best replicated candidate genes for ADHD (Gizer et al., 2009). A crucial role for the DA transporter (DAT) in ADHD is further supported by the fact that it is a principal site of action for psychostimulants, the most common pharmacological treatment for ADHD patients.

[1]Hospital Universitario Quirón, Madrid, Spain
[2]CADE, Madrid, Spain
[3]Universidad Autónoma de Madrid, Spain
[4]Univesidad Rey Juan Carlos, Madrid, Spain
[5]Centro Neuromottiva, Madrid, Spain
[6]Centro de Salud Doctor Cirajas, Madrid, Spain
[7]Hospital Gómez Ulla, Madrid, Spain

Corresponding Author:
Alberto Fernandez-Jaén, Unidad de Neurología Pediátrica, Hospital Universitario Quirón Madrid, C/Diego de Velazquez, 1, 28223 Pozuelo de Alarcón, Madrid, Spain.
Email: aferjaen@telefonica.net

Concretely, methylphenidate inhibits DA re-uptake by binding to the DAT, thereby increasing the levels of available DA in the synapse.

The DA system and its projections, which include the striatum, prefrontal, and cingulate cortices, play an important role in modulating motivation, emotion, and high-order cognitive process such as response inhibition, selective attention, and error monitoring. Within this system, the protein encoded by the *DAT1* gene is responsible for taking released DA back up into presynaptic terminals and terminating dopaminergic neurotransmission. *DAT1* is located on chromosome 5p15, contains 15 exons, and carries a variable number of tandem repeated (VNTR) polymorphisms in the 3' untranslated region. The number of repeats ranges between 3 and 13, with 9- and 10-repeats (9R and 10R) the most frequent in population. These VNTRs have been shown to affect DAT expression. Specifically, increased levels of *DAT1* expression have been associated with the number of 10R alleles in children and adolescents, with greatest expression in the homozygous 10/10 genotype (Brookes et al., 2007; in adults, it seems that the 9R allele rather than the 10R is associated with increased DAT activity: Faraone, Spencer, Madras, Zhang-James, & Biederm, 2014). Notably, meta-analyses of molecular genetic studies indicate an overrepresentation of the 10R allele in children and adolescents with ADHD (Faraone & Mick, 2010; Faraone et al., 2014). *DAT1* is expressed abundantly in midbrain and striatum (Durston, 2010). In prefrontal and cingulate cortices, it is found in low abundance and primarily at a distance from synaptic sites of DA release (Lewis et al., 2001). However, several studies suggest that individuals homozygous for the 10R allele showed abnormal activation in a number of cortical regions during high-order cognitive tasks, suggesting the *DAT1* effects on DA function affect other regions beyond the striatum, including prefrontal and cingulate cortices (Bertolino et al., 2006; Cummins et al., 2012). These findings therefore indicate that DAT influences neural activity of cortical regions, probably through a combination of direct effects on DA function within these regions and indirect effects on the function of striatum, which regulates activity within the cortico-striatal-thalamo-cortical pathway (Newman & Grace, 1999).

In a complex and heterogeneous disorder such as ADHD, imaging genetics (the study of the effects of genes on brain morphology and function) can help to bridge the gap between genes and clinical symptoms. Such an intermediate or endophenotype approach has obvious advantages: structural or functional brain measures are causally closer to genes and gene expression than behavior, and imaging phenotypes provide a useful way to identify the neural mechanisms by which gene variants affect behavior (Durston, 2010). Although only a few imaging genetic studies of ADHD have been published to date, and most of these investigations were focused on striatum (Durston et al., 2005; Durston et al., 2008; Shook et al., 2011) and prefrontal cortex (Fernández-Jaén et al., 2015), there is some evidence that *DAT1* genotype affects the functioning of CC in ADHD patients (Bédard et al., 2010; Braet et al., 2011; Brown et al., 2010). However, as far as we know, there is no study that has directly examined whether CC anatomy is influenced by *DAT1* genotype in ADHD.

This study was designed to complement these previously published functional imaging genetic studies by examining the influence of *DAT1* on the structural anatomy of CC. To this end, cortical thickness measures from MRI were obtained in a large sample of patients with ADHD. On the basis of previous findings, we explored the possibility that patients with ADHD show differences in cortical thickness depending on the genetic polymorphism of the *DAT1* gene.

Method

Participants

Ninety-eight patients with ADHD (6-17 years) were recruited from the Child Neurology Unit of the Quiron University Hospital, Madrid. ADHD diagnosis was made by a multidisciplinary team according to the *DSM-IV-TR* (American Psychiatric Association, 2000) using the Kiddie-Schedule for Affective Disorders and Schizophrenia-Present and Lifetime (K-SADS-PL; Kaufman et al., 1997), Spanish version (Ulloa et al., 2005). Forty-five patients met *Diagnostic and Statistical Manual of Mental Disorders* (4th ed.; *DSM-IV*; American Psychiatric Association, 1994) criteria for the combined and 53 for inattentive subtype of ADHD. To receive a diagnosis of predominantly inattentive, patients must display a minimum of six of the nine symptoms from the inattentive domain in a minimum of two settings. If the patient had six or more symptoms in both domains (inattentive or hyperactive/impulsive), then patient received a diagnosis of combined-type ADHD (American Psychiatric Association, 2000). The clinical diagnosis of ADHD was made by an experienced neurologist, a child and adolescent psychiatrist, and a clinical psychologist. ADHD patients with comorbid oppositional defiant disorder (ODD; $n = 23$), learning disability ($n = 25$), anxiety ($n = 8$), and depression ($n = 6$) were included. ADHD is associated with comorbidities in a vast majority of cases (Kadesjö & Gillberg, 2001; Larson, Russ, Kahn, & Halfon, 2011), being the pure form of ADHD (without comorbidity) very atypical. Exclusion criteria were (a) significant motor or perceptive alterations; (b) intellectual disability, pervasive developmental disorder, schizophrenia, or psychosis; and (c) known neurological diseases, epilepsy, drug abuse, or dependency.

Symptom severity of ADHD was assessed with a short version of the Conner's rating scale adapted and validated for the Spanish population (EDAH; Farré & Narbona, 2003). Intellectual functioning was measured using the full version of the *Wechsler Intelligence Scale for Children, fourth edition* (WISC-IV). Forty patients were medication näive at the time of testing. The remainder were taking methylphenidate (n = 53; mean dose: 1 mg/kg; duration of treatment: 26.41 ± 20.38 months) or atomoxetine (n = 5; mean dose: 1.1 mg/kg; duration of treatment: 25.4 ± 10.64 months). The study was conducted in accordance with the ethical principles of the Declaration of Helsinki and Good Clinical Practice standards. Informed consent was obtained from parents, with the child giving assent. As described later, patients were divided into those with homozygosity for the 10R allele at *DAT1* (n = 46) and those with a single copy or no copy of the allele (n = 52).

Genotyping

Genomic DNA was extracted from peripheral blood leukocytes using the *Maxwell*® 16 Blood DNA Purification Kit (Promega, Madison, WI, USA). The *DAT1* 40-bp VNTR polymorphism was genotyped by polymerase chain reaction (PCR; Vandenbergh et al., 1992). PCR amplification of 100 ng of genomic DNA was performed in 10 µl reactions containing 0.2 mM dNTPs, 0.15 µM of forward and reverse primers, 1X PCR Buffer (Invitrogen), 1.5 mM $MgCl_2$, 5% dimethyl sulfoxide (DMSO), and 0.4 U of Platimun Taq DNA polymerase (Invitrogen). The primer sequences used were Forward, 5'-TGTGGTGTAGGGAACGGCCTGAG-3' and Reverse, 5'-CTTCCTGGAGGTCACGGCTCAAGG-3'. Thermocycler conditions were DNA was denatured at 94°C for 3 min, followed by 35 cycles of 30 s denaturation at 94°C, 30 s of annealing at 58°C, and 1 min extension at 72°C, followed by a final extension at 72°C for 7 min.

DAT1 PCR product was resolved on a 3130 Genetic Analyzer using standard company protocols without modification and analyzed with GeneMapper 3.7 software (Life Technologies, Carlsbad, USA). The presence of the *DAT1* 10-repeat allele was analyzed. For further analyses, patients were divided into those with homozygosity for the 10R allele at *DAT1* (10R/10R; n = 46) and those with a single copy (10R/9R) or no copy (9R/9R) of the allele (n = 52). Grouping patients by *DAT1* genotype in this manner is justified for several reasons, including the differential gene expression as a function of VNTR alleles (individuals with 10R allele seem to have higher transporter densities than those with one or no copies of the allele) and the low frequency of non-10R alleles in European population groups (Bellgrove, Hawi, Kirley, Gill, & Robertson, 2005; Brookes et al., 2007; Cheon, Ryu, Kim, & Cho, 2005; Mill, Asherson, Browes, D'Souza, & Craig, 2002).

MRI Acquisition, Image Preprocessing, and Cortical Thickness Analysis

All participants were scanned by the same team of staff, on the same scanner, and with identical scanning parameters. Data were acquired using a General Electric-Signa 1.5 T equipment with standard head coil. T1-weighted images were obtained using a 3D spoiled gradient echo pulse sequence of the entire head in the axial plane. The acquisition parameters were the following: time to echo = 6 ms, repetition time = 20 ms, flip angle = 30º, field of view = 280 mm, slice thickness = 1.4 mm, matrix size = 224 × 224, number of excitations = 1, voxel size = 0.55 × 0.55 × 1.4, and duration = 9.37 min. Images were carefully inspected by experienced technicians who were blinded to the participants' group assignments for artifacts and poor contrast between gray and white matter boundaries.

Image preprocessing and cortical thickness analysis were carried out using the same sequential, semi-automated procedure used in previous studies (Fernández-Jaén et al., 2015; Fernández-Jaén et al., 2014). Briefly, it entails three successive steps: (a) automated removal of non-brain tissue using FMRIB´s Software Library brain extraction tool (BETv2.1; Smith, 2002). BET uses a deformable model to separate brain from non-brain tissue. BET2 command with default setting and a default fractional intensity threshold of 0.5 was used. (b) Automatic segmentation of selected brain tissue into tissue types including gray matter, white matter, or cerebrospinal fluid using the FSL's FAST tool (v4.1; Zhang, Brady, & Smith, 2001). FAST also corrects spatial intensity variations or non-homogeneities. The underlying method is based on a hidden Markov model and associated anticipation-maximization algorithm, and (c) measurement of cortical thickness for each participant using the Laplace method (Jones, Buchbinder, & Aharon, 2000), as implemented in BrainVoyager (Goebel, Esposito, & Formisano, 2006). Prior to this, anatomical data of each participant was resampled and transformed into anterior commissure-posterior commissure (ACPC) plane, and subsequently normalized in Talairach standard space. Once individual cortical thickness maps were calculated, reconstructed cortices were aligned into a spherical representation to improve the spatial correspondence across participants' brains. The improved alignment of corresponding cortical structures results in clearer average brain structure. This high-quality alignment procedure is fundamental for the later exact comparison of cortical thicknesses between groups (Haller et al., 2009).

Group differences were then mapped using a region-of-interest (ROI) approach (i.e., comparing averaged thickness values from selected ROIs across participants). Structural ROIs were defined using the tools in BrainVoyager. According to the aim of the study, the cortical surface of CC was parceled into gyrus- and sulcus-based regions as well

Table 1. Demographic and Clinical Characteristics of ADHD Patients With 10-Repeat *DAT1* Allele (Homozygous Group) and ADHD Patients With a Single or No Copy of the Allele (Non-Homozygous Group).

	10R *DAT1* non-homozygous group	10R *DAT1* homozygous group	Statistics
Number of participants	52	46	—
Continuous variables (group means and standard deviations)			
Age (years)	11.21 (2.48)	10.72 (2.81)	$t(96) = 0.92$, $p = .36$
FSIQ (WISC-IV)	105.58 (11.17)	104.22 (12.87)	$t(96) = 0.56$, $p = .58$
Hyperactivity/impulsivity (EDAH)	5.67 (3.74)	5.72 (4.13)	$t(96) = -0.06$, $p = .96$
Inattention (EDAH)	10.98 (2.27)	10.67 (2.49)	$t(96) = 0.64$, $p = .52$
Categorical variables (number of participants)			
Gender (male/female)	40/12	35/11	$\chi^2 = 0.01$, $p = .92$
ADHD subtype (combined/ inattentive)	25/27	20/26	$\chi^2 = 0.21$, $p = .65$
Medication status (näive/no näive)	19/33	21/25	$\chi^2 = 0.84$, $p = .36$
Comorbid ODD (yes/no)	11/41	12/34	$\chi^2 = 0.33$, $p = .56$
Comorbid learning disability (yes/no)	15/37	10/36	$\chi^2 = 0.65$, $p = .42$
Comorbid anxiety (yes/no)	5/47	3/43	$\chi^2 = 0.31$, $p = .58$
Comorbid depression (yes/no)	2/50	4/42	$\chi^2 = 0.99$, $p = .32$

Note. FSIQ = full-scale intelligence quotient measured using WISC-IV; WISC-IV = *Wechsler Intelligence Scale for Children, fourth edition*; EDAH = scale for assessment of attention deficit hyperactivity disorder, a short version of the Conner's rating scale adapted and validated for the Spanish population; ODD = oppositional defiant disorder.

as into Brodmann-based areas. Then, individual cortical thickness values (means) from selected ROIs were exported to SPSS v20 to perform parametric statistical analyses.

Statistical Analysis

The comparison of cingulate cortical thickness variations between groups was tested with mixed analyses of variance (ANOVAs). Significant interactions were further evaluated using simple effects with Bonferroni correction for multiple comparisons. Significant ANOVA results were confirmed using age and mean cortical thickness across the entire cortex as covariates. There were no outliers in the cortical thickness data (>1.5 times the interquartile range from the upper/lower limit of the interquartile range). Demographic and clinical data were analyzed using chi-square tests (categorical variables) and independent *t* tests (continuous variables).

Results

The two genotype groups were matched for age, gender, full-scale intelligence quotient (FSIQ), ADHD subtype, symptom severity, comorbidity, and medication treatment status—näive vs. no naive (Table 1). Similarly, the direct comparison of groups did not reveal significant differences in overall mean cortical thickness between them (Table 1). Further, we performed additional analyses on medicated patients to exclude possible group differences related to type, amount, and duration of treatment. Remarkably, there were no differences between medicated patients with two copies of the 10-repeat allele compared with those with one or no copies of the allele with respect to type of treatment (methylphenidate: $n = 23$ and $n = 30$, respectively; atomoxetine: $n = 2$ and $n = 3$; $\chi^2 = 0.21$, $p = .88$), treatment dose (methylphenidate: 0.99 ± 0.22 and 1 ± 0.23 mg/kg, $t(51) = 0.24$, $p = .81$; atomoxetine: 1.15 ± 0.7 and 1.07 ± 0.21 mg/kg, $t(3) = -0.52$, $p = .64$), and treatment duration (methylphenidate: 26.43 ± 20.97 and 26.4 ± 20.27 months, $t(51) = -0.01$, $p = .99$; atomoxetine: 32 ± 12.72 and 21 ± 8.54 months, $t(3) = -1.19$, $p = .32$).

Gyrus/Sulcus-Based Analysis

The mixed ANOVA showed a significant three-way interaction between group, hemisphere, and sulcal/gyral pattern anatomy of CC, $F(1, 96) = 4.17$, $p < .05$, $\eta_p^2 = .04$. Post hoc *t* tests of simple effects with Bonferroni correction comparing the group effect within each hemisphere and type of sulcal/gyral pattern revealed greater cortical thickness in the right cingulate gyrus in patients with two copies of the 10-repeat allele, relative to those with one or no copies of the allele (corrected $p < .05$; Figure 1). By contrast, no differences were found in the right cingulate sulcus between the two groups (corrected $p = .65$). In the left hemisphere, we detected greater cortical thickness in the cingulate sulcus in patients with two copies of the 10-repeat allele, relative to those with one or no copies of the allele (corrected $p = .03$), whereas no differences between groups were found in the cingulate gyrus (corrected $p = .08$). Neither the interaction of group and hemisphere, $F(1, 96) = 0.06$, $p = .8$, nor the interaction of group and sulcal/gyral pattern, $F(1, 96) = 1.02$, $p = .31$, was significant. When age and overall mean cortical thickness were included as covariates in the analysis, $F(1, 94) = 4.37$, $p < .05$, $\eta_p^2 = .04$, the group differences

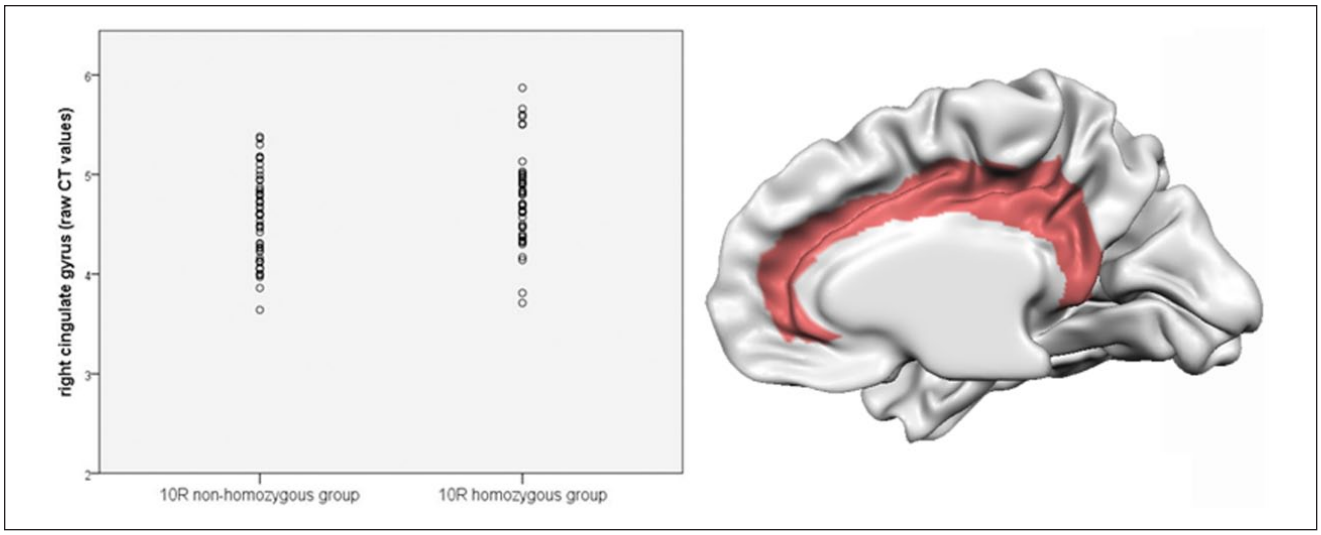

Figure 1. Scatter plot of individual cortical thickness (raw) values extracted from right cingulate gyrus within each *DAT1* genotype group (Left). Medial view of the cortical surface showing the right cingulate gyrus with differences in cortical thickness between patients with the 10-repeat *DAT1* allele and patients with 1 or 0 copies of the allele (Right).
Note. Created with the help of BrainVoyager Brain Tutor (with Rainer Goebel's permission).

Table 2. Means and Standard Deviations for Cortical Thickness Measures (mm) of Cingulate Cortex.

	10R *DAT1* non-homozygous group	10R *DAT1* homozygous group
Overall mean cortical thickness (mm)	3.35 (0.37)	3.4 (0.35)
Sulcal/gyral-based analysis		
Right cingulate gyrus	**4.54 (0.43)**	**4.75 (0.46)**
Right cingulate sulcus	3.97 (0.46)	4.01 (0.44)
Left cingulate gyrus	3.69 (0.33)	3.81 (0.35)
Left cingulate sulcus	3.68 (0.34)	3.84 (0.37)
Brodmann area (BA)–based analysis		
BA24 right (anterior-ventral)	**3.84 (0.49)**	**4.05 (0.41)**
BA32 right (anterior-dorsal)	4.39 (0.62)	4.44 (0.51)
BA23 right (posterior-ventral)	4.76 (0.57)	4.94 (0.75)
BA31 right (posterior-dorsal)	4.06 (0.53)	4.28 (0.61)
BA24 left (anterior-ventral)	3.72 (0.33	3.84 (0.33)
BA32 left (anterior-dorsal)	3.65 (0.35)	3.78 (0.37)
BA23 left (posterior-ventral)	3.85 (0.46)	3.99 (0.47)
BA31 left (posterior-dorsal)	3.74 (0.44)	3.74 (0.42)

Note. Significant differences between groups are in bold (statistics are reported in the "Results" section).

in the right cingulate gyrus remained significant (corrected $p = .02$). By contrast, differences between groups in the left cingulate sulcus did not remain statistically significant (corrected $p = .06$). Means and standard deviations for cortical thickness measures are presented in Table 2.

Brodmann Area (BA)–Based Analysis

The mixed ANOVA showed a significant four-way interaction between group, hemisphere, anterior/posterior, and ventral/dorsal, $F(1, 96) = 4.18$, $p < .05$, $\eta_p^2 = .04$. Post hoc t tests of simple effects with Bonferroni correction showed greater cortical thickness in the right BA24 (anterior-ventral region of CC) in patients with two copies of the 10-repeat allele relative to those with one or no copies of the allele (corrected $p = .02$; Figure 2). When age and overall mean cortical thickness were included as covariates in the ANOVA, $F(1, 94) = 3.96$, $p < .05$, $\eta_p^2 = .04$, the group differences in the right BA24 remained significant (corrected $p = .02$). No other BA region showed significant differences between groups (all corrected ps $> .05$). No other interaction reached statistical significance.

Finally, partial correlations between cortical thickness values of right cingulate gyrus/right BA24 and ADHD

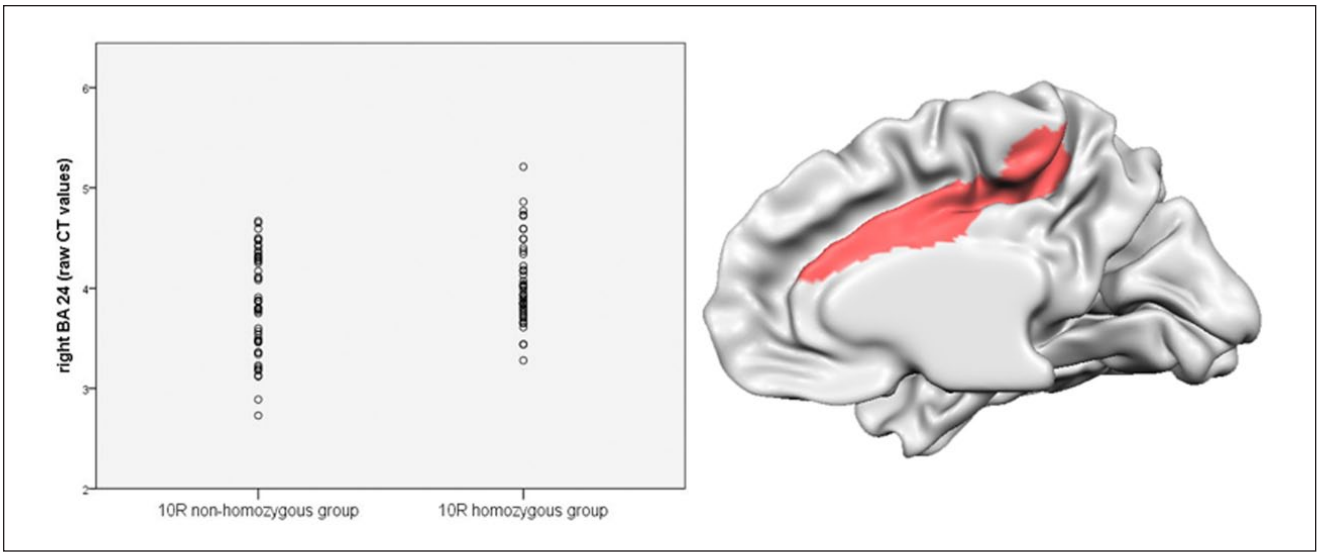

Figure 2. Scatter plot of individual cortical thickness (raw) values extracted from right Brodmann area (BA) 24 within each *DAT1* genotype group (Left) and Medial view of the cortical surface showing the right BA 24 with differences in cortical thickness between patients with the 10-repeat *DAT1* allele and patients with 1 or 0 copies of the allele (Right).
Note. Created with the help of BrainVoyager Brain Tutor (with Rainer Goebel's permission).

symptoms across the whole sample were computed. Neither inattention, $r(95) = .03$, $p = .76$ and $r(95) = -.03$, $p = .8$, respectively, nor hyperactivity/impulsivity, $r(95) = .01$, $p = .88$ and $r(95) = -.01$, $p = .96$, was associated with cortical thickness of the right cingulate gyrus or right BA 24.

Discussion

The key and novel finding of this study was that *DAT1* gene variation is associated with structural differences in the CC among ADHD patients. Specifically, greater cortical thickness in right cingulate gyrus/right BA24 were observed in patients homozygous for the 10R allele relative to those with a single or no copy of the allele. These results complement previous imaging genetic studies that examined the effect of *DAT1* genotype on striatum and prefrontal cortex (Bédard et al., 2010; Braet et al., 2011; Brown et al., 2010; Durston et al., 2005; Fernández-Jaén et al., 2015; Shook et al., 2011).

There are a number of published studies assessing volume or area differences of brain regions between ADHD patients and controls. Although data from fMRI studies underscore the importance of CC in ADHD (Bush, 2011), structural MRI studies of CC are scarce and reveal contradictory results. Relative to controls, some adults with ADHD have significantly smaller volume or thinner anterior CC (ACC; Makris et al., 2010). These data are inconsistent with studies of children, in which volumetric reductions have been generally found in prefrontal cortex but not in ACC (Kates et al., 2002; Mostofsky, Cooper, Kates, Denckla, & Kaufmann, 2002). However, a case-control study revealed that right ACC was significantly smaller for the ADHD group compared with the control group, but this difference was significant only in naïve patients (Semrud-Clikeman, Pliszka, Lancaster, & Liotti, 2006). Volumetric analysis of posterior CC (PCC) has also provided inconsistent results: one of the previously mentioned studies found no differences in PCC volume between ADHD and controls, whereas the study performed by Overmeyer and colleagues (2001) showed a significant smaller volume in right posterior cingulate gyrus. Remarkably, the fact that in the present study the *DAT1* 10R genotype was associated with thickness variation of right cingulate gyrus and right BA 24 suggests that the inconsistent structural findings on CC differences in ADHD patients may be due to the underlying genetic differences.

The *DAT1* is one of the most replicated risk gene for ADHD (Durston, 2010). At least in children and adolescents, the 10R allele has been associated with greater levels of dopamine transporter protein than the 9R form (Brookes et al., 2007; Faraone et al., 2014), so homozygous carriers of the 10R allele are thought to display lower levels of synaptic dopamine in the synaptic cleft than non-homozygous carriers. Although *DAT1* is mainly expressed in the striatum, activity and anatomy of cortical regions (including prefrontal and cingulate cortices) have shown to be influence by *DAT1* polymorphisms, both in healthy and ADHD samples (Bédard et al., 2010; Bertolino et al., 2006; Braet et al., 2011; Brown et al., 2010). Thus, two previous studies conducted with ADHD patients showed increased inhibition-related activation in prefrontal and cingulate regions in patients who were homozygous for the 10R allele (Bédard

et al., 2010; Braet et al., 2011). This enhanced activation was observed in absence of behavioral differences between groups (homozygous vs. non-homozygous), which was interpreted as reflecting a more effortful or less efficient inhibition for patients with two copies of the 10R allele. In the same vein, some neuropsychological studies have reported a detrimental effect of the 10R allele on performance during inhibition and attention tasks (Cornish et al., 2005).

As ADHD patients manifest heterogeneity in phenomenology, pathophysiology, etiology and clinical course of the disorder, imaging genetics can help to clarify the different causal pathways operating between the genetic, brain anatomy and behavioral levels. Only a few structural imaging genetic studies have been carried out on ADHD patients to date. For example, Durston and colleagues (2005) reported a reduction in caudate nucleus volume as a function of *DAT1* genotype in children and adolescents with ADHD, their unaffected siblings, and control participants. Specifically, individuals homozygous for the 10R allele were found to be those who had smaller caudate nucleus volumes. These results were subsequently replicated by Shook and colleagues (2011). Variation of striatal activation among ADHD patients as a function of *DAT1* genotype has also been identified in several fMRI studies, albeit with mixed results (i.e., hypo- or hyperactivation associated with 10R/10R genotype; Bédard et al., 2010; Braet et al., 2011; Durston et al., 2005; Durston et al., 2008).

Notably, the results presented here together with evidence from some previous investigations suggest that *DAT1 3'UTR* polymorphism may modulate the anatomy of other regions beyond the striatum. For instance, in a previous study carried out on another sample of ADHD patients and focused on prefrontal regions, Fernández-Jaén and colleagues (2015) found that patients with two copies of the 10R allele exhibited a decreased thickness in dorsolateral prefrontal cortex relative to those with one or few copies of the allele. In light of these previous imaging genetic studies showing a reduction in caudate volume and thinning in dorsolateral prefrontal cortex in ADHD patients homozygous for the 10R allele, the increased cingulate thickness observed in this study for the homozygous group might be interpreted within a neural compensation framework. The caudate and dorsolateral prefrontal cortex have strong anatomical and functional connections with CC (Beckmann, Johansen-Berg, & Rushworth, 2009; Robinson et al., 2012). Furthermore, ADHD has been shown to be characterized not only by neural hypoactivity but also neural hyperactivity in several brain regions, which may relate to compensatory brain and behavioral functioning (Fassbender & Schweitzer, 2006). Indeed, several fMRI studies have shown greater CC activation (both in anterior and posterior) in parallel to prefronto-striatal hypofunction in children with ADHD compared with healthy comparison controls during cognitive tasks (Schulz et al., 2005; Vaidya et al., 1998). In any case, further research is needed to determine the exact meaning of the increased cingulate cortical thickness found in ADHD patients homozygous for the 10R allele.

The results of the present study should be interpreted in light of its limitations. First, although this experiment revealed an association between 10R allele of *DAT1* gene and cortical thickness of CC, the molecular pathways that lead to anatomical variations in this brain structure remain unknown. Second, patients in our study had a wide range of age, potentially confounding our data with distinct gene expression across development. However, it should be noted that differential influence of *DAT1* on ADHD has been mainly reported between children and adults with the disorder but not across childhood and adolescence. Moreover, present results remained significant after accounting for confounding effects of age. Third, similar to previous imaging genetic studies (Bédard et al., 2010; Brown et al., 2010), this investigation was limited to ADHD patients. Although interesting conclusions can be drawn from this study, further research on the effect of *DAT1* on cingulate thickness in patients and controls will be important to know whether this effect is limited to ADHD or can be extended to healthy participants. Likewise, as in previous imaging genetic studies conducted only with ADHD patients (Bédard et al., 2010; Braet et al., 2011; Fernández-Jaén et al., 2015; Shook et al., 2011), we failed to find symptom differences between groups and significant correlations between ADHD symptomatology and cortical thickness values. The limited variability in symptom expression across patients is probably the reason of the absence of such differences. Further research employing children with and without ADHD is needed to examine the relationships between ADHD symptomatology and thickness of the CC.

In conclusion, this study provides new evidence supporting that thickness of CC (particularly, right cingulate gyrus and right BA 24) is conditioned by *DAT1* genotype in children and adolescents with ADHD. These findings complement prior data showing reduced striatal volume and dorsolateral prefrontal thinning in ADHD patients who were homozygous for the *DAT1* 10R risk allele. In an emerging field such as imaging genetics of ADHD, results of this study add relevant data to previous investigations using a large sample of ADHD patients.

Acknowledgments

Image reconstruction was performed using BrainVoyager Brain Tutor software (with the permission of Dr. R. Goebel).

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

References

American Psychiatric Association. (1994). *Diagnostic and statistical manual of mental disorders* (4th ed.). Washington, DC: Author.

American Psychiatric Association. (2000). *Diagnostic and statistical manual of mental disorders* (4th ed., text rev.). Washington, DC: Author.

Beckmann, M., Johansen-Berg, H., & Rushworth, M. F. (2009). Connectivity-based parcellation of human cingulate cortex and its relation to functional specialization. *The Journal of Neuroscience*, *29*, 1175-1190.

Bédard, A. C., Schulz, K. P., Cook, E. H., Fan, J., Clerkin, S. M., Ivanov, I., . . . Newcorn, J. H. (2010). Dopamine transporter gene variation modulates activation of striatum in youth with ADHD. *Neuroimage*, *53*, 935-942.

Bellgrove, M. A., Hawi, Z., Kirley, A., Gill, M., & Robertson, I. H. (2005). Dissecting the attention deficit hyperactivity disorder (ADHD) phenotype: Sustained attention, response variability and spatial attentional asymmetries in relation to dopamine transporter (DAT1) genotype. *Neuropsychologia*, *43*, 1847-1857.

Bertolino, A., Blasi, G., Latorre, V., Rubino, V., Rampino, A., Sinibaldi, L., . . . Dallapiccola, B. (2006). Additive effects of genetic variation in dopamine regulating genes on working memory cortical activity in human brain. *The Journal of Neuroscience*, *26*, 3918-3922.

Braet, W., Johnson, K. A., Tobin, C. T., Acheson, R., McDonnell, C., Hawi, Z., . . . Garavan, H. (2011). fMRI activation during response inhibition and error processing: The role of the DAT1 gene in typically developing adolescents and those diagnosed with ADHD. *Neuropsychologia*, *49*, 1641-1650.

Brookes, K. J., Neale, B. M., Sugden, K., Khan, N., Asherson, P., & D'Souza, U. M. (2007). Relationship between VNTR polymorphisms of the human dopamine transporter gene and expression in post-mortem midbrain tissue. *American Journal of Medical Genetics, Part B: Neuropsychiatric Genetics*, *144*, 1070-1078.

Brown, A. B., Biederman, J., Valera, E. M., Doyle, A. E., Bush, G., Spencer, T., . . . Seidman, L. J. (2010). Effect of dopamine transporter gene (SLC6A3) variation on dorsal anterior cingulate function in attention-deficit/hyperactivity disorder. *American Journal of Medical Genetics, Part B: Neuropsychiatric Genetics*, *153*, 365-375.

Bush, G. (2011). Cingulate, frontal, and parietal cortical dysfunction in attention-deficit/hyperactivity disorder. *Biological Psychiatry*, *69*, 1160-1167.

Castellanos, F. X., & Proal, E. (2012). Large-scale brain systems in ADHD: Beyond the prefrontal–striatal model. *Trends in Cognitive Sciences*, *16*(1), 17-26.

Cheon, K. A., Ryu, Y. H., Kim, J. W., & Cho, D. Y. (2005). The homozygosity for 10-repeat allele at dopamine transporter gene and dopamine transporter density in Korean children with attention deficit hyperactivity disorder: Relating to treatment response to methylphenidate. *European Neuropsychopharmacology*, *15*(1), 95-101.

Cornish, K. M., Manly, T., Savage, R., Swanson, J., Morisano, D., Butler, N., . . . Hollis, C. P. (2005). Association of the dopamine transporter (DAT1) 10/10-repeat genotype with ADHD symptoms and response inhibition in a general population sample. *Molecular Psychiatry*, *10*, 686-698.

Cortese, S., Kelly, C., Chabernaud, C., Proal, E., Di Martino, A., Milham, M. P., . . . Castellanos, F. X. (2012). Toward systems neuroscience of ADHD: A meta-analysis of 55 fMRI studies. *The American Journal of Psychiatry*, *169*, 1038-1055.

Cummins, T. D. R., Hawi, Z., Hocking, J., Strudwick, M., Hester, R., Garavan, H., . . . Bellgrove, M. A. (2012). Dopamine transporter genotype predicts behavioural and neural measures of response inhibition. *Molecular Psychiatry*,*17*, 1086-1092.

Durston, S. (2010). Imaging genetics in ADHD. *Neuroimage*, *53*, 832-838.

Durston, S., Fossella, J. A., Casey, B. J., Pol, H. H., Galvan, A., Schnack, H. G., . . . Van Engeland, H. (2005). Differential effects of DRD4 and DAT1 genotype on fronto-striatal gray matter volumes in a sample of subjects with attention deficit hyperactivity disorder, their unaffected siblings, and controls. *Molecular Psychiatry*, *10*, 678-685.

Durston, S., Fossella, J. A., Mulder, M. J., Casey, B. J., Ziermans, T. B., Vessaz, M. N., . . . Van Engeland, H. (2008). Dopamine transporter genotype conveys familial risk of attention-deficit/hyperactivity disorder through striatal activation. *Journal of the American Academy of Child & Adolescent Psychiatry*, *47*(1), 61-67.

Faraone, S. V., & Mick, E. (2010). Molecular genetics of attention deficit hyperactivity disorder. *Psychiatric Clinics of North America*, *33*, 159-180.

Faraone, S. V., Spencer, T. J., Madras, B. K., Zhang-James, Y., & Biederman, J. (2014). Functional effects of dopamine transporter gene genotypes on in vivo dopamine transporter functioning: A meta-analysis. *Molecular psychiatry*, *19*, 880-889.

Farré, A., & Narbona, J. (2003). *EDAH: Evaluación del trastorno por déficit de atención con hiperactividad*. Madrid, Spain: TEA.

Fassbender, C., & Schweitzer, J. B. (2006). Is there evidence for neural compensation in attention deficit hyperactivity disorder? A review of the functional neuroimaging literature. *Clinical Psychology Review*, *26*, 445-465.

Fernández-Jaén, A., López-Martín, S., Albert, J., Fernández-Mayoralas, D. M., Fernández-Perrone, A. L., de La Peña, M. J., . . . Muñoz-Jareño, N. (2015). Cortical thickness differences in the prefrontal cortex in children and adolescents with ADHD in relation to dopamine transporter (DAT1) genotype. *Psychiatry Research: Neuroimaging*, *233*, 409-417.

Fernández-Jaén, A., López-Martín, S., Albert, J., Fernández-Mayoralas, D. M., Fernández-Perrone, A. L., Tapia, D. Q., . . . Calleja-Pérez, B. (2014). Cortical thinning of temporal pole and orbitofrontal cortex in medication-naïve children and adolescents with ADHD. *Psychiatry Research: Neuroimaging*, *224*(1), 8-13.

Gizer, I. R., Ficks, C., & Waldman, I. D. (2009). Candidate gene studies of ADHD: A meta-analytic review. *Human Genetics*, *126*(1), 51-90.

Goebel, R., Esposito, F., & Formisano, E. (2006). Analysis of functional image analysis contest (FIAC) data with brainvoyager QX: From single-subject to cortically aligned group general linear model analysis and self-organizing group independent component analysis. *Human Brain Mapping*, *27*, 392-401.

Haller, S., Borgwardt, S. J., Schindler, C., Aston, J., Radue, E. W., & Riecher-Rössler, A. (2009). Can cortical thickness asymmetry analysis contribute to detection of at-risk mental state and first-episode psychosis? A Pilot Study 1. *Radiology*, *250*, 212-221.

Jones, S. E., Buchbinder, B. R., & Aharon, I. (2000). Three-dimensional mapping of cortical thickness using Laplace's Equation. *Human Brain Mapping*, *11*(1), 12-32.

Kadesjö, B., & Gillberg, C. (2001). The comorbidity of ADHD in the general population of Swedish school-age children. *Journal of Child Psychology and Psychiatry*, *42*, 487-492.

Kates, W. R., Frederikse, M., Mostofsky, S. H., Folley, B. S., Cooper, K., Mazur-Hopkins, P., . . . Kaufmann, W. E. (2002). MRI parcellation of the frontal lobe in boys with attention deficit hyperactivity disorder or Tourette syndrome. *Psychiatry Research: Neuroimaging*, *116*(1), 63-81.

Kaufman, J., Birmaher, B., Brent, D., Rao, U. M. A., Flynn, C., Moreci, P., . . . Ryan, N. (1997). Schedule for affective disorders and schizophrenia for school-age children-present and lifetime version (K-SADS-PL): initial reliability and validity data. *Journal of the American Academy of Child & Adolescent Psychiatry*, *36*, 980-988.

Larson, K., Russ, S. A., Kahn, R. S., & Halfon, N. (2011). Patterns of comorbidity, functioning, and service use for US children with ADHD, 2007. *Pediatrics*, *127*, 462-470.

Lewis, D. A., Melchitzky, D. S., Sesack, S. R., Whitehead, R. E., Auh, S., & Sampson, A. (2001). Dopamine transporter immunoreactivity in monkey cerebral cortex: Regional, laminar, and ultrastructural localization. *Journal of Comparative Neurology*, *432*, 119-136.

Makris, N., Seidman, L. J., Valera, E. M., Biederman, J., Monuteaux, M. C., Kennedy, D. N., . . . Faraone, S. V. (2010). Anterior cingulate volumetric alterations in treatment-naïve adults with ADHD: A pilot study. *Journal of Attention Disorders*, *13*, 407-413.

Mill, J., Asherson, P., Browes, C., D'Souza, U., & Craig, I. (2002). Expression of the dopamine transporter gene is regulated by the 3' UTR VNTR: Evidence from brain and lymphocytes using quantitative RT-PCR. *American Journal of Medical Genetics*, *114*, 975-979.

Mostofsky, S. H., Cooper, K. L., Kates, W. R., Denckla, M. B., & Kaufmann, W. E. (2002). Smaller prefrontal and premotor volumes in boys with attention-deficit/hyperactivity disorder. *Biological Psychiatry*, *52*, 785-794.

Nakao, T., Radua, J., Rubia, K., & Mataix-Cols, D. (2011). Gray matter volume abnormalities in ADHD: Voxel-based meta-analysis exploring the effects of age and stimulant medication. *The American Journal of Psychiatry*, *168*, 1154-1163.

Newman, J., & Grace, A. A. (1999). Binding across time: The selective gating of frontal and hippocampal systems modulating working memory and attentional states. *Consciousness and Cognition*, *8*, 196-212.

Overmeyer, S., Bullmore, E. T., Suckling, J., Simmons, A., Williams, S. C. R., Santosh, P. J., . . . Taylor, E. (2001). Distributed grey and white matter deficits in hyperkinetic disorder: MRI evidence for anatomical abnormality in an attentional network. *Psychological Medicine*, *31*, 1425-1435.

Robinson, J. L., Laird, A. R., Glahn, D. C., Blangero, J., Sanghera, M. K., Pessoa, L., . . . Fox, P. T. (2012). The functional connectivity of the human caudate: An application of meta-analytic connectivity modeling with behavioral filtering. *Neuroimage*, *60*, 117-129.

Semrud-Clikeman, M., Pliszka, S. R., Lancaster, J., & Liotti, M. (2006). Volumetric MRI differences in treatment-naive vs chronically treated children with ADHD. *Neurology*, *67*, 1023-1027.

Schulz, K. P., Tang, C. Y., Fan, J., Marks, D. J., Newcorn, J. H., Cheung, A. M., . . . Halperin, J. M. (2005). Differential prefrontal cortex activation during inhibitory control in adolescents with and without childhood attention-deficit/hyperactivity disorder. *Neuropsychology*, *19*(3), 390-402.

Shook, D., Brady, C., Lee, P. S., Kenealy, L., Murphy, E. R., Gaillard, W. D., . . . Vaidya, C. J. (2011). Effect of dopamine transporter genotype on caudate volume in childhood ADHD and controls. *American Journal of Medical Genetics, Part B: Neuropsychiatric Genetics*, *156*(1), 28-35.

Smith, S. M. (2002). Fast robust automated brain extraction. *Human Brain Mapping*, *17*, 143-155.

Ulloa, R. E., Ortiz, S., Higuera, F., Nogales, I., Fresan, A., Apiquian, R., . . . De La & Peña, F. (2005). Interrater reliability of the Spanish version of Schedule for Affective Disorders and Schizophrenia for School-Age Children–Present and Lifetime version (K-SADS-PL). *Actas Españolas de Psiquiatría*, *34*(1), 36-40.

Vaidya, C. J., Austin, G., Kirkorian, G., Ridlehuber, H. W., Desmond, J. E., Glover, G. H., . . . Gabrieli, J. D. (1998). Selective effects of methylphenidate in attention deficit hyperactivity disorder: A functional magnetic resonance study. *Proceedings of the National Academy of Sciences*, *95*(24), 14494-14499.

Vandenbergh, D. J., Persico, A. M., Hawkins, A. L., Griffin, C. A., Li, X., Jabs, E. W., . . . Uhl, G. R. (1992). Human dopamine transporter gene (DAT1) maps to chromosome 5p15.3 and displays a VNTR. *Genomics*, *14*, 1104-1106.

Willcutt, E. G. (2012). The prevalence of DSM-IV attention-deficit/hyperactivity disorder: A meta-analytic review. *Neurotherapeutics*, *9*, 490-499.

Zhang, Y., Brady, M., & Smith, S. (2001). Segmentation of brain MR images through a hidden Markov random field model and the expectation-maximization algorithm. *IEEE Transactions on Medical Imaging*, *20*(1), 45-57.

Author Biographies

Alberto Fernández-Jaén, MD, is the Head of the Pediatric Neurology Unit, Hospital Universitario Quirón, Madrid. He is also the Director of the Neurology section of CADE, and member of the scientific committee of the Spanish Federation of Children with ADHD. He is an Associate professor in the Facultad de Medicina, Universidad Europea de Madrid.

Jacobo Albert, PhD, is a Professor and Researcher at the Department of Health and Biological Psychology, Faculty of Psychology, Universidad Autónoma de Madrid.

Daniel Martín Fernández-Mayoralas, MD, is a pediatric neurologist at the Pediatric Neurology Unit, Hospital Universitario Quirón, Madrid. He is also member of the Neurology section of CADE, Madrid.

Sara López-Martín, PhD, is a researcher and a child and adolescent psychologist. Director of the Neuromottiva clinical and research center, Madrid. She is currently a Professor at the Faculty of Health Science, Univesidad Rey Juan Carlos, Madrid.

Ana Laura Fernández-Perrone, MD, is a pediatric neurologist at the Pediatric Neurology Unit, Hospital Universitario Quirón, Madrid.

Mar Jimenez de la Peña, PhD, is a radiologist at the Magnetic Resonance Unit, Hospital Universtario Qurión, Madrid.

Beatriz Calleja-Pérez, MD, is a pediatrician at the Centro de Salud Doctor Cirajas, Madrid.

Manuel Recio Rodríguez, MD, is a radiologist at the Magnetic Resonance Unit, Hospital Universtario Qurión, Madrid.

Sonia López Arribas, MD, is a child psychiatrist in the Child-Adolescent Psychiatry Unit, Hospital Gómez-Ulla, Madrid.