NeuroImage 104 (2015) 52–58

Contents lists available at ScienceDirect

NeuroImage

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

Brain anatomical substrates of mirror movements in Kallmann syndrome☆

R. Manara [a,*,1], A. Salvalaggio [b,1], V. Citton [c], V. Palumbo [d], A. D'Errico [e], A. Elefante [e], C. Briani [b], E. Cantone [f,g], G. Ottaviano [h], M.T. Pellecchia [i], N.A. Greggio [j], L. Weis [c], G. D'Agosto [k], M. Rossato [m], E. De Carlo [m], E. Napoli [k], G. Coppola [n], F. Di Salle [a], A. Brunetti [e], G. Bonanni [l], A.A. Sinisi [d], A. Favaro [o]

[a] Neuroradiology, Dept. of Medicine and Surgery, University of Salerno, Italy
[b] Neurology, Dept. of Neurosciences, University of Padova, Italy
[c] IRCCS S. Camillo, Venezia, Italy
[d] Dept. of Clinical and Experimental Medicine and Surgery, Endocrinology and Medical Andrology Section, Second University of Napoli, Italy
[e] Neuroradiology, Dept. of Scienze Biomediche Avanzate, Federico II University, Napoli, Italy
[f] Ent. Section, Dept. of Neurosciences, "Federico II" University, Napoli, Italy
[g] Dept. of Molecular and Cellular Biology and Pathology, "Federico II" University, Napoli, Italy
[h] Otolaryngology Section, Dept. of Neurosciences, University of Padova, Italy
[i] Neurology, Dept. of Medicine and Surgery, University of Salerno, Italy
[j] UOS di Endocrinolgia Pediatrica e Adolescentologia, D.A.I.S. per la Salute della Donna e del Bambino, Azienda Ospedaliera — University of Padova, Italy
[k] Medicanova, Diagnostic Center, Battipaglia (SA), Italy
[l] Unità di Endocrinologia, Dept. of Medicine (DIMED), University of Padova, Italy
[m] Clinica Medica III, Dept. of Medicine (DIMED), University of Padova, Italy
[n] Child and Adolescent Neuropsychiatry, University of Salerno, Italy
[o] Psychiatry, Dept. of Neurosciences, University of Padova, Italy

ARTICLE INFO

Article history:
Accepted 29 September 2014
Available online 6 October 2014

Keywords:
Kallmann syndrome
Mirror movements
MRI
Brain
Motor cortex

ABSTRACT

Among male patients affected by Kallmann syndrome, a genetically determined disease due to defective neural migration leading to hypogonadropic hypogonadism and hypo/anosmia, about 40% present the peculiar phenomenon of mirror movements, i.e. involuntary movements mirroring contralateral voluntary hand movements. Several pathogenic hypotheses have been proposed, but the ultimate neurological mechanisms are still elusive. The aim of the present study was to investigate brain anatomical substrates of mirror movements in Kallmann syndrome by means of a panel of quantitative MRI analyses. Forty-nine male Kallmann syndrome patients underwent brain MRI. The study protocol included 3D-T1-weighted gradient echo, fluid attenuated inversion recovery and diffusion tensor imaging. Voxel-based morphometry, sulcation, curvature and cortical thickness analyses and tract based spatial statistics were performed using SPM8, Freesurfer and FSL. All patients underwent a complete physical and neurological examination including the evaluation of mirror movements (according to the Woods and Teuber criteria).

Kallmann syndrome patients presenting with mirror movements (16/49, 32%) displayed the following brain changes: 1) increased gray matter density in the depth of the left precentral sulcus behind the middle frontal gyrus; 2) decreased cortical thickness in the precentral gyrus bilaterally, in the depth of right precentral sulcus and in the posterior portion of the right superior frontal gyrus; and 3) decreased fractional anisotropy in the left hemisphere involving the temporal lobe and peritrigonal white matter. No differences were shown by cortical curvature and sulcation analyses.

The composite array of brain changes observed in Kallmann syndrome patients with mirror movements likely represents the anatomical–structural underpinnings leading to the peculiar derangement of the complex circuitry committed to unilateral hand voluntary movements.

☆ All authors read and approved the final manuscript.

* Corresponding author at: Neuroradiology, University of Salerno, Via S. Allende 1, Baronissi 89081 (SA), Italy. Fax: +39 0498213673.
E-mail addresses: rmanara@unisa.it (R. Manara), salvalaggio.a@gmail.com (A. Salvalaggio), valentinacitton@gmail.com (V. Citton), dott.vincenzopalumbo@hotmail.it (V. Palumbo), ariannaderrico@gmail.com (A. D'Errico), andrea.elefante@unina.it (A. Elefante), chiara.briani@unipd.it (C. Briani), elenacantone@libero.it (E. Cantone), giancarlo.ottaviano@unipd.it (G. Ottaviano), mpellecchia@unisa.it (M.T. Pellecchia), greggio@pediatria.unipd.it (N.A. Greggio), luca.weis@ospedalesancamillo.net (L. Weis), g.dagosto@istitutodam.it (G. D'Agosto), marco.rossato@unipd.it (M. Rossato), eugedicarlo@tiscali.it (E. De Carlo), elvinapoli@libero.it (E. Napoli), gcoppola@unisa.it (G. Coppola), fdisalle@unisa.it (F. Di Salle), brunetti@unina.it (A. Brunetti), guglielmo.bonanni@unipd.it (G. Bonanni), antonioagostino.sinisi@unina2.it (A.A. Sinisi), angela.favaro@unipd.it (A. Favaro).

[1] These authors equally contributed to the study and should be considered first authors.

http://dx.doi.org/10.1016/j.neuroimage.2014.09.067
1053-8119/

Introduction

Kallmann syndrome (KS) is a rare inherited disorder clinically characterized by the association of hypogonadropic hypogonadism and hypo/anosmia (Kallmann et al., 1944). At conventional brain imaging, KS patients are featured by olfactory bulb hypo/aplasia and typical forebrain and anterior skull base morphological changes (Quinton et al., 1996; Maione et al., 2013). Among male KS patients, about 40% (Quinton et al., 2001) present with a peculiar phenomenon consisting of insuppressible involuntary movements that mirror voluntary contralateral hand movements (mirror movements, MMs) (see online video). Though MMs have been anecdotally reported in FGFR1 or CHD7 gene mutations (Koenigkam-Santos et al., 2010; Costa-Barbosa et al., 2013), MMs are predominantly detected in KS patients with KAL1 or, with minor frequency, PROK2/PROKR2 mutations (Quinton et al., 2001; Costa-Barbosa et al., 2013). KAL1 mutations also result in more severe reproductive phenotype while PROK2 and PROKR2 mutations are associated with variable reproductive impairment, thus highlighting a striking genotypic–phenotypic correlation (Costa-Barbosa et al., 2013).

Although MMs in KS have usually scarce clinical impact, their existence is intriguing as they unveil a genetically determined derangement of the complex circuitry committed to planning and execution of unilateral voluntary hand movement. Mild MMs are considered a physiological phenomenon before the age of 10, likely as a result of an incomplete brain myelination (Beaulé et al., 2012). Non physiological MMs have been reported in congenital (e.g. KS, Klippel–Feil disease, congenital cerebral palsy, corpus callosum agenesis, Joubert syndrome) (Farmer et al., 1990, 2004; Mayston et al., 1997; Kuhtz-Buschbeck et al., 2000; Ferland et al., 2004) and acquired conditions (Parkinson's disease, corticobasal syndrome, essential tremor, focal hand dystonia, Creutzfeld–Jakob disease, Huntington disease, stroke) (Poisson et al., 2013; Espay et al., 2005; Park et al., 2009; Chollet et al., 1991), thus highlighting the heterogeneous spectrum of diseases that might present this phenomenon. So far, the main pathogenic hypotheses of MM are: 1) abnormal persistence of the ipsilateral cortico-spinal tract, 2) abnormal interhemispheric transcallosal inhibition between the two motor cortices and/or 3) functional alteration of motor planning and motor execution (Galléa et al., 2011). All these mechanisms should entail morphological, volumetric or ultrastructural brain changes such as cervical spine neuroschisis, corpus callosum volume reduction due to defective transcallosal inhibitory fibers, or cortical–subcortical gray matter structure abnormalities.

Indeed, despite the early and rich description of MM since the 1930s (Bauman, 1932; Guttmann et al., 1939), the underlying neurological pathogenic mechanisms remain elusive and likely differ according to the associated disease. In KS, several authors suggest an involvement of the cortico-spinal tract (Conrad et al., 1978; Cox et al., 2012) mostly because cortico-spinal tract abnormalities have been observed in other inherited disorders, such as Klippel–Feil disease and Joubert syndrome, or the involvement of transcallosal networks based on the increased detection of midline abnormalities in KS (e.g. corpus callosum agenesis). The evidence for both hypotheses is however scarce (Koenigkam-Santos et al., 2008, 2010; Krams et al., 1999).

In the last two decades, a few MRI studies investigated more closely the anatomical and functional underpinnings of MM in KS, challenging some of the previous hypotheses. By means of conventional MRI, KS patients presenting with MM (KSMM+ patients) did not show hypertrophic or hypoplasic corpus callosum compared to KS patients without MM (KSMM−) (Quinton et al., 1996). Voxel-based morphometry (VBM) showed bilateral cortico-spinal tract hypertrophy in KSMM+ patients (Krams et al., 1999) that was not confirmed in a subsequent study on a larger series (Koenigkam-Santos et al., 2008). In contrast, the same cohort showed magnetization transfer ratio (MTR) and T2 relaxation time changes within the white matter of some portions of the right cortico-spinal tract suggesting possible ultrastructural white matter abnormalities coexisting with the MM phenomenon (Koenigkam-Santos et al., 2010). Functional studies puzzled the pathogenic MM hypotheses showing bilateral cortical activation during unilateral voluntary hand movement, thus advocating a more complex brain involvement (Krams et al., 1997; Leinsinger et al., 1997).

On a large KS cohort, we recently showed with novel MRI techniques (including VBM, cortical thickness, curvature, sulcation and DTI based analyses), that KS patients present with specific structural forebrain cortical changes consistent with a localized profound derangement of cortex development (Manara et al., 2014). In the present study, besides all the abovementioned techniques, we applied MRI-based analyses focused on the motor cortical and subcortical brain structures to investigate whether KSMM+ patients show specific brain anatomical and ultrastructural changes able to shed light on the intriguing phenomenon of MM.

Materials and methods

Subjects

Forty-nine male patients older than 14 years (mean-age 29.9 years; range: 15–55) affected with KS underwent brain MRI (e-Table in supplemental data). All patients met the diagnostic criteria for KS based on clinical findings and smell analysis (hypogonadotropic hypogonadism and hypo/anosmia). Forty-four patients (patients #1–44) also participated in a previous MRI study on brain changes in KS patients compared to healthy controls (Manara et al., 2014).

All KS patients underwent a complete physical and neurological examination including the evaluation of handedness (according to the Edinburgh Handedness Inventory; Oldfield, 1971) and the evaluation of MM according to the Woods and Teuber criteria (Woods and Teuber, 1978). In particular MM were scored as follows: 0 (absent); 1 (barely discernible but repetitive movements); 2 (either slight but sustained movement or stronger but briefer repetitive movement); 3 (strong and sustained repetitive movement); 4 (movement equal to that observed in the intended hand).

Subjects were divided into two groups: KS patients with MM ≥ 2 (KSMM+), KS patients without MM (KSMM−). Patients with MM $= 1$ were excluded from either the KSMM+ and KSMM− subgroups as this MM score might be observed also in normal subjects.

The study was approved by the local Ethics Committee and written informed consent was obtained from patients or their parents.

MRI acquisition

All MRI scans were performed in two centers (University Hospital of Padova and Medicanova Diagnostic Center, Battipaglia, Salerno) equipped with the same 1.5 T MRI scanner (Achieva, Philips Medical Systems, Best, the Netherlands) with a standard quadrature head coil. The MRI study protocol included:

- 3D T1-weighted imaging (repetition time/echo time: 20/3.8 ms; flip angle: 20°; slice thickness: 1 mm, acquired pixel size: 1 × 1 mm; reconstructed pixel size: 0.66 × 0.66 mm; acquisition matrix 212 × 210; reconstructed matrix 320 × 320; acquisition-time: about 7 min);
- Fluid-attenuated inversion-recovery (FLAIR, repetition time/echo time/inversion time: 10,000/140/2800 ms; echo train length: 53; flip angle: 90°; slice thickness: 5 mm; interslice gap: 0.5 mm; acquisition pixel: 0.90 × 1.15 mm; reconstructed pixel: 0.9 × 0.9 mm; acquisition-time: 3 min 20 s);
- Diffusion tensor images (DTI) were acquired with a single-shot echo planar diffusion weighted imaging (repetition time/echo time: 11114/80 ms; acquisition matrix: 112 × 110, echo train length: 59; reconstructed matrix: 128 × 128; acquisition pixel: 2 × 2 mm; reconstructed pixel: 1.75 × 1.75 × 2 mm, SENSE p reduction: 2; slice thickness: 2 mm without gap; number of excitations: 2;

acquisition-time: 12 min 24 s). The axial sections covered the whole brain including the cerebellum. The diffusion sensitizing gradients were applied along 32 non-collinear gradient encoding directions with maximum b = 800 s/mm^2. One additional image without diffusion gradients (b = 0 s/mm^2) was also acquired.

No patients required sedation during MRI acquisition.

Image processing

Data processing of volumetric images and diffusion tensor imaging

The imaging processing methods are presented in extenso in the online supplementary material.

We utilized the optimized VBM protocol (DARTEL) available in the Statistical Parametric Mapping software (SPM8, www.fil.ion.ucl.ac.uk/spm) (Ashburner and Friston, 2000). For statistical analyses, the parametric *t*-test as implemented by SPM8 was applied, using age, total intracranial volume and lateralization as covariates of no interest. Results for gray matter were considered significant for $p < 0.05$ cluster-based FWE-corrected. Statistical analyses were first performed in the whole-brain and then in the left and right motor and premotor cortices as regions of interest (ROI). Masks for motor and premotor cortices to be used for ROI analyses were obtained with the MarsBaR tool for SPM.

All 3DT1 weighted data were processed by Freesurfer (http://surfer.nmr.mgh.harvard.edu/) to derive quantitative estimates of sulcation, curvature and cortical thickness. The sulcation conveys information on how far a particular surface vertex point is from a hypothetical "mid surface" that exists between the sulci and gyri. The curvature conveys information on the curvature (not distance) at a specific vertex point. The higher the value (negative or positive), the sharper the curve. The color conveys the sign, and is just an arbitrary choice. Sulcation, curvature and cortical thickness were estimated for the whole brain (Dale et al., 1999; Fischl et al., 1999; Fischl and Dale, 2000) and in those brain areas in which a significant difference emerged in analyzing gray matter volumes. Group analysis was performed using GLM models, including age as a nuisance variable. Results were considered significant for $p < 0.05$ FDR-corrected.

Using the automated segmentation procedure implemented by the Freesurfer software, we obtained volumes of subcortical nuclei (caudate, putamen, thalamus, pallidum) and corpus callosum (divided into 5 sections). With this method, each voxel in the MRI volume was automatically assigned a neuroanatomical label, based on probabilistic information estimated automatically from a manual training set (Fischl et al., 2002).

All DTI data were preprocessed by means of the Oxford Center for Functional MRI of the Brain (FMRIB)'s Diffusion Toolbox (FDT) within FMRIB's Software Library (FSL; http://www.fmrib.ox.ac.uk/fsl) (Smith et al., 2004). The Trait-Based Spatial Statistics method has been used (for details see supplementary materials). For group comparisons concerning fractional anisotropy (FA) and diffusivity values (mean, radial, and axial diffusivity; MD, RD and L1), data were fed into the voxel-wise statistical analysis which was based on nonparametric permutation testing (5000 permutations) to account for multiple comparison corrections across space (Smith and Nichols, 2009) combined with the threshold-free cluster enhancement procedure (TFCE). Age and sites of MRI scan acquisition were entered into the analysis as covariates.

As some of the theories on MM pathogenesis include changes in the structure of the cortico-spinal tract or corpus callosum, TBSS analysis was also restricted to these structures by applying dedicated masks taken from the JHU white-matter tractography atlas (Mori et al, 2005) as implemented by FSL.

Results

The study group (e-Table 1) consisted of 16 KSMM+ patients (mean-age 33.8 years; age-range 16–48), 29 KSMM− patients (mean-age 28.0 years; age-range 15–55) and four patients with MM = 1. MM = 1 patients were not considered in subsequent MRI analyses.

Seven KS patients were left-handed; three of them were KSMM+, four were KSMM− ($p = 0.69$, Fisher's exact test).

One KSMM− patient presented with midline brain abnormalities and one KSMM− patient showed striking multiple sclerosis-like white matter lesions. These two patients were excluded from 3D-T1 and DTI based analyses to avoid the interference of malformative or acquired factors on the quantitative parenchymal and morphological evaluation.

Five KS patients (two KSMM+, and three KSMM−) were excluded from TBSS analysis because of DTI protocol deviation (longer repetition time).

Therefore, 43 patients (16 KSMM+) underwent analyses of 3D-T1 data (VBM, cortical thickness, sulcation, curvature), and 38 (14 KSMM+) underwent also DTI analysis (TBSS).

Voxel-based morphometry (VBM)

The total amount of gray and white matter did not differ between KSMM+ and KSMM−: gray matter = 0.907 ± 0.143 mL vs 0.951 ± 0.132 mL; white matter = 0.686 ± 0.094 mL vs 0.687 ± 0.099 mL, respectively.

By whole brain analysis no gray or white matter density differences were found between subgroups. By hypothesis-driven gray matter ROI analysis (motor and premotor areas), KSMM+ showed a single cluster of significantly increased gray matter density in the depth of the left precentral sulcus behind the middle frontal gyrus (cluster size 37 voxels, peak MNI coordinates −32, 4, 46) compared to KSMM− (see Fig. 1A). In addition, the analysis of hypothesis-driven white matter ROI (cortico-spinal tract and corpus callosum) did not reveal significant differences.

Automated segmentation analyses of corpus callosum and subcortical nuclei.

Compared to KSMM−, KSMM+ showed a slightly increased volume in the medium-posterior region of the corpus callosum ($p = 0.029$, adjusted for total intracranial volume) but the difference was not significant after correction for multiple comparisons.

In addition, KSMM+ patients disclosed a volume decrease of the globus pallidus, bilaterally (left: $p = 0.043$, adjusted for total intracranial volume, uncorrected for multiple comparison, Bonferroni threshold for significance $p = 0.006$; right: $p = 0.052$, adjusted for total intracranial volume, uncorrected for multiple comparison, Bonferroni threshold for significance $p = 0.006$); no other significant differences were found among subcortical gray matter structures.

Sulcation, curvature and cortical thickness whole brain analyses

Regarding curvature and sulcation analyses, there were no differences between KSMM+ and KSMM−.

By whole brain cortical thickness analysis, a few areas of decreased thickness were detected in the precentral gyrus bilaterally, in the depth of the right precentral sulcus and in the posterior portion of the right superior frontal gyrus (Fig. 1B and Table 1). The cortical thickness did not differ between KS subgroups in the region of gray matter volume increase.

Tract based spatial statistics analysis (TBSS)

The analysis did not reveal any significant difference between KSMM+ and KSMM− regarding MD, RD and L1. In contrast, KSMM+ presented several clusters of decreased FA in the left hemisphere (Fig. 1C and Table 2) involving the temporal lobe and peritrigonal white matter. Notably, TBSS analysis did not reveal any significant difference at the level of the cortico-spinal tract and the corpus callosum.

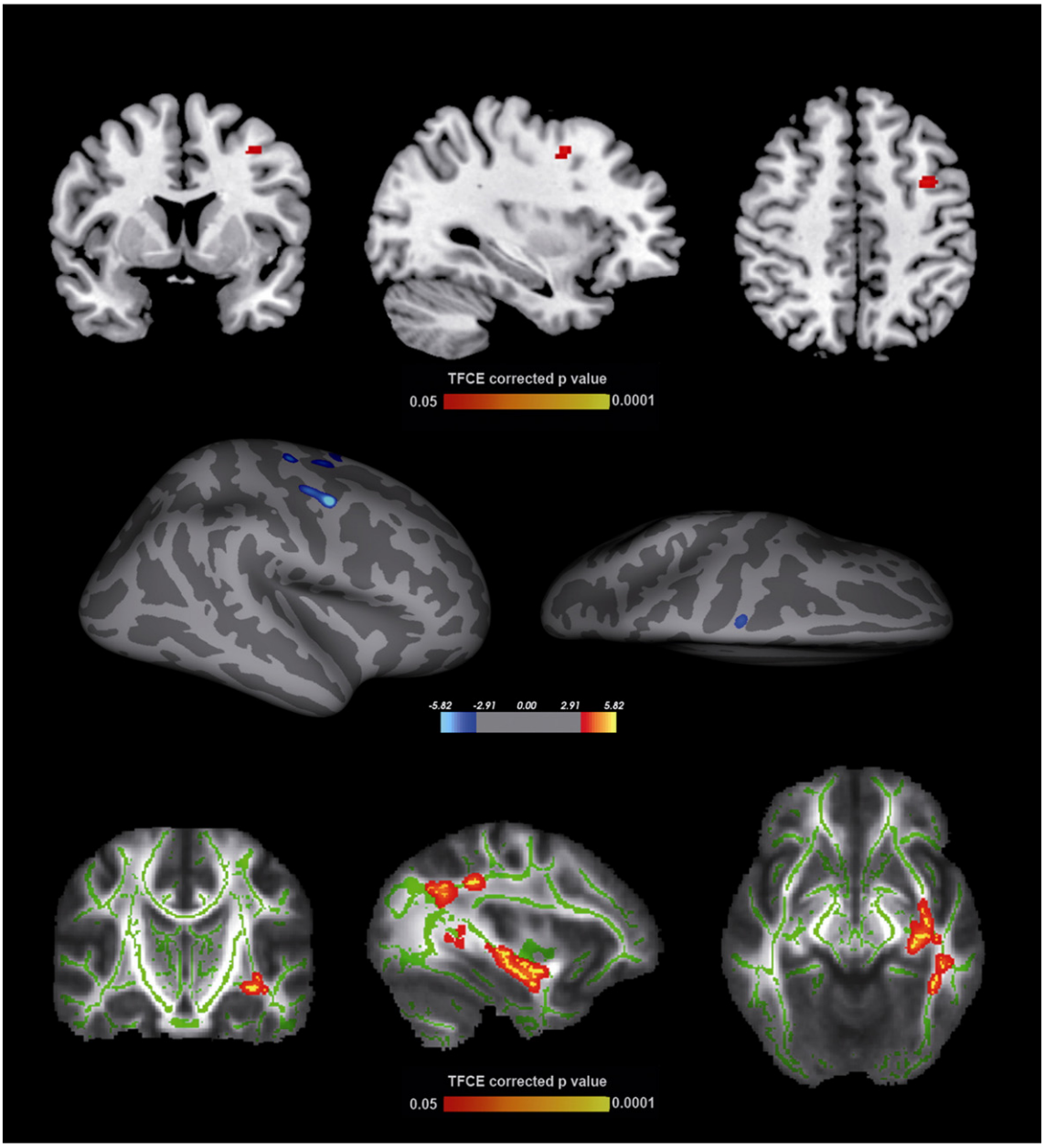

Fig. 1. Anatomical and structural brain changes in Kallmann syndrome patients presenting with mirror movements compared to Kallmann syndrome patients without mirror movements. A) Whole brain voxel-based morphometry analysis showing a cluster of increased gray matter density (red area) in the depth of the left precentral sulcus behind the middle frontal gyrus in Kallmann syndrome patients with mirror movements. B) Whole brain analysis showing areas of decreased cortical thickness located in the precentral and superior frontal cortex (blue areas) of Kallmann syndrome patients with mirror movements. There were no areas of increased cortical thickness. C) Whole brain tract based spatial statistics analysis revealing several clusters of decreased fractional anisotropy in the left hemisphere (yellow-red areas) of Kallmann syndrome patients with mirror movements; the cortico-spinal tracts were not involved. Mean, radial and axial diffusivity did not differ between Kallmann syndrome subgroups.

Table 1
Cortical areas > 30 mm^2 with significant differences between Kallmann syndrome patients with mirror movements and Kallmann syndrome patients without mirror movements by cortical thickness analysis.

Hemisphere	Size (mm^2)		Local maximum, Talairach			FDR threshold	Cerebral region
			X	Y	Z		
Left	36.79	↓	−17.1	−21.5	72.1	4.46487	Precentral
Right	200.06	↓	47.2	−3.2	47.7	3.57396	Precentral
Right	66.10	↓	31.8	−19.8	67.1	3.57396	Precentral
Right	69.38	↓	22.1	−9.6	52.4	3.57396	Precentral
Right	57.97	↓	22.9	0.2	63.0	3.57396	Superiorfrontal

↓ = Kallmann syndrome patients with mirror movements showed decreased cortical thickness.

Table 2
Clusters of significant fractional anisotropy (FA) differences between Kallmann syndrome patients with mirror movements and Kallmann syndrome patients without mirror movements by TBSS analysis.

Hemisphere	Size (voxel)		Local maximum, Talairach		
			X	Y	Z
Left	1096	↓	−33	−16	−11
Left	845	↓	−32	−53	27
Left	152	↓	−25	−59	27
Left	37	↓	−26	−68	24
Left	30	↓	−29	−59	−1

↓ = Kallmann syndrome patients with mirror movements showed decreased FA values.

Discussion

The present study showed that KS patients with MM phenomenon have distinctive cortical and subcortical changes, mostly in regions involved in motor function (globus pallidus and both primary and secondary motor areas) by VBM and cortical thickness analyses. As the neural anatomical and functional underpinnings of MM have not yet been completely understood and several pathogenic hypotheses have been proposed (Galléa et al., 2011), we will discuss which theory best fits with the presence of MM in KS, according to the literature and our findings.

(i) Abnormal persistence of the ipsilateral cortico-spinal tract

In Klippel–Feil disease, the absence of the pyramidal decussation of the cortico-spinal tract has been –shown both by pathologic (Gunderson and Solitare, 1968) and imaging studies (Royal et al., 2002). Nonetheless, the lack of cortico-spinal tract decussation leads to voluntary movements ipsilaterally to the activated cortex and does not justify by itself the presence of contralateral MM unless other still unidentified brain and/or spine anomalies coexist. Pathologic evidence of decussation abnormalities has not been reported in other pathologic conditions presenting with congenital MM. Moreover, abnormal cortico-spinal tract decussation is not supposed to be present in acquired MM disorders (e.g. Parkinson disease, Creutzfeldt–Jakob disease, stroke) (Cox et al., 2012) thus suggesting different pathogenic mechanisms and anatomical basis in different conditions.

Cortico-spinal tract hypertrophy in KSMM+ vs KSMM− was reported by Krams et al (1999) but subsequent VBM analysis on larger KS cohorts did not confirm these findings (Koenigkam-Santos et al., 2008 and the present study). Higher magnetization transfer ratio was observed in the pyramidal decussation of KS vs healthy controls, but no difference was found when comparing KSMM+ vs KSMM−, thus weakening its role in MM pathogenesis (Koenigkam-Santos et al., 2010). The same study revealed unilateral significantly decreased magnetization transfer ratio in the right internal capsule posterior limb and in the right cerebral peduncle together with an increased T2 relaxation time in the right internal capsule posterior limb, despite the presence of bilateral MM. These findings suggested the presence of primary or secondary structural cortico-spinal tract abnormalities consistent with axonal fiber microstructural disarrangement or damage, and the authors solicited DTI analysis to better characterize cortico-spinal tract white matter abnormalities. However, the present study, which included TBSS DTI analysis, did not identify ultrastructural abnormalities at cortico-spinal tract level not supporting cortico-spinal tract abnormalities as the anatomical substrate of MM. Therefore, other mechanisms acting at the cortical or spinal levels should have a role in generating MM in KS.

(ii) Abnormal inter-hemispheric inhibition between the two motor cortices

Unilateral voluntary movements require the suppression of the contralateral primary motor cortex by means of complex transcallosal inhibitory pathways. The absence of these inhibitory mechanisms in KSMM+ might be revealed by morphological and ultrastructural changes of corpus callosum. Studies on corpus callosum in KS have however produced contradictory findings. Corpus callosum underdevelopment has been reported in the seminal study by De Morsier (De Morsier, 1954) and it was claimed to be a typical feature of KS. This finding was not confirmed in subsequent larger series (Quinton et al., 1996) and even in our KS cohort only one patient presented with partial agenesia of corpus callosum. Interestingly, this patient did not present the MM phenomenon. Early studies in the late 90s detected both corpus callosum normal size or hypertrophy in KSMM+ patients (Quinton et al., 1996; Krams et al., 1999) thus leaving unresolved the issue on the role of corpus callosum in the genesis of MM. In a large cohort of KS patients, magnetization transfer ratio was found to be bilaterally decreased in the splenium of the corpus callosum and T2 relaxation time resulted to be increased in the left splenium of the corpus callosum, thus suggesting significant ultrastructural abnormalities in the posterior corpus callosum (Koenigkam-Santos et al., 2010). Moreover, the abovementioned magnetization transfer ratio and T2 relaxation time changes were restricted to the splenium, i.e. the portion of the corpus callosum that connects cortical areas that should not be significantly involved in voluntary movements. In the same cohort, VBM analysis did not reveal any change in corpus callosum white matter density (Koenigkam-Santos et al., 2008). Our study confirmed the latter finding on a larger cohort (no significant VBM white matter differences between KSMM+ and KSMM− patients). In contrast to previous studies, by TBSS analyses we did not find significant ultrastructural changes, as FA, MD, RA and L1 did not differ in corpus callosum both between KS patients and controls (Manara et al., 2014) or between KSMM+ and KSMM− patients.

(iii) Functional alteration of motor planning and motor execution

Unilateral voluntary movement requires a complex multistep activity which encompasses cortical activation and inhibition of both the primary and secondary motor cortices. Functional imaging studies disclosed an abnormal recruitment of both motor cortices in patients with MM while performing unilateral voluntary hand movements (Krams et al., 1997; Leinsinger et al., 1997).

In the present study we applied a panel of structural quantitative MRI analyses aimed at identifying subtle gray matter abnormalities in KSMM+ patients. A previous VBM study did not detect significant gray matter density changes between KSMM+ and KSMM− patients (Koenigkam-Santos et al., 2008). In contrast, VBM analysis in our KS cohort showed a cluster of increased gray matter density in the depth of the precentral sulcus. Interestingly, this area corresponds to the border between the left primary motor cortex and the contiguous premotor cortex, an area that is crucial for motor planning and execution control. The detection of this cluster in the present study might be due to several factors including the increased sample size and the exclusion from analysis of patients with age <14 years and with subtle or uncertain MM, such as those with score = 1. On the other hand, the dominant role of the left hemisphere might explain this asymmetric finding, which could represent both an anatomical substrate of MM or a secondary cortex rearrangement.

According to the other analyses applied in this study and specifically investigating cortical abnormalities, KSMM+ did not show sulcation or curvature abnormalities that could unveil an impaired developmental gyrification of motor areas, as occurs in the forebrain of KS patients (Manara et al., 2014). In contrast, this study revealed areas of decreased cortical thickness strikingly localized in the precentral gyri and in the contiguous secondary motor cortex in the left hemisphere. The areas in the motor cortical strip are located slightly inferior-laterally to the precentral hook that is usually identified with the hand representation of the motor homunculus (Yousry et al., 1997). Interestingly, the sole task-related fMRI image during voluntary finger tapping in a KSMM+ patient present in the literature seems to display bilateral motor cortex activation slightly inferior-lateral to the precentral hook (Leinsinger et al., 1997). If this observation is just a coincidence or reflects a true relocation of cortical motor areas slightly inferior-lateral to the precentral hook (thus locating the thickness changes exactly within the motor hand cortex) will be unraveled only by associating cortical thickness and functional imaging studies in an adequate KSMM+ cohort.

Interestingly, cortical areas presenting with thickness changes are relatively close to the area of increased gray matter density. These findings might appear conflicting (increased gray matter volume without cortical thickness changes and decreased cortical thickness without gray matter volume changes and, above all, areas of decreased cortical thickness close to a region of increased gray matter volume) but they

likely reflect a rather complex cortical reorganization as a possible anatomical substrate of MM. As KS is secondary to an abnormal migration of neurons from the olfactory placode to the hypothalamus (Schwanzel-Fukuda et al., 1989) involving also neurons of the forebrain (Manara et al., 2014), we might hypothesize that an analogous process involves the primary and secondary motor cortices. In particular, a subpopulation of inhibitory neurons might fail to reach the cortex causing a morphological relative decrease of cortical thickness and a functional lack of suppression of the physiological contralateral activation.

Two other extra-cortical findings have to be considered while discussing the abovementioned cortical changes. First, the trend of volume reduction of the globus pallidus observed in KSMM+ patients underlines the concomitant profound involvement of the extrapiramidal motor circuit in the phenomenon of mirror movement. Whether these changes are primitive or secondary to the motor dysfunction is unclear, but they seem to strengthen the link between mirror movements and those conditions where the basal ganglia are primarily involved (Poisson et al., 2013; Espay et al., 2005; Park et al., 2009). Second, though the association is weak and needs to be confirmed, the selective volume increase of the medium-posterior portion of the corpus callosum in KSMM+ patients seems to highlight the role of the cortex-to-cortex connectivity in the pathogenesis of MM.

All these data need validation but, more importantly, warrant further implementation with functional studies investigating the integrity of the motor neural network and the effective connectivity among cortical and subcortical structures.

Structural changes outside the motor circuitry in KSMM + patients

Previous quantitative MRI studies showed ultrastructural and volumetric differences between KSMM+ and KSMM− patients in regions not strictly involved in motor function (e.g. increased T2 relaxation time in the left frontal lobe and left corpus callosum splenium, decreased magnetization transfer ratio in the frontal lobe and corpus callosum splenium bilaterally and increased left parahippocampal white matter density) (Koenigkam-Santos et al., 2008, 2010). These data suggested a brain involvement in KSMM+ patients that spreads beyond the areas of motor function possibly related to the MM phenomenon. By whole brain VBM, we did not confirm white matter density changes in a larger cohort. Nonetheless, TBSS analysis disclosed several large clusters of increased FA in the left hemisphere. The lack of significant changes in MD, L1 and RA in the same regions seems to indicate that these changes are due to increased fiber crossing without concomitant significant myelin or axonal derangement. As the MM phenomenon is genetically linked to KAL1 and, less frequently, to PROK2/PROKR2 mutations (Quinton et al., 2001; Costa-Barbosa et al., 2013), we might infer that the involvement of these specific genes might determine other concomitant brain changes. Indeed, KAL1 mutations seem to be associated with a more severe impairment of a reproductive phenotype, thus a potential influence of early hormonal deficiency on this neurological pathway development might be hypothesized. As the left hemisphere has a key role in language functions and no data are available in the literature on language performance in KSMM+ patients, neuropsychological studies are warranted to investigate specific subtle cognitive alterations among KS subgroups.

Limits of the study

The phenomenon of mirror movement likely involves several aspects of brain anatomy (neurons, axons, cortical structure, white matter bundle anatomy) that are best studied with different neuroimaging techniques (VBM, TBSS, etc.). Each technique is not able by itself to evaluate exhaustively all the possible brain changes. VBM detects gray and white matter density changes, but does not provide any information about white matter ultrastructural or cortical developmental changes, that might coexist and contribute to the mirror movement phenomenon. Similarly, TBSS investigates the integrity of specific white matter bundles but might not detect concomitant hyper-/hypotrophy of the same structures, while cortical thickness changes do not necessarily identify concomitant regional gray matter density abnormalities. All these neuroimaging analyses apply different analytic strategies for multiple comparisons thus providing a non-uniform method for investigating brain structures. Moreover, we did not apply an overall correction for multiple comparisons, as it would have been extremely conservative with the risk of increasing the number of false negatives. In addition, we focused on the presence/absence of mirror movements in this disease, without taking into account the underlying genetics. Hypothetically, different gene mutations might imply different mechanisms leading to mirror movements, even among KS patient subgroups. This study likely represent a first step towards a more precise characterization of the significant anatomical underpinnings underlying the phenomenon of mirror movements in Kallmann syndrome but further studies applying selected analysis and genetically specific KS patient subgroups are needed to validate or enrich our findings.

Conclusions

The results of our multimodality MRI study revealed a composite array of brain changes in KSMM+ that might represent the anatomical substrate of the mirror movement phenomenon in Kallmann syndrome. Regarding the theories present in the literature our data: 1) seem to contrast the hypothesis of cortico-spinal tract abnormalities, 2) do not support the existence of abnormal transcallosal inhibitory pathways, and 3) sustain the presence of a rather complex motor (primary and secondary) cortical and subcortical (globus pallidus) reorganization that warrants focused functional studies to unravel the fascinating phenomenon of mirror movements.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.neuroimage.2014.09.067.

Conflict of interest

All authors report no disclosures.

References

Ashburner, J., Friston, K.J., 2000. Voxel-based morphometry—the methods. NeuroImage 11 (6 Pt. 1), 805–821.

Bauman, G.I., 1932. Klippel–Feil syndrome. JAMA 98, 129–132.

Beaulé, V., Tremblay, S., Théoret, H., 2012. Interhemispheric control of unilateral movement. Neural Plast. 2012, 627816.

Chollet, F., DiPiero, V., Wise, R.J., Brooks, D.J., Dolan, R.J., Frackowiak, R.S., 1991. The functional anatomy of motor recovery after stroke in humans: a study with positron emission tomography. Ann. Neurol. 29 (1), 63–71.

Conrad, B., Kriebel, J., Hetzel, W.D., 1978. Hereditary bimanual synkinesis combined with hypogonadotropic hypogonadism and anosmia in four brothers. J. Neurol. 218, 263–274.

Costa-Barbosa, F.A., Balasubramanian, R., Keefe, K.W., Shaw, N.D., Al-Tassan, N., Plummer, L., et al., 2013. Prioritizing genetic testing in patients with Kallmann syndrome using clinical phenotypes. J. Clin. Endocrinol. Metab. 98 (5), E943–E953.

Cox, B.C., Cincotta, M., Espay, A.J., 2012. Mirror movements in movement disorders: a review. Tremor Other Hyperkinet Mov. 2 (pii: tre-02-59-398-1).

Dale, A.M., Fischl, B., Sereno, M.I., 1999. Cortical surface-based analysis. I. Segmentation and surface reconstruction. NeuroImage 9 (2), 179–194.

De Morsier, G., 1954. Études sur les dysraphies crânio-encephaliques. I. Agénésie des lobes olfactifs (télencéphaloschizis latéral) et des commissures calleuse et antérieure (télencéphaloscizis median). La dysplasie olfacto-génitale. Schweiz. Arch. Neurol. Psychiatr. 74, 309–361.

Espay, A.J., Li, J.Y., Johnston, L., Chen, R., Lang, A.E., 2005. Mirror movements in parkinsonism: evaluation of a new clinical sign. J. Neurol. Neurosurg. Psychiatry 76 (10), 1355–1358.

Farmer, S.F., Ingram, D.A., Stephens, J.A., 1990. Mirror movements studied in a patient with Klippel–Feil syndrome. J. Physiol. 428, 467–484.

Farmer, S.F., Harrison, L.M., Mayston, M.J., Parekh, A., James, L.M., Stephens, J.A., 2004. Abnormal cortex–muscle interactions in subjects with X-linked Kallmann's syndrome and mirror movements. Brain 127 (Pt 2), 385–397.

Ferland, R.J., Eyaid, W., Collura, R.V., Tully, L.D., Hill, R.S., Al-Nouri, D., et al., 2004. Abnormal cerebellar development and axonal decussation due to mutations in AHI1 in Joubert syndrome. Nat. Genet. 36 (9), 1008–1013.

Fischl, B., Dale, A.M., 2000. Measuring the thickness of the human cerebral cortex from magnetic resonance images. Proc. Natl. Acad. Sci. U. S. A. 97 (20), 11050–11055.

Fischl, B., Sereno, M.I., Dale, A.M., 1999. Cortical surface-based analysis. II: inflation, flattening, and a surface-based coordinate system. NeuroImage 9 (2), 195–207.
Fischl, B., Salat, D.H., Busa, E., Albert, M., Dieterich, M., Haselgrove, C., et al., 2002. Whole brain segmentation: automated labeling of neuroanatomical structures in the human brain. Neuron 33, 341–355.
Galléa, C., Popa, T., Billot, S., Méneret, A., Depienne, C., Roze, E., 2011. Congenital mirror movements: a clue to understanding bimanual motor control. J. Neurol. 258 (11), 1911–1919.
Gunderson, C.H., Solitare, G.B., 1968. Mirror movements in patients with the Klippel–Feil syndrome. Neuropathologic observations. Arch. Neurol. 18 (6), 675–679.
Guttmann, E., Maclay, W.S., Stokes, A.B., 1939. Persistent mirror-movements as a heredofamilial disorder. J. Neurol. Psychiatry 2 (1), 13–24.
Kallmann, F.J., Schonfeld, W.A., Barvera, S.E., 1944. The genetic aspects of primary eunuchoidism. Am. J. Ment. Defic. 48, 203–236.
Koenigkam-Santos, M., Santos, A.C., Borduqui, T., Versiani, B.R., Hallak, J.E., Crippa, J.A., et al., 2008. Whole-brain voxel-based morphometry in Kallmann syndrome associated with mirror movements. AJNR Am. J. Neuroradiol. 29 (9), 1799–1804.
Koenigkam-Santos, M., de Castro, M., Versiani, B.R., Diniz, P.R., Santos, A.C., 2010. Kallmann syndrome and mirror movements: white matter quantitative evaluation with magnetic resonance imaging. J. Neurol. Sci. 292 (1–2), 40–44.
Krams, M., Quinton, R., Mayston, M.J., Harrison, L.M., Dolan, R.J., Bouloux, P.M., et al., 1997. Mirror movements in X-linked Kallmann's syndrome. II. A PET study. Brain 120 (Pt 7), 1217–1228.
Krams, M., Quinton, R., Ashburner, J., Friston, K.J., Frackowiak, R.S., Bouloux, P.M., et al., 1999. Kallmann's syndrome: mirror movements associated with bilateral corticospinal tract hypertrophy. Neurology 52 (4), 816–822.
Kuhtz-Buschbeck, J.P., Sundholm, L.K., Eliasson, A.C., Forssberg, H., 2000. Quantitative assessment of mirror movements in children and adolescents with hemiplegic cerebral palsy. Dev. Med. Child Neurol. 42 (11), 728–736.
Leinsinger, G.L., Heiss, D.T., Jassoy, A.G., Pfluger, T., Hahn, K., Danek, A., 1997. Persistent mirror movements: functional MR imaging of the hand motor cortex. Radiology 203 (2), 545–552.
Maione, L., Benadjaoud, S., Eloit, C., Sinisi, A.A., Colao, A., Chanson, P., et al., 2013. Computed tomography of the anterior skull base in Kallmann syndrome reveals specific ethmoid bone abnormalities associated with olfactory bulb defects. J. Clin. Endocrinol. Metab. 98 (3), E537–E546.
Manara, R., Salvalaggio, A., Favaro, A., Palumbo, V., Citton, V., Elefante, A., et al., 2014. Brain changes in Kallmann syndrome. Am. J. Neuroradiol. 30 (PMID:24788131).
Mayston, M.J., Harrison, L.M., Quinton, R., Stephens, J.A., Krams, M., Bouloux, P.M., 1997. Mirror movements in X-linked Kallmann's syndrome. I. A neurophysiological study. Brain 120 (Pt 7), 1199–1216.
Mori, S., Wakana, S., Nagae-Poetscher, L., Zijl, P.V., 2005. MRI Atlas of Human White Matter. Elsevier, Amsterdam, The Netherlands.
Oldfield, R.C., 1971. The assessment and analysis of handedness: the Edinburgh inventory. Neuropsychologia 9 (1), 97–113.
Park, I.S., Song, I.U., Lee, S.B., Lee, K.S., Kim, H.T., Kim, J.S., 2009. Mirror movements and involuntary homolateral limb synkinesis in a patient with probable Creutzfeldt–Jakob disease. Clin. Neurol. Neurosurg. 111 (4), 380–383.
Poisson, A., Ballanger, B., Metereau, E., Redouté, J., Ibarolla, D., Comte, J.C., et al., 2013. A functional magnetic resonance imaging study of pathophysiological changes responsible for mirror movements in Parkinson's disease. PLoS One 8 (6), e66910.
Quinton, R., Duke, V.M., de Zoysa, P.A., Platts, A.D., Valentine, A., Kendall, B., et al., 1996. The neuroradiology of Kallmann's syndrome: a genotypic and phenotypic analysis. J. Clin. Endocrinol. Metab. 81 (8), 3010–3017.
Quinton, R., Duke, V.M., Robertson, A., Kirk, J.M., Matfin, G., de Zoysa, P.A., et al., 2001. Idiopathic gonadotrophin deficiency: genetic questions addressed through phenotypic characterization. Clin. Endocrinol. 55 (2), 163–174.
Royal, S.A., Tubbs, R.S., D'Antonio, M.G., Rauzzino, M.J., Oakes, W.J., 2002. Investigations into the association between cervicomedullary neuroschisis and mirror movements in patients with Klippel–Feil syndrome. Am. J. Neuroradiol. 23 (4), 724–729.
Schwanzel-Fukuda, M., Bick, D., Pfaff, D.W., 1989. Luteinizing hormone-releasing hormone (LHRH)-expressing cells do not migrate normally in an inherited hypogonadal (Kallmann) syndrome. Brain Res. Mol. Brain Res. 6, 311–326.
Smith, S.M., Nichols, T.E., 2009. Threshold-free cluster enhancement: addressing problems of smoothing, threshold dependence and localisation in cluster inference. NeuroImage 44 (1), 83–98.
Smith, S.M., Jenkinson, M., Woolrich, M.W., Beckmann, C.F., Behrens, T.E.J., Johansen-Berg, H., et al., 2004. Advances in functional and structural MR image analysis and implementation as FSL. NeuroImage 23 (S1), 208–219.
Woods, B.T., Teuber, H.L., 1978. Mirror movements after childhood hemiparesis. Neurology 28 (11), 1152–1157.
Yousry, T.A., Schmid, U.D., Alkadhi, H., Schmidt, D., Peraud, A., Buettner, A., et al., 1997. Localization of the motor hand area to a knob on the precentral gyrus. A new landmark. Brain 120, 141–157.