Multimodal MRI Reveals Structural Connectivity Differences in 22q11 Deletion Syndrome Related to Impaired Spatial Working Memory

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Abstract: *Introduction:* Impaired spatial working memory is a core cognitive deficit observed in people with 22q11 Deletion syndrome (22q11DS) and has been suggested as a candidate endophenotype for schizophrenia. However, to date, the neuroanatomical mechanisms describing its structural and functional underpinnings in 22q11DS remain unclear. We quantitatively investigate the cognitive processes and associated neuroanatomy of spatial working memory in people with 22q11DS compared to matched controls. We examine whether there are significant between-group differences in spatial working memory using task related fMRI, Voxel based morphometry and white matter fiber tractography. *Materials and Methods:* Multimodal magnetic resonance imaging employing functional, diffusion and volumetric techniques were used to quantitatively assess the cognitive and neuroanatomical features of spatial working memory processes in 22q11DS. Twenty-six participants with genetically confirmed 22q11DS aged between 9 and 52 years and 26 controls aged between 8 and 46 years, matched for age, gender, and handedness were recruited. *Results:* People with 22q11DS have significant differences in spatial working memory functioning accompanied by a gray matter volume reduction in the right precuneus. Gray matter volume was significantly correlated with task performance scores in these areas. Tractography revealed extensive differences along fibers between task-related cortical activations with pronounced differences localized to interhemispheric commissural fibers within the parietal section of the corpus callosum. *Conclusions:* Abnormal spatial working memory in 22q11DS is associated with aberrant functional activity in conjunction with gray and white matter structural abnormalities. These anomalies in discrete brain regions may increase susceptibility to the development of psychiatric disorders such as schizophrenia. *Hum Brain Mapp* 37:4689–4705, 2016. © 2016 Wiley Periodicals, Inc.

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INTRODUCTION

Velo-cardio-facial syndrome or 22q11.2 deletion syndrome (22q11DS), one of the most common genetic syndromes, occurs in approximately 1 in 5,000 live births [Botto et al., 2003] and is associated with a microdeletion of chromosome 22q11 [Shaikh et al., 2000]. Although considerable phenotypic variability occurs, common clinical features include characteristic facial dysmorphology, cleft palate, congenital heart defects, and high rates of psychiatric disorders, particularly schizophrenia [Kalsi et al., 1999]. Indeed, aside from being the child of two parents with schizophrenia or the monozygotic twin of an affected individual, deletion of 22q11 represents the highest known risk factor for the development of schizophrenia [Murphy, 2002].

Several studies of people with 22q11DS have reported a range of neuropsychological deficits including specific deficits in spatial working memory (SWM) [Fleming et al., 1997; Heinrichs and Zakzanis, 1998; O'Connor et al., 2009]. Similarly, widespread neuropsychological deficits have also been reported in people with schizophrenia and their unaffected relatives [Heinrichs and Zakzanis, 1998; O'Connor et al., 2009]. Of these, impaired SWM features are thought to be one of the fundamental cognitive deficits in people with schizophrenia [Fleming et al., 1997; Park and Holzman, 1992; Tek et al., 2002]. In addition, as SWM deficits have also been observed in schizotypal personality disorder [Park and McTigue, 1997] and in the unaffected biological relatives of people with schizophrenia [O'Connor et al., 2009], SWM deficits are considered to be a candidate endophenotype for schizophrenia indicative of genetic liability to the disorder [Gottesman and Gould, 2003]. Although both 22q11DS and schizophrenia share SWM dysfunction, it is unclear whether deficits in SWM are associated with the subsequent development of schizophrenia in deleted individuals. Indeed, to date, very little is known about the neuroanatomical substrate of SWM dysfunction in people with 22q11DS.

Studies of the neural basis for SWM in the general population report that SWM tasks activate a cortical pathway

extending bilaterally from prefrontal cortex to anterior cingulate and parietal regions in both children and adults [Klingberg et al., 2002; Kwon et al., 2002; Thomas et al., 1999, 2009]. An fMRI study of SWM in eight children with 22q11DS, reported that controls activated parietal and occipital regions significantly more than those with 22q11DS but there was no significant between-group difference in dorso-lateral prefrontal cortex (DLPFC) [Azuma et al., 2009; Montojo et al., 2014]. However, group differences in DLPFC may not have been detected because of the small sample size. Indeed, very little is known about specific abnormalities in SWM neural networks in 22q11DS or schizophrenia [Kang et al., 2011]. Some studies have suggested that cortico-striatal hypo-functionality in SWM is expressed by greater inefficiency of the fronto-striatal response in people with schizophrenia [Callicott et al., 2000; Jansma et al., 2004] and in those at increased risk for the disorder [Callicott et al., 2003; Whitfield-Gabrieli et al., 2009]. More recently, Kang et al. [2011] reported that prefrontal-posterior functional connectivity associated with the maintenance and control of visual information is central to SWM, and that disruption of this functional network underlies SWM deficits in schizophrenia [Broome et al., 2010; Chua et al., 2007; Cocchi et al., 2009; Lee et al., 2008; Meyer-Lindenberg et al., 2001; Mitelman et al., 2005; Pantelis et al., 2003]. Yet, does the same pattern of SWM functional disruption exist in 22q11DS?

Numerous MRI studies of people with 22q11DS have reported global brain volumetric reductions in both gray and white matter (WM) with specific reductions in the cerebellum, hippocampus, temporal and parietal lobes and relatively preserved or enlarged frontal lobe volume [Jalbrzikowski et al., 2013; Kates et al., 2001; Tan et al., 2009; van Amelsvoort et al., 2001], some of which are areas utilized during SWM processes. Studies by van Amelsvoort et al. [2004a,b] reported that adults with 22q11DS and schizophrenia had significant global brain volumetric reductions in gray and white matter compared with 22q11deleted individuals without schizophrenia and healthy controls. They also suggested that frontal and temporal lobe volume reductions may be associated with the presence of psychotic symptoms. Thus, even though individuals with 22q11DS and those with schizophrenia share SWM dysfunction, the characteristics of this dysfunction may differ (in specific frontal and temporal regions for example), thus providing valuable insight regarding SWM in individuals with 22q11DS and those with schizophrenia. Hence, further studies are vital to better describe and clarify the underlying structural and functional neural circuitry of SWM in 22q11DS and thus facilitate further

Abbreviations

CSD	constrained spherical deconvolution
DTI	diffusion tensor imaging
FA	fractional anisotropy
MD	mean diffusivity
ROI	region of interest
SWM	spatial working memory

comparisons between people with a genetic vulnerability associated with increased risk for schizophrenia and those with the disorder.

In the current study, we employed a multimodal MRI approach to examine the neuroanatomical basis of spatial working memory deficits in people with 22q11DS by the assessment of functional activations and associated WM structural connectivity compared to healthy controls. In conjunction with SWM-associated cognitive activations, we utilized a region of interest (ROI) volumetric analysis localized at the activation sites to examine regional cortical GM volume to probe the structure/function relationship.

In addition, we used constrained spherical deconvolution (CSD) based tractography using the fMRI defined ROIs as seed regions to quantify the SWM associated white matter (WM) neural network of individuals with 22q11DS. We attempt to isolate the white matter tracts between the cortically active regions and suggest that a selective portion or number of the underlying white matter fibers form part of a structural connectivity network utilized and or specialized to facilitate the active network of cortical regions engaged in the performance of the SWM task. We identify this as the “WM SWM fiber bundle”. Visual inspection of the identified white matter connections between the fMRI activations, or the “WM SWM fiber bundle”, was performed. Informed by Wakana’s human white matter fiber tract atlas [Wakana et al., 2004], a subsequent and independent additional tractography analysis was performed using inclusion AND gate pairs to independently extract known fiber tracts that were identifiable and clearly present within the SWM bundle. Using these approaches, we tested the following hypotheses that compared to controls:

1. People with 22q11DS have significantly reduced activation of DLPFC, parietal, and occipital lobes during a SWM task.
2. People with 22q11DS have reduced volumes of DLPFC, parietal and occipital lobes in regions that show reduced activation during a SWM task.
3. People with 22q11DS have abnormal microstructural organization of WM fiber tracts linking brain activations utilized during SWM processes.
4. People with 22q11DS have abnormal microstructural organization of specific known WM fiber tracts identified within the SWM fiber bundle.

METHODS

Participants

Twenty-six individuals with 22q11DS and 26 controls, matched for age, gender, and handedness, were recruited from the National Centre for Medical Genetics and the 22q11 Ireland support group, 15 controls were siblings of

TABLE I. Demographic and clinical data

Measure	22q (N = 26)	Controls (N = 26)	P value
Age (years)			0.64
Mean (SD)	20.25 (10.8)	21.6 (9.8)	
Range (yrs)	10-52	8-46	
Gender (M/F)	11/15	12/14	0.785
Handedness (R/L)	22/4	22/4	0.99

the 22q11DS participants and the remaining 11 were selected from a generic control database accumulated by the School of Psychology, Trinity College Dublin.

Participants with 22q11DS had a confirmed deletion of chromosome 22q11 using fluorescence *in situ* hybridization (FISH) (Oncor, Gaithersburg, MD, USA). Participants with a history of brain trauma, substance abuse, contraindications to MRI, and under the age of 8 years, were excluded. Participants with the clinical phenotype of 22qDS but without the large 3 Mb 22q11.2 deletion, those with a clinically detectable medical disorder known to affect brain structure (e.g., epilepsy, or hypertension) and control participants with a history of neurological or psychiatric disorders were also excluded from the study (Table I).

Participants gave written informed consent or written parental consent in the case of children under the age of 18 years, in accordance with ethical approval from Beaumont Hospital Ethics committee, Dublin and the School of Psychology Ethics Committee, Trinity College Dublin.

MRI Scanning Parameters

Scanning was conducted on a Philips Achieva 3.0 T scanner equipped with an eight channel head coil with mounted mirror to view the display projected onto a 640 × 480 panel placed behind the subjects’ head, outside the magnet. Prior to scanning, participants underwent computerized training sessions including fMRI task familiarization, and acclimatisation in a mock scanner. The image protocol commenced with clinical/structural scans, followed by diffusion imaging and ended with the fMRI task. The anatomical scan acquired 180 axial high-resolution T1-weighted anatomic SPGR images (TE = 3.8 ms, TR = 8.4 ms, FOV 230 mm, 0.898 × 0.898 mm² inplane resolution, slice thickness 0.9 mm, flip angle $\alpha = 8^\circ$). Diffusion weighted images were acquired using spin-echo echo-planar imaging (TE = 52 ms, TR = 11,260 ms, flip angle $\alpha = 90^\circ$), FOV 224 mm, 60 axial slices 1.75 × 1.75 mm² inplane resolution, slice thickness 2 mm, *b* value = 1,500 s mm⁻² in 61 noncollinear directions, preceded by a nondiffusion-weighted volume (*b*₀ image). The fMRI scanning protocol comprises 32 noncontiguous (10% gap) 3.85 mm axial slices covering the entire brain collected using a T2* weighted echo-planar imaging sequence (TE = 35 ms, TR = 2,000 ms, FOV 220 mm, 80 × 80 mm matrix size in-plane resolution).

fMRI Spatial Working Memory Task

The SWM task was adapted from a paradigm described elsewhere [Jonides et al., 1993]. In brief, participants viewed stimuli of varying degrees of complexity ("1Dot", "3Dot" and "No delay") followed after a brief interval by a second stimulus of a red circle on a black background and required participants to determine if the spatial location of the dots and red circle matched. The "No Delay" condition presented the dot and circle simultaneously and required the participant to indicate if the spatial location of the objects matched. The task consisted of 24 trials for each condition (presented in random order) with half of each being a match (refer to Supporting Information Fig. 1 for details).

Image Processing

Volumetric data analysis at the functionally defined SWM task dependent cortical activation locations were obtained using a gray matter tissue [voxel-based morphometry (VBM)] strategy performed with FSL_VBM (www.fmrib.ox.ac.uk/fsl) [Ashburner and Friston, 2000; Good et al., 2001; Smith et al., 2004]. Diffusion data analysis was performed using ExploreDTI software (<http://www.exploredti.com/>). Functional MRI were analyzed using AFNI software tools (<http://afni.nimh.nih.gov>).

Detailed descriptions of each component of the multimodal analysis are found in the subsequent paragraphs. Briefly, an overview of our strategy consisted of determining fMRI task related group differences in response to the SWM paradigm, using these regions as mask regions to determine the WM fibers between these regions of cortical activation, constructing the SWM fiber bundle via CSD tractography [Jeurissen et al., 2011] and performing systematic analysis on the descriptive diffusion measures for the SWM fiber bundle. A secondary additional tractography analysis derived from the visual inspection of the white matter SWM fiber bundle, was used to identify known white matter tracts within the SWM fiber bundle. Anatomical placements of robust inclusion AND gate pairs and exclusion NOT gates were used for systematic tract delineation and diffusion metric extraction for investigation. A population atlas based tractography approach [Emsell et al., 2013; Lebel and Beaulieu, 2011; Van Hecke et al., 2008] was then employed to accurately select the left and right superior longitudinal fasciculus (SLF), the cerebellar peduncle, the genu of the corpus callosum, and the posterior parietal section of the corpus callosum, independently for investigation. Similar methods have been used previously to subdivide other tracts, such as cingulum, corpus callosum, and corticospinal tract [Emsell et al., 2013; Jones et al., 2013; Szczepankiewicz et al., 2013].

In addition, an ROI analysis was performed at each predetermined fMRI SWM cluster and GM volumes were extracted per ROI. Pearson's partial correlation analysis (with age correction) was performed to assess the

relationship between task performance scores, functional activation levels, and GM volume per cluster.

fMRI Analysis

The fMRI dataset consisted of a balanced design of 26 people with 22q11DS and 26 matched controls. A block analysis was performed to estimate the activation for the "1DOT" and "3DOT" conditions separately. The on-off block regressors were convolved with a standard hemodynamic response to accommodate the lag time of the blood oxygen level-dependent (BOLD) response. Following image reconstruction, motion correction was performed using 3-D volume registration (least-squares alignment of three translational and three rotational parameters) and edge detected. Multiple regression analysis was then used to determine the average level of block activation relative to the "No Delay" condition (baseline). The percentage change map (block activation) voxels were resampled at 1 mm³ resolution, warped into standard Talairach space and spatially blurred with a 3-mm isotropic rms Gaussian kernel. Separate 1DOT and 3DOT group differences activation maps were determined with voxelwise *t* tests. Significant voxels passed a voxelwise statistical threshold ($t = 2.94$, $P < 0.005$, $N = 26$ controls and 26 individuals with 22q11DS) and were required to be part of a larger 286 mm³ cluster of contiguous significant voxels. Thresholding was determined through 5000 Monte Carlo simulations and resulted in a 5% probability of a cluster surviving due to chance and corrected for multiple comparisons. Each between-group voxelwise comparison was corrected for age effects using age as a covariate. Finally, the between-group thresholded activation maps for each condition (1DOT and 3DOT), were combined to produce a single composite "OR map" that included all significant between-group voxels of activation in any of the constituent thresholded condition maps. The mean activation level per cluster in this combined map was extracted for each task condition. A one-way multivariate analysis of covariance (MANCOVA with age as covariate) was conducted for 1DOT and 3DOT conditions independently with mean fMRI condition activations per cluster as the dependent variables and group as the fixed factor. Correction for multiple comparisons was applied using Bonferroni correction at $0.05/12 = 0.004$ in SPSS 18.

Gray Matter ROI Volumetrics

Structural T1 data was analyzed with FSL-VBM [Douaud et al., 2007], an optimized VBM protocol [Ashburner and Friston, 2000] and [Good et al., 2001] carried out with FSL tools [Smith et al., 2004]. First, structural images were brain-extracted using the FSL brain extraction tool (BET) featuring the options of bias field correction (*B*), robust brain extraction (*R*) providing more robust brain centre estimations, and finally eye and optic nerve clean

up (S). Next, all brain-extracted images were segmented into gray (GM), white (WM), and CSF partitions. The gray matter-segmented images were averaged and flipped along the x axis to create a left-right symmetric, study-specific gray matter template before being transformed to MNI 152 standard space using FSL's nonlinear registration tool, FNIRT [Andersson et al., 2007b]. Nonlinear registration rather than affine registration is recommended where the data features different populations (clinical and control cohorts and in this case across a large age range), not typically captured in the adult based MNI 152 standard template formation. The registered images were then "modulated" [multiplied by the Jacobian of the warp field as outlined in Good et al., 2001] to correct for local expansion (or contraction) due to the nonlinear component of the spatial transformation. Finally, the gray matter images were smoothed with an isotropic Gaussian kernel of 3 sigma, ~ 8 mm FWHM. The fMRI OR activation mask (in MNI 152 standard space) was registered to the FSL_VBM gray matter structural data (GM_mod_merge_s3) using the standard FSL registration tools FLIRT and FNIRT. The alignment of the functional ROIs was inspected and subsequently, subject specific gray matter volume measures at the 12 functionally defined ROI locations were extracted and entered into a MANCOVA analysis (with age as a covariate) with an adjusted Bonferroni alpha level of 0.004. The reader is advised that only the GM ROI measures are included in this study, to provide gray matter quantifications at the regions predefined by the SWM task. The structural whole brain analysis forms part of on-going independent analyses in our lab.

Whole-Brain Diffusion Data Preprocessing

Whole brain high angular resolution diffusion imaging (HARDI) data were analyzed using the diffusion toolbox "ExploreDTI" [Leemans et al., 2009] featuring subject motion and distortion correction including B-matrix rotation [Leemans and Jones, 2009]. The tensor model was fitted to the data using the Robust Estimation off Tensors by Outlier Rejection (RESTORE) approach [Chang et al., 2005] which uses a process of iteratively reweighted least-square regression for outlier identification and subsequent removal to minimize artefacts (e.g., artefacts associated with cardiac pulsation and subject motion). Whole-brain white matter tracts were reconstructed using Constrained Spherical Deconvolution (CSD) based tractography. In brief, CSD was used to extract the fiber orientation distribution (FOD) from the diffusion signal in each voxel. Seed point resolution for this whole-brain construction was set at $2 \times 2 \times 2$ mm. For each step during the tract propagation, the FOD peak direction was extracted and the trajectory was advanced with a 1 mm fixed step size along the identified peak direction. Tracking was terminated when the FOD peak magnitude fell below a fixed threshold (0.2), or when an angle threshold of 30° was exceeded, thus limiting

unrealistic bends in fiber orientation and prevent the tract from doubling back on itself. From this whole brain tractography result, the subsequent pathways of interest were selected using fMRI defined ROIs or inclusion AND gate ROI pairs.

To facilitate the multimodal component of the analysis (fMRI combined with WM tractography), the motion and distortion corrected diffusion images were aligned with the fMRI mask of significant clusters (in MNI 152 standard space) using the FLIRT and FNIRT registration tools within the FSL toolbox [Andersson et al., 2007a; Jenkinson et al., 2002; Jenkinson and Smith, 2001]. The aligned mask of significant fMRI clusters was subsequently transformed back to each individuals native diffusion space diffusion image for the purpose of SWM WM CSD based tractography.

WM Tractography

The fMRI SWM activation ROIs aligned to the native space diffusion images were used as composite seed regions to extract the associated network of white matter fibers linking the fMRI ROIs using constrained spherical deconvolution (CSD) [Jeurissen et al., 2011] based deterministic (streamline) tractography in diffusion native space.

White Matter SWM Tract Based Analysis in 22q11DS

Group differences for each independent tract measure (included macroscopic measures for fiber bundle volume, mean tract length, the total number of tracts in the fiber bundle and microscopic diffusion measures across the SWM fiber bundle for mean FA, mean diffusivity (MD), axial diffusivity (AD), radial diffusivity (RD), λ_2 and λ_3 the second and third eigenvalues of the tensor respectively, and Westin measures [Westin et al., 2002] linear (C_L), planar (C_P), and spherical diffusion coefficients (C_S) were examined using independent t tests. The Westin measures C_L , C_P , and C_S are alternative measures of diffusion anisotropy that were used to provide a more specific interpretation of the shape of the diffusion ellipsoid (linear, planar, or spherical respectively). These metrics can be beneficial when interpreted with associated FA differences and help clarify the anatomical features of the underlying tissue [Westin et al., 2002]. Additionally, MANCOVA (with diffusion measures as dependent variables, group as the independent grouping variable and age entered as a covariate) was used to control for age effects and Bonferroni correction was applied at $P = 0.004$ (0.05/12).

WM SWM Derived Secondary Tractography of Known Tract Formations

Following visual inspection of the WM SWM fiber bundle, robust anatomical placements of two inclusion AND gates, where a fiber tract streamline had to pass through

both AND gates to be included in the tract formation, in conjunction with exclusion NOT gates to restrict spurious and unwanted fibers, were used for systematic tract delineation. A population atlas based tractography approach [Emsell et al., 2013; Lebel and Beaulieu, 2011; Van Hecke et al., 2008] was then employed to accurately select the left and right superior longitudinal fasciculus (SLF), the cerebellar peduncle, the genu of the corpus, and the posterior parietal section of the corpus callosum, independently for investigation. Diffusion metrics were extracted for systematic statistical analyses.

Correlation Analysis

The relationship between 3DOT performance scores and (1) fMRI "OR map" ROIs with extracted 3DOT mean activation levels, (2) GM volume per ROI, (3) diffusion metrics from the extracted SWM WM fiber bundle (number of tracts, tract bundle volume, tract bundle length, FA, MD, AD, λ_2 , λ_3 , RD, C_L , C_P , C_S), (4) diffusion metrics from the observed identified known tract formations within the SWM WM fiber bundle namely the parietal region of the corpus callosum, the genu of the corpus callosum, the cerebellar peduncle and bilateral SLF tracts (number of tracts, tract bundle volume, tract bundle length, FA, MD, AD, RD, C_L , C_P , C_S) were assessed independently using Pearson's partial correlation analysis (with age correction). A reduced alpha level of 0.05/12 fMRI ROIs = 0.004 for the fMRI "OR" map ROIs and $P = 0.05/12$ diffusion measures = 0.004 for the tract based diffusion metrics respectively, were adopted to account for the multiple tests performed.

In all analyses, the data was systematically examined for the presence of extreme and outlier data points. Identified outliers were removed prior to statistical testing to ensure all data fell within the 95% confidence interval of the mean values. Data outliers are shown on the boxplot representations for each diffusion group specific mean measure.

RESULTS

Demographics

There was no significant group difference for age, gender, or handedness between the 22q11DS and their matched control group (Table I).

fMRI Task Performance

Performance scores for the three conditions of the SWM task revealed "mean% correct" scores for controls of 94, 85, and 92% and 22q 11.2 DS of 73, 56, and 59% for 1DOT, 3DOT, and no delay respectively. ANCOVA per condition revealed significant between-group differences of $P \leq 0.0008$ for 1DOT, $P \leq 0.00001$ for 3DOT and $P \leq 0.00003$

Figure 1.

Spatial working memory performance scores detailing the three conditions (1DOT, 3DOT, and No Delay) for controls and 22q11DS individuals. Significant between-group differences are indicated by asterisks.

for No Delay, with age as a covariate and corrected for multiple comparisons applying Bonferroni correction at $P = 0.05/3$ ($P = 0.017$) (Fig. 1).

fMRI SWM Analysis

Significant between-group differences were observed in nine regions for the 3DOT task condition with 22q11DS participants showing reduced activation levels in the bilateral inferior parietal, right middle frontal, left superior frontal gyrus and right supramarginal regions. Greater mean activations were observed bilaterally in the superior frontal gyrus and the right cuneus (Supporting Information Table I). Eight significant regions of between-group differences were identified for the 1DOT version with 22q11DS showing reduced mean activations bilaterally in the precuneus, the right inferior parietal lobule, and the left cuneus/lingual gyrus and greater activations in the left superior frontal gyrus and the left temporal gyrus (Supporting Information Table II). The composite OR map included all significant clusters from both constituent condition maps and resulted in 12 ROIs (Supporting Information Table II). A one-way MANCOVA (with age as a covariate), with the 12 ROIs as the dependent variables and group as the independent variable, identified a significant difference for group on the combined dependent variables, $F(12,22) = 3.97$, $P = 0.002$; Wilks' Lambda = 0.32; partial eta squared = 0.68. *Post hoc* univariate tests revealed significant between group differences. The 22q11DS group had reduced 3DOT activations in six areas including bilateral inferior parietal, right superior parietal, right middle frontal, and the right precuneus. In contrast, they showed greater activations in four areas including bilateral superior frontal gyrus, right cuneus/lingual gyrus and left inferior or temporal gyrus (Table II). These four regions revealed negative BOLD signals for controls in contrast to positive BOLD activations for the 22q11DS group, possibly suggesting hyper-activation or a failure to deactivate in the patient group. Similar patterns of effect were observed

TABLE II. fMRI SWM defined “or map” (3DOT activation levels per ROI)

ROI	Size (mm ³)	Talairach X Y Z	Location (gyrus/lobe)	fMRI 3DOT (P value)	GM vol. (P value)	DWI metric (P value, r)				
						Tracts	Volume	AD	RD	C _P
1	3286	38, -37, 39	Right inferior parietal BA 40	0.0002 ↓ *	NS					
2	1313	26, -6, 54	Right middle frontal BA6	0.0009 ↓ +	0.05 ↓ +					
3	1169	-29, -46, 39	Left inferior parietal	0.0007 ↓ *	NS					
4	857	-21, -95, -3	Left lingual BA18	0.01 ↑ -	0.04 ↓ +					
5	639	-16, 37, 49	Left superior frontal/BA8	0.002 ↑ -	0.03 ↑ -					
6	577	22, -98, -2	Right cuneus/lingual BA18	0.01 ↑ -	NS					
7	544	27, -50, 28	Right precuneus	0.004 ↓ +	0.01 ↓ +	0.038-0.3	0.042 - 0.3			
8	474	16, 63, 59	Left superior parietal BA7	0.0005 ↓ *	NS					
9	472	19, -55, 43	Right precuneus BA7	0.01 ↓ +	0.0005 ↓ +					
10	413	19, 62, 9	Right superior frontal BA10	0.00005 ↑ -	NS			0.03 - 0.3		
11	367	-48, -8, -30	Left inferior temporal BA20	0.001 ↑ -	NS			0.033 - 0.3		0.037 - 0.3
12	354	-35, -36, -10	Right middle frontal BA11	0.006 ↓ +	NS	0.01 + 0.36	0.036 - 0.3		0.016 - +0.34	

Functionally defined “OR” map clusters with mean 3DOT activations per ROI. Arrows indicate the effect direction relative to controls. Bonferroni correction applied at $P = 0.004$. The bold text indicates survives Bonferroni correction. NS = not significant. Significant Pearson’s partial correlations (age corrected) are identified with an asterisk. The direction of correlation is indicated by “+” meaning positive correlation and “-” meaning negative correlation.

for the 1DOT activation levels (Supporting Information Table III).

Correlation Analysis (Performance Scores Versus fMRI Activations)

Pearson’s partial correlation analysis (with age correction) between 3DOT performance scores and 3DOT fMRI mean activation levels for each of the 12 ROIs revealed positive correlations in the ROI’s with greater mean activations in controls. Negative correlations were identified in the ROI’s with greater mean fMRI activation levels in the 22q11DS group. Three regions showed a significant positive correlation with Bonferroni correction applied at $P \leq 0.004$, (Table II). The 1DOT performance versus fMRI activations showed a similar pattern (Supporting Information Table III). Again, the direction of correlation followed the direction of the fMRI activations as indicated above.

ROI Volumetrics (SWM Defined ROIs)

A one-way MANCOVA (with age as a covariate), with the 12 functionally defined ROIs as the dependent variables and group as the independent variable identified a significant difference for group on the combined dependent variables, $F(12, 22) = 3.97$, $P = 0.002$; Wilks’ Lambda = 0.32; partial eta squared = 0.68. *Post hoc* univariate tests revealed GM volume reductions in the 22q group in four regions, namely the right middle frontal gyrus ($P = 0.05$), left lingual gyrus ($P = 0.04$), and two regions in right precuneus ($P = 0.01$ and $P = 0.0005$ respectively). Only one region survived Bonferroni correction at 0.004, namely the right precuneus. GM ROI volume increases were found for the 22q11DS group in one region, the left superior frontal gyrus ($P = 0.03$) (Table II and Fig. 2), but failed to survive Bonferroni correction ($P = 0.004$).

Correlation Analysis (Performance Scores Versus GM ROI Volumes)

Pearson’s partial correlation analysis identified a positive correlation between 3DOT performance scores and GM ROI volume in the right precuneus ($r = 0.41$, $P < 0.007$) (Table II). No correlation was found between mean fMRI activation levels and GM volume within each cluster.

White Matter SWM Fiber Bundle Tract Measures

Results from WM SWM associated tract analyses are presented in Table III and Figures 3 and 4 detailing the white matter fiber connections linking the functionally defined activation ROIs. Independent t tests of the 12 diffusion measures investigated, AD and C_L failed to show

Figure 2.

Functionally defined composite “OR” map determined using a Boolean OR operation to combine the two condition activation maps. Detailed are the ROIs showing significant gray matter volume differences in (a) right middle frontal gyrus ($xyz = 26 -6 54$), (b) right precuneus ($xyz = 27 -50 28$), (c) right precuneus ($xyz = 19 -55 43$), and (e) left superior frontal gyrus ($xyz = -16 37 49$). * indicates $P \leq 0.05$. [Color figure can be viewed at wileyonlinelibrary.com]

TABLE III. SWM WM network tract measures

Fiber bundle measure	<i>t</i> test (<i>P</i> value)	ANCOVA (<i>P</i> value)	3DOT performance Pearson's partial correlation (<i>P</i> value, <i>r</i> , age corrected)
No. of tracts	0.023	0.029	NS
Mean tract volume	0.004	0.002*	0.014, 0.356
Mean FA	0.047	0.017	0.037, -0.305
Mean diffusivity	0.003*	0.001*	NS
Mean tract length	0.046	0.046	NS
Mean AD (λ_1)	0.06	0.031	NS
Mean λ_2	0.005	0.004*	0.048, 0.294
Mean λ_3	0.001*	0.002*	NS
Mean RD ($\lambda_{\perp}$)	0.002*	0.001*	0.048, -0.258
CL	0.56	0.37	NS
C_P	0.003*	0.001*	NS
C_S	0.023	0.007	0.04, 0.3

Diffusion measures for the "SWM defined" fiber bundle as determined by independent *t* tests and corresponding *post hoc* pairwise ANCOVA (with age as a covariate) comparisons for the same fiber bundle. Pearson's partial correlation (age corrected) between each tract measure and 3DOT performance scores are given with *P* values and corresponding *r* values respectively. The asterisk indicates survives Bonferroni correction at *P* = 0.004. NS = not significant.

significant between-group differences (*P* = 0.05). MANCOVA (with age as a covariate), including the 12 diffusion measures as the dependent variables and group as the independent variable, identified a significant difference for group on the combined dependent variables, $F(11,35) = 2.83$, *P* = 0.009; Wilks' $\lambda = 0.53$; partial eta squared = 0.47. *Post hoc* independent univariate tests (with age as a covariate) revealed significant between group differences in six of the measures, using a Bonferroni adjusted alpha level of 0.004, namely, MD (*P* = 0.001), λ_2 (*P* = 0.004), λ_3 (*P* = 0.002), and RD (*P* = 0.001), C_P (*P* = 0.001), and tract volume (*P* = 0.002) with C_S showing a strong trend towards significance at *P* = 0.007 (Table III). Significant age effects were not identified for any diffusion measure within the WM SWM fiber bundle.

WM SWM Derived Secondary Tractography of Known Tract Formations

The main fiber tracts identified within the visualized WM SWM derived fiber bundle were, the parietal region of the corpus callosum, the genu of the corpus callosum, the cerebellar peduncle and bilateral SLF tracts. Representative fiber tracts are shown in Figure 5a and b. ANCOVA (with age as a covariate), for each diffusion metric below *P* = 0.05 threshold, are detailed for each fiber tract independently. Bonferroni correction was applied at *P* = 0.004 for each tract to account for multiple comparison corrections. Of the tracts investigated, the posterior (parietal) section of the corpus callosum, showed the greatest between-group differences with seven diffusion metrics surviving stringent Bonferroni's correction. Of particular note, increased FA accompanied by significant planar and trend level (0.005) spherical shape characteristics in the 22q group were identified indicating reduced fiber

complexity and or reduced crossing fibers in this region (see Fig. 5a).

Figure 3.

A representation of the fiber tracts connecting regions activated by the SWM task (shown in red). Tracts rendered with increased transparency and subsampled for clarity are displayed on the typical DWI FA background. All diffusion metrics were extracted for the entire fiber tract bundle and represent the mean values for each measure over the tract bundle. [Color figure can be viewed at wileyonlinelibrary.com]

Figure 4.

Macrostructural measures (a) the number of tracts within the SWM bundle (b) mean volume of the bundle, (c) mean tract length and microstructural measures (d) FA, (e) MD, (f) AD, (g) λ_2 , (h) λ_3 , (i) RD $[(\lambda_2 + \lambda_3)/2]$, (j) C_L , (k) C_B (l) C_S for the SWM fiber bundle. Asterisks indicates survives Bonferroni correction at $P = 0.004$. Charts detail MANCOVA (with age as a covariate) post hoc UNIVARIATE analyses (age corrected).

Figure 5.

(a) A typical representation of (i) the commissural posterior parietal fiber tracts of the corpus callosum, (ii) the cerebellar peduncle, and (iii) the genu of the corpus callosum. Boxplots detail between group differences at $P = 0.05$ and where applicable, the outlier values are shown. UNIVARIATE analyses (age corrected) P values are reported on each boxplot and asterisks. Asterisk indicates that the diffusion measure survives Bonferroni correction applied at $P = 0.004$. (b) (iv) Left SLF and (v) right

SLF respectively and associated extracted diffusion metrics. Boxplots detail between group differences at $P = 0.05$ and where applicable, the outlier values are shown. UNIVARIATE analyses (age corrected) P values are reported on each boxplot and asterisks * indicates that the diffusion measure survives Bonferroni correction applied at $P = 0.004$. [Color figure can be viewed at wileyonlinelibrary.com]

Correlation Analysis (Performance Scores Versus WM SWM Fiber Tract Metrics)

Pearson's partial correlation analysis identified positive correlations between 3DOT performance scores and WM fiber tract bundle metrics for tract bundle WM volume ($r = 0.356$, $P = 0.014$), and C_S ($r = 0.3$, $P = 0.04$), and negative correlations for FA ($r = -0.305$, $P = 0.037$) and RD ($r = 0.293$, $P = 0.048$). When Bonferroni correction was applied at $P = 0.05/12 = 0.004$, none of the correlation remained significant (Table III).

Correlation Analysis (Performance Scores Versus Tractography of Known Tract Formations within the WM SWM Fiber Bundle)

Pearson's partial correlation analysis identified highly significant Bonferroni corrected positive correlations between 3DOT performance scores and the posterior parietal corpus callosum tract bundle metrics for C_S ($r = 0.59$, $P = 0.00002$), and negative correlations for FA ($r = -0.56$, $P = 0.00006$). All other identified tracts failed to identify any significant correlation that survived Bonferroni Correction applied at $P = 0.004$.

Correlation Analysis (fMRI Activations Versus WM SWM Fiber Tract Metrics)

Finally, to further utilize the multimodal component of this study, we performed Pearson's partial correlation analysis between the fMRI-determined activation ROIs and the 12 diffusion metrics extracted from the WM SWM fiber bundle. Positive correlations between 3DOT activation measures and the number of fiber tracts within the tract bundle ($r = 0.360$, $P = 0.01$) and tract bundle WM volume ($r = 0.36$, $P = 0.01$) were identified and negative correlations for C_S ($r = -0.296$, $P = 0.037$) in ROI 12 (right middle frontal gyrus). ROI11 (Left Inferior temporal gyrus) showed positive correlation for C_P ($r = 0.341$, $P = 0.016$) and negative correlations for tract bundle WM volume ($r = -0.36$, $P = 0.01$) and RD ($r = -0.302$, $P = 0.033$). ROI 10 (right superior frontal gyrus) identified a negative correlation between AD and fMRI 3DOT activity ($r = -0.31$, $P = 0.03$). Finally ROI6 (right cuneus/lingual gyrus) identified negative correlations between 3DOT fMRI mean activity and the number of fiber tracts within the tract bundle ($r = -0.296$, $P = 0.038$) and tract bundle WM volume ($r = -0.289$, $P = 0.042$). When Bonferroni's correction was applied at $P = 0.05/12 = 0.004$, none of these remained significant (Table II).

DISCUSSION

The present study employs a novel multimodal MRI approach to specifically examine the structural and functional underpinnings of spatial working memory in

individuals with 22q11DS compared to healthy matched controls rather than examining a range of cognitive or behavioral measures. We identify significant differences in SWM functioning (via fMRI) and show that the mean activations located at these ROIs correlate with task performance scores. In addition, we show reduced GM volume in two of these functionally defined regions and that GM volume correlated positively with task performance scores. Finally, we detail significant WM differences within the network of fiber tracts associated with the functionally defined SWM cortical activations. These findings clarify the relationship between the underlying white matter microstructure and cognitive differences associated with the characteristic SWM performance deficits observed in 22q11DS and are of interest as these regions are also implicated in the pathophysiology of schizophrenia.

Our analysis strategy combining SWM BOLD activations and regional GM volumetrics clearly identifies group specific differences for both measures. GM ROI analyses at the SWM cortically active regions revealed reduced GM volume in right precuneus, right superior and right middle frontal regions in 22q11DS individuals, all key regions identified in the body of schizophrenia literature and possibly indicating increased vulnerability in people with 22q11DS. The precuneus is known to be involved in episodic memory and retrieval processes [Fernandes et al., 2005] and spatial location encoding, key components of spatial working memory processing, shown to be a highly sensitive potential marker for schizophrenia. We suggest that the precuneus may be a brain region specifically targeted and influenced during the developmental trajectories of people with 22q11DS, similar to individuals who develop schizophrenia. More specifically, a failure of the precuneus to deactivate in first episode psychosis [Guerrero-Pedraza et al., 2012], gray and white matter morphology being predictive of poor symptom relabeling ability and poor insight [Antonius et al., 2011; Cooke et al., 2008; Morgan et al., 2010] and its involvement in "monitoring the world around us" [Gusnard et al., 2001] have all been reported. This evidence together with the characteristic GM morphologies shown to play a role in the transition to psychosis [du Boisgueheneuc et al., 2006; Kikinis et al., 2010; Pridmore and Bowe, 2011], suggest that the precuneus's role in SWM dysfunction is central to and possibly shared between individuals with 22q11DS and people with schizophrenia.

Our tractography findings are intriguing in this respect. The WM SWM fiber bundle demonstrated microstructural white matter differences and revealed that an extensive proportion of this network of fibers are localized to the parietal region. The secondary tractography analyses detailed highly significant fiber tract differences in the commissural parietal tract of the corpus callosum. These interhemispheric connections allow transfer of information between the two sides of the brain and facilitate functional integration of motor, perceptual, and cognitive functions

as utilized in episodic memory, retrieval, and spatial location and memory encoding processes. Our findings identify these fiber tracts with the most pronounced differences of the tracts investigated in this study and are further supported by the significant correlations between SWM performance scores and extracted FA and C_s measures along these fibers. These commissural pathways appear to be particularly targeted in individuals with 22q11DS compared to controls and could partly explain the anatomical underpinnings of the characteristic SWM deficits associated with the syndrome. It is possible that increased callosal FA may be related to reduced WM complexity in the 22q11DS group with less organization and crossing of fibers.

SWM and Underlying White Matter Connectivity

We hypothesized that 22q11DS individuals may demonstrate structural changes extending to WM regions and evident in the fiber tracts utilized during SWM processing. Such changes may prove sensitive to regions associated with the increased risk of schizophrenia in this population. Our findings show a distinct pattern of WM tissue disorganization in associated SWM processing regions, detailed by numerous diffusion metrics that suggest a divergence in the white matter developmental trajectories of individuals with 22q11DS. In the broader context, these findings are consistent with previous studies, which clearly demonstrate the sensitivity of SWM and associated white matter changes in schizophrenia, even in clinically high-risk teenager samples [Cocchi et al., 2009; Lee et al., 2008; Simon et al., 2007; Smith et al., 2006; Wood et al., 2003]. White matter alterations in adult schizophrenia typically present as FA decreases [Clark et al., 2011; Hoptman et al., 2008; Kubicki et al., 2005; Lee et al., 2008; Mitelman et al., 2006; Peters et al., 2008; Schlosser et al., 2007] although increased FA has been reported in young ‘at-risk’ and ‘early onset’ samples with substance misuse [Bava et al., 2009; De Bellis et al., 2008; Peters et al., 2009] suggesting the directionality of FA may reflect the presence of vulnerability or disease state. We tentatively suggest that SWM FA increases may appear during early formative developmental stages in 22q11DS and render these individuals with increased vulnerability to schizophrenia. It has also been reported that regions of decreased white matter complexity identified via increased FA can arise due to the presence of crossing fibers. Thus, the identified FA increase may be suggestive of WM disorganisation and less crossing fibers.

Age Related White Matter Change and Diffusion Metrics

Previous studies suggest that individuals with 22q11DS demonstrate an atypical developmental trajectory [Simon, 2007]. Normative studies of age effects on diffusion measures such as FA and MD are consistently reported in the

literature [Barnea-Goraly et al., 2005; Giorgio et al., 2008; Qiu et al., 2008; Tamnes et al., 2010; Westlye et al., 2010; Zhang et al., 2005] with FA increasing and MD decreasing during the phase of brain maturation [Tamnes et al., 2010]. Our findings show increased FA in 22q11DS across the entire age sample of this cohort with no significant areas of decreased FA being identified in the modelled SWM network and again indicating a divergent developmental trajectory in 22q11DS. Increased FA suggested as impaired WM fiber branching in ADHD [Davenport et al., 2010], may also be a contributing factor to these observed FA increases due to the high incidence of comorbidity with 22q11DS.

Strengths and Limitations

The age range of our sample is undeniably large but unavoidable due to the small pool of people with 22q11 available, thus restricting the possibility of statistical comparisons based on closely matched distinct age ranges limiting statistical power. For this reason, every effort was made to systematically examine the data and remove any identified outliers thus minimising potential sources of associated variability and ensuring the data conformed to a 95% confidence interval around the mean of each measure explored prior to statistical analysis. In addition, age effects were controlled for in each statistical test thus minimising age-associated variability.

Similar patterns of hemispheric BOLD activations during spatial working memory have been reported between adults and children [Casey et al., 2000; Thomas et al., 1999; Thomason et al., 2009]. Children demonstrate a lack of maturity in the ability to apply greater cognitive load with increased SWM complexity, thus the SWM paradigm used, limited the complexity of task to exclude conditions with high cognitive load and difficulty thus minimising the potential age related differences associated with fMRI task difficulty. The neural substrate of SWM processing prior to puberty has been reported as similar to that observed in adults at appropriate levels of cognitive burden [Nelson et al., 2000], thus the reliability of the SWM fMRI activation patterns appropriately minimize age related variations of our sample.

Acquisition and Methodological Considerations

Data acquisition parameters must be carefully considered to facilitate direct comparisons to other studies. The 61 gradient direction 3T diffusion dataset featured, allows high angular resolution diffusion imaging (HARDI) and CSD based tractography, as DTI tensor model cannot routinely model complex white matter architecture in regions of multiple fiber orientations [Jones, 2011; Tournier et al., 2011] thus providing a more accurate representation of the underlying WM cytoarchitecture. Our methodology routinely employed B-matrix rotation correction and robust

estimation of tensors by outlier rejection, to minimize registration inaccuracies [Chang et al., 2005; Leemans and Jones, 2009] and known to influence FA values. Decreased and increased FA differences have been reported in the 22q11DS literature [Barnea-Goraly et al., 2005; da Silva Alves et al., 2011; Jalbrzikowski et al., 2014; Sundram et al., 2010; Villalon-Reina et al., 2013] yet we identify regions of significantly increased FA only. This study focused specifically on SWM and hence the reported FA measures relate to averaged values across the modelled SWM fiber bundle only and not whole brain voxelwise measures or variations along a fiber tract and hence could mask localized regions of reduced FA within the SWM fiber bundle, a pattern previously observed along the uncinate and inferior fronto-occipital fasciculi in Williams syndrome [Arlinghaus et al., 2011]. The use of HARDI based CSD tractography, which better model more complex crossing fiber orientations than its DTI counterpart, together with the inclusion of Westin diffusion metrics used here, provide greater clarity to the features of the underlying WM tissue. Increased FA, as identified in our 22q11 group, may suggest reduced white matter complexity as regions with multiple crossing fibers can decrease FA as observed in our control sample compared to the 22q11 cohort. When considered with the associated significantly increased C_p and decreased C_s , relative to controls, suggesting a more planar disc shaped diffusion ellipsoid, typically caused by two crossing fiber groups whereas the increased C_s with associated reduced FA in controls is more characteristic of more complex multiple crossing fiber orientations.

In conclusion, the present results identified cognitive, cortical and subcortical SWM associated differences in 22q11DS. We provide further evidence showing reduced WM microstructural complexity [Jbabdi et al., 2010] and compromised or atypical network connectivity via sophisticated HARDI based CSD tractography when compared to controls. These targeted regions may render 22q11DS individuals more susceptible to the development of schizophrenia but further research is needed to clarify the role of SWM in the formative mechanisms related to schizophrenia in both the general population and individuals with 22q11DS.

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