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Research report

# Evidence for alterations of the right inferior and superior longitudinal fasciculi in patients with social anxiety disorder

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ABSTRACT

The aim of our study was to detect white matter (WM) regions being involved in the pathophysiology of SAD. We applied diffusion tensor imaging in 22 consecutive adult patients (11 women and 11 men) with SAD and 22 age and sex-matched healthy control subjects. We examined white matter (WM) alterations between the patients with social anxiety disorder (SAD) and healthy controls by a whole-brain analysis. We found that fractional anisotropy (FA) was reduced in patients with SAD compared with controls in the temporal part of right inferior longitudinal fasciculus (ILF) and the occipito-temporal part of the right superior longitudinal fasciculus (SLF). We also identified that in these regions FA was negatively correlated with the severity of anxiety. Our results suggest that the lateral temporal and occipito-temporal WM microstructure plays a role in mediating social interactions, and a pattern of WM abnormality in the right ILF and SLF may be implicated in the pathophysiology of SAD plausibly through leading to deficits in face processing mechanisms.

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## 1. Introduction

Social anxiety disorder (SAD) is a common anxiety disorder characterized by marked fear or anxiety of social situations, in which the individual may be scrutinized or negatively evaluated by others (American Psychiatric Association, 1994).

Neuroimaging studies using positron emission tomography (PET) have also reported increased blood flow in the amygdala and/or hippocampus during anticipation of public speaking (Tillfors et al., 2002) and stressful speaking (Tillfors et al., 2001) tasks.

MRI-based structural and functional connectivity methods have increased our understanding of the nature of neuronal interactions that underlie specific brain functions (Park and Friston, 2013). While functional magnetic resonance imaging (fMRI) studies have suggested increased activity in the fear processing network, including the amygdala, hippocampus, insula, anterior cingulate cortex, and medial prefrontal cortex, together with top-down cognitive control of arousal and anticipated fear/perceived threat in patients with SAD (Brühl et al., 2014; Etkin and Wager, 2007; Hattingh et al., 2013), structural brain networks described in terms of fiber bundles interconnecting discrete gray matter volumes by means of diffusion tensor imaging (DTI) (Lim and Helpern, 2002) may provide further information regarding the neurobiological basis of SAD.

Although WM structural properties of SAD patients have been assessed using DTI method in a number of studies (Baur et al., 2011, 2013; Liao et al., 2011; Phan et al., 2009; Qiu et al., 2014), both methodological approaches and results show significant divergence. In a study based on whole-brain analysis of voxel-based DTI values, it has been reported that patients with SAD, compared with healthy controls, had significantly lower FA only in the right uncinate fasciculus (UF) (Phan et al., 2009), while Baur et al. (2011) with a voxel-wise analysis reported lower FA values near the left UF and left superior longitudinal fasciculus (SLF) (Baur et al., 2011), which they later could confirm for left UF by means of tractography results (Baur et al., 2013). On the other hand, Liao et al. (2011) reported enhanced WM connectivity in the genu of corpus callosum in line with increased right medial prefrontal cortex (MPFC) volume, while Qiu et al. (2014) showed significantly decreased FA values in the WM of the left insula, left inferior frontal gyrus, left middle temporal gyrus, and left inferior parietal gyrus.

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A recent *meta*-analysis conducted by Brühl et al. (2014) reports that prefrontal regions, which have been associated with emotion regulative function, are more active in SAD, however, do not exert their controlling/regulating effect upon the amygdala, and suggests that this effect occurs due to disturbed structural connectivity between the prefrontal and amygdalar/limbic regions mainly due to the reduced WM integrity of UF. By this way the review proposes a new network-based model for SAD, which extends the previous model based on increased activation in brain regions known as the fear circuit (Etkin and Wager, 2007), by also including increased activations in parietal and medial occipital brain regions, which are decoupled from the amygdalar/limbic, the cingulo-opercular and the ventral attention network at the level of functional and structural connectivity.

While an important part of the above mentioned DTI studies on SAD preferentially focus on structural connectivity changes in prefronto-amygdalar connections, main objective of this study was to examine WM alterations in SAD by a whole-brain analysis which would allow also to observe possible connectivity changes between occipito-parietal, temporal and prefrontal regions foreseen in the new network model of SAD proposed by Brühl et al. (2014). As a secondary goal, we aimed to determine whether the severity of SAD has any impact on FA.

**Table 1**
Demographic characteristics of the sample.

| | SAD (N = 22) | Healthy controls (N = 22) | *t* | *p* |
|---|---|---|---|---|
| Age (mean ± sd) | 28.72 ± 6.58 | 28.72 ± 6.57 | 0 | 1 |
| Years of education (mean ± sd) | 13.59 ± 2.82 | 12.86 ± 3.80 | 0.72 | 0.48 |
| Gender (females/males) | 11/11 | 11/11 | | |

SAD: patients with social anxiety disorder, sd: standard deviation.

**Table 2**
Means and standard deviations of clinical scales in the SAD group.

| Clinical Scale | N (SAD) | *mean* | *sd* |
|---|---|---|---|
| HAM-anxiety | 19 | 6.42 | 5.10 |
| HAM-depression | 20 | 4.10 | 4.49 |
| SDS-work | 14 | 6.00 | 2.66 |
| SDS-social life | 14 | 6.50 | 2.68 |
| SDS-family life | 14 | 2.57 | 3.44 |
| LSAS-total | 21 | 73.86 | 28.52 |
| LSAS-avoidance | 21 | 34.10 | 14.48 |
| LSAS-anxiety | 21 | 39.76 | 15.13 |

HAM-anxiety: Hamilton Anxiety Rating Scale, HAM-depression: Hamilton Depression Rating Scale, LSAS: Liebowitz Social Anxiety Scale, SAD: patients with social anxiety disorder, SDS: Sheehan Disability Scale, sd: standard deviation.

## 2. Results

The SAD and control groups were similar with respect to age (28.72 ± 6.58 years and 28.72 ± 6.57 years, respectively, t = 0, p = NS). Female ratios were identical (50.0% for both groups). The patient and control groups were not significantly different in terms of years of education (13.59 ± 2.82 and 12.86 ± 3.80, respectively, t = 0.72, p = 0.48). Demographic characteristics of the sample are summarized in Table 1.

The mean (±SD) age at onset was 15.00 (±3.86) years; the mean duration of illness was 14.41 (±6.72) years; the mean LSAS-fear score was 39.76 (±15.13); the mean LSAS-avoidance score was 34.10 (±14.48); the mean LSAS-total score was 73.86 (±28.52); the mean HAM-A score was 6.42 (±5.10); the mean HAM-D score was 4.10 (±4.49); the mean SDS-work/school score was 6.00 (±2.66); the mean SDS-social life score was 6.50 (±2.68) and the mean SDS-family life score was 2.57 (±3.44) in the SAD group.

For the SAD group, means and standard deviations of all scales are listed in Table 2, while correlation coefficients among the scores of all clinical scales and sub-scales are displayed in Table 3. As a high correlation exists between HAM-A and HAM-D scores, between SDS-social life and family life scores as well as among the LSAS total, anxiety and avoidance scores, we selected HAM-A, SDS-social life, SDS-work and the LSAS-total scores in addition to "disease duration" as representative clinical variables to be tested for correlations with the DTI findings.

### *2.1. Local group differences of fractional anisotropy in the whole-brain analysis*

There was no significant difference in head motion between the two groups (SAD: 2.21 ± 0.88 mm, healthy controls: 2.17 ± 1.34 mm, t = 0.115, p = 0.91).

The SPM two-sample *t*-test using previous antidepressant use, age and gender as co-variates revealed that patients with SAD display reduced FA compared to healthy subjects in WM of right temporal lobe that according to the JHU White Matter Tractography Atlas (Hua et al., 2008) implemented in FSL extends laterally throughout the temporal part of the inferior longitudinal fasciculus (ILF) into the occipito-temporal part of the SLF (cluster-forming threshold p < 0.001; cluster-level FWE-corrected t = 4.84, p = 0.033; see Table 4 and Fig. 1). There were no regions with

**Table 3**
Pearson correlation coefficients among the scores of clinical scales.

| | HAM-A | HAM-D | SDS-social life | SDS-family life | SDS-work | LSAS-total | LSAS-avoidance | LSAS-anxiety |
|---|---|---|---|---|---|---|---|---|
| HAM-A | | ***p < 0.001, r = 0.863*** | NS, r = 0.497 | NS, r = −0.021 | NS, r = 0.470 | NS, r = 0.354 | NS, r = 0.231 | NS, r = 0.447 |
| HAM-D | | | NS, r = −0.170 | NS, r = −0.018 | NS, r = −0.008 | NS, r = 0.193 | NS, r = 0.059 | NS, r = 0.308 |
| SDS-social life | | | | ***p < 0.05, r = 0.593*** | NS, r = 0.129 | ***p < 0.05, r = 0.631*** | ***p < 0.05, r = 0.603*** | ***p < 0.05, r = 0.621*** |
| SDS-family life | | | | | NS, r = −0.008 | NS, r = 0.440 | NS, r = 0.379 | NS, r = 0.476 |
| SDS-work | | | | | | NS, r = 0.197 | NS, r = 0.094 | NS, r = 0.291 |
| LSAS-Total | | | | | | | ***p < 0.001, r = 0.961*** | ***p < 0.001, r = 0.965*** |
| LSAS-avoidance | | | | | | | | ***p < 0.001, r = 0.855*** |
| LSAS-anxiety | | | | | | | | |

HAM-anxiety: Hamilton Anxiety Rating Scale, HAM-depression: Hamilton Depression Rating Scale, LSAS: Liebowitz Social Anxiety Scale, SDS: Sheehan Disability Scale. NS: non-significant. Significant results are displayed in bold.

**Table 4**
Whole-brain voxel-vise group comparison of Fractional Anisotropy (social anxiety disorder < healthy subjects) using previous age, gender and antidepressant use as co-variates.

| Cluster | Number of voxel | MNI Peak Coordinates | | | *T* | Cluster level $p_{FWE}$[a] |
|---|---|---|---|---|---|---|
| | | x | y | z | | |
| ILF Right and SLF Right | 734 | 49 | −27 | −17 | 4.84 | 0.033 |

[a] $p_{FWE}$ = p-value after family wise error correction for multiple comparisons.

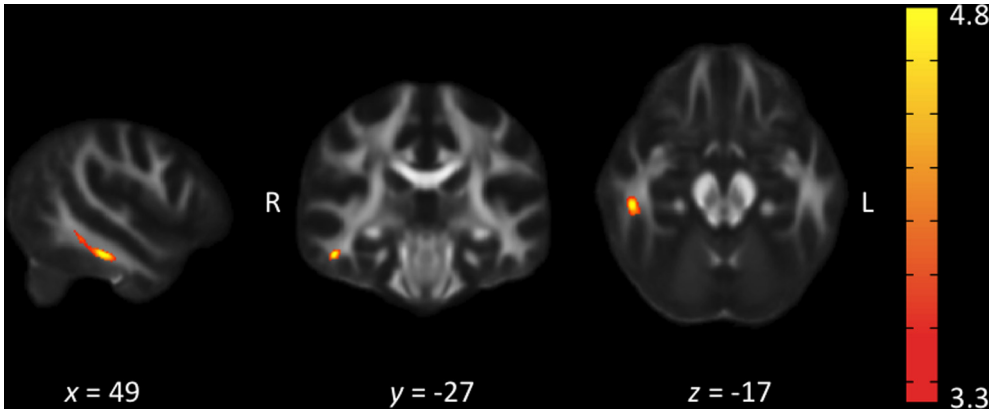



**Fig. 1.** Voxel-vise group comparison of fractional anisotropy (FA): Reduced FA (height threshold uncorrected p < 0.001, cluster level FWE corrected p = 0.033) in SAD patients in right inferior longitudinal fasciculus (ILF) and right superior longitudinal fasciculus (SLF). Clusters are superimposed on the FMRIB58_FA (1 mm) image (MNI peak coordinate: 49-27-17; cluster size = 734 voxels). From left to right; lateral sagittal (right side), coronal and axial views are shown at the peak coordinates of the significant cluster. L: Left, R: Right. Color bar indicates the t values.

**Table 5**
ROI-based between-group comparisons of Axial Diffusivity, Radial Diffusivity, and Mean Diffusivity examined by multivariate analysis of covariance (MANCOVA) with age, gender and previous antidepressant use as covariates.

| Diffusion Parameters | Mean ± sd | | *F* | *p* |
|---|---|---|---|---|
| | Control | SAD | | |
| Axial Diffusivity | 1.18E−03 ± 0.07E−03 | 1.12E−03 ± 0.07E−03 | 12.423 | <0.001 |
| Radial Diffusivity | 0.56E−03 ± 0.04E−03 | 0.62E−03 ± 0.04E−03 | 20.722 | <0.001 |
| Mean Diffusivity | 0.76E−03 ± 0.04E−03 | 0.78E−03 ± 0.03E−03 | 1.426 | 0.240 |

SAD: Patients with social anxiety disorder, sd: standard deviation.
*p*-values were adjusted for multiple comparisons using the Bonferroni correction.

increased FA in patients with SAD compared to healthy subjects. In the whole-brain comparisons, MD, AD and RD values showed no significant differences between the groups.

Although the effect of previous antidepressant use was excluded by introducing a covariate in the main analysis, the results were verified by comparing the patients with and without medication history in a separate *t*-test. This supplementary analysis revealed no significant WM differences between these patient sub-groups.

### 2.2. Region-of-interest analysis

For further elaboration of the significant FA difference, mean values of the AD, RD and MD values within the ROI that is determined by the significant cluster of the voxel-wise FA analysis were compared between the two groups. Although comparison of these values did not reach statistical significance threshold in the voxel-vise analyses carried out on the whole-brain AD, RD and MD data, the FA-directed ROI-based analysis may provide more sensitive results as it does not require any correction for multiple comparisons. Additionally, calculation of the mean values on unsmoothed images avoids partial volume effects due to neighboring gray matter voxels.

MANCOVA results with mean AD, RD and MD values of the ROI as dependent variables, group as the main factor and age, gender and previous antidepressant use as covariates revealed a significant overall effect of group factor ($F(3,37) = 16.597$, $p < 0.001$), while none of the covariates produced any significant effect. Analysis of each individual dependent variable, using a Bonferroni adjusted alpha level of 0.017, showed that there was no significant MD difference between the two groups ($F(1,39) = 1.426$, $p = 0.24$) while the SAD group showed significantly smaller AD ($F(1,39) = 12.423$, $p < 0.001$) and larger RD values ($F(1,39) = 20.722$, $p < 0.001$) compared with the healthy controls (Table 5).

Mean FA, AD, RD and MD values of the ROI were further tested for significant correlations with the clinical scores of patients. Pearson correlation analyses carried out within the SAD group with a Bonferroni adjusted alpha level of 0.01, revealed no significant correlations, except the negative correlation between the mean FA of the ROI and the HAM-A score ($r = -0.584$, $p = 0.009$), which is illustrated in the scatterplot in Fig. 2. Because one of the medication naïve cases with very high HAM-A score also displayed a very low FA value compared with the rest of the patients, we additionally applied Spearman's rank-based correlation analysis, which marginally supported the negative correlation between FA and HAM-A score found in the previous parametric analysis ($r = -0.369$, $p = 0.06$).

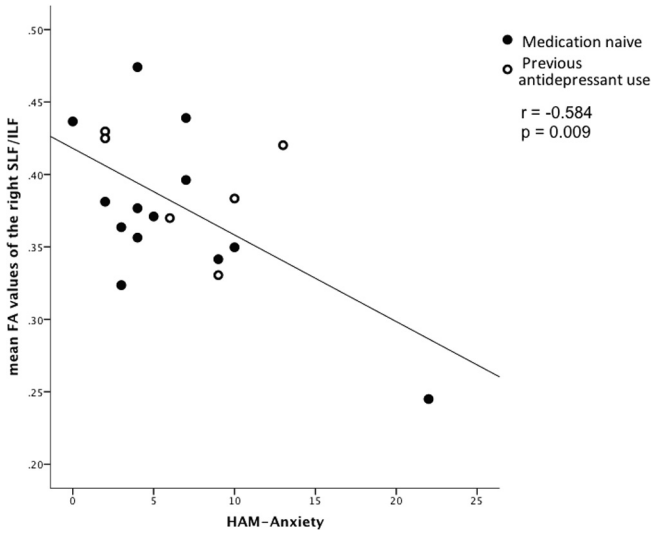



**Fig. 2.** Results of the Pearson correlation analysis between HAM-Anxiety scores and mean FA of the right SLF/ILF in the SAD group. The scatter diagram shows the negative correlation of HAM-Anxiety scores with the mean FA values of right SLF/ILF in SAD patients. White and black dots represent medication naïve patients and those who used antidepressant previously. Because one of the medication naïve cases with very high HAM-A score also displayed a very low FA value compared with the rest of the patients, we additionally applied Spearman's rank-based correlation analysis, which marginally supported the negative correlation between FA and HAM-A score found in the Pearson correlation analysis ($r = -0.369$, $p = 0.06$). HAM-Anxiety: Hamilton Anxiety Rating Scale, ILF: Inferior longitudinal fasciculus, SLF: Superior longitudinal fasciculus

## 3. Discussion

The aim of our study was to detect structural connectivity changes being involved in the pathophysiology of SAD. The main finding of the voxel-wise comparison was reduced FA in the right temporal lobe WM extending laterally throughout the ILF and in the occipito-temporal part of the SLF of patients with SAD compared with the healthy controls. There were no regions in which FA was higher in SAD compared to healthy subjects.

To better explain the WM changes responsible for the significant reduction of the FA, which is a composite measure of WM integrity, the difference of the mean axial, radial and mean diffusivity values in the FA map-driven ROI were compared between the two groups. The ROI-based analysis, although missing the spatial selectivity of the whole-brain comparisons, has a higher sensitivity for group differences, since it does not necessitate correction for multiple comparisons applied in voxel-wise analyses. Additionally, it gives the possibility to use the unsmoothed images to calculate ROI means, which reduces partial volume effects due to neighboring gray matter voxels. Using these two advantages of the ROI-based analysis, it was possible to show that the reduced right temporal FA in the SAD group depends on significantly reduced AD and increased RD values, while MD of this area did not differ between the two groups.

While AD corresponds to the largest, RD to the mean of the two smaller and MD to the mean of all eigenvalues of the diffusion tensor, the FA value reflects the overall prolateness of the diffusion ellipsoid. Various patterns of these diffusion parameters may point to different architectural properties of the WM. While animal studies show that increased RD mostly correlates with de- or dysmyelination (Song et al., 2002), it also depends on the diameters and density of the axons (Alexander et al., 2011). The AD correlates with the maturation of WM, but is also strongly affected by axonal injury, while MD mostly correlates with the cellularity, edema and necrosis (Alexander et al., 2011). According to a developmental study by Geng and Coworkers (2012), joint instead of univariate evaluations may provide a better view of the WM structure. Within this context, our WM finding of reduced AD and increased RD within the reduced FA-region may point to a low degree of maturation in ILF and SLF of the SAD patients.

Several DTI studies have previously reported WM abnormalities in patients with SAD. Phan et al. (2009) reported that patients with SAD, compared with healthy controls, had significantly (uncorrected) lower FA close to the right UF WM near the orbitofrontal cortex (OFC), while the peak coordinate reported corresponds to OFC gray matter rather than UF. In another study by Baur et al. (2011), patients with SAD had reduced FA compared to healthy subjects near to the left UF and the left SLF, but the authors did not discuss the SLF finding but focused on the UF in their discussion, while they accepted that the cluster is not located within the main trajectory of the UF but at the border of gray matter of the left OFC. The authors applied a relatively low threshold of 10% of the global mean FA for excluding the gray matter and CSF from solution space, which might be the reason of a significant finding at the gray matter border of UF. To avoid such uncertainties, we increased the FA threshold commonly applied as 20% of the global mean FA in the literature to 30%, which according to the histogram distributions in the fundamental study of Alexander et al. (2007) should exclude the possibility of gray matter or CSF contribution to the results, although it may lead to loss of some WM voxels. Hence, our analyses are significantly more conservative compared with the previous work that might be the reason for the missing UF difference in our results.

Same authors have used quantitative fiber tractography to investigate differences of volume and FA between patients with SAD and healthy subjects in the UF and inferior fronto-occipital fasciculus (IFOF) (Baur et al., 2013). They found that volume and FA of the left UF were reduced in patients with SAD, whereas there were no statistically significant group differences bilaterally for the IFOF. However, the authors clearly pronounced that their results do not exclude changes in other prominent fiber tracts as the analyses were limited to UF and IFOF on the basis of their working hypothesis.

In contrast to these SAD studies, which focus on changes in the structural connectivity between the central structures of the "fear processing network" such as UF that connects higher-level control areas in the orbitofrontal cortex with amygdala, our DTI results point to the significance of changes in ILF and SLF in SAD.

Liao et al. (2010) reported decreased effective connectivity from the inferior temporal gyrus, which is involved in visual perception and object recognition (Ishai et al., 1999; Herath et al., 2001), to the amygdala, and suggested that this may underlie the bias towards negative social information processing in the cognitive models of SAD. On the other hand, in a recent study we found significantly increased gray matter volumes in the right inferior and middle temporal regions, which were positively correlated with the social fear, social avoidance and total social anxiety scores of the SAD patients (Tükel et al., 2015). Considering that the affected WM region in the present study covers parts of ILF and SLF that connect inferior and middle temporal regions with limbic and prefrontal areas (Latini, 2015), the gray matter increases in the right inferior and middle temporal regions might result from hyperactivation of these areas to compensate their reduced structural connectivity to fronto-limbic structures.

ILF, an important source of fibers afferent to the amygdala, connects the temporal polar cortex with visual areas in the occipital cortex, inferior and middle temporal gyri and the fusiform gyrus (Kiernan, 2012; Latini, 2015), which according to a comprehensive meta-analysis of PET and fMRI findings take part in a common brain mechanism in anxiety disorders and normal fear (Etkin and Wager, 2007). On the other side, right ILF along with the IFOF constitutes a major portion of face processing (Gschwind et al., 2012).

A series of fMRI studies on emotional face processing in SAD (Frick et al., 2013; Danti et al., 2010; Gentili et al., 2008) have reported changes in the connectivity between the fusiform gyrus and amygdala, which are correlated with the severity of SAD. Through the ROI analysis, we also identified negative correlation of FA with the HAM-A scores in patients with SAD. Our finding of reduced FA in ILF, which is negatively correlated with anxiety scores, may explain the structural basis of abnormal functional connectivity of the fusiform and inferior temporal gyrus with amygdala and insula as reported in these functional connectivity studies on face processing.

The recognition of familiar faces activates transmodal nodes, including those in the lateral midtemporal cortex, which therefore may act as gateways for binding the additional associations that lead to the recognition of familiar faces (Mesulam, 1998). Correct identification of people, typically achieved by faces, is important for successful social interactions. On the other hand, social anxiety may cause reduced exposure or attention to people's faces, and thus to poor development of face recognition mechanisms (Davis et al., 2011). Considering the slow age-related changes in the volume and FA of the ILF, observed in individuals aged 6–23 years tightly linked with the increasing size of the fusiform face area (FFA), that shows a progressive right lateralization (Behrmann and Plaut, 2013), our right lateralized ILF findings raise the possibility that right inferior/middle temporal WM microstructure plays a role in mediating social interactions and may be implicated in the pathophysiology of SAD.

In addition to the face processing mechanism, the overlap of the area with reduced FA in SAD with both ILF and SLF may also support the SAD network model recently proposed by Brühl et al. (2014), which emphasizes the role of the connectivities of parietal and medial occipital regions with the fear circuitry and prefrontal areas of emotional regulation in the pathophysiology of the disorder.

This study has some limitations. Firstly, our findings must be interpreted cautiously because of the relatively small size of our sample. Another limitation of the study is the recruitment of patients, who have taken psychotropic medications before a wash-out period of 6 weeks. Since the sample size is critical for reliable results, these 8 patients were included in the study, but previous antidepressant use was included as a covariate in the group comparisons and the patients with and without previous medication were also compared using a separate *t*-test. On the other hand, comorbidity, which is a common confound in SAD patient groups was carefully excluded in our study.

## 4. Conclusion

In conclusion, our finding of reduced FA in the temporal part of the right ILF and in the occipito-temporal part of the right SLF in SAD patients is further supported by negative correlation of the FA values with the severity of anxiety. These findings raise the possibility that the pattern of WM abnormality in the right SLF and ILF may be implicated in the pathophysiology of SAD in terms of leading to cognitive deficits in social interactions, plausibly through dysfunction of face processing mechanisms.

More research on WM pathology has to be done in future to clarify the role of the inferior/middle temporal lobe and related structures in social anxiety and associated interpersonal difficulties.

## 5. Material and methods

### 5.1. Subjects

A total of 22 consecutive patients (11 women and 11 men) with SAD, who fulfilled Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) (APA, 1994) diagnostic criteria, and seen in the Outpatient Clinic of the Psychiatry Department of Istanbul Faculty of Medicine, between September 2011, and December 2012, were included in this study. Twenty-two healthy control subjects who were screened for the absence of psychiatric disorders using the Structured Clinical Interview for DSM-IV/Non-patient Edition (SCID-I/NP) (First et al., 2002) as well as any family history of psychiatric disorder were recruited on a voluntary basis and were matched to the patients for age and sex. All the patients and controls are right-handed.

Inclusion criteria were 1) SAD diagnosed using the Structured Clinical Interview for DSM-IV/Clinical Version (SCID/CV) (First et al., 1996); 2) aged between 18 and 50 years; 3) being medication-free for at least the previous 6 weeks. Exclusion criteria were 1) any current psychiatric disorder other than SAD diagnosed with the SCID-I/CV; 2) history of alcohol or drug abuse/dependence; 3) any serious concomitant general medical condition or neurologic disease; 4) pregnancy or lactation. Exclusion criteria for the patients and control subjects also included any contraindication for magnetic resonance (MR) imaging, and any history of neurodegenerative disease, seizure, central nervous system infection, cerebrovascular disease, diabetes mellitus, and head trauma causing loss of consciousness that lasted more than 30 min or that required hospitalization.

Among our patients, 14 (63.6%) were treatment-naïve; 8 (36.4%) patients had taken different types of antidepressants, with different dosages, before the 6-week drug-free period.

The ethics committee of Istanbul University Istanbul Faculty of Medicine approved the study. Written informed consent was obtained from all participants after the procedures had been fully explained. This study adheres to the Declaration of Helsinki.

### 5.2. Clinical assessment

Diagnosis was made during the initial interview by trained psychiatrists. The Liebowitz Social Anxiety Scale (LSAS) (Liebowitz, 1987), the Hamilton Anxiety Rating Scale (HAM-A) (Hamilton, 1959), the Hamilton Depression Rating Scale (HAM-D) (Hamilton, 1967) and the Sheehan Disability Scale (SDS) (Sheehan, 1984) were administered to the patients during the second interview. A semi-structured interview form prepared by the authors was used to evaluate the demographic features of the subjects.

For investigation of the correlations between the scores of the clinical scales and the DTI variables, first the correlations among the scores of the clinical sub-scales were calculated. Among the sub-scales that were highly correlated with each other, representative scores were selected for final correlation analyses with the DTI parameters.

### 5.3. MRI data acquisition

All MRI scans were acquired on a single 1.5 T clinical scanner (Philips Achieva, Philips Medical Systems, The Netherlands) equipped with an 8-channel head coil. Axial high-resolution T1-weighted MR images were acquired using a fast-spin echo sequence with voxel size 1x1x1 mm (155 axial slices, TR/TE = 25/4.6 ms, acquisition matrix $240 \times 240$). The diffusion-weighted images were obtained using single-shot echo-planar acquisition from 32 gradient directions with $112 \times 110$ acquisition matrix with 60 slices, 120 mm field of view, $1.75 \times 1.75 \times 2$ mm$^3$ voxels, 80.261 ms TE, 9.613 s TR, and b factor of 800 s/mm$^2$.

### 5.4. Data preprocessing

Image preprocessing was carried out using the FSL software (version 5.0.6; http://www.fmrib.ox.ac.uk/fsl). Diffusion images

were corrected for eddy current distortions and head motion using FMRIB's Diffusion Toolbox (Smith et al., 2004). Head motion of each subject was quantified based on root mean square deviation of all image voxels for each of the 32 gradient directions relative to the reference image (B0) by using *rmsdiff* utility of FSL. Maximum displacement value of each subject among acquisitions from 32 gradient directions was subjected to Student's *t*-test for testing any significant difference between the head motions of both groups.

Brain tissue was segregated from non-brain tissue, and individual binary brain masks were created on non-diffusion weighted images using Brain Extraction Tool (Smith, 2002). Restricted within this mask, a diffusion tensor was fitted for each voxel in the brain image space and FA and mean diffusivity (MD) maps as well as the primary ($\lambda1$), secondary ($\lambda2$) and tertiary ($\lambda3$) eigenvalues were computed using FSL's DTIFit. By this way, axial (AD = $\lambda1$), radial (RD = ($\lambda2 + \lambda3$)/2) and mean diffusivity (MD = ($\lambda1 + \lambda2 + \lambda3$)/3) values were included in the analyses.

### 5.5. Whole-brain voxel-wise analysis

All preprocessed FA and MD maps were normalized into the Montreal Neurological Institute space (MNI http://www.bic.mni.mcgill.ca). For this purpose, non–diffusion weighted image (b0) from each subject was normalized to the SPM EPI template using SPM8's (Wellcome Department of Imaging Neuroscience, Institute of Neurology, University College London; http://www.fil.ion.ucl.ac.uk/spm) nonlinear co-registration method, with a resampling resolution of $2 \times 2 \times 2$ mm$^3$. The transformation matrix derived from the normalization of the b0 image was applied to FA, MD, AD and RD maps. All normalized FA maps were visually checked for misregistration and smoothed using an isotropic 6 mm, full-width at half-maximum Gaussian kernel to reduce the variance due to individual anatomical variability.

A whole-brain voxel-wise analysis was performed using the normalized and smoothed FA, MD, AD and RD maps with 2-sample *t*-test of SPM8 to compare the 22 control participants and 22 patients using the previous antidepressant use, age and gender as covariates. In order to exclude voxels of cortical gray matter and cerebrospinal fluid (CSF), whole-brain analyses were restricted to voxels with a minimum FA of 0.3 (Smith et al., 2006; Müller et al., 2016). Voxel-wise height threshold of $p < 0.001$ combined with a cluster-extent family-wise error (FWE) correction with $p < 0.05$ for multiple comparisons was applied to determine the significant differences between the two groups.

Statistically significant between-group differences were superimposed on the FMRIB58_FA (1 mm) image in MNI space and the corresponding WM structures were determined using the JHU White Matter Tractography Atlas (Hua et al., 2008) implemented in FSL.

### 5.6. Region of interest (ROI) analysis

Since FA is a composite measure of DTI parameters, which is determined by the ratio of axial to radial diffusivity, any significant FA difference in the whole-brain analysis was further elaborated by comparing the means of more elementary DTI parameters, namely the axial (AD), radial (RD) and mean (MD) diffusivity within ROIs derived from clusters of significant FA differences created by using MarsBaR software (http://marsbar.sourceforge.net/) (Brett et al., 2002). Individual mean values within the ROIs were computed on normalized but unsmoothed images to avoid partial volume effects due to neighboring gray matter voxels.

ROI results were statistically tested by using the SPSS statistical analysis software (IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp). MD, AD and RD values of the ROIs were compared between the groups by using the multivariate analysis of covariance (MANCOVA) test that includes age, gender and previous use of antidepressants as covariates. Correlations among the psychiatric symptom scales and the FA, MD, AD and RD values of ROIs were tested. Statistical results of the multiple univariate tests in MANCOVA and multiple correlation tests were reported after Bonferroni correction.

## Contributors

Author RT and KA designed the study and wrote the protocol. Author EE and AK recruited and screened the participants. Author KA performed image processing. Author CUY and EK analyzed the DTI data and undertook the statistical analysis. Author RT wrote the paper. All authors critically reviewed content and approved final version for publication.

## Conflict of interest

All authors report no biomedical financial interests or potential conflicts of interest.

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