CHAPTER 3

CAN KLINEFELTER SYNDROME SERVE AS A MODEL FOR SCHIZOPHRENIA? DIFFERENCES IN LATERALIZATION OF BRAIN NETWORKS DURING LANGUAGE PROCESSING

SUBMITTED

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ABSTRACT

Both schizophrenia and Klinefelter syndrome (47,XXY) have been associated with disturbed language processing and reduced hemispheric lateralization. Klinefelter syndrome has also been associated with a higher risk of developing psychotic symptoms. A hypothesized cerebral dominance gene on the X chromosome may be responsible for the development of the brain in a lateralized manner. This may play a role in both Klinefelter syndrome and schizophrenia. By applying a network approach, we analyzed lateralization profiles underlying language processing in both patient groups and those of healthy male control participants. Similarities in lateralization between the two patient groups would strengthen the idea that Klinefelter syndrome could serve as a model to understand schizophrenia. A language task (involving semantic and phonological evaluation of words) was performed by men with schizophrenia (N=39), men with Klinefelter syndrome (N=11), and healthy male control participants (N=45) during fMRI. A task-related independent component analysis was executed to identify the networks that were involved in task performance. For each participant, lateralization of network contribution was calculated within relevant brain networks, using a combined bootstrap/histogram analysis approach. Differences in lateralization were analyzed with Univariate Analyses of (Co)variance. Men with schizophrenia demonstrated a normal bilateral network contribution within the salience network, whereas men with Klinefelter syndrome showed a small rightward lateralization in this same network. Moreover, men with Klinefelter syndrome showed a tendency towards a decreased contribution of the left hemisphere to the bilateral fronto-temporal network, whereas men with schizophrenia resembled healthy control participants. Within the schizophrenia group, lateralization indices in the bilateral fronto-temporal network were negatively correlated with PANSS positive and general psychopathology subscales. Men with Klinefelter syndrome showed different lateralization profiles of network involvement in a language task compared to male controls, but this was not the case in men with schizophrenia. The lack of similar task performance and lateralization profiles in both patient groups suggest that different pathophysiological mechanisms might be involved in both patient groups. These findings do not support Klinefelter syndrome as a model for schizophrenia when it comes to possible etiological pathways in the realm of language lateralization.

INTRODUCTION

Anomalies in hemispheric specialization have consistently been reported in patients with schizophrenia. For example, whereas the general population is predominantly right-handed, a less explicit right-hand preference is observed in patients with schizophrenia and non-clinical individuals with schizotypal personality traits (Somers et al., 2009; Sommer et al., 2001a). Moreover, brain activation related to language processing shows a more equal distribution over both hemispheres in patients with schizophrenia (Sommer et al., 2001b; Walter et al., 2003; Weiss et al., 2006). Anatomical differences may be underlying functional abnormalities, such as smaller gray matter volumes in the planum temporale and sylvian fissure in the left temporal cortex (Sommer et al., 2001a). Reduced language lateralization and deficits in language processing have been applied to the explanation of positive, as well as negative symptoms of schizophrenia (Crow, 2008). It is hypothesized that hemispheric specialization is mediated by a dominant allele referred to as the right shift factor (Annett, 1972). Crow (1989) argued that this cerebral dominance gene might be located on the pseudo-autosomal region of the sex chromosomes. Excessive expression of this hypothesized gene may cause a lack of development of the left hemisphere (Crow, 1989). In favor of the hypothesis of reduced lateralization are the gender-specific differences observed in patients with schizophrenia. The prevalence of schizophrenia is higher in males than in females (DeLisi et al., 1989), with a more favorable illness course in females (Riecher-Rossler & Hafner, 2000). Moreover, in males, the first signs of the disorder are often presented during adolescence or early adulthood (Hafner et al., 1992), when levels of the reproductive hormone testosterone should be peaking (Spear, 2000). However, support for the hypothesis of reduced expression of a cerebral dominance gene in patients with schizophrenia is sparse.

Studying individuals with sex chromosome aneuploidies may enhance the understnding of the influence of sex chromosomes on brain development. In this respect, Klinefelter syndrome has been suggested as a model to understand psychopathology of schizophrenia (DeLisi et al., 2005; van Rijn et al., 2006). This genetic disorder, that affects only men, is caused by a supernumerary X chromosome (47,XXY). Dosage changes of X chromosomal (X-linked) genes may result in defective testosterone signaling, which is observed in early childhood, as well as in adolescence in these males (Aksglaede et al., 2007; Lahlou et al., 2004; Salbenblatt et al., 1985), and may be related to various abnormalities. Besides physical characteristics such as tall stature, hypogonadism, infertility and gynecomastia (Lanfranco et al., 2004), men with Klinefelter syndrome may also demonstrate abnormalities that are observed in patients with schizophrenia. It is well-reported that men with Klinefelter syndrome experience difficulties in language and social cognition (Mandoki et al., 1991; Rovet et al., 1996). Also, brain imaging studies reported atypical structural and functional lateralization in these males (Itti et al., 2006; Netley & Rovet, 1984; Savic, 2014; van Rijn et al., 2008). Interestingly, men with Klinefelter syndrome demonstrate a four- to five-fold increased risk to develop psychotic disorders, compared to the general population (Cederlof et al., 2014; DeLisi et al., 1994). All these factors have led to the suggestion that Klinefelter syndrome could serve as a genetic model to study the psychopathology of schizophrenia (DeLisi et al., 2005; van Rijn et al., 2006).

In previous studies, frontal and temporal brain regions have been related to reduced structural and functional lateralization in patients with schizophrenia and men with Klinefelter syndrome (Sommer et al., 2001a; Sommer et al., 2001b; van Rijn et al., 2008; Walter et al., 2003; Weiss et al., 2006). However, brain regions do not function in isolation. With the increasing awareness that the brain is a complex system of regions in interaction, advances were made

to define schizophrenia as the result of disrupted functional integration of specialized brain systems (Andreasen et al., 1996; Friston, 1998). Hence, also in lateralization studies, a network approach might provide valuable information. Moreover, a direct comparison of neural correlates of patients with schizophrenia and men with Klinefelter syndrome has not been performed as yet. The present study therefore aimed to investigate the lateralization of the spatial contribution of brain regions to networks underlying processing of language. To study the neural substrate of the evaluation of visually presented words, participants performed a task during fMRI scanning. A data-driven independent component analysis was performed to identify the brain networks that were most invoked by the task. Lateralization indices were calculated within these networks, and comparisons were made between a group of men with schizophrenia, a group of men with Klinefelter syndrome and a group of healthy male control participants. We hypothesized that in both patient groups language processing would be compromised, reflected in less lateralized brain networks (i.e., more bilateral involvement), as compared to male controls. Confirmation of this hypothesis would strengthen the idea that the pathogenesis of symptomatology of schizophrenia can partly be explained in terms of decreased hemispheric specialization, which may be related to genetic mechanisms.

METHODS

Participants

The current analyses were performed on a combined data set from three different studies with patients with schizophrenia and one study with men with Klinefelter syndrome. Each study consisted of a patient group and a control group that were matched one age, education and handedness. All subjects performed a word evaluation task during fMRI. One study was an rTMS treatment trial for auditory verbal hallucinations, that included 51 patients with schizophrenia (Bais et al., 2014; Vercammen et al., 2009). Of these participants, 34 completed fMRI scanning before treatment. As the aim of the current study was to compare male individuals, female participants (N=17) were left out the analyses, thus of this first data set, 17 men with schizophrenia were considered for analyses. Another study among patients with schizophrenia was an fMRI study on neural correlates of emotion (Curcic-Blake et al., 2013). Twenty patients with schizophrenia participated in this study, of which four were female. Hence, of this second data set, 16 male patients were included for analyses in the current study. The third group consisted of 15 men with schizophrenia that were specifically recruited for current study. Thus, a total of 48 men with schizophrenia were included for analyses. The patients with schizophrenia were referred to the studies by professionals of local mental health care institutions (University Medical Center Groningen, Lentis, GGz Drenthe). All patients in the schizophrenia group had to meet the DSM-IV criteria for schizophrenia, as confirmed with a diagnostic interview (Schedule for the Clinical Assessment in Neuropsychiatry interview (SCAN; Giel & Nienhuis, 1996). Current symptoms were determined with the Positive and Negative Syndrome Scale (PANSS; Kay et al., 1987).

Fifteen men with Klinefelter syndrome were recruited at the Department of Endocrinology of the University Medical Center Groningen (UMCG), and affiliated hospitals, or through the website of the Dutch Klinefelter Association. The diagnosis of Klinefelter syndrome had to be confirmed by karyotyping, and all men had to meet the criterion of complete 47,XXY karyotype. Men with the so-called mosaic syndrome were excluded. Men with Klinefelter syndrome were also excluded if they had a current significant psychiatric condition, assessed with a SCAN interview (Giel & Nienhuis, 1996).

A total of 47 male control subjects participated in the four studies. Advertisements in local newspapers and supermarkets were used to recruit these men. They reported to be physically healthy, and without diagnosis of any DSM IV axis I disorder, as confirmed with a SCAN interview (Giel & Nienhuis, 1996).

All participants were native Dutch speakers. They were checked for MRI contra-indications. Handedness was administered using the Edinburgh Handedness Inventory (Oldfield, 1971). The demographic characteristics of the participants that were included in the analyses are summarized in Table 1.

Written informed consent was given after the study procedures had been fully explained. The study was approved by a licensed local medical ethical committee (University Medical Center Groningen, The Netherlands; METc protocol numbers: 2006.052; 2007.234; 2008.051) and conducted in accordance with the latest version of the Declaration of Helsinki.

fMRI paradigm

The word evaluation task comprised two experimental conditions (Aleman et al., 2005). In the phonetic condition, visually presented bisyllabic words had to be evaluated, which required phonological processing of the stimuli. Dutch is a stress-timed language in which strong and weak syllables can be distinguished. Participants had to imagine hearing the words and indicate the syllable that carried the metrical stress (e.g. in the word 'chapter' the first syllable carries the stress: CHAP-ter and not chap-TER). The semantic condition required semantic evaluation of bisyllabic words by rating them in terms of positive or negative emotional content (e.g. the word 'summer' has a positive emotional content and the word 'cancer' is negative). The semantic condition is identical to the phonetic condition in terms of visual word presentation and the two-choice task configuration. The word stimuli were presented for 2000 ms, after which a fixation cross appeared for 3000 ms. Participants were allowed to respond by choosing the correct response button during the presentation of the stimulus and during the appearance of the fixation cross. Reaction times and accuracies were logged. The task consisted of eight blocks in fixed order, with four blocks for each condition. Each block consisted of 12 trials that were presented in random order. Consequently, a total of 96 trials were presented. Active task blocks were alternated with 30000 ms rest blocks, allowing the BOLD response to return to baseline. During rest blocks the participants were instructed to lie still and fixate on a central cross on the screen.

Image acquisition

Imaging data were acquired on a Philips 3 Tesla Intera magnetic resonance scanner (Best, The Netherlands) with a SENSE-8 channel head coil, located at the University Medical Center Groningen. First, a 3-D T1-weighted anatomical image was acquired, covering the whole brain (TR=9 ms; TE=3.5 ms; matrix: 256x231; voxel size: 1x1x1 mm; 170 slices). Functional images were acquired using a T2*-weighted gradient echo EPI sequence (TR = 2500 ms; TE = 30 ms; voxel size = 3.5 x 3.5 x 3.5 mm3; 322 slices).

Data analysis

Analyses on demographical and behavioral data were performed with SPSS (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). Group differences in age and years of education were tested with analysis of variance (ANOVA). Tukey's post-hoc paired tests were performed in case of significant results. Chi-square tests were used to test for group differences in handedness.

Participants that scored below chance level during the fMRI task were excluded from analyses. Group differences in accuracy and reaction time were analyzed with Univariate Analysis of Variance (ANOVA). Additionally, we performed an Analysis of Covariance (ANCOVA), as groups differed in age. Differences were considered significant with a p-value below 0.05, two-tailed.

Preprocessing of fMRI data

fMRI data were preprocessed and analyzed using SPM8 (Statistical Parametric Mapping, version 8; The Wellcome Department of Imaging Neuroscience, London, UK). All functional images were slice time corrected and realigned, and then co-registered with the anatomical T1 image. Images were spatially normalized to standard stereotactic space (MNI T1 template) and smoothed with a 3D isotropic Gaussian kernel (FWHM 8 mm) to increase signal-to-noise ratio. Subjects were excluded from further analysis if their head movement was greater than 3 mm in any direction.

First and second level analysis

A full-factorial model was created in SPM8, with group and condition as factors, to test for differences in brain activation between the two conditions and for interaction effects between group and condition. All analyses were thresholded at $p<0.05$, whole brain corrected for Family Wise Error (FWE). As the second level tests revealed that there was no main effect of condition or interaction effect between group and condition, we concluded that both task conditions did not significantly differ in elicited brain activation. Hence, we decided to consider the phonetic and semantic condition as one condition in further analyses.

Independent component analysis

Independent Component Analysis (ICA) was performed with The Group ICA of fMRI Toolbox (GIFT; version 3.0a, MIALab Software) implemented in Matlab version 7.8.0 (Calhoun et al., 2001). Using Maximum Description Length (MDL) and Akaike's criteria, the number of independent components was estimated at 30. For data reduction, principal component analysis (PCA) was done at participant and group level. For all participants, images were decomposed into 30 spatially independent components using the Infomax algorithm. Subsequently, independent component stability was validated by running a group level ICA with the ICASSO approach, which was repeated 20 times (Himberg et al., 2004). For back-reconstruction to participant-specific independent components, spatial-temporal regression was applied.

Lateralization index

For every component, a mask was created by thresholding the mean component images at Z>5. These images were subsequently binarized. As the calculation of lateralization requires symmetrical selection of brain regions, the binarized masks were flipped over the sagittal axis. The original and flipped images were summed and the resulting symmetrical masks were used for lateralization calculation. The individual-specific, component-specific images were entered in the Lateralization Toolbox designed by Wilke & Schmithorst (2006), implemented in SPM 8. This toolbox calculates a lateralization index (LI) for each participant, applying a combined bootstrap/histogram analysis approach (Wilke & Schmithorst, 2006). This approach yields a reliable quantitative estimate of lateralization that does not rely on visual inspection, or arbitrary thresholding. In previous literature (Everts et al., 2009; Wilke & Schmithorst, 2006), functions have been considered left-lateralized at LI values higher than 0.2, and right-lateralized at LI's lower than -0.2. We tested whether group means for each component deviated significantly from zero by performing one sample T-tests. Group differences in lateralization index were calculated with Univariate Analysis of Variance (ANOVA). For each of the six components, the LI was added as dependent variable, and patient group as independent grouping variable. Tuckey's post-hoc paired tests were performed in case of significant results. Univariate analysis of covariance (ANCOVA) was performed on the same data, with age added as a covariate, as the three groups differed in mean age. The uncorrected p-level for significance was 0.05 (two-tailed), and the p-level for significance corrected for multiple comparisons (Bonferroni) was 0.008 (two-tailed).

RESULTS

Demographic data

Of the 48 men with schizophrenia that were considered for analyses, five showed too much head movement, and another four did not perform the task correctly, leaving 39 men in the schizophrenia group. Four men in the Klinefelter group showed too much head movement, which resulted in data inclusion of 11 men with Klinefelter syndrome. Of the 47 control participants, two showed too much head movement, leaving 45 participants in the control group. After merging the four groups and their matched controls, the three groups differed in age. The men with Klinefelter syndrome were older than the men with schizophrenia and male controls. Demographic and clinical data of the subjects are presented in Table 1.

Task performance

Univariate analysis of accuracy showed a trend significant effect of group ($F(2,91)=2.44$, $p=0.093$). Post hoc analysis revealed that men with Klinefelter syndrome tended to perform worse than the control participants ($p=0.090$). Reaction time demonstrated differences between the groups ($F(2,91)=3.97$, $p=0.022$). Post hoc analysis showed that men with schizophrenia were slower than the control participants ($p=0.017$; see Figure 1). After controlling for age, group differences in accuracy became significant ($F(2,90)=3.40$, $p=0.038$), and the differences in reaction time remained significant ($F(2,90)=3.91$, $p=0.024$).

Table 1. Demographic and clinical characteristics of the three subgroups.

	Men with schizophrenia (n=39)	Men with Klinfelter (n=11)	Male controles (n=45)	p-value
Age (years)	33.5 (8.0)	46.2 (5.8)	35.9 (10.6)	<0.001
Education (years)	13.8 (16)	12.6 (1.9)	13.3 (2.0)	0.132
Handedness (R/L)	33 / 6	10 / 1	41 / 4	0.626
PANSS Positive symptoms	13.7 (4.9)	-	-	
PANSS Negative symptoms	13.5 (3.7)	-	-	
PANSS General psychopathology	28.0 (7.3)	-	-	

Data are means (+/- standard deviation) or number of participants; Education level was rated according to the scale defined by Verhage (1984); R: right; L: left; PANSS: Positive and Negative Syndrome Scale (Kay et al., 1987). There was a significant group difference in age; men with Klinefelter were older than men with schizophrenia and male controls.

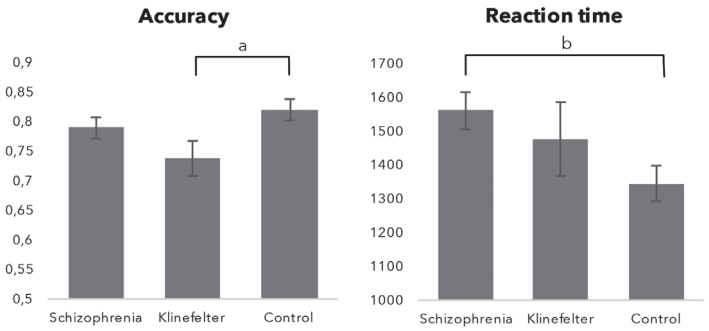

Figure 1. Mean accuracy and reaction time per group; error bars represent standard errors; a: p=0.090; b: p=0.017.

Independent Component Analysis

In total, thirty independent task-related network components were estimated. The six components that showed the highest correlation with the task, based on temporal sorting, were selected for further analysis (Supplementary Table 1). Other components in the temporal sorting were related to artifacts, such as head motion, physiological and scanner noise, cerebrospinal fluid and white matter. The six selected components resemble components identified in previous resting state studies (Allen et al., 2011; Damoiseaux et al., 2006; Raichle et al., 2001; Smith et al., 2009). The first component, the default mode network (DMN; r=0.18), showed a pattern of cortical midline structures, as well as the bilateral angular gyrus and inferior frontal areas. The second component, the auditory-sensorimotor network (ASM; r=0.19), revealed a bilateral network of superior temporal gyrus (including Heschl's gyrus), insula, pre- and post-central gyri, and parietal areas. The third component, the salience network (SAL; r=0.17), included bilateral insula, superior frontal regions, as well as the anterior cingulate cortex. The fourth component, the left fronto-parietal network (LFP; r=0.22), comprised primarily left middle and inferior frontal regions, as well as inferior

and superior parietal gyri, the supramarginal and angular gyri. The fifth component, the right fronto-parietal network (RFP; r=0.13), revealed a pattern of right middle and inferior frontal regions, as well as superior and inferior parietal gyri, the supramarginal and angular gyri. The sixth component, the bilateral fronto-temporal network (BFT; r=0.17) comprised a bilateral network of primarily the inferior frontal areas, including the insula, as well as superior and middle temporal gyri. The symmetrical masks that were created for each component are depicted in Figure 2.

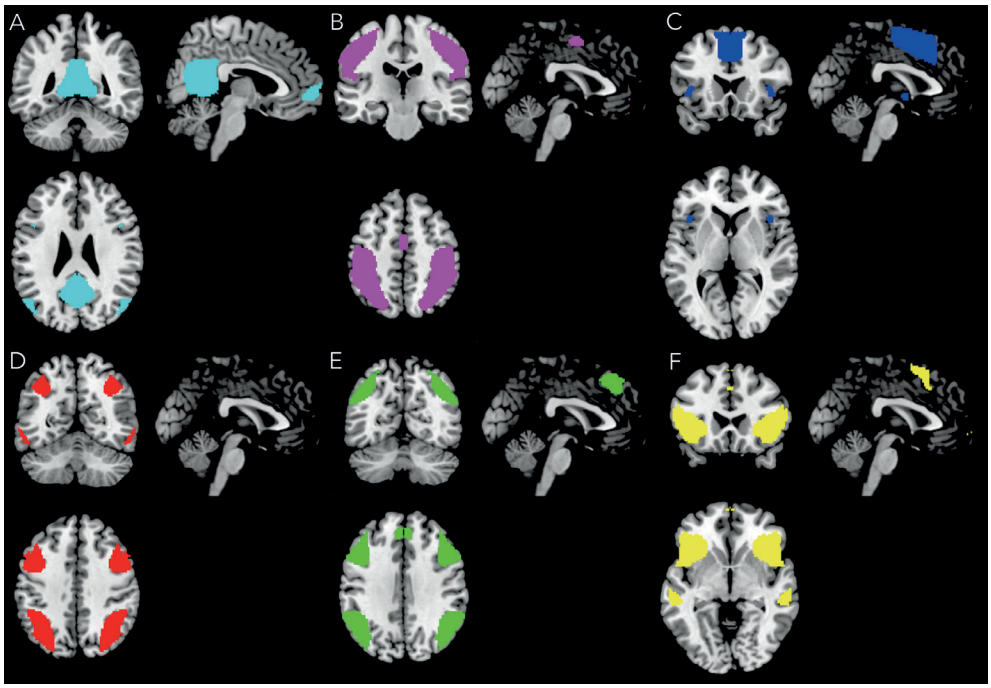

Figure 2. Symmetrical masks were created of each component and used for lateralization calculation. A: Default mode network (0,-46,25); B: Auditory-sensorimotor network (0,-22,52); C: Salience network (0,20,4); D: Left fronto-parietal network (0,-58,48); E: Right fronto-parietal network (0,-63,34); F: Bilateral fronto-temporal network (0,23,-6).

Lateralization index

Lateralization indices (LI) are presented in Table 2 and Figure 3. For the men with schizophrenia, lateralization indices deviated significantly from zero in the default mode, auditory-sensorimotor, left-fronto-parietal, right fronto-parietal, and bilateral fronto-temporal networks ($t(38)=-2.08$, $p=0.044$; $t(38)=7.49$, $p<0.001$; $t(38)=18.07$, $p<0.001$, $t(38)=-38.18$, $p<0.001$, $t(38)=16.42$, $p<0.001$, respectively). For the men with Klinefelter syndrome, lateralization indices deviated significantly from zero in the default mode, salience, left-fronto-parietal, right fronto-parietal, and bilateral fronto-temporal networks ($t(10)=-2.79$, $p=0.019$; $t(10)=-2.40$, $p=0.037$; $t(10)=8.52$, $p<0.001$, $t(10)=-25.69$, $p<0.001$, $t(10)=3.29$, $p=0.008$, respectively). For the male controls, lateralization indices deviated significantly from zero in the auditory-sensorimotor, left-fronto-parietal, right front-parietal, and bilateral fronto-temporal networks ($t(44)=8.82$, $p<0.001$; $t(44)=37.81$, $p<0.001$; $t(44)=-51.32$, $p<0.001$, $t(44)=28.83$, $p<0.001$, respectively).

Univariate test results showed group differences in LI in the salience network ($F(2;92)=3.99$, $p=0.022$). Men with Klinefelter syndrome demonstrated a small lateralization towards the right hemisphere, whereas both men with schizophrenia and male controls showed a bilateral network contribution. This difference remained significant after controlling for age ($F(2,91)=3.44$, $p=0.036$). Trend significant group differences were present in the bilateral fronto-temporal network ($F(2,92)=3.02$, $p=0.54$), which was caused by a difference between men with Klinefelter syndrome and male controls. This group difference approached significance after adding age as a covariant ($F(2,91)=3.07$, $p=0.51$). The (trend) significant group differences did not survive correction for multiple comparisons.

Table 2. Lateralization indices for the six components, for each group.

	Men with schizophrenia (n=39)	Men with Klinefelter (n=11)	Male controls (n=45)
Default mode network	-0.08 (0.23)	-0.16 (0.19)	-0.08 (0.26)
Auditory-sensorimotor network	0.42 (0.35)	0.22 (0.34)	0.38 (0.29)
Salience network	0.03 (0.23)	-0.17 (0.23)	0.04 (0.21)
Left fronto-parietal network	0.75 (0.26)	0.72 (0.28)	0.79 (0.14)
Right fronto-parietal network	-0.87 (0.14)	-0.85 (0.11)	-0.87 (0.11)
Bilateral fronto-temporal network	0.63 (0.24)	0.51 (0.51)	0.71 (0.16)

Data are means (+/- standard deviation).

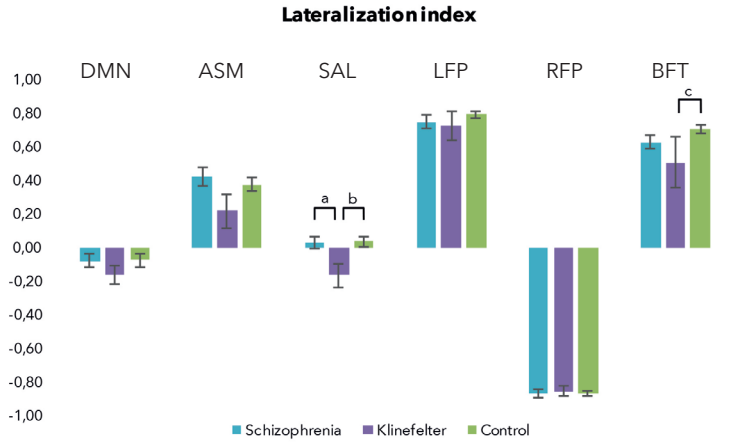

Figure 3. Distribution of lateralization indices (Y axis) per component and per group. Error bars represent standard errors. DMN: Default mode network; ASM: Auditory-sensorimotor network; SAL: Salience network; LFP: Left fronto-parietal network; RFP: Right fronto-parietal network; BFT: Bilateral fronto-temporal network. a: p=0.027; b: p=0.021; c: p=0.055.

Correlation between lateralization and symptoms

Correlation analysis within the schizophrenia group revealed a significant negative correlation between lateralization indices in the bilateral fronto-temporal network and the scores on the positive symptom scale and the general psychopathology scale of the PANSS (r=-0.35, p=0.030; r=-0.33, p=0.046, respectively). Lower PANSS scores were associated with stronger leftward lateralization.

DISCUSSION

In this fMRI study, we compared network lateralization of men with schizophrenia with that of men with Klinefelter syndrome and male controls during a word evaluation task. We hypothesized that compromised language processing and decreased network lateralization would characterize both patient groups. Lateralization indices were calculated within six brain networks that demonstrated involvement during word evaluation. Contrary to expectations, men with schizophrenia demonstrated a normal leftward lateralization profile within the bilateral fronto-temporal network, whereas leftward lateralization in men with Klinefelter syndrome in this same network was reduced. Moreover, the contribution to the salience network showed a normal bilateral distribution in men with schizophrenia, but an increased contribution of the right hemisphere in men with Klinefelter syndrome. These results suggest that different pathophysiological mechanisms might be involved in the language and social communication problems that characterize both patient groups. Hence, the hypothesis that Klinefelter syndrome could serve as a model for understanding symptoms in patients with schizophrenia was not supported.

The absence of decreased network lateralization in men with schizophrenia during word evaluation is surprising, since reduced lateralization during language processing in these patients appears a well-established finding (Alary et al., 2013; Dollfus et al., 2005; Royer et al., 2015; Sommer et al., 2001b). Structural abnormalities in laterality at the level of the temporal and frontal lobes (Crow, 2004; Sommer et al., 2001a) presumably underlie deviations in functional lateralization. It is also conceivable that the extent of functional lateralization is related to task demands and compensation strategies. The schizophrenia group in the present study was able to perform equally as the control group, but at the cost of speed. This pattern is known as speed-accuracy trade-off (Heitz, 2014; Henmon, 1911). It has been shown in patients with schizophrenia that brain activation increases during task performance, but decreases when the task becomes too complex (Callicott et al., 2003). Applied to our findings, patients with schizophrenia may have been able to compensate compromised task performance by using more left-hemispheric resources. Perhaps, higher task demands may have led to lower accuracy and a more bilateral distribution as a result of less activation in the left hemisphere. Studies in schizotypal adults found no indications of reduced lateralization either, as measured with dichotic listening and language tasks with and without fMRI scanning (Carlin & Lindell, 2015; Castro & Pearson, 2011; Park & Waldie, 2016). As these individuals are generally well-functioning, a lack of reduced lateralization may also be explained in terms of adequate compensation strategies. However, we also observed an inverse relationship between PANSS positive and general psychopathology scores and lateralization, an association that has previously been reported (Alba-Ferrara et al., 2013; Berenbaum et al., 2008; Sommer et al., 2001). It could thus be argued that reduced functional lateralization in patients with schizophrenia may not only depend on task complexity and the ability to address compensatory mechanisms, but also on symptom severity.

Lateralization profiles in men with Klinefelter syndrome, on the other hand, did corroborate previous reports (Itti et al., 2003; Lenroot et al., 2009; Lentini et al., 2013; Steinman et al., 2009; van Rijn et al., 2008). This group recruited the left hemisphere less than male controls in the bilateral fronto-temporal network, a network that is composed of brain regions involved in language processing (Tyler & Marslen-Wilson, 2008). Earlier studies also found deviations in language areas, which is understandable given the language problems that men with Klinefelter syndrome often experience (Mandoki et al., 1991; Rovet et al., 1996). Itti et al. (2003) contrasted resting state regional cerebral blood flow of men with Klinefelter syndrome against that of matched controls, and observed increases in the right supramarginal and angular gyri, and other areas in the right hemisphere, leading to relatively symmetrical cerebral perfusion. An fMRI study that used language tasks, found reduced asymmetry in men with Klinefelter syndrome as compared to male controls, which was presumably caused by increased activation in the right supramarginal and superior temporal gyri (van Rijn et al., 2008). Contrary to the two previous studies, another study reported that reduced activation in the left hemisphere during a word generation task might have resulted in reduced leftward lateralization in men with Klinefelter syndrome compared to male controls (Steinman et al., 2009). Moreover, substantial evidence has emerged from structural imaging studies that men with Klinefelter syndrome generally show decreased volumes in comparison to controls, for a range of discrete brain regions (Lenroot et al., 2009; Lentini et al., 2013), possibly related to the observed symmetrical functional patterns. The Klinefelter group in our study was less accurate than the control group, but equally fast. This indicates that Klinefelter men, contrary to men with schizophrenia, were less able to compensate for language dysfunction, and demonstrated a degree of impulsivity instead. Indeed, men with Klinefelter syndrome have been described as being impulsive (Geschwind & Dykens, 2004; Wakeling, 1972). Although speculative, the different recruitment of the salience network as compared to the other two groups might in part account for this impulsivity. The salience network is typically involved in the interplay between detection of salient stimuli from the external world and internally oriented cognition, and as such functions as a switch between the default mode and fronto-parietal networks (Menon & Uddin, 2010). Disturbed functioning of the salience network may result in the assignment of too much attention towards task-related information, causing a heightened, though less efficient reactivity to those stimuli.

The lack of a similar task performance and functional lateralization profile in men with schizophrenia and men with Klinefelter syndrome question the hypothesis that Klinefelter syndrome could serve as a model for the pathogenesis of symptoms of schizophrenia. If X-linked reduced lateralization underlies pathophysiology in both disorders, then presumably additional X-linked commonalities would be observed, such as decreased testosterone production or susceptibility to it, and shared genetic profiles. Reduced testosterone levels have been reported in Klinefelter individuals at several sensitive periods of brain development: at birth (Sorensen et al., 1981), during infancy (Lahlou et al., 2004), and during adolescence (Salbenblatt et al., 1985). Given the role testosterone plays in brain development (Arnold & Breedlove, 1985), it may be assumed that brain abnormalities already exist early in life. In psychotic men and male adolescents at ultra-high risk for psychosis, relatively low levels of testosterone have been observed (Huber et al., 2005; van Rijn et al., 2011), which have been related to negative symptoms (Akhondzadeh et al., 2006) and cognitive-emotional processing (Vercammen et al., 2013). However, to the best of our knowledge, it has not been investigated yet whether men with schizophrenia were exposed to low testosterone levels in perinatal life or during infancy, and how this may have influenced brain development and cognition. Moreover, differential developmental pathways are reflected in the observation that cognitive problems are already present

during early life in Klinefelter individuals (Mandoki et al., 1991; Rovet et al., 1996), whereas problems typically manifest themselves during adolescence or young adulthood in schizophrenia (Hafner et al., 1992). In addition, the X-linked androgen receptor gene that is found to predict physical abnormalities in men with Klinefelter syndrome (Zitzmann et al., 2004), is not among the 108 identified genes that have been associated with schizophrenia (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014). Hence, with the available knowledge, it is too early to conclude that X-linked aspects are a common underlying factor for Klinefelter syndrome and schizophrenia.

Our study has several limitations. We found a deviating lateralization profile in men with Klinefelter syndrome. However, the sample size of this group was limited. More studies with larger sample sizes are warranted to substantiate the findings. As deviating testosterone levels may contribute to pathogenic mechanisms, it would also be interesting to assess serum testosterone level in all participants at time of study participation, and to correlate this level with performance and functional lateralization. Furthermore, the distribution of left-handed subjects was equal over all groups, however, it may have confounded the results, since left-handedness has been associated with reduced language lateralization in healthy subjects (Szaflarski et al., 2002). Finally, although controlled for, mean age was not equal in all groups.

This is the first functional MRI study that directly compared men with schizophrenia to men with Klinefelter syndrome and healthy controls during language processing. Deviations in lateralization may underlie disturbances in language processing that are often found in both patient groups. Comparable language problems and similarities in lateralization profiles would strengthen the hypothesis that Klinefelter syndrome could serve as a model to understand the pathogenesis of schizophrenia. Men with schizophrenia however, revealed normal lateralization profiles, whereas men with Klinefelter syndrome demonstrated lateralization profiles that deviated from the healthy control group. These findings, in combination with the sparse evidence for the influence of testosterone or X-linked genes as common underlying factors, may suggest that Klinefelter syndrome is not an informative model helping to understand the pathophysiology of schizophrenia, at least not with regard to language lateralization. However, the influence of state versus trait characteristics in schizophrenia on lateralization and possibly related symptom severity deserves further investigation.

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Supplementary Table 1: The composition of the six independent components (p_{fwe}<0.05, k>20).

IC	Brain area	k	T	Z	x	y	z
A: Default mode	Middle Cingulate Cortex (L/R) / Posterior Cingulate Cortex (L/R) / Precuneus (L/R) / Calcarine Sulcus (L/R) / Cuneus (L/R) / Lingual Gyrus (L/R) / Middle Occipital Gyrus (L/R) / Middle Temporal Gyrus (L/R) / Angular Gyrus (L/R) / Vermis / Cerebelleum 4/5 (L/R)	12219	47.12	inf	-6	-60	12
	Medial Frontal Gyrus (L/R) / Anterior Cingulate Cortex (L/R)	1156	16.10	inf	2	56	-2
	Middle Occipital Gyrus (L) / Middle Temporal Gyrus (L)	689	14.03	inf	-46	-72	24
	Superior Frontal Gyrus (R) / Middle Frontal Gyrus (R)	489	13.49	inf	26	30	50
	Inferior Frontal Gyrus (R)	979	11.47	inf	48	32	18
	Insula (L) / Middle Temporal Gyrus (L) / Rolandic Operculum (L)	1672	10.80	inf	-40	-4	0
	Thalamus (L)	388	9.67	inf	-8	-12	6
	Middle Temporal Gyrus (R)	99	9.53	inf	58	-8	-16
	Thalamus (R)	221	8.52	7.31	10	-12	6
	Inferior Parietal Lobule (L)	220	8.28	7.16	-52	-28	44
	Middle Cingulate Cortex (L/R)	217	8.19	7.10	-2	4	32
	Middle Frontal Gyrus (L)	240	7.37	6.53	-22	22	48
	Inferior Temporal Gyrus (L)	35	6.43	5.84	-52	-56	-14
B: Auditory-sensorimotor	Precentral Gyrus (L) / Postcentral Gyrus (L) / Supramarginal Gyrus (L) / Superior Temporal Gyrus (L) / Heschl's Gyrus (L) / Rolandic Operculum (L) / Superior Frontal Gyrus (L) / Superior Parietal Gyrus (L) / Inferior Parietal Gyrus (L) / Angular Gyrus (L) / Rolandic Operculum (L)	16887	38.07	inf	-44	-34	44
	Precentral Gyrus (R) / Postcentral Gyrus (R) / Supramarginal Gyrus (R) / Superior Temporal Gyrus (L) / Heschl's Gyrus (L) / Rolandic Operculum (R) / Superior Frontal Gyrus (R) / Superior Parietal Gyrus (R) / Inferior Parietal Gyrus (R) / Angular Gyrus (R) / Rolandic Operculum (R)	10068	31.87	inf	52	-26	46
	Supplementary Motor Area (L/R)	907	16.21	inf	0	-2	54
	Inferior Temporal Gyrus (R)	334	9.18	7.73	52	-64	-4
	Medial Frontal Cortex (L/R)	669	9.00	7.63	0	60	6
	Precentral Gyrus (R)	136	8.42	7.25	58	12	32
	Thalamus (L)	393	8.25	7.13	-10	-24	10
	Cerebellum 6 (L)	108	7.99	6.96	-26	-52	-26
	Insula (L) / Amygdala (L) / Hippocampus (L)	534	7.68	6.75	-32	16	-18
	Hippocampus (R) / Parahippocampal Cortex (R)	157	7.53	6.64	24	-14	-18
	Inferior Frontal Gyrus (R)	168	7.18	6.39	40	22	-16
	Insula (R)	24	5.69	5.26	38	4	12
C: Salience	Superior Frontal Gyrus (L/R) / Anterior Cingulate Cortex (L/R) / Middle Cingulate Cortex (L/R) / Supplementary Motor Area (L/R) / Precentral Gyrus (L/R) / Middle Frontal Gyrus (L/R) / Rolandic Operculum (L) / Insula (L) / Superior Temporal Pole (L) / Postcentral Gyrus (L) / Inferior Frontal Gyrus (L)	18100	43.25	inf	-4	8	54
	Cerebellum 6 (R) / Cerebellum Crus 1 (R)	1651	16.73	inf	26	-54	-28
	Insula (R) / Inferior Frontal Gyrus (R)	1116	14.27	inf	40	18	4
	Precuneus (L/R) / Cuneus (L/R) / Calcarine Sulcus (L/R) / Lingual Gyrus (L/R)	3517	13.43	inf	14	-66	10
	Fusiform gyrus (L) / Inferior Occipital Gyrus (L)	517	12.09	inf	-40	-72	-14
	Cerebellum 6 (L) / Cerebellum Crus 1 (L)	363	10.32	inf	-32	-50	-26
	Rolandic Operculum (L) / Postcentral Gyrus (L)	127	7.13	6.36	-48	-20	16
	Middle Temporal Gyrus (R)	250	6.80	6.12	64	-48	14
	Superior Temporal Pole (R)	29	6.27	5.72	42	14	-20
	Vermis	21	6.24	5.69	4	-42	-38

IC: Independent Component; L: left hemisphere; R: right hemisphere

Supplementary Table 1, continued: The composition of the six independent components (p_{fwe}<0.05, k>20).

IC		k	T	Z	x	y	z
D: Left Fronto-Parietal	Precentral Gyrus (L) / Inferior Frontal Gyrus (L) / Middle Frontal Gyrus (L) / Frontal Orbital Gyrus (L) / Insula (L) / Supplementary Motor Area (L/R)	8290	36.96	inf	-46	20	26
	Angular Gyrus (L) / Inferior Parietal Gyrus (L) / Supramarginal Gyrus (L) / Middle Occipital Gyrus (L) / Superior Occipital Gyrus (L) / Precuneus (L) / Cuneus (L)	7095	24.96	inf	-32	-56	42
	Inferior Frontal Gyrus (R)	1848	17.84	inf	54	24	30
	Supramarginal Gyrus (R) / Angular Gyrus (R) / Inferior Parietal Gyrus (R) / Middle Occipital Gyrus (R)	899	12.43	inf	34	-68	44
	Anterior Cingulate Cortex (R)	525	12.11	inf	-2	0	32
	Cerebellum Crus 1 (R) / Cerebellum Crus 2 (R) / Cerebellum 6 (R) / Vermis	478	11.16	inf	14	-74	-32
	Cerebellum 4, 5 (L) / Vermis	876	9.88	inf	-4	-48	6
	Middle Cingulate Cortex (L/R)	137	9.60	inf	-2	-34	34
	Caudate (R)	289	9.27	7.78	10	12	6
	Caudate (L)	233	7.95	6.94	-8	16	6
	Ligual Gyrus (L)	42	6.67	6.02	-16	-90	-10
	Paracentral Lobule (R)	97	6.45	5.86	2	-30	58
	Heschl's Gyrus (R)	32	5.81	5.36	52	-14	4
	Middle Temporal Gyrus (L)	22	5.78	5.33	-52	-6	-14
E: Right Fronto-Parietal	Supramarginal Gyrus (R) / Inferior Parietal Gyrus (R) / Angular Gyrus (R) / Superior Occipital Gyrus (R) / Precuneus (R) / Cuneus (R) / Superior Parietal Gyrus (R) / Middle Temporal Gyrus (R)	6916	36.57	inf	48	-48	40
	Middle Frontal Gyrus (R) / Frontal Orbital Gyrus (R) / Insula (R) / Inferior Frontal Gyrus (R) / Superior Frontal Gyrus (R) / Supplementary Motor Area (R) / Precentral Gyrus (R)	12823	26.63	inf	4	36	42
	Cerebellum Crus 1, 2 (L)	1220	17.94	inf	-10	-78	-30
	Inferior Parietal Lobule (L) / Superior Parietal Lobule (L)	1413	16.60	inf	-44	-54	48
	Precuneus (R)	406	10.91	inf	8	-46	8
	Superior Temporal Cortex (L) / Heschl's Gyrus (L)	610	10.45	inf	-46	-14	2
	Cerebellum 3 (R)	778	9.93	inf	14	-30	-24
	Middle Frontal Gyrus (L)	178	9.46	inf	-38	50	6
	Paracentral Lobule (L/R) / Precentral Gyrus (R)	677	8.62	7.38	-6	-30	60
	Insula (L)	87	7.27	6.46	-34	20	-2
	Precentral Gyrus (L)	51	6.91	6.20	-54	12	38
	Lingual Gyrus (L/R)	258	6.89	6.19	4	-70	0
	Caudate (L)	94	6.29	5.73	-16	-10	24
	Thalamus (L)	59	5.93	5.45	-14	-12	6
F: Bilateral Fronto-Temporal	Frontal Orbital Gyrus (L) / Inferior Frontal Gyrus (L) / Insula (L) / Inferior Temporal Gyrus (L) / Middle Temporal Gyrus (L) / Superior Temporal Pole (L),	3460	32.24	inf	-46	24	-8
	Inferior Frontal Gyrus (R) / Frontal Orbital Gyrus (R) / Insula (R)	1781	19.90	inf	48	20	-4
	Middle Temporal Gyrus (L)	1660	16.35	inf	-56	-26	-6
	Superior Medial Frontal Gyrus (L/R) / Supplementary Motor Area (L/R)	2131	16.20	inf	0	8	60
	Cerebellum Crus 1 (R)	158	8.49	7.30	40	-66	-28
	Middle Cingulate Cortex (L/R)	81	7.69	6.75	0	-14	36
	Vermis	66	7.23	6.43	4	-82	-20
	Postcentral Gyrus (R)	73	7.18	6.39	26	-30	58
	Precentral Gyrus (L)	132	7.05	6.30	-44	0	48
	Superior Temporal Gyrus (L) / Middle Temporal Gyrus (L)	202	6.91	6.20	58	-26	-2
	Thalamus (L/R)	58	6.90	6.19	-2	-16	8
	Cuneus (L/R)	134	6.41	5.82	2	-80	16
	Medial Orbital Frontal Gyrus (L)	59	6.04	5.53	-4	60	-4

IC: Independent Component; L: left hemisphere; R: right hemisphere