X marks the spot: Structural and functional brain mapping in a genetically defined group at high risk of autism symptoms (47,XXY), and a comparison with idiopathic autism spectrum disorder

Goddard, M.N.

Chapter 5

Goddard, M.N., Van Rijn, S., Rombouts, S.A.R.B., & Swaab, H. *(submitted)*

Intrinsic functional brain connectivity in boys at high genetic risk of autism symptoms, and a comparison with idiopathic autism spectrum disorder

Abstract

Klinefelter syndrome (47,XXY) is a genetic condition affecting physical, cognitive and behavioral development. It is associated with compromised brain structure and function, which may be at the root of cognitive and behavioral problems reported in 47,XXY. This study assessed intrinsic functional brain connectivity (FC) in boys with 47,XXY, compared with controls. Because 47,XXY is associated with increased risk of developing autism spectrum disorders (ASD), FC in boys with 47,XXY was also compared to boys with ASD. ASD symptoms were assessed using the Social Responsiveness Scale. FC was assessed using resting state fMRI, corrected for multiple comparisons using threshold-free cluster enhancement, and pubertal maturation as a confound regressor. Boys with 47,XXY and boys with ASD had significantly elevated levels of autism symptoms compared with controls. Increased FC was found between the right precuneus/cingulate gyrus and frontoparietal network in 47,XXY relative to controls. Relative to boys with ASD, boys with 47,XXY showed decreased FC between the left precentral gyrus/middle frontal gyrus and auditory network. The frontoparietal network is associated with language functions, and the precuneus and cingulate gyrus are important for emotion processing. Increased FC might signify inadequate neural compensatory mechanisms underlying problems in these domains in 47,XXY. The difference between 47,XXY and ASD might signify phenotypical characteristics specific for idiopathic ASD, or different neural mechanisms underlying similar types of social dysfunction. These results underline the need for studies focused on delineating the neurobiological basis of social dysfunction, and may give direction to research on gene-brain-behavior relationships.

Introduction

47,XXY (also known as Klinefelter syndrome) is a chromosomal condition in males that has widespread effects on development, but to varying degrees. Physical consequences include tall stature, decreased motor tone, and fertility problems (Lanfranco, Kamischke, Zitzmann, & Nieschlag, 2004), but individuals with 47,XXY have a typical appearance. The presence of an extra X chromosome influences various aspects of development, and overexpression of genes that escape inactivation may substantially impact brain development and function, as the X chromosome contains genes coding for neural development (Ropers & Hamel, 2005). The finding of decreased brain volume in both males and females with an extra X chromosome supports the hypothesis of an effect of X chromosome genes on brain development (Lenroot, Lee, & Giedd, 2009). This is corroborated by findings of deviations in the anatomical architecture of the brain in 47,XXY. Decreased brain volume in the caudate nucleus, cerebellum,

amygdala, insular cortex, hippocampus, cingulate, temporal pole, inferior frontal lobe and superior temporal gyrus have all been reported in males with 47,XXY (Bryant et al., 2011; DeLisi et al., 2005; Giedd et al., 2007; Itti et al., 2006; Shen et al., 2004; Skakkebaek et al., 2013; Steinman, Ross, Lai, Reiss, & Hoeft, 2009).

It is to be expected that the neural consequences of 47,XXY are not limited to the structural architecture of the brain, but also affect brain function. Indeed, differences in brain function, which may be even more closely related to cognition and behavior than brain structure, have also been reported in 47,XXY. Functional imaging studies have shown deviant neural activation in a range of brain areas during specific cognitive tasks, including the inferior frontal gyrus, middle frontal gyrus, inferior temporal gyrus, middle temporal gyrus, superior temporal gyrus, superior temporal sulcus, inferior occipital gyrus, middle occipital gyrus, parahippocampal gyrus, supramarginal gyrus, amygdala, insula, and fusiform gyrus in individuals with 47,XXY (Brandenburg-Goddard, Van Rijn, Rombouts, Veer, & Swaab, 2014; Steinman et al., 2009; Van Rijn, Aleman, et al., 2008; Van Rijn et al., 2012).

Deviant brain development may be one of the mechanisms contributing to cognitive deficits and the increased risk of psychopathology that has been reported for individuals with 47,XXY, including psychotic disorders, bipolar disorder, ADHD, and autism spectrum disorders (ASD) (Bishop et al., 2011; Bruining, Swaab, Kas, & Van Engeland, 2009; Cederlof et al., 2014; DeLisi et al., 1994; Tartaglia, Cordeiro, Howell, Wilson, & Janusz, 2010), but the underlying neural mechanisms have not yet been clearly delineated. Deviations in neural structure and function may be an important missing link between genetics, cognition, and behavior. Understanding these gene-brain-behavior pathways provides a more specific characterization of the nature of observable deficits. In addition, distinct underlying neural mechanisms may differentially affect susceptibility to interventions aimed at ameliorating cognitive and behavioral impairments. This highlights the need for studies focusing on these neural mechanisms in 47,XXY, in order to obtain more optimal and tailored clinical care.

So far, studies regarding brain function in 47,XXY have focused on task-related brain activation. Although very informative, fMRI tasks are aimed at specific cognitive domains, limiting the number of brain regions that are likely to activate. Knowledge of spontaneous neural interactions at rest may be of the utmost importance, as it provides insight into the brain's default information processing systems. Deviations in these systems may be at the core of observable cognitive and behavioral problems in 47,XXY. A way of studying functional connectivity in the entire brain irrespective of cognitive performance, is resting state functional MRI (RS-fMRI). RS-fMRI may provide unique information regarding fundamental differences in the functional architecture of neural

networks in the brain, as there are no task demands for participants. Therefore, results from RS-fMRI analysis are not related to performance on a specific task but represents the brain's intrinsic functional connectivity. This may be especially relevant for clinical groups, as performance related variability in activation patterns is an important potential confounding factor in task-related fMRI in these groups. No resting state studies focusing on 47,XXY have been published, making the current study the first to map intrinsic functional connectivity in 47,XXY.

Smith et al. (2009) have identified ten robust, primary resting state networks that have been shown to be related to specific cognitive domains. This is interesting because it allows for the study of functional connectivity, and its association with cognition, in clinical populations with known cognitive impairments. For example, the frontoparietal network is associated with language functions, while the executive control network corresponds to inhibition. Considering the profile of impairments often reported in 47,XXY, including theory of mind deficits (Van Rijn, Stockmann, Van Buggenhout, Van Ravenswaaij-Arts, & Swaab, 2014; Van Rijn, Swaab, Aleman, & Kahn, 2006), impairments in (complex) motor skills and coordination (Ross et al., 2008; Samango-Sprouse & Rogol, 2002), language problems (Bishop et al., 2011; Boada, Janusz, Hutaff-Lee, & Tartaglia, 2009; Leggett, Jacobs, Nation, Scerif, & Bishop, 2010), and abnormalities in language lateralization (Van Rijn, Aleman, et al., 2008), the hypothesis for the current study was that 47,XXY is associated with deviations in intrinsic functional connectivity in specific networks. For this reason, the focus of the current study was on differences in functional connectivity at rest between boys with 47,XXY and non-clinical boys, using RS-fMRI analysis of six of the resting state networks identified by Smith et al. (2009): the default mode, sensorimotor, auditory, executive control, and two frontoparietal networks.

Because RS-fMRI allows for the investigation of functional connectivity independent of task execution and performance, it is particularly useful to assess differences and similarities in the architecture of functional networks across clinical conditions, irrespective of group differences in task performance. Conditions of particular interest in comparison to 47,XXY, are autism spectrum disorders (ASD), because many individuals with 47,XXY experience difficulties in social functioning, including a number of symptoms associated with ASD. They are at increased risk of ASD symptoms and diagnosis (Bishop et al., 2011; Bruining et al., 2009; Cederlof et al., 2014; Cordeiro, Tartaglia, Roeltgen, & Ross, 2012; Tartaglia et al., 2010; Van Rijn, Swaab, Aleman, & Kahn, 2008). However, in spite of this overlap in behavioral symptomatology, boys with 47,XXY show a specific cluster of autism symptoms, and are on average more socially anxious than boys with ASD (Bruining et al., 2010; Van Rijn et al., 2014). In addition, theory of mind deficits present in both conditions are related to executive dysfunction in 47,XXY, while being related to language and face recognition

problems in ASD (Van Rijn et al., 2014). Lastly, task related functional MRI results from the same sample as used in the current study, suggests the neural mechanisms underlying social cognition in 47,XXY and ASD may also differ (Brandenburg-Goddard et al., 2014). These findings illustrate the necessity for clear delineation of various paths to social dysfunction. In addition to being scientifically relevant, this also may influence the development of mental health care strategies tailored to individual differences. These differences may extend beyond observable symptoms to neural systems and possibly differential susceptibility to treatment.

RS-fMRI offers the unique opportunity to assess to what degree the functional architecture of the brain in individuals with 47,XXY differs from that of individuals with ASD. For this reason, a separate and secondary aim of this study was to compare intrinsic functional connectivity in boys with 47,XXY to boys with ASD. Previous RS-fMRI studies in ASD point towards altered functional connectivity in the sensorimotor cortex, superior parietal lobule, insula, fusiform gyrus, superior temporal gyrus, precuneus, parahippocampal gyrus, intraparietal sulcus, prefrontal cortex, anterior cingulate cortex, superior temporal sulcus, inferior frontal gyrus, middle frontal gyrus, postcentral gyrus, amygdala, and core areas of the DMN, compared with control participants (Anderson et al., 2011; Assaf et al., 2010; Cherkassky, Kana, Keller, & Just, 2006; Di Martino et al., 2014; Nielsen et al., 2013; Paakki et al., 2010; Redcay et al., 2013; Starck et al., 2013; Von Dem Hagen, Stoyanova, Baron-Cohen, & Calder, 2013). Significant differences between boys with 47,XXY and ASD in the current study, who share some of the core symptoms of autism, would provide unique information regarding the specificity of social dysfunction.

Materials and methods

Participant characteristics

This study had to distinctly separate aims: a comparison of functional connectivity at rest between 47,XXY and non-clinical controls, and a comparison of functional connectivity at rest between 47,XXY and ASD. As differences between non-clinical controls and the ASD group were not relevant to the research question, these groups were not compared. Results from analyses pertaining to these two aims are reported separately.

First aim: 4,XXY versus non-clinical controls

In order to address our first aim, twelve boys with 47,XXY [M_{AGE}=13.92 (*SD*=2.85)] and twenty-two non-clinical, male controls [M_{AGE}=11.56 (*SD*=2.43)] were included in analyses. ANOVA revealed a significant effect of group on age [$F(2,48)=3.787$, $p=.030$], with the 47,XXY group having a higher mean age ($p=.009$). IQ data were available for ten boys in the 47,XXY group [M_{IQ}=81.2 (*SD*=13.16)], and twenty-two in the control group [M_{IQ}=102.7

(*SD*=15.07)]. ANOVA revealed a significant effect of group on IQ [$F(2,44)=6.599$, $p=.003$], with the control group having a higher mean IQ than the 47,XXY group ($p=.001$).

Second aim: 47,XXY versus ASD

In order to address our second aim, twelve boys with 47,XXY [M_{AGE}=13.92 (*SD*=2.85)], and seventeen boys with ASD [M_{AGE}=12.13 (*SD*=2.01)] were included in analyses. ANOVA did not reveal a significant effect of group on age. IQ data were available for ten boys in the 47,XXY group [M_{IQ}=81.2 (*SD*=13.16)], and seventeen boys in the ASD group [M_{IQ}=98.9 (*SD*=17.34)]. ANOVA revealed a significant effect of group on IQ [$F(2,44)=6.599$, $p=.003$], with the ASD group having a higher mean IQ than the 47,XXY group ($p=.007$).

Recruitment

The 47,XXY group was recruited using various strategies, to avoid recruitment bias as much as possible. The sample consisted of children who were actively followed up after prenatal diagnosis with the help of clinical genetics departments in the Netherlands and Belgium, as well as children whose parents actively sought information about the condition of their child (recruited through support groups and calls for participants, with the help of the Dutch Klinefelter Association), and those who were seeking help for developmental problems (recruited through pediatricians, psychologists, psychiatrists and clinical genetics departments). The ASD group was recruited through the Center for Autism, a pediatric psychiatric outpatient department in the Netherlands. All boys with ASD were classified according to the DSM-IV criteria (A.P.A., 1994) using the Autism Diagnostic Interview-Revised (ADI-R) (Lord, Rutter, & Le Couteur, 1994), parental questionnaires, parental interviews, developmental history and family history, information from primary physicians as well as elaborate expert clinical observations. All ASD diagnoses were reached through consensus among a multidisciplinary team of mental health professionals, including board-certified pediatric psychiatrists with experience in the field of autism. Non-clinical controls were recruited through schools in the western part of the Netherlands and screened for psychopathology. None scored in the clinical range (>70) on the Child Behaviour Checklist (Achenbach, 1991).

Inclusion criteria for all participants were Dutch as primary language and age between ten and eighteen years. Exclusion criteria were a recent history of substance abuse, intellectual disability (<60 IQ points), scan or motion artifacts, as well as neurological conditions (e.g. structural brain damage due to prenatal/birth complications, tumors, strokes or diseases affecting the central nervous system). All participants and their parents received a complete description of the study and provided written informed consent prior to participation, in accordance with the Helsinki Declaration of 1975, as revised in

2008. All children received a gift card for participation, and travel costs were reimbursed. The experiment was approved by the Ethical Committee of the Leiden University Medical Center, Leiden, The Netherlands.

Procedure

All scans were administered in one morning or afternoon at the Leiden University Medical Center (Leiden, the Netherlands). Upon arrival, participants were screened for metals or other dangerous physical conditions using the MRI safety check list. Subsequently, they were escorted to the mock scanner, which was used to acclimate participants to the scanner environment. Participants were allowed to spend as much time as needed in the mock scanner.

MRI Data Acquisition

Scanning was performed on a 3-Tesla Philips Achieva whole body MRI scanner (Philips Healthcare, Best, The Netherlands), using an 8-channel SENSE receiver head coil. T1-weighted anatomical scans [TR = 9.75 ms, TE = 4.60 ms, flip angle = 8°, 140 transverse slices, 1.167 mm x 1.167 mm x 1.200 mm, FOV = 224.000 x 177.333] were obtained while participants watched an animated cartoon. All anatomical scans were reviewed and cleared by a radiologist. No anomalous findings were reported. RS functional brain images were obtained after participants completed an fMRI task. For the RS functional brain images, 160 T2*-weighted gradient-echo echo planar imaging volumes were acquired [TR = 2,220 ms, TE = 30 ms, flip angle = 80 °, 38 transverse slices with a 2.75 mm^2 voxel size, 2.72 mm slice thickness, 0.25 mm slice gap, FOV = 220 x 220 mm]. Participants were instructed to lie still with their eyes closed, without falling asleep. The total RS acquisition time was 7.5 minutes.

Outcome measures

Pubertal development

To control for developmental effects on brain maturation, pubertal maturation was assessed using the Pubertal Development Scale (PDS), a measure that shows good reliability and validity (Bond et al., 2006; Petersen, Crockett, Richards, & Boxer, 1988). Participants were classified as being pre-pubertal, early pubertal, mid-pubertal, late-pubertal, or post-pubertal, according to the Puberty Category Score (PCS) of the PDS.

Autism spectrum symptoms

The Social Responsiveness Scale (SRS) (Constantino & Gruber, 2005) is a 65-item parent-report questionnaire that was used to assess the degree of autism spectrum symptoms. It includes items that ascertain social awareness, social cognition, social communication, social motivation, and autistic mannerisms.

Higher scores indicate stronger autism traits. A validation study (Constantino et al., 2003) indicated that the SRS was highly correlated with the ADI-R (Lord et al., 1994). Coefficients were higher than 0.64 between SRS scores and all ADI-R scores. Total SRS scores were used as an indication of autism spectrum symptoms.

RS-fMRI analysis

Preprocessing

The RS-fMRI data of all participants were preprocessed using FEAT (FMRI Expert Analysis Tool), part of FSL (FMRIB's Software Library, http://fsl.fmrib.ox.ac.uk/fsl/fslwiki) version 5.0.4 (Jenkinson, Beckmann, Behrens, Woolrich, & Smith, 2012). The following preprocessing steps were conducted: nonbrain-tissue removal (Smith, 2002); motion correction (Jenkinson, Bannister, Brady, & Smith, 2002); spatial smoothing with a 5-mm fullwidth-at-half-maximum Gaussian kernel; high-pass temporal filtering using a 0.01 Hz cutoff to remove low-frequency artifacts; Multivariate Exploratory Linear Optimized Decomposition into Independent Components (MELODIC) Independent Component Analysis (ICA) data exploration; registration of the RS data to the T1-weighted anatomical image (Jenkinson et al., 2002), as well as registration of the T1 image to the 2-mm Montreal Neurological Institute (MNI) standard space image (Jenkinson & Smith, 2001). Both registration matrices were combined into a single matrix describing the transformation from the RS data to MNI standard space, and its inverse matrix was calculated. To automatically denoise the resting state data, FIX (v1.06 beta, FMRIB's ICA-based X-noiseifier; additionally requiring MATLAB (Statistics and Signal Processing Toolbox) and R) was conducted with a fully automated noise detection algorithm on first-level FEAT outputs. Given a set of independent components, FIX classifies components as "signal" or "noise" (effects of motion, non-neuronal physiology, scanner artifacts and other nuisance sources), the latter then being removed from the data (Griffanti et al., 2014; Salimi-Khorshidi et al., 2014).

Functional connectivity analysis

Individual timeseries and spatial maps were extracted for each template of the ten RS networks provided by Smith et al. (2009), using dual regression (Filippini et al., 2009). First, for each subject, the spatial maps of the ten RS networks were regressed (as spatial regressors in a multiple regression) into the subject's 4D space-time dataset. This resulted in a set of subject-specific timeseries, one per RS network spatial map. Next, these timeseries were regressed (as temporal regressors, again in a multiple regression) into the same 4D dataset, resulting in a set of subject-specific spatial maps, one per RS network spatial map. As the first three networks of the RS networks provided by (Smith et al., 2009) are

related to the visual system/visual perception, and there is no literature supporting an association between 47,XXY and deficits in these domains, these networks were excluded from group-wise analysis to reduce the number of comparisons. Additionally, because the scanner field of view excluded a large part of the cerebellum, the cerebellum network was also excluded. The remaining six networks were tested for group differences using FSL's Randomise permutation-testing tool (Nichols & Holmes, 2002; Winkler, Ridgway, Webster, Smith, & Nichols, 2014), with two general linear models including two contrasts (47,XXY>CON and 47,XXY<CON for the first aim, 47,XXY>ASD and 47,XXY<ASD for the second aim), and PCS as a confound regressor, correcting for multiple comparisons across space using threshold-free cluster enhancement (TFCE, $p<0.05$) (Smith & Nichols, 2009). A gray matter mask was created using the Atlas tool. The frontal, occipital, parietal and temporal lobes, as well as subcortical gray matter structures (caudate, putamen, thalamus and insula) were located using the MNI structural atlas. These individual structures were combined and binarized to create a gray matter mask, which was used in permutation testing. The regions that showed significant between-group differences in functional connectivity, were used to extract beta-values from participants' individual spatial maps.

Results

First aim: 47,XXY versus non-clinical controls

Pubertal development

Pubertal category scores were available for all participants in the 47,XXY and control groups. ANOVA revealed a significant effect of group on PCS [$F(2,48)=3.232$, $p=.048$], with the 47,XXY group having a higher mean PCS than the control group. Results are summarized in Table 1.

Autism spectrum symptoms

A significant effect of group on SRS total scores was found [$F(2,43)=34.484$, $p<.001$], with the 47,XXY group ($N=9$) having a higher mean SRS total score than the control group ($N=22$). Results are summarized in Table 1.

	47,XXY			**Controls**			
	N	*mean*	*SD*	*N*	*mean*	*SD*	*p*
PCS	12	4.08	2.19	22	2.32	2.72	.027
SRS	9	72.1	28.9	22	25.2	13.5	<.001

Table 1. Mean Pubertal Category Scores and mean Social Responsiveness Scale total scores of the participants related to the first aim: 47,XXY versus non-clinical controls

Second aim: 47,XXY versus ASD

Pubertal development

Pubertal category scores were available for all participants in the 47,XXY and ASD groups. ANOVA did not reveal a significant effect of group on PCS score. Results are summarized in Table 2.

Autism spectrum symptoms

No significant differences between the 47,XXY (*N*=9) and ASD (*N*=15) groups in SRS total scores were found. For the 47,XXY group, T-scores suggested two participants scored in the normal range, four scored in the mild to moderate range, while three scored in the severe range. For the ASD group, T-scores suggested two participants scored in the normal range, four scored in the mild to moderate range, while nine scored in the severe range. Results are summarized in Table 2.

	47,XXY			**ASD**			
	N	*mean*	*SD*	*N*	*mean*	*SD*	*p*
PCS	12	4.08	2.19	17	3.65	0.93	NS
SRS	9	72.1	28.9	15	91.5	33.6	NS

Table 2. Mean Pubertal Category Scores and mean Social Responsiveness Scale total scores of the participants related to the second aim: 47,XXY versus autism spectrum disorders

RS-fMRI analysis

Confounding factors

As the PDS is designed to assess pubertal maturation, PCS may provide a more precise indication of pubertal development than chronological age. Research suggests pubertal stage might play a more important role in adolescent brain maturation than chronological age (Blakemore, Burnett, & Dahl, 2010). For this reason, and because PCS correlates highly with chronological age, PCS was used as a confound regressor in RS-fMRI analysis.

Pre-processing: motion parameters

As motion effects are a point of concern in (f)MRI research in children, FIX was used to denoise the resting state data. There were no significant group differences in mean absolute or relative displacement in millimeters. However, both measures decreased significantly after using FIX on the resting state data. Results from analysis of the motion parameters are summarized in Table 3.

	Whole group	47,XXY	ASD	Controls	*p*
Abs. (pre-FIX)	0.74(1.15)	0.53(0.58)	1.01(1.66)	0.65 (0.87)	NS
Abs. (post-FIX)	0.04 (0.03)	0.04 (0.04)	0.04(0.02)	0.03 (0.02)	NS
Rel. (pre-FIX)	0.25 (0.49)	0.22 (0.34)	0.39(0.77)	0.15 (0.14)	NS
Rel. (post-FIX)	0.03 (0.02)	0.03 (0.02)	0.03(0.01)	0.02 (0.01)	NS
Abs. pre-FIX versus post-FIX (whole group)					<.001
Rel. pre-FIX versus post-FIX (whole group)					.002

Table 3. Mean absolute (Abs.) and mean relative (Rel.) displacement in millimeters (including standard deviations in parentheses) before and after using FMRIB's ICA-based X-noiseifier (FIX)

First aim: 47,XXY versus non-clinical controls

Dual regression revealed a cluster of significant between-group functional connectivity differences. Results are summarized in table 3 and depicted in figure 1A. The 47,XXY group showed increased functional connectivity between the frontoparietal network and the right precuneus/cingulate gyrus, compared with the control group (p=0.028). No significant between-group differences in other networks were found.

Second aim: 47,XXY versus ASD

Dual regression revealed a cluster of significant between-group functional connectivity differences. Results are summarized in table 4 and depicted in figure 1B. The 47,XXY group showed decreased functional connectivity between the auditory network and the left precentral gyrus/middle frontal gyrus, compared with the ASD group (p=0.018). No significant between-group differences in other networks were found.

Contrast	Network	# voxels	Max *t*	Location
47,XXY>CON	Frontoparietal	193	4.44	R precuneus/cingulate gyrus
47,XXY<ASD	Auditory	14	6.84	L precentral gyrus/middle frontal gyrus

Table 4. Characteristics of significant between-group differences per contrast

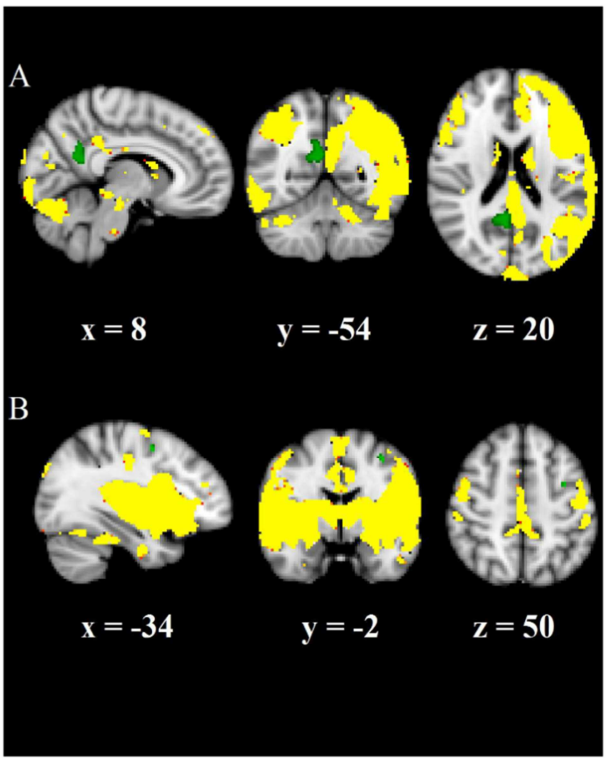

Figure 1. Significant functional connectivity between-group differences (green) overlaid on the respective resting state networks (yellow), including x,y,z (mm) coordinates. **A.** Increased connectivity between the right precuneus/cingulate gyrus and the frontoparietal network (47,XXY vs. controls). **B.** Decreased connectivity between the left precentral gyrus/middle frontal gyrus and the auditory network (47,XXY vs. ASD).

Discussion

In this neuroimaging study, intrinsic functional brain connectivity was investigated in boys with Klinefelter Syndrome (47,XXY) compared with non-clinical boys, and boys with autism spectrum disorders (ASD), using resting state functional magnetic resonance imaging (RS-fMRI). Analysis of six resting state networks identified by Smith et al. (2009) showed that, compared with non-clinical boys, boys with 47,XXY have *increased* functional connectivity between the right precuneus/cingulate gyrus and the frontoparietal network. Compared with boys with ASD, boys with 47,XXY show *decreased* functional connectivity between the left precentral gyrus/middle frontal gyrus and the auditory network. No significant differences between these groups in other networks were found.

The frontoparietal network is highly associated with language functions (Smith et al., 2009), which are consistently reported to be impaired in 47,XXY (Bishop et al., 2011; Boada et al., 2009; Leggett et al., 2010). It is therefore not surprising

that boys with 47,XXY show deviations in this network. Interestingly, these deviations seem to be located specifically in connections between the frontoparietal network and the precuneus, thought to be involved in emotional awareness (Van der Velde et al., 2013), and the cingulate gyrus, a central part of the limbic system (Kotter & Meyer, 1992). 47,XXY is not only associated with language problems but also with emotion processing deficits, which range from emotion recognition difficulties to impairments in the identification and labeling of own emotions (Aleman, Swart, & Van Rijn, 2008; Van Rijn et al., 2006). In addition, a recent neuroimaging study suggests deviant neural activation during verbal labeling of emotions in language-related regions in 47,XXY (Brandenburg-Goddard et al., 2014). Taken together, this leads to the hypothesis that individuals with 47,XXY may have problems in the link between language and emotion. This hypothesis is supported by previous studies that suggest a connection between language skills and emotion processing abilities in typically developing individuals (Cutting & Dunn, 1999; Jablonka, Ginsburg, & Dor, 2012; Lindquist, Barrett, Bliss-Moreau, & Russell, 2006; Pons, Lawson, Harris, & de Rosnay, 2003), which might be compromised in 47,XXY. The fact that in the current study an increase, rather than decrease, in functional connectivity was found between a network mainly associated with language, and structures specifically involved in emotion processing, may imply the existence of an inadequate compensatory mechanism underlying problems in these domains in 47,XXY. This could be a mechanism similar to neural compensation in individuals with brain pathology, who are hypothesized to show activation in brain regions not used by individuals with intact brains due to reorganization of neural information processing mechanisms, aimed at attaining optimal performance levels (Stern, 2009). Future studies are necessary to investigate the association between emotion processing and language impairments in 47,XXY more directly, for example by assessing if connectivity in the frontoparietal network predicts emotion processing impairment severity.

The auditory network is broadly associated with auditory processing, converting language to speech, and execution of speech (Smith et al., 2009). Delays in speech development have been reported in children with ASD (Shriberg, Paul, Black, & van Santen, 2011) as well as in males with 47,XXY, specifically those with an extra paternal X (Stemkens et al., 2006). As RS-fMRI is based on the Blood Oxygenation Level Dependent signal, increased functional connectivity might imply an increased metabolic rate. This could suggest more mental effort is being exerted. The decrease in functional connectivity found in boys with 47,XXY compared with boys with ASD, may therefore indicate that boys with ASD devote more resources to this language-to-speech network than boys with 47,XXY. The specific location of this deviation, i.e. the left precentral gyrus/middle frontal gyrus (the latter including the premotor cortex), provides additional support to this hypothesis, since these regions are heavily involved in planning and execution of speech (Brendel et al., 2010; Sato, Tremblay, & Gracco, 2009). Although boys with 47,XXY and boys with ASD participating in

the current study both showed increased levels of autism traits, they might differ in terms of language-to-speech functional networks. Hypothetically, these differences may account for aspects of the phenotype that are specific to ASD but not typical for 47,XXY. Alternatively, these functional differences may reflect different underlying mechanisms of a similar behavioral deficit. Both hypotheses are in line with research suggesting widespread abnormalities in intrinsic functional connectivity in individuals with ASD in comparison with typically developing individuals (Anderson et al., 2011; Assaf et al., 2010; Cherkassky et al., 2006; Di Martino et al., 2014; Nielsen et al., 2013; Paakki et al., 2010; Redcay et al., 2013; Starck et al., 2013; Von Dem Hagen et al., 2013), that appear to be absent in individuals with 47,XXY. However, this may be a distorted view of reality as many studies have assessed intrinsic functional connectivity in ASD, while the current study is the first to investigate this in individuals with 47,XXY. Although these hypotheses are speculative, the current study does show the importance of understanding brain-behavior relationships for the delineation of different mechanisms underlying similar types of social dysfunction in various conditions. It is therefore of the utmost importance to continue using novel techniques (such as RS-fMRI) to work towards identification of biomarkers of social dysfunction. The current study may give direction to such studies.

A limitation of the current study was the relatively small sample size, especially in the 47,XXY group, which may have led to a lack of power and prevented correlational analysis between RS-fMRI results and cognitive data. In addition, the ASD group consisted of relatively high functioning individuals, due to the demands associated with imaging studies (e.g. lying very still in a confined space, and high levels of noise). While the 47,XXY group had a significantly higher mean age and pubertal category score than controls, this was controlled for by using pubertal category score as a confound regressor in RS-fMRI analysis. Because this was the first study assessing intrinsic functional connectivity differences between these populations, no correction was applied for the fact that six networks were investigated. However, the current study was the first to demonstrate deviations in intrinsic functional connectivity in 47,XXY, both in comparison with non-clinical controls, and boys with ASD. It has generated a number of relevant hypotheses that give direction to future studies within these populations.

In conclusion, results from the current study suggest that boys with 47,XXY show increased intrinsic functional connectivity compared with non-clinical boys, between areas related to language functions and areas related to emotion processing. Additionally, boys with 47,XXY appear to have decreased intrinsic functional connectivity compared with boys with ASD, in a language-to-speech network. These results may stimulate further research into gene-brain-behavior interactions, as well as represent an important step towards elucidating separate and specific pathways to social dysfunction.

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