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

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Chapter 1

General introduction

Social behavior

Human beings are inherently social creatures. Although all primate brains are unusually large for body size as a result of the interaction between genetic mutations and the demands of living in complex social systems (Dunbar, 2009), our social interactions are infinitely more intricate. However, certain developmental conditions prevent individuals from adequately mastering social skills. This can lead to problems in adequately recognizing and interpreting social cues. Social skills develop gradually throughout childhood under the influence of both individual characteristics, and environmental factors. Social functioning involves cognitive abilities necessary to process social cues, communicative skills to successfully navigate social interactions, and the ability to use these skills across many domains in order to adapt to the social environment (e.g. at home, at work, or at social events) (Beauchamp & Anderson, 2010). Impairments in these skills significantly impact the ability to navigate our relatively fast-paced, complex social world, which is why it is important to investigate possible causes of impairments in social development. An example of a developmental disorder that has social dysfunction as its core feature, is autism spectrum disorder (ASD). The core symptoms of ASD include clinically significant deficits in social communication and interaction, and restricted, repetitive behavioral patterns. The individual manifestations of these deficits vary greatly, depending on the severity of the disorder, developmental level, and age (A.P.A., 2013). Full diagnostic criteria for autism spectrum disorder according to the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (A.P.A., 2013) are cited in Box I.

Box I. DSM-5 diagnostic criteria for autism spectrum disorder

Autism spectrum disorder

A. Persistent deficits in social communication and social interaction across multiple contexts, as manifested by the following, currently or by history:
  1. Deficits in social-emotional reciprocity;
  2. Deficits in nonverbal communicative behaviors used for social interaction;
  3. Deficits in developing, maintaining, and understanding relationships.

B. Restricted, repetitive patterns of behavior, interests, or activities, as manifested by at least two of the following, currently or by history:
  1. Stereotyped or repetitive motor movements, use of objects, or speech;
  2. Insistence on sameness, inflexible adherence to routines, or ritualized patterns of verbal or nonverbal behavior;
  3. Highly restricted, fixated interests that are abnormal in intensity or focus;
  4. Hyper- or hyporeactivity to sensory input or unusual interests in sensory aspects of the environment.

C. Symptoms must be present in the early developmental period (but may not become fully manifest until social demands exceed limited capacities, or may be masked by learned strategies in later life).

D. Symptoms cause clinically significant impairment in social, occupational, or other important areas of current functioning.

E. These disturbances are not better explained by intellectual disability (intellectual developmental disorder) or global developmental delay.

Research on impaired social development has mainly focused on ASD, as social dysfunction is the main characteristic of this condition. These types of studies are very useful and important for gaining insight into the mechanisms behind problematic social development, but new perspectives may provide new insights. Studying genetic conditions may also help uncover different pathways to social dysfunction that are more difficult to uncover by studying idiopathic ASD. ASD is behaviorally defined, and may therefore include a wide variety of underlying mechanisms leading to social dysfunction (Mottron, Belleville, Rouleau, & Collignon, 2014; Muhle, Trentacoste, & Rapin, 2004). For this reason it may be useful to study populations defined by a genetic condition that often leads to social problems, and in some cases autistic symptomatology. Studies focused on sex chromosome aneuploidies such as Klinefelter syndrome may aid in determining developmental risks for social dysfunction. Social development is often compromised in 47,XXY. Their genetic profile is known and they have a relatively homogeneous endophenotype. This is of considerable benefit when looking for (neuro)biological markers of social dysfunction, because it allows for investigation of the relationship between genetic, neural,

cognitive, and behavioral outcomes. Therefore, by studying this population, gene-brain-behavior pathways to social dysfunction may be delineated.

Klinefelter syndrome

Typically, human cells contain 23 pairs of chromosomes. 22 of those pairs are autosomes, existing in both males and females, while the last pair consists of sex chromosomes that determine the sex of an individual. This means that in typical male development, a 46,XY karyotype is present. In 1942 Harry Klinefelter described a syndrome in males, characterized by gynecomastia (i.e. enlarged breast tissue), and a specific type of hypogonadism (i.e. diminished functionality of the gonads) (Klinefelter, Reifenstein, & Albright, 1942). In 1959 it was found that these individuals had an extra X chromosome, leading to the 47,XXY karyotype (Jacobs & Strong, 1959). This syndrome was coined Klinefelter syndrome. The prevalence of this condition is about 1 in 650 newborns (Bojesen, Juul, & Gravholt, 2003), and over the years research has shed light on its physical, behavioral, and cognitive ramifications.

Physical, cognitive, and behavioral consequences of Klinefelter syndrome

The physical phenotype of Klinefelter syndrome (47,XXY) is variable and often quite subtle. Gross facial or physical abnormalities are absent. Individuals with this condition typically have disproportionally long limbs, deviating distribution of body fat including gynecomastia, decreased growth of pubic and facial hair, hypogonadism, which may influence fertility, and deviations in testicular and penile development (Bojesen et al., 2003). Additionally, individuals with 47,XXY may have diminished muscle strength (Ross et al., 2008). Current treatment for the physical symptoms includes implementation of supplemental testosterone treatment at the beginning of puberty. This may contribute to, for example, improved muscle mass and increased growth of pubic and facial hair.

Behaviorally, 47,XXY is associated with impairments ranging from subtle to pronounced. Individuals with 47,XXY are at increased risk of developing internalizing behavioral problems, shyness and withdrawal (Tartaglia, Cordeiro, Howell, Wilson, & Janusz, 2010). Additional problems include hyperactivity, impulsivity, and an increased risk of a clinical diagnosis of attention-deficit/hyperactivity disorder (Bruining, Swaab, Kas, & Van Engeland, 2009; Cederlof et al., 2014; Tartaglia et al., 2010). Individuals with 47,XXY are also more vulnerable to psychotic-like symptoms, and they are at increased risk of developing psychotic disorders (Cederlof et al., 2014). Furthermore, within the study by Cederlof et al. (2014), 5-25% of males with 47,XXY were diagnosed with ASD. Even in the absence of a formal ASD diagnosis, prominent social behavioral difficulties may be present. Individuals with 47,XXY often display autistic symptomatology such as social anxiety, reduced assertiveness, impaired emotion regulation, and communicative difficulties (Bishop et al., 2011;

Cederlof et al., 2014; Cordeiro, Tartaglia, Roeltgen, & Ross, 2012; Tartaglia et al., 2010; Van Rijn et al., 2014).

47,XXY is associated with a wide range of cognitive problems. Typically, intellectual functioning is at the lower end of the normal range, and males with 47,XXY often have language and reading difficulties (Boada, Janusz, Hutaff-Lee, & Tartaglia, 2009). Additionally, executive dysfunction (i.e. problems with higher order processes responsible for purposeful, goal-directed problem-solving) has been reported in 47,XXY (Boada et al., 2009; Boone et al., 2001; Lee et al., 2011; Van Rijn & Swaab, in press). Lastly, social cognitive deficits may be present, particularly in the interpretation of facial expressions and tone of voice, identification and verbalization of emotions, and theory of mind (Van Rijn et al., 2007; Van Rijn, Stockmann, Van Buggenhout, Van Ravenswaaij-Arts, & Swaab, 2014; Van Rijn, Swaab, Aleman, & Kahn, 2006).

The gene-brain-behavior hypothesis of social dysfunction

From a gene-brain-behavior perspective, impairments in cognitive skills may lead to social behavioral problems. For 47,XXY, this gene-brain-behavior relationship could hypothetically be as depicted in Figure 1. X-linked genes are overexpressed as a result of the presence of an additional X chromosome. Certain genes on this additional X chromosome may escape the inactivation (lyonization) that occurs in normal development. Previous studies have shown that even in normal development, 8-15% of human genes consistently escape this inactivation process, while 10-13% escape X inactivation to a certain degree (Berletch et al., 2015). In the case of 47,XXY, genes that are of particular interest are the ones that not only escape inactivation, but also have a homologue on the Y chromosome, as this implies triple expression of these specific genes. This overexpression may lead to dysregulation of typical genetic mechanisms. Because many of the X-linked genes are present in brain tissue, dysregulation of these genes influences sexually dimorphic brain development, and thus disrupts typical development of the structural and functional architecture of the brain (Raznahan et al., 2014; Vawter, Harvey, & DeLisi, 2007). The presence of an additional X chromosome is associated with decreased volume of brain regions associated with social information processing (Raznahan et al., 2014). These deviations in neural architecture may contribute to dysfunctional development of cognitive skills. These cognitive dysfunctions, in turn, influence how effective incoming information is processed. Reduced effectiveness of information processing negatively influences how adequately individuals deal with, and respond to, information from their environment. The output of cognitive processing is a behavioral response. This means that the existence of cognitive dysfunction may contribute to the development of behavioral problems. Additionally, it means there may be various pathways to social dysfunction, which may show both overlap and differences between 47,XXY and idiopathic ASD.

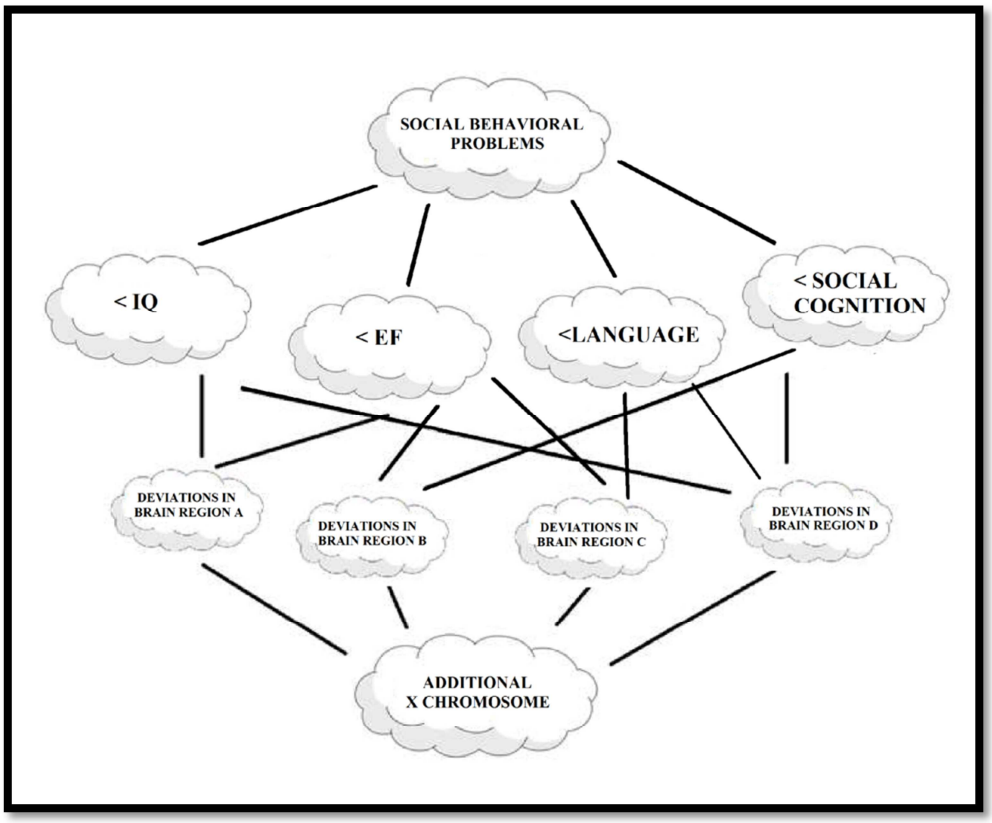

Figure 1. The gene-brain-behavior hypothesis of social dysfunction in 47,XXY

The neuroscience of 47,XXY

Although most aspects of the cognitive and behavioral phenotype associated with 47,XXY are relatively clearly delineated, less is known about the neural mechanisms underlying problems in these domains. Neural mechanisms are an important factor in the gene-brain-behavior hypothesis of social dysfunction in 47,XXY, as they represent the gateway through which genetic characteristics are expressed in behavior. This makes knowledge regarding these mechanisms essential for understanding the cognitive and behavioral issues associated with this condition. There are several ways of studying brain structure and function, but MRI allows for non-invasive, in vivo investigation of neural architecture. Structurally, gray matter and white matter can be investigated separately. For gray matter, voxel-based morphometry (VBM) offers the possibility of assessing differences in local gray matter volume, without a priori hypotheses regarding the exact location of potential differences (Good et al., 2001). For white matter, tract-based spatial statistics (TBSS) can be used to localize changes in the integrity of fiber tracts that connect different brain regions (Smith et al., 2006). Functionally, it is possible to investigate the activation of groups of neurons when an individual is performing a task (task-related brain activation), using

functional MRI (fMRI). It is also possible to assess neural activation patterns when an individual is at rest (intrinsic functional connectivity between brain regions), using resting state fMRI (RS-fMRI). RS-fMRI allows for the investigation of brain function independent of task execution and performance. This makes it particularly useful for investigation of the impact of an additional X chromosome on intrinsic functional brain connectivity. As cognitive skills are highly interrelated, it is likely impairments in one specific domain will influence performance (and neural activation patterns) in other domains. Using RS-fMRI, it is possible to assess differences and similarities in the architecture of functional networks across clinical conditions, irrespective of cognitive skills or group differences in task performance. This provides insight into deviations in intrinsic functional brain connectivity that may underlie some of these cognitive impairments. Using these different methodologies (VBM, TBSS, and (RS-)fMRI) in one sample provides essential information about brain structure and function. This may aid in further delineating the neural mechanisms underlying cognitive and behavioral problems associated with 47,XXY. This is especially important considering the vulnerability for autism symptoms in 47,XXY, which may arise as a consequence of different underlying neural abnormalities than in children with idiopathic ASD.

However, research on the structural and functional architecture of the brain in 47,XXY is limited. Only a handful of structural neuroimaging studies have been performed. These suggest that this condition is associated with gray matter volume reductions in the cerebellum, insular cortex, cingulate, inferior frontal lobe, caudate nucleus, amygdala, hippocampus, temporal pole, and superior temporal gyrus, as well as enlarged ventricles (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). White matter integrity in 47,XXY has been investigated in only one study. The results from this study suggest reduced integrity of fiber tracts in the left posterior limb of the internal capsule, bilateral anterior cingulate, and left arcuate bundle (DeLisi et al., 2005). To date, only three functional magnetic resonance imaging (fMRI) studies have been conducted. All studies were focused on task-related brain activation, meaning intrinsic functional connectivity between brain regions (independent of task performance) has not yet been investigated. Current findings indicate the presence of functional abnormalities in the amygdala, insula, fusiform gyrus, and superior temporal sulcus, related to social information-processing (Van Rijn et al., 2012). Deviations in brain function in the left middle temporal gyrus, left inferior frontal gyrus, cerebellum, middle occipital gyrus, inferior occipital gyrus, inferior temporal gyrus, parahippocampal gyrus, fusiform gyrus, superior temporal gyrus and supramarginal gyrus have been associated with language processing in 47,XXY (Steinman et al., 2009; Van Rijn et al., 2008).

Rationale for the research questions in the present thesis

In order to increase our understanding of the cognitive impairments and social dysfunction associated with 47,XXY, it is of the utmost importance to gain insight into the neural mechanisms underlying these problems. They are an essential component of the gene-brain-behavior hypothesis of 47,XXY. This is not only to the benefit of science, as it aids in understanding the neurobiology of social dysfunction, but may also have important implications for the diagnosis and treatment of these social problems. In line with this aim, a number of important and distinct differences between social dysfunction in 47,XXY and idiopathic ASD have been reported. For example, males diagnosed with 47,XXY and ASD are generally more socially anxious than males with idiopathic ASD, and they show a specific cluster of ASD symptoms that differs from individuals with idiopathic ASD in the domains of communication and reciprocal social interaction (Bruining et al., 2010; Van Rijn et al., 2014). This means that the behavioral component of the gene-brain-behavior hypothesis of social dysfunction appears to be slightly different for 47,XXY than it is for idiopathic ASD. Additionally, while theory of mind deficits are associated with both conditions, these deficits seem to be driven by different disturbances in the underlying cognitive mechanisms. In 47,XXY they are suggested to be related to executive dysfunction, while being associated with language and face recognition impairments in ASD (Van Rijn, Stockmann, Van Buggenhout, Van Ravenswaaij-Arts & Swaab, 2014). These findings strongly suggest that the cognitive component of the gene-brain-behavior hypothesis of social dysfunction may also differ between these two populations. It is currently unclear if similarities and differences in the cognitive and behavioral characteristics of 47,XXY and idiopathic ASD also extend to the neural phenotypes of both conditions. There are no neuroimaging studies directly comparing these groups, meaning it is unknown whether or not the brain component of the gene-brain-behavior hypothesis of social dysfunction differs between individuals with 47,XXY and ASD, and individuals with idiopathic ASD. However, since both populations may experience severe social impairments that require treatment, it is essential to gain insight into various pathways to social dysfunction. If the neural pathways to problematic behaviors differ between these groups, it implies the mechanisms leading to social dysfunction in 47,XXY are different from idiopathic ASD. In other words, they 'think differently'. These differences may be a valid starting point for the development and selection of tailored mental health care strategies that aim to more effectively ameliorate severe social problems.

Neuroimaging is a sensitive tool to assess the architecture of, and cooperation between, neural networks in these conditions. However, to date, no comprehensive (f)MRI studies have been conducted, using different MRI techniques in one sample of individuals with 47,XXY. Additionally, there is a lack of studies focused on children/adolescents with this condition, with only

one published (preliminary) study to date (Steinman et al., 2009). This means it is currently unclear if deviations in neural structure and function develop early in life. This is an important factor to assess, as it aids in determining the appropriate moment for preventive and/or early interventions for behavioral issues. For this reason, the present thesis reports on studies using MRI to exploratively assess gray matter volume, white matter integrity, task-related brain activation, and intrinsic functional brain connectivity in children/adolescents with 47,XXY. Because of the reported social problems in this population, and the increased risk of developing ASD, imaging results from the 47,XXY sample were not only contrasted with typically developing boys, but also with a sample of boys with idiopathic ASD.

Outline of the present thesis

Participants and design

All studies in the present thesis included three samples of participants: one group consisted of boys with 47,XXY, one group consisted of boys with idiopathic ASD, and one group consisted of non-clinical, male controls. All participants were between the ages of nine and eighteen at the time of the studies. The 47,XXY group was recruited using various strategies, to avoid recruitment bias as much as possible. The sample consisted of boys who were actively followed up after prenatal diagnosis with the help of clinical genetics departments in the Netherlands and Belgium, as well as boys 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). A number of boys with 47,XXY received supplemental testosterone treatment at the time of the study. Detailed information regarding characteristics of the 47,XXY group is reported in the respective chapters.

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 any of the scales of the Child Behaviour Checklist (CBCL) (Achenbach, 1991).

Inclusion criteria for all participants were Dutch as primary language, and an age between nine and eighteen years. Exclusion criteria were a recent history of substance abuse, intellectual disability (<60 IQ points), scan or motion artifacts (i.e. mean displacement >5 mm), as well as neurological conditions (e.g. structural brain damage due to prenatal/birth complications, tumors, strokes or diseases affecting the central nervous system). Details regarding the procedure for each study are provided in the respective chapters.

In **chapter two** we report on the volume of gray matter structures related to social information processing in all three samples, using voxel-based morphometry. Based on earlier findings, the brain regions of interest were the superior temporal cortex, amygdala, orbitofrontal cortex, insular cortex, and medial frontal cortex. In **chapter three** we evaluate white matter microstructure, using tract-based spatial statistics, to assess if deviations in brain structure also extend to white matter. Fractional anisotropy (an expression of the directionality of white matter tracts), radial diffusivity (an indication of myelination), axial diffusivity (an indication of axonal integrity), and mean diffusivity (the average diffusion of water within white matter tracts) were assessed. Taken together, these measures provide a quantification of white matter integrity. In **Chapter four** we focus on brain activation during two aspects of social-cognitive information processing: recognizing/matching facial expressions, and assigning a verbal label to facial expressions. Although both 47,XXY and ASD are associated with deficits in processing facial expressions this study used fMRI to specify this impairment, by assessing differences in neural activation patterns during these two different aspects of facial affect processing. In **chapter five** we assess intrinsic functional brain connectivity, using resting state fMRI. This allows for investigation of the presence of deviations in intrinsic functional brain connectivity that may underlie some of the cognitive and behavioral impairments associated with 47,XXY. In **chapter six** the main findings are summarized and integrated. The scientific and clinical implications of deviations in brain structure and function in 47,XXY, and differences in brain structure and function between 47,XXY and ASD, are discussed. Lastly, limitations and directions for future research are provided.

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Chapter 2

Goddard, M.N., Swaab, H., Rombouts S.A.R.B., & Van Rijn, S. (in press). Neural systems for social cognition: Gray matter volume abnormalities in boys at high genetic risk of autism symptoms, and a comparison with idiopathic autism spectrum disorder. *European Archives of Psychiatry and Clinical Neuroscience.*

Neural systems for social cognition: gray matter volume abnormalities in boys at high genetic risk of autism symptoms, and a comparison with idiopathic autism spectrum disorder

Abstract

Klinefelter syndrome (47,XXY) is associated with several physical, cognitive, and behavioral consequences. In terms of social development, there is an increased risk of autism symptomatology. However, it remains unclear how social deficits are related to abnormal brain development and to what degree underlying mechanisms of social dysfunction in 47, XXY are similar to, or different from, those in idiopathic autism (ASD). This study was aimed at investigating the neural architecture of brain structures related to social information processing in boys with 47,XXY, also in comparison to boys with idiopathic ASD. MRI scans of sixteen boys with 47,XXY, sixteen with ASD, and sixteen non-clinical, male controls were analyzed using voxel-based morphometry (VBM). A region of interest mask containing the superior temporal cortex, amygdala, orbitofrontal cortex (OFC), insular cortex, and medial frontal cortex was used. The Social Responsiveness Scale (SRS) was used to assess degree of autism spectrum symptoms. The 47,XXY group could not be distinguished from the ASD group on mean SRS scores, and their scores were significantly higher than in controls. VBM showed boys with 47,XXY have significant gray matter volume reductions in the left and right insula, and the left OFC, compared with controls and boys with ASD. Additionally, boys with 47,XXY had significantly less gray matter in the right superior temporal gyrus than controls. These results imply social challenges associated with 47,XXY may be rooted in neural anatomy, and autism symptoms in boys with 47,XXY and boys with ASD might have, at least partially, different underlying etiologies.

Introduction

Klinefelter syndrome (47,XXY) is a genetic condition in which boys have an additional X chromosome, leading to the 47,XXY chromosomal pattern. Physical consequences include gonadal hormone deficiency, infertility, and long extremities (Bojesen, Juul, & Gravholt, 2003). Many studies have focused on the physical, cognitive, and behavioral consequences of 47,XXY (Boada, Janusz, Hutaff-Lee, & Tartaglia, 2009; D. H. Geschwind, Boone, Miller, & Swerdloff, 2000; Leggett, Jacobs, Nation, Scerif, & Bishop, 2010). In recent years, attention has also shifted to the neural basis underlying cognitive and behavioral characteristics of 47,XXY. Structural imaging studies thus far indicate that compared with non-clinical controls, males with 47,XXY have decreased total brain volume, enlarged ventricles, as well as smaller caudate, cerebellar, temporal and frontal volumes (Giedd et al., 2007; Giltay & Maiburg, 2010; Itti et al., 2006; Patwardhan, Eliez, Bender, Linden, & Reiss, 2000; Steinman, Ross, Lai, Reiss, & Hoeft, 2009; Warwick et al., 1999). Other studies have reported regions

of decreased gray matter in the amygdala, insular cortex, hippocampus, cingulate, occipital lobe, parietal lobe, temporal pole, inferior frontal lobe and superior temporal gyrus (STG) in males with 47,XXY (Bryant et al., 2011; DeLisi et al., 2005; Shen et al., 2004; Skakkebaek et al., 2013; Steinman et al., 2009). Additionally, functional MRI studies have reported deviant neural activation in the superior temporal gyrus, superior temporal sulcus, supramarginal gyrus, amygdala, insula, fusiform gyrus, and middle frontal gyrus (Brandenburg-Goddard, Van Rijn, Rombouts, Veer, & Swaab, 2014; Van Rijn et al., 2008; Van Rijn et al., 2012).

One would expect that these brain abnormalities give rise to cognitive difficulties such as language and reading disorders, executive dysfunction, and social cognitive deficits. These problems have indeed been reported in 47,XXY (Boada et al., 2009; Lee et al., 2011; Van Rijn, Aleman, De Sonneville, & Swaab, 2009; Van Rijn et al., 2007; Van Rijn, Stockmann, Van Buggenhout, Van Ravenswaaij-Arts, & Swaab, 2014; Van Rijn, Swaab, Aleman, & Kahn, 2006). A few of the main social cognitive deficits associated with 47,XXY are difficulties in facial affect recognition, identification and verbalization of emotions, theory of mind, and interpreting tone of voice (Van Rijn et al., 2007; Van Rijn, Stockmann, Van Buggenhout, et al., 2014; Van Rijn et al., 2006) Such neural and cognitive deficits may help explain an increased risk of developmental psychopathology that is reported in individuals with 47,XXY, including psychotic disorders, bipolar disorder, ADHD, and autism spectrum disorder (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; Van Rijn & Swaab, 2011). A formal ASD diagnosis requires clinically significant and persistent deficits in social communication and social interaction, as well as restricted, repetitive patterns of behavior, interests, or activities (A.P.A., 2013). However, even in the absence of a formal ASD diagnosis prominent social and communicative difficulties may be present. Difficulty in social interactions, shyness, social withdrawal, problems with assertiveness, and increased levels of autistic traits (as measured with the Social Responsiveness Scale) (Constantino & Gruber, 2005) have been reported (Bishop et al., 2011; Tartaglia et al., 2010; Van Rijn, Stockmann, Borghgraef, et al., 2014; Van Rijn et al., 2006; Van Rijn et al., 2012). As the development of social competence during childhood is essential for successful participation in a relatively complex and fast-paced society as an adult, it is important to gain insight into the mechanisms underlying social dysfunction in 47,XXY.

Although there is overlap in social behavioral problems between individuals with 47,XXY and individuals with ASD, recent studies have highlighted variability in the underlying mechanisms driving these problems. For example, boys with 47,XXY show more social anxiety than boys with ASD (Van Rijn, Stockmann, Borghgraef, et al., 2014), and they display a specific cluster of autism symptoms that differs from idiopathic ASD in the ability to employ

social verbalization/chat, offering to share objects, and interest in other children (Bruining et al., 2010). Additionally, theory of mind impairments in 47,XXY appear to be related to executive dysfunction, while these impairments appear to be related to language and face recognition problems in boys with ASD (Van Rijn, Stockmann, Van Buggenhout, et al., 2014). In line with these results, both boys with 47,XXY and boys with ASD show deviations in neural networks associated with facial affect labeling. However, while these deviations are located in the amygdala in boys with ASD, boys with 47,XXY show deviant frontal activation (Brandenburg-Goddard et al., 2014). It is important to also study the underlying mechanisms in terms of brain development. In this respect, it would be relevant to know to what degree neural architecture (in terms of gray matter volume) of structures related to social functioning is affected in boys with 47,XXY, and to assess to what degree morphological deviations are similar to, or different from, boys with ASD.

Many different brain structures are involved in decoding social stimuli. For instance, the basic processing of facial information (e.g. gaze shifts and mouth movements, important aspects of emotional expressions), and the recognition of biological motion, involve the superior temporal cortex (i.e. both the superior temporal gyrus and sulcus). The amygdala is important for more complex social judgments based on facial information, assessing the significance of social information, and recognizing threat or danger (Adolphs, 2003). The orbitofrontal cortex (OFC) is involved in many aspects of social competence and social-cognitive information processing including the evaluation of sensory stimuli, reward and punishment related behavior, social decision making, theory of mind, self-reflection, and the representation of facial expressions and identity (Noonan, Sallet, Rudebeck, Buckley, & Rushworth, 2010; Rolls, 2004; Rolls & Grabenhorst, 2008). The insular cortex mediates recognition of, and responses to, emotional stimuli, regulation of autonomic states related to emotional processes, as well as the representation of one's internal state (Adolphs, 2003). Lastly, a structure involved in more complex interpretation of social-cognitive stimuli including face familiarity, theory of mind, and the distinction between self and other, is the medial frontal cortex (Amodio & Frith, 2006).

The current study is based on the hypothesis that the anatomical maturation of this network may be adversely affected in boys with an extra X chromosome. Imaging techniques may help uncover different etiologies of risk of social dysfunction. Nevertheless, structural imaging studies focusing on males with 47,XXY are relatively sparse . In contrast, many studies have addressed structural brain abnormalities in regions related to social functioning in individuals with ASD. A recent review evaluated structural imaging studies in ASD. Relevant to the current study, lower gray matter volume in the superior temporal cortex and higher gray matter volume in the medial frontal cortex were systematically reported in boys with ASD compared with non-clinical, age-matched, male controls (Chen, Jiao, & Herskovits, 2011). However, to date, no

studies have assessed regional morphologic brain differences between boys with 47,XXY and boys with ASD, which would be very relevant considering the differences in brain activation during social information processing between boys with 47,XXY and boys with ASD that were reported in a recent fMRI study (Brandenburg-Goddard et al., 2014).

The main aim of this study was to assess the neural architecture of brain regions associated with social functioning in boys with 47,XXY and boys with ASD, using voxel-based morphometry. The focus was on the superior temporal cortex, amygdala, OFC, insular cortex, and medial frontal cortex. Significant differences would suggest social dysfunction in Klinefelter syndrome may be anchored in anatomical brain deviations**.** The secondary aim was to investigate gray matter volumetric differences between boys with 47,XXY and boys with ASD in these structures, to assess if neural architecture of brain areas important for social information processing are differentially affected.

Methods

Participants

Sixteen boys with 47,XXY, sixteen boys with ASD, and sixteen non-clinical, male controls between the ages of nine and eighteen were included in analyses. Analysis of variance (ANOVA) did not reveal a significant effect of group on age [$F(2,45)=.573$, $p=.568$]. Participants completed the Block Design and Vocabulary subtests of the Dutch adaptations of the Wechsler Scales (WAIS-III and WISC-IV) (Wechsler, 1997, 2005). The subtest Vocabulary measures the degree to which one has learned, is able to comprehend, and verbally expresses vocabulary. The subtest Block Design measures spatial perception, visual abstract processing, and problem solving. These two subtests form the V-BD short form. The V-BD short form is often used to estimate full scale IQ (FSIQ) according to the algorithm (2.9*(sum of normed scores)+42) (Campbell, 1998). The V-BD short form correlates highly with WISC full scale IQ ($r=.88$) (HerreraGraf, Dipert, & Hinton, 1996) and has been found valid for the estimation of intelligence, with good reliability ($r=.91$) and validity (.82) (Campbell, 1998). There was a significant effect of group on IQ [$F(2,44)=11.73$, $p<.001$], with the 47,XXY (n=15) group having a significantly lower IQ than both controls (n=16) and the ASD group (n=16) ($p<.001$ in both instances). There was also a significant effect of group on internalizing problems [$F(2,36)=10.66$, $p<.001$], externalizing problems [$F(2,36)=9.47$, $p<.001$], and total problems [$F(2,36)=17.76$, $p<.001$] (as measured by the Child Behaviour Checklist (Achenbach, 1991)). The 47,XXY (n=14) and ASD (n=11) groups had significantly more internalizing problems than controls (n=14) ($p=.001$ in both instances), significantly more externalizing problems than controls ($p=.027$ and $p<.001$, respectively), and significantly more total problems than controls ($p<.001$ in both instances). Background variables are summarized in table 1. In

the 47,XXY group four participants received testosterone replacement therapy at the time of the study, while twelve did not. In the ASD group none of the participants used psychiatric medication at the time of the study.

	Age	IQ	CBCL int.	CBCL ext.	CBCL tot.
47,XXY	13.2(2.3)	80(12.6)	18.64(8.4)	10.6(8.6)	55.6(20.1)
ASD	12.8(1.7)	102(16)	19.09(13.8)	15.3(8)	68.1(34.9)
Control	12.4(2.4)	103(16.1)	4.71(3.9)	3.4(2.9)	16.7(10.5)

Table 1. Summary of means and standard deviations on background variables. CBCL int.=total score on 'internalizing problems' scale of Child Behaviour Checklist, CBCL ext.=total score on 'externalizing problems' scale of Child Behaviour Checklist, CBCL tot.=total score on 'total problems' scale of Child Behaviour Checklist.

The 47,XXY group was recruited using various strategies, to avoid recruitment bias as much as possible. The sample consisted of nine children who were actively followed up after prenatal diagnosis with the help of clinical genetics departments in the Netherlands and Belgium, as well as seven 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 any of the scales of the Child Behaviour Checklist (CBCL) (Achenbach, 1991).

Inclusion criteria for all participants were Dutch as primary language and an age between ten and eighteen years. Exclusion criteria were a recent history of substance abuse, intellectual disability (<60 IQ points), scan or motion artifacts (i.e. mean displacement >5 mm), 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 Declaration of Helsinki. 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. Participants underwent anatomical scanning while watching an animated cartoon.

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 a movie. All anatomical scans were reviewed and cleared by a radiologist. No anomalous findings were reported.

Outcome measures

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 Autism Diagnostic Interview-Revised (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.

Voxel-Based Morphometry

Structural data was analyzed using FSL-VBM (Douaud et al., 2007) (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSLVBM), an optimized VBM protocol (Good et al., 2001) carried out with FSL tools (Smith et al., 2004). First, structural images were brain-extracted and gray matter-segmented before being

registered to the MNI 152 standard space using non-linear registration (Andersson, Jenkinson, & Smith, 2007). The resulting images were averaged and flipped along the x-axis. The mirror images were then averaged to create a left-right symmetric, study-specific gray matter template. Second, all native gray matter images were non-linearly registered to this study-specific template and "modulated" to correct for local expansion (or contraction) due to the non-linear component of the spatial transformation. The modulated gray matter images were then smoothed with an isotropic Gaussian kernel with a sigma of 3 mm. Finally, voxel-wise GLM was applied using permutation-based non-parametric testing, correcting for multiple comparisons across space using threshold-free cluster enhancement (TFCE). Because of the specific hypotheses regarding volumetric differences in brain structures involved in social-cognitive information processing, a region of interest mask consisting of the superior temporal cortex, amygdala, OFC, insular cortex, and medial frontal cortex was used. All groups were mutually compared, meaning six contrasts were set up: 47,XXY<HC, 47,XXY>HC, 47,XXY<ASD, 47,XXY>ASD, ASD<HC, and ASD>HC.

Results

Autism spectrum symptoms

SRS total scores were available for fourteen boys in the control group [M_{srs}=23.4 (*SD*=12.7)], thirteen in the 47,XXY group [M_{srs}=73.8 (*SD*=25.0)] and fifteen in the ASD group [M_{srs}=94.2 (*SD*=39.5)]. A significant effect of group on SRS scores was found [$F(2,37)=23.59$, $p<.001$], for which Bonferroni post-hoc testing showed this was due to significantly higher mean scores in both the 47,XXY ($p<.001$) and ASD ($p<.001$) groups compared with controls. No significant difference in mean scores between the 47,XXY and ASD groups was found.

Voxel-based morphometry

Significant TFCE-based thresholding results are summarized in Table 1. The 47,XXY group had significantly less gray matter in the left and right insular cortices ($p<0.001$), the left OFC ($p<0.01$), and the right STG ($p<0.05$), compared with controls (Figure 1). Additionally, the 47,XXY group had significantly less gray matter in the left (p=0.001) and right insular cortices ($p=0.002$), as well as in the left OFC ($p=0.001$), compared with the ASD group (Figure 2). Other contrasts did not result in significant differences between groups.

Contrast	# voxels	Volume (mm³)	Max t	Location
47,XXY<NCC	1414	11312	5.83	Left insular cortex
	1197	9576	6.02	Right insular cortex
	450	3600	4.01	Left OFC
	26	224	4.39	Right STG
47,XXY<ASD	1429	11936	5.17	Left OFC
				Left insular cortex
	340	2720	5.64	Right insular cortex

Table 2. Characteristics of clusters of significantly differing gray matter volume. OFC=orbitofrontal cortex; STG=superior temporal gyrus; NCC=non-clinical controls; ASD=autism spectrum disorders

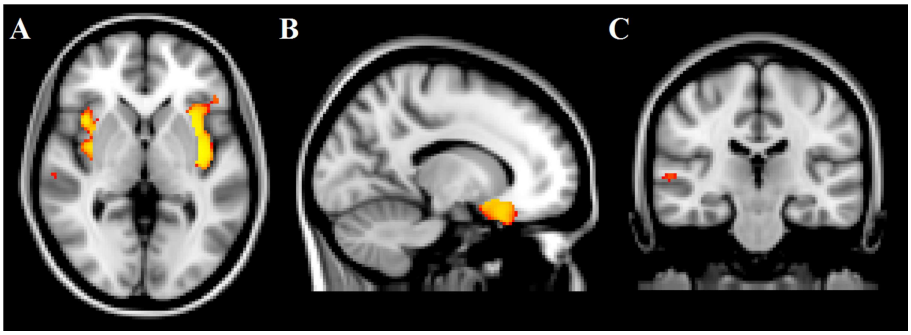

Figure 1. Clusters of significantly deviating gray matter volume. A. 47,XXY<controls left and right insular cortices; B. 47,XXY<controls left orbitofrontal cortex; C. 47,XXY<controls right superior temporal gyrus.

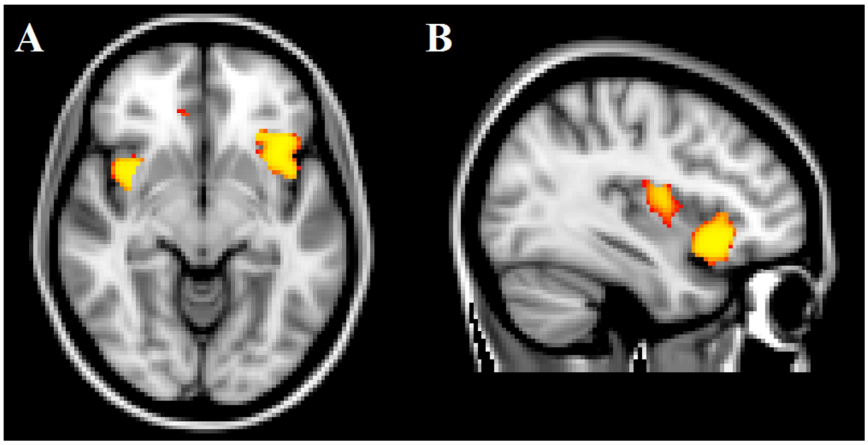

Figure 2. Clusters of significantly deviating gray matter volume. A. 47,XXY<ASD left and right insular cortices; B. 47,XXY<ASD left orbitofrontal cortex. ASD=autism spectrum disorders.

Discussion

In this imaging study the neural architecture of brain regions associated with social functioning, i.e. the superior temporal cortex, amygdala, orbitofrontal cortex (OFC), insular cortex, and medial frontal cortex, was investigated in boys with 47,XXY, compared with boys with ASD and non-clinical, male controls. The results showed that boys with 47,XXY have significant gray matter volume reductions in the left and right insular cortices, as well as the left OFC, compared with healthy controls. These structures were also found to be significantly smaller in boys with 47,XXY than in boys with ASD. Additionally, boys with 47,XXY had significantly smaller gray matter volumes in the right superior temporal gyrus (STG) compared with controls.

In general, the OFC is associated with the evaluation of sensory stimuli, reward and punishment related behavior, social decision making, and the representation of facial expressions and identity (Noonan et al., 2010; Rolls, 2004; Rolls & Grabenhorst, 2008). Importantly, the OFC is also involved in processing mental state concepts and self-reflection (Baron-Cohen et al., 1994), related to both theory of mind and emotional literacy. Theory of mind impairments and alexithymia have both been reported in boys with 47,XXY (Van Rijn, Stockmann, Van Buggenhout, et al., 2014; Van Rijn et al., 2006). Although speculative, the OFC volume reductions reported in the current study might be one of the anatomical underpinnings of these problems. Previous studies have not reported volume reductions in the OFC specifically, but reduced gray matter in frontal areas has been found repeatedly (DeLisi et al., 2005; Giedd et al., 2007; Giltay & Maiburg, 2010; Skakkebaek et al., 2013; Steinman et al., 2009). Additionally, one study reported reductions in white matter regions near the OFC in their sample (Bryant et al., 2011).

The insular cortex is involved in recognition of and responses to emotional stimuli, regulation of autonomic states related to emotional processes, as well as the representation of one's internal state (Adolphs, 2003). The finding of deviating gray matter volume in the insular cortex fits with the finding that individuals with 47,XXY show problems in identification and verbalization of emotional states, are more easily emotionally aroused than controls, and are significantly more influenced by their emotional states than controls when making decisions (Van Rijn, Barendse, Van Goozen, & Swaab, 2014; Van Rijn et al., 2006). Another study suggests the insular cortex is functionally involved in decision making processes (Sanfey, Rilling, Aronson, Nystrom, & Cohen, 2003), and an fMRI study using a social judgment task reported less neural activation in the insula in individuals with 47,XXY (Van Rijn et al., 2012). The bilateral reductions in insular cortex volume found in the current study are in line with results from earlier structural studies (Bryant et al., 2011; Shen et al., 2004; Skakkebaek et al., 2013), and might be an anatomical underpinning of these functional findings.

The STG is involved in the processing of facial information, e.g. small changes in facial expression and gaze direction (Adolphs, 2003). The finding of reduced right STG volume is in line with earlier studies reporting impairments in face processing in individuals with 47,XXY (Van Rijn et al., 2006; Van Rijn et al., 2012). Additionally, Wernicke's area and Heschl's gyri are part of the Wernicke-Geschwind model of language processing, and are located in the STG, making it a key structure in this model (Bigler et al., 2007; N. Geschwind, 1972). Although language functions are usually located in the left hemisphere, a functional MRI study reported decreased functional asymmetry in the STG during a language task in individuals with 47,XXY (Van Rijn et al., 2008), due to increased activation in the right hemisphere. This suggests individuals with 47,XXY may have decreased left-sided dominance for language, which could imply the Wernicke-Geschwind model of language processing also involves right-sided brain regions in this condition. The reported deviations in STG function (Van Rijn et al., 2008) support the suggestion of a relationship between anatomical deviations and altered functionality. Earlier structural MRI studies consistently reported abnormalities in the temporal lobe as a whole in individuals with 47,XXY (Giedd et al., 2007; Giltay & Maiburg, 2010; Itti et al., 2006; Steinman et al., 2009). In line with other studies (Bryant et al., 2011; DeLisi et al., 2005; Patwardhan et al., 2000; Shen et al., 2004), the current study suggests these abnormalities may be located specifically in the STG.

There has been much debate within the field of neuroscience about the relationship between brain anatomy and cognitive and behavioral function in general. This debate also extends to the field of 47,XXY, with the largest reported study by Skakkebaek et al. (2013) finding no association between specific differences in gray matter volume and the neuropsychological profile of 47,XXY. It is important to continue research in this area, to gain more insight into the impact of individual brain structures on behavioral function. In addition to assessing linear relationships between volume differences in individual brain structures and specific cognitive or behavioral problems, it may also prove essential to focus on neural networks. The deviations found in the current study (that focused on specific regions of interest) may very well be part of a larger network of structural and functional brain deviations, that together lead to the cognitive and behavioral issues associated with 47,XXY. Imaging studies like this may have important theoretical implications, as they offer the opportunity for understanding the underlying mechanisms along a gene-brain-behavior pathway. The results are in line with the notion that a high number of X chromosome genes are expressed in the brain (Lenroot, Lee, & Giedd, 2009). It has been suggested that X chromosome genes are involved in sexually dimorphic brain development (Raznahan, Probst, Palmert, Giedd, & Lerch, 2013), making it more likely that individuals with sex chromosome disorders, such as 47,XXY, show deviations in brain anatomy. Another possibility is that these deviations are the result of gene-gene interactions, or hormonal influences on brain development. Future studies focused on these mechanisms may aid in

disentangling these complex issues. However, environmental factors may also have a marked influence on shaping the brain (McEwen, 2012), stressing the notion that a deterministic approach towards brain development in 47,XXY is not useful. Instead, early positive experiences and interventions (e.g. Theory of Mind training (Steerneman, Jackson, Pelzer, & Muris, 1996) or social skills training) may have a positive influence on brain development and may even (partially) ameliorate or prevent severe social dysfunction later in development. Neuroimaging may not only help in identifying targets for treatment, but may also offer sensitive tools to design and assess the effectiveness of such interventions in 47,XXY.

The secondary aim of this study was to investigate gray matter volumetric differences between boys with 47,XXY and boys with ASD in brain regions associated with social functioning. The SRS scores suggest boys with 47,XXY have increased levels of autism symptoms and they could not be distinguished from boys with ASD in terms of overall level of these symptoms, but they showed significant reductions in left and right insular cortex volume, as well as the left OFC, compared with boys with ASD. These findings support the notion that the autism spectrum may be extraordinarily heterogeneous. Various neural etiologies may result in similar behavioral symptoms that are classified as autistic. Because boys with 47,XXY represent a genetically homogenous high risk group for ASD, these findings might help in identifying biomarkers of social dysfunction related to X chromosomal genes, although much more research is needed in this area. The current findings may stimulate such lines of research.

A limitation of the current study was the relatively small sample size. Additionally, the 4,XXY sample appeared to consist of boys with more severe behavioral issues than samples from earlier studies, e.g. Tartaglia et al. (2010), as demonstrated by higher scores on the SRS. It is possible that parents of more severely affected boys with 47,XXY were more motivated to participate in neuroimaging research. However, the behavioral characteristics of the current sample were similar to those of the larger cohort from which they were drawn (for an extensive behavioral description of this cohort, see Van Rijn, Stockmann, Borghgraef, et al. (2014)). In addition, there may be cultural differences in the prevalence of behavioral issues associated with 47,XXY, due to differences in mental health care systems, including protocols for interventions or support over the course of development. Voxel-based morphometry has some inherent limitations, e.g. individual differences in cortical folding patterns influencing the results. The results of the current study are in line with those from earlier imaging studies on 47,XXY, with the exception of a lack of significant differences in amygdala volume. These have been reported in earlier neuroimaging studies (Skakkebaek et al., 2013; Steinman et al., 2009), but were not present in our sample. It is possible this is due to age-related changes in brain morphology. However, the fact that the significant differences that were found in the current study are similar to those from earlier studies in adult

individuals with 47,XXY, seems to suggest deviations in brain anatomy develop early and are relatively stable over time. It is therefore possible that differences in imaging analysis methodology or lack of statistical power have led to discrepancies between volume deviations in the amygdala in earlier studies, and a lack of such deviations in the current sample. Furthermore, the lack of significant volumetric differences between the ASD group and controls may imply they were relatively high functioning. IQ scores seem to corroborate this suggestion. A problem inherent to participating in imaging research is that typically, only a selective subgroup of ASD participants (e.g. those with high functioning autism) are included in a study. Additionally, findings regarding structural brain abnormalities in ASD are often conflicting and depend on many (study specific) factors, such as inclusion and exclusion criteria, MR acquisition parameters, and details of the image processing pipeline (Chen et al., 2011). In the current study small sample size may have played a role, although the 47,XXY group was equally small but did show significant volume reductions. Lastly, because this study was explorative in nature and the number of contrasts was limited to six, no correction for multiple comparisons was applied. However, all but one significant result would have survived such a correction.

In sum, the current study suggests boys with 47,XXY with overt social behavioral problems have abnormalities in neural architecture in specific brain regions associated with social functioning, i.e. the insular cortices, OFC, and STG. This implies the X chromosome may significantly impact brain morphology, and the social problems individuals with 47,XXY encounter may be rooted in neural anatomy. Additionally, the differences between boys with 47,XXY and boys with ASD highlight the idea that for understanding developmental risk of social dysfunction in children with 47,XXY, it is important to not only rely on behavioral observations. Brain-behavior relationships are very complex, and social problems may arise as a consequence of different dysfunctions, not only in children with 47,XXY, but possibly also in children with idiopathic ASD. This suggests it is essential to also gain understanding of neuropsychological and neurobiological mechanisms underlying social problems.

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Chapter 3

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

White matter microstructure in a genetically defined group at high risk of autism symptoms, and a comparison with idiopathic autism

Abstract

Klinefelter syndrome (47,XXY) is associated with physical, behavioral, and cognitive consequences. Deviations in brain structure and function have been reported, but structural characteristics of white matter have barely been assessed. This diffusion tensor imaging study assessed white matter microstructure in boys with 47,XXY compared with non-clinical, male controls. Additionally, both similarities and differences between 47,XXY and autism spectrum disorders (ASD) have been reported in cognition, behavior and neural architecture. To further investigate these brain-behavior pathways, white matter microstructure in boys with 47,XXY was compared to that of boys with ASD. Fractional anisotropy (FA), radial diffusivity (Dr), axial diffusivity (Da), and mean diffusivity (MD) were assessed in 47,XXY (n=9), ASD (n=18), and controls (n=14), using tract-based spatial statistics. Compared with controls, boys with 47,XXY have reduced FA, coupled with reduced Da, in the corpus callosum. Boys with 47,XXY also have reduced Dr in the left anterior corona radiata and sagittal striatum compared with controls. Compared with boys with ASD, boys with 47,XXY show reduced Da in the right inferior fronto-occipital fasciculus. Reduced white matter integrity in the corpus callosum may be a contributing factor in the behavioral problems associated with 47,XXY. Reduced Dr implies enhanced myelination, which could hypothetically be the result of hormone treatment, or the additional X chromosome. If so, it would be an important protective factor associated with 47,XXY that warrants further research. Differences between boys with 47,XXY and those with ASD, provide additional evidence for possible variability in mechanisms underlying similar behavioral problems.

Introduction

Boys with 47,XXY (often referred to as Klinefelter syndrome) are born with an extra X chromosome. It affects approximately 1 in 650 newborns (Bojesen, Juul, & Gravholt, 2003) and although it is not associated with gross facial or physical abnormalities, a range of physical, behavioral, and cognitive consequences may be present, to varying degrees (Giltay & Maiburg, 2010; Groth, Skakkebaek, Host, Gravholt, & Bojesen, 2013). Physical consequences include tall stature, fertility problems, and endocrinological imbalances (Lanfranco, Kamischke, Zitzmann, & Nieschlag, 2004; Ross et al., 2005). Behaviorally, an increased risk of psychopathology is often reported. To illustrate, elevated incidences of bipolar disorder, attention-deficit/hyperactivity disorder, and autism spectrum disorders are found among individuals with 47,XXY (Bishop et al., 2011; Bruining, Swaab, Kas, & Van Engeland, 2009; Cederlof et al., 2014; Tartaglia, Cordeiro, Howell, Wilson, & Janusz, 2010; Van Rijn & Swaab, 2011). Cognitive

problems associated with 47,XXY are heterogeneous and range from subtle to quite pronounced. Intellectual functioning at the lower end of the normal range, language impairment, and executive dysfunction are among the most often reported cognitive characteristics of 47,XXY (Boada, Janusz, Hutaff-Lee, & Tartaglia, 2009; Geschwind, Boone, Miller, & Swerdloff, 2000; Leggett, Jacobs, Nation, Scerif, & Bishop, 2010; Verri, Cremante, Clerici, Destefani, & Radicioni, 2010). However, it has remained largely unclear what the neural mechanisms are that underlie cognitive impairment and the increased risk of behavioral problems in 47,XXY.

From a neuroscientific perspective, deviations in structure and function of the brain are central to the understanding of both psychopathology and cognitive impairment. It is likely that the genetic effects of 47,XXY on cognition and behavior are mediated by the structure and function of the brain, underlining the importance of studying the neural mechanisms associated with this condition. In recent years, advances in magnetic resonance imaging (MRI) analysis methodology have led to increased knowledge of brain structure and function in 47,XXY (Mueller, 2013; Reiss, Eliez, Schmitt, Patwardhan, & Haberecht, 2000). This condition is associated with deviations in gray matter volume, and functionality of language and social-cognitive brain regions appears to be abnormal (Brandenburg-Goddard, Van Rijn, Rombouts, Veer, & Swaab, 2014; Bryant et al., 2011; Skakkebaek et al., 2013; Steinman, Ross, Lai, Reiss, & Hoeft, 2009; Van Rijn et al., 2008; Van Rijn et al., 2012). These types of studies provide better understanding of the neural mechanisms associated with the cognitive and behavioral consequences associated with 47,XXY, contributing to the aim of understanding gene-brain-behavior pathways.

While knowledge regarding deviations in gray matter volume associated with 47,XXY has become increasingly extensive, there is a growing awareness among neuroscientists of the importance of insight into connections between brain areas. Studying neural connectivity is crucial to understanding how information is processed in the brain, and thus to delineating the neural mechanisms associated with the cognitive and behavioral consequences of 47,XXY. One way of looking at brain connectivity, is studying the integrity of neural fiber tracts connecting neurons in different parts of the brain. This provides insight into how adequately neural signals are transmitted and thus how effectively various parts of the brain communicate. Diffusion tensor imaging (DTI) offers the possibility of measuring these tracts *in vivo*. As nearly fifty percent of the human brain is composed of white matter, which contributes substantially to both cognition and behavior (Filley, 2005), DTI may provide unique information regarding structural connectivity in 47,XXY. The only DTI study in 47,XXY thus far focused on adult males (DeLisi et al., 2005). Studying children with 47,XXY may aid in determining if deviations in brain connectivity develop early in life. Therefore, the primary aim of the current study was to exploratively assess

neural fiber tract integrity in boys with 47,XXY compared with non-clinical controls, using tract-based spatial statistics (TBSS).

Secondarily, it may be important to assess how deviations in white matter integrity in 47,XXY compare to other (neurodevelopmental) conditions. There is now substantial empirical evidence showing parallels between behavioral symptoms associated with 47,XXY, and those associated with autism spectrum disorders (ASD) (Cederlof et al., 2014; Cordeiro, Tartaglia, Roeltgen, & Ross, 2012; Tartaglia et al., 2010). However, the specific manifestation of social problems, as well as the underlying cognitive and neural mechanisms associated with social dysfunction, may be different (Brandenburg-Goddard et al., 2014; Bruining et al., 2010; Van Rijn, Stockmann, Borghgraef, et al., 2014; Van Rijn, Stockmann, Van Buggenhout, Van Ravenswaaij-Arts, & Swaab, 2014). The discrepancy between behavioral similarities and differences in underlying mechanisms between individuals with 47,XXY and individuals with ASD, highlights the need for more knowledge regarding overlap and differences in brain-behavior pathways in these conditions. In pursuit of this knowledge, the secondary aim of the current study was to compare neural fiber tract integrity in boys with 47,XXY, to that of boys with ASD. As this was the first study to assess white matter integrity in children with 47,XXY four measures (i.e. fractional anisotropy, radial diffusivity, axial diffusivity and mean diffusivity) are reported, to provide a comprehensive overview of neural fiber tract characteristics associated with this condition.

Methods and materials

Participants

DTI analyses were performed on a subsample of participants from the study by Brandenburg-Goddard et al. (2014b). Nine boys with 47,XXY [M_{age}=14.53 (*SD*=3.03)], eighteen boys with ASD [M_{age}=11.84 (*SD*=2.13)], and fourteen non-clinical, male controls [M_{age}=11.95 (*SD*=2.91)] were included in analyses. Analysis of variance (ANOVA) revealed a significant effect of group on age [$F(2,38)=3.575$, $p=.038$], for which post-hoc testing showed this was due to a significant age difference between the 47,XXY group and both the control group ($p=.027$), as well as the ASD group ($p=.016$). To control for age related differences in brain maturation, age was used as a confound regressor in DTI analyses. Within the 47,XXY group, four participants received supplemental testosterone treatment at the time of the study. Five participants did not receive supplemental testosterone treatment.

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 (CBCL) (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 Declaration of Helsinki. 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. All anatomical scans were reviewed and cleared by a radiologist. No anomalous findings were reported. DTI scans were acquired as part of an MRI

sequence including anatomical and functional scans, using a single-shot echo-planar imaging (EPI) sequence with the following parameters: TR = shortest, TE = 56 ms, flip angle 90°, b factor = 1000 s/mm², voxel dimensions = 2.3 mm isotropic, 73 slices, no slice gap. DTI scans were acquired along sixteen directions, together with a baseline imaging having no diffusion weighting (b=0). The total DTI acquisition time was approximately six minutes.

Outcome measures

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 Autism Diagnostic Interview-Revised (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.

DTI analysis

Preprocessing

The DTI data of all participants were preprocessed using FSL (FMRIB's Software Library, http://fsl.fmrib.ox.ac.uk/fsl/fslwiki) version 5.0.4 (Jenkinson, Beckmann, Behrens, Woolrich, & Smith, 2012; Smith et al., 2004a). Affine registration of each diffusion weighted image to the b=0 reference image was performed to correct for distortion and motion artifacts induced by eddy currents or head motions, followed by non-brain tissue removal (Smith, 2002). To generate individual FA, Dr, Da, and MD maps for each participant, the diffusion tensor model was fitted to each voxel using FMRIB's Diffusion Toolbox (Behrens et al., 2003). For Da, the principal eigenvalue (L1) was used, for Dr the two minor eigenvalues (L2 and L3) were averaged. MD was calculated as the average of the three eigenvalues (L1, L2, and L3).

TBSS

Fractional anisotropy (FA), an expression of the directionality of white matter tracts, radial diffusivity (Dr), an indication of myelination, axial diffusivity (Da), an indication of axonal integrity, and mean diffusivity (MD), the average diffusion of water within white matter tracts, together provide a quantification of white matter integrity. Voxelwise statistical analysis of the FA data was carried out using TBSS (Smith et al., 2006), part of FSL (Smith et al., 2004b). First, FA images were created by fitting a tensor model to the raw diffusion data, and then brain-extracted (Smith, 2002). All participants' FA data were then aligned into a

common space using the nonlinear registration tool (Andersson, Jenkinson, & Smith, 2007), which uses a b-spline representation of the registration warp field. Next, the mean FA image was created and thinned to create a mean FA skeleton which represents the centres of all tracts common to the group. The mean FA skeleton was thresholded at an FA value of ≥0.35 to exclude peripheral tracts and minimize partial voluming. Each participant's aligned FA data was then projected onto this skeleton. Similarly, Dr, Da, and MD data were projected onto the skeleton using FA registration and skeleton projection parameters. The resulting FA, Dr, Da, and MD data were fed into voxelwise permutation-based analysis, using Randomise (Nichols & Holmes, 2002; Winkler, Ridgway, Webster, Smith, & Nichols, 2014), with a general linear model including four contrasts (47,XXY<CON and 47,XXY>CON for the first aim; 47,XXY<ASD and 47,XXY>ASD for the second aim), age as a confound regressor, and 5000 permutations, correcting for multiple comparisons across space using threshold-free cluster enhancement (TFCE, p<0.05) (Smith & Nichols, 2009).

Results

Autism spectrum symptoms

A significant effect of group on SRS scores was found [$F(2,31)=23.61$, $p<.001$], with mean scores in both the 47,XXY [$N=6$; $M_{srs}=74.2$ ($SD=32.5$)] ($p=.005$) and ASD [$N=14$; $M_{srs}=98.3$ ($SD=34.1$)] ($p<.001$) groups being significantly higher than in controls [$N=14$; $M_{srs}=27.6$ ($SD=15.0$)]. No significant difference in mean scores between the 47,XXY and ASD groups was found. In the 47,XXY group, T-scores suggested two participants scored in the normal range, one scored in the mild to moderate range, while three scored in the severe range. In the ASD group, T-scores suggested one participant scored in the normal range, three scored in the mild to moderate range, while ten scored in the severe range.

TBSS: 47,XXY versus controls

As summarized in Table 1 and depicted in Figure 1, whole-brain TBSS analysis revealed that, compared with controls, the 47,XXY group had significantly lower FA values in the body of the corpus callosum, coupled with significantly lower Da values in the genu of the corpus callosum. In addition, the 47,XXY group had significantly lower Dr values in the left anterior corona radiata and sagittal striatum. There were no significant differences in MD values between the 47,XXY group and controls.

TBSS: 47,XXY versus ASD

As summarized in Table 2 and depicted in Figure 2, whole-brain TBSS analysis revealed that, compared with the ASD group, the 47,XXY group had significantly lower Da values in the right inferior fronto-occipital fasciculus.

There were no significant differences in FA, Dr or MD values between the 47,XXY and ASD groups.

	#voxels	*p*	Max *t*	x,y,z	Location
FA	58	.016	4.56	11,-14,29	Body of corpus callosum
	51	.019	4.10	-7,-11,27	Body of corpus callosum
Dr	3027	.031	3.79	-18,16,32	L anterior coronaradiata
	730	.032	4.51	-43,-24,-15	Sagittal striatum
Da	242	.030	4.84	16,23,24	Genu of corpus callosum

Table 1. Characteristics of clusters of significantly reduced fractional anisotropy (FA), radial diffusivity (Dr), and axial diffusivity (Da) in 47,XXY relative to controls (TFCE corrected $p<0.05$)

	#voxels	*p*	Max *t*	x,y,z	Location
Da	241	.042	5.23	25,-80,10	Right inferior fronto-occipital fasciculus

Table 2. Characteristics of a cluster of significantly reduced axial diffusivity (Da) in 47,XXY relative to autism spectrum disorders (TFCE corrected $p<0.05$)

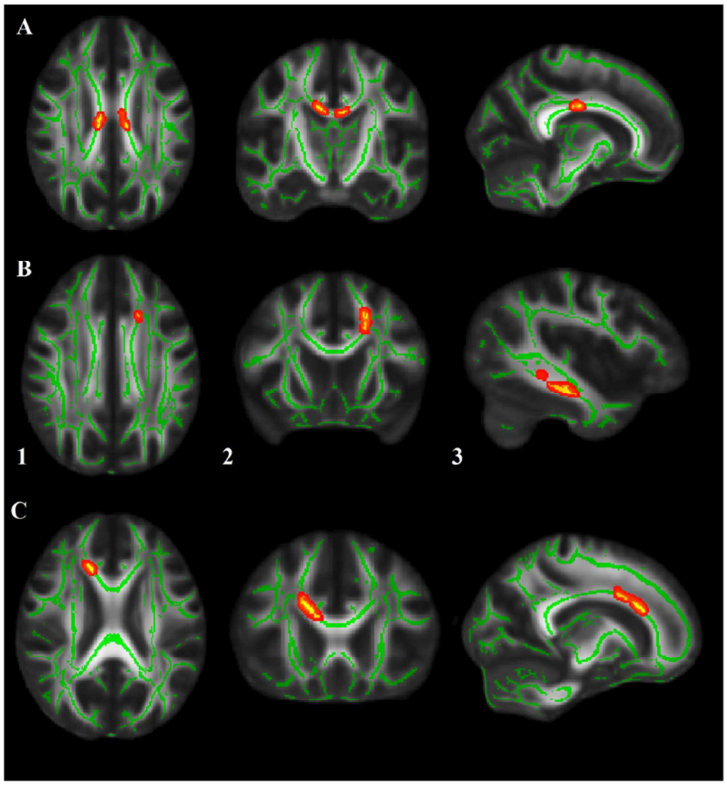

Figure 1. Whole-brain tract-based spatial statistics results, overlaid on axial, coronal and sagittal sections of the white matter skeleton (green), showing clusters (yellow/orange) of: A. reduced fractional anisotropy in 47,XXY relative to controls in the body of the corpus callosum; B. reduced radial diffusivity in 47,XXY relative to controls in (1+2) the left anterior corona radiata and (3) the sagittal striatum; C. reduced axial diffusivity in 47,XXY relative to controls in genu of the corpus callosum. For better visibility, results were thickened using the 'tbss-fill' command.

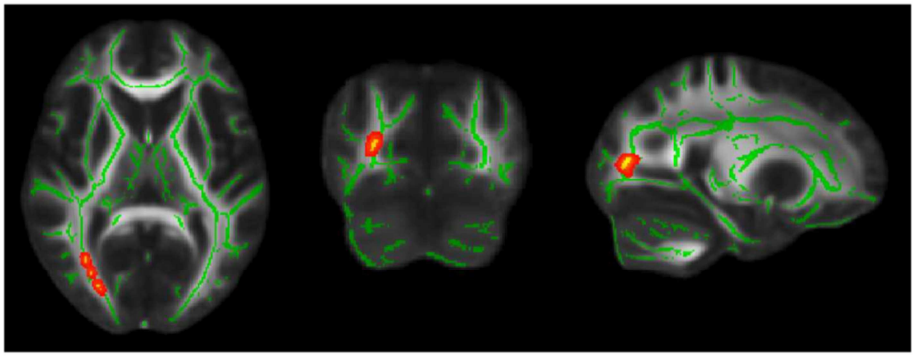

Figure 2. Whole-brain tract-based spatial statistics results, overlaid on axial, coronal and sagittal sections of the white matter skeleton (green), showing a cluster (yellow/orange) of reduced axial diffusivity in 47,XXY relative to autism spectrum disorders, in the right inferior fronto-occipital fasciculus. For better visibility, results were thickened using the 'tbss-fill' command.

Discussion

This diffusion tensor imaging (DTI) study used tract-based spatial statistics (TBSS) (Smith et al., 2006) to exploratively assess white matter integrity in boys with 47,XXY (also known as Klinefelter syndrome) relative to non-clinical controls, as well as boys with autism spectrum disorders (ASD). The results show that boys with 47,XXY have significantly reduced fractional anisotropy (FA) in the body of the corpus callosum compared with controls, coupled with significantly reduced axial diffusivity (Da) in the genu of the corpus callosum. In addition, boys with 47,XXY show significantly lower radial diffusivity (Dr) in the left anterior corona radiata, and sagittal striatum. No significant differences in mean diffusivity (MD) between boys with 47,XXY and controls were found. In comparison with boys with ASD, boys with 47,XXY show significantly reduced Da in the right inferior fronto-occipital fasciculus. No significant differences in FA, Dr, or MD were found between these groups.

The finding of reduced FA and Da in the corpus callosum in 47,XXY is important, as several hypotheses can be formulated regarding possible mechanisms underlying deficits associated with this condition, based on this result. In previous studies an association was found between reduced FA in the corpus callosum and reduced performance on bimanual motor coordination (Gooijers & Swinnen, 2014). Research in individuals with 47,XXY suggests motor dexterity is one of the domains of impairment associated with this condition (Boone et al., 2001). As this is a cognitive domain that is particularly related to the ability for cooperation between hemispheres, the current finding of reduced FA in the corpus callosum is in line with the behavioral phenotype associated with 47,XXY. Callosal abnormalities might be part of the mechanism leading to this deficit. In addition, callosal lesions have been associated with alexithymia and language problems (Devinsky & Laff, 2003). Although reduced FA and Da do not equal damaged white matter tracts, it is an indication of reduced integrity of white matter, and thus diminished efficiency of neural connections. Hypothetically, the alexithymia and language problems that have been reported in individuals with 47,XXY (Boada et al., 2009; Leggett et al., 2010; Van Rijn, Swaab, Aleman, & Kahn, 2006) might be related to reduced efficiency of interneuronal communication. In support of this hypothesis, in the intact brain the corpus callosum plays an important role in language functions, possibly through facilitation of interhemispheric communication between the left and right plana temporale (Bloom & Hynd, 2005; Van der Knaap & Van der Ham, 2011). Interestingly, the superior temporal gyri (part of the plana temporale) show decreased functional asymmetry in 47,XXY during language processing (Van Rijn et al., 2008). The results from the current study suggest this functional abnormality may be mediated by abnormalities in callosal integrity, and are an important starting point for future studies focusing on specific tract structures and their association with cognitive and behavioral measures.

The reductions in Dr seem counterintuitive at first, as clinical conditions are often accompanied by reduced myelin integrity expressed by increased Dr. However, steroid hormones enhance myelination in the human brain (Peper, Van den Heuvel, Mandl, Hulshoff Pol, & Van Honk, 2011). Hypothetically, testosterone treatment in individuals with 47,XXY could result in increased myelination, making it an important potential protective factor associated with this condition. Unfortunately, in the current study the 47,XXY group was too small to assess differences between boys who did receive treatment, and those who did not. However, the finding of reduced Dr in this group warrants further research focused on the potential neural benefits of testosterone treatment in 47,XXY. Alternatively, the gene for the myelin proteolipid protein has been assigned to the X chromosome (Willard & Riordan, 1985). Many factors are involved in determining the exact consequences of the presence of an extra X chromosome in 47,XXY (e.g. the pattern of gene inactivation), but the extra X in 47,XXY might influence myelin proteolipid protein function. Previous studies (Hodes 2000) suggest that additional copies of this protein may lead to severe somatic conditions. It is therefore unlikely that the enhanced myelination is a result of this gene simply escaping gene inactivation. However, studies in mice indicate that this gene may partially escape inactivation, which could hypothetically lead to overexpression. These results demonstrate the value of interdisciplinary research (e.g. neuroscience and clinical genetics) to further explore the possible effect of the additional X chromosome on the functioning of this protein. However, this hypothesis is strictly speculative, as studies investigating these genetic mechanisms in sex chromosome aneuploidies are currently lacking. The results from the current study may inspire future studies in this domain.

A cluster of significantly reduced Da was found in the right inferior fronto-occipital fasciculus in boys with 47,XXY, compared with boys with ASD. The function of this structure is subject to debate, but it has been implicated in a multitude of domains (e.g. sensory-motor integration, as well as semantic and emotional processing) (Sarubbo, De Benedictis, Maldonado, Basso, & Duffau, 2013). Although both boys with 47,XXY and boys with ASD may have similar social difficulties, results from the current study add to previous research suggesting there may be crucial differences in the specific manifestation of social problems and underlying cognitive and neural mechanisms (Brandenburg-Goddard et al., 2014; Bruining et al., 2010; Van Rijn, Stockmann, Borghgraef, et al., 2014; Van Rijn, Stockmann, Van Buggenhout, et al., 2014), one of which being white matter microstructure. These differences illustrate that it is important to study underlying (neural) mechanisms of social dysfunction. This not only aids in specifying the type of deficit, and in creating awareness that social dysfunction may arise as a consequence of various types of dysfunctions, but it may also have important clinical implications. Children with different pathways to social dysfunction require tailored treatment, which may be developed based on findings from these types of studies. Further research in this

area is necessary, preferably combining behavioral, neuroscientific, and (neuro)physiological data, to establish separate and specific (endo)phenotypes that could serve as starting points for intervention studies.

A limitation of the current study was that the ASD group was relatively high functioning due to the demands associated with participating in imaging research, such as having to lie very still in a confined space. Additionally, sample size was small, especially of the 47,XXY group. However, all results from DTI analysis were corrected for multiple comparisons, meaning significant results were powerful enough to show up even in these small groups. Because this was the first study assessing structural connectivity differences between these populations, no correction was applied for the fact that four measures of white matter integrity were used. However, this study has garnered important results that may give clear direction to future studies in this area.

Taken together, the results from the current study suggest 47,XXY is associated with a combination of both risk factors (because of the reduced fractional anisotropy and axial diffusivity that may underlie the reported cognitive and behavioral problems) and potential protective factors (because of the reduced radial diffusivity, suggesting enhanced myelination). Additionally, the finding of reduced axial diffusivity compared with boys with ASD, adds to existing literature suggesting that even though individuals with 47,XXY and ASD share a number of defining characteristics, distinct differences are also present. This not only confirms the presence of individual variability in underlying mechanisms, and therefore different routes to social dysfunction, but in time it may also benefit the development of tailored interventions. These aspects underline the need for additional studies focused on disentangling these complex gene-brain-behavior relationships.

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Chapter 4

Brandenburg-Goddard, M.N., Van Rijn, S., Rombouts, S.A.R.B., Veer, I.M., & Swaab, H. (2014). *Social Cognitive and Affective Neuroscience, 9*(12), 1926-1933.

A comparison of neural correlates underlying social cognition in Klinefelter syndrome and autism

Abstract

Klinefelter Syndrome (KS) is a genetic syndrome characterized by the presence of an extra X chromosome that appears to increase the risk of psychopathology, such as autism symptoms. The current study used functional MRI to determine underlying mechanisms related to this risk, with the aim of gaining insight into neural mechanisms behind social-cognitive dysfunction in KS and autism, and understanding similarities and differences in social information processing deficits. Fourteen boys with KS, seventeen boys with autism spectrum disorders (ASD) and nineteen non-clinical male controls aged 10-18 were scanned while matching and labeling facial expressions (i.e. face processing and affect labeling, respectively). No group differences in neural activation were found during face processing. However, during affect labeling the ASD group showed increased activation in the amygdala compared to controls, while the KS group showed increased activation in frontal areas compared to both controls and the ASD group. No group differences in task performance were found. Although behavioral symptoms of social dysfunction appear similar both in boys with KS and ASD, this is the first study to demonstrate different underlying etiologies. These results may aid in identifying different pathways to autism symptoms, which may help understand variability within the ASD spectrum.

Introduction

Klinefelter syndrome (KS) is characterized by the presence of an extra X chromosome in men, leading to an XXY karyotype. Prevalence estimates vary from 1:500 to 1:1000. In addition to physical consequences such as above average height, endocrinological imbalances and infertility, the presence of an extra X chromosome may also affect (neuro)psychological development (Herlihy et al., 2011; Sorensen, 1992). The X chromosome contains many genes that affect brain development, which may result in cognitive and behavioral impairment, although hormones may also influence the expression of KS symptoms (Bruining et al., 2011; Verri, Cremante, Clerici, Destefani, & Radicioni, 2010). While intellectual functioning appears to be at the lower end of the normal range, language disorders and reading disabilities are often reported (Boada, Janusz, Hutaff-Lee, & Tartaglia, 2009) . Additionally, a range of executive functioning deficits has been reported (Boada et al., 2009; Lee et al., 2011; Van Rijn, Aleman, De Sonneville, & Swaab, 2009). Clearly, KS affects several areas of cognitive functioning.

Moreover, KS appears to increase the risk of psychopathology. It is important to understand the underlying cognitive and neural mechanisms driving this risk. For instance, several studies have proposed an association between KS and

autism symptoms such as social anxiety, social withdrawal, reduces assertiveness, impaired emotion regulation, and communicative difficulties. Studies show that 5-25% of individuals with KS are diagnosed with ASD (Bishop et al., 2011; Bruining, Swaab, Kas, & Van Engeland, 2009; Cordeiro, Tartaglia, Roeltgen, & Ross, 2012; Geschwind & Dykens, 2004; Tartaglia, Cordeiro, Howell, Wilson, & Janusz, 2010; Van Rijn et al., in press; Van Rijn, Swaab, Aleman, & Kahn, 2008). It is important to determine what underlying mechanisms cause the heightened risk for ASD in boys with KS. Since these boys represent a genetically homogeneous high risk group for ASD, this will not only further insight into mechanisms behind social problems in children with genetic abnormalities, but findings may also have implications for understanding variability within the ASD phenotype.

In order to understand the risk mechanisms driving vulnerability for ASD, it may be important to focus on social cognition and its neural correlates. For example, the processing of affective information from faces, one of the most crucial sources of social information, appears to be affected in individuals with ASD. In individuals with KS, impairments in facial affect processing have also been found (Van Rijn, Swaab, Aleman, & Kahn, 2006). For example, adult males with KS appear to have difficulties interpreting social-emotional cues from faces, such as labeling facial expressions and detecting gaze direction (Van Rijn et al., 2006). Although both disorders are associated with impairments in social cognition, it is important to assess whether this is a different type of deficit since facial affect processing involves a number of information processing steps. For example, there may be specific deficits in face processing, the recognition and processing of faces, or deficits in higher order cognitive processes such as affect labeling, the identification and labeling of emotions. These different types of deficits may become evident in neural activation patterns during facial affect processing. Magnetic resonance imaging and especially functional magnetic resonance imaging (fMRI) studies may thus be useful in addition to neuro-cognitive and clinical research methods, as these may provide detailed insight into the processing of social information on the level of neural activation patterns. However, studies focusing on neural mechanisms mediating social problems in boys with KS are lacking.

Anatomical studies thus far indicate that individuals with KS have smaller total cerebral, frontal, temporal and caudate volumes, and the cortex in temporal and frontal regions is thinner (Giedd et al., 2007). Merely a handful of functional MRI studies have been performed, only one of which in children, which focused on language processing (Steinman, Ross, Lai, Reiss, & Hoeft, 2009). fMRI studies focusing on neural networks subserving social functioning in children with KS, and studies comparing brain activation patterns in boys with KS and boys with ASD are currently non-existent. The current study will therefore compare boys with KS, ASD, and non-clinical controls between the ages of ten

and eighteen, using two fMRI tasks: one focusing on face processing, the other focusing on affect labeling. Differences in brain activation patterns between these groups may help differentiate between different types of social dysfunction.

Materials and methods

Participants

Fourteen boys with KS [M_{age}=14.02 (*SD*=2.59)], seventeen boys with ASD [M_{age}=12.41 (*SD*=1.94)], and nineteen non-clinical male controls [M_{age}=12.03 (*SD*=2.36)] were included in analyses. Analysis of variance (ANOVA) revealed a significant effect of group on age [$F(2,48)=3.310$, $p=.045$], for which post-hoc testing showed this was due to a borderline significant difference in age between the KS group and controls ($p=.051$), with the KS group having a higher mean age.

The KS group was recruited using different 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 (APA, 1994), using the Autism Diagnostic Interview-Revised (ADI-R) (Lord, Rutter, & Lecouteur, 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 (CBCL) (Achenbach, 1991).

Inclusion criteria for all participants were Dutch as primary language and an age between ten and eighteen years. Exclusion criteria were a recent history of substance abuse, intellectual disability (<60 IQ points), scan or motion artifacts (i.e. mean displacement >5 mm), 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 Declaration of Helsinki. 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. A laptop computer was used for task instruction. Participants were allowed to practice as much as needed to fully grasp task requirements. Prior to fMRI scanning, participants underwent anatomical scanning while watching a movie.

Outcome measures

Intellectual functioning

Participants completed the Block Design and Vocabulary subtests of the Dutch adaptations of the Wechsler Scales (WAIS-III and WISC-IV) (Wechsler, 1997, 2005). The subtest Vocabulary measures the degree to which one has learned, is able to comprehend, and verbally expresses vocabulary. The subtest Block Design measures spatial perception, visual abstract processing, and problem solving. These two subtests form the V-BD short form. The V-BD short form is often used to estimate full scale IQ (FSIQ) according to the algorithm (2.9*(sum of normed scores)+42) (Campbell, 1998). The V-BD short form correlates highly with WISC full scale IQ (r=.88) (HerreraGraf, Dipert, & Hinton, 1996) and has been found valid for the estimation of intelligence, with good reliability (r=.91) and validity (.82) (Campbell, 1998).

Autism spectrum symptoms

The Social Responsiveness Scale (SRS) (J.N. 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 Autism Diagnostic Interview-Revised (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.

Social cognition fMRI task

The 'matching/labeling' fMRI task was used to assess participants' neural activation during face processing (the matching of facial expressions) and affect labeling (the labeling of facial expressions). The task consisted of 64 trials in which stimuli were to be labeled and 64 in which stimuli were to be matched. In each task, half of the stimuli (i.e. 32) consisted of faces, and half consisted of objects. This resulted in four conditions. In the face matching condition, participants were instructed to select the face that best matched the facial expression of faces at the top of the screen, from two faces at the bottom corners of the screen. In the face labeling condition, participants were instructed to select one of two semantic labels presented at the bottom corners of the screen. In the object matching condition, participants were asked to match pictures of either bags or coats at the top of the screen, with pictures of bags and coats at the bottom corners of the screen. In the object labeling condition, participants were asked to assign a semantic label ('bag' or 'coat') to pictures of bags or coats at the top of the screen. Stimuli used in the object trials were selected from the colored version of the validated Snodgrass and Vanderwart picture set (Rossion & Pourtois, 2004; Snodgrass & Vanderwart, 1980). Stimuli used in the face trials were selected from the Karolinska Directed Emotional Faces (KDEF) (Lundqvist, Flykt, & Öhman, 1998). Examples of trials from each condition are shown in Figure 1. The object matching and face matching conditions were contrasted in analysis to provide an indication of 'face processing' activation, while the face matching and face labeling conditions were contrasted to provide an indication of 'affect labeling' activation.

This task was specifically aimed at activating social networks, instead of challenging participants' cognitive abilities. Consequently, task demands were relatively low and did not mirror real life social situations, in which responses are immediate and non-dichotomous. This was done to ensure activation of social networks without involving cognitive functions related to task complexity. Prior to starting the task, an introduction was presented on the screen (lasting 128 seconds), in which task instructions were repeated. The task consisted of sixteen counter balanced blocks of eight trials (four blocks in each condition), and was divided into two halves, with a short break in between. Each stimulus remained on the screen for five seconds with a 600 millisecond inter-trial interval. Answers were provided by pushing buttons with the left and right index fingers. Task performance was saved in Eprime data files.

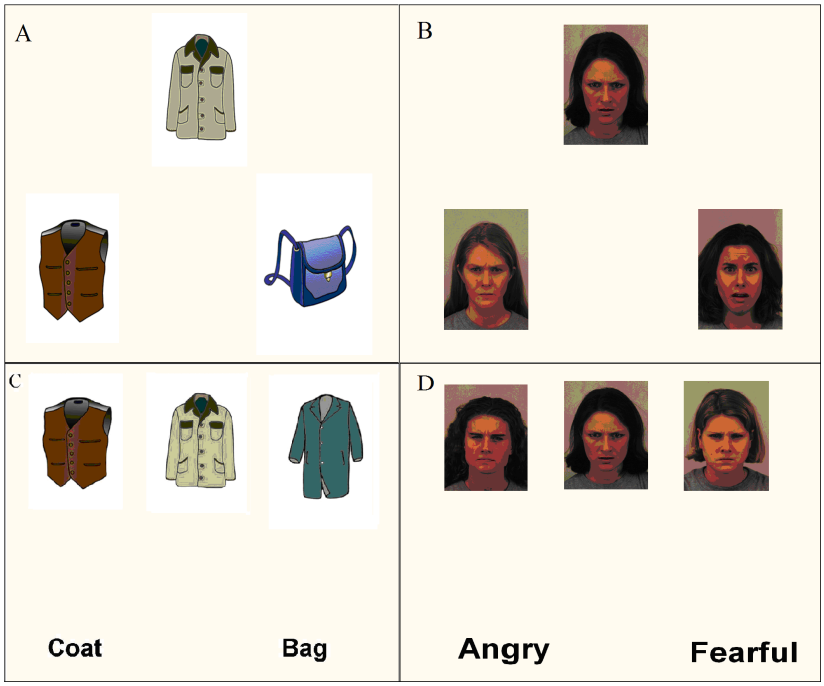


Figure 1. Examples of trials from all four conditions of the matching/labeling task. A: Object matching; B: Face matching; C: Object labeling; D: Face labeling.

fMRI Data Acquisition

Scanning was performed on a 3-Tesla Philips Achieva whole body MRI scanner (Philips Healthcare, Best, The Netherlands), using a 8-channel SENSE receiver head coil. For the fMRI task scans, a total of 326 dynamic scans (two times 163 volumes) were acquired, including two dummy scans preceding both scans to allow for equilibration of T1 saturation effects [time repetition (TR) = 2.2 s, time echo (TE) = 30 ms, flip angle = 80°, 38 transverse slices, FOV = 220 x 220, 2.75 mm isotropic voxels, 0.25 mm slice gap]. Visual stimuli were projected onto a screen that was viewed through a mirror mounted onto the head coil. For registration purposes, T1-weighted anatomical and high-resolution EPI scans were obtained prior to functional scans [T1-weighted 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; high resolution EPI scan: TR = 2.2 ms, TE = 30 ms, flip angle = 80°, 84 transverse slices, FOV = 220 x 220, in-plane resolution = 1.964 mm x 1.964 mm, slice thickness = 2 mm]. All anatomical scans were reviewed and cleared by a radiologist. No anomalous findings were reported.

fMRI Data Analysis

Preprocessing

fMRI data analysis was performed using FMRIB's Software Library's (FSL) FMRI Expert Analysis Tool (FEAT) version 4.1.6 (Jenkinson, Beckmann, Behrens, Woolrich, & Smith, 2012; Smith et al., 2004; Woolrich et al., 2009). The following settings were applied during first-level analysis: MCFLIRT motion correction (Jenkinson, Bannister, Brady, & Smith, 2002); BET brain extraction (Smith, 2002); spatial smoothing using a Gaussian kernel of FWHM 8.0 mm; 190 s high-pass temporal filtering; and FILM pre-whitening (Woolrich, Ripley, Brady, & Smith, 2001). In order to compare activity at the group level, fMRI data was registered to the high-resolution EPI image, the high-resolution EPI image to the T1-image, and the T1-image to the 2mm MNI standard space template (Jenkinson et al., 2002; Jenkinson & Smith, 2001). The resulting registration matrices were combined to describe the fMRI to MNI space transformation.

First level analysis

Two designs were created. One design excluded object matching as a regressor (using it as an implicit baseline), and one excluded face matching as a regressor. This was done for both task halves separately. Two contrasts were set up to compare neural activation patterns. In the 'face matching > object matching' [1 0] contrast, significant results indicated more brain activation during face matching than object matching, and were used as an indication of face processing activation. In the 'face labeling > face matching' [1 0] contrast, significant results indicated more brain activation during face labeling than face matching, and were used as an indication of affect labeling activation. All three groups were mutually compared on both contrasts.

Higher level analysis

First, task halves were combined to create one lower level statistical map per participant for each of the lower level contrasts, using a fixed effects analysis. At the group level a mixed effects analysis was employed (FLAME 1) (Smith et al., 2004). Correction for multiple comparisons across all brain voxels was done using cluster based thresholding, using an initial cluster-forming threshold of $Z>2.3$, and a corrected $p<0.05$. Activation clusters were labeled using the Harvard-Oxford cortical and subcortical structural atlases (Desikan et al., 2006; Frazier et al., 2005; Goldstein et al., 2007; Makris et al., 2006) as well as the Jülich histological (cyto- and myelo-architectonic) atlas (Amunts, Malikovic, Mohlberg, Schormann, & Zilles, 2000; Amunts et al., 1999; Eickhoff, Heim, Zilles, & Amunts, 2006; Eickhoff et al., 2007; Eickhoff et al., 2005). Labels with reported percentages of ≥10 were deemed relevant.

Results

Intellectual functioning

For one boy with KS, IQ data was missing. All IQ scores are presented in table 1. A significant main effect of group on overall IQ was found [$F(2,46)=10.4$, $p<.001$], due to a significant difference in IQ scores between the KS group and both the control and ASD groups, with the KS group having lower mean IQs than controls ($p<.001$) and the ASD group ($p=.003$). In a second analysis, scores on the two separate IQ subtests, Block Design and Vocabulary, were compared across groups. Multivariate analysis showed a significant main multivariate effect of group [$F(4,92)=45.0$, $p<.001$]. The univariate results showed no significant main effect of group on Block Design ($p=0.13$). There was however a significant main effect of group on Vocabulary [$F(2,46)=12.5$, $p<0.001$], which was solely driven by lower scores in the KS group as compared to the control group ($p<0.001$) and the ASD group ($p=0.001$). To prevent group differences in aspects of IQ from confounding fMRI task performance results, Block Design and Vocabulary scores were used as covariates in analysis of task performance.

	Overall IQ	**Block Design**	**Vocabulary**
Controls (n=19)	103.9 ± 3.5	10.3 ± 0.71	11.0 ± 0.83
KS (n=13)	79.0 ± 4.2	8.1 ± 0.85	4.5 ± 1.0
ASD (n=17)	96.5 ± 3.7	9.7 ± 0.75	9.0 ± 0.88

Table 1. Mean overall IQ scores and mean norm scores for IQ subtests, including standard errors.

Autism symptoms

SRS scores were available for nineteen boys in the control group [M_{srs} = 29.8 (SD = 22.9)], ten in the KS group [M_{srs} = 75.7 (SD = 26.4)] and fourteen in the ASD group [M_{srs} = 96.5 (SD = 37.6)]. A significant effect of group on SRS scores was found [$F(2,40)=22.37$, $p<.001$], with mean scores in both the KS ($p=.001$) and ASD ($p<.001$) groups being significantly higher than in controls. No significant difference in mean scores between the KS and ASD groups was found.

fMRI activation patterns

'Face processing': Face matching vs. Object matching

Controls

Mean activation results for controls are summarized in table 2. Significant activation in three clusters was found, i.e. right-sided frontal regions (figure 2A), the amygdala (figure 2B) and fusiform cortex (figure 2C).

Clinical groups

No significant between-group differences in brain activation were found, i.e. the ASD and KS groups did not show activation that was deviant from controls.

'Affect labeling': Face labeling vs. Face matching

Controls

Mean activation results for controls are summarized in table 3. Significant activation in two clusters was found, i.e. occipital regions (figure 3A), and the left temporal pole (figure 3B).

Clinical groups

The ASD and KS groups showed significantly different activation patterns as compared to controls. Activation results for the KS and ASD groups are summarized in table 3. Higher level analysis comparing the face labeling > face matching contrast across all groups resulted in one significant cluster of deviating neural activation in each group. Asking participants to label rather than match faces, resulted in significantly more activity in the right middle frontal gyrus (including Broca's area) (figure 3C) in the KS group than in both controls and the ASD group. In contrast, the ASD group showed more right amygdala activation (figure 3D) than controls. To illustrate these significant group effects, uncorrected z-values for all groups on the coordinates showing significant group differences are depicted in figure 4 for both activation clusters.

Mean activation controls

No. of Voxels	Corrected p	Z value	x, y, z	Structures
2240	<.001	4.42	40, 4, 32	R precentral gyrus, middle frontal gyrus, inferior frontal gyrus/pars opercularis (Broca)
2089	<.001	4.17	22, -2, -17	R amygdala
1860	<.001	5.17	42, -48, -24	R temporal occipital fusiform cortex

Activation KS deviant from controls

non-significant

Activation KS deviant from ASD

non-significant

Activation ASD deviant from controls

non-significant

Table 2. Activation clusters for 'face processing' (face matching>object matching)

Mean activation controls

No. of Voxels	Corrected p	Z value	x, y, z	Structures
5633	<.001	4.89	4,-88, 4	Intracalcarine cortex, occipital pole (V1), supracalcarine cortex, lingual gyrus
2109	<.001	4.36	-50, 14, -20	Left temporal pole

Activation KS more than controls

No. of Voxels	Corrected p	Z value	x, y, z	Structures
1009	0.008	3.77	48, 16, 36	Right middle frontal gyrus (Broca)

Activation KS more than ASD

No. of Voxels	Corrected p	Z value	x, y, z	Structures
1684	0.00024	4.57	48, 16, 36	Right middle frontal gyrus (Broca)

Activation ASD more than controls

No. of Voxels	Corrected p	Z value	x, y, z	Structures
929	0.012	3.66	16, 0, -22	Right amygdala, parahippocampal gyrus (anterior)

Table 3. Activation clusters for 'affect labeling' (face labeling>face matching)

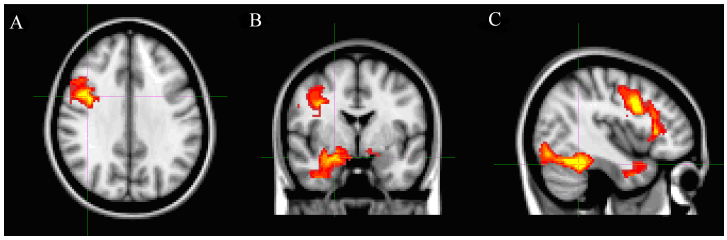


Figure 2. Mean activation clusters in controls for 'face processing' (face matching>object matching). A: Frontal activation; B: Amgydala activation; C: Temporal activation.

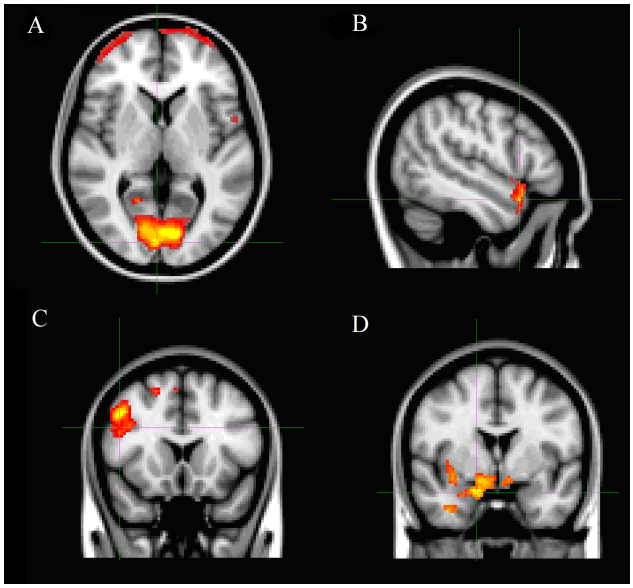
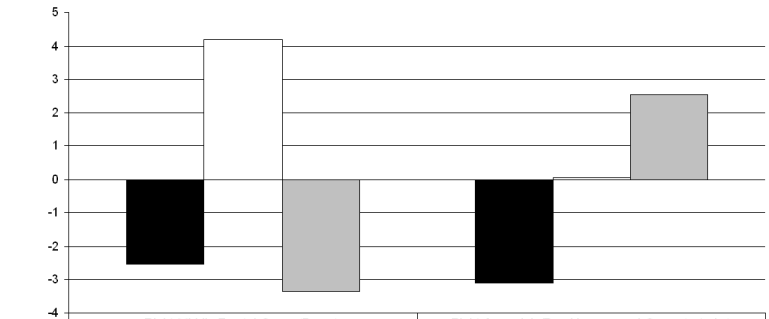


Figure 3. Activation clusters for 'affect labeling' (face labeling>face matching). A: Occipital activation controls; B: Temporal activation controls; C: Frontal activation KS group more than controls and ASD; D: Amygdala activation ASD group more than controls.



	Right Middle Frontal Gyrus (Broca)	Right Amygdala/Parahippocampal Gyrus (anterior)
Controls	-2,52	-3,09
KS	4,18	0,06
ASD	-3,33	2,55

Figure 4. Z-values in significant activation clusters for 'affect labeling' (face labeling>face matching).

Task performance

Task performance is summarized in table 4. Three controls were removed from analysis due to Z-scores >2.5, as it could not be excluded that these participants used the response box incorrectly. MANCOVA, covarying for Block Design and Vocabulary, showed no significant main multivariate effects of group (p=0.33), Blockdesign (p=0.34) or Vocabulary (p =0.07), on task performance. Accordingly, none of the univariate effects of group, Block Design or Vocabulary were significant.

	Object matching	**Face matching**	**Face labeling**
Controls (n=16)	30.4 ± 1.2	28.9 ± 1.4	29.3 ± 1.4
KS (n=13)	26.5 ± 1.4	24.1 ± 1.6	24.8 ± 1.6
ASD (n=17)	27.7 ± 1.1	24.9 ± 1.3	25.0 ± 1.3

Table 4. Estimated marginal means (corrected for covariates) for the fMRI task. Scores indicate mean number of correct answers and standard errors.

Discussion

This fMRI study compared brain activation patterns in boys with an extra X-chromosome (XXY karyotype, Klinefelter syndrome, KS), autism spectrum disorders (ASD), and non-clinical controls during a social-cognitive (facial affect) processing task. During face processing, in which participants were instructed to match visually presented faces based on expressions, increased activation in right-sided frontal regions, the amygdala and fusiform gyrus was found in controls. These results suggest these areas show increased activation specifically during face processing. The amygdala and fusiform cortex have been implicated in face processing in multiple studies (Adolphs & Spezio, 2006; Critchley et al., 2000; Morris et al., 1998; Pizzagalli et al., 2002). No significant differences in brain activation were found between controls and the KS and ASD groups. This suggests that, compared to controls, face processing is not accompanied by more or less brain activation in these clinical groups than processing socially neutral information.

However, interesting group differences were found during affect labeling, i.e. the higher order processing of facial information. In controls, affect labeling was associated with significantly increased activation in occipital regions (V1) and the left temporal pole. Increased activation in the occipital cortex may be explained by the theory that processing socially relevant information may boost activation in a neural system involving the primary visual cortex (Lang et al., 1998). Moreover, the temporal pole is implicated in social semantic processing, which requires knowledge regarding social concepts (Ross & Olson, 2010). Facial affect labeling requires such knowledge. Additionally, linguistic studies

have implicated the left temporal pole in proper names processing, referring to naming specific entities instead of general classes of entities (in the current study, 'angry' or 'sad' as opposed to 'emotional') (Semenza, 2011). This suggests a degree of higher order processing in controls during affect labeling, that is not present during face processing. Assigning a semantic label to a facial expression may be more demanding than matching facial expressions, as the latter could conceivably be accomplished by merely visually locating differences in perceptual features, as opposed to actually understanding the significance of facial expressions.

It was affect labeling that led to deviant activation patterns in the clinical groups. The ASD group showed significantly more activation in the right amygdala than controls. In contrast, the KS group showed more activation in the right middle frontal gyrus (including Broca's area) than controls. Crucial to the aim of the current study, this significant group effect also extended to the KS-ASD contrast, dissociating the KS group from the ASD group based on frontal activation. These results suggest more involvement of specific brain regions in the ASD and KS groups than controls during affect labeling. However, this concerned involvement of very different parts of the brain: the amygdala in the ASD group and frontal areas in the KS group. Possibly, boys with KS solve social issues through increased involvement of frontal functions, relying heavily on reasoning abilities, while boys with ASD seem to rely more on basic social networks involving the limbic system.

Taken together, the following conclusions can be drawn regarding neural activation in the ASD group. During face-specific processing, controls showed significant amygdala activation, suggesting the meaning of facial expressions is processed relatively automatically in non-clinical individuals. However, no significant differences in neural activation between controls and the ASD group were found, meaning boys with ASD do not show more or less amygdala activation. However, during affect labeling the ASD group did show increased amygdala activation compared to controls. This boost in amygdala activation may be explained by the hypothesis that during face processing, they apply a more perceptual feature based approach ('spotting the differences') which is impossible during affect labeling. The latter requires social information processing, leading to an increase in amygdala activation.

For the KS group results are different from the ASD group, and lead to the conclusion that affect labeling is associated with deviant neural activation in more frontal areas of the brain. The current finding of increased activation in the middle frontal gyrus may signify compensatory mechanisms involving a higher order reasoning approach to social information processing. As this frontal structure also includes Broca's area, language functions may play a role in these mechanisms. Possibly, boys with KS do not label incoming social information intuitively, but rather attempt to use a 'reasoning' or more rational

approach. These findings are in line with a study in adults with KS, in which increased activation in language related areas in the right hemisphere was found during a language processing task (Van Rijn, Aleman, et al., 2008). However, others have found that boys with KS showed significantly reduced brain activation in areas associated with language and reading during a language related task (Steinman et al., 2009).
It would be interesting to further assess the role of language skills in social cognitive processing in individuals with KS. As is typically found, the KS group had lower IQ scores than the other groups, specifically on Vocabulary, which measures the degree to which one has learned, is able to comprehend and verbally expresses vocabulary. This is important, as one would expect language skills to play a role in affect labeling. However, in spite of lower Vocabulary scores, there were no group differences in task performance during affect labeling when controlling for Vocabulary performance. Also, Vocabulary performance did not significantly contribute to task performance in the scanner. This implies that despite the verbal nature of the fMRI task, all groups were equally skilled in performing the scanner task. However, the finding of increased activation in Broca's area does imply compensatory mechanisms in the language domain during affect labeling. This supports a link between language and social cognitive processing in boys with KS, which should be studied more thoroughly in future studies in which more complex affect labeling skills are assessed.

Regarding limbic and temporal lobe networks associated with social information processing, decreased activation in the amygdala, fusiform gyrus, superior temporal sulcus and insula was found in adults with KS during a task focusing on judging face trustworthiness (Van Rijn et al., in press). It would be interesting to assess if such a complex social-cognitive task also involves more frontal activation, as frontal activation may inhibit limbic activity (Berkman, Burklund, & Lieberman, 2009). Taken together, these studies point towards abnormalities in the fronto-amygdala emotion regulation circuitry.

A limitation of the current study was that the KS group had a significantly lower mean IQ and higher mean age. However, separate aspects of IQ (both spatial and verbal) were used as covariates in analyzing task performance. Additionally, task demands were intentionally kept to a minimum to ensure activation of social neural networks in all groups irrespective of intellectual level. Significant age differences may be of influence on the results, especially in children. However, from a developmental perspective, this could only result in an advantage for the KS group. Another limitation was the relatively small sample size, especially in the KS group, which may have led to lack of power in analyzing task performance and prevented correlational analysis between fMRI and cognitive data. In future studies it would be interesting to assess connectivity networks during information processing, to determine how brain areas work together in individuals with KS. Significant differences in these

connectivity networks would corroborate the hypothesis of a specific ASD phenotype in these individuals. Additionally, it would be interesting to assess whether subtypes can be identified within the autism spectrum, e.g. those with more frontal deficits versus those with more limbic deficits.

The frontal abnormalities found in this study may underlie the reported social-cognitive deficits and social-behavioral problems. What the present study may contribute is increased understanding of specific underlying etiologies of impairments in terms of neural mechanisms. Conceivably, boys with KS who have high levels of autism symptoms or an ASD diagnosis, may represent a subgroup with a specific etiology underlying social dysfunction. Although the behavioral parameters (i.e. SRS scores) indicate similar types of social dysfunction on a behavioral level, neuroimaging revealed different underlying etiologies. Studying individuals with genetic syndromes such as KS may aid in understanding and explaining variation within the broad ASD phenotype. It is important to identify specific subpopulations within the spectrum, as this contributes to tailored diagnosis and treatment (Van Rijn et al., 2012). Using neuroimaging in addition to cognitive and behavioral measures, may benefit this process.

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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

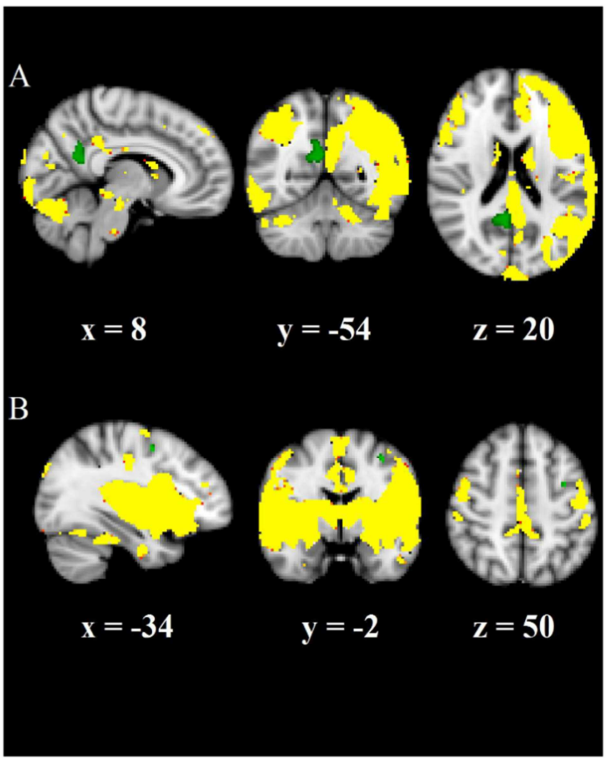

Figure 1. Significant functional connectivity between-group differences (green) overlaid on the respective resting state networks (yellow), including x,y,z (mm) coordinates. **A.** Increased connectivity between the right precuneus/cingulate gyrus and the frontoparietal network (47,XXY vs. controls). **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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Chapter 6

Summary and general discussion

Advanced social skills are necessary to successfully navigate the complexities of interpersonal communication. For example, skills as seemingly trivial as being able to engage in 'small talk' during social functions enhance social cohesiveness (Coupland, 2003), the forming of bonds with other people. There is great individual variability in social skills, and certain developmental conditions impair social functioning to a degree that necessitates intervention. Autism spectrum disorder (ASD) is the most well-known example of a disorder that involves social dysfunction. However, certain genetic conditions may also lead to social dysfunction. Klinefelter syndrome (47,XXY) is a genetic condition that occurs only in males. It is characterized by the presence of an extra X chromosome, and is associated with varying degrees of cognitive, social, and behavioral problems (Boada, Janusz, Hutaff-Lee, & Tartaglia, 2009; Visootsak & Graham, 2009). Males with 47,XXY are at increased risk of developing symptoms of psychosis, bipolar disorder, attention-deficit/hyperactivity disorder, and autism (Cederlof et al., 2014). Studying the neural phenotype of 47,XXY may provide essential information regarding mechanisms underlying the broad range of cognitive, social and behavioral problems associated with this condition. All neuroimaging studies to date focused on adult males with 47,XXY. However, studying children and adolescents with this condition may aid in determining the neural phenotype early in life.

From a gene-brain-behavior perspective, it is possible that the additional X chromosome that is present in 47,XXY leads to overexpression of X-linked genes. This overexpression may lead to dysregulation of genetic mechanisms. Many X-linked genes are involved in brain development, which indicates that an additional X chromosome may substantially influence the structural and functional architecture of the brain. Moreover, brain development appears to be modulated by the actual number of X chromosomes, specifically in regions responsible for adaptive social functioning (Raznahan et al., 2014; Vawter, Harvey, & DeLisi, 2007). These deviations in neural makeup may negatively impact cognitive development. Because of this impact on how incoming information is processed, cognitive impairments may lead to problems in the processing of, and reaction to, information from the environment. This means that disturbances in typical genetic mechanisms, through neural and cognitive pathways, may lead to problems in observable behavior. As ASD is diagnosed based on behavioral symptoms, individuals receiving this diagnosis may suffer from social problems as a result of many different underlying genetic and neural dysfunctions. In contrast, genetic conditions are characterized by a relatively homogeneous endophenotype, with substantial variation in behavioral outcomes. Therefore, studying genetic conditions such as 47,XXY may also help uncover different pathways to social dysfunction that are more difficult to uncover by studying idiopathic ASD, because of the variability in (endo)phenotypes (Mottron, Belleville, Rouleau, & Collignon, 2014; Muhle,

Trentacoste, & Rapin, 2004). Indeed, recent studies suggest there may be subtle differences between individuals with idiopathic ASD, and individuals with ASD associated with 47,XXY, in the expression of these symptoms and the underlying cognitive mechanisms (Bruining et al., 2010; Van Rijn et al., 2014; Van Rijn, Stockmann, Van Buggenhout, Van Ravenswaaij-Arts, & Swaab, 2014). Therefore, this thesis was aimed at assessing if individuals with 47,XXY and autistic symptomatology also differ from individuals with idiopathic ASD in the neural mechanisms underlying their cognitive and behavioral symptoms.

In the present thesis we used MRI to exploratively assess gray matter volume, white matter integrity, task-related brain activation, and intrinsic functional brain connectivity in individuals with 47,XXY compared with typical development, to gain insight into the mechanisms that contribute to cognitive and behavioral impairments specific to 47,XXY. In addition, we assessed similarities and differences in neural pathways to social dysfunction. As most neuroimaging studies to date focused on adult males with 47,XXY, and only a handful of pediatric neuroimaging studies have been performed, our samples consisted of boys between the ages of nine and eighteen. This made it possible to determine the neural phenotype early in life, which would open the door to early interventions when circumstances are more optimal in terms of neural plasticity.

Main findings

As described in **chapter 2**, we assessed gray matter volume of brain regions important for social information processing in boys with 47,XXY compared with typically developing boys, and boys with idiopathic ASD. Boys with 47,XXY were found to have significantly less gray matter in the right superior temporal gyrus (STG) than typically developing boys. Additionally, they have significant gray matter volume reductions in the left and right insular cortex, and the left orbitofrontal cortex (OFC), compared with both typically developing boys, and boys with ASD. In **chapter 3** we report results from diffusion tensor imaging analysis, focused on white matter microstructure. We found reduced white matter/axonal integrity in the corpus callosum in boys with 47,XXY compared with typically developing boys, as well as reduced axonal integrity in the right inferior fronto-occipital fasciculus compared with boys with ASD. However, we found reduced radial diffusivity, indicating *enhanced* myelination, in boys with 47,XXY compared with typically developing boys in the left anterior corona radiata and sagittal striatum. **Chapter 4** contains results from a task-related fMRI study focused on social-cognitive information processing. Facial affect recognition is associated with similar neural activation patterns in all three groups. However, boys with 47,XXY were found to have increased activation during facial affect labeling in the right middle frontal gyrus (including Broca's area) compared with both typically developing boys, and boys with ASD. Boys with ASD on the other hand, showed increased activation in a different brain

region, i.e. the right amygdala. In **chapter 5** we describe results from resting state functional MRI analysis, i.e. intrinsic functional brain connectivity. Boys with 47,XXY were found to have increased intrinsic functional brain connectivity in the right precuneus/cingulate gyrus and frontoparietal network relative to typically developing boys. Relative to boys with ASD however, they showed decreased intrinsic functional brain connectivity between the left precentral gyrus/middle frontal gyrus and auditory network.

Deviating neural mechanisms involved in higher order cognitive functions in 47,XXY

It is widely accepted that the frontal lobes play an essential role in higher order processes responsible for conscious reasoning, and purposeful, goal-directed problem-solving (Ardila, 2008). The increased activation in middle frontal regions during social-cognitive information processing that was found in boys with 47,XXY (**chapter 4**), implies they may rely more on reasoning (i.e. higher order cognitive functions) instead of 'social intuition' when engaging in social decision making. However, task demands in our study were intentionally kept to a minimum, because we wanted to exclude brain activation related to task complexity in favor of activation related purely to social-cognitive information processing. As higher order cognitive deficits have been reported in 47,XXY (Boada et al., 2009; Boone et al., 2001; Lee et al., 2011; Van Rijn & Swaab, in press), it is possible this higher-order reasoning approach to social-cognitive information processing will prove ineffective with increasing task demands. Although speculative, this may be one of the reasons individuals with 47,XXY experience difficulties in identifying and interpreting facial expressions (Van Rijn et al., 2014).

Deviating neural mechanisms involved in social information processing difficulties in 47,XXY

Many brain regions are known to be involved in the neural network for social information processing. For example, the OFC is important for emotion regulation, decision-making and social information processing, including the recognition of mental states (i.e. theory of mind), and the processing of facial expressions (Baron-Cohen et al., 1994; Golkar et al., 2012; Noonan, Sallet, Rudebeck, Buckley, & Rushworth, 2010; Rolls, 2004; Rolls & Grabenhorst, 2008). Inferences about cognitive and behavioral dysfunctions based on structural brain deviations must be done with caution. However, it is possible that the reduced OFC volume that was found is one of the anatomical roots of social and cognitive problems associated with 47,XXY, such as deficits in theory of mind and facial affect processing (Van Rijn, Swaab, Aleman, & Kahn, 2006). The OFC is anatomically connected with a region adjacent to the frontal lobes, the insular cortex (Jakab, Molnar, Bogner, Beres, & Berenyi, 2012). The bilateral insular cortices are also significantly smaller in boys with 47,XXY, a finding that

is in line with research in adult males with 47,XXY (Bryant et al., 2011; Shen et al., 2004; Skakkebaek et al., 2013). This region is associated with emotion regulation, recognition of, and responses to, emotional stimuli and in particular internal emotional states, as well as social decision making processes (Adolphs, 2003; Sanfey, Rilling, Aronson, Nystrom, & Cohen, 2003). Males with 47,XXY show decreased neural activation in the insular cortex when asked to make social judgments (Van Rijn et al., 2012). Our findings suggest the socio-emotional impairments found in 47,XXY might be mediated by bilateral reductions in insular cortex volume in 47,XXY that are already present in childhood, and may possibly impact the brain function in this region as well. Other studies have shown that individuals with 47,XXY have trouble identifying and verbalizing internal emotional states, they are more easily emotionally aroused in terms of psychophysiological responses, and their emotional states exert more influence on their decision making processes (Van Rijn, Barendse, Van Goozen, & Swaab, 2014; Van Rijn et al., 2006). Our MRI findings imply that abnormalities in the OFC and insular cortices may be part of the mechanism underlying these deficits in emotion regulation in 47,XXY.

The STG has been implicated in the processing of facial information, such as facial expressions and gaze directions. Impairments in the processing of facial expressions have been reported in children and adults with 47,XXY, and eye tracking suggests they are less focused on other people's eyes (Van Rijn et al., 2014; Van Rijn et al., 2014; Van Rijn et al., 2006; Van Rijn et al., 2012). Hypothetically, these impairments might be mediated by the reduced gray matter volume in the STG that was found in our sample. The increased functional connectivity at rest between the frontoparietal network and the right precuneus/cingulate gyrus, might imply more global deviations in brain function. The precuneus is thought to be involved in emotional awareness (Van der Velde et al., 2013), and the cingulate gyrus is a central part of the limbic system, otherwise known as our 'emotional epicenter' (Kotter & Meyer, 1992). Increased intrinsic functional brain connectivity between these areas and the frontoparietal network, which is primarily associated with language functions (Smith et al., 2009), may imply a compensatory mechanism for the connection between language and emotion. Hypothetically, this compensatory mechanism could consist of processes similar to neural compensation. This is a term used to describe brain activation in individuals with brain pathology, in regions not used by individuals with intact brains, intended to (subconsciously) increase cognitive performance (Stern, 2009). Based on our findings, we hypothesize that in 47,XXY this compensatory mechanism may be inadequate, resulting in a problematic connection between language and emotion. This may (partially) explain the language and emotion processing deficits, such as alexithymia and problems with emotion labeling, that are among the most often reported domains of impairment in 47,XXY (Boada et al., 2009; Boone et al., 2001; Van Rijn et al., 2006).

Deviating neural mechanisms involved in language processing difficulties in 47,XXY

The increased intrinsic functional brain connectivity between the frontoparietal (language) network and the precuneus/cingulate gyrus, may reflect an ineffective neural compensatory mechanism for the connection between language and emotion. Research in typically developing individuals suggests language skills influence emotion processing abilities (Cutting & Dunn, 1999; Jablonka, Ginsburg, & Dor, 2012; Lindquist, Barrett, Bliss-Moreau, & Russell, 2006). Based on these studies, we suggest that in 47,XXY impaired language skills may contribute to impaired emotion processing. In our fMRI study focused on the neural mechanisms underlying facial affect labeling part of Broca's area, a region essential for language processing (Davis et al., 2008), showed increased activation in boys with 47,XXY during the labeling of prototypical facial expressions of emotions. This finding gives additional support for the hypothesis of a dysfunctional neural mechanism underlying problems in the connection between language and emotion processing. Broca's area is usually active in the left hemisphere, as this is the hemisphere that is most often dominant for language. In our fMRI study the increased activation was located in Broca's area in the right hemisphere. This is in line with an earlier fMRI study in adult males with 47,XXY that reported increased activation in language-related areas in the right hemisphere during a language processing task, suggesting individuals with 47,XXY may have reduced hemispheric specialization for language (Van Rijn et al., 2008).

Although our finding of reduced white matter integrity in the corpus callosum in 47,XXY does not equal actual damage to these fiber tracts, it does imply reduced efficiency of neural connections. In typically developing individuals the corpus callosum is involved in language functions (Bloom & Hynd, 2005; Van der Knaap & Van der Ham, 2011). Lesions in the corpus callosum have been associated with alexithymia, a term used to describe deficits in the ability to identify and verbalize one's emotional state, indicating problems in both language and emotion processing (Sifneos, 1973). Increased rates of alexithymia have been reported in 47,XXY (Van Rijn et al., 2006). Diminished integrity of fiber tracts in the corpus callosum may therefore be another part of the mechanism underlying this deficient connection between language and emotion in 47,XXY.

The reduced gray matter volume in the STG that we found in boys with 47,XXY might contribute not only to the social problems associated with this condition, but also to the language impairments. Wernicke's area and Heschl's gyri are responsible for speech and auditory processing, respectively (Da Costa et al., 2011; Wise et al., 2001). They are part of the Wernicke-Geschwind model of language processing and located in the STG, making the STG a key structure in this model. The Wernicke-Geschwind model of language processing consists of

Broca's area, Wernicke's area, the superior temporal sulcus, inferior parietal lobule, middle temporal gyrus, and the arcuate fasciculus connecting these areas (Dick, Bernal, & Tremblay, 2014). Although this classic model has been deemed overly simplistic, it still serves as the basis from which language processing in the brain is studied. More importantly, most contemporary models of language processing also involve superior temporal areas (Dick et al., 2014). The reduced hemispheric specialization for language in the STG reported by Van Rijn et al. (2008) might be a functional consequence of anatomical deviations in STG development in 47,XXY. Additionally, the involvement of the corpus callosum in language processing is hypothesized to involve facilitation of interhemispheric communication between the left and right plana temporale, which include the STG (Bloom & Hynd, 2005; Van der Knaap & Van der Ham, 2011). Altogether, these findings suggest abnormal frontoparietal connectivity, abnormalities in Broca's area, gray matter volume deviations in the STG and reduced integrity of the corpus callosum may be part of the anatomical mechanism underlying language impairments in 47,XXY.

Enhanced myelination in 47,XXY

The finding of reduced radial diffusivity in the left anterior corona radiata and sagittal striatum in boys with 47,XXY may have important implications. Reduced radial diffusivity is associated with enhanced myelination of white matter (Alexander, Lee, Lazar, & Field, 2007), which enables neurons to transmit information faster and thus enhances neural communication. Clinical conditions that influence brain structure and function are usually associated with reduced myelin integrity. In our sample of boys with 47,XXY this effect appears to be reversed. Although speculative, we offer two possible explanations for this unexpected finding. The first concerns the use of steroid hormones. Individuals with 47,XXY often receive supplemental testosterone treatment to ameliorate some of the symptoms associated with this condition (e.g. decreased growth of facial and pubic hair). These hormones are known to enhance myelination in the human brain (Peper, Van den Heuvel, Mandl, Hulshoff Pol, & Van Honk, 2011). In our sample four participants received supplemental testosterone treatment at the time of the study, while five did not. If our finding of enhanced myelination is indeed a consequence of testosterone treatment, it could be an important protective factor that could potentially enhance neural communication in 47,XXY. As an already relatively small sample size prevented us from splitting our 47,XXY group into those who received testosterone treatment and those who did not, this hypothesis calls for future studies assessing the impact of hormone treatment on myelination. A second possible explanation for the enhanced myelination in 47,XXY comes from the finding that the gene for the myelin proteolipid protein lies on the X chromosome (Willard & Riordan, 1985). It is suggested that the pattern of gene inactivation is one of the mechanisms through which the extra X chromosome impacts phenotypic development in 47,XXY (Skakkebaek et al., 2014). Hypothetically,

the extra X that is present in this condition could influence functioning of the myelin proteolipid protein. Previous studies (Hodes 2000) suggest that additional copies of this protein may lead to severe somatic conditions. It is therefore unlikely that the enhanced myelination is a result of this gene simply escaping gene inactivation. However, studies in mice indicate that this gene may partially escape inactivation, which could hypothetically lead to overexpression. These results demonstrate the value of interdisciplinary research (e.g. neuroscience and clinical genetics) to further explore the possible effect of the additional X chromosome on the functioning of this protein. However, these explanations are very speculative at this point. More research is necessary to determine the exact origin of enhanced myelination in 47,XXY.

47,XXY versus idiopathic ASD

With the exception of reduced gray matter volume in the bilateral insular cortices in 47,XXY, all differences in brain structure and function between our 47,XXY and ASD groups pertain to the frontal lobes. Anatomically, boys with 47,XXY have reduced gray matter volume in the left OFC compared with boys with ASD, as well as reduced axonal integrity in the right inferior fronto-occipital fasciculus, a fiber bundle connecting the frontal lobe with the temporal and occipital lobes (Martino, Vergani, Robles, & Duffau, 2010). Functionally, boys with 47,XXY show decreased intrinsic functional brain connectivity between the auditory resting state network and the left precentral gyrus/middle frontal gyrus compared with boys with ASD. During social-cognitive information processing, boys with 47,XXY show increased brain activation in the right middle frontal gyrus compared with boys with ASD.

This is the first time brain structure and function in these populations were compared, and a detailed interpretation of the observation that the differences appear to center around the frontal lobes requires more research. However, these results do suggest that reduced intrinsic functional connectivity between different regions of the cortex is a feature that is not specific for idiopathic ASD (Di Martino et al., 2014), as our 47,XXY group showed even more decreased intrinsic functional connectivity. Hypoconnectivity of cortical regions may therefore be a feature that is shared by 47,XXY and ASD, suggesting it may have a crucial role in social dysfunction. It may therefore be deficit specific rather than disorder specific. Importantly though, when asked to label facial expressions, boys with 47,XXY have substantially increased activation in frontal regions compared with boys with ASD. This, once again, may imply a form of neural compensation in 47,XXY, that is different from idiopathic ASD. In our study boys with ASD also showed increased activation during facial expression labeling, but in a different brain region (i.e. middle frontal gyrus in 47,XXY versus amygdala in ASD). The amygdala is part of the social brain network and heavily involved in automatic screening of emotional relevance, including facial expressions, in typical development, which may imply that boys with idiopathic

ASD rely more on social intuition. The profound deficits in this domain associated with ASD, however, suggest that this form of neural compensation is ineffective. Behaviorally both groups may experience similar social problems, but our findings clearly point towards differences in underlying neural compensatory mechanisms. The bilateral reductions in insular cortex and OFC volume that were found in 47,XXY compared with typically developing boys, were also present in comparison with boys with idiopathic ASD. These brain areas are important for awareness of internal emotional states, as well as the regulation of these states. This may suggest that social dysfunction in individuals with 47,XXY is influenced more heavily by defective emotion regulation (i.e. difficulties with monitoring one's internal emotional state, and using it as a social compass) than it is in individuals with idiopathic ASD.

These findings imply distinct differences in neural structure and function between 47,XXY and ASD. In addition to differences in the cognitive and behavioral components of the gene-brain-behavior hypothesis of social dysfunction, the brain component appears to differ substantially as well. However, boys with 47,XXY could not be distinguished from boys with ASD in our sample based on behavioral measures of autism symptoms. There may therefore be a multitude of pathways to social dysfunction, including heterogeneity in underlying neural mechanisms. Knowledge regarding the exact nature of these differences aids in specifying the behavioral deficits and has important scientific and clinical implications, which we will turn to now.

Scientific and clinical implications

The current thesis marks the first time that the structural and functional architecture of the brain were assessed across multiple domains, using various MRI techniques in one sample of children/adolescents with 47,XXY. This was also the first time that brain structure and function of boys with 47,XXY, who are at increased risk of developing autism symptomatology, was compared to that of boys with idiopathic ASD. The results provide interesting and important directions for future research. The presence of an extra X chromosome in 47,XXY, and/or the hormonal consequences of this condition, appear to significantly impact brain development. Together, these factors may contribute to the increased risk of problems in 47,XXY in higher order cognitive functions, social emotional information processing, and language processing. These neural pathways to social dysfunction show specific differences from those in idiopathic ASD, especially in the domains of frontal functions and emotion regulation.

Future research in this area should focus on replication of our findings in larger samples with varying age ranges, and further specification of the exact gene-brain-behavior pathways in 47,XXY. MRI offers great potential for investigating these mechanisms, especially when combined with other neurobiological,

cognitive, and behavioral measures. The functional MRI task from our study for example, was focused on decoding facial expressions. Adding neurophysiological measures such as eye tracking, which is used to gather data regarding the focus of the participants' attention, makes it possible to determine if the increased frontal activation found in 47,XXY in our study is top-down (i.e. the incoming information is processed differently in the brain), or bottom-up (i.e. brain activation differs, because the incoming information is different, due to a deviating focus of attention). For example, eye tracking has shown that adult males with 47,XXY fixate less on the eye regions of faces than typically developing males when assessing facial expressions (Van Rijn, in press). These types of studies may be of tremendous benefit to the specification of the exact deviations in social-cognitive processing in 47,XXY.

This thesis also provides starting points for intervention research. The results suggest the existence of various pathways to social dysfunction, as demonstrated by the findings that were specific to boys with 47,XXY compared with those with idiopathic ASD. This could hypothetically mean these groups are differentially susceptible to treatment. Research on the development of tailored interventions for social dysfunction, intended to enhance treatment effect, may benefit from knowledge regarding differences in these neural mechanisms. A particularly relevant area of research in this respect, is real time fMRI neurofeedback. This technique is aimed at modifying behavior by modifying brain function. Real time information about changes in neural activation is provided to an individual during multiple training sessions, with the intention of facilitating self-regulation of this activation. The idea is that this will produce changes in brain function, which may in turn lead to changes in cognition and/or behavior (Stoeckel et al., 2014). More research is necessary to determine its effectiveness in influencing behavior by influencing brain activation. However, real time fMRI neurofeedback may potentially increase resilience in individuals at risk for social dysfunction, by correcting known dysfunctions in neural activation patterns before severe developmental problems become apparent. In 47,XXY, potential targets for treatment could for example be brain regions known to contribute to language and emotion processing in typical development, in an attempt to modify the current, ineffective, neural compensatory mechanism. Conversely, in individuals with formal diagnoses of psychiatric conditions such as ASD, real time fMRI neurofeedback may be used to directly target neural systems underlying social dysfunction in order to improve behavior (Stoeckel et al., 2014). The first studies focused on this potential next generation therapeutic tool have yielded positive results in individuals with contamination anxiety, not only in changing the functional architecture of the brain, but also in inducing behavioral changes that last for several days after the training session (Robineau et al., 2014; Scheinost et al., 2013). In the current thesis we identified differences in the specific neural systems contributing to social dysfunction. Our work could therefore be a valuable source of information, and will hopefully stimulate

research on real time fMRI neurofeedback for social dysfunction, by identifying potential targets for treatment.

From a clinical perspective, the current thesis may aid in creating awareness among clinicians of the existence of great individual variability, not only in behavioral symptoms of social dysfunction or autism, but also in the mechanisms underlying this dysfunction. This may impact the selection of mental health care strategies. For example, if individuals with 47,XXY indeed use a more rational approach (involving frontal lobe areas) during social-cognitive information processing, clinicians may be able to use this knowledge to determine how to best implement interventions in this population. For example, social skills training for 47,XXY could focus more on explanations of *why* certain behaviors are adaptive in social situations, and less on practicing social situations through role-play. In addition, the knowledge that emotion regulation impairments may be mediated by bilateral reductions in insular cortex volume specifically in 47,XXY, may aid in making clinicians aware of the possibility that the etiology of their emotion regulation problems differs from those with idiopathic ASD. This, in turn, may signify these individuals require a different type of treatment more focused on identifying and monitoring one's own internal emotional state. Lastly, our results suggest that deviating development of brain structure and function in 47,XXY is already present during childhood. This means that interventions aimed at preventing or ameliorating behavioral problems in individuals with 47,XXY, may be most effective when implemented prior to adolescence.

Limitations

Although all reported results from our study were significant and methodologically sound, there were some unavoidable limitations. Part of the (f)MRI data was of insufficient quality to include in analysis due to movement of the participants, or other artifacts. This resulted in relatively small sample sizes, which may have led to a lack of power to detect more subtle differences in neural structure and/or function, and prevented correlational analysis of cognitive and (f)MRI data. However, the (f)MRI data used in analysis was thoroughly evaluated and of good quality, making the reported results more reliable. In addition, boys with idiopathic ASD in our sample were, on average, relatively high functioning, a limitation inherent to imaging research due to the demands associated with participation (e.g. having to lie very still in a confined space). Lastly, autism spectrum symptoms in boys with 47,XXY were assessed using the Social Responsiveness Scale (Constantino & Gruber, 2005). Although this measure shows high correlations with the Autism Diagnostic Interview-Revised (Constantino et al., 2003; Lord, Rutter, & Le Couteur, 1994), no formal diagnostic assessment for ASD was conducted in our 47,XXY participants.

Concluding remarks

The current thesis provides insight into the neural mechanisms underlying cognitive and behavioral problems in 47,XXY, and differences in these mechanisms between individuals with 47,XXY (which is associated with an increased risk of autism spectrum symptomatology) and individuals with idiopathic ASD. Individuals with 47,XXY show characteristic deviations in brain structure and function associated with higher order cognitive functions, social emotional information processing, and language processing. Additionally, while boys with 47,XXY show considerable overlap with boys with idiopathic ASD in autism symptomatology, there are specific differences in the underlying neural mechanisms that revolve around the frontal lobes and insular cortices. Gene-brain-behavior relationships are extraordinarily complex, and disentangling individual aspects of these relationships will require a substantial amount of additional research. It is our hope that the hypotheses generated from our results will give direction to future studies within these populations.

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Chapter 7

Nederlandse samenvatting en discussie

Adequaat ontwikkelde sociale vaardigheden zijn essentieel om succesvol om te kunnen gaan met de complexiteit van interpersoonlijke communicatie. Ogenschijnlijk triviale vaardigheden als kunnen praten over koetjes en kalfjes in sociale situaties kunnen sociale cohesie, het aangaan van banden met andere mensen, aanzienlijk vergroten (Coupland, 2003). Er is grote individuele variabiliteit in sociale vaardigheden en bepaalde klinische aandoeningen beperken de sociale ontwikkeling dusdanig, dat interventie noodzakelijk is. Autismespectrumstoornis (ASS) is het meest sprekende voorbeeld van een aandoening die gepaard gaat met sociale disfunctie. Bepaalde genetische aandoeningen kunnen echter ook leiden tot sociale disfunctie. Het syndroom van Klinefelter (47,XXY) is een genetische aandoening die alleen voorkomt bij mannen. Het wordt gekenmerkt door de aanwezigheid van een extra X chromosoom en is geassocieerd met een variërende mate van cognitieve, sociale en gedragsproblemen (Boada, Janusz, Hutaff-Lee, & Tartaglia, 2009; Visootsak & Graham, 2009). Mannen met 47,XXY hebben een verhoogd risico op het ontwikkelen van symptomen van psychose, bipolaire stoornis, ADHD en autisme (Cederlof et al., 2014). Het bestuderen van het neurale fenotype van 47,XXY kan leiden tot essentiële inzichten in de mechanismen onderliggend aan de grote verscheidenheid aan cognitieve, sociale en gedragsproblemen geassocieerd met deze aandoening. Tot op heden richtten alle neuroimaging studies zich op volwassen mannen met 47,XXY. Het bestuderen van kinderen en adolescenten met deze aandoening kan echter bijdragen aan het bepalen van het neurale fenotype op jonge leeftijd.

Vanuit een gen-hersen-gedragperspectief is het mogelijk dat het extra X chromosoom waar sprake van is bij 47,XXY leidt tot overexpressie van X-gerelateerde genen. Deze overexpressie kan leiden tot disregulatie van genetische mechanismen. Veel X-gerelateerde genen zijn betrokken bij de hersenontwikkeling, waardoor een extra X chromosoom substantiële invloed kan hebben op de structurele en functionele opmaak van de hersenen. Daarnaast lijkt de hersenontwikkeling gemoduleerd te worden door het daadwerkelijk aanwezige aantal X chromosomen, specifiek in hersenregio's verantwoordelijk voor adaptief sociaal functioneren (Raznahan et al., 2014; Vawter, Harvey, & DeLisi, 2007). Deze afwijkende opmaak kan de cognitieve ontwikkeling nadelig beïnvloeden. Door deze impact op hoe binnenkomende informatie verwerkt wordt, kunnen cognitieve beperkingen leiden tot problemen in het verwerken van, en reageren op, informatie uit de omgeving. Dit betekent dat verstoringen in genetische mechanismen, via de hersenen en cognitie, kunnen leiden tot problemen in observeerbaar gedrag. Omdat ASS gediagnosticeerd wordt op basis van gedragscriteria, is het goed mogelijk dat mensen met deze diagnose sociale problemen ontwikkelen als gevolg van verschillende onderliggende genetische en neurale disfuncties. Genetische aandoeningen daarentegen, worden gekenmerkt door een relatief homogeen

endofenotype, met aanzienlijke variatie in gedragsmatige gevolgen. Het bestuderen van genetische aandoeningen als 47,XXY kan daarom ook helpen bij het blootleggen van verschillende paden naar sociale disfunctie die moeilijker bloot te leggen zijn door het bestuderen van idiopathische ASS, als gevolg van variabiliteit in de (endo)fenotypen (Mottron, Belleville, Rouleau, & Collignon, 2014; Muhle, Trentacoste, & Rapin, 2004). Recent onderzoek wijst inderdaad op mogelijke subtiele verschillen tussen individuen met idiopathische ASS en individuen met ASS geassocieerd met 47,XXY, in het cluster van symptomen en de onderliggende cognitieve mechanismen (Bruining et al., 2010; Van Rijn et al., 2014; Van Rijn, Stockmann, Van Buggenhout, Van Ravenswaaij-Arts, & Swaab, 2014). Om deze reden was dit proefschrift gericht op onderzoeken of individuen met 47,XXY en autistische symptomatologie ook verschillen van individuen met idiopathische ASS in de neurale mechanismen onderliggend aan hun cognitieve en gedragssymptomen.

In dit proefschrift hebben wij MRI gebruikt om exploratief het volume van de grijze stof, de integriteit van de witte stof, taakgerelateerde hersenactivatie en intrinsieke functionele hersenconnectiviteit te onderzoeken in individuen met 47,XXY in vergelijking met normaal ontwikkelende individuen. Het doel was inzicht te verkrijgen in de mechanismen die bijdragen aan cognitieve en gedragsproblemen specifiek voor 47,XXY. Daarnaast hebben wij overeenkomsten en verschillen in neurale paden naar sociale disfunctie onderzocht. Omdat de meeste neuroimaging studies tot op heden zich richtten op volwassen mannen met 47,XXY en slechts een handvol pediatrische neuroimaging studies uitgevoerd zijn binnen deze populatie, bestond ons sample uit jongens in de leeftijd van negen tot achttien jaar. Dit maakte het mogelijk net neurale fenotype op vroege leeftijd te bepalen, wat mogelijkheden biedt ten aanzien van vroege interventie, wanneer de omstandigheden optimaler zijn in termen van neurale plasticiteit.

Opbouw van het proefschrift

In dit proefschrift worden achtereenvolgens de structuur van de grijze stof, integriteit van de witte stof, taakgerelateerde hersenactivatie en intrinsieke (resting-state) functionele connectiviteit van jongens met 47,XXY, jongens met ASS en normaal ontwikkelende jongens met elkaar vergeleken.

Hoofdstuk 2 is gericht op het volume van grijze stof regio's die geassocieerd zijn met sociale informatieverwerking, gebruik makend van voxel-based morphometry. De regio's in kwestie waren de superiore temporale cortex, amygdala, orbitofrontale cortex, insulare cortex en mediale frontale cortex. **Hoofdstuk 3** beschrijft onderzoek naar de integriteit van de witte stof, op basis van tract-based spatial statistics. In deze studie hebben wij gekeken naar fractional anisotropy (een weergave van de mate waarin witte stofvezels in een specifieke richting liggen), radial diffusivity (een indicatie van de mate van

myelinisatie), axial diffusitivy (een indicatie van de integriteit van de axonen) en mean diffusivity (de gemiddelde diffusie van water binnen de witte stofvezels). Samengenomen geven deze metingen een kwantificatie van de integriteit van de witte stof. **Hoofdstuk 4** is gericht op taakgerichte hersenactivatie, ofwel functionele MRI (fMRI). Gekozen is voor een sociaal-cognitieve informatieverwerkingstaak, waarbij twee aspecten van dit cognitieve domein werden aangeboord: het herkennen/matchen van gezichtsuitdrukkingen en het toekennen van een verbaal label aan gezichtsuitdrukkingen. Eerstgenoemde omvat relatief basale sociaal-cognitieve vaardigheden, terwijl laatstgenoemde een beroep doet op complexere informatieverwerking. In **hoofdstuk 5** ten slotte, wordt onderzoek naar intrinsieke (resting-state) functionele connectiviteit beschreven. Hiervoor is gebruik gemaakt van resting-state fMRI (RS-fMRI). Het voordeel aan RS-fMRI ten opzichte van taakgerichte fMRI, is dat het functioneren van hersennetwerken in rust geëvalueerd wordt. Men voert tijdens de scan geen cognitieve taak uit. Om deze reden kunnen resultaten uit analyse van deze hersenscans onafhankelijk van taakprestatie geïnterpreteerd worden. Dit maakt het mogelijk uitspraken te doen over potentiële verschillen in functionele connectiviteit van hersenregio's, die onderliggend kunnen zijn aan eerdergenoemde cognitieve problemen.

Onderzoeksbevindingen

Zoals beschreven in **hoofdstuk 2**, bleken jongens met 47,XXY significant minder grijze stof te hebben in de rechter superiore temporale gyrus (STG) dan normaal ontwikkelende jongens. Daarnaast hadden zij, zowel in vergelijking met normaal ontwikkelende jongens, als jongens met ASS, minder grijze stof in de linker en rechter insulare cortices en linker orbitofrontale cortex (OFC). In het onderzoek beschreven in **hoofdstuk 3** vonden wij verminderde witte stof/axonale integriteit in het corpus callosum in jongens met 47,XXY in vergelijking met normaal ontwikkelende jongens. Daarnaast hadden jongens met 47,XXY verminderde axonale integriteit in de rechter inferiore fronto-occipitale fasciculus in vergelijking met jongens met ASS. Tegen de verwachtingen in, bleken jongens met 47,XXY echter verminderde radial diffusivity en dus *toegenomen* myelinisatie te hebben in de linker anteriore corona radiata en het sagittale striatum in vergelijking met normaal ontwikkelende jongens. Uit het onderzoek naar taakgerelateerde hersenactivatie (**hoofdstuk 4**) bleek dat de drie groepen jongens vergelijkbare activatiepatronen lieten zien wanneer het gaat om het herkennen van gezichtsuitdrukkingen. Wanneer gezichtsuitdrukkingen gelabeld moesten worden, lieten jongens met 47,XXY echter verhoogde activatie in de mediale frontale gyrus zien (inclusief het gebied van Broca) ten opzichte van zowel controles als jongens met ASS. Jongens met ASS daarentegen, lieten verhoogde activatie in de amygdala zien tijdens het labelen van gezichtsuitdrukkingen. De intrinsieke functionele connectiviteit (**hoofdstuk 5**) ten slotte, bleek bij jongens met 47,XXY verhoogd te zijn in de verbinding tussen de rechter precuneus/cingulate gyrus en het frontopariëtale hersennetwerk, in

vergelijking met normaal ontwikkelende jongens. In vergelijking met jongens met ASS lieten zij echter verminderde intrinsieke functionele connectiviteit zien tussen de linker precentrale gyrus/mediale frontale gyrus en het auditieve hersennetwerk.

Afwijkende neurale mechanismen betrokken bij hogere orde cognitieve functies in 47,XXY

Het is inmiddels algemeen geaccepteerd dat de frontaalkwab een belangrijke rol speelt in hogere-orde cognitieve processen betrokken bij bewuste redenering en doelgericht, probleemoplossend vermogen (Ardila, 2008). De toegenomen activatie in de mediale frontale regio's tijdens sociale informatieverwerking die gevonden werd in jongens met 47,XXY, kan impliceren dat zij redenering (i.e. hogere-orde cognitieve functies) gebruiken in plaats van 'sociale intuïtie' bij het nemen van sociale beslissingen. De taakeisen in onze studie werden echter bewust tot een minimum beperkt, omdat wij hersenactivatie gerelateerd aan taakcomplexiteit zoveel mogelijk wilden vermijden en wij uitsluitend geïnteresseerd waren in activatie gerelateerd aan sociaal-cognitieve informatieverwerking. Verschillende onderzoeken rapporteren echter beperkingen in hogere-orde cognitieve processen in 47,XXY (Boada et al., 2009; Boone et al., 2001; Lee et al., 2011; Van Rijn & Swaab, in press). Het is daarom mogelijk dat een dergelijke strategie niet werkt in complexere sociale situaties. Dit kan een van de redenen zijn dat individuen met 47,XXY moeite hebben met het identificeren en intepreteren van gezichtsuitdrukkingen (Van Rijn et al., 2014)

Afwijkende neurale mechanismen betrokken bij sociale informatieverwerkingsproblemen in 47,XXY

Verschillende hersenregio's zijn betrokken bij het neurale netwerk voor sociale informatieverwerking. De OFC is bijvoorbeeld belangrijk voor emotieregulatie, het nemen van beslissingen en sociale informatieverwerking, waaronder het herkennen van mentale staten (i.e. theory of mind) en het verwerken van gezichtsuitdrukkingen (Baron-Cohen et al., 1994; Noonan, Sallet, Rudebeck, Buckley, & Rushworth, 2010; Rolls, 2004; Rolls & Grabenhorst, 2008). Conclusies over cognitieve en gedragsproblemen gebaseerd op structurele hersenafwijkingen kunnen alleen onder voorbehoud getrokken worden. Het is echter mogelijk dat verminderd volume van de OFC een van de anatomische grondslagen is van de sociale en cognitieve problemen geassocieerd met 47,XXY, zoals beperkingen in theory of mind en de herkenning van gezichtsuitdrukkingen (Van Rijn, Swaab, Aleman, & Kahn, 2006). De OFC is anatomisch verbonden met een gebied naast de frontaalkwab, de insulare cortex (Jakab, Molnar, Bogner, Beres, & Berenyi, 2012). De bilaterale insulare cortices zijn eveneens significant kleiner in jongens met 47,XXY, een bevinding die overeenkomt met onderzoek naar volwassen mannen met 47,XXY (Bryant et al.,

2011; Shen et al., 2004; Skakkebaek et al., 2013). Deze regio wordt in verband gebracht met herkenning van, en reactie op, emotionele stimuli en interne emotionele staten, evenals het nemen van sociale beslissingen (Adolphs, 2003; Sanfey, Rilling, Aronson, Nystrom, & Cohen, 2003). Mannen met 47,XXY laten verminderde neurale activatie zien in de insulare cortex tijdens het nemen van sociale beslissingen (Van Rijn et al., 2012). Onze bevindingen suggereren dat de socio-emotionele beperkingen gevonden in 47,XXY mogelijk gemedieerd worden door bilaterale afnames in het volume van de insulare cortex in 47,XXY, die al aanwezig zijn in de kindertijd en mogelijk ook impact hebben op het functioneren van deze hersenregio. Andere studies wijzen uit dat individuen met 47,XXY moeite hebben met het identificeren en verwoorden van emotionele staten, ze laten verhoogde emotionele reactiviteit zien in termen van psychofysiologische responsen en hun emotionele staten hebben grotere invloed op de beslissingen die ze nemen (Van Rijn, Barendse, Van Goozen, & Swaab, 2014; Van Rijn et al., 2006). Onze MRI bevindingen impliceren dat afwijkingen in de OFC en insulare cortices onderdeel kunnen zijn van het mechanisme onderliggend aan tekorten in emotieregulatie in 47,XXY.

De STG wordt in verband gebracht met het verwerken van gezichtsinformatie, zoals gezichtsuitdrukkingen en kijkrichting. Beperkingen in het verwerken van gezichtsuitdrukkingen worden gevonden in zowel kinderen als volwassenen met 47,XXY en zij zijn minder gericht op andermans ogen (Van Rijn et al., 2014; Van Rijn et al., 2014; Van Rijn et al., 2006; Van Rijn et al., 2012). Deze problemen worden mogelijk gemedieerd door verminderd volume van de STG dat gevonden werd in ons sample. De toegenomen functionele connectiviteit in rust tussen het frontopariëtale netwerk en de rechter precuneus/cingulate gyrus kan echter impliceren dat ook sprake is van globalere afwijkingen in hersenfunctioneren. De precuneus is betrokken bij emotioneel bewustzijn (Van der Velde et al., 2013) en de cingulate gyrus is een kernstructuur in het limbisch systeem, ook wel ons 'emotionele epicentrum' genoemd (Kotter & Meyer, 1992). Verhoogde intrinsieke functionele hersenconnectiviteit tussen deze gebieden en het frontopariëtale netwerk, dat vooral geassocieerd wordt met taalfuncties (Smith et al., 2009), kan wijzen op een compensatiemechanisme voor de verbinding tussen taal en emotie. Dit compensatiemechanisme zou hypothetisch gesproken kunnen bestaan uit processen lijkend op neurale compensatie. Dit is een term die gebruikt wordt om hersenactivatie in individuen met hersenpathologie te beschrijven, in regio's die niet gebruikt worden door individuen met intacte hersenen, met als doel het (onbewust) verbeteren van de cognitieve prestatie (Stern, 2009). Gebaseerd op onze bevindingen, is onze hypothese dat dit compensatiemechanisme in 47,XXY ontoereikend kan zijn, wat leidt tot een problematische verbinding tussen taal en emotie. Dit kan de taal- en emotieverwerkingsproblemen zoals alexithymie en moeite met het labelen van emoties, die behoren tot de meest gerapporteerde beperkingen in 47,XXY, mogelijk (deels) verklaren (Boada et al., 2009; Boone et al., 2001; Van Rijn et al., 2006).

Afwijkende neurale mechanismen betrokken bij taalverwerkingsproblemen in 47,XXY

De toegenomen intrinsieke functionele hersenconnectiviteit tussen het frontopariëtale (taal)netwerk en de precuneus/cingulate gyrus, kan een reflectie zijn van een ineffectief neuraal compensatiemechanisme voor de verbinding tussen taal en emotie. Onderzoek in normaal ontwikkelende individuen suggereert dat het taalvermogen invloed heeft op emotieverwerkingsvaardigheden (Cutting & Dunn, 1999; Jablonka, Ginsburg, & Dor, 2012; Lindquist, Barrett, Bliss-Moreau, & Russell, 2006). Dit leidt tot de hypothese dat beperkingen in het taalvermogen in 47,XXY mogelijk bijdragen aan beperkingen in emotieverwerking. In onze fMRI studie gericht op de neurale mechanismen onderliggend aan het labelen van gezichtsuitdrukkingen vertoonde een deel van Broca's gebied, een kerngebied voor taalverwerking (Davis et al., 2008), toegenomen activatie in jongens met 47,XXY tijdens het labelen van prototypische gezichtsuitdrukkingen van emoties. Deze bevinding geeft extra ondersteuning aan de hypothese van een disfunctioneel neuraal mechanisme onderliggend aan problemen in de connectie tussen taal- en emotieverwerking. Broca's gebied is doorgaans actief in de linkerhemisfeer, aangezien deze hemisfeer meestal dominant is voor taal. In onze fMRI studie werd de toegenomen activatie gevonden in Broca's gebied in de rechterhemisfeer. Een eerdere fMRI studie in volwassen mannen met 47,XXY rapporteerde echter ook toegenomen activatie in taalgebieden in de rechterhemisfeer tijdens een taalverwerkingstaak. Dit suggereert dat individuen met 47,XXY mogelijk minder hemisferische specialisatie hebben voor taal (Van Rijn et al., 2008).

Hoewel de bevinding van verminderde witte stof integriteit in 47,XXY niet gelijk staat aan schade aan deze vezelbanen, impliceert het wel verminderde efficiëntie van neurale connecties. In de normale ontwikkeling is het corpus callosum betrokken bij taalfuncties (Bloom & Hynd, 2005; Van der Knaap & Van der Ham, 2011). Laesies in het corpus callosum worden ook geassocieerd met alexithymie, een term die gebruikt wordt om problemen met het identificeren en verwoorden van emoties aan te duiden, wat duidt op problemen in zowel taal- als emotieverwerking (Sifneos, 1973). Alexithymie is een probleem dat gerapporteerd wordt in 47,XXY (Van Rijn et al., 2006). Verminderde integriteit van vezelbanen in het corpus callosum kan daarom nog een onderdeel zijn van het mechanisme onderliggend aan de gebrekkige connectie tussen taal en emotie in 47,XXY.

Verminderd volume van de grijze stof in de STG draagt mogelijk niet alleen bij aan de sociale problemen geassocieerd met 47,XXY, maar ook aan de beperkingen in het taalvermogen. Wernicke's gebied en Heschl's gyri zijn verantwoordelijk voor respectievelijk spraak- en auditieve verwerking (Da Costa et al., 2011; Wise et al., 2001). Zij zijn onderdeel van het Wernicke-Geschwind

model van taalverwerking en liggen in de STG, wat de STG een kerngebied maakt binnen dit model. Het Wernicke-Geschwind model van taalverwerking bestaat uit Broca's gebied, Wernicke's gebied, de superiore temporale sulcus, inferiore pariëtale lobule, mediale temporale gyrus en de fasciculus arcuatus die deze gebieden met elkaar verbindt (Dick, Bernal, & Tremblay, 2014). Hoewel dit klassieke model tegenwoordig te simplistisch geacht wordt, vormt het nog steeds de basis van waaruit taalverwerking in het brein bestudeerd wordt. Daarnaast bevatten hedendaagse modellen van taalverwerking ook superiore temporale gebieden (Dick et al., 2014). De verminderde hemisferische specialisatie voor taal in de STG gerapporteerd door Van Rijn et al. (2008) is mogelijk een functionele consequentie van anatomische afwijkingen in STG ontwikkeling in 47,XXY. Het corpus callosum faciliteert daarnaast mogelijk taalverwerking door middel van interhemisferische communicatie tussen de linker en rechter plana temporale, waar de STG onderdeel van is (Bloom & Hynd, 2005; Van der Knaap & Van der Ham, 2011). Deze bevindingen leiden tot de hypothese dat structurele afwijkingen in de STG en het corpus callosum mogelijk onderdeel zijn van het anatomische mechanisme onderliggend aan taalverwerkingsproblemen in 47,XXY.

Toegenomen myelinisatie in 47,XXY

De bevinding dat jongens met 47,XXY verminderde radial diffusivity hebben in de linker anteriore corona radiata en het sagittale striatium, heeft mogelijk belangrijke implicaties. Verminderde radial diffusivity wordt geassocieerd met toegenomen myelinisatie van witte stof (Alexander, Lee, Lazar, & Field, 2007), waardoor neuronen informatie sneller kunnen doorgeven, wat neurale communicatie verbetert. Doorgaans worden klinische aandoeningen geassocieerd met verminderde integriteit van de myeline, maar in ons 47,XXY sample lijkt dit effect omgekeerd te zijn. Hoewel speculatief, geven wij twee mogelijke verklaringen voor deze onverwachte bevinding. De eerste heeft betrekking op het gebruik van steroïde hormonen. Jongens en mannen met 47,XXY ondergaan vaak testosteronbehandeling ter verlichting van de symptomen geassocieerd met deze aandoening (e.g. verminderde haargroei in het gezicht en de schaamstreek). Dergelijke hormonen bevorderen myelinisatie in het brein (Peper, Van den Heuvel, Mandl, Hulshoff Pol, & Van Honk, 2011). In ons sample ondergingen vier van de negen participanten testosteronbehandeling op het moment van de studie. Als onze bevinding van toegenomen myelinisatie inderdaad het gevolg is van testosteronbehandeling, dan zou dit een belangrijke beschermende factor zijn die mogelijk neurale communicatie in 47,XXY zou kunnen verbeteren. Een al relatief kleine sample size maakte dat wij onze 47,XXY groep niet konden opsplitsen in jongens die testosteronbehandeling ontvingen en jongens die deze behandeling niet ontvingen. Het is echter belangrijk toekomstig onderzoek te richten op de impact van behandeling op myelinisatie. Een tweede mogelijke verklaring voor toegenomen myelinisatie in 47,XXY komt voort uit de bevinding dat het gen

voor de myeline proteolipide eiwit op het X chromosoom ligt (Willard & Riordan, 1985). Er wordt gesuggereerd dat het patroon van gen-inactivatie een van de mechanismen is waardoor het extra X chromosoom fenotypische ontwikkeling in 47,XXY beïnvloedt (Skakkebaek et al., 2014). Het is mogelijk dat de extra X die aanwezig is bij deze aandoening, het functioneren van de myeline proteolipide eiwit beïnvloedt. Eerder onderzoek (Hodes, 2000) suggereert dat extra kopieën van dit eiwit kunnen leiden tot ernstige somatische aandoeningen. Het is daarom onwaarschijnlijk dat de toegenomen myelinisatie komt doordat dit eiwit simpelweg ontsnapt aan inactivatie. Muisstudies wijzen er echter op dat dit gen mogelijk deels kan ontsnappen aan inactivatie, wat hypothetisch kan leiden tot overexpressie. Deze resultaten demonstreren de waarde van interdisciplinair onderzoek (e.g. neurowetenschappen en klinische genetica) om het mogelijke effect van het extra X chromosoom op het functioneren van dit eiwit verder te verkennen. Deze verklaringen zijn op dit moment echter erg speculatief. Meer onderzoek is noodzakelijk om de exacte oorsprong van toegenomen myelinisatie in 47,XXY te bepalen.

47,XXY versus idiopathische ASS

Met uitzondering van verminderd grijze stofvolume in de bilaterale insulare cortices in 47,XXY, hebben alle verschillen in hersenstructuur en –functie tussen onze 47,XXY- en ASS-groepen betrekking op de frontaalkwab. Anatomisch hebben jongens met 47,XXY minder grijze stof in de linker OFC en zij hebben verminderde axonale integriteit in de rechter inferiore fronto-occipitale fasciculus, een vezelbundel die de frontaalkwab verbindt met de temporaal- en occipitaalkwabben (Martino, Vergani, Robles, & Duffau, 2010). Functioneel laten jongens met 47,XXY verminderde intrinsieke hersenconnectiviteit zien tussen het auditieve hersennetwerk en de linker precentrale gyrus/mediale frontale gyrus. Tijdens sociaal-cognitieve informatieverwerking, hebben jongens met 47,XXY toegenomen hersenactivatie in de rechter mediale frontale gyrus.

Dit is de eerste keer dat hersenstructuur en –functie in deze populaties zijn vergeleken. Meer onderzoek is nodig om solide hypotheses te genereren die kunnen verklaren waarom de verschillen zich voornamelijk in de frontaalkwab bevinden. Deze resultaten suggereren echter wel dat verminderde intrinsieke functionele connectiviteit tussen verschillende corticale gebieden een kenmerk is dat niet specifiek is voor idiopathische ASS (Di Martino et al., 2014), aangezien onze 47,XXY-groep nog sterker verlaagde intrinsieke functionele connectiviteit lieten zien dan de idiopathische ASS-groep. Hypoconnectiviteit van corticale regio's zou daarom mogelijk een gedeeld kenmerk van 47,XXY en idiopathische ASD kunnen zijn, wat suggereert dat het een cruciale rol kan hebben in sociale disfunctie. Het zou symptoomspecifiek in tegenstelling tot stoornisspecifiek kunnen zijn. Het is echter een belangrijke bevinding dat jongens met 47,XXY een substantiële toename in activatie in frontale gebieden laten zien ten opzichte van jongens met ASS, wanneer zij een sociaal-cognitieve taak uitvoeren. Dit kan

wederom een vorm van neurale compensatie in 47,XXY impliceren, die verschilt van idiopathische ASS. In ons onderzoek lieten jongens met ASS ook toegenomen activatie tijdens sociaal-cognitieve informatieverwerking zien, maar in een ander gebied (i.e. mediale frontale gyrus in 47,XXY, versus amygdala in ASS). De amygdala is onderdeel van een 'sociaal brein' netwerk en sterk betrokken bij het automatisch screenen van emotionele relevantie, waaronder gezichtsuitdrukkingen, in de normale ontwikkeling. Dit kan betekenen dat jongens met idiopathische ASS meer leunen op 'sociale intuïtie'. De uitgesproken beperkingen op dit gebied waar bij ASS sprake van is, suggereren echter dat deze vorm van neurale compensatie ontoereikend is. Op gedragsniveau kunnen beide groepen dus overeenkomstige sociale problemen ervaren, maar onze bevindingen wijzen op duidelijke verschillen in de onderliggende neurale compensatiemechanismen. De bilaterale afnames in het volume van de insulare cortex en OFC in 47,XXY ten opzichte van normaal ontwikkelende jongens, waren ook aanwezig in vergelijking met jongens met idiopathische ASS. Deze hersengebieden zijn belangrijk voor het bewustzijn van interne emotionele staten, evenals de regulatie van deze staten. Dit kan betekenen dat sociale disfunctie in individuen met 47,XXY meer beïnvloed wordt door verstoorde emotieregulatie (i.e. problemen met het monitoren van de eigen, interne, emotionele staat en deze gebruiken als sociaal kompas) dan in individuen met idiopathische ASS.

Deze bevindingen impliceren uitgesproken verschillen in neurale structuur en functie tussen 47,XXY en ASS. Naast verschillen in de cognitieve en gedragscomponenten van de gen-hersen-gedraghypothese van sociale disfunctie, lijkt de hersencomponent ook aanzienlijk te verschillen. Jongens met 47,XXY in ons sample konden echter niet onderscheiden worden van jongens met idiopathische ASS op basis van gedragsmaten voor autismesymptomen. Er lijken dus verschillende paden naar sociale disfunctie te zijn, waaronder heterogeniteit in onderliggende neurale mechanismen. Kennis over de exacte aard van deze verschillen draagt bij aan het specificeren van de gedragsproblemen en heeft belangrijke wetenschappelijke en klinische implicaties, waar we ons nu op zullen richten.

Wetenschappelijke en klinische implicaties

Het huidige proefschrift markeert de eerste keer dat de structurele en functionele architectuur van het brein onderzocht zijn op verschillende domeinen, met verscheidene MRI technieken, in één sample van kinderen/adolescenten met 47,XXY. Het was eveneens de eerste keer dat hersenstructuur en –functie van jongens met 47,XXY, een hoog-risico groep voor autismesymptomen, vergeleken werden met die van jongens met idiopathische ASS. De resultaten leiden tot interessante en belangrijke suggesties voor toekomstig onderzoek. De aanwezigheid van een extra X chromosoom in 47,XXY, dan wel de hormonale gevolgen van deze aandoening, lijken een

significante impact te hebben op hersenontwikkeling. Deze factoren dragen mogelijk samen bij aan het verhoogde risico op problemen in 47,XXY in de hogere orde cognitieve functies, sociaal-emotionele informatieverwerking en taalverwerking. Deze neurale paden naar sociale disfunctie verschillen van die in idiopathische ASS, vooral op het gebied van frontaalfuncties en emotieregulatie.

Toekomstig onderzoek op dit gebied zou zich moeten richten op replicatie van onze bevindingen in grotere samples met verschillende leeftijdscategorieën, evenals verdere specificatie van de exacte gen-hersen-gedragrelaties in 47,XXY. MRI biedt buitengewoon perspectief voor het onderzoeken van deze mechanismen, vooral wanneer het gecombineerd wordt met andere neurobiologische, cognitieve en gedragsmaten. De functionele MRI taak in onze studie was bijvoorbeeld gericht op het decoderen van gezichtsuitdrukkingen. Het toevoegen van neurofysiologische maten zoals eye tracking, wat gebruikt wordt om data te verzamelen met betrekking tot de aandachtsfocus van de participant, maakt het mogelijk te bepalen of de toegenomen frontale activatie in 47,XXY in onze studie top-down (i.e. de binnenkomende informatie wordt op een andere manier verwerkt in het brein) of bottom-up (i.e. hersenactivatie verschilt, omdat de binnenkomende informatie anders is, als gevolg van een afwijkende aandachtsfocus) is. Eye tracking heeft bijvoorbeeld aangetoond dat volwassen mannen met 47,XXY minder fixeren op de oogregio van gezichten dan normaal ontwikkelende mannen wanneer zij gezichtsuitdrukkingen moeten decoderen (Van Rijn, in press). Dergelijke studies kunnen buitengewoon nuttig zijn voor het specificeren van de exacte afwijkingen in sociaal-cognitieve informatieverwerking in 47,XXY

Dit proefschrift biedt ook perspectief voor interventiestudies. De resultaten suggereren het bestaan van verschillende paden naar sociale disfunctie, wat blijkt uit de verschillen tussen jongens met 47,XXY en jongens met idiopathische ASS. Dit zou kunnen betekenen dat deze groepen ook differentieel gevoelig zijn voor behandeling. Onderzoek naar de ontwikkeling van behandeling op maat voor sociale disfunctie, gericht op het vergroten van behandeleffecten, kan baat hebben bij kennis over verschillen in deze neurale mechanismen. Een specifiek relevant onderzoeksgebied in deze context is real time fMRI neurofeedback. Deze techniek is gericht op het aanpassen van gedrag door het aanpassen van hersenfunctioneren. Real time informatie over veranderingen in neurale activatie wordt voor een participant in beeld gebracht tijdens meerdere trainingssessies, met als doel het faciliteren van zelfregulatie van deze activatie. Het idee is dat dit veranderingen teweegbrengt in hersenfunctioneren, die op hun beurt kunnen leiden tot veranderingen in cognitie en/of gedrag (Stoeckel et al., 2014). Meer onderzoek is noodzakelijk om de effectiviteit van deze interventie in het beïnvloeden van gedrag door het beïnvloeden van hersenactivatie te bepalen. Real time fMRI neurofeedback kan echter mogelijk de weerbaarheid verhogen van individuen met een hoog risico op sociale disfunctie, door het corrigeren van reeds bekende disfuncties in neurale

activatiepatronen vóór ernstige ontwikkelingsproblematiek zich manifesteert. In 47,XXY zouden potentiële gebieden voor behandeling bijvoorbeeld hersenregio's kunnen zijn waarvan bekend is dat zij bijdragen aan taal- en emotieverwerking in de normale ontwikkeling. Hiermee kan getracht worden het huidige, ineffectieve neurale compensatiemechanisme aan te passen. Omgekeerd zou real time fMRI neurofeedback voor individuen met een formele psychiatrische diagnose als ASS gebruikt kunnen worden om neurale systemen onderliggend aan sociale disfunctie op een directe manier aan te pakken, om gedragsproblemen te verminderen (Stoeckel et al., 2014). De eerste onderzoeken naar deze potentiële next generation therapeutische toepassing hebben tot positieve resultaten geleid in mensen met smetvrees, niet alleen in het veranderen van de functionele architectuur van het brein, maar ook in het veroorzaken van gedragsveranderingen die aanhielden tot meerdere dagen na de trainingssessies (Robineau et al., 2014; Scheinost et al., 2013). In het huidige proefschrift hebben wij verschillen geïdentificeerd in de specifieke neurale systemen die bijdragen aan sociale disfunctie. Ons werk kan daarom een waardevolle bron van informatie zijn en stimuleert hopelijk onderzoek naar real time fMRI neurofeedback voor sociale disfunctie, door het identificeren van potentiële targetgebieden voor behandeling.

Vanuit een klinisch perspectief kan het huidige proefschrift helpen bij het creëren van bewustzijn onder clinici van het bestaan van grote individuele variabiliteit, niet alleen in de gedragssymptomen van sociale disfunctie of autisme, maar ook in de mechanismen onderliggend aan deze symptomen. Dit kan de selectie van behandelstrategieën beïnvloeden. Onze resultaten suggereren bijvoorbeeld dat individuen met 47,XXY een meer rationale aanpak gebruiken bij sociaal-cognitieve informatieverwerking (waarbij de frontaalgebieden substantieel meer activatie laten zien). Clinici kunnen deze informatie gebruiken om te bepalen hoe zij interventies het best kunnen implementeren bij jongens en mannen met 47,XXY. Sociale vaardigheidstraining voor mensen met 47,XXY zou zich bijvoorbeeld meer kunnen richten op verklaringen voor *waarom* bepaalde gedragingen gepast zijn sociale situaties en minder op het oefenen van sociale situaties middels rollenspellen. Daarnaast kan de wetenschap dat emotieregulatiebeperkingen mogelijk gemedieerd worden door bilaterale afnames in insulare cortexvolume clinici ervan bewust maken dat emotieregulatieproblemen in jongens en mannen met 47,XXY in etiologie verschillen van individuen met idiopathische ASS. Dit kan vervolgens impliceren dat zij een andersoortige behandeling nodig hebben die zich meer richt op het identificeren en monitoren van de eigen, interne emotionele staat. Ten slotte suggereren onze bevindingen dat afwijkingen in de ontwikkeling van hersenstructuur en –functie in 47,XXY al aanwezig zijn in de kindertijd. Dit betekent dat interventies gericht op het voorkomen en verbeteren van gedragsproblemen in individuen met 47,XXY mogelijk het meest effectief zijn wanneer deze voor aanvang van de adolescentie worden geïmplementeerd.

Limitaties

Hoewel alle gerapporteerde resultaten uit onze onderzoeken significant en methodologisch verantwoord waren, was sprake van enkele onvermijdelijke limitaties. Een deel van de (f)MRI data was van onvoldoende kwaliteit om te includeren in analyses, als gevolg van beweging van de participant en andere artefacten. Dit resulteerde in relatieve kleine samples, wat geleid kan hebben tot een gebrek aan power om subtielere verschillen in neurale structuur en/of functie te detecteren. Daarnaast maakte het correlationele analyse van cognitieve en (f)MRI data onmogelijk. De (f)MRI data gebruikt in de analyses was echter grondig gecontroleerd en van goede kwaliteit, wat de gerapporteerde resultaten betrouwbaarder maakt. Daarnaast waren de jongens met idiopathische ASS in ons sample gemiddeld relatief hoogfunctionerend, een limitatie die inherent is aan imagingonderzoek als gevolg van de eisen geassocieerd met deelname (e.g. erg stil moeten liggen in een kleine ruimte). Ten slotte werden autismesymptomen in jongens met 47,XXY vastgesteld door middel van de Social Responsiveness Scale (Constantino & Gruber, 2005). Hoewel dit meetinstrument hoge correlaties heeft met het Autism Diagnostic Interview-Revised (Constantino et al., 2003; Lord, Rutter, & Le Couteur, 1994), is geen formeel diagnostisch onderzoek verricht bij onze 47,XXY participanten.

Slotbeschouwing

Het huidige proefschrift verschaft inzicht in de neurale mechanismen onderliggend aan cognitieve en gedragsproblemen in 47,XXY en verschillen in deze mechanismen tussen individuen met 47,XXY (geassocieerd met een verhoogd risico op autismeproblematiek) en individuen met idiopathische ASS. Individuen met 47,XXY hebben kenmerkende afwijkingen in hersenstructuur en –functie geassocieerd met hogere-orde cognitieve functies, sociaal-emotionele informatieverwerking en taalverwerking. Hoewel jongens met 47,XXY aanzienlijke overlap vertonen met jongens met idiopathische ASS in autismesymptomen, is sprake van specifieke verschillen in de onderliggende neurale mechanismen die zich lijken te centreren rondom de frontaalkwab en insulare cortices. Gen-hersen-gedragrelaties zijn buitengewoon complex en het ontwarren van individuele aspecten van deze relaties vereist een substantiële hoeveelheid aanvullend onderzoek. Het is onze hoop dat de hypothesen gegenereerd op basis van onze resultaten richting geeft aan toekomstige studies binnen deze populaties.

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Curriculum Vitae

Marcia Naomi Goddard werd geboren op 2 september 1984 te Zaanstad. Na het afronden van de middelbare school op het Zaanlands Lyceum te Zaandam, studeerde zij enkele jaren aan de Universiteit van Amsterdam. In 2008 begon zij, na enige jaren gewerkt te hebben, aan de bacheloropleiding Psychologie aan de Universiteit Leiden. In 2011 rondde zij deze cum laude af. In 2012 studeerde Marcia cum laude af in de Neuropsychologie, eveneens aan de Universiteit Leiden. In oktober 2011 begon zij aan haar promotieonderzoek aan de Universiteit Leiden, op de afdeling Orthopedagogiek, onder begeleiding van prof. dr. Hanna Swaab en dr. Sophie van Rijn. De resultaten van dat onderzoek staan beschreven in dit proefschrift. Naast haar werk als promovenda begeleidde Marcia kinderen met ontwikkelingsproblemen via Inzowijs. Sinds maart 2015 is zij werkzaam als Universitair Docent op de afdeling Orthopedagogiek van de Universiteit Leiden. Naast haar onderzoeks- en onderwijswerkzaamheden, houdt Marcia zich bezig met wetenschapscommunicatie. In april 2015 won zij de wetenschapscommunicatiewedstrijd FameLab. Sindsdien spreekt zij regelmatig over neurowetenschappelijk onderzoek in de media en op (inter)nationale evenementen.

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