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Y chromosome gene copy number and lack of autism phenotype in a male with an isodicentric Y chromosome and absent NLGN4Y expression

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Abstract

We describe a unique male with a dicentric Y chromosome whose phenotype was compared to that of males with 47,XYY (XYY). The male Y-chromosome aneuploidy XYY is associated with physical, behavioral/cognitive phenotypes, and autism spectrum disorders. We hypothesize that increased risk for these phenotypes is caused by increased copy number/overexpression of Y-encoded genes. Specifically, an extra copy of the neuroligin gene *NLGN4Y* might elevate the risk of autism in boys with XYY. We present a unique male with the karyotype 46,X,idi(Y)(q11.22), which includes duplication of the Y short arm and proximal long arm and deletion of the distal long arm, evaluated his physical, behavioral/cognitive, and neuroimaging/magnetoencephalography (MEG) phenotypes, and measured blood RNA expression of Y genes. The proband had tall stature and cognitive function within the typical range, without autism features. His blood RNA showed two-fold increase in expression of Yp genes versus XY controls, and absent expression of deleted Yq genes, including *NLGN4Y*. The M100 latencies were similar

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to findings in typically developing males. In summary, the proband had overexpression of a subset of Yp genes, absent *NLGN4Y* expression, without ASD findings or XYY-MEG latency findings. These results are consistent with a role for *NLGN4Y* overexpression in the etiology of behavioral phenotypes associated with XYY. Further investigation of *NLGN4Y* as an ASD risk gene in XYY is warranted. The genotype and phenotype(s) of this subject may also provide insight into how Y chromosome genes contribute to normal male development and the male predominance in ASD.

Keywords

XYY; Y chromosome; autism spectrum disorder; neuroligin4Y; *NLGN4Y*; RNA expression

Introduction

We describe a unique male with a dicentric Y chromosome whose phenotype was compared to that of males with 47,XYY. The presence of a supernumerary Y chromosome, as found in the male sex chromosome aneuploidy disorder 47,XYY (XYY), is known to be a strong genetic risk factor for autism spectrum disorders (ASD). Newborn screening series demonstrate that XYY occurs in about 1 in 1,000 males, but it is diagnosed much less frequently because associated features such as tall stature or developmental delays are not distinctive (A. Robinson, Bender, Linden, & Salbenblatt, 1990). Pubertal development and testosterone levels are generally normal (Aksglaede, Skakkebaek, & Juul, 2008; Bojesen, Juul, & Gravholt, 2003; MacLean, 1961; Ratcliffe, 1982; Ratcliffe, Pan, & McKie, 1992; A. Robinson et al., 1990; Rovet, Netley, Bailey, Keenan, & Stewart, 1995; Welch, 1985). While general intelligence in XYY males is typically normal or only mildly diminished (Bender, Puck, Salbenblatt, & Robinson, 1984; Leggett, Jacobs, Nation, Scerif, & Bishop, 2010; Netley, 1986; A. Robinson, Bender, BG, Puck, MH, Salbenblatt, JA., 1985), these boys are at increased risk for neurodevelopmental phenotypes such as language-based learning disabilities, attention deficit hyperactivity disorder (ADHD) (Mulligan, Gill, & Fitzgerald, 2008), social-emotional difficulties, and seizure disorders (Bardsley et al., 2013; Bishop & Scerif, 2011; Cordeiro, Tartaglia, Roeltgen, & Ross, 2012; Geerts, Steyaert, & Fryns, 2003; Margari, Lamanna, Craig, Simone, & Gentile, 2014; A. Robinson et al., 1990; Ross et al., 2012; Ross, Zeger, Kushner, Zinn, & Roeltgen, 2009; Tartaglia, Ayari, Hutaff-Lee, & Boada, 2012). The reported prevalence of ASD in males with XYY ranges from 19-50% in cohorts diagnosed prenatally or postnatally, (Bishop et al., 2011; Cordeiro et al., 2012), and a recent evaluation of 57 boys with XYY found that 38% met DSM-5 ASD criteria (Tartaglia et al., 2017).

The mechanism by which an extra Y chromosome contributes to these neurodevelopmental clinical features is not known. Unlike supernumerary X chromosomes, which undergo transcriptional silencing, or X-inactivation, extra copies of the Y chromosome are thought to remain transcriptionally active. Originally, the Y chromosome was thought to bear only a few genes, primarily involved in sex determination and testicular function, but it is now known to contain numerous genes with diverse functions (Bellott & Page, 2009; Fukuda et al., 2010). Accordingly, we and others have hypothesized that the increased risk of ASD in XYY results from double dosage and associated overexpression of one or more of these Y

genes (Bishop et al., 2011). One Y gene in particular, *neuroligin 4Y (NLGN4Y)*, is an intriguing XYY ASD candidate (Bishop & Scerif, 2011). *NLGN4Y* is one of a pair of cognate genes on the human X and Y chromosomes that encode members of a family of trans-synaptic cell adhesion molecules which bind neuroligins and stabilize excitatory and inhibitory synaptic activity (Hoon et al., 2011; Jamain et al., 2003; Zhang et al., 2009). Some but not all (Blasi et al., 2006; Gauthier et al., 2005; Jamain et al., 2003; Vincent et al., 2004; Ylisaukko-oja et al., 2005) genetic studies implicate synaptic molecules, including neuroligins and neuroligins, in the pathogenesis of ASD. Additionally, the presence of a neuroligin 4 gene on the Y chromosome has been proposed to explain the male predominance of ASD (Baron-Cohen et al., 2011; Bishop & Scerif, 2011).

To date, few studies have explored potential pathogenic genetic mechanisms of the increased ASD risk in XYY. Similarly, neuroimaging findings related to genetic aspects of XYY have not been explored in depth. In this paper, we present the ASD phenotype, molecular, and neuroimaging results (whole cortex magnetoencephalography (MEG)) from an unusual male (pt1794) with a complex Y chromosome rearrangement (isodicentric Y) resulting in two copies of a subset of Y chromosome genes and no copy of *NLGN4Y*. Our goal was to investigate the phenotype associated with increased dosage and overexpression of selective Y chromosome genes, thereby gaining insight into the potential roles of specific Y genes in the XYY phenotype. At the same time, the phenotype(s) of this subject could suggest a function for *NLGN4Y* or another deleted Y gene in normal male development.

Materials and Methods:

The study was approved by the Human Studies Committee at Thomas Jefferson University and Informed consent and assent was obtained from the proband, 9 XYY (mean±SD age 11.6±3.2), and 6 male control subjects (mean±SD age 13.2±3.5). Chromosome microarray analysis of the proband was performed as part of his initial clinical diagnostic evaluation.

RNA-Seq and Analysis

Blood was collected from the proband, XYY, and control subjects using the PAXGene system (QIAGEN, Valencia, CA) and sent via overnight courier to UT Southwestern. RNA was purified from whole blood with Qiagen RNeasy Mini kit according to manufacturer's instruction. RNA concentration was measured by a Qubit fluorometer, and then libraries were prepared for RNA sequencing using the TruSeq Stranded mRNA LT Sample Prep Kit (Illumina), following the manufacturer's instructions, as summarized as follows: DNase-treated total RNA was used as input. Poly-A RNA was purified and fragmented before strand-specific cDNA synthesis. cDNA was then A-tailed and ligated with indexed adapters. PCR amplified libraries were purified with AmpureXP beads, and validated on the Agilent 2100 Bioanalyzer. Before being normalized and pooled, samples were quantified by Qubit (Invitrogen) and then run on an Illumina HiSeq 2500 using SBS v3 reagents to generate 150-bp paired-end reads. Reads were trimmed to remove adaptor sequences and low-quality bases using fastq-mcf (v1.1.2-806, <https://github.com/ExpressionAnalysis/eutils/blob/wiki/FastqMcf.md>), followed by mapping to human genome (hg38) using Tophat (v2.0.10, (Trapnell et al., 2012)) with igene annotations (<https://ccb.jhu.edu/software/tophat/>

[igenomes.shtml](#)). Duplicate reads were marked but not removed. Sequencing reads were counted by using FeatureCounts (Liao, Smyth, & Shi, 2014) normalized by using edgeR (M. D. Robinson, McCarthy, & Smyth, 2010) for the differential gene expression analysis. The annotated protein-coding gene transcripts are included, without noncoding RNAs. Results are reported as counts per million (CPM) values, which reflect the abundance of the RNA signal.

Neuroimaging

Magnetic Resonance Imaging (MRI)—A structural MRI was recorded from the participant on a 3T Siemens Verio scanner using a 32 channel receiver only head RF coil with a magnetization-prepared pulse sequence (MPRAGE) with the following parameters: 126 slices, $1 \times 1 \times 1$ mm voxels, field of view 256, matrix 256×256 , echo time 2.87ms, repetition Time 1,900ms, flip angle 9° , inversion time 1,050ms.

Magnetoencephalography (MEG)—MEG data was recorded, with a sampling rate of 1200Hz, using a using whole-head 275 axial gradiometer CTF system (VSM MedTech, Coquitlam, BC) and synthetic third-order gradiometer noise correction. Electrooculogram (EOG) and electrocardiogram (ECG) were recorded to correct for eye-blinks and heartbeat artifacts. Head position was continuously monitored using three head position indicator (HPI) coils attached to the scalp. Auditory stimuli, 500Hz sinusoidal tones, were presented binaurally at 45-dB sensation level above the subject's hearing threshold. Each trial consisted of a 500Hz tone (300ms duration) plus an inter-stimulus interval varied between 600ms and 2000ms. A total of 530 tones were presented. To minimize fatigue, during the auditory test the subject viewed (but did not listen to) a movie projected onto a screen.

Using BESA 6.0 (BESA®, Gräfelfing – Germany), MEG data was downsampled to 500Hz and artifact correction was applied to remove eye blink activity as outlined in Edgar et al. (Edgar et al., 2015). Artifact-free epochs were then averaged and used for source localization. Source localization was accomplished using anatomical constraints. A rigid body registration between MEG data and the structural MRI was achieved using 3 anatomical landmarks (nasion and right and left preauriculars) as well as the additional 200+ digitized head shape points. Latencies of the left and right 50ms (M50) and 100ms (M100) auditory response were estimated. The primary auditory generator was modeled by single dipole locations in the subject's left and right Heschl's gyrus, as determined visually from sMRI. Auditory dipoles were oriented separately at the maxima of the M50 and M100 response. Thus, although estimates of left and right auditory activity were obtained using an anatomical constraint, the orientation of the M50 and M100 dipoles was optimized separately.

Assessment procedures: The proband completed a physical examination, behavioral questionnaires and autism assessment. His mother completed the behavioral questionnaires.

Anthropometric Measurements: The clinical assessment included measurements of height, weight, and head circumference (HC) that were converted to z-scores using age- and gender-specific norms (Hamill et al., 1979; Marshall & Tanner, 1970). Pubertal development

was assessed by an experienced pediatric endocrinologist (JR) and included evaluation of testicular volume (Prader orchidometer (Hall, 1995)) and pubic hair development (Tanner method (Marshall WA, 1993))

Statistical methods: Descriptive results and RNA expression are presented for the proband (pt1794). For comparisons, mean $\pm$ SD results are also presented from 9 boys with XYY and 6 typically developing control boys presumed to be 46,XY on the basis of <0.5% prevalence of chromosome abnormalities in phenotypically normal men (Ravel, 2006). Based on the single index case and the small numbers of subjects, no statistical comparisons are included. **MEG:** Latencies of the M50 and M100 components of the auditory evoked response of the proband were compared to recently published data (Bloy, 2017) acquired in a cohort of 21 boys with XYY (mean $\pm$ SD age 11.9 $\pm$ 3.4) and 47 XY typically developing boys (mean $\pm$ SD age 12.8 $\pm$ 2.9). Most of the TD and XYY subjects with MEG measurements did not overlap with the subjects who had RNA analyses, except for 4 XYY subjects and 5 XY subjects. Historic data was age corrected to the proband's age (17.8 years) using a linear model including age and group. For all components, age-corrected group (TD and XYY) means and standard deviations were computed and served as a comparison for the proband's latencies.

Results

Clinical summary:

This 17.8 year-old male had a karyotype performed at age 17, based on clinical suspicion for Klinefelter syndrome, related to his tall stature. Parents were also karyotyped; the mother was 46,XX, and the father was 46,XY.

Past Medical History:

The proband was the first child of a 34 year-old mother and 30 year-old father, born after a full-term uncomplicated pregnancy and delivery. Birth weight was 8 pounds 10 ounces (z-score = 1.2), and birth length was 19 inches (z-score = -0.4). His height has been greater than 95th percentile since early childhood. He has been generally healthy without any history of seizures. He had asthma since age 4 that has improved.

Motor milestones and early language development were in the typical range. He had speech therapy for stuttering from ages 4-13. He was diagnosed with ADHD at age 3 and started methylphenidate at age 9. His current medications include lisdexamfetamine dimesylate, 70 mg daily. He was in 12th grade and planned to go to college. He had never been diagnosed with autism spectrum disorder.

Family History:

His mother's height was 165 cm (z-score= 0.4), father's height was 174.5 cm (z-score= -0.2). He had two siblings: a sister's (age 14 years) height was 157 cm (z-score= -0.5), onset of menarche at age 14, and a brother's (age 10 years) height was 154 cm (z-score= 1.3).

Physical Examination:

His anthropometric measurements included height 192 cm (6' 3.75") (z-score= 2.4), weight 117 kg (257 lb 9.6 oz) (z-score= 2.6), body mass index 31.6 kg/m² (z-score= 2.0), and head circumference 58.1 cm (z-score= 1.8). He had evidence of mild hypertelorism (inner canthal distance 3.6 cm, outer canthal distance 11.6 cm). There were no clinical findings of elbow dysplasia, 5th finger clinodactyly, or flat feet. Muscle mass appeared normal. GU exam revealed normal phallus size, pubic hair Tanner Stage IV and testes measuring 12 mL (z-score= -1.3), bilaterally. He was right handed.

Hormone measurements:

Total testosterone 216 ng/dl, LH 1.5, FSH 9.5 mIU/ml and estradiol 14 pg/ml.

Cognitive and behavioral evaluation:

The cognitive evaluation was performed by a licensed Ph.D. school psychologist, as part of a prior school evaluation to develop an IEP related to attention deficit disorder and to assist with educational and transition planning. Results included WRAT reading 110, arithmetic 106, WAIS-IV Verbal comprehension index 103, nonverbal reasoning ability measured by perceptual reasoning index was 88, Working memory 131, processing speed 89. His ability to sustain attention, concentrate and exert mental control was in the very superior range (Working memory Index, WMI=131). The autism evaluation was performed by a specialist in autism assessment for research and clinical purposes (JSM): ADOS-2 Module 3 Social Affect: communication-0, reciprocal social interaction-2, Total 2 (Non spectrum, cutoff for ASD is 7), with CSS score 1 (minimal to no evidence) of autism spectrum related symptoms. Parental questionnaire: SCQ 10 (cutoff is 15). Based on the proband's ADOS/SCQ evaluation, in terms of social communication and social interaction, he did not exhibit deficits in social-emotional reciprocity or in nonverbal communicative behaviors for social interaction. In terms of Restricted, repetitive patterns of behavior, interests, or activities, he did not exhibit stereotyped or repetitive motor movements, insistence on sameness, inflexible adherence to routines, or highly restricted, fixated interests.

Cytogenetics (Figure 1):

The karyotype was 46,X,idic(Y)(q11.22), with a normal X chromosome and a isodicentric Y chromosome consisting of a duplication of the Y short arm and proximal long arm. 20 cells were counted and analyzed, and no mosaicism was detected.

Chromosomal microarray analysis (Figure 2A, 2B):

The microarray showed Arr Yp11.32q11.22(168,547-16,171,556) x2, Yq11.22q12(16,171,557-57,772,954) x0. This result indicated an isodicentric Y chromosome with 16.17 MB terminal duplication of Ypter->Yq11.221 (Fig 2A) and a 41.60 MB terminal deletion of Yq11.221->Yqter (<https://genome.ucsc.edu>) (Fig 2B). The duplicated region (Figure 2A) contained the male sex determining gene *SRY* and the stature gene *SHOX*, but neither of these highly tissue-specific genes were expressed in blood at levels detectable via RNAseq. The deleted region contained the *DAZ* genes (*DAZ1*, *DAZ2*, *DAZ3*, *DAZ4* (also called human Y chromosomal azoospermia factors)).

Y Gene Expression results (Figure 3A)

We detected expression in peripheral blood of 11 Y genes and present four Y-specific genes, including *RPS4Y*, *UTY*, *NLGN4Y*, and *KDM5D* in the index patient, 9 boys with XYY, and 6 healthy TD male control boys. In the proband, there was increased expression of the *RPS4Y* and *UTY* genes in the duplicated region of Y and minimal or undetectable expression of the *NLGN4Y* and *KDM5D* genes in the deleted region of Y. The two-fold increase in expression of Y genes in XYY males was consistent despite widely differing absolute expression levels, as reflected by RNA-Seq read counts. For example, *RPS4Y1* transcripts were much more abundant than *NLGN4Y* transcripts, (Y axes, Fig. 3A). In addition, RT-PCR corroborated the absence of detectable *NLGN4Y* expression in the proband (data not shown).

PAR genes (Figure 3B)

We also detected expression of 15 PAR1 and PAR2 genes and present expression three PAR1 (*PLCXD1*, *ASMTL*, and *IL3RA*) and two PAR2 (*IL9R* and *SPRY3*) genes. There was dosage sensitivity with increased expression of the PAR1 genes, as expected with 3 copies in XYY versus 2 copies in XY. In contrast, for the two PAR2 genes, there was similar expression in the proband, compared to the XYY and XY groups, indicating dosage compensation of the PAR2.

Neuroimaging

Structural results—On brain MRI without contrast, the ventricular system was normal in size, shape, and configuration. There were no abnormal fluid collections or areas of abnormal parenchymal signal intensity.

Auditory Magnetoencephalography—We measured the latencies of the M50 and M100 components of the auditory evoked response in the index subject and compared to recently published data (Bloy, 2017) acquired in a separate cohort of 47 XY TD control boys and 21 boys with XYY. The proband had M50 latencies of 57ms and 53ms (Left/Right) and M100 latencies of 99ms and 97ms (Left/Right). Age corrected group (XY and XYY) means and standard deviations are shown in Table 1. For all components, except the right M50, the proband's latency was closer to mean of the XY group than the XYY group. Importantly in the left hemisphere, where significant XYY related latency prolongations (Left M50 and M100 shown in Figure 4) have been observed (Bloy, 2017), the proband's left M50 latency was in the 25th vs 17th percentile of the XY control vs. XYY populations and in the 50th vs. 2nd percentile for the left M100 latency. That is to say, both the M50 and M100 latencies for this individual more closely resembled those of TD controls than the XYY results.

Discussion

This proband had a normal X chromosome and a isodicentric Y chromosome, consisting of a duplication of the Y short arm and proximal long arm and deletion of the remaining long arm, without detectable mosaicism. Isodicentric Y chromosomes are the most commonly reported Y chromosome aberration and are associated with variable clinical features, based on frequent mosaicism for 45,X cell lines (Beaulieu Bergeron, Brochu, Lemyre, & Lemieux,

2011). Our goals in this study were to 1) report the physical and autism phenotypes in this unique patient, 2) measure the relationship between his copy number and expression levels for selected Y genes, in particular the potential Y ASD candidate gene, *NLGN4Y*, and 3) compare and contrast neuroimaging results (auditory evoked responses) in this patient with those reported in XYY and typically developing controls. This case study serves as an illustrative example of potential inferences made from relating ASD behavioral features of an individual with distinctive genetic findings to multimodal imaging findings in typical versus atypical cohorts.

Physical phenotype

The proband had tall stature and increased head circumference, as has been previously reported in males with XYY (Evans, de von Flindt, Greenberg, Ramsay, & Hamerton, 1986; Geerts et al., 2003; Nicolson, Bhalerao, & Sloman, 1998). A subset of clinical features in this patient are likely to be related to increased gene dosage of specified Y genes. For example, relative tall stature is related to the extra copy of the *SHOX* gene (Ottesen et al., 2010) in the pseudoautosomal region of the extra Y chromosome. *SHOX* is expressed selectively in growth plates, but RNA levels in blood could not be measured in the current study.

Behavioral/Cognitive/ASD phenotype

This patient had verbal abilities within the normal range and superior working memory abilities. XYY cohorts, on average, have lower verbal than nonverbal abilities and impaired performance on tests of working memory (Ross et al., 2009). Two previously reported nonmosaic cases of isodicentric Y chromosomes, with breakpoints near Yq11.21, had a history of global developmental delay and dyspraxia in one and mild language delay in the other (DesGroseilliers, Beaulieu Bergeron, Brochu, Lemyre, & Lemieux, 2006). He did not have diagnostic features of ASD by clinical opinion or results from the ADOS-2, unlike, XYY populations, in whom 38% fit criteria for ASD and up to 50% have at least some features of ASD (Tartaglia et al., 2017). The increased prevalence of ASD in XYY is likely due to a genetic rather than a hormonal effect, since puberty, testosterone levels, and fertility are generally normal in XYY (Bardsley et al., 2013). Subtle effects of androgen on brain organization and lateralization in XYY may be operational, but could not be detected in this case study. Our proband had ADHD, similar to ADHD reported in another subject with a dicentric Y chromosome (Mulligan et al., 2008). Since ADHD is common (15% of males), the significance of ADHD in these two idic(Y) males is unclear.

Neuroimaging-Auditory Magnetoencephalography phenotype

MRI structural imaging did not demonstrate any atypical findings. Quantitative structural analyses were not performed in this study. The proband did have an increased head circumference, as has previously been reported in XYY cohorts [17]. Also, neuroimaging findings previously reported in boys with XYY include increased cerebral grey matter (GM) and white matter (WM) (Bryant et al., 2012). Increased GM in XYY may be the result of reduced synaptic pruning, leading to altered synaptic function.

This proband had auditory latencies more in line with TD controls than with XYY subjects. Our recent study examining the auditory evoked responses in XYY subjects (Bloy, 2017) found significant XYY related prolongations of the M50 and M100 auditory responses of the left hemisphere. The typical findings in this subject, with duplication of part of the Y chromosome and deletion of Y genes on the distal long arm, suggests that the deleted Y genes are potentially 1) not required for the generation of typical auditory evoked response and 2) when overexpressed, as in XYY subjects, may contribute to delayed auditory processing. In disorders such as idiopathic ASD, such delays are hypothesized to arise from atypical maturation of the thalamocortical white matter pathways or from atypical synaptic transmission (Roberts et al., 2009; Roberts et al., 2013). The genetic contributions to these mechanisms remains to be directly investigated. However, the lack of delays in this proband versus the delayed auditory latencies in males with XYY, provides indirect support for the potential role for Y chromosome genes such as *NLGN4Y* or others in these developmental differences. Brain development may be negatively affected by excessive expression of *NLGN4Y* in males with XYY and needs to be studied further in depth.

Y chromosome genes

The Y chromosome is approximately 60 mb in size and contains 78 protein-coding genes, which code for 27 distinct proteins or protein families (Skaletsky et al., 2003), much fewer than other chromosomes. Despite limited number of genes, the Y chromosome is likely to influence male brain development both by direct genetic effects and by indirect sex steroid (testosterone) effects. Several Y chromosome regions have been associated with testis differentiation and fertility, including the azoospermia factor regions. In this proband, the deleted region contained the *DAZ* genes (*DAZ1*, *DAZ2*, *DAZ3*, *DAZ4* (also called human Y-chromosomal azoospermia factors)). The duplicated region also contained *USP9Y*, which may function in spermatogenesis and/or play a role in autism (SFARI website (geneSFARI.org)) (Hopps et al., 2003).

He had typical male genitalia without ambiguity. His testosterone level was in the normal range. Whether or not his testes have typical spermatogenesis is not known. Since he had pubertal levels of testosterone, his neuroimaging phenotype likely reflects specific Y genetic effects rather than testosterone deficiency or excess effects.

In this proband, we observed an approximately 2 fold increase in expression of duplicated Y genes, including *RPS4Y1* and *UTY*. In contrast, there was no detectable expression of deleted Y genes, including *NLGN4Y* and *KDM50*. Approximately 20% of Y chromosome genes are expressed in midgestation human brain (Reinius & Jazin, 2009), including 11 Y-chromosome-encoded genes ((*RPS4Y1*, *PCDH11Y*, *DDX3Y*, *USP9Y*, *NLGN4Y*, *EIF1AY*, *UTY*, *ZFY*, *TMSB4Y*, *CYorf15B*, and *PRKY*). All except *PRKY* are widely expressed in male brains. *RPS4Y1* and *ZFY* are located in the short arm (Yp) very close to *SRY*, and were not deleted in the proband, and most of the remaining genes are located in the long arm (Yq).

We also observed an approximately 1.5 fold increase in expression of the PAR1 genes *PLCXD1* and *IL3RA* and similar expression of the PAR2 genes *IL9R* and *SPRY3*, compared to the XY and XYY results. This is consistent with previous reports that PAR1 genes are

expressed from all X and Y chromosomes present (Helena Mangs & Morris, 2007), whereas PAR2 genes appears to be dosage compensated, with only one copy expressed in males and females (De Bonis et al., 2006).

Y chromosome ASD candidate genes

Y chromosome genes that contribute to the susceptibility of ASD and their mechanism in leading to neurodevelopmental changes are important for understanding the pathogenesis of ASD in XYY males and may also provide insight into the basis of the male predominance in ASD. However, there has been limited research on Y chromosome genes in ASD, and the evidence implicating individual Y genes in ASD is inconclusive (Baron-Cohen et al., 2011). Genes in the nonrecombining region (95%) of the Y chromosome cannot be studied by linkage and have generally not been included genome-wide association studies (GWAS) or focused CNV studies of ASD. The SFARI website (geneSFARI.org), lists four Y genes possibly associated with autism: *NLGN4Y*, *ASMT*, *USP9Y*, and *SHOX*, based on minimal or suggestive evidence (two lines of evidence).

Polymorphisms in the neuroligin 4Y (*NLGN4Y*) gene involved in neurodevelopment and synaptic function have been associated with ASD (Serajee & Mahbubul Huq, 2009; Yan et al., 2008), and we previously observed a positive correlation in children with XYY between *NLGN4Y* expression and symptoms of ASD but not measures of attention, anxiety, and depression (Ross, Tartaglia, Merry, Dalva, & Zinn, 2015). Mutations of the closely related X-linked gene *NLGN4X* (neuroligin 4 X) have been implicated in autism/ASD and mental retardation (Laumonnier et al., 2004; Yan et al., 2008). There is evidence that mutations in noncoding promoter sequences or copy number variation (CNV) of neuroligin genes, leading to gain of function, can alter synaptic function. For example, a neuroligin 3 duplication cosegregated with ASD in an extended family (Kaya et al., 2012); *de novo* sequence variation in the *NLGN4X* promoter was associated with increased gene expression in an ASD subject (Daoud et al., 2009); and neuroligin 1 or 2 overexpression in mice, either by transgenic manipulation or as a result of increased expression of eIF4E or knockout of eIF4E binding protein 2, led to ASD-like behavioral phenotypes (Dahlhaus et al., 2010; Gkogkas et al., 2013). That XY males express two neuroligin genes (*NLGN4X* and *NLGN4Y*), whereas XX females express only one copy of *NLGN4X* due to X-inactivation (Castagne et al., 2011; Talebizadeh, Simon, & Butler, 2006), suggests that the higher total neuroligin 4 levels in males versus females may mimic the effects of the neuroligin 3 duplication or *NLGN4X* promoter mutations, placing them nearer a threshold for synaptic dysfunction and thereby contributing to the marked male predominance of idiopathic ASD (Baron-Cohen et al., 2011; Bishop & Scerif, 2011).

Other Y chromosome candidate genes with potential neural impact include *PCDH11Y* (protocadherin 11Y) (Carrasquillo et al., 2009; Speevak & Farrell, 2011), which is located on both the X and Y chromosomes (Yp11.2) in humans and is highly expressed in fetal brain and spinal cord. The evolutionary consequence of X and Y isoforms of PCDH11 has been proposed to include the sexual dimorphism of cerebral asymmetry and the human capacity for language. *PCDH11X/Y* genes may also play a role in language development in humans, as suggested by the report of a male child with severe language delay who had loss of this

locus on both the X and Y chromosomes (Speevak & Farrell, 2011). Unfortunately, *PCDH11Y* expression was not detectable in the RNAseq analysis of any of our samples. Several other known Y genes may be important to consider in relationship to ASD risk. For example, *SRY* is well known for its important role in triggering sexual differentiation; however in some studies but not others (Reinius & Jazin, 2009), *SRY* is expressed in human brain regions and has been shown to upregulate tyrosine hydroxylase (the rate-limiting enzyme in the synthesis of catecholamines dopamine, epinephrine, and norepinephrine), beta-endorphin production, and monoamine oxidase-A (MAO-A), the enzyme that breaks down monoamine neurotransmitters such as catecholamines and serotonin. (Cohen et al., 2003; Mayer, Lahr, Swaab, Pilgrim, & Reisert, 1998; Wu, Chen, Li, Lau, & Shih, 2009). Other studies did not detect *SRY* expression in male prenatal brain (Reinius & Jazin, 2009). In this study, *SRY* expression was minimal.

PAR1 genes—PAR1 genes are expressed from both the X and Y chromosomes without dosage compensation, so 3 copies are expressed in XYY. The mild or not significantly increased ASD prevalence in XXX and XXY (Tartaglia, Howell, Sutherland, Wilson, & Wilson, 2010; Tartaglia et al., 2017) argues against increased pseudoautosomal gene dosage as the cause of increased ASD risk in XYY. However, we cannot rule out sex-limited effects, given the differences in androgen status in these three disorders. The *SHOX* gene is located in the PAR1. Tropeano found microduplications for *SHOX* are a low penetrance risk factor for ASD/NDD in both sexes (Tropeano et al., 2016). *SHOX* expression is restricted primarily to long bone growth plates, and expression in blood could not be detected by RNAseq in our study. ASD or other neurocognitive phenotypes have not been noted in subjects with dyschondrosteosis or mesomelic dwarfism due to reduced or absent *SHOX* expression (Zinn et al., 2007; Zinn et al., 2002), arguing against a critical role for *SHOX* in the nervous system. Variations in ASMT acetylserotonin O-methyltransferase were reported in a recent paper (Jonsson et al., 2014) in girls with association of microduplication with social interaction impairments. In this study, expression of *ASMT* was increased consistent with duplication of this gene in the proband.

Study strengths and limitations

Study limitations relate to this being a single case study, however single cases can raise interesting fundamental questions and generate testable hypotheses. In the case of this unusual proband, only inferences can be made about a possible association between *NLGN4Y* action and XYY-associated ASD risk. Association studies relating peripheral gene expression to behavioral phenotypes in one case do not establish *NLGN4Y* overexpression as the etiology of ASD in boys with XYY. Nonetheless, predicting behavioral and clinical outcome by integrating genetic and behavioral findings can lead to hypotheses regarding gene-behavior relationships and the neurobiological pathways underlying such associations in XYY.

We restricted our study to annotated protein-coding gene transcripts and did not include noncoding RNAs, as little is known about the function of the vast majority of noncoding RNAs and the possible effects of increased gene dosage. Another limitation of this study is that our results were obtained from peripheral blood and may not reflect expression in brain.

However, studies are beginning to relate expression of genes relevant to ASD, e.g., *FMR1*, *MECP2*, in blood-derived lymphoblastoid cell lines and peripheral blood lymphocytes (Glatt et al., 2012) to ASD phenotypes. Expression patterns of many genes in peripheral blood leukocytes and derivative lymphoblastoid cell lines are highly heritable and stable over time and can be used as intermediate phenotypes (Cheung et al., 2005). Finally, we measured RNA levels and not protein expression, so we could not determine if mRNA levels actually reflect protein levels.

XYY syndrome represents a novel, genetically homogeneous model for elucidating Y chromosome-related genetic influences on ASD. In light of evidence that sex-differentiating mechanisms play an important role in the development of ASD (Baron-Cohen et al., 2011; Gillberg, Winnergard, & Wahlstrom, 1984), the strong likelihood that increased Y-chromosome dosage and increased risk of ASD are interrelated warrants further investigation. XXY syndrome is also of great interest in understanding the influence of the sex chromosomes on male brain development and behavior because XYY is associated with normal testicular function, in contrast to males with an extra X chromosome XXY, Klinefelter syndrome, where the presence of both an extra sex chromosome and androgen deficiency/testicular failure are confounding factors.

Study strengths relate to the detailed phenotypic characterization of this proband and the multidisciplinary expertise in the interpretation of results. Future investigations will examine the role of Y chromosome genes such as *NLGN4Y* influence on brain and behavior in typically developing boys, boys with ASD, and boys with XYY.

Conclusions:

Expression of *NLGN4Y*, a gene involved in synaptic function, was undetectable in this unique proband, which may have mitigated the risk of XYY-associated cognitive and behavioral phenotypes. Studies of additional rare probands with informative partial Y chromosome aneuploidy and further investigation of *NLGN4Y* as a plausible ASD risk gene in XYY are warranted. XYY is a compelling genetic model for increased autism risk and may provide insight into the molecular basis for the male bias seen in the general ASD population. Further investigation of the neurodevelopmental effects of Y-chromosome genes is important as it will contribute to understanding both the male predominance in ASD and the increased rate of ASD in males with Y chromosome aneuploidy. Compelling unanswered questions include how structure-function relationships emerge in genetic disorders and how this developmental architecture results in autism. Future studies in informative rare subjects, XYY, and idiopathic autism cohorts may yield answers.

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— Breakpoint

Y chromosome

Figure 1:

Karyotype of proband: 46,X,Idic(Y) (q11.22), showing normal X chromosome and isodicentric Y chromosome, duplication of the Y short arm and proximal long arm

a.

Figure 2a, 2b:
Duplicated and deleted regions of the Y chromosome in proband from gene browser

Figure 3A: RNA sequence data from 4 genes on Y chromosome, *RPS4Y1* is on the duplicated portion of the short arm in proband, *UTY* is proximal long arm, on the duplicated portion of the short arm in proband, *NLGN4Y* proximal long arm, is on the deleted portion of the long arm in proband, and *KDM5D* is on the deleted portion of the long arm in proband

b.

Figure 3B:
RNA sequence data from 5 PAR genes, top three on PAR1 and bottom two on PAR2

Figure 4:

The latency of the proband's left hemisphere auditory M100 response (99ms) is shown in relation to an existing cohort of XY and XYY subjects. Group means and standard errors are shown in the left panel. The relationship between the proband's M100 latency and the M100 latency distributions for the XYY (red) and XY (blue) populations can be seen on the right.

Table 1:

Left and Right hemisphere M50 and M100 latencies for the proband, relative to the XY and XYY groups.

	Proband (XYq)	XY (N=47)	XYY (N=21)
Left M50 (ms)	57	63.0 ± 9.0	68.7 ± 12.4
Left M100 (ms)	99	98.8 ± 14.8	135.6 ± 17.7
Right M50 (ms)	53	62.5 ± 13.1	63.9 ± 8.2
Right M100 (ms)	97	98.2 ± 17.1	106.8 ± 18.9