Abnormal Motor Cortex Excitability is Associated with Reduced Cortical Thickness in X Monosomy

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Abstract: Turner syndrome (TS) is a noninherited genetic disorder caused by the absence of one or part of one X chromosome. It is characterized by physical and cognitive phenotypes that include motor deficits that may be related to neuroanatomical abnormalities of sensorimotor pathways. Here, we used transcranial magnetic stimulation (TMS) and cortical thickness analysis to assess motor cortex excitability and cortical morphology in 17 individuals with TS (45, X) and 17 healthy controls. Exploratory analysis was performed to detect the effect of parental origin of the X chromosome (X^{mat} , X^{pat}) on both measures. Results showed that long-interval intracortical inhibition was reduced and motor threshold (MT) was increased in TS relative to controls. Areas of reduced thickness were observed in the precentral gyrus of individuals with TS that correlated with MT. A significant difference between X^{mat} ($n = 11$) and X^{pat} ($n = 6$) individuals was found on the measure of long-interval intracortical inhibition. These findings demonstrate the presence of converging anatomical and neurophysiological abnormalities of the motor system in X monosomy. *Hum Brain Mapp* 34:936–944, 2013. © 2011 Wiley Periodicals, Inc.

Key words: cortical thickness; genomic imprinting; motor cortex; transcranial magnetic stimulation; turner syndrome

INTRODUCTION

X monosomy, or Turner syndrome (TS), is a genetic disorder caused by the complete or partial absence of one X chromosome that affects 1/2,000 live birth females (Sybert

and McCauley, 2004). There exists multiple TS karyotypes, the most common being 45, X, where one copy of the sexual chromosome is completely absent (Sybert and McCauley, 2004). It is believed that haploinsufficiency resulting from the absence of genes escaping inactivation is responsible for the classic TS phenotype (Bondy and Cheng, 2009), which includes short stature, gonadal dysgenesis, and a cognitive profile marked by weak visuospatial and arithmetic abilities (Temple and Marriott, 1998; Williams et al., 1991). Individuals with TS also present motor impairments that have been documented in numerous studies (Nijhuis-van der Sanden et al., 2003), the neural underpinnings of which are only beginning to emerge (Holzapfel et al., 2006).

Motor difficulties in TS can be observed from an early age (Mathisen et al., 1992) and seem to affect multiple

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aspects of motor function including feeding (Mathisen et al., 1992), articulation defects/language development (van Borsel et al., 1999), and manual strength (Haverkamp et al., 2003). Reduced motor speed has also been reported in TS and appears to be related to movement execution (Nijhuis-van der Sanden et al., 2002, 2004). While the exact cause of these impairments remains largely unknown, results from recent neuroimaging studies suggest that motor difficulties in TS may have an anatomical basis. Abnormal gray matter volume of the right precentral gyrus and of the left hemisphere has been reported in TS (Cutter et al., 2006; Molko et al., 2003). White matter abnormalities have also been found in TS. While Molko et al. (2004) did not find any area of reduced fractional anisotropy in a sample of 14 females with TS affecting the motor system, Holzapfel et al. (2006) observed substantial alterations of white matter tracts in a homogenous sample of 45X participants. These alterations included decreased fractional anisotropy and density throughout the internal capsule, presumably including sensorimotor neurons from the precentral and postcentral gyri. Taken together, these data suggest the existence of structural alterations in regions and pathways involved in motor function.

To better understand the neurophysiological and neuro-anatomical substrates of motor impairment in TS, integrity of the motor system was assessed using transcranial magnetic stimulation (TMS) in a homogeneous sample of 17 nonmosaic, monosomic individuals with TS and 17 healthy controls. In addition, whole-brain cortical thickness analysis was performed to detect the presence of morphological abnormalities in the precentral gyrus. Finally, exploratory analyses were conducted to determine the influence of X chromosome parental origin on measures of motor cortex excitability and cortical thickness, given the previous findings that this may play a role in the TS phenotype (Hamelin et al., 2006; Skuse et al., 2005).

MATERIALS AND METHODS

Participants

Participants were recruited through the Endocrinology Clinic of the Centre Hospitalier Universitaire Sainte-Justine. Written informed consent was obtained from all participants and the experimental protocol was approved by the *Comité d'éthique de la recherche du CHU- Sainte-Justine*. Participants were considered eligible to take part in the study if they met the following criteria: (1) presenting a complete X chromosome monosomy (45,X), as demonstrated by peripheral blood karyotypes with no normal cell lines, mosaicism or Y-chromosome material and an analysis of buccal epithelium DNA consistent with a 45,X karyotype; (2) willingness and availability of the biological mother to provide a peripheral blood sample; and (3) not presenting any chronic condition other than those associated with TS. After signed consent, 17 participants with TS met these criteria (all Caucasians, right handed; mean age:

TABLE I. Subject characteristics

	Controls, N = 17	TS, N = 17	X ^{mat} , X = 11	X ^{pat} , X = 6
Age	27	25	24	25
Diagnosed at birth (%)	—	10 (59%)	6 (55%)	4 (67%)
Previous GH Tx	—	6 (35%)	5 (45%)	1 (17%)
Previous oxandrolone Tx	—	2 (18%)	1 (9%)	1 (17%)
Age at puberty induction	—	13	14	13
Clinical severity score	—	18 (5–32)	18 (5–32)	17 (14–31)
Major heart defect	—	3 (18%)	2 (18%)	1 (17%)
Hypothyroidism with L-T ₄ Tx	—	6 (35%)	3 (27%)	3 (50%)

GH, growth hormone; Tx, treatment; L-T₄, levo-thyroxine.

24.5 ± 4.93; 11 X^{mat} and 6 X^{pat}). Hormone replacement status and clinical severity score of all TS subjects was obtained through medical files (Table I); all were being cycled on oral contraceptives because of gonadal failure. Seventeen healthy controls were also recruited (all Caucasians, right handed; mean age: 26.9 ± 6.78). All patients were euthyroid at the time of testing, as evidenced by normal TSH values, and all were scheduled during days 7 to 14 of their menstrual cycle (*n* = 6 controls) or days 7–10 of oral contraceptive use (all subjects with TS and *n* = 11 controls), to control for hormonal effects on cortical excitability (Smith et al., 1999).

Genetics

Duplicate peripheral blood samples were drawn from the participants and their mothers and leukocyte and buccal epithelial DNA was extracted according to methods described elsewhere (Hamelin et al., 2006). Karyotype analysis was performed on peripheral blood and showed the 45,X karyotype in all 30 cells examined with no evidence of mosaicism. Microsatellite analyses were performed on a different blood draw of peripheral blood leukocytes as well as in a sample of buccal epithelial cells. There were no conflicting results between the microsatellite analyses and the karyotype or between the two tissues on which the microsatellite analyses were performed. PCR conditions were optimized for 14 highly polymorphic X chromosome microsatellites (DXS7100, DXS1053, CYBB, DXS538, DXS1068, DXS1003, DXS1204, AR, DXS981, DXS1125, DXS986, DXS1120, DXS1047, and DXS102) chosen after their high degree of heterozygosity (mean = 78%) and their allele frequencies (≤47%). Most microsatellites were amplified with commercially available primers (MapPairs Human Markers) through Invitrogen (Burlington, Ontario, Canada) with the exception of the AR polymorphism for which the forward primer 1,5'-TCCA-GAATCTGTCCAGAGCGTGC-3', and the reverse primer

3,5'-CTCTACGATGGGCTTGGGAGAAC-3', were used. Specifications regarding allele number and size were obtained through the Genome DataBase web site (<http://www.gdb.org>).

To determine the parental origin of the X^{intact} chromosome, comparisons between mothers and their daughters were conducted for different combinations of microsatellites depending on the daughter's karyotype. For each microsatellite, the size of the allele on the X-intact chromosome was first determined using the M13mp18 plasmid sequence generated as indicated in the Sequenase version 2.0 DNA Sequencing Kit protocol (USB, Amersham Biosciences, Baie d'Urfé, Québec, Canada). Only alleles showing rare frequency (≤ 0.15 in the case of a maternal allele assignment) were retained with the aim of calculating a discrimination power ($\text{allele frequency}_1 \times \text{allele frequency}_2 \times \text{allele frequency}_n$). The discrimination power allows estimation of the probability of false assignment of parental origin. We required a discrimination power of less than 0.001 to assign maternal origin to the X chromosome (mean of nine microsatellites when only maternal blood available; five when both parents available, $n = 2$) and less than 0.01 in the case of an intact X^{pat} chromosome (mean of seven microsatellites when only maternal blood available; five when both available, $n = 3$). In none of the cases did the results from buccal DNA differ from the peripheral blood leukocyte DNA.

Transcranial Magnetic Stimulation

Subjects were seated in a comfortable chair with their arms fully supported. TMS was delivered with a Medtronic Magpro X100 with MagOption device (Medtronic, Minneapolis, MN) with a 80-mm-diameter figure-of-eight coil (MC-B70). The current waveform was biphasic and the coil was angled 45° from the midline with the handle pointing backward. All TMS measures were obtained from stimulation of the left primary motor cortex and motor evoked potentials (MEPs) were recorded from surface electrodes placed over the contralateral first dorsal interosseus (FDI) muscle. The electromyographic signal was amplified using a Powerlab 4/30 system (ADInstruments, Colorado Springs, CO), filtered with a band pass 20–1,000 Hz and digitized at a sampling rate of 4 KHz. MEPs were recorded using Scope v4.0 software (ADInstruments, Colorado Springs, CO).

Before the experimental procedure, the stimulation site eliciting MEPs of maximal amplitude was determined by moving the stimulating coil in 1 cm steps on a $7 \times 7 \text{ cm}^2$ grid drawn on a tight-fitting lycra swimcap and centered ~5 cm lateral to Cz. The position eliciting MEPs of the greatest amplitude was chosen as the stimulation site for the remainder of the experiment. This stimulation "hot-spot" was subsequently marked on each participant's swimcap by drawing the exterior edges of the stimulating coil. The stimulating coil was firmly handheld throughout

the experimental session by one experimenter while a second experimenter modified the stimulation parameters on the TMS device. Motor threshold (MT) was defined as the minimum TMS intensity required to induce MEPs of $>50 \mu\text{V}$ peak-to-peak amplitude in at least five of ten trials in the contralateral target muscle. For paired-pulse TMS, conditioning stimulus (cS) intensity was set at 80% MT, and test stimulus (tS) intensity was adjusted to induce MEPs of ~1 mV peak-to-peak amplitude. Interstimulus intervals between cS and tS were 2 and 3 ms for short-interval intracortical inhibition (SICI) and 9 and 12 ms for intracortical facilitation (ICF), and condition order was randomized. Ten MEPs were recorded for each interstimulus interval (ISI) and for tS alone. For long-interval intracortical inhibition (LICI), cS and tS intensities were adjusted to induce MEPs of ~1 mV peak-to-peak amplitude and an ISI of 100 ms was used. Because of technical difficulties (EMG traces were collected but not saved), data for LICI could not be analyzed in two participants (one in each group). LICI, SICI, and ICF were expressed as the ratio of conditioned relative to the unconditioned stimulus.

Cortical Thickness

T_1 -weighted magnetic resonance imaging (MRI) sequences were acquired for all subjects on a Siemens (Erlangen, Germany) Sonata 1.5 Tesla according to the following acquisition parameters: three-dimensional fast-field echo scan with 160 slices, 1 mm isotropic resolution, repetition time of 18 ms, echo time of 10 ms, flip angle of 30°. Data for all subjects were processed using a cortical thickness pipeline developed at the Montreal Neurological Institute (Lerch and Evans, 2005). Each T_1 -weighted image volume was corrected for signal intensity nonuniformity and linearly transformed into standard MNI-space (ICBM152 template). The transformed images were then classified into gray matter, white matter, and CSF using an automatic tissue classification algorithm (Zijdenbos et al., 2002). The white and gray matter surfaces were then extracted using CLASP (Constrained Laplacian Anatomical Segmentation using Proximities; Kim et al., 2005; McDonald et al., 2000). This step fits both surfaces using deformable spherical models, resulting in two surfaces with 40,962 vertices each. A previous study (Lee et al., 2006) showed that CLASP was representing the cortical anatomy with more accuracy than other cortical surface extraction methods. It also showed that the mesh topology optimization was better in CLASP, resulting in both an optimized global mesh density and a precise local cortical anatomy. The surface deformation algorithm works by first fitting the white matter surface, then expanding outward to find the gray matter and cerebral spinal fluid intersection. Each vertex (or point) on the white matter surface is related to its gray matter surface counterpart and cortical thickness can thus be defined as the distance between linked vertices. Cortical

Figure 1.

Cortical excitability measures. **(A)** Motor threshold values (mean and individual data) defined as the minimum TMS intensity (in % maximum stimulator output) required to induce MEPs of >50 μ V peak-to-peak amplitude in at least five of 10 trials in the contralateral target muscle. **(B)** Long-interval intracortical inhibition values (mean and individual data) representing the amplitude of the conditioned (second) MEP over the unconditioned

(first) MEP in a paired-pulse design where the two pulses were separated by 100 ms. **(C)** Short-interval intracortical inhibition and intracortical facilitation (mean values) representing the amplitude of the conditioned MEP over the unconditioned MEP in a paired-pulse design. Interpulse intervals of 2 and 3 ms inhibited the contralateral MEP, whereas interpulse intervals of 9 and 12 ms facilitated the contralateral MEP.

thickness was measured at every vertex and was blurred using a 30 mm surface-based kernel.

Statistical Analysis

For TMS measures of cortical excitability, independent samples *t*-tests were performed on MT and LICI data while ANOVA with group (controls, TS) and ISI (2, 3, 9, 12 ms) as factors was performed on paired-pulse data. Statistical analyses were performed on the cortical thickness data for both TS and control groups according to the general linear model using age as a covariate. Between-group comparisons (TS vs. control) were conducted to test for a difference in whole-brain cortical-thickness. Results were thresholded at a whole-brain level using the false discovery rate theory with $q = 0.05$ (Genovese et al., 2002). Results were considered significant at a *t* threshold of 2.5 ($P < 0.05$). To assess whether differences between

groups on TMS measures could be related to cortical thickness, regressions corrected for multiple comparisons were performed at each vertex with significant TMS data. Regression analysis was performed only in participants in whom full TMS datasets were available (16 in each group).

RESULTS

TMS

Motor threshold

There was a significant group difference for motor threshold ($t_{32} = 2.8$; $P = 0.008$; Fig. 1A). Mean resting motor threshold was 36% (SD = 6.0%) for controls and 42% (SD = 7.2%) for individuals with TS. Realized rate of current change (di/dt) at the coil was 52.18 A/ μ s for controls and 61.71 A/ μ s for individuals with TS at threshold.

Figure 2.

(A) Differences in regression of cortical thickness with MT between TS participants and controls displayed on a standardized brain. (B) In a cluster located in the precentral gyrus, MT increases with cortical thickness in TS while it decreases in controls. (C) Within the same cluster, cortical thickness is significantly reduced in TS. The coloring bar represents *P* values. **P* < 0.05. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com]

Given that the scalp-to-cortex distance is known to influence MT (McConnell et al., 2001), distance from the scalp to the hand motor cortex was calculated using the method of McConnell et al. (2001). There was no significant difference between groups ($t_{32} = 0.86$; $P = 0.67$). Mean scalp-to-cortex distance was 15.3 mm (SD = 1.6 mm) for controls and 15.0 mm (SD = 1.6 mm) for individuals with TS.

Long interval intracortical inhibition

A paired-pulse interval of 100 ms was used to assess the integrity of intracortical inhibition. Individuals with TS showed reduced LICI ($t_{30} = 2.2$; $P = 0.04$; Fig. 1B).

Short-interval intracortical inhibition and intracortical facilitation

Paired-pulse intervals of 2 and 3 ms were used to assess intracortical inhibition whereas intervals of 9 and 12 ms were used to assess intracortical facilitation. In both groups, paired-pulse at 2 and 3 ms inhibited the response to the test stimulus whereas paired-pulse at 9 and 12 ms facilitated the response to the test stimulus (Fig. 1C). A mixed ANOVA revealed no main effect of group ($F_{1,30} =$

0.1; $P = 0.73$) and no interaction between factors ($F_{1,30} = 0.7$; $P = 0.58$).

Cortical Thickness

A whole-brain group comparison between individuals with TS and controls revealed that individuals with TS had significantly thinner cortex relative to controls in a region of the left precentral gyrus ($x = -48$, $y = -4$, $z = 53$; $P = 0.004$). A regression of cortical thickness with the MT and LICI scores at each vertex, for each group separately, was performed to detect if precentral gyrus thickness correlates with dysfunctional measures of motor cortex excitability. Regression analysis revealed a significant positive correlation between MT and cortical thickness in two clusters located in the left precentral gyrus of the TS group ($x = -23$, $y = -16$, $z = 67$; $P = 0.0007$; $x = -13$, $y = -26$, $z = 79$; $P = 0.0027$). Regression analysis at each vertex also revealed the presence of a significant cluster in the left precentral gyrus where individuals with TS were more likely to have thicker cortex associated with higher MT score than controls ($x = -22$, $y = -15$, $z = 71$; $P = 0.007$; Fig. 2B). A between-groups comparison of cortical thickness in that cluster showed that individuals with TS

Figure 3.

X chromosome parent-of-origin analysis of long-interval intracortical inhibition (LICI) data showing that X^{mat} individuals display significantly less LICI than X^{pat} ($P = 0.04$) and controls ($P = 0.007$). The panel at the right shows superimposed EMG traces acquired during LICI in three TS individuals in which the protocol induced facilitation. The target symbol represents the artifact produced by the TMS stimulation. Unconditioned (first) and conditioned (second) MEPs appear ~ 25 ms after stimulation. Whereas in controls and all other TS individuals, the second MEP is smaller than the first, in three X^{mat} the second, conditioned MEP was of greater amplitude.

had significantly thinner cortex than controls ($t_{32} = 6.3$; $P = 0.018$; Fig. 2C).

Exploratory Analysis of X Chromosome Parental Origin

Despite a limited sample size, exploratory analyses were performed to detect the effect of X-chromosome parent-of-origin on measures of motor cortex excitability and cortical thickness in the precentral gyrus. For TMS measures, a significant difference was found between X^{mat} and X^{pat} individuals on measures of long-interval intracortical inhibition. A one-way ANOVA (controls, X^{mat} , X^{pat}) revealed a significant effect of group ($F_{2,29} = 6.3$; $P = 0.005$, Fig. 3). Posthoc tests revealed that X^{mat} individuals had significantly less long-interval intracortical inhibition than controls ($P = 0.007$) and X^{pat} individuals ($P = 0.04$, uncorrected). For cortical thickness, no significant group difference was found in the precentral gyrus.

DISCUSSION

This study revealed abnormal motor cortex excitability in TS in the form of increased motor threshold and reduced M1 long-interval intracortical inhibition. In TS participants, a positive correlation between MT and cortical thickness was observed in a cluster located in precentral gyrus whereas the opposite pattern was observed in

healthy controls. In that same precentral cluster, individuals with TS displayed significantly thinner cortex than controls.

Motor threshold is a measure of the minimum stimulation intensity required to excite corticospinal fibers indirectly through excitatory input from cortico-cortical projections (Ziemann, 2004). It is widely assumed that TMS preferentially activates cortico-cortical axons and as such drugs that act on axon excitability have been shown to modulate MT. It is believed that voltage-gated sodium channels are directly related to motor threshold. It has been repeatedly shown that inhibition of voltage-gated sodium channels increases MT, probably through a reduction of membrane excitability (e.g., Chen et al., 1999; Ziemann et al., 1996). Elevated MT has been associated with reduced white matter microstructure of the motor system. In healthy individuals, MT was found to inversely correlate with fractional anisotropy (FA) in white matter beneath motor and premotor cortex, including the internal capsule (Klöppel et al., 2008). Interestingly, women with TS show reduced FA and white matter density in the internal capsule (Holzapfel et al., 2006). Taken together with the present results showing increased MT in TS, these data suggest a pattern that closely matches that found in healthy controls: an elevated MT in the presence of reduced white matter in motor pathways. This converging evidence suggests that white matter and neurophysiological abnormalities in the motor cortex may underlie motor deficits found in TS. The extent to which abnormalities of the corticospinal tract and M1 dysfunction underlie the abnormal TMS values reported here is an open issue. Although MEP amplitudes and latencies were similar in the TS and control groups, reduced FA in the corticospinal tract (Holzapfel et al., 2006) could account for part of the elevated MT values. Although it is not possible to disentangle central and peripheral contributions at this point, the significant and linear correlation between cortical thickness and MT in TS participants argues in favor of specific M1 neurophysiological and neuroanatomical dysfunctions in this group.

At the cortical level, increased motor threshold in TS participants was found in conjunction with reduced cortical thickness close to the presumed M1_{hand} area (Mayka et al., 2006). This is in line with a previous study that reported reduced gray matter density in left M1 and increased gray matter density in right M1, which may be caused by displacement of the central sulcus consecutive to parietal lobe atrophy (Cutter et al., 2006). A similar pattern has been observed in individuals with dystonia, where a 4-week immobilization of the dystonic hand induced reductions in M1_{hand} volume and increases in motor threshold (Granert et al., 2011). It thus appears that a thinning of primary motor cortex can be associated with decreases in corticospinal excitability (higher MT). In the same dystonic patients the amount of gray matter reduction was positively correlated with increases in MT

after immobilization whereas the opposite relationship (the greater the reduction in MT, the greater the increase in gray matter volume) was found following 8 weeks of motor training after immobilization (Granert et al., 2011). A similar pattern was found in our control participants, where low MTs were associated with thicker cortex in the precentral gyrus whereas in TS, higher MTs correlated with thicker cortex. The present data thus add support to the notion that cortical excitability is linked to M1 volume/thickness and suggest that it can be modified by genetic factors, presumably linked to the X chromosome. Whether the reversal in correlation between cortical thickness and excitability in TS is a direct result of X monosomy or the byproduct of impaired motor function remains to be fully determined, but available data point to a complex interaction between spinal (Klöppel et al., 2008) and cortical impairments, both anatomical and neurophysiological.

In addition to elevated MT, reduced long-interval intracortical inhibition was found in primary motor cortex of women with TS. Long-interval intracortical inhibition is believed to be a measure of GABA_B receptor activity, based in part on the fact that administration of selective GABA_B agonist Baclofen increases it in healthy subjects (McDonnell et al., 2006), that LICI inhibits short-interval intracortical inhibition (SICI; Sanger et al., 2001), and that its timing is similar to facilitation of GABA_B receptor-mediated long-lasting inhibitory postsynaptic potentials (McCormick, 1989). It is important to note that reduced cortical inhibition has also been reported in a variety of neuropsychiatric and neurological disorders such as schizophrenia (Wobrock et al., 2008), Gilles de la Tourette syndrome (Orth and Rothwell, 2009) and dystonia (Di Lazzaro et al., 2009). More specifically, reduced long-interval intracortical inhibition has been found in disorders such as attention deficit hyperactivity disorders (Buchmann et al., 2007). Close examination of single subject data in this study, however, suggests a more complex picture. When parental origin of the X chromosome is taken into consideration, it becomes evident that reduced LICI is mainly driven by three X^{mat} participants that display facilitation rather than inhibition in the LICI protocol. The nature of the reversal from inhibition to facilitation in these three participants is unclear. One possibility is that exaggerated long-interval facilitation may occur, which could be assessed by testing it at numerous interpulse intervals (both shorter and longer than 100 ms). Interestingly, the three TS participants with the highest MT (higher than 50% maximum stimulator output) were also X^{mat}, and only one of them showed facilitation rather than inhibition on the LICI measure. Taken together, these data show that the presence of increased motor threshold in conjunction with reduced long-interval intracortical inhibition (turning to facilitation) is not a general feature of TS but may represent a distinct characteristic of X^{mat} individuals. However, the small sample size and uncorrected *P* value

obviously limit the conclusions that can be drawn from these exploratory analyses. As such, it cannot be ruled out that these effects do not represent a general feature of the X^{mat} genotype but rather reflect unrelated individual differences specific to these participants. Larger sample studies are needed to determine if motor cortex neurophysiology is modulated by parental origin of the X chromosome.

The mechanism by which neuroanatomical and neurophysiological abnormalities occur in TS is unclear. Genomic imprinting (as reflected in an impact of the parental origin of the remaining, intact X chromosome), karyotype (whether true X chromosome monosomy or occult mosaicism), oxandrolone history, growth hormone (GH) treatment, and X chromosome gene dosage have been suggested as possible candidates for structural differences observed in TS (Holzapfel et al., 2006; Raznahan et al., 2010). At the behavioral level, Ross et al. (1998) have suggested that impaired motor function in TS is related to estrogen levels. Indeed, estrogen treatment in girls with TS has been shown to improve spatially mediated motor function, but no effects have been found on simple motor tasks (Ross et al., 1998). In this study, great care was taken to select TS participants with a 45X karyotype in both peripheral blood leukocytes and in buccal epithelial cells, all subjects were tested during the same time relative to their last menses, whether oral contraceptive-induced or spontaneous, and every TS participant was taking oral contraceptives. Furthermore, although ovarian hormones alter cortical excitability, it has been shown that MT is not modulated by the menstrual cycle (Hattemer et al., 2007; Smith et al., 1999) and oxandrolone treatment is not associated with significant alterations of gray and white matter in TS (Cutter et al., 2006). Past GH use by individuals with TS, however, has been associated with increased gray matter volume, albeit in non-motor areas (Cutter et al., 2006). In line with previous investigations (Cutter et al., 2006; Holzapfel et al., 2010), the present data thus suggest that abnormalities in motor cortex anatomy and physiology are related to haploinsufficiency of genes escaping inactivation, and/or estrogen absence prior to pubertal induction, and/or (more hypothetically), genomic imprinting.

Finally, it must be pointed out that the lack of motor behavioral data makes it difficult to determine to which extent the observed neurophysiological and neuroanatomical abnormalities relate to motor deficits previously reported in TS. The present data make it clear, however, that neuroanatomical abnormalities found in the precentral gyrus of TS individuals (Cutter et al., 2006; Molko et al., 2003) are directly related to dysfunctions in corticospinal excitability. Considering recent evidence of reduced fractional anisotropy in the internal capsule (Holzapfel et al., 2006) and extensive data showing motor execution dysfunction (see Nijhuis-van der Sanden et al., 2003), a general pattern of widespread abnormal motor function emerges in TS.

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