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REGULAR ARTICLE

Effects of short-course androgen therapy on the neurodevelopmental profile of infants and children with 49,XXXXY syndrome

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ABSTRACT

Aim: The aim of this investigation was to ascertain whether an early course of androgen treatment (three injections testosterone enanthate, 25 mg) could have a positive impact on any domains of neurodevelopmental function in boys with 49,XXXXY.

Methods: A total of 22 boys with a karyotype of 49,XXXXY participated in a multidisciplinary assessment of neurocognition, speech and language, paediatric neurology and endocrinology evaluations. One group had received early androgen and another group did not receive any hormonal treatment prior to the evaluation. The mean age of treatment for Group 1 was 12 months with the mean age of first evaluation 74 months. The mean age of first evaluation for Group 2 was 87 months. Statistical analysis was completed to determine whether there was a positive treatment effect from androgen therapy.

Results: There was a significant positive treatment effect in speech and language domain, gestural communication and vocabulary development. No treatment effect was seen on nonverbal capacities.

Conclusion: Our findings revealed improved function in several areas of development which had been severely delayed in boys with 49,XXXXY. Continued research is underway to expand our understanding of the relationship of androgen, brain function and behavioural outcome in boys with 49,XXXXY.

INTRODUCTION

The 49,XXXXY syndrome is a rare variant of Klinefelter syndrome (47,XXY) and was first reported in 1960 (1). The prevalence is one in 85 000 to one in 100 000 male births (2) and there are few research studies completed on boys with 49,XXXXY. Congenital anomalies may be present in cardiac, skeletal, endocrine and central nervous systems (3–7). Some of the musculoskeletal findings reported are genu valgum, radioulnar synostosis and pes cavus. Endocrine abnormalities include growth retardation, diminished phallic size and androgen insufficiency at puberty. MRI brain imaging in boys with 49,XXXXY revealed morphological, volumetric and white matter differences that are associated with the deficits in neurodevelopmental performance (8,9).

Congenital abnormalities and neurodevelopmental delays usually bring these boys to the attention of medical providers early in life, which is in marked contrast to boys with a 47,XXY karyotype (Klinefelter syndrome). Neurocognitive deficits in 49,XXXXY include severe language-based learning dysfunction, marked speech delay and oral motor dysfunction (3,10). Because of the severe language dysfunction, the boys with 49,XXXXY are at risk for atypical social interactions and complex educational challenges (11).

Graphomotor deficits, balance and motor learning issues are also common in 49,XXXXY (Samango-Sprouse CA, unpublished data). Behaviourally, these boys have been described with contrasting personality features: shy and friendly, behavioural outbursts with aggression, impulsivity and distractibility (3,10).

Previous reports of neurocognitive function of boys with 49,XXXXY indicated IQ ranging from 20 to 60 typically with isolated anecdotal reports of higher cognitive capacities (3,10,11) reported on the largest cohort of boys to date with 49,XXXXY with more intact nonverbal abilities and presence of complex speech and oral motor dyspraxia.

ANDROGEN AND LEARNING IN HUMAN DEVELOPMENT

Androgens influence neurodevelopment, brain function and behavioural outcomes from as early as 16 weeks

gestation throughout adulthood (12). Foetal testosterone affects various structures of brain development including cortex and limbic systems (13,14).

It is well recognized that brain morphology as well as linear growth varies between girls and boys and hormones play a fundamental role in these differences. Hormones have a pivotal role in modulating mood, neurocognitive capacities and even the neuroaging process (15–17).

Although the effect of androgen deficiency on behaviour, neurodevelopment and cognition have not been well explored in boys or men with 47,XXY, there are several small studies suggesting the positive effects of testosterone treatment in adult males with XXY (18–20). To our knowledge, there have been no research studies on males with 49,XXXXY and androgen replacement. Androgen deficiency may be contributing to the complex neurodevelopmental issues of boys with 49,XXXXY.

METHODS

Subjects

A multidisciplinary clinic was held during six consecutive years (July 2004–2009) at the Neurodevelopmental Diagnostic Center for Young Children in Davidsonville, MD, where boys with 49,XXXXY karyotype were invited to attend. Informed consent was obtained from parents for their children to participate in the programme. Inclusion criteria included a karyotype indicating 49,XXXXY. Two foundations supported the enrolment of families to minimize ascertainment bias after the second year.

Medical records were obtained and reviewed prior to the visit. All subjects were evaluated by a paediatric endocrinologist (AR), neurologist and geneticist (AG), neurodevelopmental specialist (CASS) and speech pathologist (PLA).

Endocrinologic

Anthropomorphic measurements including height, weight and head circumference and growth velocity were assessed. Medical examination focused on genital development and presence or absence of infant hormonal replacement (Table 1).

Neurological

A routine neurological evaluation tailored to age by single neurologist/geneticist (AG). Cognitive function, cranial nerves, motor (tone, strength, coordination, and tendon stretch reflexes), sensory systems, function and gait were assessed. All subjects were screened for the presence or absence of oral motor or verbal apraxia.

Neurobehavioural and neurodevelopmental testing

Standardized testing was selected based on the subject's chronological age and the neurodevelopmental profile associated with 49,XXXXY. Testing probed multiple domains including speech and language development, neurocognition and behaviour. The Leiter International Performance Scale-Revised (LIPS-R), Preschool Language Scales-4 (PLS-4), the Receptive One Word Vocabulary Test (ROWPVT-R), Expressive One Word Picture Vocabulary Test-Revised (EOWPVT-R). Parents completed the MacArthur Communication Developmental Inventory (CDI) to assess additional dimension of the child's speech and comprehension because shyness and anxiety could compromise testing. The Gilliam Autism Rating Scale-2 (GARS-2) was completed by parents to determine the presence or absence of risk factors for autism spectrum disorder.

Table 1 Genital characteristics of the treated and untreated groups of boys with 49,XXXXY

Group 1* (N = 10)	Age	Penile length (cm)	Testes size (cc)
1	2 y 6 m	4	1
2	3 y 11 m	3.5	1
3	3 y 11 m	3	1.5
4	4 y 2 m	4.5	1
5	4 y 8 m	4.5	1
6	5 y	4	0.5
7	5 y 5 m	3	1
8	8 y 2 m	3	2
9	9 y 2 m	3	1.5
10	14 y 6 m	4	2
Mean	6 y 2 m	3.7	

Group 2* (N = 7)	Age	Penile length (cm)	Testes size (cc) L	Testes size (cc) R
1	5 y 2 m	3	1	
2	6 y 2 m	3	0.5	NF
3	6 y 5 m	1.5	0.5	1
4	7 y	4	0.5	
5	7 y 9 m	4.5	NF	1
6	7 y 11 m	3.5	2	1
7	10 y 4 m	3.5	2	
Mean	7 y 3 m	3.3		

y = years; m = months; L = Left; R = Right; NF = Not Found.
*Not all children were compliant for measurements.

Statistical analysis

Statistical procedures were utilized to measure treatment effects between the two groups and test of significance between the means of the treated group (Group 1) and untreated group (Group 2). The skewness–kurtosis test was used to assess normality. A test of significance was performed using the two-sample Wilcoxon rank-sum test or Mann–Whitney test. The null hypothesis is that there is no statistically significant difference between the means of the Group 1 (treated) and Group 2 (untreated).

Study fidelity

Subjects were evaluated by examiners who were blinded to androgen replacement therapy with the exception of the paediatric endocrinologist (AR). A data coordinator scored all standardized assessments. Data analysis was completed by a biostatistician who was not on site and did not interact with any patients directly.

RESULTS

The intent of the study was to investigate the effect of a short course of androgen replacement on neurodevelopmental performance in boys with 49,XXXXY. Group 1 received hormonal replacement in the first year of life and Group 2 had not received any hormonal therapy prior to their participation in the study. The children were treated by community paediatric endocrinologist who determined the course of treatment, timing and dosage on an individual basis.

Patient demographics

Parental education, socioeconomic background and educational levels were similar between the two groups. All parents had completed high school and greater than 60% had college degrees. There were a few parents who had postgraduate degrees as well. Children came from an equal mix of first born, second or more than three children. Maternal or paternal age was similar between the two groups. The untreated group (Group 2) weighed slightly more at 2.59 kg, while Group 1 weighed 2.45 kg. All children were identified with 49,XXXXY in the first 16 months of life because of dysmorphic features and/or developmental delay. All children received community intervention programmes shortly after receiving the diagnosis, and most children in each group received additional private speech and language therapy (Table 2).

The treated group has 11 boys ranging in age from 8 months to 14 years and 10 months who received androgen replacement at the mean age of 12 months. Ten boys received one injection per month of 25 mg testosterone enanthate for 3 months. One child received 40 mg of testosterone enanthate monthly for 3 months. No additional testosterone therapy was given to any child in the treated group. The untreated group had 11 boys ranging in age from 27 months to 9 years and 2 months who had not received androgen replacement at any time. There were ten children who were Caucasian in each group and one non-white child in each group. Two of the children, who resided outside the United States, were in the untreated group (Canada and Spain) and the one child from Honduras was in the treated group.

The genital characteristics of the boys treated and not treated with testosterone are shown in Table 1. The physical examination revealed no differences in penile length or testis size. Penile length did not differentiate the two groups.

Table 2 Patient characteristics

	Treated	Untreated
Birth weight	2.45 kg	2.59 kg
Vaginal	6	8
C-Section	5	3
Race		
White	10	10
Other	1	1
Maternal age	29.9	30.0
	R: 25–37	R: 26–43
Paternal age	32.9	32.0
	R: 22–37	R: 21–37

R = Range.

Table 3 Results of data analysis

Test type	Variable	Significance	p-value
MacArthur gestures	Early gestures	Mann–Whitney	0.0453*
	Later gestures	Mann–Whitney	0.0234*
	Total gestures	Mann–Whitney	0.0601**
PLS-4	Aud Comp	Mann–Whitney	0.0752*
	Verb Ability	Mann–Whitney	0.0085*
Picture vocabulary	EOW-PVT-R	Mann–Whitney	0.0155*
	ROW-PVT-R	Mann–Whitney	0.0275*

PLS-4 = Preschool Language Scale-4th Edition; Aud Comp = Auditory Comprehension; Verb Ability = Verbal Ability; EOW-PVT-R = Expressive One Word Picture Vocabulary-Revised; ROW-PVT-R = Receptive One Word Picture Vocabulary-Revised.
*These variables are significant at the 5% level.
**These variables are significant at the 10% level.

Standardized test results revealed that LIPS-R Fluid Reasoning and Brief IQ were not significantly different between the two groups and showed no treatment effect (Table 3). There were significant positive treatment effect for early gestures ($p = 0.04$) and later gestures ($p = 0.04$) on the MacArthur CDI. Auditory comprehension ($p = 0.0769$) and verbal ability (0.0085) on the PLS-4 showed a significant treatment effect. There was a significant positive treatment effect for expressive ($p = 0.01$) and receptive one word vocabulary development ($p = 0.02$).

DISCUSSION

To our knowledge, this is the first study in boys with this rare neurogenetic disorder, which demonstrates a favourable effect in overall speech and language ability and gestural communication from hormonal replacement. There may be a positive relationship between androgen and neurodevelopmental outcome in males with 49,XXXXY based on our findings. Our earlier study demonstrated that motor planning and motor learning were deficient in the verbal and oral motor development in boys with 49,XXXXY (3). Lenroot et al. (9) described decreased volume in Broca's area and Werdmann regions of the brain in boys with 49,XXXXY which are associated with oral and verbal motor dyspraxia (20). Our findings reveal improved function in selected aspects of verbal ability which is indicative of an improvement in oral motor and verbal praxis.

There was a positive effect in gestural communication. During infancy, gestural communication is critical to the development of spoken communication, social cognition and neurocognition in children. It provides the infrastructure for later development of advanced communication skills for children. The positive effect in auditory comprehension and receptive and expressive vocabulary development in the treatment group further supports the broad-based positive effect on overall language abilities in boys with 49,XXXXY.

These improvements in language development are consistent with smaller earlier studies in adult men with 47,XXY, which revealed improved verbal skills when testosterone treatment was given (19,21–23) as well as antedotal reports of overall improvement in boys and men with 47,XXY after hormonal replacement therapy has been instituted.

MRI brain imaging studies revealed an increased volume in grey matter of the temporal lobe of the brain of those men treated with hormones when compared to untreated individuals (19). One of the functions of the temporal lobe of the brain relates to language capabilities as well as social language skills (20). The androgen receptors in temporal lobes may explain the improvement in multiple aspects of language development. Although the neurobiological basis for this improvement is not well understood, it could possibly be a cascade effect that affects brain function over time.

The investigation of a select population of children with variant form of 47,XXY is important for several reasons. First, the encouraging treatment effect on the language domain coupled with unaffected nonverbal skills suggests a possible selective process of androgen on brain function. It links an improvement in neurodevelopmental function with a small dose of hormonal replacement in infancy in a very developmentally challenged population. Future investigations are necessary to determine whether there is a critical period or window of opportunity when androgen is most effective on neurodevelopmental outcome in children with 49,XXXXY. It is also plausible that multiple influences have a synergistic effect and positively alter neurodevelopmental outcome.

This study does have some weaknesses. Androgen levels were not taken prior to administration of the testosterone and therapy was instituted primarily based on diminished penile size. The reduced size of the phallus may reflect an androgen deficiency or perhaps there are unknown genetic influences affecting the efficacy of the androgen during gestation in some children with 49,XXXXY. The dosage was typically 25 mg; however, the timing of the androgens varied from newborn period to 30 months of age. It is plausible that timing may have a differential effect on learning and development, but additional investigation is necessary to ascertain this effect (24).

The ramifications of our study are intriguing to consider that the function of a very debilitating disorder could be improved to some degree through early androgen replacement. More studies are underway to evaluate the relationship between behavioural outcome, sensory function and neurocognition in boys with 49,XXXXY and androgen replacement in early infancy. Although 49,XXXXY is a variant form of 47,XXY, the question of androgen deficiency has not been well explored in these boys with Klinefelter syndrome and the variant disorders. These are some studies supporting androgen deficiency and improvements with androgen supplementation (23,25,26). The question of neurobiological underpinnings of androgen and neurodevelopmental function required further in-depth study in boys with Klinefelter Syndrome and the variant forms.

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APPENDIX: DISCUSSION FOLLOWING CAROLE SAMANGO-SPROUSE'S PRESENTATION

Neurodevelopmental problems in infancy and childhood

Nicole Tartaglia (Denver, USA):
Your emphasis on the effects of the family background and other background genes on the variability of mental health, behaviour and learning ability of the children with Klinefelter syndrome (KS) is very important. I would propose that more than half of the variability in the phenotype is due to the family environment and other inherited traits.

Hilgo Bruining (Utrecht, Netherlands):
Your slide showing discrepancies between two different groups of KS patients suggest that there may be two distinct phenotypes, the majority with impaired verbal IQ and the minority with impaired perceptual IQ.

Carole Samango-Sprouse:
There are actually three different phenotypes. The smallest group, <5% of all KS children, have normal IQ with equal verbal and non-verbal IQ, do not require help from special services and have only mild non-problematic reading dysfunction: they have increased activity and are good at sports, perhaps excelling if they come from a sporting family. The majority have either verbal or perceptual IQ deficiencies. Those with accelerated perceptual IQ disability are sluggish with reading and struggle tremendously. The patients with accelerated verbal IQ deficiency all had a family history of similar disability traits including high risk for dyslexia, or frank dyslexia.

Martin Ritzén (Stockholm, Sweden):
As an endocrinologist I am interested in the dosage of your androgen replacement, and what kind of testosterone was given.

Alan Rogol (Charlottesville, USA):
The children received three doses of 25 mg testosterone, mainly enanthate and less frequently cypionate, at monthly intervals (0, 4 and 8 weeks). The age of the first injection depended on age at diagnosis, typically around 4–5 months old, and no injections were given after 11 months of age.

Carole Samango-Sprouse:
The patients were seen by paediatric endocrinologists and the parents requested treatment to mimic the infantile mini puberty although they were outside of the time window for this. Pressure from parents resulted from their knowledge of the condition gleaned from the internet.

Martin Ritzén:
Did any of the boys have micropenis?

Carole Samango-Sprouse:
They had smaller than normal penises, but not true micropenis.

Niels E Skakkebæk (Copenhagen, Denmark):
You present rather soft data but we require hard data obtained from controlled trials. You examined some of your subjects blindly, but not really in a controlled fashion. Properly controlled studies must be conducted before we can use the date to produce universally accepted recommendations on therapy. We all want these children to be successful so controlled data are essential.

Carole Samango-Sprouse:
I agree that we need clinical randomized controlled trials and I would be happy to suggest how these can be set up. Our examinations were partially blinded because the examiners did not know if the children had been treated or not. We do see children whose parents inform us that they had been previously treated, and we continue to follow these children some of whom are now up to 6½ years old. The differences observed following treatment appear to be maintained. It is conceivable that therapy starts a cascade effect which helps the brain. When I examine treated children I notice that there is improved muscle tone and strength.

I hope that one of the outcomes of this meeting is that clinical controlled trials can be instigated which are both productive and protective.