HHS Public Access

Author manuscript

Am J Med Genet A. Author manuscript; available in PMC 2017 June 02.

Published in final edited form as:

Am J Med Genet A. 2010 May ; 152A(5): 1206–1212. doi:10.1002/ajmg.a.33334.

Increased Number of Sex Chromosomes Affects Height in a Nonlinear Fashion: A Study of 305 Patients With Sex Chromosome Aneuploidy

Anne Marie Ottesen[1], **Lise Aksglaede**[1,*], **Inger Garn**[1], **Nicole Tartaglia**[2], **Flora Tassone**[3], **Claus H. Gravholt**[4], **Anders Bojesen**[5], **Kaspar Sørensen**[1], **Niels Jørgensen**[1], **Ewa Rajpert-De Meyts**[1], **Tommy Gerdes**[6], **Anne-Marie Lind**[6], **Susanne Kjaergaard**[6], and **Anders Juul**[1]

[1]University Department of Growth and Reproduction, Rigshospitalet, Copenhagen, Denmark

[2]University of Colorado School of Medicine, Child Development Unit, The Children's Hospital, Aurora, Colorado

[3]Department of Biochemistry and Molecular Medicine, University of California, Davis, California

[4]Medical Department M (Endocrinology and Diabetes), Aarhus University Hospital, Aarhus, Denmark

[5]Department of Clinical Genetics, Vejle Hospital, Vejle, Denmark

[6]University Department of Clinical Genetics, Rigshospitalet, Copenhagen, Denmark

Abstract

Tall stature and eunuchoid body proportions characterize patients with 47,XXY Klinefelter syndrome, whereas patients with 45,X Turner syndrome are characterized by impaired growth. Growth is relatively well characterized in these two syndromes, while few studies describe the growth of patients with higher grade sex chromosome aneuploidies. It has been proposed that tall stature in sex chromosome aneuploidy is related to an overexpression of *SHOX*, although the copy number of *SHOX* has not been evaluated in previous studies. Our aims were therefore: (1) to assess stature in 305 patients with sex chromosome aneuploidy and (2) to determine the number of *SHOX* copies in a subgroup of these patients (n =255) these patients and 74 healthy controls. Median height standard deviation scores in 46,XX males (n =6) were −1.2 (−2.8 to 0.3), +0.9 (−2.2 to + 4.6) in 47,XXY (n =129), +1.3 (−1.8 to +4.9) in 47,XYY (n =44), +1.1 (−1.9 to +3.4) in 48,XXYY (n =45), +1.8 (−2.0 to +3.2) in 48,XXXY (n =9), and −1.8 (−4.2 to −0.1) in 49,XXXXY (n =10). Median height standard deviation scores in patients with 45,X (n =6) were −2.6 (−4.1 to −1.6), +0.7 (−0.9 to +3.2) in 47,XXX (n =−40), −0.6 (−1.9 to +2.1) in 48,XXXX (n =13), and −1.0 (−3.5 to −0.8) in 49,XXXXX (n =3). Height increased with an increasing number of extra X or Y chromosomes, except in males with five, and in females with four or five sex chromosomes, consistent with a nonlinear effect on height.

[*]Correspondence to: Lise Aksglaede, Department of Growth and Reproduction GR, Rigshospitalet Section 5064, Blegdamsvej 9, DK-2100 Copenhagen Ø, Denmark. lise.aksglaede@rh.hosp.dk.
Anne Marie Ottesen and Lise Aksglaede contributed equally to this work.

Keywords

Klinefelter syndrome; *SHOX* copy number; tall stature; X and Y chromosomes; XXY

INTRODUCTION

Patients with 47,XXY Klinefelter syndrome tend to have eunuchoid body proportions and a final height above what is predicted for mean parental height, whereas, in contrast, patients with 45,X Turner syndrome have impaired growth. Growth is relatively well described in these two syndromes, while few studies, mainly based on single case reports, describe the stature of patients with higher grade sex chromosome aneuploidies. Patients with Klinefelter syndrome are characterized by hypergonadotropic hypogonadism and this has previously been considered the main explanation for the excessive growth. However, tall stature in these patients is present from early childhood [Aksglaede et al., 2008], suggesting that hypogonadism in puberty and young adulthood cannot solely explain their tall stature. The fact that males with 47,XYY and females with 47,XXX are equally tall even though they do not have hypogonadism supports the hypothesis that other factors are involved.

Although the interaction of sex steroids and the growth hormone and insulin-like growth factor axis is important in regulating postnatal growth, multiple genetic factors, especially genes located on the sex chromosomes, also play a role. With the discovery of the sex chromosome-related short stature homeobox-containing gene (*SHOX*) located in the pseudoautosomal region (PAR1) which is not inactivated [Rao et al., 1997b], a new perspective was added to the understanding of the regulation of growth. The short stature of patients with Turner syndrome is now considered a consequence of *SHOX* haploinsufficiency, although other factors may play a role. Many have suggested that tall stature in patients with additional sex chromosomes is associated with an overdosage of the *SHOX* gene [Ogata et al., 2001; Aksglaede et al., 2008; Thomas et al., 2009], this has not been measured, except in a few case reports, which have suggested that *SHOX* overdosage may result in excessive growth [Ogata et al., 2001; Kanaka-Gantenbein et al., 2004]. However, the presence of a duplication of this genomic region does not always results in tall stature, most likely due to differences in the size and position of the duplication including flanking sequences and involvement of transcriptional enhancers [Thomas et al., 2009].

In this study of 305 patients with sex chromosome aneuploidy we aimed to compare stature in patients with different forms of sex chromosome aneuploidy, and to quantify the number of *SHOX* genes in a subgroup of these patients (n =255).

MATERIALS AND METHODS

Patients

Patients with sex chromosome aneuploidy and healthy controls were recruited from four centers: Copenhagen, Denmark (n =107), Aarhus, Denmark (n =78), California, USA (n =119), and Denver, USA (n =75). Height was measured to the nearest 0.1 cm and was available in 369 patients and controls (Table I), whereas parental heights were only available

in 155 patients. In girls with Turner syndrome, height was measured before initiating growth hormone treatment. Target height was calculated as 0.5 ×(height of mother +height of father) ×6.5 in boys and girls, respectively. All heights were expressed as SD scores (SDSs) using standard references according to age and sex [Andersen et al., 1982]. Nearly all patients were white, except 14 (Hispanic (n =6), Asian (n =4), and African American (n =4)). Patients from the USA and Denmark were equally distributed among the different karyotypes. DNA samples were obtained from a total of 306 patients and controls (Table I).

Analyses

Karyotyping—For karyotyping, routine G-banding was performed, including counting of at least 10 metaphases, three of which were fully analyzed.

DNA isolation—DNA from patients in the Copenhagen group was purified using the QuickGene-810 Nucleic Acid Isolation System (Fujifilm, Life Science-Products, Tokyo, Japan) by means of the QIAmp 96 DNA, Blood kit (Qiagen, Inc., Chatsworth, CA), or Nucleo Spin, 96 Blood kit (Macherey-Nagel, Select Science Ltd, Corston, UK). The Chemagic DNA Blood kit (Chemagen Parral-labs, Inc., Worchester, MA) was used in the Aarhus group, and the Puregene DNA isolation kit (Puregene, Gentra Systems, Inc., Minneapolis, MN) in the American group.

qPCR measurements of SHOX numbers—All qPCR measurements were carried out in Copenhagen performed on the Mx3000P platform (Stratagene, Cedar Creek, TX). The detailed protocol of the method was described previously [Ottesen et al., 2007; Juul et al., 2009]. The primers designed for *SHOX* were: forward (Fw) 5′-CTC CTA CCC GCC TGT CCA-3′ and reverse (Rev) 5′-TCC GCG CGT CTC TTT CTA CT-3′, and for the reference gene *GAPDH*: (Fw 5′-CTC CCC ACA CAC ATG CAC TTA-3′ and Rev 5′-TTG CCA AGT TGC CTG TCC TT-3′) (DNA Technology A/S, Århus, Denmark). The *SHOX* copy numbers of specimens from each center were specifically validated using a local individual control.

Multiplex ligation-dependent probe amplification (MLPA)—DNA samples from six patients with discordant results between qPCR and karyotype were analyzed by means of the MLPA technique using the SALSA MLPA kit P018-D1 SHOX probemix (MRC-Holland, Amsterdam, the Netherlands). The protocols for sample preparation and analysis were according to the manufacturer.

Fluorescence in situ hybridization analysis (FISH)—Fluorescence in situ hybridization (FISH) was performed using metaphase spreads of lymphocytes prepared according to standard protocols, or interphase leukocytes enriched by buffy coats. For analysis of the presence of the *SHOX* gene, a fluorescent DNA probe locus specific for *SHOX* (probe 1145-L00702) and a control probe for chromosome X (KB-40112, Poseidon DNA Probes, Kreatech Biotechnology B-V, Amsterdam, the Netherlands) were hybridized, and signals from 10 nuclei were scored.

Statistical Analyses

Comparison of height SDS and target height SDS was performed using a paired *t*-test (paired difference). The copy numbers of *SHOX* in each karyotype are presented as median and percentiles. All statistical calculations were performed using the statistical software SPSS (version 17; SPSS, Inc., Chicago, IL).

Ethics

The protocol was approved by the Copenhagen Ethics committee (KF-01-26 5848), Aarhus County Ethical Scientific Committee (# 20010155), the Danish Data Protection Agency, Colorado Multiple Institution Review Board (# 080953), and the UC-Davis Institutional Review Board (protocol number #200412687).

RESULTS

Correlation Between Height and Sex Chromosome Number

Heights according to age and karyotype are presented in Figure 1 and Table I. Height and final height (below and above 20 years) according to karyotypes are illustrated in Figure 2. Height was significantly greater than target height in patients with 47,XXY (n =42, P <0.0001), 47,XYY (n =27, P =0.001), 48,XXYY (n =34, P =0.001), and 47,XXX karyotypes (n =25, P =0.009), whereas girls with 45,X were significantly shorter than their target height (n =6, P =0.027). Availability of target heights was limited in patients with the remaining karyotypes, and statistical comparisons were therefore not possible.

Copy Numbers of *SHOX*

The reference ranges for one, two, three, four, and five copy numbers by qPCR were calculated as the 2.5 and 97.5 percentiles for karyotyped subjects in each category excluding the six patients with an unexpected copy number (Table II). None of the reference ranges overlapped another range. In 98.4% (251/255) of cases, the *SHOX* gene copy number was within the predicted reference range and agreed with the number of sex chromosomes observed on the karyotype. A comparison of copy numbers from the qPCR analysis of the exon 1 region in the *SHOX* gene with the MLPA analysis of exon 1 of *SHOX* (probe 1145-L00702) in the six subjects with an unexpected copy number is shown in Table III. Three of six MLPA results (a–c), and a *SHOX*-FISH result (e) confirmed the qPCR results.

FISH Analysis of *SHOX*

Hybridizations using a *SHOX*-specific DNA probe, showed three *SHOX* signals and two signals from the control probe of the Xq centromere region in 10 interphase and metaphase cells from a patient (e) with Klinefelter syndrome (47,XXY). In 8 of 10 nuclei, one of the three *SHOX* signals appeared more strongly fluorescent than the two other signals (Fig. 3) indicative of four copies, which supports the *SHOX*-qPCT analysis. The strongly fluorescent signals were most likely localized to one of the X-chromosomes deduced from the proportions of the p- and q-arm demonstrated by the DAPI staining of the metaphase preparation.

DISCUSSION

In this cohort of 305 patients with sex chromosome aneuploidy we found a nonlinear effect of the number of sex chromosomes on height. Thus, we report on increasing heights in patients with increasing number of additional sex chromosomes. However, this association became negative with the presence of four (in females) or five (in both males and females) sex chromosomes.

Increased height in patients with additional sex chromosomes is recognized, but published data on height in girls with 47,XXX, 48,XXXX, and 49,XXXXX karyotypes are sparse. In males, we and others have reported on increased stature in subjects with 47,XXY, 47,XYY, and 48,XXYY karyotypes [Schibler et al., 1974; Ratcliffe, 1999; Bojesen et al., 2006; Vorona et al., 2007; Aksglaede et al., 2008; Tartaglia et al., 2008]. In contrast, stature has only been reported in single cases of males with 48,XXXY and 49,XXXXY karyotypes [Linden et al., 1995; Peet et al., 1998; Visootsak and Graham, 2006].

The *SHOX* gene is located on the distal part of PAR1 of the sex chromosomes, a region of the X chromosome escaping X inactivation [Rao et al., 1997a]. Thus, two active copies of *SHOX* are present in both males and females [Rappold, 1993]. *SHOX* thereby exerts a dosage effect in patients with supernumerary sex chromosomes [Rao et al., 1997b] and may influence stature in these patients. *SHOX* is expressed in the zone of hypertrophic chondrocytes of the growth plate [Marchini et al., 2004], and the protein is thought to be involved in chondrocyte differentiation. In addition, *NPPB*, a gene that encodes brain natriuretic peptide (BNP) as a transcriptional target gene for SHOX, has recently been identified [Marchini et al., 2007]. Therefore, in patients with additional *SHOX* copies increased *BNP* expression may induce increased growth and skeletal abnormalities including scoliosis, as demonstrated in *BNP* transgenic mice [Suda et al., 1998]. Accordingly, we detected a minor thoracic scoliosis by X-ray in one patient with 49,XXXXY, which however, could not explain the severe short stature of this patient. Below average stature was found in all patients with five sex chromosomes.

Deficiency or mutations of the *SHOX* gene and in some cases a region downstream of the gene are associated with the skeletal dysplasias, Léri-Weill dyschondrosteosis, and Langer mesomelic dysplasia, which manifest short stature [Belin et al., 1998; Shears et al., 1998; Benito-Sanz et al., 2005; Fukami et al., 2005; Huber et al., 2006; Sabherwal et al., 2007]. *SHOX* mutations including deletions of the gene or the downstream region are also found in some cases of idiopathic short stature [Rao et al., 1997b; Benito-Sanz et al., 2005; Chen et al., 2009]. In contrast, we here observe an increase in height with increasing copy number of sex chromosomes and the *SHOX* gene although this effect was reversed with the presence of four (females) or more (both sexes) copy numbers. As seen in Figure 2, the presence of three sex chromosomes in males had the greatest impact on stature, whereas the presence of four sex chromosomes did not positively affect height any further. This observation may, however, be due to the relatively small number of subjects with four sex chromosomes examined. The curve in Figure 2 gives the impression that the effect on height is already reversed in males with four sex chromosomes. However, this is a statistical consequence of the very low height SDSs in patients with five sex chromosomes. Our finding of a nonlinear

effect of sex chromosome number on height suggests that genes or hormonal factors other than solely the number of *SHOX* copies come into play with increasing copy number. Patients with higher grade aneuploidies are most likely more severely affected at multiple organ sites, possibly due to the influence of other genes located on the sex chromosomes creating an overdosage effect, compared to patients with one or two additional sex chromosomes. However, the specific explanation for their short stature remains unknown.

One limitation of our study is the comparison of nonfinal heights of growing children with heights of adult patients. We therefore presented the height data as raw data in relation to chronological age (Fig. 1), as well as age-corrected SDSs. When expressing height as SDSs, the data become more comparable even between unequally aged populations, although an age effect cannot be excluded.

We have previously described that patients with 47,XYY syndrome are taller than the 47,XXY boys [Aksglaede et al., 2008], although this difference was not statistically significant in the present study. A difference in height in patients with 47,XYY compared to those with 47,XXY could be explained by the presence of a double dosage of potential Y-chromosome-specific growth genes in addition to a triple dosage of the *SHOX* gene. It should be emphasized that we have not measured the actual number of transcriptionally active copies of *SHOX* in our present study. A possible growth control gene located on the Y chromosome (GCY) may be linked to the pericentromeric region of the long arm of the Y chromosome, but to our knowledge no specific candidate genes have been identified [Kirsch et al., 2004]. The presence of such gene(s) could be a part of the explanation of the height difference in the sexes. This hypothesis would also explain both the reduced height of SRY-positive males with 46,XX as compared with males with 46,XY, and the height of males who are SRY-positive 46,XX being similar to that of females with 46,XX [Vorona et al., 2007; Aksglaede et al., 2008].

In conclusion, we have demonstrated an association between increased number of sex chromosomes and tall stature in this large cohort of patients with 47,XXX, 47,XXY, 47,XYY, 48,XXXY, and 48,XXYY karyotypes, but not in females with 48,XXXX and 49,XXXXX, or males with 49,XXXXY karyotypes, who are shorter than average, suggesting a nonlinear effect of the number of sex chromosomes on height.

Acknowledgments

Grant sponsor: Danish Medical Research Council; Grant sponsor: Kirsten and Freddy Johansen Foundation; Grant sponsor: Aase and Einar Danielsen Foundation; Grant sponsor: Danish Diabetes Association; Grant sponsor: UC-Davis MIND Institute; Grant sponsor: Bonfils-Stanton Foundation; Grant sponsor: Klinefelter Syndrome and Associates; Grant sponsor: University of Colorado IDDRC.

The study was supported by grants from the Danish Medical Research Council, the Kirsten and Freddy Johansen Foundation, the Aase and Einar Danielsen Foundation, the Danish Diabetes Association, the UC-Davis MIND Institute, the Bonfils-Stanton Foundation, and Klinefelter Syndrome and Associates. Dr. Tartaglia acknowledges additional support from the University of Colorado IDDRC. We acknowledge the assistance of Christa Hutaff-Lee for data management.

References

Aksglaede L, Skakkebaek NE, Juul A. Abnormal sex chromosome constitution and longitudinal growth: Serum levels of insulin-like growth factor (IGF)-I, IGF binding protein-3, luteinizing hormone, and testosterone in 109 males with 47,XXY,47, XYY, or sex-determining region of the Y chromosome (SRY)-positive 46,XX karyotypes. J Clin Endocrinol Metab. 2008; 93:169–176. [PubMed: 17940117]

Andersen E, Hutchings B, Jansen J, Nyholm M. Heights and weights of Danish children. Ugeskr Laeger. 1982; 144:1760–1765. [PubMed: 7135585]

Belin V, Cusin V, Viot G, Girlich D, Toutain A, Moncla A, Vekemans M, Le Merrer M, Munnich A, Cormier-Daire V. SHOX mutations in dyschondrosteosis (Leri-Weill syndrome). Nat Genet. 1998; 19:67–69. [PubMed: 9590292]

Benito-Sanz S, Thomas NS, Huber C, Gorbenko DB, Aza-Carmona M, Crolla JA, Maloney V, Rappold G, Argente J, Campos-Barros A, Cormier-Daire V, Heath KE. A novel class of pseudoautosomal region 1 deletions downstream of SHOX is associated with Leri-Weill dyschondrosteosis. Am J Hum Genet. 2005; 77:533–544. [PubMed: 16175500]

Bojesen A, Kristensen K, Birkebaek NH, Fedder J, Mosekilde L, Bennett P, Laurberg P, Frystyk J, Flyvbjerg A, Christiansen JS, Gravholt CH. The metabolic syndrome is frequent in Klinefelter's syndrome and is associated with abdominal obesity and hypogonadism. Diabetes Care. 2006; 29:1591–1598. [PubMed: 16801584]

Chen J, Wildhardt G, Zhong Z, Roeth R, Weiss B, Steinberger D, Decker J, Blum WF, Rappold GA. Enhancer mutations of the SHOX gene as a frequent cause of short stature—The essential role of a 250kb downstream regulatory domain. J Med Genet. 2009; 46:834–839. [PubMed: 19578035]

Fukami M, Okuyama T, Yamamori S, Nishimura G, Ogata T. Microdeletion in the SHOX 3' region associated with skeletal phenotypes of Langer mesomelic dysplasia in a 45,X/46,X,r(X) infant and Leri-Weill dyschondrosteosis in her 46,XX mother: Implication for the SHOX enhancer. Am J Med Genet Part A. 2005; 137A:72–76.

Huber C, Rosilio M, Munnich A, Cormier-Daire V. High incidence of SHOX anomalies in individuals with short stature. J Med Genet. 2006; 43:735–739. [PubMed: 16597678]

Juul A, Sorensen K, Aksglaede L, Garn I, Rajpert-De Meyts E, Hullstein I, Hemmersbach P, Ottesen AM. A common deletion in the uridine diphosphate glucuronyltransferase (UGT) 2B17 gene is a strong determinant of androgen excretion in healthy pubertal boys. J Clin Endocrinol Metab. 2009; 94:1005–1011. [PubMed: 19088161]

Kanaka-Gantenbein C, Kitsiou S, Mavrou A, Stamoyannou L, Kolialexi A, Kekou K, Liakopoulou M, Chrousos G. Tall stature, insulin resistance, and disturbed behavior in a girl with the triple X syndrome harboring three SHOX genes: Offspring of a father with mosaic Kline-felter syndrome but with two maternal X chromosomes. Horm Res. 2004; 61:205–210. [PubMed: 14752208]

Kirsch S, Weiss B, Zumbach K, Rappold G. Molecular and evolutionary analysis of the growth-controlling region on the human Y chromosome. Hum Genet. 2004; 114:173–181. [PubMed: 14579146]

Linden MG, Bender BG, Robinson A. Sex chromosome tetrasomy and pentasomy. Pediatrics. 1995; 96:672–682. [PubMed: 7567329]

Marchini A, Marttila T, Winter A, Caldeira S, Malanchi I, Blaschke RJ, Hacker B, Rao E, Karperien M, Wit JM, Richter W, Tommasino M, Rappold GA. The short stature homeodomain protein SHOX induces cellular growth arrest and apoptosis and is expressed in human growth plate chondrocytes. J Biol Chem. 2004; 279:37103–37114. [PubMed: 15145945]

Marchini A, Hacker B, Marttila T, Hesse V, Emons J, Weiss B, Karperien M, Rappold G. BNP is a transcriptional target of the short stature homeobox gene SHOX. Hum Mol Genet. 2007; 16:3081–3087. [PubMed: 17881654]

Ogata T, Matsuo N, Nishimura G. SHOX haploinsufficiency and overdosage: Impact of gonadal function status. J Med Genet. 2001; 38:1–6. [PubMed: 11134233]

Ottesen AM, Garn ID, Aksglaede L, Juul A, Rajpert-De Meyts E. A simple screening method for detection of Klinefelter syndrome and other X-chromosome aneuploidies based on copy number of the androgen receptor gene. Mol Hum Reprod. 2007; 13:745–750. [PubMed: 17720778]

Peet J, Weaver DD, Vance GH. 49, XXXXY: A distinct phenotype. Three new cases and review. J Med Genet. 1998; 35:420–424. [PubMed: 9610808]

Rao E, Weiss B, Fukami M, Mertz A, Meder J, Ogata T, Heinrich U, Garcia-Heras J, Schiebel K, Rappold GA. FISH-deletion mapping defines a 270-kb short stature critical interval in the pseudoautosomal region PAR1 on human sex chromosomes. Hum Genet. 1997a; 100:236–239. [PubMed: 9254856]

Rao E, Weiss B, Fukami M, Rump A, Niesler B, Mertz A, Muroya K, Binder G, Kirsch S, Winkelmann M, Nordsiek G, Heinrich U, Breuning MH, Ranke MB, Rosenthal A, Ogata T, Rappold GA. Pseudoautosomal deletions encompassing a novel homeobox gene cause growth failure in idiopathic short stature and Turner syndrome. Nat Genet. 1997b; 16:54–63. [PubMed: 9140395]

Rappold GA. The pseudoautosomal regions of the human sex chromosomes. Hum Genet. 1993; 92:315–324. [PubMed: 8225310]

Ratcliffe S. Long-term outcome in children of sex chromosome abnormalities. Arch Dis Child. 1999; 80:192–195. [PubMed: 10325742]

Sabherwal N, Bangs F, Roth R, Weiss B, Jantz K, Tiecke E, Hinkel GK, Spaich C, Hauffa BP, van der KH, Kapeller J, Tickle C, Rappold G. Long-range conserved non-coding SHOX sequences regulate expression in developing chicken limb and are associated with short stature phenotypes in human patients. Hum Mol Genet. 2007; 16:210–222. [PubMed: 17200153]

Schibler D, Brook CG, Kind HP, Zachmann M, Prader A. Growth and body proportions in 54 boys and men with Klinefelter's syndrome. Helv Paediatr Acta. 1974; 29:325–333. [PubMed: 4372201]

Shears DJ, Vassal HJ, Goodman FR, Palmer RW, Reardon W, Superti-Furga A, Scambler PJ, Winter RM. Mutation and deletion of the pseudoautosomal gene SHOX cause Leri-Weill dyschondrosteosis. Nat Genet. 1998; 19:70–73. [PubMed: 9590293]

Suda M, Ogawa Y, Tanaka K, Tamura N, Yasoda A, Takigawa T, Uehira M, Nishimoto H, Itoh H, Saito Y, Shiota K, Nakao K. Skeletal overgrowth in transgenic mice that overexpress brain natriuretic peptide. Proc Natl Acad Sci USA. 1998; 95:2337–2342. [PubMed: 9482886]

Tartaglia N, Davis S, Hench A, Nimishakavi S, Beauregard R, Reynolds A, Fenton L, Albrecht L, Ross J, Visootsak J, Hansen R, Hagerman R. A new look at XXYY syndrome: Medical and psychological features. Am J Med Genet Part A. 2008; 146A:1509–1522. [PubMed: 18481271]

Thomas NS, Harvey JF, Bunyan DJ, Rankin J, Grigelioniene G, Bruno DL, Tan TY, Tomkins S, Hastings R. Clinical and molecular characterization of duplications encompassing the human SHOX gene reveal a variable effect on stature. Am J Med Genet Part A. 2009; 149A:1407–1414. [PubMed: 19533800]

Visootsak J, Graham JM Jr. Klinefelter syndrome and other sex chromosomal aneuploidies. Orphanet J Rare Dis. 2006; 1:42. [PubMed: 17062147]

Vorona E, Zitzmann M, Gromoll J, Schuring AN, Nieschlag E. Clinical, endocrinologic and epigenetic features of the 46, XX male syndrome compared to 47,XXY Klinefelter patients. J Clin Endocrinol Metab. 2007; 92:3458–3465. [PubMed: 17579198]

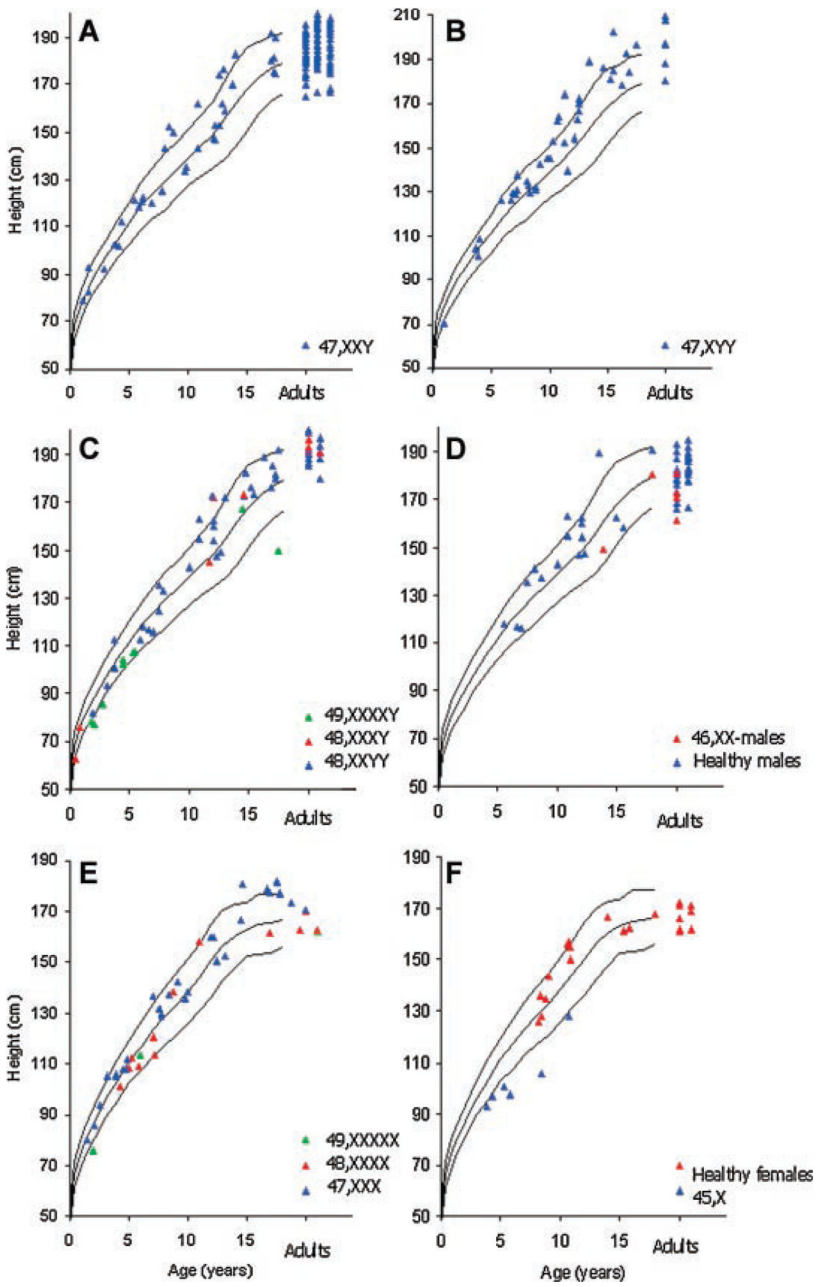

FIG. 1.

Height (cm) according to chronological age in patients with sex chromosome aneuploidy and healthy controls. A: Height in 129 patients with nonmosaic 47,XXY karyotypes, (B) height in 44 patients with 47,XYY karyotypes, (C) height in 45 patients with 48,XXYY karyotypes, 9 patients with 48,XXXY karyotypes and 10 patients with 49,XXXXY karyotypes, (D) height in 6 patients with *SRY*-positive 46,XX-male karyotypes and 43 healthy male controls, (E) height in 40 patients with 47,XXX karyotypes, 13 patients with 48,XXXX karyotypes and 3 with 49,XXXXX karyotype, (F) height in 6 patients with 45,X

karyotypes and 21 healthy female controls. Lines represent mean ± 2 SD in healthy Danish boys (A–D) and girls (E,F), respectively [Andersen et al., 1982].

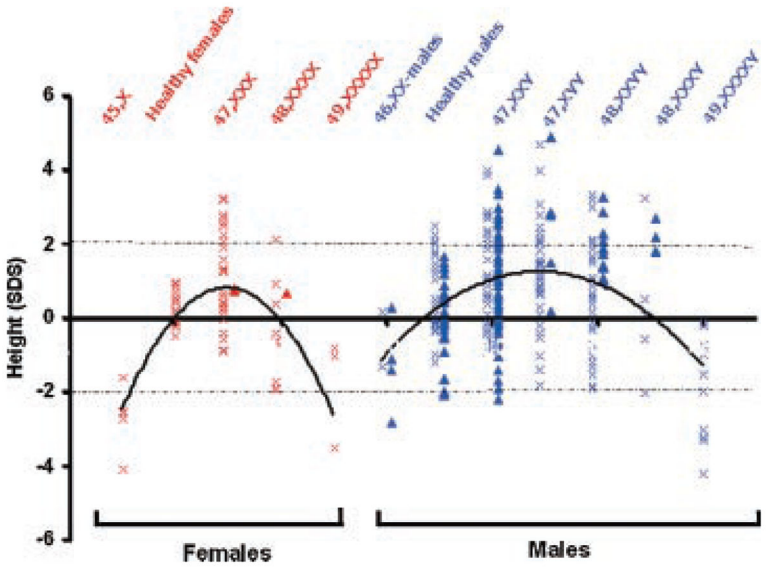

FIG. 2.

Height (expressed as SDS) in the patients and healthy controls according to karyotype; female patients in red, male patients in blue. Within each karyotype group, results are grouped according to age; <20 years of age (×), ≥20 years (▲). Curves indicate the influence of the number of sex chromosomes and hypothetically the *SHOX* copy number on height as indicated by the best-fitted polynomials for girls and boys, respectively. Note the decline in the curve according to height in males with 48,XXXY, which is an artifact due to the very low height SDS in patients with 49,XXXXY.

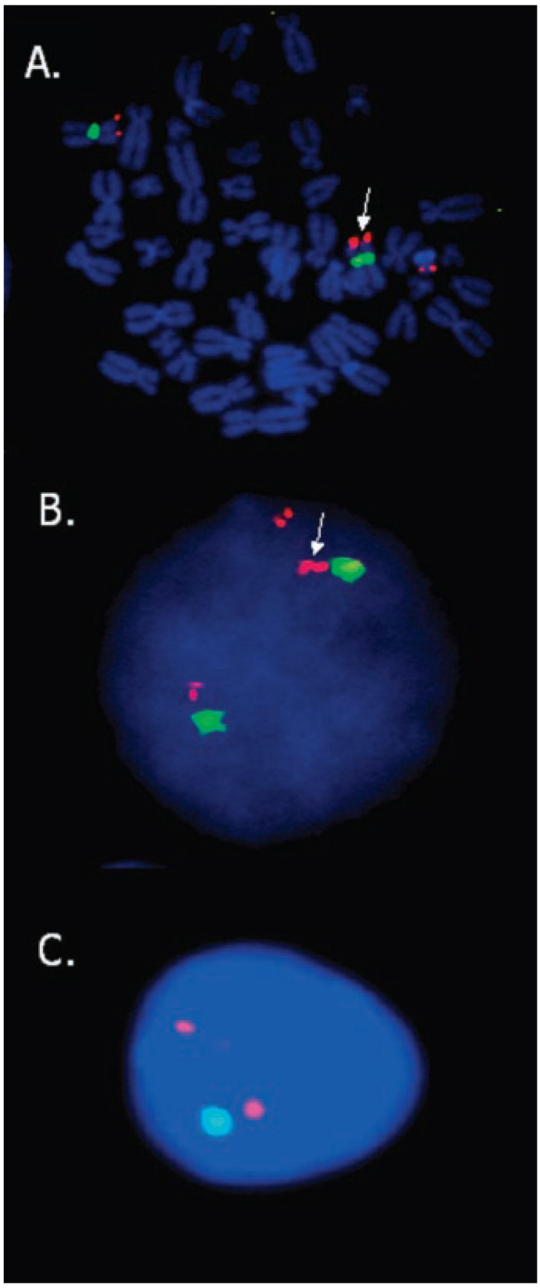

FIG. 3.

Two-color FISH analysis of gene copy number of *SHOX*. The green spots represent the signals from the control probe on chromosome X, and the red spots show the locus-specific *SHOX* probe on Xp and Yp. A: *SHOX*-FISH analysis of a metaphase of case e (47,XXY). The white arrows point to the duplication of *SHOX* in both A and B; (B) *SHOX*-FISH analysis of an interphase with split spots from the same case e; (C) *SHOX*-FISH analysis of an interphase of a normal male (46,XY).

TABLE I

Clinical Presentation of the 305 Patients With Sex Chromosome Aneuploidy and 74 Healthy Controls

Karyotype	Number of subjects (height available)	Number of SHOX measurements	Median age (years) (range)	Median height SDS (range)
45,X	6 (6)	6	5.6 (3.8–10.7)	−2.6 (−4.1 to −1.6)
Female controls (not karyotyped)	21 (21)	21	15.3 (8.2–19.9)	0.2 (−0.5 to 1.0)
47,XXX	40 (40)	18	9.9 (1.4–20.0)	0.7 (−0.9 to 3.2)
48,XXXX	13 (13)	3	7.1 (4.2–24.0)	−0.6 (−1.9 to 2.1)
49,XXXXX	3 (3)	1	5.9 (1.9–19.0)	−1.0 (−3.5 to −0.8)
SRY-pos 46,XX-males	6 (6)	6	27.2 (13.8–39.9)	−1.2 (−2.8 to 0.3)
46,XY	29 (23)	29	28.3 (3.7–56.2)	0.2 (−1.6 to 2.0)
Male controls (not karyotyped)	24 (20)	24	19.1 (18.0–28.6)	0.5 (−2.1 to 2.5)
47,XXY	129 (129)	129	27.7 (1.1–66.3)	0.9 (−2.2 to 4.6)
47,XYY	44 (44)	31	11.3 (1.0–36.5)	1.3 (−1.8 to 4.9)
48,XXXY	9 (9)	2	14.6 (0.5–31.8)	1.8 (−2.0 to 3.2)
48,XXYY	45 (45)	34	14.6 (1.9–35.3)	1.0 (−1.9 to 3.4)
49,XXXXY	10 (10)	2	4.9 (1.8–17.5)	−1.8 (−4.2 to −0.1)

SRY, sex determining region of the Y chromosome.

TABLE II

Summary of the Statistics of the qPCR Data

Number of sex chromosomes	**1**	**2**	**3**	**4**	**5**
Number of samples	6	35	178	39	3
Median copy number	0.9	1.9	2.9	3.9	4.7
2.5–97.5 percentiles	0.6–0.9	1.7–2.2	2.6–3.3	3.5–4.3	4.6–5.0

TABLE III

Copy Numbers of the *SHOX* Gene Using Two Different Techniques

Specimen	Karyotype	*SHOX* expected copies	*SHOX*-qPCR copy number	*SHOX*-MLPA copy number
a	47,XXY	3	2	1.8
b	47,XXY	3	2.2	2
c	47,XXY	3	3.8	3.6
d	47,XYY	3	4	3.4
e	47,XXY	3	4.2	3.7
f	46,XX[a]	2	3.0	2.5

[a]SRY positive.