Short report

Short stature in a girl with partial monosomy of the pseudoautosomal region distal to DXYS15: further evidence for the assignment of the critical region for a pseudoautosomal growth gene(s)

Tsutomu Ogata, Atsuko Yoshizawa, Koji Muroya, Nobutake Matsuo, Yoshimitsu Fukushima, Gudrun Rappold, Susumu Yokoya

Abstract

This report describes a 12 year 10 month old girl with short stature and a non-mosaic 46,X,Xp+ karyotype. Her height remained below −2 SD of the mean, and her predicted adult height (143 cm) was below her target height (155·5 cm) and target range (147·5 cm–163·5 cm). Cytogenetic and molecular studies showed that the Xp+ chromosome was formed by an inverted duplication of the Xp21.3-Xp22.33 segment and was missing about 700 kb of DNA from the pseudoautosomal region distal to DXYS15. The results provide further support for the previously proposed hypothesis that the region between DXYS20 and DXYS15 is the critical region for a pseudoautosomal growth gene(s).

(*J Med Genet* 1995;32:831-834)

Department of Paediatrics, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160, Japan
T Ogata
K Muroya
N Matsuo

Department of Paediatrics, Toranomon Hospital, Tokyo 105, Japan
A Yoshizawa
S Yokoya

Divison of Medical Genetics, Saitama Children's Medical Centre, Saitama 339, Japan
Y Fukushima

Institut für Humangenetik, Ruprecht-Karls-Universität Heidelberg, 69120 Heidelberg, Germany
G Rappold

Correspondence to:
Dr Ogata.

Received 24 February 1995
Revised version accepted for publication 25 May 1995

Although genotype-phenotype correlations in patients with partial monosomy of the pseudoautosomal region (PAR) suggest that the region between DXYS20 and DXYS15 is the critical region for a pseudoautosomal growth gene(s) (P growth gene(s)),[1] the assignment is primarily based on the report of a single patient with short stature and a terminal deletion of the PAR distal to DXYS15.[2] Here, we describe a Japanese girl with short stature and a rearranged X chromosome. Genotype-phenotype correlation of this girl provides further support for the assignment of the critical region for the P growth gene(s).

This girl was the 48·0 cm (mean −0·8 SD) and 2·2 kg (mean −2·2 SD) product of an uncomplicated term pregnancy. Her postnatal course was uneventful, and menarche occurred at 11 years 3 months of age. At 11 years 8 months, she was seen at Toranomon Hospital because of short stature. Physical examination showed no abnormality except for proportionate short stature. Secondary sexual development was appropriate for her age (Tanner stage: breast 4, pubic hair 3). Mental development appeared normal. Routine laboratory tests were normal, as were endocrine and radiological studies for short stature. At present, she is 12 years 10 months old and has regular menses.

The growth chart of the patient is shown in fig 1. Her height remained below −2 SD of the mean height of normal Japanese girls.[3] Furthermore, her predicted adult height (PAH, a child's final height as predicted from biological data of the child) was below her target height (TH, a child's final height as predicted from the parental height) and target range (TR, 95% confidence interval of TH). PAH was determined as 143 cm (mean −2·7 SD) by the method of Ito and Yokoya,[4] and TH and TR were obtained as 155·5 cm (mean −0·3 SD) and 147·5 cm–163·5 cm (mean −1·8 SD–mean +1·2 SD), respectively, by the equations of Ogata *et al.*[5]

The patient's karyotype was 46,X,Xp+ in all the 263 lymphocytes analysed. High resolution G banding[6] indicated that the Xp+ chromosome was formed by an inverted duplication of the Xp21.3–Xp22.33 segment (fig 2). R banding (RBG)[7] showed that the Xp+ chromosome was late replicating in 76 of 101 lymphocytes examined and the normal X chromosome was late replicating in the remaining 25 lymphocytes. Fluorescence in situ hybridisation analysis using an X chromosome specific cocktail mixture of probes covering the entire X chromosome[8] detected positive signals on the entire Xp+ chromosome. The parents had a normal karyotype.

To determine whether the Xp+ chromosome was associated with a deletion of the PAR, Southern blot analysis was performed on the patient, the mother, and normal controls. Representative results are shown in fig 3 and summarised in the table. Informative restriction fragment length polymorphism was obtained only for DXYS14; maternal bands for DXYS14 were not inherited by the patient, implying that the patient's DXYS14 was of paternal origin

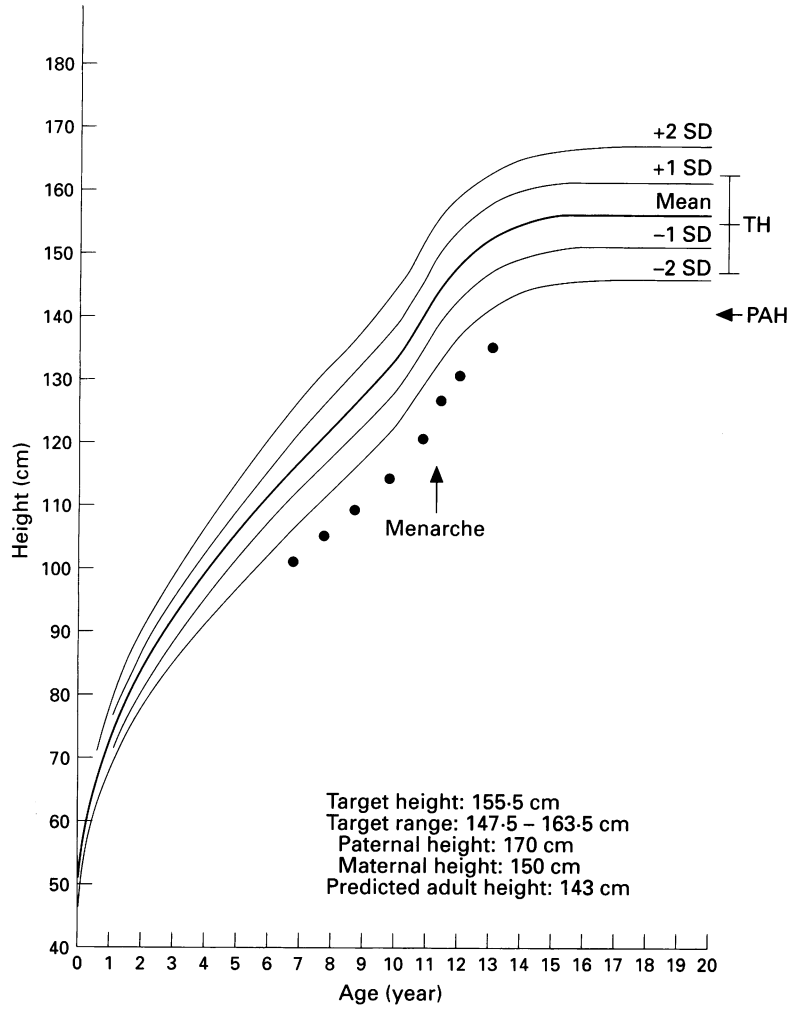

Figure 1 Growth chart of the patient. The dots represent the actual heights of the patient. The growth curves depict the longitudinal height standards for normal Japanese girls. Target height (TH) indicates a child's final height as predicted from the parental height, target range indicates 95% confidence interval of TH, and predicted adult height (PAH) indicates a child's final height as predicted from biological data of the child.

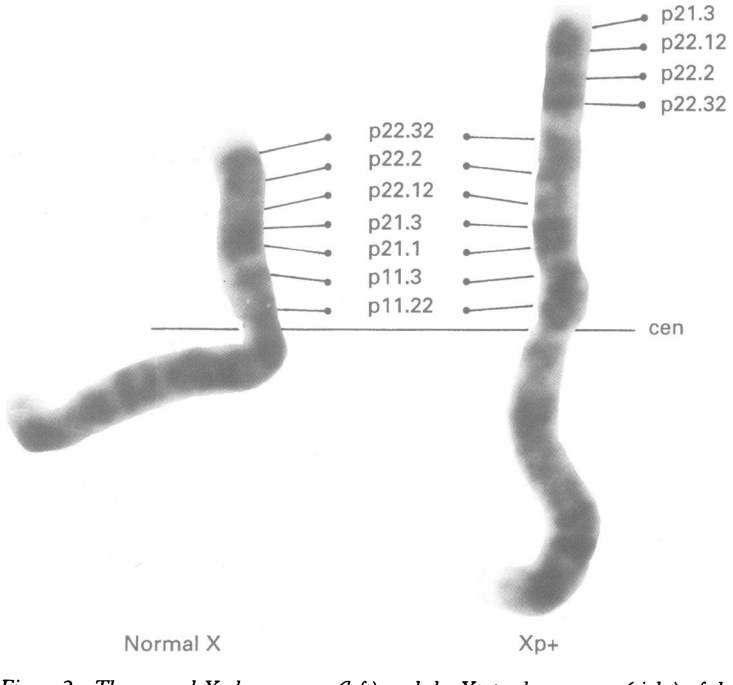

Figure 2 The normal X chromosome (left) and the Xp+ chromosome (right) of the patient. The band pattern of the extra chromosomal material is consistent with an inverted duplication of the Xp21.3–Xp22.33 segment.

and present in a single copy. The comparison of band intensity using the band for autosomal TK gene as an internal control indicated that, in the patient, DXYS60, DXYS87, DXYS161, DXYS28, and DXYS59 were present in a single copy, whereas DXYS15, CSF2RA, DXYS17, and MIC2 were present in triple copies.

The results indicate that the Xp+ chromosome was formed by an inverted duplication of the Xp21.3–Xp22.33 segment and was missing the distal part of the PAR. It is inferred that the Xp+ chromosome was generated during maternal meiosis (fig 4). Sine the Xp+ chromosome was the sole discernible abnormality, it is probable that the short stature of our patient was caused by the Xp+ chromosome.

The most pertinent explanation for the short stature would be to postulate that the P growth gene(s) is deleted or disrupted in our patient. In this context, it is noteworthy that the Xp+ chromosome is missing only about 700 kb of DNA from the PAR distal to DXYS15 (table). The result is consistent with the notion that the region from DXYS20 to DXYS15 is the critical region for the P growth gene(s).[1] Furthermore, the difference between PAH and TH of our patient is in close agreement with the adult height difference between 13 females with 46,X,del(X)(p22.32) and nine females with 46, XX in the two pedigrees reported by Curry *et al*[13] (154·1 (SD 4·9) cm *v* 166·3 (SD 4·0) cm, $p<10^{-5}$). This would also be compatible with the P growth gene(s) being impaired in our patient. Although it might be possible that the P growth gene(s) is preserved on the Xp+ chromosome but is subject to a position effect or to an abnormal spreading of X inactivation, as has been suspected for large Xq− chromosomes,[14] the possibility remains purely speculative at present.

By contrast, other factors relevant to the Xp+ chromosome are unlikely to explain the short stature adequately. First, several genes on the duplicated segment could affect stature because of the dosage effect. In this regard, genes escaping X inactivation would be present in three active copies in all somatic cells, and genes normally subject to X inactivation would be present in two active copies in somatic cells with the active Xp+ chromosome. However, height comparison between white patients with 45,X (143·3 (SD 6·4) cm, n=130), 46,XX (162·2 (SD 6·0) cm, the British standard), and 47,XXX (167·9 (SD 7·7) cm, n=19)[15] argues against the X chromosome carrying a gene(s) escaping X inactivation that has a growth suppressing effect, and there is no evidence for the presence of a gene(s) subject to X inactivation that acts as a growth suppressor. Second, chromosome imbalance would cause global non-specific developmental defects, exerting a disadvantageous effect on statural growth.[15] However, it appears difficult to ascribe the short stature to chromosome imbalance alone. (1) If the P growth gene(s) is preserved on the Xp+ chromosome, it would be present in three copies, and 47,XXX patients with three copies of the P growth gene(s) are taller than normal females.[15] Thus, unless the P growth gene(s) is impaired, somatic cells of our patient, at

The copy number of pseudoautosomal loci analysed in the present study

Locus (probe)	*Distance from telomere (kb)*	*Enzyme*	*Patient*	*Mother*	*Male*	*Female*	*Reference*
DXYS14 (29C1)	<20	*Taq*I	1	2	2	2	9
DXYS60 (U7A)	310–360	*Eco*RI	1 (0·37)	2 (0·75)	2 (0·78)	2 (0·68)	10
DXYS87 (P99)	380–420	*Taq*I	1 (1·15)	2 (2·08)	2 (1·97)	2 (1·89)	10
DXYS161 (B6)	470–476	*Eco*RI	1 (0·48)	2 (0·81)	2 (0·78)	2 (0·76)	10
DXYS28 (pDP411)	482–486	*Eco*RI	1 (0·58)	2 (0·91)	2 (0·88)	2 (0·85)	10
DXYS59 (68A)	500–650	*Taq*I	1 (1·49)	2 (2·83)	2 (2·98)	2 (2·85)	11
DXYS15 (113D)	700–760	*Taq*I	3 (2·32)	2 (1·45)	2 (1·31)	2 (1·37)	10
CSF2RA (cDNA)	1180–1300	*Eco*RI	3 (0·68)	2 (0·47)	2 (0·48)	2 (0·45)	10
DXYS17 (601)	1840–1880	*Eco*RI	3 (0·98)	2 (0·63)	2 (0·75)	2 (0·60)	10
MIC2 (19B)	2500–2600	*Taq*I	3 (3·63)	2 (2·50)	2 (2·81)	2 (2·35)	10

The copy number of DXYS14 is determined by the RFLP pattern (see fig 3), and that of the remaining loci is based on the band intensity analysis using a laser densitometer (ACD-250DX, ATTO). The values in parentheses represent the ratio of the band intensity between each locus and autosomal TK gene.[12] Similar band intensity ratios have been reproduced by other enzyme-probe combinations (data not shown). The positions of the pseudoautosomal loci are based on the reports of Henke *et al*[10] and Petit *et al*.[11]

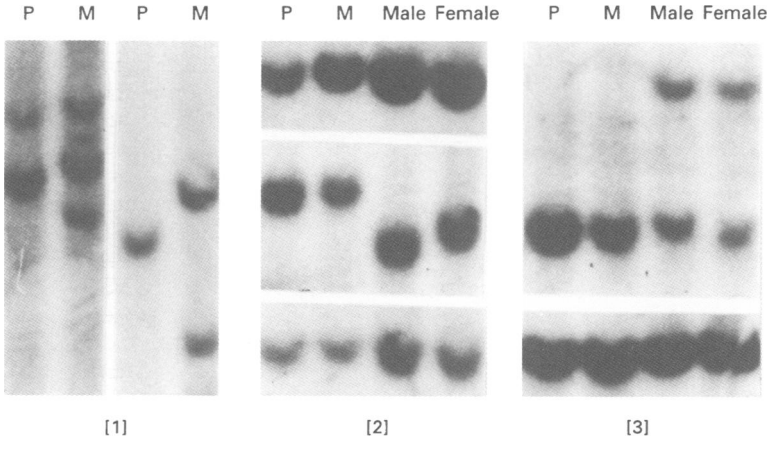

Figure 3 Southern blot analysis (P=patient; M=mother, normal male, and normal female). (1) EcoRI digests (left) and TaqI digests (right) hybridised with 29C1 (DXYS14). Maternal bands are not inherited by the patient. (2) TaqI digests hybridised with 68A (DXYS59, top panel), 113D (DXYS15, middle panel), and the probe for autosomal TK gene (bottom panel) (same filter). Although comparison of the band intensity for the TK gene indicates that the DNA loaded is similar between the patient and the mother, the band intensity for DXYS59 is weaker in the patient than in the mother and that for DXYS15 is stronger in the patient than in the mother. The band intensity pattern for the three loci is similar between the mother and the normal subjects. (3) EcoRI digests hybridised with 601 (DXYS17, upper panel) and the probe for autosomal TK gene (lower panel) (same filter). Although comparison of the band intensity for TK gene implies that the DNA loaded is similar between the four subjects, the band intensity for DXYS17 is stronger in the patient that in the mother and the normal subjects.

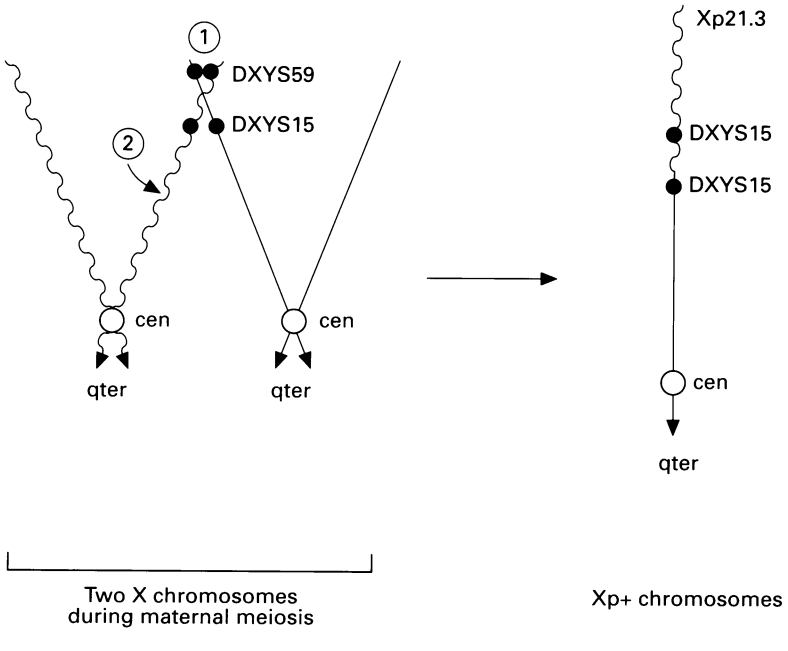

Figure 4 A schematic representation of the generation of the Xp+ chromosome. It is inferred that crossing over btween DXYS59 and DXYS15 (a normal event designated by the number 1) and chromosomal breakage at Xp21·3 (an abnormal event designated by the number 2) occurred during maternal meiosis and, subsequently, the distal Xp segment between the crossing over point and the chromosomal breakpoint was transferred onto the homologous X chromosome, resulting in the inverted duplication of the Xp21·3–Xp22·33 segment and in the deletion of the telomeric portion distal to DXYS15.

least those with the inactive Xp+ chromosome, would have sufficient growth potential. (2) Although chromosome imbalance would be severer in somatic cells with the active Xp+ chromosome than in those with the inactive Xp+ chromosome, the X inactivation pattern suggests that the deleterious effects of chromosome imbalance as well as those of perturbed gene dosage are not so different between the two types of cells. (3) Our patient lacked non-specific features attributable to chromosome imbalance such as mental retardation and somatic anomalies. This agrees with the notion that clinical effects caused by chromosomal duplication are much milder than those caused by corresponding chromosomal deletions,[15 16] and implies that the phenotypic effects of chromosome imbalance remained at mild or subclinical level in our patient. Third, if the Xp+ chromosome is inactivated preferentially, this implies the operation of a mild degree of cell selection mechanism that could cause growth failure by reducing the total number of viable cells in a person.[17] In this connection, since the inactivation pattern of lymphocytes does not necessarily reflect that of other tissues, several tissues may be associated with a very skewed inactivation pattern. However, normal adult height in white patients with 46,X,t(X;autosome) accompanied by non-random inactivation of the normal X chromosome (161·6 (SD 7·6) cm, n=17)[15] argues that the cell selection mechanism, if it occurs, does not cause growth failure.

We would like to thank Dr Jun Kusuda for the band intensity analysis, and Dr Christine Petit for probes U7A and 68A. This work was supported in part by a grant for Paediatric Research from the Ministry of Health and Welfare, a grant from Vehicle Racing Commemorative Foundation, and a grant from Keio University.

1 Ogata T, Petit C, Rappold G, Matsuo N, Matsumoto T, Goodfellow P. Chromosomal localisation of a pseudoautosomal growth gene(s). *J Med Genet* 1992;**29**:624–8.
2 Ogata T, Goodfellow P, Petit C, Aya M, Matsuo N. Short stature in a girl with a terminal Xp deletion distal to DXYS15: localisation of a growth gene(s) in the pseudoautosomal region. *J Med Genet* 1992;**29**:455–9.
3 Suwa S, Tachibana K, Maesaka H, Tanaka T, Yokoya S. Longitudinal standards for height and height velocity for Japanese children from birth to maturity. *Clin Pediatr Endocrinol* 1992;**1**:5–13.
4 Ito R, Yokoya S. Predicting adult height from skeletal age: a modified Bayley-Pinneau method for Japanese children. *Annual Report of Japan Athletic Association* 1994; No 6: 46–53.
5 Ogata T, Matsuo N, Tamai S, Osano M, Tango T. Target height and target range for the Japanese. *Jpn J Paediatr* 1990;**94**:1535–40.
6 Ikeuchi T. Inhibitory effect of ethidium bromide on mitotic chromosome condensation and its application to high-resolution chromosome banding. *Cytogenet Cell Genet* 1984;**38**:56–61.
7 Wolf S, Perry P. Differential Giemsa staining of sister chromatids and study of sister chromatid exchanges without autoradiography. *Chromosoma* 1974;**48**:341–53.

8 ONCOR. Chromosome in situ system, edition 3, Gaithersburg, 1991.
9 Cooke HJ, Brown WRA, Rappold GA. Hypervariable telomeric sequences from the human sex chromosomes are pseudoautosomal. *Nature* 1985;**317**:687–92.
10 Henke A, Fischer C, Rappold GA. Genetic map of the human pseudoautosomal region reveals a high rate of recombination in female meiosis at the Xp telomere. *Genomics* 1993;**18**:478–85.
11 Petit C, Levilliers J, Weissenbach J. Physical mapping of the human pseudo-autosomal region: comparison with genetic linkage map. *EMBO J* 1988;**8**:2369–76.
12 Lau YF, Kan YW. Direct isolation of the functional human thymidine kinase gene with a cosmid shuttle vector. *Proc Natl Acad Sci USA* 1984;**81**:414–18.
13 Curry JR, Magenis RE, Brown M, *et al.* Inherited chondrodysplasia punctata due to a deletion of the terminal short arm of an X chromosome. *N Engl J Med* 1984;**311**:1010–15.
14 Geekens C, Just W, Vogel W. Deletion of Xq and growth deficit: a review. *Am J Med Genet* 1994;**50**:105–13.
15 Ogata T, Matsuo N. Sex chromosome aberrations and stature: deduction of the principal factors involved in the determination of the adult height. *Hum Genet* 1993;**91**: 551–62.
16 Gilbert EF, Opitz JM. Developmental and other pathologic changes in syndromes caused by chromosome abnormalities. *Perspect Pediatr Pathol* 1982;7:1–63.
17 Gartler SM, Sparkes RS. The Lyon-Beutler hypothesis and isochromosome X patients with the Turner syndrome. *Lancet* 1963;**ii**:411.