Hum Genet (1995) 95:283–286

ORIGINAL INVESTIGATION

Pia Salo · Helena Kääriäinen · David C. Page
Albert de la Chapelle

Deletion mapping of stature determinants on the long arm of the Y chromosome

Received: 5 August 1994 / Revised: 14 September 1994

Abstract A gene contributing to human growth has previously been tentatively mapped to the long arm of the Y chromosome. In the present study, recently developed sequence-tagged site markers covering the entire Y chromosome were used to define deletion breakpoints in 15 males with partial deletions of Yq. By correlating the height of these individuals with their deletion breakpoints, we located a region whose presence or absence has a marked effect on stature. This critical region comprises the most proximal portion of the long arm, extending from marker sY78 in interval 4B to marker sY94 in interval 5G of the proximal long arm.

Introduction

Height is a multifactorial trait influenced by genetic and environmental factors that are difficult to separate from each other (Mueller 1986). The Y chromosome is believed to harbor genes influencing height. This can be deduced from the fact that XY males are taller than XX males, that XY females are taller than XX females, and that XYY males are taller than XY males (de la Chapelle 1972). Interestingly, when adult height is compared between female patients with XX gonadal dysgenesis (XXGD) and XY gonadal dysgenesis (XYGD), the mean adult height of XYGD patients is significantly greater than that of XXGD patients (Ogata and Matsuo 1992). Thus, it can be hypothesized that at least one Y-specific growth-promoting gene exists.

Several case reports of short males with a partially deleted or morphologically altered Y chromosome were published in the early years of cytogenetics, reinforcing the notion that loss of part of the Y chromosome can lead to shortness of stature. In particular, cases with short stature and 46,XYq– as the only cell line give support to the presence of stature determinants on the Y chromosome long arm (Bühler 1980; Kosztolányi and Trixler 1983; Langmaid and Laurence 1974; Podruch et al. 1982; Skare et al. 1990; Telfer et al. 1973; Yunis et al. 1977).

Another parameter of growth is tooth size. As the size of the permanent teeth is relatively independent of environmental factors and is closely correlated with height, tooth size was determined in two males with deletions of the long arm of the Y chromosome. The male with the more extensive-appearing deletion clearly had smaller teeth than the individual with the smaller deletion, allowing the tentative assignment of the locus to proximal Yq11 (Alvesalo and de la Chapelle 1981). This putative gene was named growth control Y, GCY (Goodfellow et al. 1985); it might be the same or distinct from the gene affecting stature.

The purpose of this study was to reexamine the existence and location of a growth control gene on the human Y chromosome. We reasoned that the recent detailed mapping of the human Y chromosome would provide us with greatly improved tools to delineate the deletions in patients with partial Y chromosomes (Foote et al. 1992; Vollrath et al. 1992). The previous literature concerning Y chromosomal long arm deletions has consisted mainly of case reports, and the molecular breakpoints have rarely been defined. This paper describes the molecular-phenotypic correlations observed in 15 patients with partial deletions of the long arm of the Y chromosome.

P. Salo (✉) · H. Kääriäinen · A. de la Chapelle
Department of Medical Genetics,
P.O. Box 21 (Haartmaninkatu 3),
FIN-00014 University of Helsinki, Helsinki, Finland

D. C. Page
Howard Hughes Research Laboratories
at Whitehead Institute and Department of Biology,
Massachusetts Institute of Technology, 9 Cambridge Center,
Cambridge, MA 02142, USA

Materials and methods

Patients with Y chromosomal deletions

All available patients in whom a deleted Y chromosome had been suspected or detected by cytogenetic analysis were included in this study. The patients were identified in three chromosome laboratories in Finland. The most common reasons for referral were infer-

tility, hypogonadism or short stature. Patients 3, 10, 11, and 12 have been reported previously (Alvesalo and de la Chapelle 1981; de la Chapelle and Hortling 1962, 1963; Kaluzewski et al. 1978). The deletion breakpoint of patient 10 has also been reported previously (Vollrath et al. 1992).

Sequence-tagged sites detected by polymerase chain reaction

The Y-chromosomal breakpoints were defined by polymerase chain reaction (PCR) detection of sequence-tagged sites (STSs). PCR was performed under the following final reaction conditions: 1 × PCR reaction buffer (10 m*M* TRIS pH 8.2, 5 m*M* NH_4Cl, 1.5 m*M* $MgCl_2$, 100 m*M* KCl), 100 µ*M* dNTPs, with 5 U *Taq* polymerase per 100 µl reaction, 80–100 ng human genomic DNA, and each primer at 1.0 µ*M*, in a reaction volume of 10–20 µl. Thirty-five cycles were performed at 94°C for 1 min and 30 s, at 55°C or 61°C for 1 min and 30 s and at 72°C for 1 min and 30 s. PCR products were subjected to electrophoresis in 3% NuSieve gels. After electrophoresis, the gels were photographed. Male and female controls were included.

The human Y chromosome can be divided into euchromatic and heterochromatic regions. The euchromatic region consists of the short arm, centromere and proximal long arm. The heterochromatic region in the distal long arm contains highly repeated sequences.

DNAs from 11 patients with known or suspected Y chromosomal deletions were tested for the presence or absence of 69 markers located thoughout the Y chromosome (see Table 1). Of these markers, none of which were pseudoautosomal, 12 were on the short arm (intervals 1–4A), one was in the centromeric region (interval 4B), 55 were in the euchromatic q11 band of the long arm (intervals 5A–6F), and one was in the distal heterochromatic q12 band (interval 7). In addition, four patients with cytogenetically detected Y chromosomal deletions were studied after the initial screening had been completed. They were examined with at least 20 markers with the aim of defining their deletion breakpoints.

Height assessment

The height of the patients was compared with the Finnish population standards (Sorva et al. 1984). Target height (*TH*, a child's height relative to Finnish population standards in SDs as predicted from the parental heights) was determined using the following equation: $TH = [(PH + MH)/2 - 170]/10$, where *PH* is the paternal height and *MH* the maternal height in centimeters.

Results

Table 1 summarizes the results of 10 nonmosaic patients and of five patients who had a 45,X cell line in addition to the cell line having a structurally abnormal Y chromosome. Only results relevant to defining the breakpoints

Table 1 Results of deletion mapping with STS markers in nonmosaic Yq-patients (patients 1–10) and mosaic Yq-patients (patients 11–15). Only a subset of all markers relevant to defining the breakpoints is shown; other markers used in the initial screening are given in the footnote. In all patients in which a sample from the father or brother was available, the father's or brother's sample showed no deletion when studied with STSs. In patients 2, 7, 11, 12, 13, and 15, a sample from the brother or father was not available for study. In patient 7, the father's chromosomes had been studied previously and showed a normal 46,XY karyotype. *ND* Not done

Interval	STS	Number of the patient														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1A1A	sY14	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3C	sY57	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3E	sY67	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4A	sY72	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4B	sY78	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
5A	sY79	+	+	+	+	+	+	+	–	–	–	+	+	+	+	+
5A	sY81	+	+	+	+	+	+	+	–	–	–	+	+	+	+	+
5B	sY82	+	+	+	+	+	+	+	–	–	–	+	+	+	ND	+
5C	sY84	+	+	+	+	+	+	–	–	–	–	+	+	+	+	+
5C	sY85	+	+	+	+	+	+	–	–	–	–	+	+	+	+	+
5D	sY87	+	+	+	+	+	–	–	–	–	–	+	+	+	+	+
5E	sY182	+	+	+	+	+	–	–	–	–	–	+	+	+	+	+
5G	sY94	+	+	–	–	–	–	–	–	–	–	+	+	+	+	+
5K	sY108	+	+	–	–	–	–	–	–	–	–	+	+	+	+	+
5M	sY113	+	–	–	–	–	–	–	–	–	–	+	+	–	–	–
5M	sY119	+	–	–	–	–	–	–	–	–	–	+	+	–	–	–
5N	sY121	–	–	–	–	–	–	–	–	–	–	+	+	–	–	–
6A	sY142	–	–	–	–	–	–	–	–	–	–	+	+	–	–	–
6D	sY147	–	–	–	–	ND	–	ND	ND	–	–	+	–	–	–	–
6E	sY155	–	–	–	–	ND	–	ND	ND	–	–	+	–	–	–	–
6F	sY157	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
7	sY159	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

sY15, sY16, sY17, sY18, sY19, sY63, sY69, sY71, sY183, sY165, sY88, sY90, sY95, sY161, sY97, sY98, sY100, sY101, sY102, sY103, sY104, sY105, sY106, sY114, sY115, sY116, sY117, sY118, sY122, sY124, sY125, sY126, sY127, sY128, sY129, sY130, sY131, sY133, sY134, sY135, sY143, sY148, sY149, sY152, sY153, sY154, sY158. Marker sY78 was weakly positive in female controls probably because of the alphoid repeat nature of the region

Table 2 Description of 10 nonmosaic Yq-patients. Height is given in centimeters and SDs with reference to Finnish population standards. Target height, a child's height as predicted from parental heights, is given in SDs. *M* Mother, *F* Father, *NA* Not available, *LGL* Patient number given in the Department of Medical Genetics (LGL)

No.	LGL no.	Age (years)	Height (cm, SD)	Target height (SD)	Parental heights (cm)
1	5167	21	178	–0.6	156 (M) 173 (F)
2	5205	35	177	NA	NA
3	825	25	181	NA	NA
4	5022	8	152, +1.7	+0.8	170 (M) 186 (F)
5	5825	15	167, –0.7	+0.2	167 (M) 176 (F)
6	5019	3	92, –1.7	–0.2	157 (M) 180 (F)
7	862	8	131, 0	+0.1	165 (M) 176 (F)
8	5860	8	133, +0.3	+0.6	167 (M) 185 (F)
9	676	15	155, –2.1	+0.2	161 (M) 182 (F)
10	658	17	160, –2.8	–0.7	150 (M) 176 (F)

Table 3 Description of five mosaic Yq-patients. Height is given in centimeters and SDs with reference to Finnish population standards. Target height, a child's height as predicted from parental heights, is given in SDs. *LGL* Patient number given in the Department of Medical Genetics (LGL)

No.	LGL no.	Age (years)	Height (cm, SD)	Karyotype
11	816	55	157	45,X/46,XYq–
12	813	28	147	45,X/46,XYq–
13	5245	26	164	45,X/46,XYq–/46,XdicYp
14	4719	12	146 –1.1	45,X/46,XYq–
15	796	33	175	45,X/46,XdicYp

are shown. All other markers gave results compatible with those shown. As far as can be judged from these results, the deletions in all 15 patients were terminal, i.e., the distal euchromatic portion and the entire heterochromatic portion of the Yq were missing.

Nonmosaic patients 1–7 were of normal height and had deletions with breakpoints distal to marker sY82 in interval 5B. Patients 8–10 had larger deletions, with breakpoints falling more proximally between markers sY78 and sY79. The deletions in these three patients comprised most if not all of the Y chromosome long arm. Patient 8 was of normal height, but patients 9 and 10 were clearly of short stature, measuring 2.3 and 2.1 SDs below their TH as predicted from parental heights and population standards (Table 2).

Of the five mosaic patients, three had deletion breakpoints distal to marker sY108 in interval 5K, whereas the remaining two breakpoints were more distal. Two of these patients were clearly of short stature, measuring 157 and 147 cm as their final adult heights, but adult patient 15 was of normal height (Table 3).

Discussion

Among the nonmosaic cases, only patients 9 and 10 are clearly of short stature, i.e., they measure at least 2 SDs below their target heights. This places the putative growth control gene on the long arm, just distal to marker sY78 in interval 4B. Thus, the growth control gene appears to be close to the centromere. In patient 10, height may have been affected by mild hypothyroidism, for which he has received thyroxin substitution treatment. Hypothyroidism frequently presents with growth failure with a rapid "catch-up" growth once thyroxin replacement therapy has commenced. Patient 10 cannot therefore be considered fully informative regarding height.

The nonmosaic cases 1–8 are of normal stature (patients 1–4, 7 and 8) or have only a slight growth deficiency (patients 5 and 6). Patients 1–3 are adult and thus are most informative with respect to genotype-phenotype correlation. Patient 3 has a deletion breakpoint between markers sY182 in interval 5E and sY94 in interval 5G. This places the growth control gene proximal to marker sY94 in interval 5G. The breakpoint in patient 8 is in the same region as in patients 9 and 10, but this patient is growing normally at the age of 8 years. Although his youth makes it difficult to evaluate his final height, his case tentatively suggests that the growth determinant resides between sY78 and sY79.

The nonmosaic patients 5 and 6 measure 0.9 and 1.5 SDs below their TH as predicted from parental heights. Patient 5 had delayed puberty as the reason for referral and might later reach normal adult height. Since patient 6 is only 3 years old, the significance of his height is also difficult to assess.

Shortness of stature in mosaic patients can be explained by the influence of the 45,X cell line on growth. Thus, patients with 46,XYq– as the only cell line are more suitable for genotype-phenotype analysis. Moreover, one cannot totally exclude the existence of a 45,X cell line in any patient. The mosaic patient 15 is however of normal height, having a deletion breakpoint distal to marker sY108 in interval 5K. This reinforces the above conclusion regarding the distal boundary of the major growth gene.

It is not yet possible to determine whether the gene related to tooth size is identical to the one related to height. Under the working hypothesis that they are identical, the previous cytogenetic assignment of GCY to proximal Yq11 (Alvesalo and de la Chapelle 1981) could be con-

firmed, as both males were restudied here. The male with the less extensive-appearing deletion and larger teeth was patient 3, and the patient with the more extensive-appearing deletion and smaller teeth was patient 10. In addition, our results conform well with a previous genotype-phenotype analysis that suggested the location of a gene involved in stature determination to Yq11.21, proximal to the azoospermia factor gene, AZF (Bardoni et al. 1991). A 13-year-old boy measuring below the 3rd centile and with a marker chromosome consisting of Y centromeric and short arm material probably represents another extreme long-arm deletion with shortness of stature (Crolla et al. 1989).

It can thus be hypothesized that the GCY gene maps to the proximal long arm of the Y chromosome, close to the centromere. The critical region comprises the most proximal portion of the long arm, extending from marker sY78 in interval 4B to marker sY94 in interval 5G. We consider this assignment tentative until additional patients with large Yq deletions can be evaluated both for molecular breakpoint and adult height. Interestingly, a gene that predisposes Turner patients with Y chromosome material to gonadoblastoma, the gonadoblastoma locus on the Y chromosome (GBY; Page 1987), also appears to map close to the Y chromosome centromere (Petrovic et al. 1992).

Acknowledgements We thank the patients, their families, and several clinical investigators for their help and cooperation, and Sinikka Lindh for collecting the samples. This study was supported by the Academy of Finland, National Institutes of Health, the Sigrid Juselius Foundation, and the Finnish Cultural Foundation, the Regional Fund of Häme. Part of the study was performed at the Folkhälsan Institute of Genetics.

References

Alvesalo L, Chapelle A de la (1981) Tooth sizes in two males with deletions of the long arm of the Y-chromosome. Ann Hum Genet 45:49–54

Bardoni B, Zuffardi O, Guioli S, Ballabio A, Simi P, Cavalli P, Grimoldi MG, Fraccaro M, Camerino G (1991) A deletion map of the human Yq11 region: implications for the evolution of the Y chromosome and tentative mapping of a locus involved in spermatogenesis. Genomics 11:443–451

Bühler EM (1980) A synopsis of the human Y chromosome. Hum Genet 55:145–175

Chapelle A de la (1972) Nature and origin of males with XX sex chromosomes. Am J Hum Genet 24:71–105

Chapelle A de la, Hortling H (1962) XY/XO mosaicism. Lancet 2:783–784

Chapelle A de la, Hortling H (1963) Cytogenetical and clinical observations in male hypogonadism. Acta Endocrinol 44:165–182

Crolla JA, Smith M, Docherty Z (1989) Identification and characterisation of a small marker chromosome using non-isotopic in situ hybridisation with X and Y specific probes. J Med Genet 26:192–194

Foote S, Vollrath D, Hilton A, Page DC (1992) The human Y chromosome: overlapping DNA clones spanning the euchromatic region. Science 258:60–66

Goodfellow PN, Davies KE, Ropers H-H (1985) Report of the committee on the genetic constitution of the X and Y chromosomes. Cytogenet Cell Genet. 40:296–352

Kaluzewski B, Jokinen A, Hortling H, Chapelle A de la (1978) A theory explaining the abnormality in 45,X/46,XY mosaicism with non-fluorescent Y chromosome. Presentation of three cases. Ann Genet 21:5–11

Kosztolányi G, Trixler M (1983) Yq deletion with short stature, abnormal male development and schizoid character disorder. J Med Genet 20:393–394

Langmaid H, Laurence KM (1974) Deletion of the long arms of the Y chromosome with normal male development and intelligence. J Med Genet 11:208–211

Mueller WH (1986) The genetics of size and shape in children and adults. In: Falkner F, Tanner JM (eds) Human growth. Plenum, New York, pp 145–168

Ogata T, Matsuo N (1992) Comparison of adult height between patients with XX and XY gonadal dysgenesis: support for a Y specific growth gene. J Med Genet 29:539–541

Page DC (1987) Hypothesis: a Y-chromosomal gene causes gonadoblastoma in dysgenetic gonads. Development 101 (Suppl):151–155

Petrovic V, Nasioulas S, Chow CW, Voullaire L, Schmidt M, Dahl H (1992) Minute Y chromosome derived marker in a child with gonadoblastoma: cytogenetic and DNA studies. J Med Genet 29:542–546

Podruch PE, Yen F-S, Dinno ND, Weisskopf B (1982) Yq- in a child with livedo reticularis, snub nose, microcephaly, and profound mental retardation. J Med Genet 19:377–380

Skare J, Drwinga H, Wyandt H, vanderSpek J, Troxler R, Milunsky A (1990) Interstitial deletion involving most of Yq. Am J Med Genet 36:394–397

Sorva R, Perheentupa J, Tolppanen E-M (1984) A novel format for a growth chart. Acta Paediatr Scand 73:527–529

Telfer M, Baker D, Rollin I (1973) Probable long-arm deletion of Y chromosome in boy of short stature. Lancet I:608

Vollrath D, Foote S, Hilton A, Brown LG, Beer-Romero P, Bogan JS, Page DC (1992) The human Y chromosome: a 43-interval map based on naturally occurring deletions. Science 258:52–59

Yunis E, García-Conti FL, Torres de Caballero OM, Giraldo A (1977) Yq deletion, aspermia, and short stature. Hum Genet 39:117–122