The sex related height difference in humans is thought to be caused mainly by two components: first, a hormonal component determined by the sex dimorphism of bioactive gonadal steroids, and, second, a genetic component attributed to a Y specific growth gene, GCY. Despite extensive mapping attempts for this gene on the human Y chromosome, its precise position remains unknown. We have recently provided evidence that inappropriate cytogenetic methodology in the characterisation of Y chromosomal terminal deletions has led to some of the difficulties in elucidating the GCY critical region. In order to circumvent these problems, we have decided to consider only patients presenting de novo interstitial deletions for GCY analysis on the Y chromosome. This approach allows the assignment of GCY to a particular chromosomal interval without excluding the presence of X0 mosaicism and/or i(Yp) and idic(Yq11) chromosomes in patients with terminal deletions. Indeed, the direct comparison of overlapping interstitial deletions in seven adult males with normal height, one male with borderline height, and one patient with short stature resulted in the confirmation of a GCY critical interval between markers DYZ3 and DYS11. This region roughly encompasses 1.6-1.7 Mb of genomic DNA. To improve the resolution in the region of interest close to the centromere, we established additional new STS markers specific for this part of the chromosome using our bacterial artificial chromosome (BAC)/P1 derived artificial chromosome (PAC) contig. Molecular deletion analysis using these new Y chromosomal STSs allowed us to exclude almost all of the Y chromosomal long arm as the putative location of the GCY growth gene and to narrow down the critical interval to a genomic region of 700 kb.

**MATERIALS AND METHODS**

**Selection of patients**

All nine patients are sterile and have deletions of either AZFa, AZFb, or AZFc or a combination thereof. They were otherwise healthy. Patients 293, JOLAR, 28, 63, and 95 have been described clinically in detail elsewhere. Patient Y0308

<table>
<thead>
<tr>
<th>Case</th>
<th>Country of origin</th>
<th>Height of patient (cm) and SDS</th>
<th>National height standard (cm)</th>
<th>Heights of family members (cm) and SDS</th>
</tr>
</thead>
<tbody>
<tr>
<td>293</td>
<td>USA</td>
<td>157 (SDS -2.9) Short</td>
<td>176.9 (SD 6.8)</td>
<td>(F) 170 (M) Normal (B) Normal</td>
</tr>
<tr>
<td>Y0308</td>
<td>USA</td>
<td>165.1 (SDS -1.7) Borderline/short</td>
<td>176.9 (SD 6.8)</td>
<td>(F) 170.1 (M) 170.1 (B) 188 (SDS +1.7) (S) 167.6 (SDS -0.4)</td>
</tr>
<tr>
<td>JOLAR</td>
<td>UK</td>
<td>168 (SDS -1.0) Normal</td>
<td>174.7 (SD 6.7)</td>
<td>(F) Normal (M) Normal (B) Normal</td>
</tr>
<tr>
<td>28</td>
<td>Italy</td>
<td>175 (SDS -0.3) Normal</td>
<td>176.7 (SD 6.5)</td>
<td>(F) Normal (M) Normal</td>
</tr>
<tr>
<td>63</td>
<td>Ethiopia</td>
<td>170 (SDS +0.3) Normal</td>
<td>168.0 (SD 7.4)</td>
<td>(F) Normal (M) Normal</td>
</tr>
<tr>
<td>95</td>
<td>Israel</td>
<td>185 (SDS +1.4) Normal</td>
<td>175.6 (SD 6.8)</td>
<td>(F) Normal (M) Normal</td>
</tr>
<tr>
<td>TM</td>
<td>Belgium</td>
<td>182 (SDS +1.3) Normal</td>
<td>173.5 (SD 6.7)</td>
<td>(F) Normal (M) Normal</td>
</tr>
<tr>
<td>1947</td>
<td>Germany</td>
<td>175 (SDS -0.8) Normal</td>
<td>179.9 (SD 6.4)</td>
<td>(F) Normal (M) Normal</td>
</tr>
<tr>
<td>1972</td>
<td>Germany</td>
<td>181 (SDS +0.2) Normal</td>
<td>179.9 (SD 6.4)</td>
<td>175 (M) 165 (S) (SDS +1.0)</td>
</tr>
</tbody>
</table>

The standard deviation score (SDS) was calculated based on the equation: SDS = (X-M)/SD, where X is a person’s adult height and M and SD are the mean adult height and the 1 SD of the normal male population, respectively. (M) mother, (F) father, (S) sister, (B) brother. Patients are over 21 years of age.
corresponds to case 1 in Pryor et al. Patients TM, 1947, and 1972 are phenotypically normal males suffering from idiopathic infertility. Genomic DNA samples were extracted from peripheral blood leukocytes (patients 28, 63, 95, Y0308, TM, 1947, and 1972) or from lymphoblastoid cell lines (patients 293 and JOLAR). DNA isolated from peripheral blood leukocytes of normal males and females served as internal controls.

Height assessment

As all the subjects are of diverse ethnic origins, height was compared to the respective national height standards (table 1). Patients were of similar age range. When possible, special attention was given to adult height comparisons between parents and sibs. Data are summarised along with the height assessment

<table>
<thead>
<tr>
<th>STS</th>
<th>Left primer</th>
<th>Right primer</th>
<th>Product size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SKY1</td>
<td>GGACATTTGGCTCAGAGAT</td>
<td>TGGCAATTGACCTTCATC</td>
<td>255</td>
</tr>
<tr>
<td>SKY2</td>
<td>TCAGGCAAAAGGCTGCTGCT</td>
<td>CGTCCTGAACTTACGG</td>
<td>167</td>
</tr>
<tr>
<td>SKY3</td>
<td>TTCTCCCTACCTCTCAAGC</td>
<td>GGCTCCTACCAATACGGA</td>
<td>202</td>
</tr>
<tr>
<td>SKY4</td>
<td>CTCCTTCACCTTCTCCTCA</td>
<td>CGCAGCITATGCGGCA</td>
<td>328</td>
</tr>
<tr>
<td>SKY5</td>
<td>TCAATGGCATGAGTCTTGTTC</td>
<td>CTTTCCATGGGGTGACATTC</td>
<td>223</td>
</tr>
<tr>
<td>SKY6</td>
<td>TTGAGTCACTGGGGATAACTG</td>
<td>ATGAGGTCAGAATTGGA</td>
<td>189</td>
</tr>
<tr>
<td>SKY7</td>
<td>AAAGTGGAATTGAAAGCAATG</td>
<td>GAAACGGCCCTGGAATCTT</td>
<td>531</td>
</tr>
<tr>
<td>SKY8</td>
<td>ATAAGGGGCAGGTTTGTCAC</td>
<td>GCTACTTATTCAGTGTTTAACTGACAC</td>
<td>329</td>
</tr>
<tr>
<td>SKY9</td>
<td>ATAAGGGGCAGGTTTGTCAC</td>
<td>GCTACTTATTCAGTGTTTAACTGACAC</td>
<td>329</td>
</tr>
<tr>
<td>SKY10</td>
<td>ATAAGGGGCAGGTTTGTCAC</td>
<td>GCTACTTATTCAGTGTTTAACTGACAC</td>
<td>329</td>
</tr>
<tr>
<td>SKY11</td>
<td>AATAAGGGAATTGAAAGCAATG</td>
<td>TTTTTGTTTGTGGCAGGTG</td>
<td>469</td>
</tr>
<tr>
<td>SKY12</td>
<td>TTGAGTCACTGGGGATAACTG</td>
<td>CTTCCTGACCTCATTCAAC</td>
<td>216</td>
</tr>
<tr>
<td>SKY13</td>
<td>GGACATTTGGCTCAGAGAT</td>
<td>CTTTTGTTGCAACAGTTTGTC</td>
<td>198</td>
</tr>
</tbody>
</table>

Markers with an asterisk amplify DNA fragments from more than one genomic locus (see section “Restriction analysis of PCR products for detail”).

Table 3

<table>
<thead>
<tr>
<th>STS</th>
<th>Restriction enzyme</th>
<th>BAC clones</th>
<th>Fragment sizes (bp) after restriction</th>
</tr>
</thead>
<tbody>
<tr>
<td>SKY10</td>
<td>Tsp509I</td>
<td>487K20 70G12 560118</td>
<td>279, 50 329 329*</td>
</tr>
<tr>
<td>SKY11</td>
<td>NolI</td>
<td>245K04 245K04 506M09</td>
<td>217, 154, 79, 19 233, 221, 15</td>
</tr>
<tr>
<td>SKY12</td>
<td>Msel</td>
<td>245K04 506M09</td>
<td>88, 57, 39, 32 145, 39, 32</td>
</tr>
<tr>
<td>SKY13</td>
<td>CoBI/TspI</td>
<td>10021 589P14 251M08</td>
<td>97, 83, 23 175, 23 97, 50, 33, 23</td>
</tr>
</tbody>
</table>

*The submitted sequence of the chromosome 1 derived BAC clone RP11-560118 (AC053222) does not show a Tsp509I restriction site within the genomic fragment amplified by the primer pair SKY10. Restriction analysis of fragments amplified from male and female genomic DNA, from a somatic cell hybrid line containing chromosome 1 as the only chromosome of human origin, and from the BAC RP11-560118 also shows two fragments of ~180 bp and ~155 bp, indicating a sequence error in the complete sequence of the BAC clone.

Restriction analysis of PCR products

PCR products were resolved on agarose gels, the appropriate gel bands cut out, and the DNA isolated with GFX DNA Purification Kit (Amersham Pharmacia Biotech) according to the manufacturer’s protocol. Fragments amplified from SKY5 and SKY6 were digested with TaqI and BsmI, respectively. To detect SFVs at SKY10, SKY11, SKY12, and SKY13, PCR products were digested with restriction enzymes as listed in table 3.

Sequencing of BAC/PAC/YAC end fragments

DNA from BAC/PAC clones selected for end sequencing was purified with the Nucleobond PC100 Kit (Macherey-Nagel) according to the manufacturer’s instructions. End fragments

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were directly sequenced using the Thermosequenase Fluorescent Labelled Primer Cycle Sequencing Kit and analysed on a Pharmacia ALF Express (Amersham Pharmacia Biotech). YAC end fragments were generated with Alu/vector polymerase chain reaction and subcloned in pCR2.1 with the TOPO-TA cloning Kit (Invitrogen). Sequencing was performed as described.

Fluorescence in situ hybridisation

Metaphase spreads were obtained either from primary blood samples or immortalised cell lines. Cosmid and plasmid DNA was labelled by nick translation with biotin-16-dUTP (Roche). Slides were hybridised, stained, counterstained, and photographed according to standard protocols.

RESULTS

Mapping of interstitial deletions

We studied the DNA of nine adult males who originally consulted reproduction centres about idiopathic infertility. Of the nine males, seven were of normal height. One patient, No 293, with a height of 157 cm, had short stature (SDS \(-2.9\)) and one, Y0308, with a height of 165.1 cm showed borderline height, being on the 3rd centile of normal US height standard (SDS \(-1.7\)). The adult height of his parents and sibs are in the normal range (table 1), his brother being 22.9 cm taller than the patient. Compared to his brother and sister on one side and to his target height (176.6 cm) and target range (166.6-186.6 cm) on the other side, he can be considered short. All men were ascertained solely on the basis of large de novo interstitial deletions on the Y chromosome. Only two of the patients had undergone previous chromosomal studies.

In our effort to localise the GCY locus, we focused on that part of the Y chromosome long arm that was delimited by the boundaries of the interstitial deletions of the patients with short stature (fig 1). Recently, a detailed physical map of the human Y chromosome incorporating 758 ordered STSs and 199 completely sequenced BAC clones has been constructed.\(^{17}\) We used a slightly modified PCR multiplex system\(^{15}\) to test the absence or presence of 28 DNA loci from the Y chromosome long arm. In patients where sufficient DNA was available for further PCR analysis, additional STSs were tested. As a result, eight of nine interstitial deletion breakpoints could be positioned (fig 1). As the deletions of patients JOLAR, 28, 63, 95, TM, and 1947, all with normal height, overlap, most of the long arm of the Y chromosome could be excluded as a critical region for GCY.

As the distal breakpoint of the deletion of patient 1972 does not reside within the specific part of the Y chromosome long arm, the nature of the deletion (terminal or interstitial) remained unclear. There was also no overlap of his deletion with the deletions of patients 1947 and TM. Relying solely on the results obtained by the STS based interstitial deletion mapping, the region distal to sY158 could not formally be excluded as a potential critical region for GCY. However, multiplex PCR analysis always showed a less intense amplification product for STS sY157 (a Y derived marker in close proximity to sY158). To address this problem, the rearranged Y chromosome of patient 1972 was investigated in more detail.

Fluorescence in situ hybridisation and sequence family variant typing of patient 1972

The overall integrity of the Y chromosome from patient 1972 was shown by FISH of cosmids LLOYNC03‘M’34F05 (PAR1) and LLOYNC03‘M’49B02 (PAR2) as well as the Y centromere specific probe Y-97 and the telomere specific probe “all human telomeres” (data not shown). On the basis of these results the deletion in patient 1972 was confirmed to be interstitial. Being aware of the complex structural organisation of the human DAZ

Figure 1  Deletion mapping on the long arm of the human Y chromosome. A diagram of the human Y chromosome with Yp telomere to the left and Yq telomere to the right is presented at the top. Shown below are the results of low resolution analysis of Y chromosomes of adult males with normal height or short stature. Along the top border, 95 Y chromosomal STSs are listed. Except for SKY3 and SKY8 (see table 2 for detail), all other STSs have been previously reported.\(^{17-19}\) Blank spaces or grey boxes indicate inferred absence or presence of markers for which assay was not performed. Asterisks indicate markers in the respective breakpoint regions which could not be tested. In all cases where previously published data of the patients were reinvestigated, the identical DNA sample used for the primary analysis was studied. (Please note that the proximal as well as the distal breakpoint of the interstitial deletion of patient 293 resides within satellite type II sequences.)
locus (fig 2A), we specifically searched for sequence family variants (SFVs). To prevent misjudging sequence errors as single nucleotide differences, PCR/restriction digest assays were developed only from SFVs present in at least two overlapping BAC clones. The localisation of these SFVs is shown in fig 2B. As these SFVs could represent allelic variants, 10 unrelated normal German males were typed. In all cases, the expected fragment pattern could be detected for the Y chromosome derived

Figure 2. Sequence family variant (SFV) typing in the human DAZ locus in distal Yq11.23. (A) Overview and amplicon structure of the human Y chromosome in the vicinity of the human DAZ cluster. Each amplicon is represented by a specific colour. Shown above are arrows indicating the orientation of each member of an amplicon family with respect to each other. The amplicon coloured orange arose from a portion of chromosome 1 that was transposed to the distal end of the DAZ cluster and partially duplicated. (B) Precise position of selected Y specific STSs and the SFVs according to the physical map of the human Y chromosome. Marker sY157 is highlighted as it was suspected to be present in only one copy by multiplex PCR analysis [see text for detail]. (C) Summary of STS and SFV analysis in patients with Y chromosomal rearrangements within the human DAZ cluster region. Grey boxes indicate inferred absence or presence of markers. (D) Sequence family variant typing of SKY10 and SKY12 in genomic DNA of patient 1972. The assay is described in table 3. Along the right are listed fragment sizes (in bp). Products were separated by electrophoresis in 3% NuSieve agarose (3:1) and visualised by ethidium bromide staining.
sequences. In contrast, the fragment pattern deduced from the genomic sequence of the chromosome 1 derived BAC clone RP11-560I18 could not be confirmed (see table 3 for detail). Each SFV specific PCR/restriction digestion was compared to the presence/absence in the corresponding BAC clones.

Typing the genomic DNA of patient 1972 for all four sequence family variants (SKY10/\textit{Ts}p509I, SKY11/\textit{Nla}III, SKY12/\textit{Ms}I, and SKY13/\textit{Cac}8I + \textit{Tfl}I) showed the absence of one Y derived non-allelic sequence variant (table 3 and fig 2C, D). In the case of SKY10 the distal copy is deleted. Not surprisingly, in all other typing experiments the more proximal copy of the respective SFVs was shown to be deleted.

Next, we investigated these SFVs in the two patients with the most distal breakpoints (Nos 95 and 1947). Using genomic DNA, we determined that both non-allelic variants of SKY11, SKY12, and SKY13 and one non-allelic variant of SKY10 were absent in patient 1947, whereas for all tested SFVs one non-allelic variant was absent in patient 95.

Taken together, these results provide evidence that the proximal breakpoint of the interstitial deletion present in the Y chromosome of patient 1972 resides within the interstitial deletion of patient 1947, thereby excluding this genomic region as a potential critical interval for \textit{GCY}.

**Refinement of the \textit{GCY} critical interval**

Based on the molecular analysis of the pericentric region of the long arm of the human Y chromosome, the physical extension of the \textit{GCY} critical region as defined by the markers

---

**Figure 3** Schematic representation of the organisation of the long arm pericentromeric region of the human Y chromosome. (A) Diagram showing the distribution of major tandem repeat blocks and general organisation of sequence homologies. Basically, the region can be subdivided into three distinct intervals: a proximal region characterised by 5 bp satellite sequences (green), a central region with high homology to chromosome 1 (orange), and a distal region composed of X/Y homologous sequences (blue). In this X/Y homologous region, a segment homologous to chromosome Xq25/10q25 (red) has been integrated. The precise position of the newly established and previously published STS markers in this region are illustrated below. At the bottom border, the PAC/BAC contig constructed with the aid of the new STS markers is shown. Prefixes RP1-3 indicate PAC clones and RP11 BAC clones, respectively. (B) Localisation of the \textit{GCY} critical interval as defined by high resolution STS mapping in patients with short stature and normal height. Black boxes indicate the presence, white boxes the absence of the respective STS. Striped boxes depict the dosage unknown regions where the breakpoint resides.
sY78 (DYZ3) and sY83 (DYS11) was estimated to constitute 1.6-1.7 Mb (fig 3A) of DNA. The most proximal 400 kb of this region consists exclusively of 5 bp satellite sequences separated from the Y centromere only by Alu sequences. This constant part of the human Y chromosome is therefore unlikely to contain coding sequences. The remainder of the GYC critical region is composed of XY homologous as well as autosomal/Y homologous sequence blocks. At the onset of this study, only limited coverage in YAC clones was available for this region. In order to refine the GYC critical interval and to generate gene finding substrates, it was necessary to establish a BAC/PAC contig of this region.

We generated 25 additional markers mainly by sequencing the end fragments of BAC, PAC, and YAC clones as well as clone internal sequences amplified by various combinations of Alu-Alu oligonucleotide primer pairs. Of these, only seven turned out to be Y specific (SKY1, SKY2, and SKY4-8) (see table 2 for detail). The BAC and PAC clones identified during the generation of the physical map are summarised in table 4. Meanwhile, some of these clones have been completely sequenced as they form part of a tiling path for sequencing the contig of this region.

Confirming the overlap between BAC RP11-295P22 and BAC RP11-322K23 appeared to be the most crucial step in the process of contig construction. Y specific markers derived from the opposite end fragments of both clones were suspected to amplify identical sized fragments from two different loci within the same 5 bp satellite region. By testing several restriction enzymes known to cut frequently within 5 bp satellites composing the consensus sequence (TGGAA)_n, we developed loci specific PCR/restriction digestion assays. Typing all BAC clones mapping to this sequence block with the appropriate PCR/restriction digestion assay allowed us to position them precisely, thereby confirming their overlaps.

In order to narrow down the critical interval for the GYC gene, we tested for the presence of the newly generated STS in patients 293, Y0308, and JOLAR. These results allowed us to define a small region for the GYC gene (fig 3B). Direct sequence comparison showed that the sequenced BAC clones RP11-322K23, RP11-75F05, RP11-461H06, RP11-333E09, RP11-558M10, CITB-298B15, and CITB-203M13 completely cover the mapped region between Y-STSs SKY8 and sY83 (DYS11), suggesting that it encompasses roughly 700 kb.

**DISCUSSION**

Since the issue of the existence of a Y specific growth gene (GYC) was first raised, there have been several attempts to define its precise location. Whereas initial studies unanimously pointed towards a common region on the Y chromosome long arm, more recent investigations have led to the identification of two non-overlapping critical intervals. FISH analyses resolved this apparent contradiction by presenting clear evidence that the patient materials used in these initial investigations contained 45,X0 cells and/or i(Yp) idic (Yq11) chromosomes. Both genetic parameters influence the adult height of a given subject, thereby rendering it impossible to predict whether such patients have lost GYC or not. Studies with patients carrying de novo interstitial deletions are, therefore, much better suited to address the localisation of GYC. In the course of reviewing published reports for patients with small interstitial deletions, in particular close to the centromere, it became clear that these patients are very rare. This prompted us to extend our search for patients carrying large de novo interstitial deletions, irrespective of their actual adult height. We examined nine adult patients, seven with normal height. Furthermore, we could show overlapping deletions, thereby excluding GYC from almost the entire long arm of the Y chromosome including the pseudoautosomal region 2 (PAR2). Two patients, 293 and Y0308, presented interstitial deletions enabling the restriction of the GYC critical region to approximately 700 kb of DNA. This region is therefore predicted to harbour one or more genes required for normal human growth.

All nine patients studied share infertility as a common phenotype, which is in agreement with their large Yq deletions. Despite extensive routine screening of infertile males in reproduction centres, only two patients were found to present borderline/short stature in combination with a confirmed large de novo deletion. We therefore conclude that cytogenetically detectable de novo deletions including the GYC gene are rare events. It is possible, however, that deletion sizes are generally small in this area and therefore undetectable using the presently available set of markers. In addition, the adult height reduction of 6-8 cm attributed to the Y specific growth gene does not necessarily result in the diagnosis of short stature in all affected males. Sex related adult height difference is determined by the level of bioactive gonadal steroids and the Y specific growth gene. In addition, the mid-parental height strongly contributes to the final height of a person. Parameters such as nutrition, infectious diseases, and secular trend are further components influencing the adult height of a given person. Therefore, high heritability for a given trait does not prevent this trait being strongly influenced by environmental conditions.

In summary, our data suggest that GYC maps to a critical interval marked by the Y derived markers SKY8 and sY83 (DYS11). This 700 kb interval, recently sequenced by the Human Genome Project, does not contain any known gene or any Y specific ESTs. Different reasons such as unusual gene structures, for example, genes consisting of only one exon, the
lack of homology to any identified gene, or spatially or temporally restricted gene expression patterns could account for this.

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REFERENCES
Localisation of the Y chromosome stature gene to a 700 kb interval in close proximity to the centromere


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