Molecular characterization of a Y-derived marker chromosome and identification of indels in the DYS1 region in a patient with stigmata of Turner syndrome

Anu Bashamboo[†], Sunita Bhatnagar[†], Anupam Kaur[#], Virinder Kaur Sarhadi[#], Jai Rup Singh[#] and Sher Ali[†,*]

[†]National Institute of Immunology, Aruna Asaf Ali Marg, New Delhi 110 067, India
[#]Centre for Genetic Disorders, Guru Nanak Dev University, Amritsar 143 005, India

Presence of the Y chromosome in human female and its absence in the male is an abnormal condition, implying a spectrum of genetic abnormalities. In this communication, we describe studies conducted on a 17-year-old patient (HK-459) with stigmata of Turner syndrome. We analysed the hormonal profile of the subject, chromosomal constitution and DNA for the five different loci encompassing both the arms of the Y chromosome. Chromosomal analysis showed mosaicism containing 45,X/46,X,+mar but no Y chromosome. The X chromosome and all the autosomes of the patient and her parents were normal. The Southern analysis of the patient's genomic DNA with probes specific to DYZ1 locus did not detect the corresponding Y-specific signal. Similarly, primers RG4, RG7 and PABY, corresponding to *SRY* gene and pseudoautosomal boundary regions respectively, failed to generate Y-specific amplicons. However, primers DYZ3 and DYS1, representing centromeric heterochromatic and euchromatic regions respectively, on the long arm of the Y chromosome uncovered Y-specific signals in the patient and her mother. Sequence analysis of DYS1-specific (710 bp) amplicon from the patient, both of her parents and two normal males showed insertion/deletion mutation in the patient. It is inferred that the Y-derived marker chromosome in the patient is of maternal origin and had undergone post-zygotic mutational events. The possible prognostic implications of this combined approach in the patient(s) with stigmata of Turner syndrome are discussed here.

A normal human female is homogametic with two X-chromosomes and the male is heterogametic with XY chromosomes. An imbalance in the sex chromosome constitution causes several genetic anomalies, including Turner syndrome. Turner's patients often have mosaic karyotype with second cell lines carrying numerical or structural aberrations of the sex chromosome[1]. A sizable body of literature is available on the aberrant Y chromosome or its complete absence in the Turner patients[2–6]. In several studies, post-zygotic mutational events and loss of the Y chromosome heterochromatin (Yqh) have been reported[7,8]. It has been suggested that Yqh plays a role in immune response during early embryonic development and control of normal male development[9]. Further, its deletion has been associated with spontaneous abortion[10] and Noonan's syndrome[11,12]. The human Y chromosome is known to carry relatively few functional genes and thus far, only 33 genes have been reported[13]. Also, more than 95% of the Y chromosome does not undergo recombination during meiosis and maintains its structural integrity. Majority of repeat elements associated with Yq region are distributed evenly along its entire length[14,15]. Thus, the quest for functional prioritization of Y-linked loci and phenotypic effects of their loss or gain in patients with stigmata of Turner syndrome, with or without marker chromosome is of relevance. In this study, we present results of our analysis on one such marker chromosome in a phenotypic female patient (HK-459) with stigmata of Turner syndrome, analysing five different loci (PABY, SRY, DYZ3, DYS1 and DYZ1) encompassing both the arms of the Y chromosome[6].

In view of the imperfect Turner features and seemingly normal parentage, we studied chromosomal and hormonal profiles of the patient. For ascertaining the presence of DYZ1 locus, Southern blot hybridization was conducted on DNA samples of the patient (HK-459), her parents and normal individuals of both sexes using synthetic oligonucleotide (OAT20Y)[16] and cloned (pSA1F) probes[6]. Remaining four loci, PABY, SRY[17,18], DYZ3 (refs 19, 20) and DYS1 (ref. 21) were analysed using PCR approach. Blood samples from all the individuals listed above were obtained with their informed consent. Ethical guidelines of the Guru Nanak Dev University, Amritsar were strictly followed during sample collection.

Chromosome preparation and G-banding were carried out following established protocols[16]. Karyotyping was done using Zeiss microscope fitted with automatic karyotyping system (Karyotec 2000, Amgenics, Israel).

DNA from the blood was isolated following standard protocols[22]. Approximately 0.5 µg of genomic DNA from each of the above-mentioned subjects was used for digestion with *Hae*III, and *Rsa*I enzymes in 20 µl independent reactions following supplier's specifications (New England Biolabs, USA). Digested DNA was electrophoresed on a 20 cm long, 1.5% agarose gel in 0.5 X TBE buffer (pH 8.2). The DNA was transferred onto the nylon membrane[23] and UV-fixed using Stratalinker (Stratagene, USA). Blot hybridization with cloned and synthetic oligo probes representing DYZ1 locus and autoradiography were conducted following standard protocols[16,24].

For assessing hormonal profiles, standard protocols were followed[16].

A set of oligo primers for the loci mentioned earlier was used for amplification of genomic DNA from two

*For correspondence. (e-mail: sherali@nii.res.in)

Table 1. Y chromosome-related primers representing different loci used for PCR amplification

Loci	Set of primers	Annealing temperature (°C)	Amplicon size (bp)	Reference
SRY	(i) 5′GGTCAAGCGACCCATGAAYGCNTT 3′ (ii) 5′GGTCGATACTTATAGTTCGGGTAYTT 3′	55	231	29
PABY	(i) 5′GTACTACCTTTAGAAAACTAGTATTTTCCC3′ (ii) 5′GAATTCTTAACAGGACCCATTTAGGATTAA3′	54	970	19
DYZ3	(i) 5′ATGATAGAAACGGAAATATG3′ (ii) 5′AGTAGAATGCAAAGGGCTCC3′	54	120	19
DYS1	(i) 5′ AATAGAGCCTTATCAGCAGA3′ (ii) 5′ AGTCAGTCTGGATGTTTCAG3′	54	710	19

(i) and (ii) represent forward (5′) and reverse (3′) primer sequences respectively.
Reproducibility of the result was confirmed by conducting PCR amplification at least thrice.

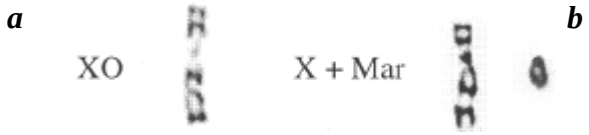

Figure 1. G-banded partial metaphases from the patient showing XO (***a***) and X,+mar chromosomes (***b***).

normal males and females, the patient (HK-459) and both her parents, following standard protocols[25]. Details about these primers, their annealing temperatures, size of the expected amplicons and their corresponding references are given in Table 1.

The 710 bp band(s) amplified using DYS1-specific-primers and DNA samples from two normal males, the patient and both her parents were resolved on the agarose gel and processed independently for purification and cloned in pGEM-T easy vector (Stratagene Inc., USA) following manufacturer's instructions. From the resultant clones, one each of all the samples – patient's mother (pAS1), patient (pAS2), patient's father (pAS3) and two normal males (pAS4) and (pAS5) respectively, was fully sequenced using Sequenase kit (Amersham, version 2.0) following standard method[26]. The sequences were deposited in GenBank with the following accession numbers: pAS1-AJ344999; pAS2-AJ345000; pAS3-AJ344998; pAS4-AJ344996; pAS5-AJ344997.

Multiple alignment of 710 bp sequences from all the clones (pAS1–5) was conducted on ebi server (http://www2.ebi.ac.uk) using Clustal W program and default parameters to uncover possible gross sequence modulation.

The patient suffering from primary amenorrhea was born following full-term, uneventful pregnancy and normal vaginal delivery. Although face appearance and carrying angle were normal, the body build was short, but proportionate. No webbing of the neck was detected. During the onset of puberty, breast development and menstruation was induced following hormonal treatment. Ultrasound analysis showed normal kidneys with respect to size, shape, outline and echo texture. The external genitalia were normal, but pubic hair showed sparse growth. The uterus was small and infantile and ovaries were not detected.

Hormonal profile showed an abnormal level of E2 (0.01 pg/mg), which was less than that observed in children (0.12–0.04 pg/mg). The FSH was found to be 52.33 mIU/ml, which is equivalent to the levels (28–130 mIU/ml) of a post-menopausal female. LH showed a value of 23.37 mIU/ml, which corresponds to that of a female (25–64 mIU/ml) during the ovulation phase of her cycle.

A total of 90 G-banded metaphases was analysed. Both the parents were normal with respect to their chromosome constitutions, but the patient (HK-459) showed mosaicism, 45,X/46,X,+mar chromosomes. Nearly 50% of the metaphases showed 45,X chromosome constitution (Figure 1 *a*) and the remaining had a small chromosome (46,X,+mar) without its homologue. This was referred to as the marker chromosome (Figure 1 *b*).

As mentioned earlier, DYZ1 locus was analysed using synthetic oligo and cloned probes that revealed a 3.4 kb band with *Hae*III-digested DNA samples in normal males[16,27]. The remaining Y chromosome-linked loci (PABY, SRY, DYZ3 and DYS1), shown tentatively in the schematic presentation (Figure 2) were assessed for their presence by employing PCR approach.

Southern hybridization showed that the patient and her mother were both negative for heterochromatic region DYZ1 with oligo probe OAT20Y[27] and genome-derived cloned probe pSA1F[6]. The male-specific 3.4 kb band was observed only in normal male and the patient's father with OAT20Y probe (Figure 3 *a*). The cloned probe pSA1F with *Hae*III enzyme also showed male-specific bands in the patient's father and normal males but not in the patient DNA or that of her mother (not shown). Similarly, typing of DNA samples with *Rsa*I enzyme and pSA1F clone showed several discernible bands specific to normal males and the patient's father, which were absent in the patient, her mother and normal females (Figure 3 *b* arrow heads). Thus, DYZ1 locus in the patient was not detected.

Following PCR amplification with SRY-related primers RG4 and RG7, a 231 bp band of expected size was observed in normal males and the patient's father, whereas the same was absent in normal females, the patient and her mother (Figure 4 *a*). Similarly, primers specific to PABY region produced a 970 bp band in normal males and the patient's father but not in the patient, her mother and normal female DNA samples (Figure 4 *b*). Thus, both the patient and her mother were negative for SRY and PABY loci. On the other hand, primers corresponding to DYZ3 and DYS1 loci of the Y chromosome uncovered 120 bp and 710 bp bands respectively, in the patient, both her parents and normal males (Figure 4 *c*, *d*) that are absent in normal females. Thus, both the patient and her mother were positive for DYZ3 and DYS1 loci of the Y chromosome.

Sequencing of the recombinant clones specific to DYS1 locus representing pAS1 (patient's mother), pAS2 (the patient), pAS3 (patient's father), pAS4 and pAS5 (two normal males) was conducted. A comparison of the autoradiogram showed marked deletion/addition (mutation) in the range of nt 172–225 in the patient but not in other samples, in addition to several point mutations (Figure 5). Multiple sequence alignment of these clones using Clustal W (http://www2.ebi.ac.uk) program showed insertion of the 33 nucleotides (5′CAG TCG AAG TTA TTT CTT ATG CCC ACC AGT CAG 3′) within the region 588–625 nt and deletion of 44 nucleotides (5′CCT GGT GAA TGT GGA TAA GCA GAG TAA GCC TGA AAA TAA AGT TT 3′) within the region 361–440 nt in the patient (Figure 6). A detailed search on the origin of these sequences was conducted using default server http://www.ncbi.nlm.nih.gov./BLAST. No homology of the inserted sequences was found with any of the entries in GenBank and thus the origin of these sequences remained unclear.

The homozygous (XX) or heterozygous (XY) sex chromosome constitution is essential for survival of the conceptuses during early embryogenesis and subsequent normal development[28]. Patients with 45,X/46,X,+mar or other mosaic conditions survive owing to the presence of critical sequences on the homologous loci. However, due to haploinsufficiency, their ensuing post-natal normal

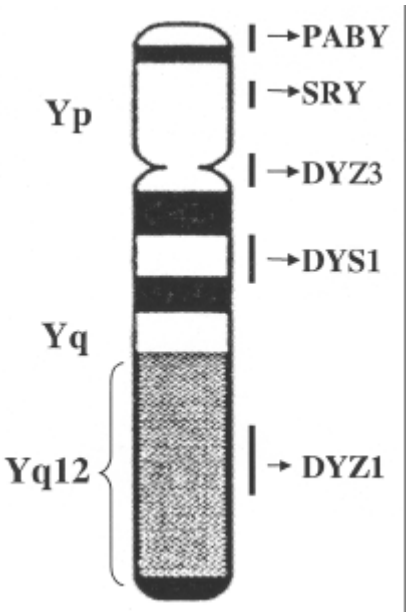

Figure 2. Y chromosome map (partial) showing tentative positions of the five loci analysed in the present study (see text for details).

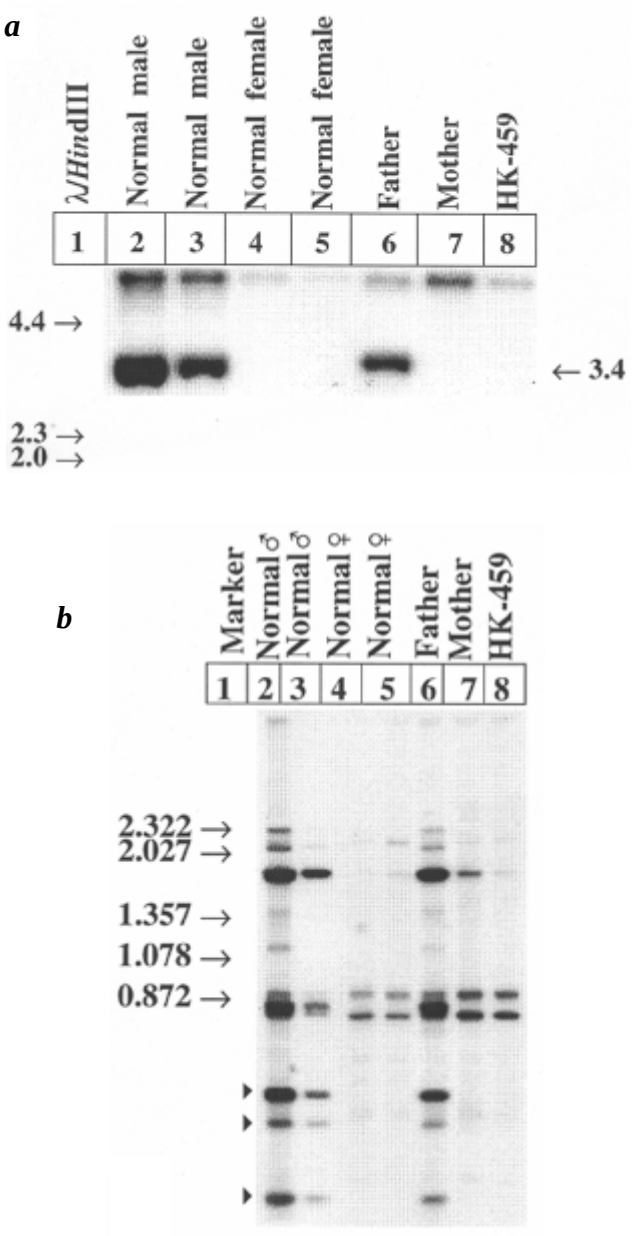

Figure 3. Typing of genomic DNA from normal individuals, patient and her parents with *Hae*III enzyme and oligo probe OAT20Y (***a***) and *Rsa*I enzyme and cloned probe pSA1F (***b***). Lane 1 has molecular size marker in kb. Note the absence of Y-specific band(s) in the patient, her mother and normal females and the presence of the same in the father and normal males.

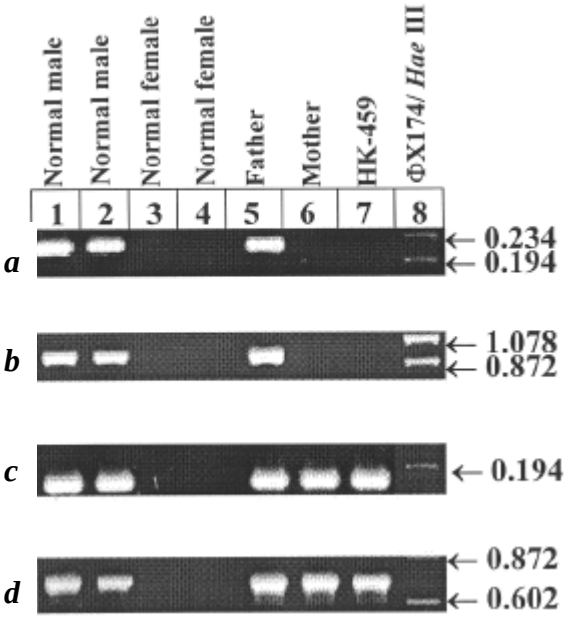

Figure 4. PCR amplification of genomic DNA from normal individuals, patient and her parents with different sets of defined primers related to: ***a***, *SRY*; ***b***, PABY; ***c***, DYZ3 and ***d***, DYS1 loci. Male-specific signals corresponding to DYZ3 and DYS1 loci in the patient and her mother are shown in panels ***c*** and ***d*** respectively.

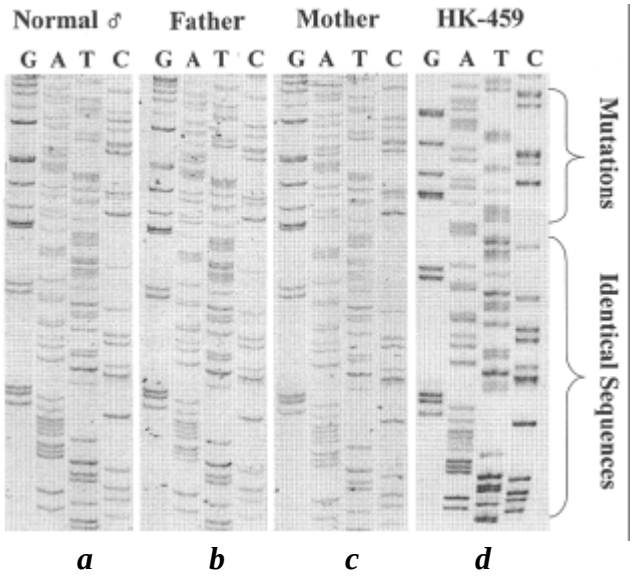

Figure 5. Comparative autoradiograms of the DYS1 locus (partial sequence) showing mutational region in the patient (HK-459) compared to other samples (see text for details).

development and eventual fertility status are seriously affected. The Y-derived marker chromosome inherited from the mother seems to contribute towards genetic imbalance, though this alone may not be responsible for haploinsufficiency. This is due to the fact that only about 50% of the cells in the proband had X chromosome. The present study seems to be a rare one since no such gross deletion/insertion in the DYS1 region has been reported earlier. The indels reported herein may serve as a reference point for comparison of the possible sequence modulation within this region in subsequent studies on more number of samples.

The marker chromosome analysed in the patient was positive for DYZ3 and DYS1 loci, which were also observed in her mother. How then was the mother normal is not clear at this stage. From the data, it is inferred that owing to non-disjunction of the X chromosome during oogenesis in mother, the patient inherited normal X from her father and the marker chromosome from her mother. Further, during post-zygotic sequence modulation, marked deletion/insertion within the DYS1 region took place in the patient. In addition, perhaps several other important loci were also affected resulting in Turner syndrome in the proband. Although ultrasonography did not detect ovaries or any kind of streak gonads, partial gonadal dysgenesis in the patient may not be ruled out because in the absence of *SRY* gene, streak gonads are observed. Although it is difficult to ascertain the parental origin of the marker chromosome unequivocally, it is more likely that the same originated from the mother since she was positive for DYS1 and DYZ3 loci. If the maternal origin of the marker chromosome is assumed, it involved only insertion/deletion of the sequences in the DYS1 locus. However, if the marker chromosome is construed to be of paternal origin, this would have involved a great deal of post-zygotic mutational events leading to a sizable part of the deletion of the father's Y chromosome followed by insertion/deletion of the sequences. This seems less likely, also because during chromosome analysis, remarkably, none of the cells in the patient had the father's (normal) Y chromosome. Thus, marker chromosome seems to be of maternal origin.

In Turner cases, due to mosaicism or gross aberration of the Y chromosome, the latter may be under-represented and escape detection during routine karyotyping. A combined molecular and cytogenetic approach to trace the origin of such chromosomes is of diagnostic relevance. In the present study, marker chromosome representing an aberrant Y has retained the centromeric (DYZ3) and euchromatic (DYS1) sequences. In the absence of *SRY* and other regulatory sequences, one may infer that the development of male gonads will be suppressed. This, in turn, would also exclude the possibility of its neoplastic development. However, with the Yp deletion resulting in the loss of *SRY* gene, gonadoblastoma may still occur because genes responsible are putatively located in the pericentromeric regions of the Y chromosome[4]. In this context, analysis of more number of Y-derived marker chromosome(s) would enable to ascertain preferential involvement of any of the above-mentioned loci in the Turner cases. The present study complements routine diagnosis of the aberrant Y or its derived marker

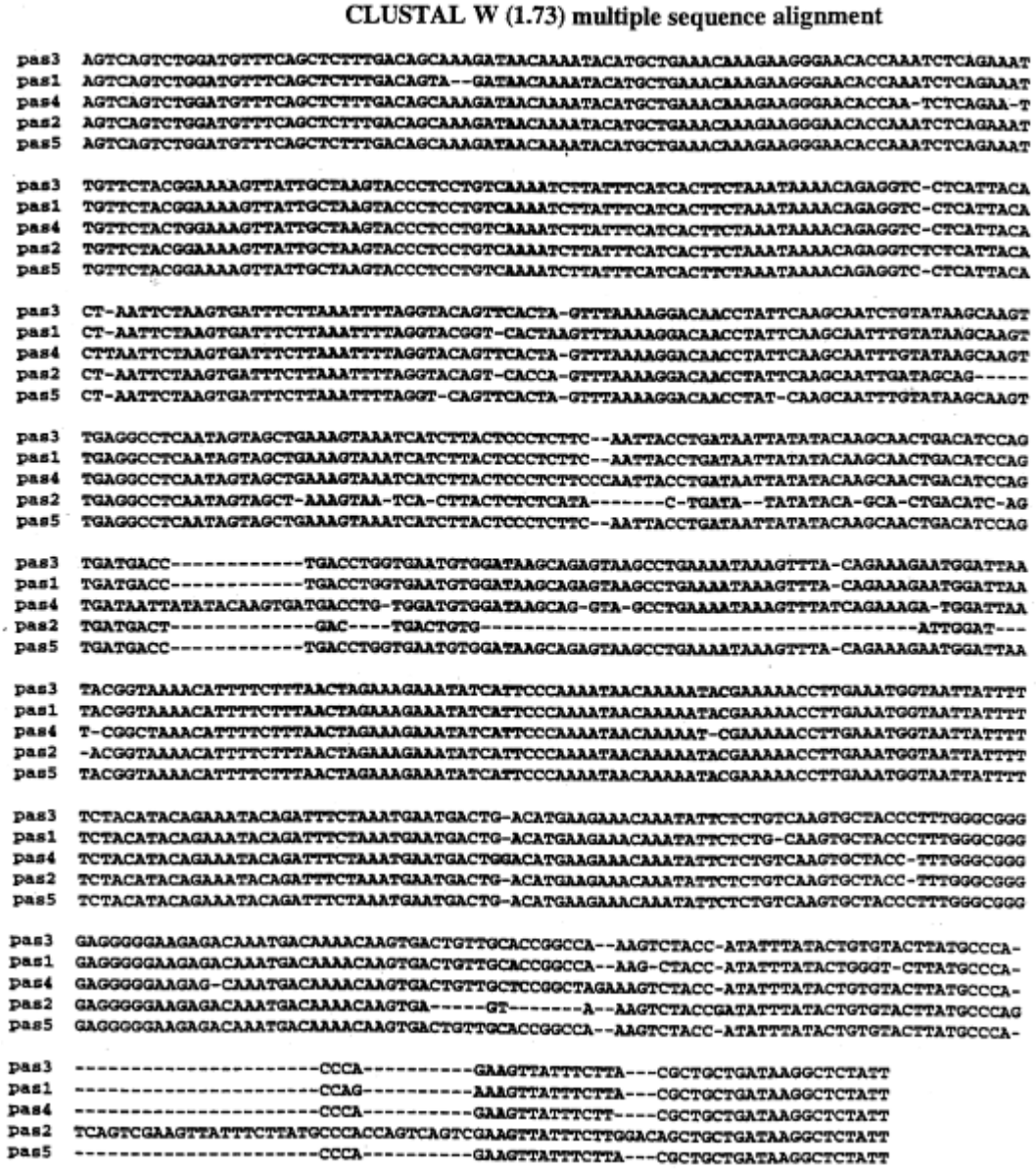

Figure 6. Multiple alignment of recombinant clones representing subset sequences of DYS1 locus from two normal males, patient and both her parents using Clustal W software. Clones pas denote: 1, Mother; 2, Patient; 3, Father; 4 and 5, Normal males. Deletion/addition of sequences in patient is shown compared to other samples (see text for details).

chromosome, facilitating genetic counselling and clinical management of the patient with stigmata of Turner syndrome.

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*For correspondence. (e-mail: ngrigravity@yahoo.com)