length determinations using the Genescan software on an ABI 373A automatic sequencer. The procedure contains a PCR with one primer labelled with fluorescence dye.

For the statistical comparison of the distributions of CAG copies for the three samples, the p value from the exact procedure for contingency tables was calculated using StatXact's Monte Carlo approach with 1 000 000 replications. Thus the width of the 99% confidence interval for the p value could be reduced to 0·001 indicating that the p value given is exact at least to the second decimal.

The distribution of repeat sizes in the DRPLA gene in 47 male and female controls (94 chromosomes) varies between seven and 25 CAG copies with a maximum for $[CAG]_{15}$ in 29% of the chromosomes. In two cases (4%) homozygosity has been observed.

In 35 patients with questionable diagnoses of SCA1 without expansions in the ataxin gene, seven to 21 CAG copies in the DRPLA gene have been found, including 26 of 70 alleles with $[CAG]_{15}$ (37%). No significant differences in the trinucleotide repeat frequency of the DRPLA gene between controls and patients with ataxia could be ascertained. For seven patients only one allele could be amplified, indicating 20% homozygosity. This result may be explained by the small number of cases. Insufficient amplification of an expanded repeat appears improbable since DNA analyses of two affected persons, kindly provided by T Warner and A Harding, London, have been successful.

Investigating the DNA of 73 patients suspected of having HD but negative for the mutation in the IT15 gene, alleles ranging from $[CAG]_{7-23}$ in the DRPLA gene have been identified. Two homozygous DNA samples (3%) were present. Surprisingly, in this collection the second most frequent allele was $[CAG]_{10}$, the most frequent being $[CAG]_{15}$ (30% of chromosomes). Twenty-six of the 146 chromosomes investigated (18%) contain 10 CAG copies representing about 36% of the patients with symptoms of HD. Controls and patients shared a common ethnic background and, therefore, differences between populations[5] cannot account for this result. Furthermore, the allele distribution in our controls corresponds to data of the white population. Surprisingly, the allele $(CAG)_{10}$ is overrepresented in the Japanese population, too.[5]

Allele distributions in the DRPLA gene in the three groups are summarised in the figure. Neither in our patients with chorea nor in patients with symptoms of ataxia could an expansion of the CAG repeat on chromosome 12, to explain the affected status, be found. Our data confirm the low frequency of the DRPLA mutation in the European population. Interestingly, the incidence of allele $[CAG]_{10}$ is significantly increased in patients with a questionable diagnosis of HD. The exact p value for the comparison of the three distributions of CAG copies is calculated as $p=0{\cdot}031$ which indicates a significant discrepancy between the samples. This result may be because of linkage disequilibrium between the disease and a mutation on chromosome 12 or, possibly, a further gene causing a progressive neurodegenerative disorder is located in the DRPLA region. Extended analyses with polymorphic markers are necessary to define a high risk haplotype for this genetically uncharacterised disease. Investigations of pedigrees with affected persons with the allele $[CAG]_{10}$ may detect the presence of a gene responsible on chromosome 12.

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1 Koide R, Ikeuchi T, Onodera O, *et al.* Unstable expansion of CAG repeat in hereditary dentatorubral-pallidoluysian atrophy (DRPLA). *Nature Genet* 1994;**6**:9–13.
2 Nagafuchi S, Yanagisawa H, Sato K, *et al.* Dentatorubral and pallidoluysian atrophy: expansion of an unstable CAG trinucleotide on chromosome 12p. *Nature Genet* 1994;**6**:14–18.
3 The Huntington's Disease Collaborative Research Group. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. *Cell* 1993;**72**:971–83.
4 Orr HT, Chung M, Banfi S, *et al.* Expansion of an unstable trinucleotide CAG repeat in spinocerebellar ataxia type 1. *Nature Genet* 1993;**4**:221–6.
5 Burke JR, Ikeuchi T, Koide R, *et al.* Dentatorubral-pallidoluysian atrophy and Haw River syndrome. *Lancet* 1994;**344**:1711–12.

Detection of Y mosaicism in patients with Turner's syndrome

The very high intrauterine loss rate in Turner's syndrome and the observation that there is a higher ratio of mosaic karyotypes to monosomy X in liveborns compared with aborted fetuses has led to the speculation that all liveborn patients are mosaic for a critical cell line.[1]

Experimental evidence has shown that 70 to 80% of patients with Turner's syndrome retain the maternal X chromosome.[2] Therefore there is the theoretical possibility of Y mosaicism occurring in 35 to 40% of Turner's syndrome patients.[3] This has not been shown in cytogenetic studies where only 5% of cases have Y mosaicism[4] and another 3% have unidentified markers. However, low level mosaicism may be missed by conventional cytogenetic techniques and over 100 cells would need to be examined to exclude 5% mosaicism with 99% confidence.[5] This fact is also a limitation in the use of FISH which has an established role in the identification of fragments.[6]

Identification of Y chromosomal mosaicism is of clinical relevance because of the high risk of tumour formation in the dysgenetic gonads of patients with entire or structurally abnormal Y chromosomes present, which may be as high as 30%.[7] Page[8,9] has hypothesised the presence of a "gonadoblastoma" locus which is probably located in interval 4b–5.

Several studies on relatively small numbers of patients have investigated Y mosaicism in Turner's syndrome using Southern blotting with Y specific probes,[3] PCR with one or two Y primer sets,[10,11] and even Southern blotting of amplified DNA[12] and have suggested that occult Y mosaicism occurs in 0 to 33% of patients. In view of this lack of consensus, we have used PCR with a panel of Y primers from the whole length of the Y chromosome and Southern blotting of amplified DNA in a large unselected cohort of patients with Turner's syndrome to determine the frequency of occult Y mosaicism.

As part of a total ascertainment study, 100 patients with Turner's syndrome were recruited from the four growth clinics and from adult Turner's syndrome clinics in Scotland. The patients had the following karyotypes: 45,X in 48, 45,X/46,XiXq in 14, 45,X/46,Xr(X) in 10, 45,X/46,XX in seven, 45,X/46,XY in three, 45,X/46,Xi(Yp) in one, 45,X/46,X,+M in nine, and other karyotypes in eight.

DNA was extracted from peripheral lymphocytes of all patients by the same normal female (CEC, karyotype 46,XX) and PCR was carried out with nine sets of primers from the Y chromosome: PABY from the pseudoautosomal region which amplifies X and Y specific products of different sizes,[13] SRY1,[14] amelogenin[15] which amplifies X and Y specific products with a size difference, Y centromere,[16] sY85,[17] sY117,[17] sY146,[17] and sY160,[17] Y1.1.[18] An additional primer set, sY159,[17] was used in patients who were positive with Y1.1. sY146 and sY160 were amplified in a multiplex reaction. All PCR products were resolved on 1–2% ethidium bromide stained agarose gels with both normal male and normal female controls on every gel.

Southern blotting of amplified DNA was also carried out on 84 of the patients who had been shown to be negative on direct visualisation of PCR products. Three primer sets were used initially, SRY1, Y centromere, and sY85, and a fourth XES10/11[19] from the SRY gene was used on positive cases. Great care was taken to prevent contamination either by PCR products or genomic DNA when setting up the PCR reaction or running gels. All the experiments were carried out by the same female (46,XX karyotype). Separate pipettes were used for setting up the PCR and running gels, autoclaved solutions were used throughout, and all solutions were subjected to UV light for 10 minutes before use. The PCR products were run out in a separate laboratory one floor below that used for setting up the PCR. All doubtful cases were repeated and in two cases DNA was extracted from fresh blood samples. In five other cases different DNA samples were used.

Forty-six normal females were also screened using the SRY1 primers. In 40 of these the DNA was extracted by female laboratory staff and in six by a male scientist.

Probes used for the Southern blotting were gene cleaned PCR products amplified from normal males. These were labelled with ^{32}P dCTP by the random labelled method.

Parental origin of the normal X chromosome was determined either by use of Southern blotting with probe M27β or by PCR with primers from a repeat sequence in the androgen receptor gene, as previously described.[20] Concurrent analysis of X inactivation patterns in patient 9 assisted in the identification of the normal X chromosome.

Nine patients were positive for one or more Y regions on direct visualisation of ethidium bromide stained gels with the results shown in table 1 along with the deletion interval of the primers used. Three of these were already known to have a Y chromosome cytogenetically and were positive for all regions tested (patients 3, 4, and 5, table 1). One of these subjects (patient 3) had a prophylactic

Table 1 Turner's syndrome patients positive for Y sequences

		Y chromosome interval									
		1		3	4b	5		6	7		
Pt	Original karyotype	PABY	SRY	AMEL	CEN	85	117	146	160	Y1.1	159
1	45,X/46,X+M	+	+	+	+	+	+	+	+	+	+
2	45,X/46,X+M	−	−	−	+	+	+	+	+	+	+
3	45,X/46,XY	+	+	+	+	+	+	+	+	+	+
4	45,X/46,XY	+	+	+	+	+	+	+	+	+	+
5	45,X/46,XY	+	+	+	+	+	+	+	+	+	+
6	45,X/46,Xi(Yp)	+	+	+	+	+	+	+	−	−	−
7	45,X	−	−	−	+	+	+	+	+	+	+
8	45,X	−	−	−	−	−	−	−	−	+	+
9	45,X/46,Xi(Xq)	−	−	−	−	−	−	−	−	+	+

gonadectomy at the age of 6 years and was found to have a gonadoblastoma in the dysgenetic gonads.

Patient 1 presented at the age of 17 years with primary amenorrhoea and was found to have a 45,X line and also a second cell line containing a small unidentifiable marker. She had no features of virilisation. She was positive for all primer sets tested as were the gonads which were removed after the identification of the Y material.

Patient 2 had a 45,X/46,X,+M karyotype. She had no virilisation features and no secondary sexual characteristics. Gonadectomy was carried out and no evidence of gonadoblastoma was found in the streak gonads.

Patient 6 had a 45,X/46,Xi(Yp) karyotype and presented at birth with ambiguous genitalia. Prophylactic gonadectomy was performed at the age of 1 year and a testis was identified on the right and a streak gonad on the left with normal female internal organs. There was no trace of tumour found in the gonads. On PCR testing this patient had positive Y sequences down to and including sY146 (interval 6).

Patient 7 was originally thought to have a 45,X karyotype. She had no signs of virilisation. The karyotype was reviewed in the light of the PCR result and a total of 95 cells were then counted before an i(Yq) was found in 10% of cells. FISH analysis was also carried out but again was negative until the third review of the slides. She underwent gonadectomy at the age of 9 years and no evidence of tumour was found on histology.

Patient 8 was reported to have a 45,X karyotype and had the features of Turner's syndrome with no additional features of note. She developed a primary lung carcinoma with cerebral metastases at the age of 16 years and died two years later. She had no evidence of ovarian pathology on CT scan. She had only distal Yq sequences present on PCR analysis.

Patient 9 had a 45,X/46,Xi(Xq) karyotype and has features of Turner's syndrome but had spontaneous puberty. She is positive for distal Yq sequences.

After the finding of distal Yq sequences in patients 8 and 9, DNA from the mothers of both these patients was analysed for Y sequences. The mother of patient 8 was also positive for the same distal Yq sequences but not any other Y sequences and the mother of patient 9 was negative for all sequences tested.

Using the method of Southern blotting of amplified DNA, no patients were consistently positive for either sY85 or Y centromere sequences. Seventeen (17/84, 20%) Turner patients were positive for SRY1 sequences. The signals obtained varied in intensity and some were only visible after overnight exposure. Fourteen of the normal females (14/46, 30%) also showed positive signals using this primer set and the signals also varied in intensity. Only one of the samples extracted by a male scientist showed a positive signal.

A separate experiment using XES10/11 primers was then carried out for those patients and controls positive with the SRY1 primers. This set of primers is within the coding region for SRY but both primers are outside the HMG box. None of the samples showed positive signals. Serial dilution of male DNA showed that this method was able to detect 0·0001 μg male DNA.

Parental blood was available from all patients except patient 2 and the normal X chromosome was found to be maternal in origin for all the other patients.

We have shown that 9% (9/100) of Turner's syndrome patients from a large unselected group have Y mosaicism, fewer than half of whom were identified by cytogenetic analysis. If patients with known Y material and unidentified markers are excluded from the analyses, 3·4% (3/87) of patients in this cohort were found to have unsuspected Y mosaicism. This is a slightly higher figure than that found in other studies to date (table 2) and probably reflects the fact that in this study multiple primer sets were used.

The present study highlights several different points. Firstly PCR can detect low level mosaicism missed by conventional cytogenetic analysis of relatively large numbers of cells, as in patient 7. Secondly, more than one area of the Y chromosome should be screened. Many of the previous studies have used SRY primers and the use of such primers alone would have missed four of our nine positive cases (patients 2, 7, 8, and 9). Also by using multiple primer sets we were able to define the content of the Y isochromosome in patient 6. This patient may have been thought to be at low risk of tumour formation as she presumably has mainly short arm material (i(Yp)). However, using PCR it has been shown that a significant amount of long arm material is present in the isochromosome including the at risk area of interval 5 (table 1). Thirdly, Y material may be present even in patients with other mosaic karyotypes (patient 9, table 1). This confirms findings which have been noted in two other patients in different reports. Medlej *et al*[23] found SRY sequences on PCR with direct visualisation in a patient with a 45,X/46,XX karyotype in streak ovaries and Rosamund *et al*[24] described a patient with an XY translocation seen on cytogenetic analysis and a 45,X/46,Xi(Xq)/46,XX karyotype. The significance of the Y material detected in patients 8 and 9 is uncertain as it probably represents only distal Yq sequences. The finding of these distal sequences was confirmed by using a second primer set (sY159) from the same region. We have so far been unable to ascertain the location of these sequences using FISH but this may be because the sequences are beyond the limit of resolution of this technique. An explanation for the Y long arm sequences present in these patients may be that although it is generally supposed that pairing of the X and Y chromosomes during meiosis only involves the distal third of the X short arm and almost all the Y short arm up to the centromere, studies of the XY bivalent by electron microscopy have shown that it may in fact be much more extensive.[25] This hypothesis is supported by the fact that the mother of patient 8 was also positive for the distal Yq sequences thus indicating that these may represent a familial translocation.

Although the repetitive sequences DYZ2 have been thought to map to the heterochromatic region only and thus represent genetically inert material, Young *et al*[26] found these sequences in the non-fluorescent band Yq11 so that they may have some effect. At present, patient 9 is being observed and gonadectomy has not been suggested because she has functioning ovaries. Unfortunately patient 8 has died but had no evidence of gonadoblastoma at the time of death.

Direct visualisation of PCR products is rapid and sensitive, being capable of detecting 0·01% male DNA on a female DNA background.[11] Kocova *et al*[12] proposed an alternative strategy involving Southern blotting of amplified PCR products and found a surprisingly high incidence of patients positive for SRY sequences (6/18). In our study a similar number (17/84, 20%) of patients were

Table 2 Summary of studies looking for Y mosaicism in liveborn patients with Turner's syndrome

Study	Approach	No tested	No +ve(%)
Ostrer and Clayton[3]	SB, 2 Y probes	11	0
Jacobs *et al*[21]	SB, 2 Y probes	31	0
Tho *et al*[22]	SB, 3 Y probes	7	0
Lorda-Sanchez *et al*[2]	SB, 1 Y probe	40	1 (2·5%)
Medlej *et al*[23]	PCR, 1 Y locus (SRY)	40	1 (2·5%)
Witt *et al*[10]	PCR, 2 Y loci (SRY and Y cen)	18	0
Yankowitz *et al*[11]	PCR, 2 Y loci (SRY and Yq)	43	0
Kocova *et al*[12]	PCR with SB, 2 Y loci (SRY and Y cen)	18	6 (33·3%)
Present study	PCR, 9 Y loci	87	3 (3·4%)

SB = Southern blotting, cen = centromere.

positive with one set of SRY primers (SRY1) but all were negative for Y centromere and sY85 sequences and a similar percentage of normal female controls (14/46, 30%) were also positive using this method of analysis. Southern blotting of amplified DNA is extremely sensitive and very prone to contamination; however, great care was taken during all our experiments to prevent contamination. The positive results cannot be the result of contamination by genomic DNA as only one locus was affected and positive cases were retested on different DNA samples. Contamination by PCR products is also extremely unlikely, firstly because different pipettes were used to set up the PCR from those used to run out the products, the laboratory used to run out products was situated on a different floor from that used to set up the experiment, and, most importantly, the intensity of the positive signals seen in both the patients and controls varied from person to person.

The explanation for the positive signals in these patients and controls may be that the PCR primers used by Kocova *et al*[12] and ourselves amplify a 270 bp sequence representing a DNA binding motif known as the high mobility group box (HMG box). This motif shares homology with a DNA binding motif present in non-histone proteins related to HMG1 and HMG2[27] and a DNA binding domain of hUBF (human upstream binding factor)[27] and possibly other regions of the genome as well. It is possible that the signals detected in Turner's syndrome patients and normal female controls represent amplification of partially homologous sequences from these other regions. This theory is supported by our finding that patients and controls previously positive for SRY1 were negative using primer sets XES10/11 which are still within the coding sequence for SRY but are located well outside the HMG box. These patients were negative in spite of the sensitivity of this particular assay which detects down to 0·0001 µg of male DNA. Southern blotting of amplified DNA did not identify any patients not previously picked up using direct visualisation of PCR products and we would therefore not advocate its routine use. However, it may be useful in confirming ambiguous results if great care is taken in the experimental methodology.

The clinical significance of occult Y mosaicism is unknown but in our experience PCR (without Southern blotting of amplified DNA) is more effective than routine cytogenetic analysis for its detection and may be further enhanced by screening multiple tissues.

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1 Hook EB, Warburton D. The distribution of chromosomal genotypes associated with Turner's syndrome: livebirth prevalence rates and evidence for diminished fetal mortality and severity in genotypes associated with structural X abnormalities or mosaicism. *Hum Genet* 1983;**64**:24–7.
2 Lorda-Sanchez I, Binkert F, Maechler M, Schinzel A. Molecular study of 45,X conceptuses: correlation with clinical findings. *Am J Med Genet* 1992;**42**:487–90.
3 Ostrer H, Clayton CM. Y chromosome mosaicism in 45,X Turner syndrome. *Am J Med Genet* 1989;**34**:294–6.
4 Magenis RE, Breg WR, Clark KA, *et al*. Distribution of sex chromosome complements in 651 patients with Turner's syndrome. *Am J Med Genet* 1980;**32**:79A.
5 Hook EB. Exclusion of chromosomal mosaicism: tables of 90%, 95% and 99% confidence limits and comments on use. *Am J Med Genet* 1977;**29**:94–7.
6 Lindgren V, Chen C, Bryke CR, Lichter P, Page DC, Yang-Feng TL. Cytogenetic and molecular characterization of marker chromosomes in patients with mosaic 45,X karyotypes. *Hum Genet* 1992;**88**:393–8.
7 Verp MS, Simpson JL. Abnormal sexual differentiation and neoplasia. *Cancer Genet Cytogenet* 1987;**25**:191–218.
8 Page DC. Hypothesis: a Y chromosomal gene causes gonadoblastoma in dysgenetic gonads. *Development* 1987;**101**(suppl):151–5.
9 Page DC. Y chromosomal sequences in Turner's syndrome and risk of gonadoblastoma or virilisation. *Lancet* 1994;**343**:240.
10 Witt M, Michalczak K, Latos-Bielenska A, Jaruzelska J, Kuczora I, Lopez M. An improved, non-isotopic method of screening cells from patients with abnormalities of sexual differentiation for Y chromosomal DNA content. *J Med Genet* 1993;**30**:304–7.
11 Yankowitz J, Neely EK, Hajdu K, Norton ME, Rosenfeld RG, Golbus MS. Screening of Turner subjects by PCR of occult Y chromosomal DNA. *Am J Hum Genet* 1993;**53**: 1766A.
12 Kocova M, Siegal SF, Wenger SL, Lee PA, Trucco M. Detection of Y chromosomal sequences in Turner's syndrome by Southern blot analysis of amplified DNA. *Lancet* 1993; **342**:140–3.
13 Ellis N, Taylor A, Bengtsson BO, Kidd J, Rogers J, Goodfellow P. Population structure of the human pseudoautosomal boundary. *Nature* 1990;**344**:663–5.
14 Nakahori Y, Hamano K, Iwaya M, Nakagome Y. Sex identification by polymerase chain reaction using X-Y homologous primers. *Am J Med Genet* 1991;**39**:472–3.
15 Nakagome Y, Seki S, Fukutani K, Nagafuchi S, Nakahori Y, Tamura T. PCR detection of distal Y sequences in an XX true hermaphrodite. *Am J Med Genet* 1991;**41**:112–4.
16 Witt M, Erickson RP. A rapid method for detection of Y-chromosomal DNA from dried blood specimens by the polymerase chain reaction. *Hum Genet* 1989;**82**:271–4.
17 Vollrath D, Foote S, Hilton A, *et al*. The human Y chromosome: a 48-interval map based on naturally occurring deletions. *Science* 1992; **258**:52–9.
18 Kogan SC, Doherty M, Gitschier J. An improved method for prenatal diagnosis of genetic diseases by analysis of amplified DNA sequences. *N Engl J Med* 1987;**317**:985–90.
19 Hawkins JR, Taylor A, Berta P, Levilliers J, Van der Auwera B, Goodfellow PN. Mutational analysis of SRY: nonsense and missense mutations in XY sex reversal. *Hum Genet* 1992; **88**:471–4.
20 Chu CE, Donaldson MDC, Smail PJ, *et al*. Possible role of imprinting in the Turner phenotype. *J Med Genet* 1994;**31**:840–2.
21 Jacobs PA, Betts PR, Cockwell AE, *et al*. A cytogenetic and molecular reappraisal of a series of patients with Turner's syndrome. *Ann Hum Genet* 1990;**54**:209–23.
22 Tho SPT, Behzadian A, McDonough PG. Screening of seven putative 45X subjects with DNA probes to detect low mosaicism for Y cell lines. *Am J Obstet Gynecol* 1990;**162**:59–60.
23 Medlej R, Lobaccaro JM, Berta P, *et al*. Screening for Y-derived sex determining gene SRY in patients with Turner syndrome. *J Clin Endocrinol Metab* 1992;**75**:1289–92.
24 Rosamund Pinto M, Marques C, Magalhaes Faria R. X/Y translocation in a Turner syndrome. *Am J Hum Genet (Suppl)* 1991;**274**: 1514.
25 Chandley AC, Goetz P, Hargreaves TB, Joseph AM, Speed RM. On the nature and extent of XY pairing at meiotic prophase in man. *Cytogenet Cell Genet* 1984;**38**:241–7.
26 Young KE, Willard HF, Smith KD. Molecular and chromosomal organisation and analysis of DNA methylation of the human Y chromosome-specific 2·1-kb *Hae* III DNA fragment. *Am J Hum Genet* 1981;**33**:60A.
27 Sinclair AH, Berta P, Palmer MS, *et al*. A gene from the human sex-determining region encodes a protein with homology to a conserved DNA-binding motif. *Nature* 1990;**346**:240–4.
28 Jantzen HM, Admon A, Bell SP, Tjian R. Nucleolar transcription factor hUBF contains a DNA-binding motif with homology to HMG proteins. *Nature* 1990;**344**:830–6.