Xq–Yq interchange resulting in supernormal X-linked gene expression in severely retarded males with 46,XYq- karyotype

Bruce T. Lahn[1], Nancy Ma[2], W. Roy Breg[3], Robert Stratton[4], Urvashi Surti[5] & David C. Page[1]

The critical importance of dosage compensation is underscored by a novel human syndrome ("XY_{Xq} syndrome") in which we have detected partial X disomy, demonstrated supernormal gene expression resulting from the absence of X inactivation, and correlated this overexpression with its phenotypic consequences. Studies of three unrelated boys with 46,XYq- karyotypes and anomalous phenotypes (severe mental retardation, generalized hypotonia and microcephaly) show the presence of a small portion of distal Xq on the long arm of the Y derivative. Cells from these boys exhibit twice-normal activity of glucose-6-phosphate dehydrogenase, a representative Xq28 gene product. In all three cases, the presence of Xq DNA on a truncated Y chromosome resulted from an aberrant Xq–Yq interchange occurring in the father's germline.

[1]*Howard Hughes Research Laboratories at Whitehead Institute, and Department of Biology, Massachusetts Institute of Technology, 9 Cambridge Center, Cambridge, Massachusetts 02142, USA*
[2]*Department of Human and Molecular Genetics, Collaborative Research, Inc., Waltham, Massachusetts 02154, USA*
[3]*Departments of Genetics and Pediatrics, Yale University School of Medicine, New Haven, Connecticut 06510, USA*
[4]*South Texas Genetics Center, 7922 Ewing Halsell Drive, San Antonio, Texas 78229, USA*
[5]*Departments of Pathology and Genetics, Magee Women's Hospital, University of Pittsburgh, Pittsburgh, Pennsylvania 15213, USA*

Correspondence should be addressed to D.C.P.

It is widely accepted that X inactivation occurs in humans to ensure that X-linked gene expression in females is equal to rather than twice the level found in males. It follows, as many have argued, that failure to dosage-compensate X-linked genes could be the cause of certain anomalous human phenotypes[1–7]. If so, one should be able to demonstrate supernormal X-linked gene expression in association with these phenotypes. We have had an opportunity to test this prediction while exploring the origins of apparent terminal deletions of the Y chromosome long arm (that is, the 46,XYq- karyotype), which are among the most common chromosomal disorders in human populations.

At least one in 1,000 males lacks about half of the Y chromosome, including the quinacrine-bright, heterochromatic region[8]. The 46,XYq- karyotype can be associated with short stature and azoospermia[9,10], but more severe phenotypes have been reported, including profound mental retardation, hypotonia and dysmorphic features[11,12]. While exploring a possible genetic basis for the phenotypic variability observed in XYq- males, we have discovered that a subset of such males delineate a syndrome, which we refer to as XY_{Xq} syndrome. We show here, at the levels of gene expression and organismal phenotype, the consequences of failing to dosage-compensate X-linked genes that are present in two copies per cell. We have also discovered Xq–Yq counterparts to the aberrant Xp–Yp exchanges that have been so thoroughly studied in human XX males and XY females[13–19]. Finally, we consider the factors that may predispose Xq and Yq to recombine aberrantly in the paternal germline.

XYq– phenotypes and deletion breakpoints

Our study focused on ten males with 46,XYq- karyotypes. Samples from these individuals were received from various medical centers for Y chromosome analysis. All patients had been ascertained postnatally and karyotyped previously because of phenotypic abnormalities.

The phenotypes of these ten males varied dramatically (Table 1). Three individuals ("Group I") were severely mentally retarded, microcephalic boys with little or no ability to speak or comprehend words. They had very poor muscle tone, were unable to stand without assistance, and had suffered nonfebrile seizures. The three Group II individuals were school-age boys with mild to moderate learning disabilities and delays in speech and motor development. Both Group I and Group II boys had mild facial dysmorphism, and four of the six boys had undescended testes. The four Group III individuals had no history of delayed cognitive or motor development. The two youngest Group III males had mild facial dysmorphism. All ten individuals were short in stature, and the two adults (both Group III) were infertile (azoospermic).

All ten individuals lacked the distal long arm of the Y chromosome, as initially revealed by cytogenetic analyses conducted at the referring medical centers. Eight of the ten patients had been tested previously by PCR for the presence of specific Y-linked loci, resulting in localization of their Yq breakpoints[20]. We extended these studies by testing all ten individuals for 80 loci distributed across the euchromatic portion of the Y. The results allowed us to rule out interstitial deletions within the portions of the Y retained. The positions of the Yq breakpoints among these ten individuals are quite heterogeneous, with at least

eight distinct breakpoints detectable (Fig. 1).

Although the results of Y chromosome DNA analysis were consistent with simple terminal deletions of Yq in all ten cases, there were reasons to suspect that the chromosomal anomalies in at least some of these patients might be more complex. There was no correlation between the size of the deletion and the severity of the phenotype (Fig. 1). One of the smallest deletions (WHT2277) was found in a severely affected (Group I) patient whereas two of the largest deletions (LGL658 and WHT1157) were found in the mildly affected (Group III) individuals. This suggested that much of the phenotype could not be attributed to loss or disruption of specific Yq genes. Indeed, deletion of Y chromosomal genes would appear unlikely to cause severe mental retardation, hypotonia and microcephaly, as these traits are uncommon in 45,X individuals, who for purposes of this argument can be seen as having lost the entire Y chromosome. Also, it is probable that Yq- chromosomes would be stable only if the Yq telomere were retained or replaced by another telomere. Further, distal Yq and Xq form synaptonemal complexes and recombine during normal male meiosis[21,22], providing a possible opportunity for aberrant Xq–Yq recombination, perhaps producing what appears to be a Yq- chromosome. In this respect, the behaviour of distal Yq and Xq might resemble that of distal Yp and Xp, where aberrant, grossly misaligned X–Y recombination occasionally occurs, giving rise to XX males and XY females[13–19]. Of particular relevance here are the rare females whose karyotypes were originally described[23–25] as 46,XYp- (with terminal deletions of the short arm), but whose Y derivatives were subsequently found to be products of aberrant Xp–Yp interchange (ref. 18; D.C.P. *et al.*, unpublished results). By analogy, we speculated that

Table 1 Phenotypes of ten unrelated 46,XYq– males

	Patients	Age at last examination	Cognitive development	Motor development	Seizures	Height[a]	Other characteristics
Group I Severely affected	WHT1278	8 years	Severely retarded; unable to speak	Hypotonia w/ hyper-extensible joints; unable to stand without assistance	Partial complex seizures (confirmed by EEG)	119 cm (5–10%)	Microcephaly; prominent ears; hypoplastic midface; high, narrow palate; small feet; prominent keloids from surgery; undescended testes
	WHT1373	8 years	Severely retarded; unable to speak	Hypotonia; unable to stand without assistance	Repeated seizures	80 cm (<<2%)	Microcephaly; widely spaced eyes; beaked nose; high, narrow palate; small jaw; broad thumbs; undescended left testis; small right testis; repeated unexplained fevers
	WHT2277	5.5 years	Severely retarded; unable to speak	Hypotonia w/ hyper-extensible elbows; unable to stand without assistance	One prolonged grand mal seizure	100 cm (<5%)	Microcephaly with flat occiput; low posterior hairline; small upturned nose; narrow mouth; short neck; small feet; bilateral camptodactyly of 4th fingers; livedo reticularis
Group II Moderately affected	WHT1829	7.5 years	Mild but persistent speech delay; marked learning disability	Mild gross and fine motor clumsiness	None	119 cm (10–25%)	Prominent ears; unilateral amblyopia; small atrial septal defect
	WHT1832	10.5 years	Speech delay corrected by therapy	Mild fine motor clumsiness	None	125 cm (<5%)	Atypical facies with prominent low-set ears, partial ptosis; mild pulmonic stenosis; undescended testes
	WHT1876	10 years	Moderate speech and learning disabilities	Mild hypotonia and motor clumsiness	None	95 cm at 5 yrs (<5%) (before growth hormone treatment)	Nasal bridge slightly broadened; widely spaced nipples; undescended right testis
Group II Mildly affected	WHT1983	5.7 years	Normal	Normal	None	99 cm (<5%)	Microcephaly[b]; prominent ears; long eyelashes; bushy eyebrows w/mid-fusion; thin lips; small feet; hypospadias
	LGL658[c]	17 years	Normal	Normal	None	160 cm (<5%)	High-arched palate; small mandible; small teeth; mild hypothyroidism
	WHT1157	28 years	Normal	Normal	None	159 cm (<5%)	Azoospermia
	WHT2168	32 years	Normal	Normal	None	165 cm (5%)	Azoospermia

[a]Heights are expressed in cm and as age-adjusted percentiles.
[b]WHT1983's head circumference was proportionately greater than his height; given normal cognitive and motor development, his "microcephaly" may simply reflect a generalized growth delay.
[c]LGL658's clinical features are as reported by P. Salo, *et al.* (ref. 55)

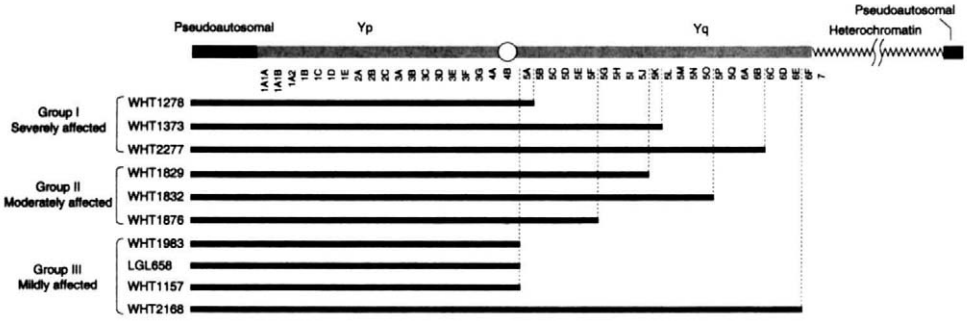


Fig. 1 Poor correlation of Yq breakpoint with phenotypic severity in ten unrelated XYq- males. Schematic representation of the Y chromosome, with Yp and Yq pseudoautosomal regions and heterochromatic region labelled. Immediately below are listed 43 deletion intervals (1A1A through 7), as defined[20]. The black bars below indicate the portions of the Y chromosome found to be present in the XYq- males by testing for 80 Y-specific STSs. Results for all patients except WHT1983 and WHT2277 were reported previously[20].

some XYq- males might be the result of grossly misaligned exchanges between Yq and Xq.

Aberrant Xq–Yq interchange

To test this possibility we typed all ten patients and, if available, their parents, for two genetic markers mapping near the Xq telomere. *DXS1108*, a strictly X-linked marker, lies about 60 kilobases (kb) proximal to the long-arm pseudoautosomal region[22,26]. *DXYS154* , a long-arm pseudoautosomal marker, is located about 140 kb from the Xq/Yq telomere[22,26].

In three patients the results suggested aberrant Xq–Yq interchange in the paternal germline. WHT1278, WHT1373 and WHT2277 exhibited two alleles at both loci, and in each case, comparison with parental genotypes revealed that one allele was maternally derived and the other paternally derived (Fig. 2). Thus, these XYq- males inherited not only one maternal X chromosome but also the distal long arm of the paternal X chromosome.

A fourth patient, WHT1829, inherited a paternal and a maternal allele for the pseudoautosomal marker *DXYS154* but only a maternal allele for the more proximal, strictly X-linked marker *DXS1108* (Fig. 2*b*). These findings suggest either an interstitial deletion on the Y chromosome or an aberrant Xq–Yq interchange with the X breakpoint falling between *DXYS154* and *DXS1108*.

By contrast, the six remaining patients showed no evidence of Xq–Yq exchange. WHT1832 and WHT1876 exhibited single maternal alleles for both markers. Single alleles for both markers were also observed in WHT1983, LGL658, WHT1157 and WHT2168; these are likely to be of maternal origin, but in the absence of parental samples, we cannot exclude the possibility that identical alleles were transmitted from both parents. (In these four cases, further evidence against the presence of a second copy of Xq28 — the most distal band — was obtained by typing for highly polymorphic markers at the Factor VIII and *GABRA3* loci. In no case were two alleles observed; data not shown.)

For the three individuals in whom analysis of Xq markers strongly suggested aberrant Xq–Yq interchange (WHT1278, WHT1373 and WHT2277), we searched for more evidence by generating human-hamster somatic

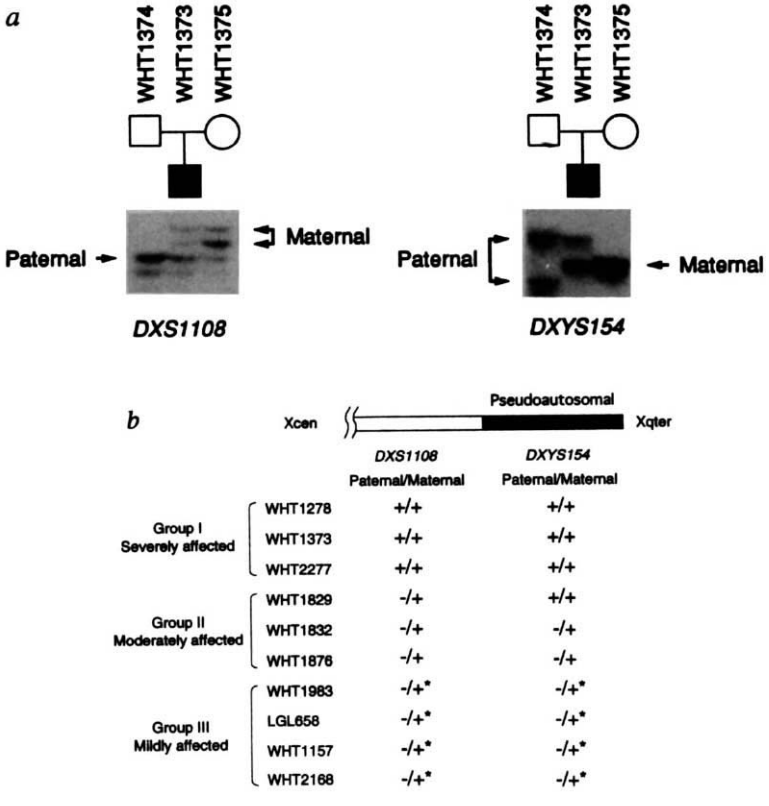


Fig. 2 *a*, Inheritance of CA-dinucleotide repeat polymorphisms at *DXS1108* (left) and *DXYS154* (right) in the family of XYq- male WHT1373. WHT1373 inherited a paternal (WHT1374) and a maternal (WHT1375) allele at each of the two loci. At *DXS1108*, an Xq28-specific locus, WHT1373 inherited the single paternal allele and maternal upper allele (indicated by upper arrow; lower arrow denotes second allele, not transmitted to WHT1373). At *DXYS154*, an Xq/Yq pseudoautosomal locus, WHT1373 inherited the paternal upper allele and the allele for which mother is homozygous. *b*, Transmission to XYq- males of paternal alleles at *DXS1108* and *DXYS154* . +/+, XYq- male inherited a paternal allele and a maternal allele; –/+, XYq- male inherited no paternal allele (only a maternal allele); –/+*, XYq- male exhibited only one allele (presumably of maternal origin), but parents were not available for testing.

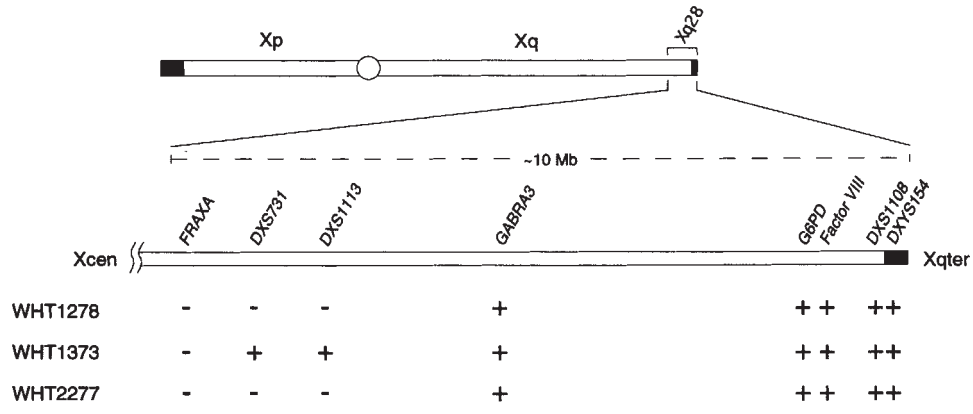
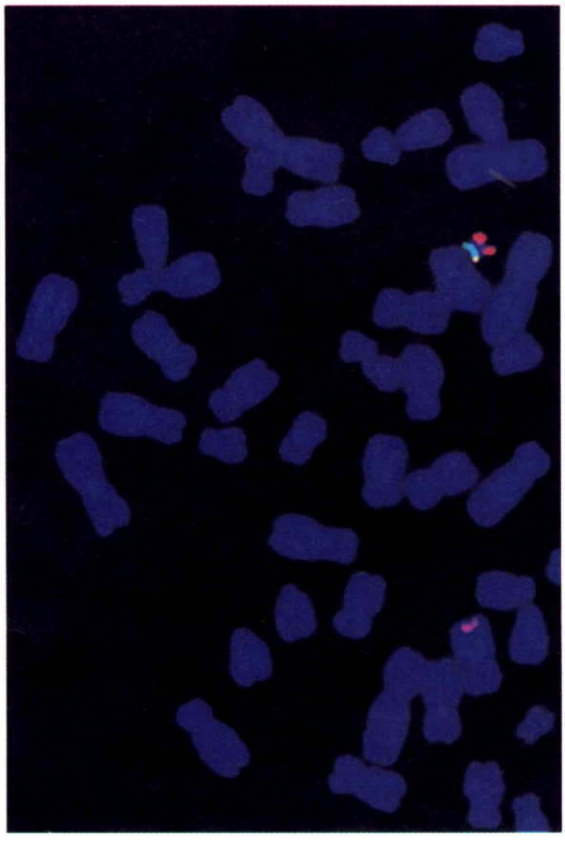


Fig. 3 Terminal portions of Xq for which the three Group I XYq- males are disomic. For each patient, somatic cell hybrids retaining the derivative Y but lacking the intact X were tested for presence (+) or absence (–) of seven X-specific (*FRAXA* through *DXS1108*) loci and one Xq–Yq pseudoautosomal (*DXYS154*) locus. Based on physical mapping of Xq (ref. 53), we estimate the size of the disomic regions to be 5–7 Mb in WHT1278 and WHT2277 and 8–10 Mb in WHT1373.

cell hybrids retaining the human Yq- chromosomes but lacking human X chromosomes. The Xq–Yq interchange model predicts that such Yq- hybrids would carry paternally derived alleles for *DXYS154* and *DXS1108*. This was the case for Yq- hybrids from all three patients (data not shown). Testing of these hybrids with other Xq DNA loci (Fig. 3) revealed the presence of variably sized terminal portions of the chromosome, with breakpoints in Xq28 (between *DXS1113* and *GABRA3* in WHT1278 and WHT2277 and between *FRAXA* and *DXS731* in WHT1373), again consistent with Xq–Yq interchange in the paternal germline.



To assess directly whether distal Xq DNA had been transferred to the truncated long arm of the Yq- chromosomes in WHT1278, WHT1373 and WHT2277, we performed fluorescence *in situ* hybridization (FISH) to metaphase chromosomes from all three patients. The FISH probe was derived from human Factor VIII, an Xq28 gene we had detected in Yq- hybrids prepared from all three individuals (Fig. 3). In normal males and females, the Factor VIII probe hybridized *in situ* only to Xq28 (data not shown). In each of the three patients, the Factor VIII probe hybridized both to the distal long arm of the intact X chromosome and to one end of the Yq- chromosome (Fig. 4). Thus, all three of these XYq- males have two copies of the Factor VIII gene, the second copy being located on the derivative Y. In each case, a second probe, specific to Yp and labelled with a different fluorescent dye, hybridized to the opposite end of the Yq- chromosome (Fig. 4). Thus, it was the truncated long arm of the derivative Y to which Xq28 DNA had been transferred in all three individuals.

In conclusion, genetic marker and FISH studies provided strong evidence that, in three unrelated XYq- males, aberrant Xq–Yq interchange in the father's germline had produced "Y_{Xq}" chromosomes that lost a terminal portion of Yq in exchange for a terminal portion of Xq (Fig. 5). There is a striking correlation with phenotype: among ten XYq- males studied, the most severe phenotypes were found in the three males with unequivocal evidence of Xq–Yq interchange. These three patients form a coherent set that is chromosomally and phenotypically distinct from the other XYq- males studied. We will refer to these three individuals as "XY_{Xq} males".

Fig. 4 *In situ* hybridization of *Factor VIII* probe to derivative Y chromosome of XYq- male WHT1278. Factor VIII plasmid p482.6 (red) and Yp-specific plasmid pDP1335 (green) localized to opposite ends of derivative Y chromosome. Factor VIII probe also localized to distal long arm of intact X. Similar results obtained with XYq- males WHT1373 and WHT2277 (not shown).

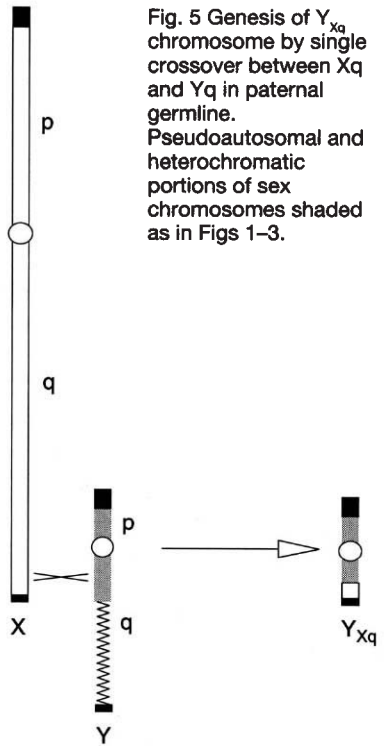


Fig. 5 Genesis of Y_{Xq} chromosome by single crossover between Xq and Yq in paternal germline. Pseudoautosomal and heterochromatic portions of sex chromosomes shaded as in Figs 1–3.

Supernormal expression of *G6PD*

The correlation between chromosomal constitution and phenotype strongly suggested a cause-and-effect relationship. As argued earlier, the severe mental retardation, hypotonia and microcephaly of the XY_{Xq} males are unlikely to be the result of the absence of specific Yq genes. Neither are the XY_{Xq} phenotypes likely to be the result of truncation, fusion or other rearrangement of specific Yq genes, since these phenotypically similar males display widely different Yq breakpoints (Fig. 1).

If the phenotypes are not readily accounted for by Yq deletion *per se*, then perhaps they can be explained by the presence of Xq28 DNA on the Y_{Xq} chromosomes. Specifically, the severe phenotypes might be the result of twice-normal expression of Xq28 genes that are normally X-inactivated when present in two copies (in normal females) but that fail to be dosage-compensated here. The X inactivation centre has been mapped to Xq13, and it must be present in two (or more) copies per cell for X inactivation to occur[27,28]. As the duplicated portion of Xq (that is, the portion of the X present on the Y_{Xq} chromosome) does not include Xq13, the cells of the XY_{Xq} males carry only one X inactivation centre and X inactivation should not occur. Thus, Xq28 genes present in two copies should not be dosage-compensated and supernormal expression of these genes might result in the severe phenotypes observed.

To examine these questions, we studied expression of the glucose-6-phosphate-dehydrogenase gene (*G6PD*), a representative Xq28 gene normally dosage-compensated via X inactivation[29], in cells from the XY_{Xq} males. *G6PD* is present on the Y_{Xq} chromosomes in all three of these individuals (Fig. 3). We quantitated G6PD enzymatic activity in lysates of cultured lymphoblastoid cells from the patients and their parents by spectrophotometry (Fig. 6). The G6PD activities in the five parents tested fell within a narrow range, while each of the three XY_{Xq} males exhibited G6PD activity approximately twice that of his parent(s).

The spectrophotometric results are consistent with the presence in the XY_{Xq} males of two actively expressed *G6PD* genes per cell. To test directly whether G6PD is expressed from the derivative Y, we assayed human G6PD activity in human-hamster somatic cell hybrids retaining Y_{Xq} but lacking intact human X chromosomes. Since human and rodent G6PD proteins have different gel mobilities, we could detect the human isoform by staining for enzymatic activity after non-denaturing electrophoresis of total protein. On non-denaturing gels, G6PD protein exists as a dimer. As shown in Fig. 7, a control hybrid containing an inactive human X chromosome expressed only the rodent isoform, while a control hybrid retaining an active human X chromosome exhibited three G6PD bands, corresponding to human homodimer, human-rodent heterodimer and rodent homodimer. (The heterodimer reflects the synthesis of human and rodent isoforms within the same cell.) Hybrids retaining Y_{Xq} chromosomes from WHT1278, WHT1373 or WHT2277 exhibited the three G6PD bands observed in the active-X hybrid. Since these Y_{Xq} hybrids contain no human X chromosome, the Y_{Xq} chromosomes must be the source of the human G6PD activity. We conclude that, in each of the three XY_{Xq} males, G6PD is expressed from both the intact X and Y_{Xq} chromosomes, resulting in twice-normal levels of expression.

Discussion

Origin of XY_{Xq} males. The most distal portions of Xp and Yp are extraordinarily recombinogenic during male meiosis. As a result, the nucleotide sequences of these regions are indistinguishable, and their inheritance is "pseudoautosomal" rather than strictly sex-linked[30,31]. Most human XX males and some human XY females are

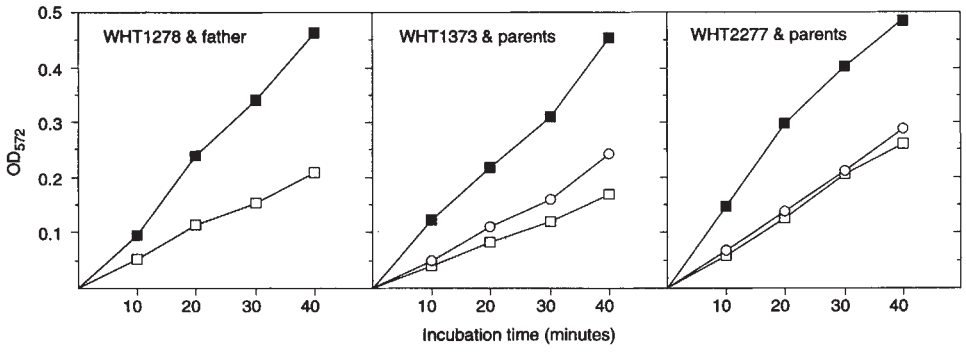


Fig. 6 Doubled G6PD activity in XY_{Xq} males compared with their parents. Conversion of glucose 6-phosphate to 6-phosphogluconolactone by lymphoblastoid extracts was measured as an increase in OD_{572} (see Methodology). Substrate was not limiting, as demonstrated by linearity of product present after 10, 20, 30 and 40 minutes of incubation. —■—, Patient; —□—, father; —○—, mother.

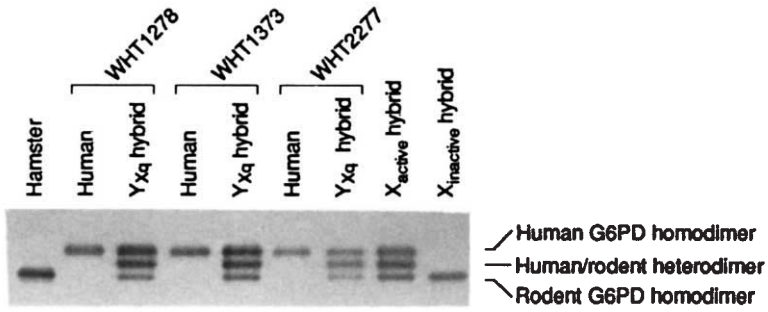


Fig. 7 Human G6PD enzymatic activity in somatic cell hybrids retaining Y_{Xq} chromosomes. Gel stained for G6PD activity after non-denaturing electrophoresis of total protein from the following cultured cells (left to right): RJK (hamster fibroblast)[50], WHT1278 (XY_{Xq} male lymphoblastoid), WHT2656 (human–hamster hybrid retaining Y_{Xq} chromosome but not intact X chromosome from WHT1278), WHT1373 (XY_{Xq} male lymphoblastoid), WHT2666 (human–hamster hybrid retaining Y_{Xq} from WHT1373), WHT2277 (XY_{Xq} male lymphoblastoid), WHT2667 (human–hamster hybrid retaining Y_{Xq} from WHT2277), WHT2660 (human–hamster hybrid retaining active X chromosome from WHT2281), 37-26R-D (human–mouse hybrid retaining inactive human X)[54]. Similar results were obtained with other Y_{Xq} hybrids prepared from WHT1278, WHT1373 and WHT2277.

the result of aberrant exchanges of terminal portions of Xp and Yp in the paternal germline[13–19]. These Xp–Yp exchanges may represent aberrant byproducts of the highly recombinogenic pairing of distal Xp and Yp during male meiosis.

During male meiosis, synaptonemal complexes are formed not only between the distal portions of Xp and Yp but also between the most distal portions of Xq and Yq (ref. 21). The nucleotide sequences of the most distal 320 kb of Xq and Yq are indistinguishable[26], and their inheritance, like that of distal Xp and Yp, is "pseudoautosomal"[22]. We speculate that the Xq–Yq exchanges giving rise to XY_{Xq} males (Fig. 5) represent aberrant byproducts of the recombinogenic pairing of distal Xq and Yq during male meiosis. (We cannot exclude the possibility that the aberrant Xq–Yq exchanges giving rise to XY_{Xq} males occurred during mitosis rather than meiosis in the paternal germline. This is also true for the aberrant Xp–Yp exchanges giving rise to XX males and XY females.) In the case of human XX males, Xp–Yp exchange can be the result of a single crossing-over between grossly misaligned X and Y chromosomes, with homologous recombination occurring at sites of local sequence similarity between Xp and Yp (refs 17,19). If some XY_{Xq} males result from a single crossing-over between Xq and Yq, then that crossing-over must also occur between grossly misaligned X and Y chromosomes (as depicted in Fig. 5). It will be of interest to learn whether aberrant Xq–Yq exchanges, like the aberrant Xp–Yp exchanges, occur at sites of local X–Y sequence similarity.

Thus, the relationship between the long-arm pseudoautosomal region and XY_{Xq} males may be analogous to that between the short arm pseudoautosomal region and XX males (and some XY females). The Xp–Yp exchange products found in such XY females are roughly reciprocal to those found in XX males[13–19,32]. By analogy, we might predict the existence in human populations of Xq–Yq exchange products reciprocal to those found in XY_{Xq} males. Indeed, three females with 46,X,der(X)t(X;Y)(q;q) karyotypes have been reported[33–35]. Though these cases, to our knowledge, have not been studied with genetic markers, the available information is consistent with their X derivatives having resulted from aberrant Xq–Yq interchange in the paternal germline.

It will be of interest to determine the nature of the derivative Y chromosomes in the seven XYq- males in whom we did not demonstrate Xq–Yq exchange, especially since these seven males display considerable phenotypic diversity (Table 1). Perhaps some of these males will prove to be the result of Xp;Yq translocation, as recently demonstrated by Bardoni and colleagues[7], or of Yq; autosome translocations

Critical importance of dosage compensation. It is generally agreed, in a teleological sense, that the purpose of X inactivation is to ensure that expression of X-linked genes in females is equal to rather than twice that in males. It is implicitly understood that failure to inactivate X-linked genes present in two copies per cell would be harmful to the organism. Indeed, some aberrant human phenotypes have been interpreted as likely resulting from supernormal expression of X-linked genes that would normally be dosage-compensated via X inactivation. These phenotypes, all associated with partial X disomy (and monosomy for the X inactivation centre) include: (i) gonadal sex reversal in 46,XY individuals with partial Xp duplications[1–3], (ii) mental retardation and dysmorphic features atypical of Turner syndrome in females with mosaic 45,X/46,X,r(X) karyotypes[4,5], (iii) severe phenotypes associated with X;autosome translocations[6] and (iv) psychomotor retardation and dysmorphic features in three males with 46,X,der(Y)t(X;Y)(p21.3 or p22.1;q11) karyotypes[7]. In each of these cases, supernormal X-linked gene expression is among the most likely explanations for the abnormal phenotype. However, to our knowledge, supernormal expression has not been directly demonstrated in any of these cases. (Three reports have described single patients with tandem Xq duplications and twice-normal activity of an X-linked enzyme[36–38], but in those cases it remains unclear whether supernormal expression of X-linked genes caused the patients' phenotypic abnormalities).

We have described three unrelated boys in whom DNA marker and FISH studies revealed similar 46,X,der(Y)t(X;Y)(q28; q11) karyotypes. All three boys exhibited severe mental retardation, generalized hypotonia and microcephaly, and these phenotypes are almost certainly due to overexpression of certain genes in the portions of Xq28 for which these boys are disomic. We have demonstrated twice-normal activity of a representative Xq28 gene product, G6PD, in cells from each of the three boys, and it is reasonable to suppose that other Xq28 products would show similar behaviour. The portions of the X for which these boys are disomic measure 5 to 10 megabases (Mb) and are likely to contain more than a hundred genes. The phenotypically critical, dosage-sensitive genes probably constitute a subset of these and may or may not include *G6PD*.

Turner syndrome (typically 45,X) and Klinefelter syndrome (47,XXY) are better-known disorders associated with abnormal sex chromosome dosage. As in the XY_{Xq} syndrome described here, the Turner and Klinefelter phenotypes are due, at least in part, to quantitatively abnormal expression of sex chromosomal genes. However, the dosage-sensitive genes critical to the XY_{Xq} syndrome should differ systematically and fundamentally from the dosage-sensitive genes critical to the Turner or Klinefelter syndromes. The Turner phenotype probably results, at least in part, from haploinsufficiency of genes that are common to the X and Y chromosomes and that escape X inactivation[39,40]. The Klinefelter phenotype probably results, at least in part, from overexpression of genes that escape X inactivation. (These "Klinefelter genes" may or may not have Y counterparts). By contrast, we predict that the dosage-sensitive genes critical to the XY_{Xq} syndrome are strictly X-linked, have no functional equivalents on the Y chromosome, and normally are subject to X inactivation.

The perplexing range of XYq- phenotypes. Males with 46,XYq- karyotypes exhibit a wide range of phenotypes, extending from normal development and normal fertility[41] to short stature and azoospermia[9,10] to severe mental retardation and dysmorphic features[11,12]. The unpredictability of the phenotype creates real dilemmas for families and those counseling them.

Our studies represent a step toward resolution of this perplexing situation. Among the ten patients with 46,XYq- karyotypes studied, we found evidence of Xq–Yq exchange in the three most severely affected individuals. We note two previously reported XYq- boys[11,12] whose phenotypes are quite similar to those of the XY_{Xq} males described here. We would not be surprised if these two boys were found to carry products of aberrant Xq–Yq interchange. One might predict that XYq- fetuses with evidence of Xq–Yq exchange (for example, from FISH studies or assay of G6PD activity) would develop severe phenotypes like those seen in our three XY_{Xq} patients. However, there is undoubtedly bias toward referral of severely affected individuals to our laboratory, and this may result in our set of XYq- patients constituting a skewed sample.

Among XY_{Xq} males, one might expect phenotypic severity to increase with the extent of Xq disomy. Among our three XY_{Xq} boys, WHT1373 displayed both the largest region of Xq disomy (Fig. 3) and the most profound growth retardation (height 80 cm, weight 35 lbs at 8 years). Conversely, we note that one XYq- male in whom an Xq;Yq translocation has been detected (cited as unpublished result in ref. 7) displays a much milder phenotype than seen in our patients (see description of case 7 in ref. 7); perhaps he is disomic for a smaller region of Xq than our patients. Further phenotypic and genotypic comparisons of these and similar cases will be of value.

Methodology

PCR analysis of DNA markers. Patient DNAs were tested for the presence of Y-chromosomal sequence-tagged sites; oligonucleotide primers, agarose gel electrophoresis, and ethidium-bromide detection were as described[20]. Thermocycling conditions: 3 min at 94 °C followed by 30 cycles of 1 min at 94 °C, 1 min at 58 °C, and 1 min at 72 °C. Similar methods were used to test human-rodent somatic cell hybrid DNAs for the presence of the following pseudoautosomal or X-linked loci:

Locus	Primers	Ref.
DXYS154	5'–GGCCTGAATTCATTTATTATTCTAATAG–3'	22
	5'–GAACAGGCAAAGATGCCCACTCTC–3'	
DXS1108	5'–ACTAGGCGACTAATACAGTGGTGC–3'	22
	5'–GTGAATTCATCATATGTGATTTCC–3'	
Factor VIII	5'–TGCATCACTGTACATATGTATCTT–3'	42
	5'–CCAAATTACATATGAATAAGCC–3'	
G6PD	5'–CCTCTATGTGGAGAATGAGAG–3'	43
	5'–CACTGCTGGTGGAAGATGTCG–3'	
GABRA3	5'–TCCTGAGGGCAGGGTCTCTGATT–3'	44
	5'–GGGTTCAGGAGACTGCACAGCAA–3'	
DXS1113	5'–ACCTGTGGAGGATAGTAGTCTGACT–3'	45
	5'–GGGAGCTTTAGAGATTTTGGTAAAC–3'	
DXS731	5'–CTCACCATTGGGTCTTCATACA–3'	46
	5'–TATGATAGGCATGAATTGTGTCTG–3'	
FRAXAC2	5'–GACTGCTCCGGAAGTTGAATCCTCA–3'	47
	5'–CTAGGTGACAGAGTGAGATCCTGTC–3'	

Patients and parents were typed for CA-dinucleotide repeat polymorphisms using radioactively labelled primers (listed above) and polyacrylamide gel electrophoresis, as described[48].

Construction of human–hamster somatic cell hybrids. Hybrids were generated as described[49] by fusing human lymphoblastoid lines with RJK (thymidine-kinase-deficient) hamster fibroblasts[50] in the presence of polyethylene glycol-4000; subsequent culture in HAT medium supplemented with glycine. After isolation and propagation of adherent clones, DNA was extracted and tested for the presence of human X-specific and Y-specific sequence tagged sites.

Fluorescence in situ hybridization (FISH). Chromosome spreads were prepared from lymphoblastoid cell lines cultured in the presence of 0.1 μg ml^{-1} colcemid for 60 min. *In situ* suppression hybridization and two-colour fluorescent detection were performed as described[51]. In brief, plasmid p482.6 (ATCC #57202), whose 9.6-kb insert derives from intron 22 of the human Factor VIII gene[52], was labelled by nick translation with biotin-14-dATP and visualized using avidin-Texas Red. Plasmid pDP1335, whose 19-kb insert derives from interval 1A1A on distal Yp (ref. 20) was labelled with digoxygenin-11-dUTP and visualized using fluorescein-conjugated antibody. Slides were counterstained with DAPI. FISH images were captured using a CCD camera and electronically processed.

Quantitation of G6PD activity. Pelleted lymphoblastoid cells were lysed in 10 volumes of water by vortexing. After microcentifugation, total protein concentration in the supernatant was measured by Lowry reaction and adjusted to 0.15 mg ml^{-1}. We then added 10 μl of adjusted supernatant to 100 μl of G6PD staining solution (0.1 M Tris pH8, 1 mg ml^{-1} Na_2-glucose-6-phosphate, 80 μg ml^{-1} $NADP^+$, 100 μg ml^{-1} Methylthiazolium tetrazolium [MTT], 40 μg ml^{-1} phenazine methosulfate[PMS]) at room temperature. Reactions were stopped after 0, 10, 20, 30, or 40 min by adding 10 μl of 10% SDS and 380 μl water. G6PD catalyzes conversion of glucose-6-phosphate to 6-phosphogluconolactone and the coupled reduction of $NADP^+$ to NADPH. In the presence of PMS, oxidation of NADPH drives conversion of MTT to formazan, whose increasing concentration was followed by monitoring optical density at 572 nm.

Electrophoretic detection of G6PD activity. Cultured cells were pelleted and lysed in an equal volume of 50 mg ml^{-1} DTT, 5 mg ml^{-1} $NADP^+$. After microcentrifugation, 10 μl of supernatant from each sample was subjected to electrophoresis on a non-denaturing 4% polyacrylamide gel. The gel was treated with G6PD staining solution for 10 min at room temperature and photographed.

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