Familial X/Y translocations associated with variable sexual phenotype

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T rue hermaphroditism, defined clinically as the presence of both male and female gonadal tissue in the same individual usually accompanied by ambiguous genitalia, is a genetically heterogeneous condition.^{1–3} Less than 10% of true hermaphrodites with an apparent 46,XX constitution are *SRY*-positive, usually resulting from translocation between Xp and Yp.^{4–7} Previously we described two true hermaphrodites (AK and PG) of Polish origin, both of whom carry X/Y translocations with *SRY* translocated onto distal Xp.⁶ Here, we show that the same X/Y translocation is also carried by fertile male and female relatives of the hermaphrodite probands, and identify varying sexual phenotype in individuals with the same chromosome abnormality.

AK was ascertained aged 17 with ambiguous internal and external genitalia. Biopsy revealed a left ovary and a right testis with no signs of spermatogenesis. PG was ascertained at 6 months of age with ambiguous internal and external genitalia. She was found to have a right ovary and a left ovotestis, which was removed and upon microscopical examination showed signs of dysgenesis in the testicular part. Circulating testosterone levels were <0.1 ng/ml, rising to 0.2 ng/ml following hCG stimulation. Now aged 15, her remaining ovary has developed apparently normally, she menstruates regularly, and thus may be fertile. There was no family history of sex discrepancy in either pedigree, with no indication of abnormalities of sexual development in any of the relatives examined.

By PCR of STS markers, breakpoints on the Y chromosome were localised 6–7 kb proximal to *SRY* in both AK and PG (data not shown). In order to map breakpoints on the X chromosome we obtained parental blood samples, extracted DNA, and amplified microsatellite markers located in distal Xp. Results for DXYS228X, located within the proximal region of the Xp/Yp pseudo-autosomal region (PAR1) approximately 80 kb from the pseudo-autosomal boundary (<http://genome.cse.ucsc.edu/>), are shown in fig 1. Both AK and PG have three alleles, indicating that the X chromosome breakpoint lies distal to this locus, resulting in a der(X) chromosome carrying two copies of the proximal part of PAR1.⁸ Because individuals with an *SRY*-positive 46,XX constitution are almost invariably infertile, and, by definition, translocations involving the Y chromosome must be of paternal origin, we expected the der(X) in AK and PG to be paternal and de novo in origin. Much to our surprise however, results for DXYS228X show that both the father of AK and the mother of PG also possess three copies of this locus, suggesting that they also carry the der(X).

In order to confirm both the presence and inheritance of the der(X) in these individuals, we performed SSCP analysis of a single nucleotide polymorphism 1.5 kb 5' of *SRY* (rs2534636 in dbSNP, <http://www.ncbi.nlm.nih.gov/SNP/>). Results are shown in fig 2, and demonstrate that both the hermaphrodites AK and PG possess a single *SRY* allele, while the mother of AK is *SRY*-negative, as expected. However, the father of AK has two *SRY* alleles, suggesting he possesses a

Key points

- We describe two families in which an *SRY*-positive X/Y translocation segregates through at least three generations causing variable sexual phenotype.
- This phenotypic variation may be due to reduced or variable *SRY* expression resulting from either (i) spreading of X inactivation into the translocated Yp segment, or (ii) a position effect due to the close proximity of a translocation breakpoint.
- Polymorphism and junction fragment analyses indicate both families are descended from a common ancestor.
- Based on our observations we propose a mechanism by which X/Y translocations could be silently transmitted for many generations.

normal Y chromosome and the der(X). This was confirmed by positive amplification of several STS markers mapping to proximal Yp and Yq (data not shown). In addition, the mother of PG also carries *SRY*. As her *SRY* allele is identical to that in PG but different from that in PG's father, this verifies both the presence of the der(X) in the mother of PG and its maternal transmission. Furthermore, use of STS markers in the mother of PG localised the Yp breakpoint to the same interval 6–7 kb proximal to *SRY* as observed in PG, suggesting that there was no gain or loss of Yp material between generations (data not shown). Analysis of DNA obtained from the maternal aunt of PG also yielded identical results, indicating that she has the same 46,X,der(X) karyotype. The presence of the der(X) in both the mother and maternal aunt of PG indicates that it must also be carried by one of the maternal grandparents. Similarly, as the father of AK carries a normal Y chromosome which was presumably paternally inherited, the der(X) must therefore also be present in the paternal grandmother of AK, although we were unable to formally prove this. Unfortunately no further family members were available for analysis. Pedigrees of AK and PG are shown in fig 3.

These data demonstrate the presence of an *SRY*-positive X/Y translocation associated with male, female, and hermaphrodite development in different individuals within the same pedigree. Despite possessing an intact copy of *SRY*, both the mother and maternal aunt of PG have developed as normal, fertile females. This same X/Y translocation has then been inherited by PG, causing hermaphroditism and ambiguous genitalia. Similarly, the hermaphrodite AK carries the same der(X) that is present in his father, a normal fertile male. As AK's father also possesses an intact Y chromosome, the paternal grandmother of AK (a fertile and reputedly normal female) must also carry the der(X). Thus, our observations

Figure 1 Results of PCR of the Xp/Yp PAR microsatellite DXYS228X. Both AK (track 1) and her father (track 3) carry the der(X) and are thus trisomic at this locus, as indicated by the presence of three separate alleles, confirming paternal inheritance. Similarly, the increased relative intensity of the 202 bp allele in both PG (track 4) and her mother (track 5) suggests that both carry the der(X), indicating maternal inheritance. Quantitative analysis of the intensity of 202 bp alleles in PG and her mother showed they are a mean of 41% and 54% greater than that in control individuals with identical allele distributions, respectively, strongly suggesting that both are trisomic at this locus. Figures below each allele represent size in base pairs and peak height, respectively.

fulfil the prediction made by Ferguson-Smith (1966) to account for the familial occurrence of X/Y translocations.⁹

Because of the clear similarities in the associated phenotype, geographical origin, and chromosomal breakpoints of the der(X) between the families of AK and PG, we hypothesised that they may be derived from a single common ancestor. Although the two families were not aware of any shared ancestry, this hypothesis makes two testable predictions: (i) haplotypes on the Y-specific fragment of the der(X) should be shared between AK and PG, and (ii) the X and Y breakpoints on the der(X) should also be identical. Unfortunately, due to the close proximity of the Y-breakpoint to the boundary of the pseudo-autosomal region, <20 kb of Y-specific material remains on the der(X), containing very few known polymorphisms. Although far from conclusive, analysis of one available SNP does show the presence of a common allele between AK and PG, consistent with identity by descent (fig 2). In order to identify the X/Y junction fragment on the der(X) we performed Southern analysis using a probe specific to the 5' region of *SRY*, generated by PCR using primers *SRY2A* gcaggtcaccaagacacaa and *SRY2B*

Figure 2 Results of SSCP analysis of a c/t SNP 1.5 kb 5' of *SRY*. DNA amplified from homo- or hemizygous individuals migrates as two bands specific to either the "c" or "t" allele, while heterozygotes show both "c" and "t" alleles, represented by three bands. The hermaphrodites AK and PG each possess a single *SRY* allele, while the mother of AK is *SRY*-negative, as expected. However, the father of AK has two *SRY* alleles, suggesting he possesses both a normal Y chromosome and the der(X). The mother of PG is also *SRY*-positive and carries the same *SRY* allele as PG, confirming both the presence of the der(X) in the mother of PG and its maternal transmission. Note the common *SRY* allele in AK and PG, consistent with identity by descent.

tgaatcacatttctactggga. Results are shown in fig 4. Both AK and PG show an abnormal hybridisation pattern, confirming the presence of the translocation breakpoint 6–7 kb 5' of *SRY*. However, the probe identifies an apparently identical translocation junction fragment in both patients, suggesting that they share the same X and Y breakpoints, providing strong evidence of identity by descent.

Although it is quite possible that the two families could be unaware of their shared ancestry, both also reported that there was no family history of sexual discrepancy. This observation can be easily reconciled by examination of the probable architecture of the der(X), as described in fig 5. Its unusual structure, with the *SRY*-bearing Y-specific fragment sandwiched between two portions of PAR1, means that any X/Y recombination that occurs in the proximal PAR1 segment will transpose *SRY* from the der(X) onto distal Yp, creating a der(Y) carrying two copies of *SRY*. This der(Y) would cause normal male development and could be transmitted without phenotypic effect through the paternal lineage for many generations. However, because of this unusual structure, the additional copy of *SRY* is predisposed to revert back onto distal Xp by further X/Y recombination in the proximal PAR1 segment, recreating the original der(X) and unmasking the second "silent" *SRY* allele, potentially many generations after the original translocation event. Although we have no direct evidence to support this hypothesis, it provides a simple explanation to link the families described here which is consistent with the probable architecture of the der(X).

There have been three previous reports of inherited X/Y translocations associated with variable sexual phenotype. Abbas *et al* (1993) reported a pedigree similar to that of AK, with the father carrying both a der(X) and a normal Y chromosome.¹⁰ However, in this family the same der(X) was present in both a 46,X,der(X) male and his true hermaphrodite sibling. Although the only carrier male in the families

Figure 3 Pedigrees of (A) AK and (B) PG. Carriers of the der(X) are represented by dotted symbols. Although the paternal grandmother of AK is an obligate carrier of the der(X), we were unable to formally prove her karyotype, indicated by a grey dot. Percentages shown represent the X inactivation ratios (percentage of peripheral blood cells in which the der(X) is inactive), N/I denotes analysis was uninformative.

we describe also possesses an intact Y chromosome which has presumably conferred his normal male phenotype, given the observations of Abbas *et al*, it seems likely that the der(X) present in AK and PG may also be capable of conferring overtly male development. Mücke *et al* (1995) described a mother and her hermaphrodite offspring, both of whom presented with MIDAS syndrome and carried the same SRY-positive der(X).¹¹ Jakubowski *et al* (2000) described a further true hermaphrodite who had inherited an X/Y translocation from his father, although in this case the der(X) was present in mosaic form in the gonads of the hermaphrodite, accounting for intersex development.¹² Finally, similar variation in sexual phenotype has also been observed in the *Sxr* mouse.^{13 14}

What could account for the variable sexual development associated with these X/Y translocations? Somatic mosaicism for an SRY-bearing chromosome is associated with variability in phenotypic sex, but the familial nature of the der(X) makes this explanation highly unlikely. X inactivation spreading into the translocated Yp segment and variably

Figure 4 Results of Southern analysis using probe SRY2 hybridised to *Ban*I digested DNA. Both AK (track 1) and PG (track 2) show an abnormal hybridisation pattern, with the same novel fragment of ~6.7 kb replacing the expected 4.9 kb fragment seen in the male control (track 3). This identifies an apparently identical translocation junction fragment in both families, indicating they share the same X and Y breakpoints.

silencing the *SRY* gene has also been proposed as a possible mechanism,^{6 9 15} and studies of the *Sxr* mouse support this suggestion.^{13 14} In order to investigate this hypothesis, we analysed X inactivation ratios in the pedigrees of AK and PG by methylation analysis at the *AR* locus, as described previously.¹⁶ Results are shown in fig 3. Despite the possibility of recombination between the *AR* locus (Xq12) and the translocated *SRY* gene (distal Xp22.3) making the direction of X inactivation skewing ambiguous, we did not find preferential silencing of the der(X) in female carriers as predicted by the hypothesis. However, it is important to note that while there was no apparent correlation between sexual phenotype and the proportion of cells in which the der(X) was inactive, the only tissue available for analysis was peripheral blood which may not reflect the situation in the gonads,¹⁶ and thus these results should be treated with caution.

Alternatively, incomplete masculinisation in carriers of X/Y translocation may be due to position effects, with chromosomal rearrangements of distal Yp disrupting *SRY* expression. In support of this hypothesis, the majority of reported SRY-positive 46,XX true hermaphrodites that have been studied molecularly have been found to have breakpoints very close to *SRY*.^{6 7 10 17 18} The female and hermaphrodite development seen in carriers of the same X/Y translocation may represent a position effect disrupting *SRY* with variable penetrance. A similar phenomenon has been described previously in large family with a 7;16 translocation associated with extensive phenotypic variation in Williams-Beuren syndrome.¹⁹ There is evidence that *SRY* and other genes involved in the sex

Figure 5 Proposed mechanism by which X/Y translocations could be silently transmitted for many generations. (A) Probable structure of the der(X;Y) associated with variable sexual phenotype. The X breakpoint is distal to DXYS228X, resulting in a der(X) chromosome carrying two copies of the proximal part of PAR1.⁶ X/Y recombination occurring in the proximal PAR1 segment in a male 46,Y,der(X) carrier would transfer the SRY-bearing portion onto distal Yp, creating a der(Y) carrying two copies of SRY, as shown in (B). In this state, the der(Y) would cause normal male development and could be transmitted without phenotypic effect through the paternal lineage for many generations. However, the architecture of this der(Y) means that the additional copy of SRY is predisposed to revert back onto distal Xp by further X/Y recombination in the proximal PAR1 segment in a 46,X,der(Y) carrier. (C) This results in an SRY-positive der(X) associated with variable sexual phenotype, potentially many generations after the original translocation event.

determination pathway, such as *SOX9* and *DAX1*, are dosage sensitive and only function once a critical threshold level is reached.²⁰ Thus, the translocation in the families described

here may reduce *SRY* expression to this critical threshold such that variation in the expression level of interacting genes, the genetic background, or stochastic factors could result in variation in testis-determination and sexual phenotype.

Our observations also have implications for the construction of human phylogeny based on Y chromosome haplotypes. Previous studies have invoked that the c/t SNP 1.5 kb 5' of *SRY* is subject to either recurrent mutation and/or reversion to the ancestral state in order to account for certain haplotype variants.^{21–23} However, the occurrence of fertile males carrying two independent *SRY* alleles in families such as we describe here introduces the potential for allelic exchange between Y chromosomes by gene conversion. This mechanism could lead to haplotype variants being transferred to different phylogenetic backgrounds, and thus appearing as recurrent mutations in the phylogenetic tree.

In conclusion, our findings indicate the existence of a large Polish pedigree in which an X/Y translocation segregates associated with variable sexual phenotype. Although likely to be very rare, our findings underline the familial nature of some X/Y translocations, particularly those involving rearrangements close to *SRY*.

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