European Journal of Human Genetics (2000) 8, 805–808

www.nature.com/ejhg

SHORT REPORT

A reinvestigation of non-disjunction resulting in 47, XXY males of paternal origin

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We have used polymorphisms within the Xp/Yp pseudoautosomal region (PAR 1) to determine the frequency and location of recombination in 80 paternally derived 47, XXY males. Of 64 informative results, there were 10 single cross-overs, one double cross-over and 53 without a cross-over. Therefore 2/3 of 47, XXY males of paternal origin result from meiosis in which the X and Y chromosomes fail to recombine. This failure was not associated with the presence of an increase in recombination in the smaller Xq/Yq pseudoautosomal region (PAR 2) or with the presence of microdeletions within PAR 1. *European Journal of Human Genetics* (2000) **8**, 805–808.

Keywords: sex-chromosome non-disjunction; recombination; PAR 1; PAR 2; microdeletion

Introduction

Trisomy is the most common class of human chromosome abnormality. Among autosomal trisomies the great majority of cases are attributable to errors in maternal meiosis.[1] In contrast, the proportion of paternally derived cases is much higher for sex chromosome aneuploidy. Non-disjunction of the paternal sex chromosomes is responsible for approximately 50% of 47, XXY males[2,3] and 74% of 45, X females.[4]

The XY bivalent is particularly susceptible to errors in meiosis because homology between the X and Y chromosomes, the sequence available for recombination and synapse formation, is limited to two regions of 2.5 Mb and 0.33 Mb located at the distal tips of the short and long arms. These are referred to as pseudoautosomal regions (PAR) 1 and 2, respectively. Although sequence exchange may occur in both PAR 1 and PAR 2, the nature and extent of recombination is very different between the two regions. During male meiosis a single cross-over normally occurs within PAR 1 to ensure proper disjunction.[5] Genetic maps have established a linear gradient of recombination within PAR 1, with markers close to the telomere displaying 50% recombination.[6]

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Received 29 February 2000; revised 17 May 2000; accepted 6 June 2000

In contrast, electron microscopy[7] and molecular studies[8,9] have demonstrated that exchanges within PAR 2 are rare. Further PAR 2 recombination is thought neither sufficient nor necessary for normal disjunction to occur.[9,10] Very rarely a chiasma has been observed at both PAR 1 and PAR 2, with the bivalent forming a ring-like structure.[11] Of four cases identified molecularly that recombined within PAR 2, two simultaneously recombined within PAR 1.[8] Therefore it is possible that recombination within PAR 2 could interfere with normal pairing at PAR 1.

Around 1 in 1000 newborn males have a 47, XXY constitution.[12] Cases that are of paternal origin must have arisen from an error during meiosis I. Failed or altered meiotic recombination plays a significant role in the aetiology of human trisomies and has been demonstrated for paternal 47, XXYs.[13] Only 15% recombination was detected with two polymorphic markers close to the telomere and the resultant genetic map was significantly shorter in 47, XXY males than normal males. We wished to confirm these findings in a larger sample using improved fluorescent PCR for allele scoring. We also tested three polymorphisms within PAR 2 to investigate whether decreased recombination in PAR 1 was associated with recombination in PAR 2.

Complete loss of PAR 1 in males, either through a deletion[14] or a translocation[15] results in failure of X–Y pairing. Submicroscopic deletions encompassing the SHOX gene within PAR 1 have recently been identified as the causative

mutation in 12 families with Leri-Weill dyschondrosteosis (LWD), a dominantly inherited skeletal dysplasia.[16,17] Such deletions would reduce the extent of X/Y homology and could have a similar effect on pairing or recombination. To test the hypothesis that PAR 1 deletions would increase the risk of sex chromosome non-disjunction, we have screened our series of paternal 47, XXYs for PAR 1 microdeletions using a total of seven microsatellites across the region.

Therefore this study has two principle aims:

(1) to confirm that the absence of detectable recombination between the paternal X and Y chromosomes is more common in 47, XXY males than normal males;

(2) to investigate whether this absence of recombination is associated with either increased recombination within PAR 2 or microdeletions within PAR 1.

Materials and methods

Subjects

A total of 80 47, XXY individuals of paternal origin were available for analysis, comprising 25 from the study by Hassold[13] for which DNA was still available, and an additional 55 previously unreported cases. Of the new cases, 21 were diagnostic referrals including three prenatal samples referred for advanced maternal age, four were ascertained through a screen of newborns for abnormal karyotypes and the remaining 30 were recruited as part of a study into the effect of the parental origin of the additional sex chromosome on behavioural and cognitive phenotypes. DNA samples were available from both parents of the 25 cases of Hassold, and from 35 of the new cases, but from only one parent for the remaining 20.

Molecular studies

DNA was amplified using seven microsatellites within PAR 1; marker order: telomere – DXYS233, CA-SHOX, DXYS201, DXS6814, DXYS234, DXYS228, DXYS232 – centromere. CA-SHOX was taken from Shears *et al*,[17] otherwise all primer sequences and conditions are available from the Genome Data Base (www.gdb.org). Within PAR 2 we used one diallelic polymorphism DXYS225[9] and two CA repeats, DXYS1107[8] and SKK-1.[10] DXYS1107, SKK-1 and DXYS225 are located 140 kb, 135 kb and 120 kb from the telomere respectively. One primer from each pair was fluorescently labelled to enable visualisation of PCR products.

The proband's alleles were scored as follows: 'N' not reduced, ie heterogygosity in the father is maintained in the proband; 'R' reduced, ie heterogygosity in the father is reduced to homozygosity in the proband; '–' non-informative, ie homozygous father; 'X' diallelic intercross (ab × ab) resulting in proband with aab or abb; '.' not tested. The number of alleles scored for the proband at each locus was also recorded to screen for the presence of microdeletions.

Table 1 PAR 1 recombination results for previously unpublished cases

	DNA available from both parents (n = 35)						
Sample	*233*	*Shox*	*201*	*6814*	*234*	*228*	*232*
S9	–	N	N	.	N	N	.
S10	N	N	N	N	.	–	.
S11	R	N	N	N	X	N	N
S18	–	N	.	N	N	N	.
S20	–	N	.	N	N	N	.
S22	X	N	.	N	N	N	N
S30	N	N	N	N	N	N	N
S31	N	N	N	–	–	–	N
S32	N	N	N	N	N	N	N
S38	R	R	R	R	N	–	N
S44	–	R	R	N	N	–	N
Y166	–	N	N	N	–	N	N
KS1	N	N	N	–	N	N	N
KS2	N	N	.	–	–	N	N
KS3	N	–	–	–	N	–	–
KS4	N	N	.	N	N	–	N
KS8	N	N	N	N	N	N	N
KS10	N	N	N	N	–	N	N
KS11	X	N	N	–	N	N	N
KS12	–	N	N	N	N	N	N
KS16	N	N	N	N	N	N	N
KS18	–	X	X	N	N	–	N
KS21	N	N	N	N	N	N	N
KS25	X	N	N	N	N	–	N
KS27	–	–	–	N	.	X	N
KS28	N	N	N	N	N	–	N
KS35	N	–	–	X	–	N	N
KS36	N	N	N	N	N	N	N
KS39	N	N	N	N	N	–	N
KS41	N	R	R	–	R	–	N
KS50	R	R	R	–	R	N	N
KS51	N	N	.	X	N	–	N
KS54	N	N	.	N	N	N	N
KS55	N	N	.	N	N	N	N
KS56	N	N	N	N	–	N	.

Non-disjunction mapping

A standard map of the region was created using pairwise lods from CEPH version 8.2 and analysed using the *map+* program.[18] The typing error frequency was estimated at 0.003 and the interference parameter P in the Rao function[19] was 0.37. This suggests that interference is incomplete which is surprising, given that the region has only 2.5 Mb. A non-disjunction map of PAR 1 was also prepared using the *map+* program[18] assuming the same interference and error frequency as the standard map as described in Bugge *et al*.[20]

Results

Recombination within PAR 1

The results for the 35 new cases for which both parents were available are given in Table 1 and combined with the cases from Hassold *et al*[13] in Table 2. Of 64 cases informative for the most distal marker used, there are 10 single recombinants. For case KS41 we identified a double cross-over between DXYS233/CA-SHOX and DXYS234/DXYS232.

Table 2 Summary of PAR 1 recombination results for all cases informative at DXYS233

Data source	*No.*	*No. informative for most distal marker*	*No. of recombinants*	*% recombinants*
Hassold 1991, not retyped	16	16	3	18.8
Hassold 1991, retyped	25	23	3	13.0
New cases[a]	35	25[b]	5[b,c]	20.0
Total	76	64	11	17.2

[a]includes results only from probands with both parents; [b]includes case S44 which was uninformative at DXYS233, but displayed recombination at CA-SHOX; [c]includes one double recombinant KS41.

The most distal marker DXYS233 is located less than 25 kb from the telomere and for interpretation of our results we assume that the assay will detect recombination in 50% of the cases in which it has occurred. Thus in normal male meiosis 32 recombinants would be expected from 64 cases, and the observed number of 11 represents a highly significant reduction ($\chi^2 = 27.6$; $P < 0.001$). The program Exchange[20] was used to reconstruct the chiasma distribution from the transitions observed. There are 53, 10 and 1 examples of zero, one and two transitions, respectively in the 64 cases. This suggests that 67.06% of tetrads are achiasmate.

PAR 1 non-disjunction map

A total of 48 informative cases were used in the analysis comprising 23 retyped Hassold cases[13] and 25 new cases. Only the markers DXYS233 and DXYS234 are common to both maps. The distance between them is 36.19 cM in the standard map (standard error 5.53 cM) compared with 13.84 cM in the non-disjunction map, a reduction of 62% over this region.

Recombination in PAR 2

The three markers used were all approximately the same distance from the telomere and therefore the results from each marker were combined. No combination was observed in the 60 informative cases. Of the remaining 20 cases, recombination could not be excluded in seven for whom only one parent was available.

Screen for microdeletions

There was no evidence for the presence of any microdeletions within PAR 1 in the 80 cases analysed. At a small number of loci, the proband had only a single allele because of inheritance of two copies of the same allele from a homozygous father. Three alleles (abc, abb, aab) were present at all other loci tested. Deletions in the intervals between markers or which included only one marker could not be excluded.

Parental ages

Parental ages for the previously unreported cases, with the exception of the three prenatal diagnostic referrals, are given in Table 3. As a control we have used parental ages of maternal 47, XXYs identified from the same populations, but excluding nine diagnostic cases ascertained prenatally because of advanced maternal age, so that our results can be directly compared with those of Lorda-Sanchez *et al*.[3] We found no evidence for any effect of increased paternal age. The number of cases was too small to identify an age difference between paternal 47, XXYs derived from recombined and achiasmate mechanisms (data not shown).

Discussion

The results confirm the previous finding of an association between absence of recombination and non-disjunction in paternal 47, XXYs.[13] Multiple cross-overs within PAR 1 are rare[21] and, assuming that normally a single chiasma is present in the X/Y bivalent during meiosis, half the paternal XXYs would be expected to show evidence of recombination, while in the other half either both recombined or both non-recombined meiotic products would be recovered and the exchange would go unnoticed. Recombination was observed in only 11 out of 64 informative cases, a highly significant reduction from the expected number of 32. Thus 67% of paternal 47, XXYs result from meiosis in which the X and Y chromosomes did not recombine. In mice a small proportion of germ cells with unrecombined sex chromosomes are able to complete meiosis and form aneuploid sperm which result in sex chromosome trisomy. In humans reduced recombination within PAR 1 has also been reported in disomic 24, XY sperm compared to normal sperm.[22]

The use of a number of polymorphisms makes it possible to determine the location as well as the frequency of exchanges.

Table 3 Parental ages

		Maternal age			*Paternal age*	
Sample origin	*No.*	*Mean*	*S.D.*	*No.*	*Mean*	*S.D.*
Paternal cases	48	28.71	5.97	35	30.69	4.61
Maternal cases	50	30.49	6.74	40	33.58	8.34

In total 10 single and one double cross-over were identified. Multiple cross-overs within PAR 1 are rare and only one double cross-over was reported from the analysis of meiosis in 330 males.[21] In normal meiosis the probability of an exchange event is approximately uniform throughout PAR 1 and the 12 cross-overs identified appeared to be distributed evenly.

Our assay is unable to distinguish between non-disjunction caused by absence of pairing (and consequently absence of recombination) or by absence of recombination despite pairing of the X and Y chromosomes. Complete absence of pairing causes infertility associated with arrested germ cell development.[11,14,15] However, the effect of pairing failure in a single chromosome in a small proportion of cells is unknown and could be compatible with germ cell development.

Our results indicate that the decrease in recombination in PAR 1 is not associated with increased recombination in PAR 2. In normal male meiosis recombination in PAR 2 is detected in only 2% of cases[8] and we were unable to detect any recombination in 60 informative cases. Recombination could have occurred between our markers and the telomere and so be missed. However, recombination does not occur uniformly in PAR 2, rather there is a recombination hotspot proximal to DXYS1107.[9] Therefore there appears to be no increase in recombination in this region in 47, XXYs compared with normal males.

The identification of PAR 1 microdeletions in patients with LWD[16,17] suggests a possible mechanism to explain some cases of paternal sex chromosome non-disjunction. However, we could find no evidence for the presence of any deletions as large as those reported in LWD, although the presence of much smaller deletions could not be excluded.

While the association of non-disjunction and increased maternal age is well established, the relationship, if any, between increasing paternal age and trisomy is unclear, with conflicting results from epidemiological and molecular studies.[1] Previously Lorda-Sanchez *et al*[3] reported a significant increase in the paternal age of paternally derived 47, XXY cases compared to maternally derived cases. In contrast, in this study there was no evidence for any increase in paternal age among paternal 47, XXYs.

In summary, absence of recombination is associated with 2/3 of paternal 47, XXYs. The reasons for the failure to recombine are unknown; in our series of patients it was not the result of an increase in recombination in PAR 2 or because of microdeletions within PAR 1. The remaining 1/3 of paternal 47, XXYs arose from an X/Y bivalent that had recombined but failed to disjoin properly. Amongst these cases the pattern of recombination appeared normal. Therefore paternally derived 47, XXYs result from at least two different mechanisms.

Acknowledgements

We are very grateful to Professor David Skuse for providing patient samples and to Professor Peter Scambler for helpful discussions and proposing the hypothesis of PAR 1 microdeletions. This work was funded by the Wellcome Trust.

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