Hum Genet (1988) 79:29–35

Human Genetics

Molecular characterization of a Y;15 translocation segregating in a family

Tiina Alitalo[1,2], Jari Tiihonen[3], Panu Hakola[3], and Albert de la Chapelle[1,2]

[1] Department of Medical Genetics, University of Helsinki, Haartmaninkatu 3, and [2] Folkhälsan Institute of Genetics, SF-00290 Helsinki, Finland
[3] Department of Psychiatry, University of Kuopio, Niuvanniemi Hospital, SF-70211, Kuopio, Finland

Summary. We have used Y-specific and Y-derived DNA probes for in situ hybridization and Southern blotting analysis to characterize a Y;15 translocation showing normal Mendelian inheritance in a family. Cytogenetically there appeared to be an unbalanced translocation of Yqh to 15p; this translocation may be considered as a prototype of those translocations between Yq and the short arm of an acrocentric chromosome which have a population incidence of approximately 1 in 2,000. Our molecular studies showed that, in all probability, the breakpoints were near the border between Yq11.23 and Yq12, and in 15p11, respectively; the translocation is abbreviated t(Y;15)(q12;p11). Using the Y-specific probe pY431 in a quantitative Southern hybridization assay, normal females had no hybridization, female carriers and normal men had the same amount, and male carriers had twice that amount. Cytogenetic analysis and quantitative in situ hybridization using probes pY431 and pY3.4 were consistent with the hypothesis that the portion of Yq translocated to 15p comprised all of Yq12 and none of Yq11. The absence of Southern hybridization with probes specific for Yp and Yq11 confirmed this observation. Even though the family was ascertained through two brothers who both had schizophrenia and were carriers of the translocation, the clinical evaluation of a total of nine individuals with the translocation and five without it did not suggest its association with an abnormal phenotype.

Introduction

The short arms and satellite regions of the human acrocentric chromosomes are highly heteromorphic with respect to size and staining properties (Harnden and Klinger 1985). When such a region is large and brilliantly fluorescent by quinacrine staining, it resembles the distal part of the long arm of the Y chromosome and raises the question as to whether it might represent material translocated from Yq12. Using Y-specific probes such Y;acrocentric translocations have been shown to exist both by Southern hybridization (Cooke and Noel 1979; Schmidtke and Schmid 1980; Burk et al. 1983; Schmid et al. 1983; Vergnaud et al. 1986) and by in situ hybridization (Lau 1985; Lau et al. 1985).

According to Nielsen and Rasmussen (1976a) the frequency of Y;autosome translocations in the general population is approximately 1 in 2000. Two distinctly different types of translocations occur. In the more common form the distal portion of Yq is translocated to the short arm of an acrocentric chromosome. Those forms in which the Y material is translocated onto a non-acrocentric chromosome are much rarer and may involve any part of the Y chromosome (Smith et al. 1979). Translocations belonging to the first group, most commonly t(Y;15), have been seen in normal individuals and are therefore thought to be compatible with a normal phenotype including fertility (Nielsen and Rasmussen 1976a; Cohen et al. 1981; Fryns et al. 1985). Translocations of the second type often lead to an abnormal phenotype, notably including sterility (Bühler 1980, 1985; Davis 1981; Fryns et al. 1985; Goodfellow et al. 1985).

In this study we have made use of Southern blotting and in situ hybridization using Y chromosome specific probes to characterize a t(Y;15) segregating in a family. We correlate the findings with the phenotypic features of individuals carrying and not carrying the translocation chromosome. Finally, our in situ hybridization data provide information about the regional distribution of the probes we have used on the normal Y chromosome.

Offprint requests to: A. de la Chapelle

Materials and methods

Cytogenetic analysis

Chromosome preparations from phytohaemagglutin-stimulated whole blood cultures (72 h) were obtained by standard methods. Banding was done by the GTG-technique according to Seabright (1971), QFQ-banding according to Caspersson et al. (1971) and CBG-banding according to Sumner (1972).

DNA probes

Repeated and single-copy DNA sequences specific for the Y chromosome or recognizing fragments localized on the Y chromosome and elsewhere were used as molecular probes. Probe pY431 (recognizing Y-specific 2.1-kb *Hae*III repeat DNA) was obtained from Kirby Smith (unpublished). Probe pY3.4 (recognizing Y-derived 3.4-kb *Hae*III repeat DNA) was obtained from Yun-Fai Lau and Yuet Wai Kan (Lau et al. 1984). Probe 50f2 was obtained from Jean Weissenbach (Guellaen et al. 1984). Probe pDP105 was a gift from David Page. It detects Y-specific *Taq*I fragments of 2.5 kb (pDP105/A) and 5.2 kb (pDP105/B) (unpublished results). In those Southern blotting experiments in which the intensity of the bands was measured by densitometry we used the probe NJ-3 (Tsipouras et al. 1983), localized on chromosome 7 and obtained from Petros Tsipouras, as a control.

In situ hybridization and Southern blotting

Slides were aged for 1 week at room temperature prior to in situ hybridization. The probes were nick-translated with ^{3}H-dCTP (57.1 Ci/mmol, New England Nuclear) and ^{3}H-dTTP (105.4 Ci/mmol). In situ hybridization was carried out according to the method of Harper and Saunders (1981). The chromosome preparations were hybridized with ^{3}H-labelled probe pY431 (specific activity 1.1×10^7 cpm/μg) at a concentration of 40–50 ng/ml or with the ^{3}H-labelled probe pY3.4 (specific activity 6×10^6 cpm/μg) at a concentration of 5–30 ng/ml. The slides were coated with Kodak NTB-2 emulsion and exposed for 3–11 days at 4°C. Following developing and fixation of the photographic emulsion the chromosomes were stained with 0.25% Wright's stain. Complete metaphase spreads were photographed and, after trypsin treatment (Popescu et al. 1985), karyotyped and analysed for silver grain localization.

DNA was extracted from whole blood in EDTA using standard methods (Page and de la Chapelle 1984). DNA samples (2 μg) were digested with *Hae*III and 5-μg DNA samples with *Taq*I, the fragments separated on 0.7% agarose gels, transferred to nitrocellulose filters (Schleicher and Schuell, BA85), denatured and hybridized to radioactively labelled denatured probes. The probes were radiolabelled by nick-translation to a specific activity of 1.8×10^8 to 2.1×10^8 cpm/μg, using ^{32}P-CTP (3000 Ci/mmol, Amersham). After hybridization, the filters were washed and exposed to Fuji X-ray films with intensifying screens.

Clinical description

The family was ascertained through the proband (individual III-11 indicated on the pedigree, Fig. 1). Clinical data on the family members were obtained by general somatic, neurological, and psychiatric examination, and psychological tests. Some symptomless family members were not studied; data on their physical and mental health were obtained through interviews with those relatives that we studied. Below is a brief summary of the findings.

Male translocation carriers

II-1 and III-1 were somatically and psychically normal. Individual II-2 was somatically normal at age 76. Based on psychological tests and psychiatric examination his mental status was considered to be borderline normal. Individual III-11 has suffered from a psychotic disorder diagnosed previously as paranoid schizophrenia since the age of 27. Individual III-12 had bradycephaly. He has acted strangely and violenty since the age of 17 and has showed on-going florid paranoid delusions. Both III-11 and III-12 are treated in a state high security hospital because of their impulsive and aggressive behaviour.

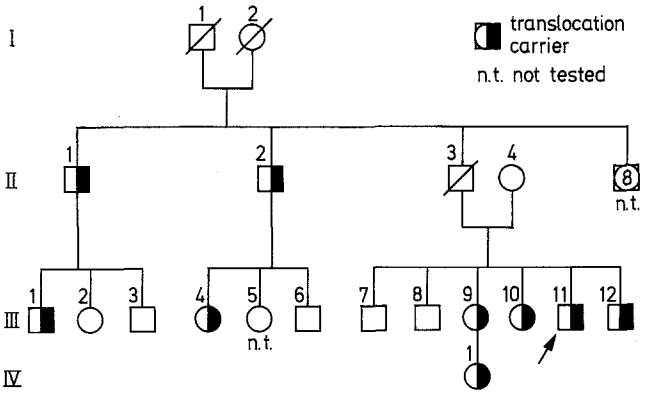


Fig. 1. Pedigree of the family

Males without translocation

Individuals III-3 and III-6 were mentally and physically normal. Individual III-7 served a 3-month prison sentence for assault and battery at the age of 34. He has not been in psychiatric care and is physically normal. Individual III-8 was hospitalized twice for schizophrenia at the age of 25.

Females

No evidence was obtained indicating mental or physical abnormalities in females III-4, III-9, III-10 and IV-1 with the translocation. Females II-4 and III-2 without the translocation, as well as female III-5 (chromosomes or DNA not studied) were also mentally and physically normal.

Results

Cytogenetic analysis

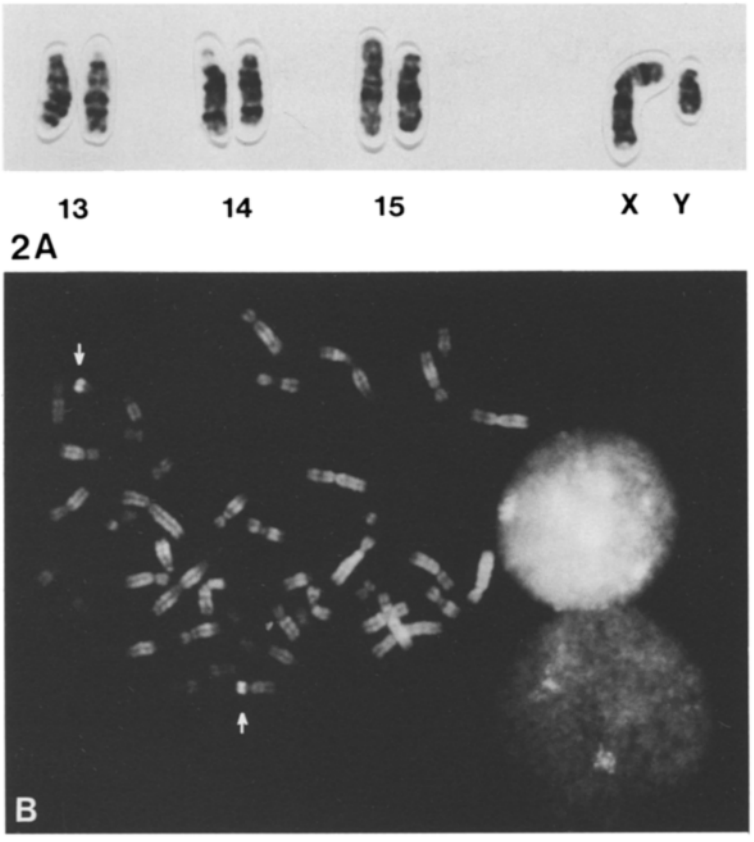
Of those individuals whose karyotypes were studied, nine had one chromosome 15 with a very large short arm here referred to as 15p+. The 15p+ chromosome appeared identical in all individuals in whom it was found. The abnormal short arm stained lightly on G-banded and brilliantly on Q-banded preparations (Fig. 2). Not shown in Fig. 2 is C-banding by which the 15p+ region stained darkly. Using the nomenclature of Harnden and Klinger (1985) the designation of this chromosome is var(15)(p1,GTG53,CBG51,QFQ55). The morphology and staining properties of this chromosome were compatible with it carrying a piece of Yq12 on its short arm. The segregation shown in Fig. 1 is compatible with normal dominant inheritance.

In situ hybridization

To characterize the 15p+ chromosome further we examined the chromosomes from a male and a female family member (II-2 and III-9, respectively) by in situ hybridization with the Y-specific probe pY431. Metaphases from II-2 were also examined with probe pY3.4. The brightly quinacrine-fluorescent regions in Yq12 and 15p+ were heavily labelled with both probes (Fig. 3).

With probe pY431, hybridized to metaphases from the male translocation carrier II-2, 24% of the chromosomal grains were located on the Y chromosome and 15% on the 15p+ chromosome (Fig. 4B). In the female translocation carrier III-9, 39% of all chromosomal grains were localized on the 15p+ chromosome (Table 1). The remaining grains were distributed throughout the chromosome complement without any accumulation of grains over any other chromosome or region, as shown in Fig. 4A.

With probe pY3.4, hybridized to metaphases from II-2, 27% of the chromosomal grains were on the Y chromosome and 20% on the 15p+ chromosome (Fig. 5B). Considerable numbers of grains also occurred in the centric regions of the chromosomes 9; 17% of all grains were on the two homologues of this chromosome. The short arms of the other ac-



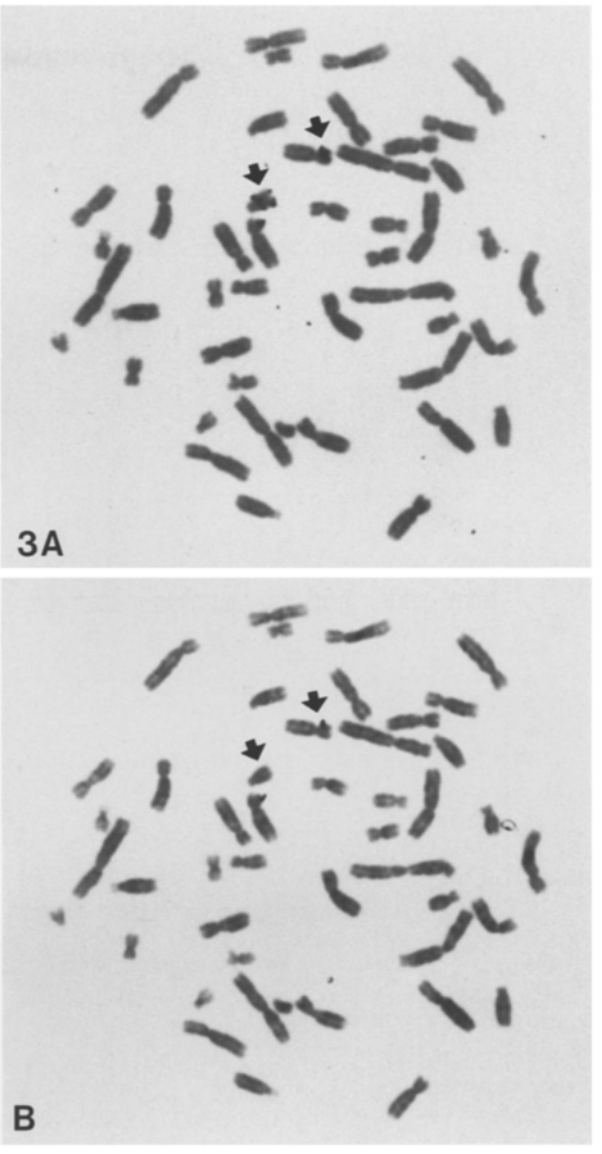


Fig. 2A, B. G- and Q-banded chromosomes. **A** Partial karyotype 46,XY,15p+ (G-bands) from individual III-12. **B** Metaphase showing 46,XY,15p+ (Q-bands) from individual III-11. Intensely fluorescent regions in 15p+ and Y indicated by *arrows*

Fig. 3. A, B. In situ hybridization of probe pY3.4 to a 46,XY,15p+ metaphase from individual II-2. **A** After Wright staining showing grains *(arrows)*. **B** After destaining and trypsin treatment followed by Wright staining yielding G-bands. *Arrows* indicate 15p+ and Y chromosomes

Table 1. Grain counts obtained by in situ hybridization. NA, Not applicable

Individual	Karyotype	Probe	No. of mitoses	Mitoses with grains on						Total no. of grains on all chromosomes	Grains on				
				Y		15p+		9			Y		15p+		9	
				No.	(%)	No.	(%)	No.	(%)		No.	(%)	No.	(%)	No.	(%)
II-2	46,XY,15p+	pY431	67	45	(67%)	30	(45%)	3	(4%)	308	75	(24%)	45	(15%)	3	(1%)
III-9	46,XX,15p+	pY431	80	NA		69	(86%)	7	(9%)	279	NA		108	(39%)	7	(3%)
II-2	46,XY,15p+	pY3.4	51	47	(92%)	42	(82%)	29	(57%)	295	80	(27%)	58	(20%)	49	(17%)

rocentric chromosomes, and the centromeric region of chromosome 5 were also labelled, but to a much lesser degree (Fig. 4C).

Southern hybridization

Autoradiograms of Southern blots of *Hae*III-digested DNA from normal male III-8 (lane 1), normal female II-4 (lane 2), female translocation carrier III-9 (lane 3) and male translocation carrier II-2 (lane 4) hybridized simultaneously with the probes pY431 and NJ-3 are shown in Fig. 6. Two prominent bands of hybridization are seen at 2.1 kb and 3.0 kb. The band produced by probe NJ-3 recognizing sequences on chromosome 7 was used as a reference to estimate the amount of DNA available for hybridization in each lane. Densitometric tracings were made in which the amount of hybridizing DNA

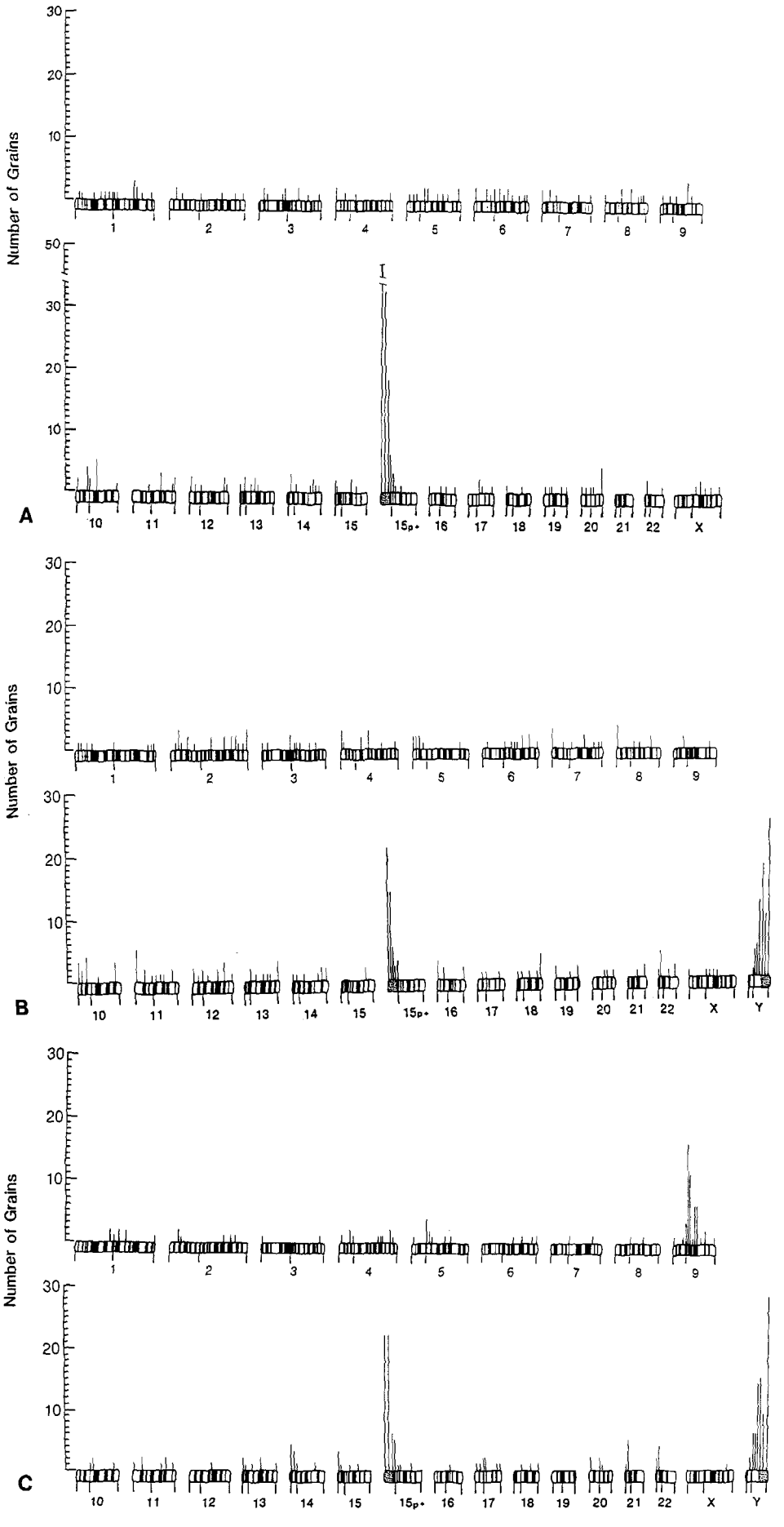


Fig. 4.A–C. Histograms showing the localization of grains on the chromosomes. **A** Probe pY431, female translocation carrier III-9. **B** Probe pY431, male translocation carrier II-2. **C** Probe pY3.4, male translocation carrier II-2

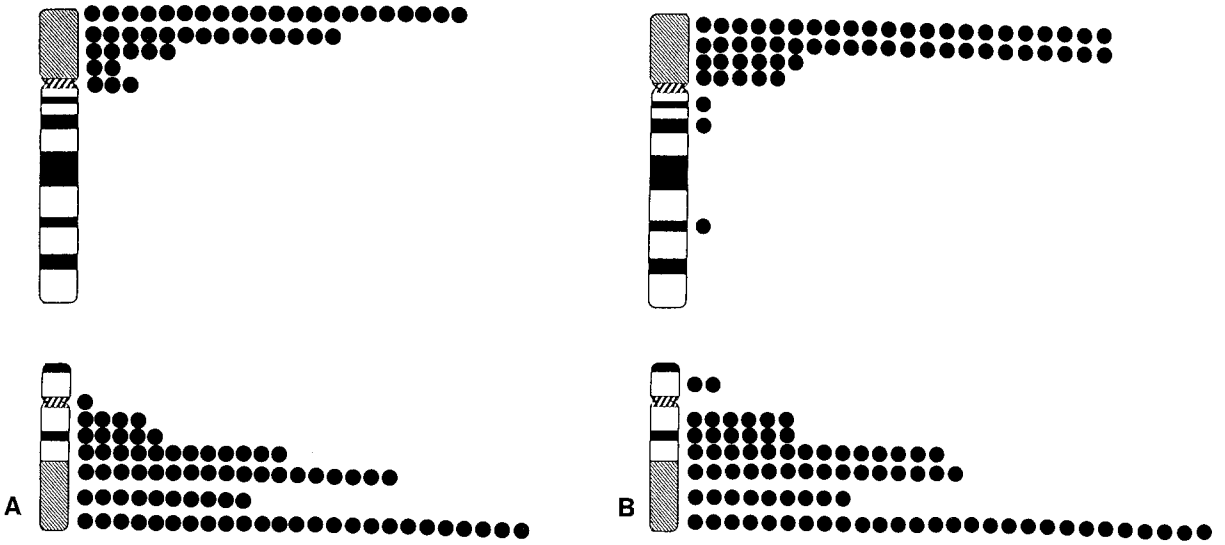


Fig. 5A, B. Schematic representation of grain distribution on the 15p+ and Y chromosomes. **A** Probe pY431, individual II-2. **B** Probe pY3.4, individual II-2

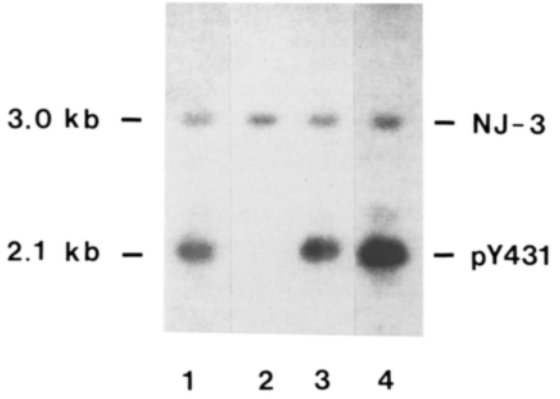


Fig. 6. Autoradiograph of Southern blot after hybridization with the probes pY431 (2.1 kb) and NJ-3 (3.0 kb). *lane 1,* normal male III-8; *lane 2,* normal female II-4; *lane 3,* translocation carrier female III-9; *lane 4,* translocation carrier male II-2

in the 2.1-kb band (Y-specific) was calculated relative to the amount present in the chromosome-7-specific band at 3.0 kb. If the value for the control female was 0, then the normal male and the female translocation carrier had values of 1.02 and 1.14, respectively, while the male translocation carrier had the value 2.28.

To find out exactly what portion of the Y chromosome was included in the translocation, we performed Southern blotting experiments with the Y chromosome specific probes pDP105 and 50f2. The results shown in Table 2 indicate that none of the DNA segments recognized by these probes were present on the translocation chromosome since DNA from female carriers did not hybridize with these probes. Referring to the map of the Y chromosome published by Vergnaud et al. (1986) these results indicate that at least intervals 3,4 and 6 were not included. Interval 3 is in the short arm, interval 4 is in the centromeric region and interval 6 is in the distal part of the euchromatic region of the long arm. Thus the breakpoint must have been either in distal Yq11.2 or in the heterochromatic region Yq12. We conclude that there is no evidence for any euchromatic part of the Y chromosome being involved.

Discussion

Our findings using in situ hybridization and Southern blotting confirmed the cytogenetic interpretations that the 15p+ carries material from Yq and also provided quantitative evidence to show that the heterochromatic band Yq12, present on the 15p+ chromosome, is of the same size as that of the Y chromosome segregating in this family. We demonstrate the absence of any of the Y-specific fragments recognized by probes localized in the euchromatic portion of the Y, including Yp, the pericentromeric region, and Yq11. On the deletion map of Yq (Vergnaud et al. 1986) fragment 50f2/C,E was assigned to interval 6, the most distal of those intervals that map to the euchromatic region of Yq. Since probe 50f2/C,E is not present on the 15p+ chromosome we conclude that the breakpoint on

Table 2. Summary of the results of Southern hybridization with the probes pDP105, 50f2 and pY431

Subject	Sex	Presence of t(Y;15)	Fragments of probe pDP105		Fragments of probe 50f2				Probe pY431
			A	B	A or B	C	D	E	
III-7, III-8	M	Absent	+	+	+	+	+	+	+
II-1, III-11	M	Present	+	+	+	+	+	+	+
II-4, normal control	F	Absent	–	–	–	–	–	–	–
III-9, IV-1	F	Present	–	–	–	–	–	–	+

the ancestral Yq was probably close to the end of the euchromatic region. The same conclusion can be made from the results obtained by Vergnaud et al. (1986) in a t(Y;15) female whose translocation breakpoint on the Y was similar to the one we report here.

We have not been able to use molecular methods to help determine the breakpoint on chromosome 15. However, since the 15p+ has a centromere which is not that of the Y (based on the absence of probes localized in intervals 3 and 4), the breakpoint must be in the short arm. The cytogenetic evidence clearly suggests 15p11. Thus the abnormality can be described as a translocation t(Y;15)(q12;p11). The translocation is unbalanced since it entails the loss of most of 15p and the gain of Yq12. We show by several methods that females with the 15p+ have one copy of Yq12 while males have two copies. The full abbreviation of the karyotype in male and female carriers is 46,XY or 46,XX,−15,+der(15),t(Y;15)(q12;p11).

We wish to draw attention to the localization of grains on the structurally normal Y chromosome shown in Fig. 5A, B. With probe pY431, 71% of all the grains on the Y chromosome, and with probe pY3.4, 65% respectively, were on Yq12 while 29% and 35%, respectively, were on the euchromatic portion (mostly Yq11). Moreover, in the case of pY3.4 we counted 49 grains on the two chromosomes 9, which almost equals the number observed on Yq12. In comparison, the numbers of grains on the short arms of the other acrocentric chromosomes were small. With the exception of the paper by Szabo et al. (1979) our results provide the first quantitative approach to the chromosomal localization of those repeated sequences that are recognized by these widely used probes (Lau et al. 1985). The data we provide for the two probes were obtained using identical hybridization conditions and are therefore directly comparable. However, in the case of these highly repetitive related satellite DNAs, different conditions of stringency will produce quite different hybridization results. For extensive comparison between probes, several stringencies should probably be used. We wish to emphasize the great inter-individual variability that exists in mitotic heterochromatin size for, e.g. chromosomes 9 and Y as documented cytogenetically (Harnden and Klinger 1985). Experiments on individuals with different expressions of these heteromorphisms are needed to characterize this variation further in molecular terms.

The t(Y;15) described here can probably be regarded as a prototype of those translocations that occur between Yq and the short arm of the acrocentric chromosomes. Such familial translocations have been described for each of the other acrocentric chromosomes except for chromosome 21 (only one de novo case; Fryns et al. 1985). Adult and fertile de novo translocation carrier males have not been described so these translocations evidently represent ancestral events that are regularly transmitted to the offspring by virtue of their lack of phenotypic effect. As far as we know these familial translocations and so-called satellited Y chromosomes (Schmid et al. 1984) are the only well-documented human ones known to date that have no phenotypic effect even when unbalanced. Carriers have a loss of Dp and an extra dose of Yq12 or vice versa. The lack of phenotypic effect is not surprising, as it is well established that the loss of one or even several short arms of acrocentric chromosomes has no deleterious consequences (Chandley et al. 1975; Nielsen and Rasmussen 1976b) and that the presence of two entire Y chromosomes often has no effect (Thompson et al. 1967; Chandley et al. 1976).

Several members of the family we describe are mentally abnormal. While it appears unlikely that the clinical features are related to the cytogenetic and molecular abnormality seen in some family members we are continuing our clinical and psychiatric investigations with the aim of elucidating the aetiological mechanisms involved.

Acknowledgements. We thank Drs. Y. W. Kan, Y.-F. Lau, D. C. Page, K. Smith, P. Tsipouras, and J. Weissenbach for the probes and Dr. J. Kere for help with the densitometric tracings. This work was supported by grants from the Sigrid Jusélius Foundation and the Academy of Finland.

References

Bühler EM (1980) A synopsis of the human Y chromosome. Hum Genet 55:145–175

Bühler EM (1985) Clinical and cytologic impact of Y-chromosome abnormalities. In: Sandberg AA (ed) The Y chromosome, part B: Clinical aspects of Y chromosome abnormalities. Liss, New York, pp 61–93

Burk RD, Stamberg J, Young KE, Smith KD (1983) Use of repetitive DNA for diagnosis of chromosomal rearrangements. Hum Genet 64:339–342

Caspersson T, Lomakka G, Zech L (1971) The 24 fluorescence patterns of the human metaphase chromosomes-distinguishing characters and variability. Hereditas 67:89–102

Chandley AC, Edmond P, Christie S, Gowans L, Fletcher J, Frackiewics A, Newton M (1975) Cytogenetics and infertility in man. 1. Karyotype and seminal analysis. Ann Hum Genet 39:231–252

Chandley AC, Fletcher J, Robinson JA (1976) Normal meiosis in two 47,XYY men. Hum Genet 33:231–240

Cohen MM, Frederick RW, Balkin NE, Simpson SJ (1981) The identification of Y chromosome translocation following distamycin A treatment. Clin Genet 19:335–342

Cooke HJ, Noel B (1979) Confirmation of Y/autosome translocations using recombinant DNA. Hum Genet 50:39–44

Davis RM (1981) Localization of male determining factors in man: a thorough review of structural anomalies of the Y chromosome. J Med Genet 18:161–195

Fryns JP, Klecykowska A, Van den Berghe H (1985) Clinical manifestations of Y/autosome translocations in man. In: Sandberg AA (ed) The Y chromosome, part B: Clinical aspects of Y chromosome abnormalities. Liss, New York, pp 213–243

Goodfellow P, Darling S, Wolfe J (1985) The human Y chromosome. J Med Genet 22:329–344

Guellaen G, Casanova M, Bishop C, Geldwerth D, Andre G, Fellous M, Weissenbach J (1984) Human XX males with Y single-copy DNA fragments. Nature 307:172–173

Harnden DG, Klinger HP (eds) (1985) An international system for human cytogenetic nomenclature. Report of the standing committee on human cytogenetic nomenclature. Karger, Basel New York

Harper ME, Saunders GF (1981) Localization of single copy DNA sequences on G-banded human chromosomes by in situ hybridization. Chromosoma 83:431–439

Lau Y-F (1985) Detection of Y-specific repeat sequences in normal and variant human chromosomes using in situ hybridization with biotinylated probes. Cytogenet Cell Genet 39:184–187

Lau Y-F, Dozy AM, Huang JC, Kan YW (1984) A rapid screening test for antenatal sex determination. Lancet I:14–16

Lau Y-F, Ying KL, Donnell GN (1985) Identification of a case of Y;18 translocation using a Y-specific repetitive DNA probe. Hum Genet 69:102–105

Nielsen J, Rasmussen K (1976a) Y/autosomal translocations. Clin Genet 9:609–617

Nielsen J, Rasmussen K (1976b) Autosomal reciprocal translocations and 13/14 translocations: a population study. Clin Genet 10:161–177

Page DC, Chapelle A de la (1984) The parental origin of X-chromosomes in XX males determined using restriction fragment length polymorphismis. Am J Hum Genet 36:565–575

Popescu NC, Amsbaugh SC, Swan DC, DiPaolo JA (1985) Induction of chromosome banding by trypsin/EDTA for gene mapping by in situ hybridization. Cytogenet Cell Genet 39:73–74

Schmid M, Schmidtke K, Kruse K, Tolksdorf M (1983) Characterization of a Y/15 translocation by banding methods, distamycin A treatment of lymphocytes and DNA restriction endonuclease analysis. Clin Genet 24:234–239

Schmid M, Haaf T, Solleder E, Schempp W, Leipoldt M, Heilbronner H (1984) Satellited Y chromosomes: structure, origin, and clinical significance. Hum Genet 67:72–85

Schmidtke J, Schmid M (1980) Regional assignment of a 2.1-kb repetitive sequence to the distal part of the human Y heterochromatin. Hum Genet 55:255–257

Seabright M (1971) A rapid banding technique for human chromosomes. Lancet II:971–972

Smith A, Fraser IS, Elliot G (1979) An infertile male with balanced Y;19 translocation. Review of Y;autosome translocations. Ann Genet 22:189–194

Sumner AT (1972) A simple technique for demonstrating centromeric heterochromatin. Exp Cell Res 75:304–306

Szabo P, Kunkel L, Yu LC, George D, Smith KK (1980) Chromosomal distribution of DNA sequences derived from the human Y chromosome in human higher primates. Cytogenet Cell Genet 25:212–213

Thompson H, Melnyk J, Hecht F (1967) Reproduction and meiosis in XYY. Lancet II:831

Tsipouras P, Myers JC, Ramirez F, Prockop D (1983) RFLP associated with the proalpha2(I) gene of human type I procollagen. J Clin Invest 72:1262–1267

Vergnaud G, Page DC, Simmler M-C, Brown L, Rouyer F, Noel B, Botstein D, Chapelle A de la, Weissenbach J (1986) A deletion map of the human Y chromosome based on DNA hybridization. Am J Hum Genet 38:109–124

Received August 28, 1987 / Revised November 6, 1987